

Analysis of relevant technical issues and deficiencies of the existing sensors and related initiatives currently set and working in marine environment. New generation technologies for cost-effective sensors

Deliverable D2.1 of the COMMON SENSE project

Jose A. Saez¹, Sergio Martinez Navas¹, Jordi Salat², Jaume Veciana², Joaqui Ballabrera Poy², Emilio Garcia-Ladona², Jaume Piera Fernández², Jordi Sorribas², Paolo Magni³, Andrea Satta³, Fabio Antognarelli³, Maurizio Avella³, Roberto Avolio³, Gennaro Gentile³, Mireno Borghini³, Katrin Schroeder³, Pablo Fanjul Bolado⁴, John Cleary⁵, Margaret McCaul⁵, Dermot Diamond⁵, Anita Grozdanov⁶, Fabio Confalonieri⁷, John Barton⁸, Ashad Mustufa⁸, Saskia Heckmann⁹, Emma Moynihan¹⁰, Eoin Murray¹⁰, Mike Challis¹¹, Alberto Ribotti^{3*}

¹ Leitat, Spain

² CSIC, Spain

³ CNR, Italy

⁴ DropSens, Spain

⁵ DCU, Ireland

⁶ FTM-UCIM, Macedonia

⁷ IDRONAUT, Italy

⁸ Tyndall, Ireland

⁹ SubCtech, Germany

¹⁰ TELLAB, Ireland

¹¹ CEFAS, UK

* corresponding author: Alberto Ribotti, alberto.ribotti@cnr.it



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REQUIREMENTS

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Deliverable 2.1

Analysis of relevant technical issues and deficiencies of the existing sensors and related initiatives currently set and working in marine environment. New generation technologies for cost-effective sensors

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Authors:	Jose Saez ¹ , Sergio Martinez Navas ¹ , Jordi Salat ² , Mireno Borghini ² , Jaume Veciana ² , Joaqui Ballabrera Poy ² , Emilio Garcia-Ladona ² , Jaume Piera Fernández ² , Jordi Sorribas ² , Paolo Magni ³ , Andrea Satta ³ , Fabio Antognarelli ³ , Maurizio Avella ³ , Roberto Avolio ³ , Gennaro Gentile ³ , Mireno Borghini ³ , Katrin Schroeder ³ , Pablo Fanjul ⁴ , John Cleary ⁵ , Anita Grozdanov ⁶ , Fabio Confalonieri ⁷ , John Barton ⁸ , Ashad Mustufa ⁸ , Saskia Heckmann ⁹ , Emma Moynihan ¹⁰ , Eoin Murray ¹⁰ , Mike Challis ¹¹ , Alberto Ribotti ³ ¹ Leitat, ² CSIC, ³ CNR, ⁴ DropSens, ⁵ DCU, ⁶ FTM-UCIM, ⁷ IDRONAUT, ⁸ UCC, ⁹ SubCtech, ¹⁰ TELAB, ¹¹ CEFAS
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EXECUTIVE SUMMARY

The last decade has seen significant growth in the field of sensor networks, which are currently collecting large amounts of environmental data. This data needs to be collected, processed, stored and made available for analysis and interpretation in a manner which is meaningful and accessible to end users and stakeholders with a range of requirements, including government agencies, environmental agencies, the research community, industry users and the public.

The COMMONSENSE project aims to develop and provide cost-effective, multi-functional innovative sensors to perform reliable in-situ measurements in the marine environment. The sensors will be easily usable across several platforms, and will focus on key parameters including eutrophication, heavy metal contaminants, marine litter (microplastics) and underwater noise descriptors of the MSFD.

The aims of Tasks 2.1 and 2.2 which comprise the work of this deliverable are:

- To obtain a comprehensive understanding and an up-to-date state of the art of existing sensors.
- To provide a working basis on “new generation” technologies in order to develop cost-effective sensors suitable for large-scale production.

This deliverable will consist of an analysis of state-of-the-art solutions for the different sensors and data platforms related with COMMONSENSE project. An analysis of relevant technical issues and deficiencies of existing sensors and related initiatives currently set and working in marine environment will be performed. Existing solutions will be studied to determine the main limitations to be considered during novel sensor developments in further WP's.

Objectives & Rationale

The objectives of deliverable 2.1 are:

- To create a solid and robust basis for finding cheaper and innovative ways of gathering data.

This is preparatory for the activities in other WPs:

for WP4 (Transversal Sensor development and Sensor Integration),

for WP(5-8) (Novel Sensors) to develop cost-effective sensors suitable for large-scale production, reducing costs of data collection (compared to commercially available sensors), increasing data access availability

for WP9 (Field testing) when the deployment of new sensors will be drawn and then realized;

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1 INTRODUCTION

1.1 Background

Reliable, high quality, and high resolution information about water quality is essential for water management and for improving the quality of water resources. However, existing monitoring practices are unsatisfactory for a variety of reasons. Monitoring of environmental waters is still predominantly based on manual sampling followed by laboratory analysis using standard techniques such as colorimetry, atomic absorption spectroscopy, mass spectrometry and ion chromatography. While this approach yields high quality and reliable data (assuming that the appropriate protocols are followed during collection, transport, storage and analysis of the sample), the cost per sample is significant due to the manpower requirement for sample collection as well as the cost of analysis. This approach is therefore incapable of meeting the demand for monitoring at the much higher temporal frequencies and geographical densities which are envisaged under, for example, the Water Framework Directive. This is particularly true in relation to oceanographic monitoring, due to the additional challenges associated with deploying sensor technology in the marine environment. These include:

- High biofouling potential.
- The corrosive nature of seawater.
- Complex and variable sample matrix.
- Wave/tidal action.
- Pressure and temperature effects.
- Communications range constraints.
- Access limitations.

The COMMON SENSE project aims to support the implementation of European Union marine policies such as the Marine Strategy Framework Directive (MSFD) and the Common Fisheries Policy (CFP). The project has been designed to directly respond to requests for integrated and effective data acquisition systems by developing innovative sensors that will contribute to our understanding of how the marine environment functions.

The core project research will focus on increasing the availability of standardised data on: eutrophication; concentrations of heavy metals; microplastic fraction within marine litter; underwater noise; and other parameters such as temperature and pressure. This will be facilitated through the development of a sensor web platform, called the *Common Sensor Web platform*.

2 METHODOLOGY

This deliverable is a review of scientific literature, existing sensors and relevant commercially available sensors for in-situ monitoring. It is an aseptic and exhaustive review and analysis of existing sensors and related projects and initiatives currently set and working in marine environments in EUROPE. This deliverable consists of an analysis of state-of-the-art solutions for the different sensors and data platforms related with the COMMONSENSE project. Existing solutions will be studied to determine main limitations to be considered during each sensor's developments.

The COMMONSENSE consortium offers a good balance between RTD and industrial partners. It gathers a multidisciplinary and multi-sectorial team with relevant background and scientific expertise in different fields including GOOS, INSPIRE, in-situ ocean monitoring, sensor technology, multifunctional sensors, data collection, web based services for accessing and viewing data, sensors' networks, oceanographic and marine knowledge, marine observation, knowledge of diverse platforms (research vessels, ocean yachts, buoys, etc), new generation technologies, technology transfer, EU legislation (MSFD, WFD, IMP, etc.).

In consultation with the WP2 task leaders and the other WP Leaders, a comprehensive matrix was generated of topics to be reviewed for this deliverable. Some of these topics have been moved to





D1.4 to avoid significant overlaps. Each topic was then assigned a partner and a named 'champion' to perform the research. The 11 partners who contributed to D2.1, experts in their own fields, have used that domain knowledge to perform an exhaustive review of scientific literature, existing sensors and relevant commercially available sensors.

The deliverable has been obtained thanks to a thorough search on the web and a peer review of the publications available up to date from the most important marine research institutes and from the most important industries presently active in the marine monitoring and survey market.

3 RESULTS

The result of the deliverable for a best interpretation has been subdivided in following main headings:

- Existing sensors
- Novel Sensors
- Antifouling
- Remote sensing
- Communication and positioning systems
- Sensor network operating systems
- Miniaturization
- Energy storage and usage



4 DISCUSSION

4.1 Existing sensors

As in many other fields of technology, the ideal sensor or transducer does not exist. In fact, every specific transducer produced is always a compromise between the performance specifications needed, the technologies available and the affordable manufacturing cost. Apart from this simple concept, how successful a sensor is mainly depends on the capability to design and manufacture the small quantities required by this rather small research orientated market at the right time.

4.1.1 Nitrates

Excessive input of nutrients into natural water is a major pollution problem in the world today, hence the need for low cost monitoring sensors. It is important to control and monitor this nutrients level in order to reduce pollution and protect aquatic life. The variety of sensors currently available in the market differ in so many ways related to the sensor design and this in turn affects the accuracy and precision of the measurements they produce in different types of natural waters. However, to design an effective sensor that will be deployed for use in harsh and remote environments important consideration must be given to the modification of the sensors such as rugged housing and components, efficient power and heat handling, internal data loggers and controllers, few moving parts, antifouling components and data processing. Having all of these elements in place allows in situ monitoring to be done accurately and in real time. Other important parameters include accuracy, precision, resolution and deployment time.

There are three common methods for measuring nitrate and nitrite in natural waters. This includes:

- 1) UV (Optical)
 - HACH Nitratax
 - Satlantic SUNA
 - TRIOS
 - ProPS
- 2) Wet chemical sensor
 - The Wiz probe
 - DPA probe
- 3) Ion selective electrode (Based on electrodes)
 - Vernier
 - Idronaut
 - HACH NISE
 - YSI (EXO)
 - FOXCROFT 300 Sensor

Each of these methods has its various advantages and disadvantages and it is briefly discussed below.

4.1.1.1 Ultraviolet Nitrate Sensors

UV nitrate sensors have been used during the past few decades for wastewater monitoring as well as for coastal and oceanographic studies [DV10; RLK08]. According to the US Geological Survey, nearly all the UV nitrate sensors operate based on the same principle using the property of dissolved nitrate to absorb ultraviolet light i.e. the absorbance of light by nitrate at a specific wavelength [PBD13]. The absorbed light is measured by a spectrophotometer and converted to a nitrate concentration. The instrument consists of a light source (deuterium lamp), collimating optics, a light path through the sample water, and a spectrometer with a photo detector. The resulting absorption spectra can then be analyzed using either an on board computer or after data recovery [JC02]. The simple UV method offers several advantages when compared to the other available nitrate sensors such as the wet

chemical nitrate sensors and ion –selective electrodes. The primary factor determining the measureable range of nitrate concentration is the optical path length – the path length must be short enough for adequate light to reach the detector but be long enough for measurable difference between the incident and transmitted light. Sensors with longer path length typically have a lower measureable range of nitrate concentration, whereas sensors with shorter path length allow for measurements over a greater range of concentrations. There are instruments currently available with path lengths of less than 1mm to more than 100mm, with 2 – 10 mm being most common path length in instruments used for freshwater and coastal deployments while longer path lengths can be used for clear water [PBD13]. The detection limits on the UV sensors depends solely on the optical absorption path. The detection limits of the current generation of UV nitrate sensors ranges from less than 0.01 to 1.0mg/L as N.

Specifications

- Pathlengths 1, 2, 5 mm
- Wavelength 220, 350
- Housing materials Stainless steel/titanium
- Lamp type: Xenon/deuterium
- Window Quartz
- LDL 0.01 – 1.0
- UDL (mg/L as N) 20 – 100

Advantages of UV Sensors

- High Resolution and precision
- Chemical free
- Fast response time (on the order of 1s)
- Large nitrate range
- Gives additional optical information in spectra

Disadvantages UV Sensors

- Expensive
- High power requirement
- High maintenance cost
- Subject to a range of optical interferences

Nitratax UV Nitrate sensors

The HACH Nitratax is a very simple, accurate and economical nitrate analyser. It uses advanced ultraviolet absorption technology to continuously measure the UV light absorbed by nitrate. It eliminates the need for reagents, sampling and does not require frequent calibration. It works based on the principle that molecular bonds i.e. NO_3 and NO_2 absorb UV light. As the concentration of nitrate increases the UV absorbance also increases. The nitrate sensor is a continuous reading sensor that utilizes a 2-beam ultraviolet absorption technology with a 1, 2 or 5mm path length. The built in photometer measures the primary beam, while a second beam of UV light provides the reference standard and it can be corrected for interference caused by turbidity or organic matter. The measurement range of the instrument is 0.1

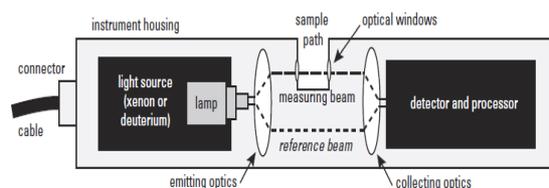


Figure 4.1.1 General design and key components of a field deployable ultraviolet (UV) nitrate sensors [LGPO4]



Figure 4.1.2. Showing the HACH Nitratax [HAC14 (a)]

to 100mg/L NO₂-N +NO₃-N. The UV sensor also has the ability to self-clean itself due to the built in wiper in the analyser and it retains a life- long factory calibrations.

Satlantic SUNA

The Submersible Ultraviolet Nitrate Analyser (SUNA) is a chemical free UV nitrate sensor based on the ISUS (In-situ Ultraviolet Spectroscopy) UV nitrate measurement. The SUNA V2 is very efficient in measuring nitrate over a wide range of environmental conditions. It comprises of a reduced 5mm pathlengths for high turbidity environment and an antifouling control measure. The SUNA can be used in both freshwater and sea water.



Figure 4.1.3. SUNA V2 [SAT14]

Specification

- Limit of detection: 0.5µM
- Detection Range: 3000 µM(10mm path length)
4000 µM(10mm path length)
- Path lengths: 10mm and 5mm
- Wavelength: 190 – 370nm
- Method: Sea water, freshwater
-

TriOS optical sensor

LISA is the new low-cost SAC254 probe in the TriOS family. The key features of the probe are longevity with innovative UV-LED technology, the rugged and smart housing for maximum flexibility of use as well as the novel and intuitive user interface via a Web browser. Its advantages include low-cost, ease of use, long lifetime, UV LED, robust, stainless steel or titanium, integrated temperature sensor



and compressed air cleaning. It is possible to exchange its path length. Also, window replacement by customer is possible with its modern technology.

Figure 4.1.4. TriOS optical sensor [TRI14]

ProPS - UV

The **ProPS-UV** is a submersible UV process photometer, which combines high precision UV transmission measurements with mathematical spectral analysing software in order to provide single substance concentrations from natural or artificial mixed samples. The system can determine simultaneously and online NO₃, NO₂, TOCeq, CODEq or many others parameters without chemical reagents. ProPS-Kits are cost-effective and innovative setups for the online measurement of nitrate. The individual kits include everything required for the measurement: ProPS-UV sensor, TriBox2



Figure 4.1.5. ProPS UV [TRI14]

controller, 10m sensor cable and 10 m tube for air cleaning. All systems are delivered ready-to-measure, with an installed pre-calibration for the ordered parameters, which works in most applications.

Key advantages include: newest anti fouling technology with nanocoating (no pollution of the medium), low maintenance costs (no chemical reagents or cleaning solvents needed), small sized, wide range of applications, easy upgrade options (new parameters can be added to an existing system) and adjustable path length.

UV Nitrate Sensors Comparison

Table 1. Comparison between the UV Nitrate sensors.

Parameter	TrioS ProPS	Satlantic SUNA	Nitratax
Pathlengths available (mm)	1 – 60 (Semi-fixed)	5, 10 (fixed)	1, 2,5 (Fixed)
Wavelengths measured (mm)	190 - 360	190 - 370	220 - 350
Maximum operating depth (m)	500	100	5
Lower detection limit (mg/L as N)	0.005 – 0.5	0.007	0.1 – 1.0
Upper detection limits (mg/L as N)	8.3 – 500	28 – 56	***
Accuracy	±2% or ±0.155mg/L	±10% of reading	±2% or 0.155mg/L
Maximum sampling Interval	120	1	60
Precision (mg/L as N)	0.03	0.028	0.1 – 0.5

4.1.1.2 Wet Chemical Nitrate Sensors

Most in-situ nutrient sensors use wet chemical techniques based on laboratory methods. A variety of this wet chemical nutrient analysers exist on the market. The analysis requires the addition of chemical reagents with nitrate to determine the concentration of target nutrients which can then be measured by photometry (JC02). The resulting solution develops a particular property (e.g. color) depending on the concentration of the target nutrient, which then can be measured. In some cases, heating of the solution is required to speed up the development. Parameters limiting the deployment time of wet chemical analysers are reagent consumption, reagent degradation time, available electrical energy (batteries) and bio fouling. A distinct advantage of the wet-chemical analyser is their capability to conduct in-situ calibrations by piping a blank or standard solution of known concentration into the analyser instead of the sample. Using this method any instrument drift can be detected and the measurements can be corrected for the drift.

Advantages

- High resolution, accuracy and precision
- In situ calibration can be done
- Fast response time

Figure 4.1.6. The WIZ probe[SYS14 (a)]

Disadvantages

- High power requirement
- High potential for fouling
- Requires reagents (generates wastes)
- High maintenance waste

The WIZ Probe (Water-In-Situ Analyser)

The WIZ probe is the state of the art portable in situ probe that can effectively measure automatically up to four chemical compounds in surface and sea water. It is designed in such a way that field deployment and easy handling is possible by a single user. The four main nutrients compounds N-NH₃, PO₄, NO₃ +NO₂ and NO₂ can be detected at very low levels, making the Wiz probe the most advanced analytical probe for field applications. The Wiz probe works based on spectrophotometric wet chemistry methods and advanced fluorometric methods.



DPA Pro (Deep-Sea Probe Analyser)

The DPA (Deep-see Probe Analyser) is a state of the art in-situ probe analyser, which can measure automatically up to four chemical compounds in surface and sea water. The DPA is designed in such

an innovative way allows for easy installation in coastal or floating platform and it can detect at trace concentration level the main four nutrients compounds: ammonia ($\text{NH}_3\text{-N}$) orthophosphate ($\text{PO}_4\text{-P}$) nitrate + nitrite ($\text{NO}_3 + \text{NO}_2$), nitrite ($\text{NO}_2\text{-N}$). It uses spectrometric wet chemistry method for the determination of the nutrients and advanced fluorometric method for ammonia measurements.



Figure 4.1.7. Deep-sea probe analyser (DPA) [SYS14 (b)]

Specifications

Parameters: $\text{NH}_3\text{-N}$, $\text{PO}_4\text{-P}$, $\text{NO}_3 + \text{NO}_2$, $\text{NO}_2\text{-N}$

Depth Rating: 30 meters

Operating Temperature: 4° to 40°C .

Sample rate: 30 minutes for a full four parameters sampled.

Wavelength: Fluorometric: excitation 370nm, emission 420 /470, 1cm.

4.1.1.3 Ion Selective Electrode

An Ion selective electrode also known as a specific ion electrode (ISE) is a transducer or a sensor that converts the activity of a specific ion dissolved in a solution into an electrical potential which is then measured by a voltmeter or a pH meter. The sensing part of the electrode is usually made as an ion-specific membrane coupled with a reference electrode. As the name implies, the ISE works based on a high degree of selectivity and the selectivity is simply determined by the composition of the membrane. When the membrane of the electrode is in contact with a solution containing the specific ion, a voltage, dependent on the level of that ion in solution, develops at the membrane. The membrane allows the uptake of only one specific ion which could either be an anion or cation into it. The transportation of an ion from a high conc. to a low one through selective binding with some sites within the membrane which creates the potential difference.

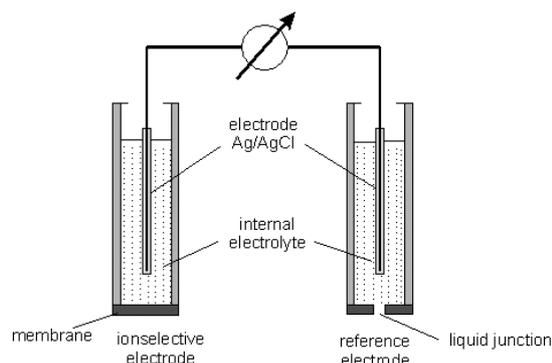


Figure 4.1.8. (ISE Set up)
www.ch.pw.edu.pl/~dyko/csrg/tutorials/ise/index.html

The ISE has four main types of membrane

- **Glass membrane electrode:** the glass membrane electrode is the most common of the three types of electrode due to its high selectivity and it is composed of a silicate glass. It determines the H^+ , NA^+ and Cd^{2+} activity or the pH. This glass membrane is very efficient in working in aggressive media and it has excellent durability.
- **Liquid membrane electrode:** this consists of a liquid ion exchanger incorporated in a PVC membrane.
- **Polymer membrane electrodes:** the polymer membrane uses a polymeric membrane which consists of a polymer such as polyvinylchloride (PVC) and an ion carrier or exchanger. It is an alternative to wet liquid membranes. Its' response is very selective and it has been used to replace many liquid membrane electrodes. The polymer membrane can be used to determine ions such as K^+ , Ca^{2+} , NO_3^- and Cl^-

- **Solid state membrane electrode:** The solid state electrode consists of an insoluble inorganic salt. (E.g. F^- ion electrode which uses a Eu^{2+} doped LaF_3 crystal in order to improve conductivity).

Advantages of ISE

- Non contaminating
- They can be used very rapidly and easily when interfering ions are not present.
- Short response time results obtained in sec or min.
- ISEs can be used in aqueous solutions over a wide temperature range.
- Unaffected by color or turbidity.
- Linear response: over 4 to 6 orders of magnitude.
- Non-destructive: no consumption of analytes.
- Low purchase and operation cost.
- Rugged and durable.

Disadvantages

- Interference by other ions is a major problem limiting the use of ISE.
- Precision is rarely better than 1% i.e. precise degree of interference depends on many factors.
- Electrodes can have limited shelf life and are very fragile.
- Electrodes can be fouled by organic solutes.

Vernier Nitrate ISE

The Vernier Nitrate ISE is used to qualitatively determine the concentration of specific ions like Nitrate (NO_3^-) in aqueous samples. The Vernier Ion-Selective Electrode uses the Solid Polymer membrane which is a porous plastic disk which is permeable to the ion exchanger but not to water. It has a combination-style non-refillable, gel-filled electrode. Like all other PVC ISE membranes, the membrane on the ISE has a limited life expectancy

It has a replaceable module which allows users to simply discard the used membrane module, and replace it with a new one, making the ISE very economical to use. Others species that can be measured include Chloride (Cl^-), Ammonium (NH_4^+) and Calcium (Ca^{2+}).

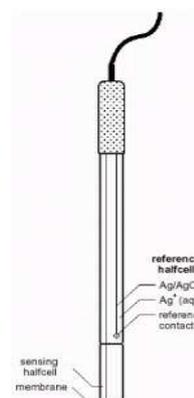


Figure 4.1.9. The Vernier ISE [VER14 (A)]

Idronaut Ion- Selective Electrodes (ISE)

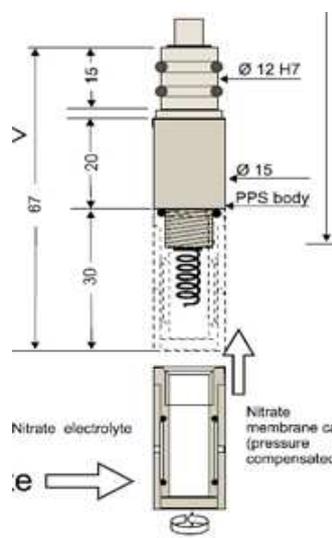


Figure 4.1.10. Nitrate Sensor Design [IDR09]

The Idronaut nitrate sensor belongs to the family of Liquid Membrane Ion Sensitive Electrodes. According to [IDR09], the nitrate ion selective electrode is the only ISE that is able to operate up to 200 bar pressure because its membrane cap is provided with a unique pressure compensation system which protects and avoids the breakage and stressing of the measuring membrane. The active membrane consists of an organic solvent which contains the ion exchanger. The ion exchanger is incorporated in a special PVC membrane, which is glued on top of the screwed membrane cap. It is very important that the membrane cap of the Nitrate sensor is filled with Nitrate electrolyte before applying pressure otherwise the measuring membrane will irreparably break when pressure is applied. Its' measuring range is from 0 to 100mg/l-N.

As mentioned previously, all ISE's including nitrate ISEs suffer a great deal from interference from other ions, such as chloride, bromide, bicarbonate, perchlorate and chlorate. Even though the ISE sensor is most selective to nitrate, other ions when found in high concentrations, can dominate the sensor response. Significant interference is not likely to be found in water with conductivity below $1.000\mu\text{S}$, but in sea water, which contains over 18.000mg/l of chloride ion, a nitrate sensor would read over 70mg/l-N for nitrate concentration, even in the absence of nitrate, because of its sodium interference. The nitrate sensor also performs poorly in salt water due to interference from chloride ions. Ag Ag/Cl electrode is common to Nitrate sensors and the sensor can be used in conjunction with the Idronaut solid gel reference sensor which is able to operate up to 700bar.

Advantage

- Inexpensive
- Easy to use
- Fast response time
- Large nitrate range
- Not influenced by color or turbidity

Disadvantages

- Low resolution
- Accuracy and precision
- Subject to ionic interference
- High instrument drift

Specification

- Range $0.10 - 14000\text{mg/L}$ or ppm ($7 * 7 \times 10^{-6} \text{ M}$ to 1.0 M)
- Low scale reading: 0.10 ± 0.0007
- High scale reading: $14,000 \pm 98 \text{ mg/L}$
- Reproducibility: $\pm 5\%$ of reading
- Temperature range (can be placed in): 0 to 50°C (no temperature compensation)
- pH Range: 2.5 to 11 , Interfering Ions: ClO_4^- , I^- , ClO_3^- , CN^- , BF_4^-

HACH NISE

The HACH Nitrate ISE is a probe that sets new standards in nitrate measurement using (ISE) to make continuous in-situ measurements. It offers a simple and reliable long term measurement through a catrical plus cartridge. It is calibration free with automatic chloride compensation thereby eliminating cross sensitivity between nitrate and chloride. No reagent is required. The catrical plus cartridge replaces the three separate electrodes and makes handling as simple as possible.



Figure 4.1.11. HACH Nitrate ISE [HAC14 (b)]

Specifications

- Measuring range: $0 - 1000\text{mg/L NO}_3 - \text{N}$
- Lower limit of detection: $0.5\text{mg/L NO}_3 - \text{N}$
- Response time: $<3\text{min}$
- pH range: $5 - 9$
- Flow: $4 < \text{m/s}$

YSI (DS5X Multiparameter Sonde) Sensor

The YSI sensor is an extremely versatile and rugged sensor that can be used for monitoring multiple parameters simultaneously. It is used for monitoring parameters such as ammonia, nitrate, and chloride. It is a type of ISE that has its reference electrode immersed in a solution of fixed ion concentration separated by a



Figure 4.1.12. The YSI and EXO Sensor [YSI14]

membrane containing a chemical compound which interacts with the ion of interest. It monitors ammonia or nitrate by tracing movement of point or non-point pollutants and by surveying aquaculture for excessive waste concentrations.

The YSI is designed in such a way that it incorporates a user replaceable sensor tip and a reusable base that houses the processing electronics memory, and wet-mate connector. The YSI Sonde sensor is most suitable for freshwater monitoring up to a maximum depth of 15 meters. The YSI has an improved power management and an anti-fouling component that is used to prolong deployment and improve data accuracy.

Advantages

- Long battery life and power management to extend deployment periods.
- Minimal power consumption.
- Field-replaceable sensors to save you time.
- High-impact plastic and titanium resists breaking.
- Long-term continuous monitoring.

YSI Sensors Comparison

Table 2: Comparison between the nitrate and ammonium YSI ISE sensor.

Sensor	Parameter	Range	Accuracy	Response	Resolution
Ammonium with PH sensor	Ammonium (fresh water only)	0 to 200 mg/L (0 to 30°C)	±10% of reading	T65 < 30sec	0.01mg/L
Nitrate	Nitrate(fresh water only)	0 to 200mg/l-N (0 to 30°C)	±10% of reading	T65 < 30sec	0.01mg/L

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4.1.2 Nitrites

Nitrite is an inorganic compound that is widely found in natural water, soil, fertilizers and food materials. In water, nitrite can react with secondary amines to form carcinogenic as well as mutagenic N-nitrosoamines. Nitrate also when present at high concentrations can be considered a pollutant since it can be reduced to nitrite and can be present as an intermediate step in the oxidation of ammonia [ZAE99]. The methods that have been described in literature for nitrite determination includes spectrofluorometry, chromatography, electrochemical detection, capillary electrophoresis and flow by injection [SM12]. Most of these methods are not suitable for routine determination of nitrite because some of them require expensive instruments or reagents and others are involved in difficult and time-consuming separation procedures. On the other hand, many analytical methods have also been proposed for the detection of nitrite and the majority of these methods employed for the detection of nitrite in water is based on colorimetric determination with diazocoupling reaction [OSL07]. Standard methods for nitrite analysis include colorimetric and ion selective methods.

4.1.2.1 Colorimetric method (The Griess method)

According to [DMN12], Griess proposed the reaction of sulfanilic acid 1-naphthylamine in the presence of nitrites and sulfuric acid and since 1879, the Griess method has been the most widely used colorimetric method for the determination of nitrites. Unlike nitrate, nitrite is usually present at a concentration lower than 0.01mg/L.

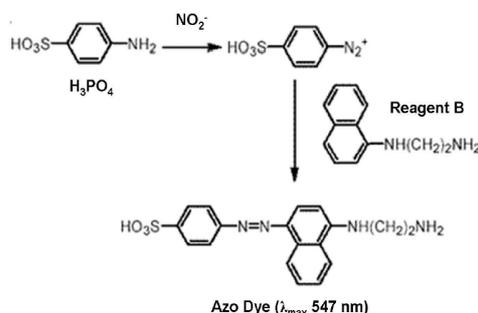


Figure 4.1.13. Showing the mechanism of nitrite detection using the Griess reaction method.

TresCon Nitrite Analyser

The TresCon online nitrite analyser works based on the azo dye method. A reagent reacts with nitrite to change the sample solution color to pink. The intensity of the pink color is proportional to the nitrite concentration in the sample and this is measured by a double beam reference photometer. The analyser is capable of monitoring pollution in natural waters and it can be used in weakly polluted water without sample preparation.

Figure 4.1.14. Trescon nitrite analyser. [WTW14]



Nitrite Colorimetric Online Analyser CL112

The CL112 is a microprocessor controlled online analyser that is specifically designed by AWA instruments for automatic nitrite monitoring on several types of water matrix.

This analyser is very user friendly and has low operating cost and maintenance. It is rugged and reliable very ideal for deployment and ensures the highest level of robustness in its electronics, mechanics and hydraulics components.

Figure 4.1.15. Nitrite CL112 analyser.[AWA14]



Specification

Colorimeter used: LED, photo detector

Measurement time: Approx. 15mins

Range: 0-0.36; 0-6.0; 0-30.0mg/l

Detection limit: <2% of full scale

Reagents consumption: 2500 measurement /liter

Weight: Approx. Kg 17kg

Fx-300 Online Monitor and Controller

The Foxcroft Fx-300 uses the ISE technology to monitor and control ammonia NH₃ and ammonium (NH₄⁺), Nitrate (NO₃) and Nitrite (NO₂) in fresh water. The FX-300 is designed to be a simpler, lower cost alternative to other conditioning analyser. It comprises of a unique membrane technology which does not need rebuilding or maintenance and it provides increased selectivity over interfering ions.

Advantages of the Fx 300 controller include:

- Very rugged ISE Sensors that require no reagents.
- Monitor nitrate or nitrite without interference from turbidity or chemical oxygen demand that can be problematic for optical sensors.
- No sample preparation is needed.
- No rebuilding.

Specifications for Nitrite FX-300 Sensor

- Concentration Range: 1 to 5 X 10⁻⁵ Molar, (46,000 to 0.460 ppm)
- Lowest Limit of Detection: 0.276 ppm (276 ppb)
- pH Range: 4 to 9.0 pH
- Temperature Range: 5 to 40° C Pressure Range: 1 to 10 psig
- Body Materials: CPVC, Ultem Junction: HDPE
- Interfering Ion: SCN⁻, ClO₄⁻, Br⁻, NO₃⁻, Cl⁻



Figure 4.1.16. Fx-300 Nitrite and Nitrate ISE Sensor. [FOX14]

4.1.2.2 Nitrite References

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4.1.3 Phosphates

Eutrophication as already known, is a major pollutant of the ecosystem. Therefore the monitoring of phosphate level is a key step in the protection of natural water and aquatic life. The concentration of phosphate in solution is found in small amounts because the inorganic phosphate in the water sample changes as a result of biological processes. Hence the need for a cost effective sensor that is both specific for phosphate in a complex medium and robust enough to withstand field usage in order to monitor the eutrophication process effectively.

In order to replace the acidification problems of the traditional method of colorimetric analysis being carried out on phosphate, various other detection strategies have been discovered for monitoring the concentration of phosphate online in order to allow for rapid detection and quantitative site evaluations. These methods includes electrochemical methods such as phosphate Ion selective Electrode based on potentiometric technique, Indirect voltametric detection based on the reaction of phosphate with various metals and associated complexes and the development of sensors exploiting enzymatic reactions [VMV09]. The main limitation in the development of phosphate sensors however is selectivity.

Types of Online Phosphate Analyser

- Electrochemical method
 - Potentiometric
 - Voltametric
 - Amperometric
- Colorimetric Method
 - Microfluidic based analyser
 - HaCH Lange 5000
 - AutoLab 4 Analyser (EnviroTech Instruments)
 - Mini Analyser (stevenswater)
 - ChemScan Uv-6100 Analyser System
 - Aztec 600 phosphate analyser
 - Cycle phosphate sensor

4.1.3.1 Electrochemical Method

Potentiometric, amperometric and voltametric sensing approaches are widely used in the measurement of water nutrients. The properties of the sensor changes as a result of the interaction with the different components being measured. The species of interest is either oxidized or reduced at the working electrode causing a transfer of electrons, thus generating a measurable signal

[KMA12] Potentiometric detection is the earliest direct electrochemical approach taken to the detection of phosphate in natural water [VMV09]. It works based on the operational qualities of other Ion selective electrode (ISE) sensors. It is inexpensive to operate, highly portable but however, it possesses some disadvantage such as poor selectivity and sensitivity below 0.1ppm. The measurement of phosphate concentration in a sample depends on the change in potential as the phosphate sample is added but its poor selectivity is a major drawback because related ions affects its potential and also its analytical signal. Interfering ions include chloride, bromide, iodide and sulphide [CMA97]. According to [VMV09], phosphate determination based on cobalt/cobalt oxide electrode has shown to be capable of detecting phosphate to 0.1ppm whilst retaining its high selectivity.

4.1.3.2 Colorimetric Methods (Molybdenum Blue method)

The standard method for the determination of phosphate is colorimetric which involves the addition of ammonium molybdate to orthophosphate sample with ascorbic acid and antimony (III) which then produces a blue coloured phosphomolybdate complex. The concentration is then determined optically by a spectrophotometer at a wavelength of 880nm. This method however suffers from interference from sulphides, silicates, arsenates and oxidizing agents. Another disadvantage is that acidification of samples may lead to changes in phosphate content due to hydrolysis of other phosphorus containing compounds and to the desorption of phosphate from suspended particles [ENG98].

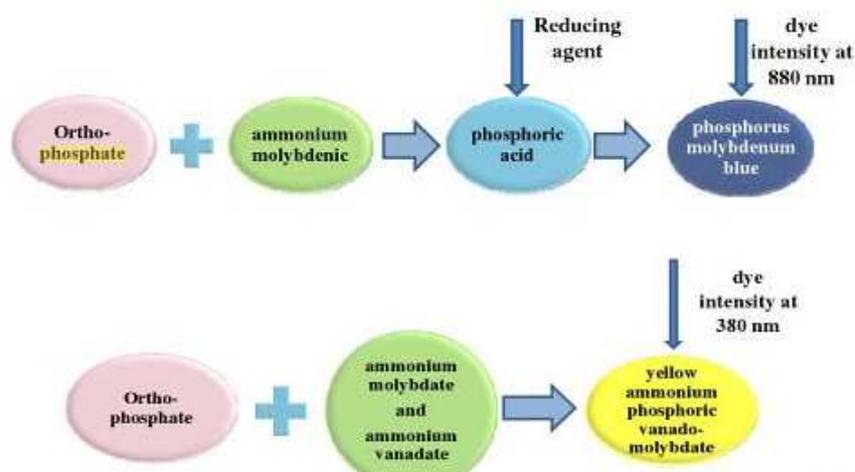
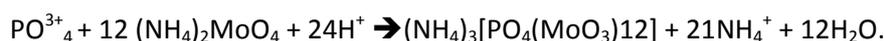


Figure 4.1.17. Diagram showing a standard UV-Vis optical methods: 1) Molybdenum blue method, 2) Molybdate yellow method [KMA12]

An example of this method is the Trescon phosphate analyser.

TresCon OP 510 (Blue Method)

The TresCon OP 510 is an automatic monitoring analyser that uses a 2-point calibration and high degree of accuracy. It uses the blue method for the determination of phosphorous in all natural waters. Its measuring principle consists of two units; in the first unit the (digestion unit), the sample undergoes a chemical thermal digestion whereby all the phosphorous compounds contained in the sample are converted to orthophosphate and this is determined photometrically.

In the second unit, the total phosphorous content is determined. Analysis using the molybdenum blue method is carried out whereby the sample is mixed with a molybdate reagent which reacts with phosphate via an intermediate chemical step to form the blue coloration. The intensity of this coloration is then measured photometrically.

4.1.3.3 Microfluidics Based Analyser (Molybdate Yellow Method)

The microfluidic analysis systems offer the ability to perform rapid analysis of samples while keeping the amounts of samples, reagents, waste storage and power to a minimum level. This analyser is developed such that it combines low power wireless communication systems with low cost analysis which can provide reliable information on the state of art of natural waters over a long period of time. The analyser is based on the molybdenum yellow method which is a simple colorimetric method. It involves the formation of vanadomolybdophosphoric acid when a phosphate containing sample is mixed with an acidic reagent containing ammonium molybdate and ammonium metavanadate [CSD09]. The absorbance of phosphate is measured at 370nm using a LED light source and a photodiode detector. The major advantage of this method is the stability of the reagent which can be used for up to a year without significant loss of activity therefore allowing longer deployment to be done without the need to change or replace the reagent solution. Other advantages include: portability, low cost and low power consumption.



Figure 4.1.18. The prototype phosphate analyser system [CSD09].

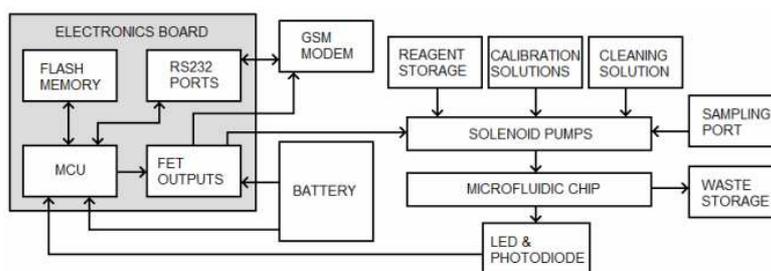


Figure 4.1.19. Schematic diagram of the analyser system, showing various components [CSD09].

Trescon Phosphate analyser (PO_4) yellow method

The Trescon PO_4 analyser works based on the vanadate/molybdate method (yellow method) for determining the orthophosphate content in natural water. A reagent reacts with phosphate in the sample to colour the sample solution yellow. The intensity of the colour is then recorded photometrically and evaluated as a measure of the phosphate content.



(a)



(b)

Figure 4.1.20. Trescon phosphate analyser using the 3(a) yellow method and 3(b) blue method [WTM14]

Limitations of the optical methods of detection

The advantages of phosphate sensors over traditional sampling techniques are fourfold and fall into the following categories - cost, time, accuracy, and mobility. According to [KMA12] the majority of the commercially available sensors that are based on colorimetric UV spectral suffer from a number of limitations such as sample handling and the acquisition of reference spectra and calibration process are necessary for sample of different origin.

4.1.3.4 HACH Series 5000 Phosphate Analyser

The HACH phosphate analyser is a continuous analyser that comes in two forms low range (LR) and high range (HR). The (LR) instrument is suited to monitor wastewater, drinking water, boiler water etc. and it gives continuous reading using ascorbic acid colorimetric method of analysis for measurement of phosphate at a wavelength of 880nm. It measures from a range of 0 to 5000µg/L. On the other hand, the (HR) uses molybdovanadate colorimetric method of analysis for measurement of orthophosphate at a wavelength of 480nm within the range of 0 to 50 milligrams/litre. The two analysers can work for 90 days continuously using only two litres of reagent.



Figure 4.1.21. The HaCH Series 5000 Phosphate Analyser [HAC14]

Specifications

	Series 5000 Low Range Phosphate Analyser	Series 5000 High Range Phosphate Analyser
Range	0 to 5000 µg/L as PO ₄	0.0 to 50.0 mg/L as PO ₄
Accuracy	±4 µg/L or ±4% of reading whichever is greater (typical)	±0.5 mg/L or ±5% of reading whichever is greater (typical)
Minimum Detection Limit	Less than 4 µg/L	Less than 0.2 mg/L
Precision	±1% of reading	±0.5 mg/L or ±5% of reading whichever is greater
Step Response Time	15 minutes	11 minutes

*Operating Conditions: Temperature: 10 to 50°C (50 to 122°F)

The Hach phosphate analyser auto zero on each sample analysis in order to prevent turbid and colored samples from interfering with analysis.

4.1.3.5 CL201 Phosphate Colorimetric Analyser

The CL201 is a microprocessor controlled online analyser specifically designed for automatic phosphate monitoring on several types of water. It has a dual compartments enclosure to ensure complete separation between electronics and hydraulics. The CL201 is very rugged and reliable and it is designed for industrial and environmental on-line applications. It has a low maintenance and low operating cost.

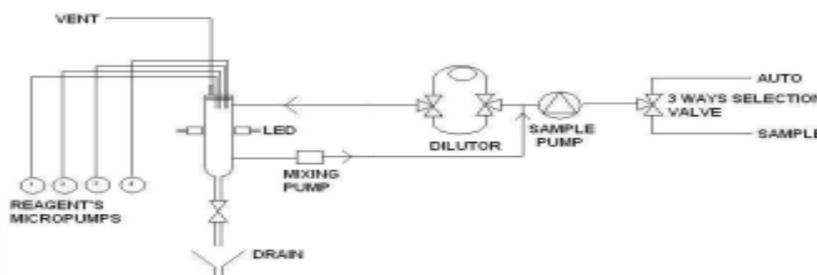


Figure 4.1.22. Mode of operation of the CL201 analyser [AWA14]

4.1.3.6 Autolab 4 Non Submersible Automatic Analyser (Envirotech Instruments)

The AutoLAB 4 system uses established wet-chemistry methods to determine nutrient concentrations through standard analytical techniques which allows a direct comparison with lab data and avoids interferences and fouling problems associated with optical systems. The AutoLAB is a pumped field station nutrient analyser that is capable of measuring up to multiple nutrients such as (ammonia phosphate, nitrate or silicate) simultaneously. It can run unattended for long periods thereby making it useful for long field deployment. It consists of high frequency sampling and integral self-calibration which makes it very effective and provides reliable



Figure 4.1.23. AutoLab4 Remote station nutrient/chemical analyser [KMA12]

nutrient data. The aqua 4 analyser is very cost effective, gives real-time nutrients measurements and can be used in riverine or freshwater monitoring [KMA12]. Nitrate is detected at a range of 0 - 5mg/L at 543nm and phosphate 0.08 mg/L at 880nm both with a sensitivity of 0.003 mg/L.

4.1.3.7 Mini Analyser (Stevenwater)



The mini analyser uses a colorimetric method to measure the phosphate level in water. It works based on the Molybdenum Blue method. Reagents added to the sample react with the dissolved phosphate present to produce a blue color. The higher the intensity of the color the more phosphate present in the sample. Measurements are carried out by using a spectrophotometer.

Figure 4.1.24. Mini Analyser (STE02)

4.1.3.8 Chemscan Uv-6100 Analyser System (Applied Spectrometry Associates (Asa))

The ChemScan UV-6100 is an online UV spectrometer capable of measuring the concentration of multiple dissolved chemical constituents of an aqueous sample with a single analyser [KMA12]. It operates by transmitting UV light through the sample and a portion of the transmitted light is absorbed by the chemical constituents and the analyser splits the resulting light into different individual wavelengths ranging from 200 to 450nm. Chemometrics is then used to solve and calculate the concentration parameters. Operating range for phosphate is from 0.01 mg/l to 5.0mg/l.



Figure 4.1.25. Chemscan Analyser System [KMD04]

4.1.3.9 Aztec 600 Phosphate Analyser

The Aztec 600 colorimetric series is an online-colorimetric analyser that has been designed specifically for measurements of phosphate in both potable water and raw water systems. It offers a reliable and accurate online analysis of phosphate up to 50ppm PO₄. All samples and chemical fluid handling used for measurements, mixing and disposal is controlled by the Aztec fluid handling system

that cleans the measuring cell with every movement. The analyser is designed in such a way that it can measure up to 4 samples per hour using the standard molybdate blue chemical method.

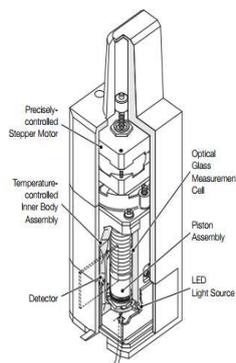


Figure 4.1.26. Aztec 600 phosphate analyser [ABB12]

The advantages of the Aztec 600 phosphate analyser includes:

- Real-time process control
- Improve process reliability
- Increase plant efficiency
- Continual monitoring of remote or unstaffed sites

4.1.3.10 Cycle Phosphate Sensor

The cycle phosphate sensor is designed for unattended long term operations. The cycle line combines precision micro-fluidics with state-of-the-art optics to provide unparalleled precision and accuracy in operational; in-situ monitoring of nutrients. The cycle PO_4 features automated, on-board calibration of each measurement sample against a standard solution.



Figure 4.1.27. Cycle Phosphate Sensor [WET14]

Specifications

Accuracy: 50 nM

Wavelength: Phosphate absorption 870nm

Sample rate: 2 per hour

Dimension: 56cm *18cm

Power supply: 9.5 to 18 VDC

Parameters: Designed for long term deployment.

4.1.3.11 Phosphate References

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4.1.4 Ammonia Sensors

The measurement of the concentration of ammonia is very important in freshwater and seawater monitoring. Ammonia is a chemical combination of elemental hydrogen (H) and nitrogen (N) and it occurs extensively in nature. The physical state of ammonia is dependent on temperature and pH, but pH is the determining factor. At a high pH, ammonia is expressed as NH_3 and it is referred to as free ammonia. In this state, ammonia NH_3 is a colorless gas that is readily soluble in water. At a low pH it is completely soluble in water and forms ammonium ions (NH_4^+) and other anions. The types of ammonia analyser listed below detect ammonia using different analytical methods.

Types of online ammonia Analysers

- Ion Selective Electrode
 - JUMO
 - Thermo Scientific
 - Q45N Dissolved Ammonia Monitor
 - Fx-300 Online Monitor and Controller
 - Idronaut
 - YSI Ammonia sensor
 - TresCon NH_4 analyser (WTW Xylem Group)
- Colorimetric Method
 - Aztec 600 ammonia analyser
- UV or multiple wavelength UV absorbance Spectrophotometer.
 - Nessler Regents
 - Berthelot's

4.1.4.1 Ion Selective Electrode (ISE) Ammonia Analyser

The online ISE ammonia analysers are probe type analysers that use a reference electrode and an ammonia ISE analyser. The ISE analyser feed sample through a flow cell whereby a reagent such as sodium hydroxide (NAOH) is added to the sample to raise its pH in order to convert all ammonia to free ammonia NH_3 . All the free ammonia released then penetrates into the ISE ammonia analyser membrane cap. The ISE analyser measures the change in pH of the membrane cap. The ISE is the cheapest method of analyser but it is not suitable for low-level concentrations, amines interfere with it and it is not applicable to some samples, such as sea water.

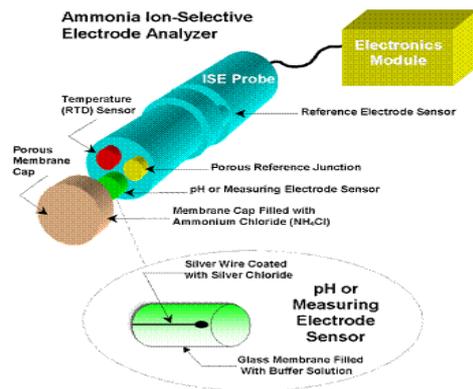


Figure 4.1.28: General Diagram of an ISE Ammonia Analyser [ITA10]

Disadvantage: ISE too insensitive to ammonium ions. They are subject to unacceptably high interference from sodium and potassium.

JUMO ISE

The JUMO ammonia sensor uses a fast and simple way to measure ammonia (NH_3) in aqueous solutions by using a membrane covered gas sensitive sensor that operates based on potentiometric principle. Ammonia when in an aqueous solution is in pH-dependent equilibrium with ammonium ions. If these ammonium ions are converted into ammonia by the addition of an alkali, then the sensor will detect the resulting ammonia gas. However, the ammonium ions themselves will not be detected. The JUMO sensor consists of a glass pH electrode and a reference electrode which are surrounded by a common electrolyte that is separated from the liquid being measured by a hydrophobic, gas permeable membrane. One of the advantages of the JUMO sensor is that measurement can be made directly in the liquid medium thereby eliminating unnecessary time consuming sample preparation. Also, a pre-fabricated membrane cap is available for the JUMO ammonia probe which makes the probe easy to quickly replace. The JUMO is very efficient in measuring seawater, fresh and salt water.

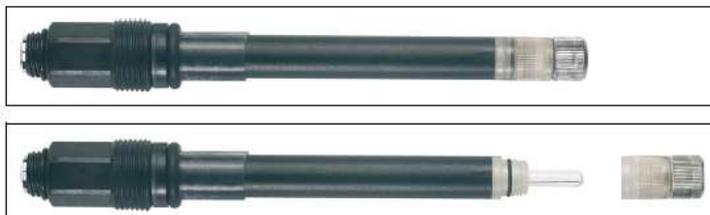


Figure 4.1.29. Showing a typical JUMO NH_3 Sensor. [JUM14]

Specifications

Measuring range

- 0.01 to 20000ppm (mg/l) NH_3 .
- Temperature range: 0 to 50°C
- Accuracy: +/- 2%
- Length: 120mm
- Diameter: 12mm

Thermo Scientific Gas sensing electrode

The gas sensing electrode works by measuring a pH change caused by diffusion of gas through a hydrophobic but porous membrane. The membrane is permeable only to gas thereby allowing only gas to pass through until partial pressure is equal on the both sides.



Figure 4.1.30. Thermo Scientific Gas sensing Electrode [THE14]

Advantages include: Direct measurement can be done on many samples

Precision is + or – 2%

Rapid measurement

Disadvantages include:

Interferences by ions: although this can be overcome by using an Ionic Strength Adjuster (ISA).

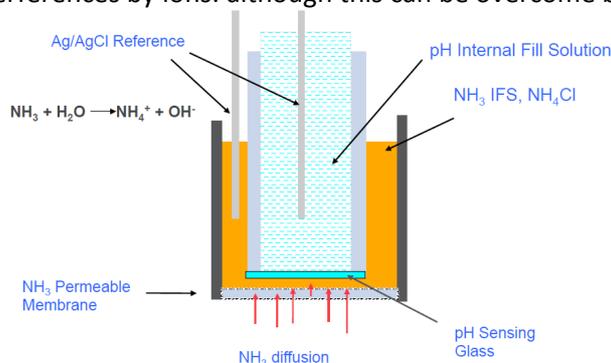


Figure 4.1.31. Ammonia Electrode structure

ATI Q45N Dissolved Ammonia Monitor

The dissolved ammonia monitor is a new low cost monitoring method which uses reaction chemistry that converts ammonia in solution to a stable monochloramine compound which is equivalent in concentration to the original ammonia level. The concentration of the chloramine is then measured with a unique amperometric sensor that responds linearly to chloramines while getting rid of interferences from excess free chlorine in solution.

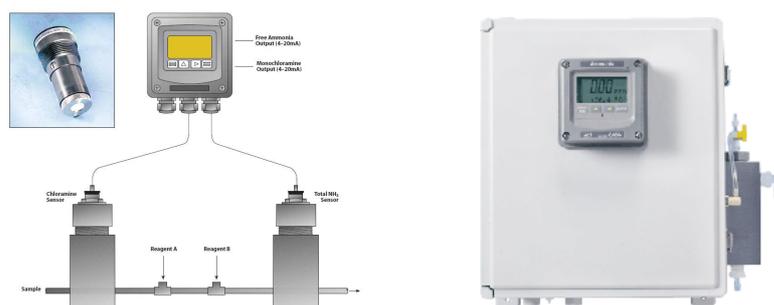


Figure 4.1.32. (a) Chloramination process monitoring and (b) ATI Dissolve ammonia monitor [ANA14]

Advantages

The dissolved ammonia monitor is very stable compared to typical ISE sensors which are subject to significant drift problems. The amperometric sensor provides excellent results which is repeatable and can be deployed over a long period of time. Furthermore, the ATI Q45N is both simple to operate and economical to purchase selling at 50% of the cost of many competitive ammonia monitors.

Specifications

Measurement: Total ammonia (as $\text{NH}_3 - \text{N}$)

Sensor type: Amperometric membrane cell

Ammonia Range: 0 – 2.000 ppm minimum, 0 – 10.00 ppm maximum

Monochloramine Range: 0 – 10.00 ppm

Response Time: 90% in 120 seconds

Accuracy: +/- 0.1 ppm or 2%

Fx-300 Online Monitor and Controller

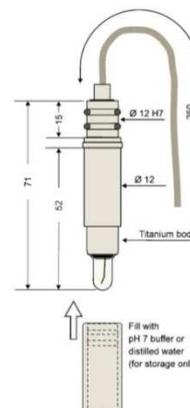
See Section 4.1.2.1 and Figure 4.1.16 for description.

The FX-300 NH_4 is capable of measuring ammonia in the presence of 20 to 30ppm potassium ions as low as 1ppm in the presence of up to 400ppm chloride ions while still maintaining its linearity and stability.

Figure 4.1.33. The Idronaut Ammonium sensor design

The Idronaut Ammonium Sensor

The Idronaut Ammonium, very similar to the Idronaut nitrate sensor is based on the same ion selective electrode mechanism as the nitrate ISE sensor. The ammonium ion selective electrode is the only ISE that is able to operate up to 200 bar pressure because its membrane cap is provided with a unique pressure compensation system which protects and avoids the breakage and stressing of the measuring membrane. The active membrane consists of an organic solvent which contains the ion exchanger. The ion exchanger is incorporated in a special PVC membrane, which is glued on top of the screwed membrane cap. It is very important that the membrane cap of the ammonium sensor is filled with a special ammonium electrolyte (NH_4Cl 0.01M) before applying pressure otherwise the measuring membrane will irreparably break when pressure is applied. Its measuring range is from 0 to 100mg/l-N.



YSI Ammonia Sensor



Figure 4.1.34. Showing a typical YSI sensor.

The YSI ammolyt plus sensor is an in-situ ammonium sensor with potassium compensation. It is economic, cost effective and calibration free. It has a long term stability and short response time. Measurement is carried out directly in the medium without sample preparation.

The YSI ammolyt plus sensor is an in-situ ammonium sensor with potassium compensation. It is economic, cost effective and calibration free. It has a long term stability and short response time. Measurement is carried out directly in the medium without sample preparation.

The sensor consists of a Ag/AgCl wire electrode in a custom filling solution. For the determination of nitrate the internal solution is separated from the sample by a polymer membrane, which selectively interacts with nitrate ions. For the determination of ammonia the internal solution is separated from the sample medium by a non-actin membrane, which selectively binds with ammonium ions. The sum of the two forms of ammonias (i.e. unionized and ammonium ions) is then reported in form of total ammonia. See Section 4.1.1.3 and Table 2 for more information

TresCon Ammonium analyser (NH_4)

The TresCon NH_4 analyser used for the continuous determination of ammonium is based on potentiometric measuring principle with a gas-sensitive NH_3 electrode. NaOH is added to the thermostated sample to convert the ammonium dissolved in the medium into undissociated ammonia gas. The gaseous ammonia alters the pH registered by the measuring electrode this alteration is then used for direct measurement of the ammonia concentration in the sample. Calibration of the analyser is done automatically. The TresCon has a short response time and continuous monitoring is possible.

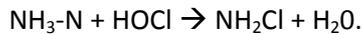


Figure 4.1.35. TresCon NH_4 Analyzer [DIR14]

4.1.4.2 COLORIMETRIC ANALYSERS

A colorimeter is a light intensity meter capable of measuring the intensity of light at a specific wavelength. Colorimetric ammonia analysers use this colorimeter to measure the color intensity of sample solutions. The analyser is set at a wavelength of 645nm –

655nm. The color is produced by the addition of reagents to the sample and its intensity is proportional to the free ammonia concentration in the sample. The free ammonia in the sample is first converted to monochloramine by the addition of hypochlorous acid.



Disadvantage: Colorimetric test kits are inaccurate at low levels and impractical for field use.

The Aztec 600 ammonia

The Aztec 600 ammonia is an online colorimetric analyser used in the monitoring of ammonia. It comprises of a unique fluid handling system with the latest electronics platform. It is very compact yet very reliable in continual monitoring of ammonia in potable water applications. The Aztec 600 can measure up to 4 samples per hour using the indophenol blue chemistry to measure ammonia and ammonium ions up to 3ppm. "The samples and chemical fluid is controlled precisely by the patented Aztec fluid handling system that cleans the measuring cell with every movement"

Specifications

Accuracy: < ±5% of reading or ±0.005ppm
 Repeatability: < ±5% of reading or ±0.005ppm
 Resolution: 0.001ppm or 1ppb
 Calibration: 2-point, automatic calibration
 Sample flow: Continuous

4.1.4.3 UV or multiple wavelength UV absorbance Spectrophotometer

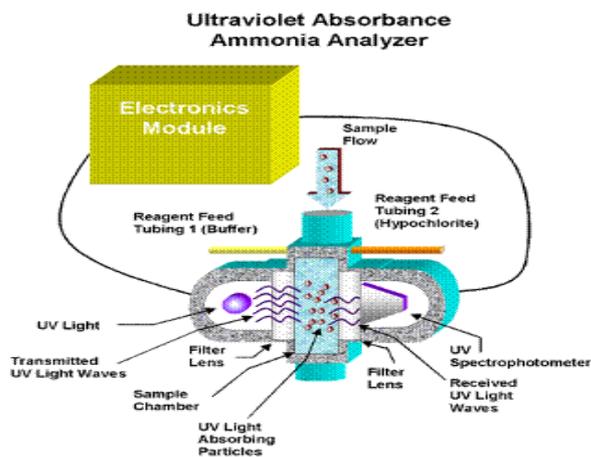


Figure 4.1.38. Ultraviolet Absorbance Spectrophotometer Ammonia analyzers [ITA10]

analyser measures the difference in the transmitted UV light versus the light that passes through the UV spectrophotometer. Some UV ammonia analyser uses multiple paths of UV light to adjust for turbidity or other interferences.

Disadvantages of the UV ammonia analyser however is interference from turbidity and some inorganic ions like chlorine.

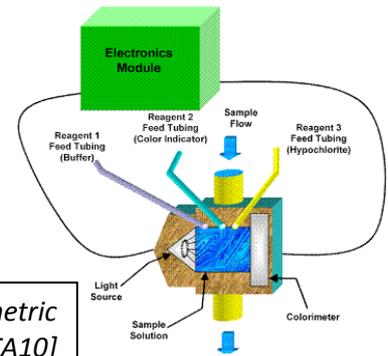


Figure 4.1.36. Colorimetric Ammonia [ITA10]

of ammonia. It

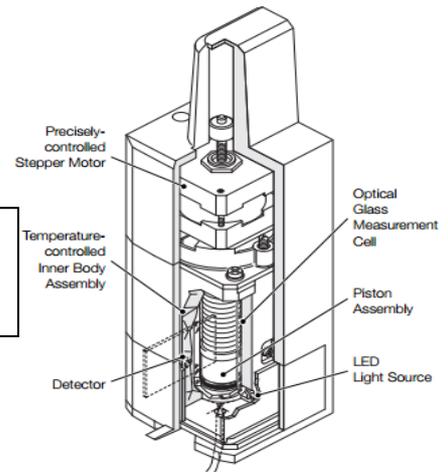


Figure 4.1.37. A typical Aztec ammonia analyzer[ABB12]

Ultraviolet absorbance spectrophotometer uses ultraviolet light source to measure the absorbance of UV light waves passing through a sample. Generally, the UV light absorbance analysers is calibrated to measure the wavelength of UV light within a range of 200nm – 450nm. Measurements of ammonia concentration is done by collecting a sample and the addition of reagent to the sample that acts as a buffer. Hypochlorite solution is also added and this reacts with free ammonia in the sample to form monochloramine. As the UV light strikes the sample, some of the light is

absorbed by the monochloramine concentration of the sample and the remaining UV light passes through. The ammonia

Spectrophotometry is a technique where a specific reaction causes a coloration of an analyte [TOV05]. There are two classical examples of spectrophotometric method for ammonia determination namely:

- Nessler's reaction
- Berthelot indophenol reaction

Nessler's Reaction: The Nessler reaction method is readily available and it is mostly used for determining the total ammonia concentration in water. It is based on the reaction between ammonia and tetraiodomercuriate (II), the latter being formed on site from mercury (II) iodide in alkaline potassium iodide medium. A colloidal dispersion is formed as a result and this is detected by absorbance measurements at 400 and 425nm. The reagent used is, however, very toxic hence the use of the Nessler is very limited due to its toxicity.

Berthelot's Reaction: This is the second coloration method mostly used to measure ammonia concentrations in aqueous solution. The reaction is less toxic compared to the Nessler method as the reagents used are less dangerous and are water soluble [TOV05]. One disadvantage of the Berthelot however, is that the kinetics is rather slow. The detection limit is about 5µM of NH₄ in water. The solid-state ammonia sensor is an example of a sensor based on Berthelot's reactions which requires relatively slow reactions between ammonia and phenol in the presence of chlorine (sodium hypochlorite) from indophenol blue. The time required for an uncatalyzed reaction may take over an hour, therefore elevated temperatures have been used to speed up the colour development in order for results to be obtained in a much faster rate [LED04].

Bulk Acoustics Wave sensor

The acoustic wave sensor is a type of sensor that is currently being proposed to replace the traditional methods of Berthelot's spectrophotometric reaction. It uses a piezoelectric quartz crystal coated with a sensitive film [AGN10]. This sensor has a highly sensitive response compared to conventional methods and it can be applied to sample solutions which contains a large amount of unreacted foreign electrolytes. When the sensor probe comes in contact with a sample, gaseous ammonia will diffuse across the gas-permeable membrane and dissolve into the internal electrolyte solution until the ammonia partial pressures in the sample and in the thin film of the internal electrolyte solution are equal [XLC96].

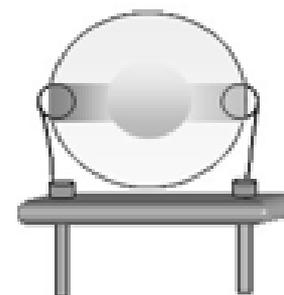
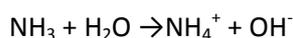


Figure 4.1.39. Schematic view of the piezoelectric quartz crystal with gold electrodes.

As the concentration of ammonia in the sample increases the amount of ammonia present in the internal electrolyte solution increases also and this in turn increases the conductance of the internal electrolyte solution.

One drawback of the acoustic wave sensor is interference from volatile species like amines [XLC96]. The acoustics sensor when compared to potentiometric ammonia sensor has a higher detection limit of 1×10^{-7} and a quicker response time and it does not require an electrode.

Table 3: Comparison of Acoustic Wave and Potentiometric Sensors.

	Detection Limit	Response	Reference Electrode
Acoustics Wave sensor	1×10^{-7}	70	No
Potentiometric sensor	Ca. 10^{-7}	110	Ag/AgCl

*NH₄ concentration changes from 1×10^{-6} to 1×10^{-7}

4.1.4.4 Ammonia References

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4.1.5 Chlorophyll Sensors

Chlorophyll concentration is traditionally used as an indicative index of the phytoplankton abundance in the sea water, although there cannot be a general conversion from chlorophyll concentration to phytoplankton biomass, since the amount of chlorophyll per unit of biomass largely varies from one to another species and according to other environmental conditions. Chlorophyll concentration could also be indicative of the primary production by phytoplankton, although in addition to the above mentioned limitation, the amount of C fixed depends on many other factors such as light availability and physiological status of each phytoplankton cell [AME02]. Moreover, there are other primary producers such as bacteria that contribute, sometimes in a relevant fraction, to the total primary production [LF95]. So therefore, why is this such a popular measurement? Because *it is relatively easy to obtain a semiquantitative indication of the environmental conditions of the waters*. For example, if at a given location the environment is clearly oligotrophic, then chlorophyll concentration must be low, but if in a survey, chlorophyll concentration is found high, then it is a clear indication of eutrophication. It can also be used to monitor the effectiveness of restoration plans in human perturbed marine environments and is a key parameter for monitoring according to the EU directives: [EU00] and [EU08]. At sea, chlorophyll will only be found at depths where light is able to penetrate. In particular a segment of the light spectrum that is called Photosynthetic Active Radiation (PAR) which has two peaks: ~440 and ~665 nm (Fig. 1). Since water is absorbing the different components of light more or less rapidly, according to the extinction curve of PAR, there is no chance for chlorophyll to get any energy from light below 200m depth.

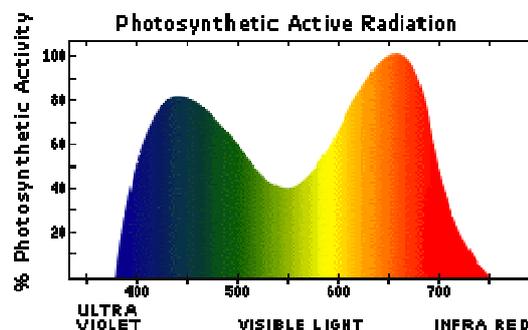


Figure 4.1.40. PAR spectrum showing the two peaks of maximum absorption. Note that the green colour corresponds to the minimum absorption

However since water transparency also depends on the presence of particles, the higher phytoplankton concentration in the upper layers the lower is the penetration of light in what is typically called self-shadow of phytoplankton. In stratified waters, far from any nutrient source from surface such as a river or sewage, or any other form of eutrophication, since there is no renewal of water, nutrients at the surface are being depleted.

Thus, phytoplankton can only survive concentrated in a thin deep layer that can still be reached by the light, at the edge of the dark nutrient rich layer. This feature is very common in oligotrophic seas during summer (the hot season) and is known as Deep Chlorophyll Maximum (DCM) [Est85]. Where there is a natural source of nutrients at surface, from upwelling to riverine discharge, as well as when there is eutrophication, most of the chlorophyll will be found at the surface.

4.1.5.1 Measuring chlorophyll

Since chlorophyll is not a dissolved matter but a component of phytoplankton and other living microorganisms, it must be measured from suspended particulate matter. It can be done either by extracting this component from particulate matter, or by measuring some property of the particles that could be related to the chlorophyll. We will refer to as *in-vivo* chlorophyll this last method in opposition to the extracted chlorophyll in the first case. To obtain the extracted chlorophyll, particles must be first trapped and concentrated in a filter. In that case, filters could also be used to obtain other relevant information as well as a proxy of the biomass. Moreover, if different mesh size filters are being used, chlorophyll values can also be referred to groups of organisms sharing a size range. For example, diatoms and coccoliths (two of the most abundant phytoplankton groups) can be easily separated, so also their relative contributions to total chlorophyll [SG06]. Once obtained, filters are processed and chlorophyll is extracted with acetone or methanol and measured by spectrophotometry, fluorometry or high performance liquid chromatography (HPLC), [JH75], [YM63] and [Lat96], respectively.

The measure of *in-vivo* chlorophyll is based either on the light spectrum absorbance, when a sufficient amount of water is being observed, or on the fluorescence response under UV excitation pulse. The first method being used are remote sensed measurements, typically images in the visible range of light from satellites or aircrafts, but also from cameras on top of towers, lighthouses, etc. Although this information may cover large areas it is restricted to the surface and upper layer chlorophyll while the contribution to the total chlorophyll by the deep layers such as the DCM cannot be recorded by this way. In particular, in oligotrophic environments during summer, total chlorophyll remotely sensed will be much underestimated. The second method, the fluorescence response, of measuring *in-vivo* chlorophyll is the most widely used for *in-situ* measurements, and will be presented and discussed below in the present report.

4.1.5.2 Fluorescence and *in-vivo* chlorophyll.

Chlorophyll is a molecule that is able convert light into chemical energy by changing its properties with the light action. The mechanism is too complex [KW91] to be detailed here but it can be summarized saying that the chlorophyll molecule will present two different states, stand-by and busy, as follows: We will say that chlorophyll is in stand-by when it is ready to receive photons, and busy otherwise, that is, when the energy obtained from photons is used to mobilise electrons.

When chlorophyll is in stand-by, a pulse of UV light will cause a fluorescent emission however if chlorophyll is busy fluorescence will not be emitted. Then, fluorescence can be used as an indicator of the chlorophyll assuming that it is in stand-by during most of the time. This is obviously true when there is no light in the environment, for example, during the night. However, in the presence of light, a fraction of the total chlorophyll would be busy and fluorescence measurement will underestimate the total chlorophyll. Under most of the conditions the fraction of busy chlorophyll used to be relatively low, but not always negligible.

[EMS96] found, comparing situations under similar light, that there can be differences in reaction because there is an additional quenching effect on the chlorophyll. The authors found that under

certain conditions the underestimation may be as high as 20% but in most cases it is not higher than 5%. In oceanographic cruises, typically fluorometers are used attached to CTDs or other oceanographic instruments, working on water pumped from surface. In all these applications, fluorometers have been calibrated from phytoplankton cultures before the cruise starts.

Then, during the cruise simple regression is used between *in-vivo* fluorescence and random samples where chlorophyll is directly measured from filters by extraction at certain points and depths to control the response in the natural environment. In any case, the best accuracy that can be obtained usually is not better than 5% because of the above mentioned problems in addition to the patchiness of the distribution of suspended particles.

Therefore, the problem of underestimation using fluorometers is not crucial for general purpose monitoring of environmental conditions regarding eutrophication.

4.1.5.3 Instruments

The fluorometers adapted to measure *in-vivo* chlorophyll consisted of a UV source, traditionally a Xn lamp, that now is being substituted by a LED source, especially in autonomous devices powered by batteries, but also those mounted on CTDs online to reduce the power supply required. A filter covering the captor cell ensures the correct wavelength.

Sensitivity used to be controlled by windows of different size that may change automatically, according to the light measured, or have to be set manually according to the characteristics of the zone to be sampled. All these characteristic are common for laboratory and submersible fluorometers. Laboratory instruments (Fig. 2) are able to measure *in-vivo* fluorescence use to be the same as for measuring extracted chlorophyll, adapted to the corresponding fluorescence levels. They can also be adapted for a continuous flow of water circulating instead of from samples. Submersible fluorometers have the light source and the captor forming a square such as captor cannot receive any direct light from the source (Fig. 3). Although functional chlorophyll can only be present in shallow waters, since the instruments are to be lowered in an array with other sensors (*e.g.* CTDs) to cover the entire water column, they have to be mounted in pressure resistant housings to permit different depth ranges. Shallow submersible fluorometers in a plastic housing will not be able to go down 100 or 200m, while housing made with titanium can safely reach any oceanic depth.

There are several manufacturers of oceanographic fluorometers. Turner Designs is considered the standard for laboratory use. Submersible fluorometers are manufactured by several companies such as: Turner Designs, Sea-Tech, Seapoint, WetLabs, Chelsea, among others. Although CTDs are prepared to admit anyone of them, there are some preferences and better adaptations.



Figure 4.1.41. Classic 10-AU Field and Laboratory fluorometer [TD11]. It is a field-portable instrument that can be set up for continuous-flow monitoring or discrete sample analyses with exceptionally high sensitivity

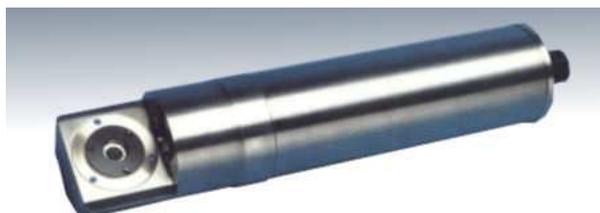


Figure 4.1.42. AquaTracka III (Chelsea), a compact, lightweight, submersible fluorometer for the detection of chlorophyll a, dye tracing or turbidity. Manufactured in titanium, it is rated to 6000m.

4.1.5.4 Concluding remarks

Fluorescence methods are the most widely used, either for chlorophyll monitoring, *in-vivo* or extracted. Fluorescence is a relative measure that needs to be calibrated with a known standard obtained from phytoplankton cultures. *In-vivo* measurements have the advantage of being quick and permitting a wide coverage, vertical or horizontal, if fluorometers are respectively attached to a CTD or connected to a water pump on a ship. Accuracy of the method however is relatively low because of physiological restrictions or interferences with other substances. For a more detailed information on fluorometric methods, there is a complete and comprehensive report on chlorophyll measurements in sea water [TD11], with a complete list of scientific references, that has been produced by Turner Designs, a specialist manufacturer of fluorometers for sea water.

4.1.5.5 Chlorophyll References

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4.1.6 Heavy metals – Cd, Pb, Zn, Cu, Hg

The release of different pollutants into environment has increased noticeably as a result of industrialization, and thereby, lowered the quality of the environment to alarming levels. Of such pollutants, heavy metals are the most important because of their non-biodegradability, with lead and cadmium ions being among the most toxic and hazardous. The presence of the trace elements such as copper, zinc, cadmium and lead in seawater may be a result of either human activity or due to the natural causes.

Important sources of seawater pollution by heavy metals in the coastal and bay areas include industrial wastes and liquid effluents and paint degradation of naval ships. Such metals as copper, zinc and cadmium at the low concentrations are necessary for both plants and animals as micronutrients and are involved in the several biochemical processes. In contrast at slightly higher concentrations they become toxic for the aquatic biota, e.g. copper and zinc are used as toxicant in the antifouling coating. Therefore, simultaneous and real-time detection of the content and bioavailability of multiple heavy metals in the coastal areas and industrial effluents is required.

Two types of water quality criteria are employed for the evaluation of the element toxicity to the biota namely acute and chronic water criteria [EPA03]. Chronic values are usually defined as the geometric mean of the highest concentration of a toxic substance at which no adverse effect is observed and the lowest concentration of the toxic substance that causes an adverse effect. The significance of the observed effects is determined by statistical tests comparing responses of organisms exposed to the low-level (control) concentrations of the toxic substance against responses of organisms exposed to elevated concentrations. Acute criterion is defined as concentration associated with 50% mortality for a fixed exposure. Data from several species are necessary for the definition of the both criteria.

Though water criteria are usually presented as total concentrations of an element in question, it has been established that free concentrations or activities of heavy metals and not their total concentrations primarily determine their biological availability and consequently toxicity [Sun83, Cam95]. Such metals as copper, zinc, lead and cadmium possess high affinity to the inorganic and organic ligands and sorptive surfaces abundant in the seawater. Due to this property activity of these metal ions in natural waters is several orders of magnitudes lower than their total concentrations and controlled by the “metal buffering capacity” of the system [BAF90]. Therefore, activity of the metal cations in the seawater is an indispensable parameter for the evaluation of their toxicity. Currently toxicity of a particular metal is estimated using several parameters such as total metal concentration, pH, total organic carbon, etc.

Usually, the presence of trace amounts of heavy metals in environmental samples is determined by spectrophotometric techniques [RHR84, RBC86]. However, the direct analysis of some complex samples like seawater presents some difficulties due to the high salt content, causing matrix interference and insufficient precision. Therefore, in such cases, a typical dilution of the sample may be necessary before the analysis, which in turns can aggravate the problem and leads to poor results.

4.1.6.1 Commercial portable heavy metal analysers

Water pollution is a worldwide problem affecting developing and developed countries alike. Heavy metal contaminants are one prevalent type of water pollutant. They are persistent in the environment once discharged and removal from source waters is necessary to ensure a clean drinking water supply. The problem of heavy metal pollution arises from several sources. Heavy metals such as uranium can naturally exist in ground water. Lead can be present as a result of lead solder in copper piping. Mercury and cadmium can be a result of power plant emissions. Additionally, a variety of industrial processes can produce problematic heavy metal concentrations in discharged water from factories that are harmful to humans and can contaminate agricultural land.

Limits on heavy metals in waste water and drinking water are often stated in parts per billion (ppb) and are heading in only one direction: down. Current techniques for detection of heavy metals in the low ppb range, such as inductively coupled plasma – mass spectrometry (ICP-MS), are expensive, requiring a six figure investment. These instruments are operated by a trained scientist and remain stationary once installed. Extensive sample preparation may be required and a single mislabelled sample can result in extensive downtime. These workhorse instruments excel at metals analysis, however a method simpler in implementation is advantageous.

A portable alternative would allow for on-site analysis in real time without expensive sample transportation and preparation. Contaminants could be monitored on a regular basis and high contaminant concentrations can be detected before harm is done. Such metals test kits are currently available. The most of them are based on voltammetry technology but there are too analysers based on colorimetric principles.

Addition of multiple reagents may be necessary, the test procedure for each metal is different, and the detection limits are usually not single ppb level. The USEPA’s (US Environmental Protection Agency) Maximum Contaminant Limits for Lead and Mercury (inorganic) are currently 15 ppb



Figure 4.1.43.
PDV6000 [MOD14]

and 2 ppb, respectively. Many colorimetric test kits fail to test below these levels.

PDV 6000 plus Portable Heavy Metal Analyser

The PDV6000 *plus* is an easy to use portable instrument designed to identify and measure the concentration of heavy metal ions in a wide range of sample types, both in the laboratory and out on site. Typical quantification limits are 5 parts per billion in the sample solution. The PDV6000 *plus* comprises a small analytical cell assembly and controller used together to provide a very convenient and portable tool for field screening for most heavy metals. The controller can be programmed with up to 10 analytical methods to detect 10 different metals. The results of the analysis are displayed on the controller screen. The PDV6000 *plus* is capable of better than 10% precision and accuracy used as a standalone instrument. When used in conjunction with VAS, a Windows software application provided with the kit, the PDV6000 *plus* is capable of highly accurate and reproducible metal ion analysis in the laboratory, with precision and accuracy better than 5%. The principle of analysis used by the PDV6000 *plus* is Anodic Stripping Voltammetry (ASV).

Table 4. PDV6000 Specifications

Analyser specifications	Include as Standard	Chemical specifications
Glassy carbon or gold working electrode	Waterproof carry case	Detection Limit with VAS 5 ppb
Silver/silver chloride reference electrode	Rechargeable NiMH battery pack	Detection Limit without VAS 10ppb
Platinum counter electrode	Battery charger	Resolution 1 ppb
10 Programmable working electrode analysis menus	Reference electrode plating accessory	Precision +/- 5% at 100 ppb using VAS
5 Programmable conditioning menus	110/240 V power supply	Standard Applications include: As (III), total As, Cd, Pb, Cu, Zn, Hg, Cr and Ni
RS232 interface	Cell stand	
On screen instructions	Resource CD containing application notes, manuals in PDF format and step by step guides	
DC motor and stirrer	15 Disposable analysis cups	
9V power supply via battery or main supply	VAS software	
Splash proof	Manuals	

It is realistic to expect the PDV6000 *plus* to obtain data in the field that is within 20% of the actual value. For this reason when used on site it is best to use the PDV6000 *plus* to classify samples as “above a threshold concentration” or “below a threshold concentration”. For example, using Pb, with a limit of 20 ppb in drinking water. The PDV6000 *plus* should be calibrated with a 20 ppb Pb standard and any result that is above 20 ppb less 20%, i.e. 16 ppb, should be considered as potentially being above the 20 ppb limit.

Portable Water Quality (Heavy Metals HM-3000P) Analyser [SKY14]

Portable Water Quality (Heavy Metals) Analyser is based on the authority-approved standard method, anodic stripping voltammetry (ASV), which features low cost and high precision and are replacing traditional atomic absorption method in Europe. It is widely applied on on-site environment detection, tap water detection, waste water testing of electroplate and surface processing industries, waste water monitoring of food, medicines, and hospitals. American EPA and other authorities have listed this method, such as EPA7063 and 7472. This instrument not only can be used for on-site application in urgent cases



Figure 4.1.44.
Heavy Metals HM-3000P

(for instance, on-site testing of contaminated water), but also can be applied for precise heavy metals detection in labs.

Advantages

- Rapid testing: 30secs to 5mins testing time
- Wide testing range: typical measurement ions include copper, cadmium, lead, zinc, mercury, arsenic, chromium, nickel, manganese, and thallium, etc
- High precision: analytical precision accounts to 1ppb and detection limit is less than 0.5ppb
- Electrode advantages: special make-up ensures better stability and it is easy to change and maintain
- Intelligent: intelligent operation program guide users finishing operation easily
- Low cost: cheap reagents and little amount of use
- Safe operation: non-toxic reagents ensure safety of users.

Table 5. HM3000P Technological Specifications

Sample name	Minimum detection limit (ppb)	Maximum detection limit (ppb)
Cu	0.1	60.0
Cd	0.1	60.0
Pb	0.1	30.0
Zn	0.5	60.0
Hg	0.1	25.0
As	0.1	30.0
Cr	0.5	20.0
Ni	0.5	30.0
Mn	0.1	10.0
Tl	0.1	30.0
Fe	0.1	30.0
Co	0.1	30.0

Application Fields

- On-site application in urgent cases (For instance, on-site testing of water pollution)
- Precise heavy metals detection in labs
- Water quality monitoring to surface water, underground water, seawater, industrial wastewater, drinking water and other waters
- Heavy metals testing in soil, food, and other waste solids (analytes should be extracted from solids before testing)

Nice Technology ASV3000 Portable heavy metal detector [NIC13]

Instrument suitable for the detection of heavy metal in water and soil based on anodic stripping voltammetry.

Product features

1. Large-screen color display, guide-type operation.
2. Can detect zinc, cadmium, lead, copper, mercury, arsenic, chromium, nickel, manganese, thallium, iron and other heavy metal ions.
3. Detection accuracy is better than PPB class, the fastest detection time is less than 30 seconds



Figure 4.1.45. ASV3000 Portable Heavy Metal detector

4. The unique portable workbench design, easy for field testing.
5. USB communication interface, supporting professional analysis software for online testing.
6. Nanometer process Electrode, stable performance and easy maintenance.
7. Professional analysis, design and package. It is suitable for a variety of water conditions.
8. Waterproof and durable.

Applications

For the determination of drinking water, surface water, beverage, environmental water, sea water, industrial waste and other heavy metals in the water column; this method is widely used to check the soil, food, solid matter content of heavy metals.

Metalyser HM1000 [TRA14]

The Metalyser HM1000 has been designed specifically to allow easier, cost-effective monitoring of heavy metals most commonly associated with health and environmental problems. Developed and manufactured solely in the UK, the Metalyser offers a breakthrough in terms of a simple-to-use field instrument that offers high levels of accuracy at an attractive price. Analysis of heavy metals in water has traditionally been difficult in the field. By combining the proven method of Anodic Stripping Voltammetry (ASV) along with an innovative sonde design and a simple buffer delivery system, Trace₂o has developed an instrument that can finally deliver reproducible results on site. The design of the Metalyser allows for the addition of future parameters, without the need to upgrade the instrument. 10 of the more common parameters are currently available for analysis using the HM1000. (Patent No. GB2481541).



Figure 4.1.46. Metalyser HM1000

Specifications

- Analytical principle: Anodic and Cathodic stripping voltammetry using disc working electrodes
- Parameters measured: Arsenic (III), Total Arsenic, Cadmium, Chromium, Copper, Lead, Manganese, Mercury, Nickel and Zinc
- Operating Temperature: -20°C to +70°C
- Results obtained in 5 minutes
- Internal memory: 1000 data sets with facility to enter sample number, time and date
- Transfer via USB to PC
- New application methods can be downloaded to the unit via USB
- LCD full graphics backlit display: 128 x 128 pixels
- Joystick cursor control
- Menu driven software
- Rechargeable battery providing in excess of 50 tests per charge
- Alternative power supply via mains adaptor or vehicle cigarette lighter
- Waterproof to IP67; CE Mark
- Dimensions: 470mm (L) x 370mm (W) x 170mm (D) Net Weight: 9kg

Table 6. Metalyser HM1000 Limits of detection (fresh water)

Parameter	Lower limit (ppb)	Upper limit (ppb)	WHO guideline value (ppb)
As (III)	5	500	<10
As (Total)	10	500	<10
Cd	3	500	<3
Cr (VI)	50	500	<50
Cu	5	500	<2000
Pb	5	500	<10
Mn	5	200	<100
Hg	5	500	<6
Ni	10	100	<70
Zn	5	500	<4000

Metalyser Deluxe HM2000 [TRA14]

Similar to the above analyser but voltammetric and photometric technologies (metalyser and metalometer) have been combined into a compact portable solution, enabling to provide the user with simple, cost-effective testing of heavy metals. Now the dual instrumentation provides the ability to measure 12 metals from ppb to ppm concentrations: Al, As, B, Cd, Cr (VI), Cu, Fe, Hg, Mn, Ni, Pb, Zn

Figure 4.1.47. Metalyser HM1000



Table 7. Metalyser HM2000 Limits of detection

Parameter Metallometer	Lower limit (ppb)	Upper limit (ppb)
Al	10	250
B	100	2000
Fe	20	3000
Cr (VI)	20	2000
Cu	50	5000
Mn	100	18000
Ni	100	10000

Figure 4.1.48. Istran Ltd PCA

Istran Ltd PCA (Portable Coulometric Analyser) [IST14]

PCA uses the principle of flow-through electrochemistry, coulometry and in-electrode coulometric titrations. The measurement is automatic and is controlled by a microprocessor.

Samples and sample preparation

- *Water samples (process-, tap-, surface-, underground- and waste water):* Simple sample pretreatment, just mix the sample with the reagent solution and inject.
- *Beverages and fruit- and vegetable-juice for determination of ascorbic acid or iodine:* Filter
- *Foods and food additives for determination of ascorbic acid or iodine:* To solve or leach and filter

Determined species

- Heavy metals in waters (Ni, Cu, Cr(VI), Pb, As...)
- Nitrates, phosphates, acids, bases, chlorides, ...in waters
- Dissolved oxygen in waters



- Ascorbic acid in beverages, food, food additives, fruit and vegetable
- Iodide in enriched beverages, food and food additives and others
- Determination limits below 1 mg/l.

Analysis

PCA is a portable instrument rechargeable battery operated. This enables simple and quick analyses in the field or in the laboratory. The sample is aspirated into the disposable syringe containing the reagent solution and on mixing the content is injected into the measuring cell. The calibration is carried out in a similar way. The result is then shown on the graphical display and stored in the memory. Up to 20 results can be stored and then they can be transferred to a PC.

Advantages

- Portable instrument for fast analyses in the field and the laboratory
- Broad linear concentration range
- Simple handling, sample-preparation and dosing
- No problems with coloured solutions
- Communication with PC enables download of software for determination of different species
- Communication with PC enables simple data transfer
- Advantageous price and low running costs

IDRONAUT VIP Voltammetric InSitu Probe [IDR14]

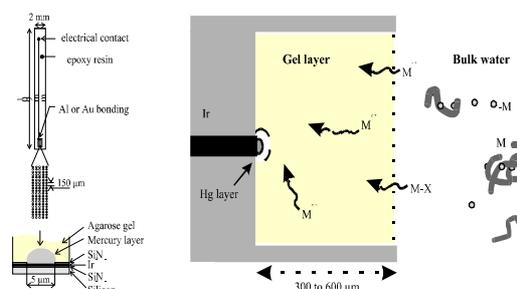
IDRONAUT submersible VIP allows reproducible and reliable in situ, continuous monitoring/profiling of trace elements in natural aquatic ecosystems can be performed. The whole VIP system consists of several units: the submersible voltammetric probe based on a unique microsensor, a submersible OCEAN SEVEN316Plus multiparameter probe, a calibration deck unit, a surface deck unit and a management software. It allows simultaneous measurements of Cu(II), Pb(II), Cd(II) and Zn(II) with a sensitivity at the ppt level, as well as Mn(II) and Fe(II) with a sensitivity at the ppb level (extension to other elements and organic compounds are foreseen). It is usable in sea and freshwater down to 500 m. It is controlled either by an operator from the surface, or in autonomous mode, under pre-programmed sequence. The heart of the VIP is its sensor. It measures a signal intensity, produced by the chemical reaction at its surface, which is proportional to the concentration of the analytes. However, to perform automatic measurements over extended periods in complex media such as natural waters, most of the currently available sensors are not reliable nor sensitive enough for monitoring very low concentration of chemical compounds.



Figure 4.1.49. Idronaut VIP

Figure 4.1.50. Idronaut VIP

In addition, fouling problem, due to the adsorption of organic and inorganic matters at the sensor surface, is an important limitation of direct voltammetric measurements in complex matrices. The VIP sensor is a unique microsensor which has been developed by CABE (University of Geneva-CH) and IMT (University of Neuchatel-CH) to solve all the above problems. It is produced by thin film technology on chips and photolithographic technique. It consists of an array of 5 x 20 interconnected Iridium microdiscs,



having a diameter of 5 μm and a centre to centre spacing of 150 μm , coated by Hg layers and covered by an hydrophilic protective gel membrane. Measurements with this gel-integrated microsensor are performed in two successive steps: a) equilibration of the gel with the test solution (typically 5 min for a membrane thickness of 300 μm) and b) voltammetric analysis inside the gel.

ANDalyze, Inc AND1000 fluorometer [AND14]

ANDalyze, Inc. has developed heavy metals tests based on catalytic DNA. Using catalytic DNA technology it is possible to reach single digit ppb level limits of detection and up to million-fold specificity over other metals. Colorimetric methods, though well developed, cannot easily match these capabilities. The selectivity and specificity comes from the ability of DNA strands, obtained through a process known as *in vitro* selection, to bind one metal preferentially over another.

Catalytic activity of the DNA strands or rapid binding to metal ions leads to very quick testing times – *e.g.* under one minute for Lead. To allow for portable heavy metals analysis, the selectivity of DNA has been paired with fluorescence detection – a very sensitive technique – in the form of a custom designed portable fluorometer. The AND1000 fluorometer is designed to work specifically with ANDalyze sensors.

Combining catalytic DNA chemistry with fluorescence-based detection results in detection limits well below the USEPA and other international regulatory limits for dissolved metals in water. Current metals that are detectable include Copper, Lead, Mercury, Zinc, Uranium with several other metals in development. The combination of catalytic DNA and fluorescence-based



Figure 4.1.51. AND1000 Fluorometer

detection was developed and patented at the University of Illinois at Urbana-Champaign. Catalytic DNAs (also called DNAzymes or DNA enzymes) are DNA sequences that catalyze the cleavage of another DNA strand (the substrate) in the presence of a specific cofactor. Active DNA sequences are incorporated into a porous material using a proprietary method and then placed in a plastic housing that allows for liquid flow from a syringe. During testing, the housing is placed over a cuvette and a buffered sample is passed through the housing using a disposable 1 mL syringe.

The sample flows through the housing and passes through the porous material containing the DNA. The amount of DNA in each sensor housing is so small that it cannot be seen with the naked eye, though it is easily detected using fluorescence. As soon as the DNA mixes with the liquid sample, an almost instantaneous process, the DNA begins reacting with metal ions in solution. Each metal ion leads to a cleavage event and a release of the substrate strand from the enzyme strand. This release also separates the fluorophore from the quencher, leading to a fluorescence signal that is directly proportional to the metal ion concentration. The AND1000 Fluorometer is a portable self-contained instrument, though it may be connected to a computer via USB if desired to download data. The AND1000 Fluorometer is used in conjunction with metal-specific kits from ANDalyze, and yet the fluorometer may be utilized independently as a research grade instrument. Fluorescence detection was chosen by ANDalyze due to the inherent high sensitivity of the technique and the sensitivity was enhanced further by using a photomultiplier tube (PMT) as a detector, which is uncommon in portable fluorometers. Excitation is accomplished using a light emitting diode (LED) and an excitation filter. An emission filter is located next to the PMT completes the setup. The AND1000 Fluorometer is recharged via USB or a wall adapter, is water resistant with an IP54 enclosure rating, and is CE marked.

Advantages of Catalytic DNA Technology

- Platform technology – analysis of metals as well as other analytes is possible using the same system with different cartridges and buffers for each target

- Low detection limits – all sensors currently for sale test at or below the USEPA limits for drinking water
- High selectivity - many classic metal analysis reagents react with multiple metals, whereas catalytic DNA has much higher selectivity
- Highly portable
- Reagents are not hazardous
- No contamination issues as kits are disposable
- Low initial investment compared to other solutions

Table 8. ANDalyze Limits of detection

ANDalyze Limit of Quantification (LOQ) versus USEPA Maximum Contaminant Level (MCL)			
Metal	USEPA MCL ¹	ANDalyze LOQ	ANDalyze Detection range
Lead	15 ppb	2 ppb	2-100 ppb
Copper	1300 ppb	40 ppb (low range) 600 ppb (high range)	40-200 ppb (low range) 600-3000 ppb (high range)
Uranium	30 ppb	2 ppb	2-60 ppb
Mercury	2 ppb	2 ppb	2-50 ppb

¹ Drinking water contaminants: <http://water.epa.gov/drink/contaminants/index.cfm>

Applications

ANDalyze test kits may be utilized in a wide variety of matrices from drinking water to industrial wastewaters and new applications are constantly emerging.

ANDalyze test kits were originally developed for testing drinking water and do not require sample preparation. Drinking water may include water from a utility or municipality, bottled water, and treated spring and well water. In short, any water intended for human consumption.

The utility of ANDalyze test kits has been expanded to include waters such as:

- Environmental fresh waters (rivers, lakes, streams, and ground water)
- Seawater
- Industrial process wastewater
- Municipal final effluent

4.1.6.2 Heavy Metal References

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4.1.7 MicroPlastic

4.1.7.1 Introduction

Due to the massive use of plastic materials from the second half of the twentieth century, a significant portion of the waste generated by the population is formed by these materials. Plastic debris dumped at sea comprises a variety of sizes from a few microns to several meters. Microplastics, usually ranged at >5mm, are plastic particles originated from industrial precursors (pellets) or by fragments of larger plastic parts and fibers caused by erosion. Although long-term effects of microplastics are still unknown, their observation is crucial because of its extensive presence in seas and oceans and its ingestion by marine biota.

For these reasons, in recent years efforts to monitor these microplastic debris have been intensified. In order to standardize obtained data, the EU Marine Strategy Framework Directive includes protocols for sampling and classification of microplastics. Following, a summary of the main current methods for sampling and characterization of microplastics and their main limitations is presented.

4.1.7.2 Sampling methods

Sampling methods for microplastics are strongly dependent of monitoring objectives (size range, spatial distribution or temporal variability) and the location of targeted microplastics (sea surface, water column or sediment). Within the Common Sense project, focus will be on seawater sampling for determination of microplastics concentration.

Different methods for seawater sampling have been reported in bibliography depending on required sample depth.



Figure 4.1.52. Net for microplastics sampling in seawater surface. The net is towed by a vessel for collecting solid samples. Courtesy of NOAA Okeanos Explorer Program

Sea Surface Trawls

For sea surface samples (up to 25cm depth) the most common method is dragging with different types of Surface trawls. The most commonly used nets for sampling the water surface are: Neuston nets, horizontal Bongo nets and Manta trawls [JRC11]. They are typically nets with medium-sized apertures which are designed to be trawled at (generally) low speeds behind the ships. Although different mesh sizes are reported (ranging from 80 μm to 2 mm), most of the studies have been done with mesh sizes in the range of 330 μm , to optimize trawling speed, and because those dimensions are also used to sample for plankton. The nets are usually combined with a flow-meter in order to

assess accurately the volume of water filtered during the sampling process. Once the sampling is done, the nets are lifted and their contents are taken to the laboratory for examination. The main advantage of this method is the capability of sampling large water volumes retaining only volume-reduced samples.

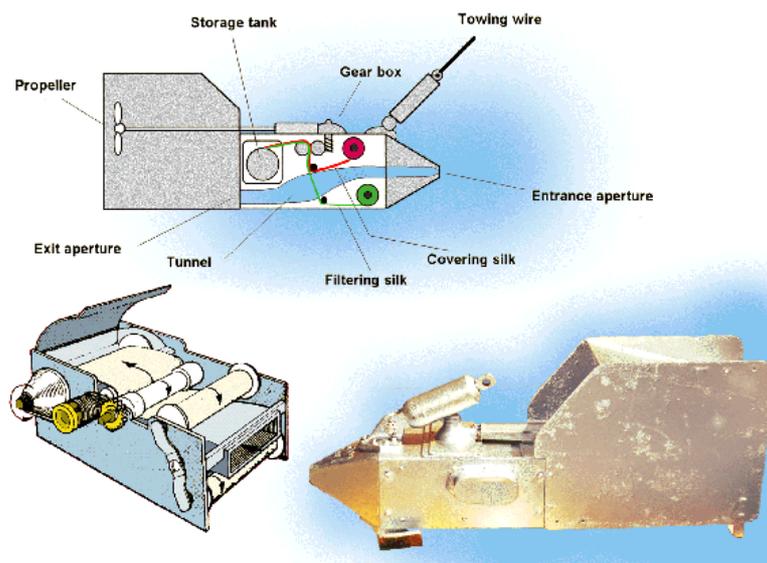
Rotating Drum Samples:

In some cases where samples are restricted to surface micro-layers (up to 1000 μm), a rotating drum sampler is used. This sampler consists of a partly immersed rotating glass cylinder with a clean hydrophilic surface, using capillary force to sample water from the surface micro-layer. Finally, for

seawater column sampling (up to 200m. depth), circular zooplankton nets with opening diameter between 0.8m to 1.6m and lengths ranging from 1m to 8m are commonly used [LVK11].

Continuous plankton recorder (CPR)

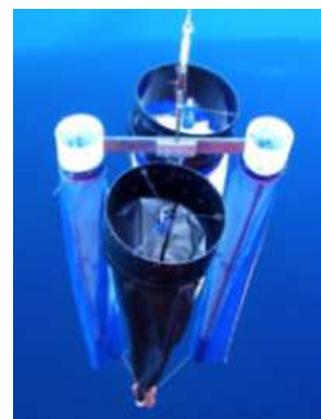
This instrument is used to sample plankton and is designed to be towed at a depth of approximately 10 m from merchant ships performing their normal activities. It works by filtering plankton and small pieces of debris from the water over long distances. The water enters the device by a 127mm² entrance located on the front of the device, and the plankton and other material are collected on a moving band of silk, with a mesh size of 270 µm. The silk bands are then viewed under the microscope and plastic pieces identified and separated.



Bongo/Plankton nets

Bongo nets are paired mesh nets attached to a metal frame which can be used to take samples throughout the water column, either under continuous operation while being towed, thereby filtering water at all depths, or, if provided with a remotely closable mouth, taking samples at only selected depths. As in the other two cases, once the sampling finished, the nets are taken to the labs for inspection.

Although those methods allow volume-reduced samples, none of them holds sampled water. This could be an advantage in those studies focusing just on microplastics but, in wider focus studies where cross-relations between different parameters are required, is a clear restriction. Furthermore, there is the risk of losing particles or collecting other types of materials in case the nets' mesh is not properly chosen, requiring sample preparation to avoid wrong results (under/over estimation).



Vertical Bongo Net

4.1.7.3 Sample preparation and characterization.

The methods described above allow particle selection according to their size by selecting different nets mesh. However, this method does not differentiate between different types of particles so it is necessary to apply additional steps to separate microplastics from other types of particles (sand, biota, etc.).

Again, the chosen method will depend on sample characteristics. For samples mixed with different types of sediment, the most widely used method is to separate the particles by density. For this, sampled particles are immersed in a highly saturated solution (with NaCl or SPT). While sediments will settle to the bottom, the lower density of microplastics will cause them to remain floating near

the surface, allowing separation by filtration or sieving. If samples are not mixed with sediments, it is possible to directly apply filtering and sieving methods to separate microplastics from other types of particles. Next step consists in visual sorting to remove organic marine debris or other elements. This step can be performed by naked eye or with the aid of a microscope. For this purpose, it is necessary to standardize the identification parameters of microplastics in terms of size, morphology, color, etc. Another possibility for sample characterization is to apply infrared spectroscopy techniques. These techniques identify microplastic comparing the IR spectrum of an unknown sample with the known spectrum of certain plastics. The advantages of these methods are that they avoid human error during visual sorting, and allow identifying and quantifying the different types of plastics [HGT12]. The different steps described for samples' preparation and characterization require the intervention of qualified specialist and transport of the samples to a laboratory. This, not only makes microplastics monitoring in the marine environment difficult and costly, it also prevents continuous and prolonged monitoring, hindering the obtaining of data about microplastics drift.

4.1.7.4 Analytical methods.

As mentioned above, there are different analytical methods for the identification and quantification of microplastics. Most of them are based on two spectroscopy techniques: Raman spectroscopy and Infrared (IR) spectroscopy.

Raman spectroscopy is based on inelastic scattering of monochromatic light. This means that the frequency of photons in monochromatic light changes upon interaction with a sample. When a sample is irradiated with a monochromatic light, photons are absorbed and reemitted by the sample with a different frequency than original monochromatic light. This is called the Raman Effect and can be used to study gas, liquid and solid samples. Typical Raman spectroscopy equipment is formed by the following elements:

1. Excitation source (usually a Laser).
2. Sample illumination system and light collection optics.
3. Wavelength selector (Filter or Spectrophotometer).
4. Detector (Photodiode array, CCD or PMT).

Main advantages of this technique are simple sample preparation and compatibility with wet samples and normal ambient. However, Raman spectrometers are designed for in lab operation and require skilled people to perform measurements. [Las01]

Infrared spectroscopy is based on molecular absorption of specific frequencies (resonant frequencies) that are characteristic of their structure (molecular potential energy surfaces, masses of the atoms and associated vibronic coupling). In IR spectroscopy analysis an infrared light beam is passed through a sample. When the IR frequency matches the sample resonant frequency, absorption occurs. By recording the sample's IR spectrum, it is possible to detect the resonant frequencies of the different elements forming the sample. Analyzing peaks at different frequencies (Fourier transform) it is possible to determine the composition of the sample [UC14].

The main advantage of IR spectroscopy is a relatively simple operation and the possibility of performing FT analysis using microprocessors. This characteristic makes this technique especially suitable to be used on mobile/portable equipment. On the other hand, its' application is restricted to samples with covalent bonds.

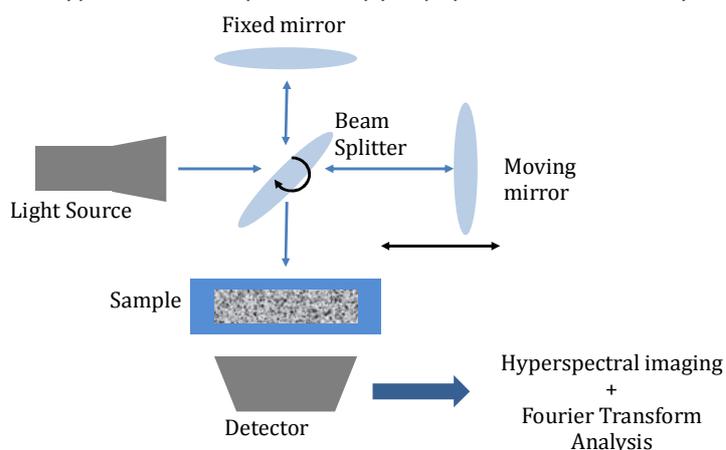


Figure 4.1.53. FT IR spectrometer scheme

4.1.7.5 Main existing limitations.

According to existing bibliography about microplastics monitoring in marine environment, the following main limitations in state of the art methods and technologies have been identified:

1. There is no standardized method for microplastics sampling and identification. Existing methods require human visualization for the sorting, and it is often quite difficult, in very small particles to distinguish plastic from other types of materials, like paints, wood, or organic fibers. This is especially difficult when no FTIR is used for the identification of the sorted material. This is expected to be partially addressed in Marine Strategy Framework Directive.
2. Most common sampling methods just discriminate particles by size, which cause a risk of “under estimation” in case that mesh size is not properly chosen. This is of particular concern for two reasons. Firstly, because, whenever an accurate sampling of small particles has been carried out, it has been observed that they are in fact much more abundant than larger particles. Norén & Naustall [Nor11], for example, found 100.000 times more particles in the same location when using a mesh size of 80 µm than when using a mesh size of 450 µm, and the concentrations were even higher for smaller mesh sizes. The other major concern with the particle size issue is that it would seem that, with microplastics, the smaller the particle size (and thus the surface to volume ratio), the higher the potential for toxicity [WGT13].
3. Sample preparation must be done in a laboratory by skilled personnel, causing possible loss of information due to sample transport and manipulation. Additionally, the lab processing is very time consuming, and the final identification of the material with FT-IR requires expensive equipment and a highly trained operator. This prevents in situ measurements of microplastics concentration.
4. Current sampling techniques are not well suited to evaluate the microplastic distribution along the water column, as shown by the conspicuous lack of data gathered to date on the subject. This is, however, a very important issue, as available time series do not show convincing trends in microplastic concentrations, implying that we are missing important issues, such as particle sinking due to particle fouling [GES12]. Modelling of microplastics behavior with ageing and the corresponding sinking rates is essential to understanding their impact on the marine fauna and the transport of alien species throughout the globe [LVK11]

From available sources of information, it is possible to conclude that there is a real need for monitoring the concentration of microplastics in the marine environment in order to determine their movement patterns (drifts) and its influence on marine biota. However, there is a lack of methods and technologies specifically designed for in situ continuous monitoring of microplastics.

Current image acquisition hardware should be perfectly adequate to the task, so the main challenges to be solved are:

1. Adequately presenting the sample so that the particles are clearly visible to the imaging system and it can easily discriminate between them and the background, even in cases where the plastic particles are clear or translucent. This would also require developing adequate optics and illumination systems.
2. Developing a software and corresponding data library to allow the system to discriminate between plastic particles and other type of suspended matter, such as plankton, paint chips, wood bits and organic fibers. Different strategies can be implemented to do this, from morphology criteria such as color, transparency and shape discrimination, to analyzing the reflectance/absorption of the particles at different illumination wavelengths.

Such an automatic imaging system would have clear advantages over current methods: it would be much cheaper than all other alternatives and could conceivably be used without requiring highly qualified personnel. With an adequate compromise between speed and resolution it could allow for the detection of much smaller particles than those that are currently being counted. Obviously it

would not be as reliable for identifying types of plastic as FTIR lab measurements, but it would be very useful to perform cheap, exploratory surveys at sea, rapid identification of hotspots and accumulation zones, which would then be used to plan future monitoring programs. A sufficiently advanced device, coupled to a simple sampling system, could in future be installed on commercial ships and operated during their normal activities.

Those points will be addressed in Common Sense project to improve data collection about microplastics by developing a new cost-affordable, small size microplastics sensor based on spectroscopy techniques requiring minimal sample preparation to allow its integration in different marine platforms (vessels, buoys) to enable in situ continuous monitoring of microplastics concentration at seawater surface.

4.1.7.6 Microplastics References

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4.1.8 Noise monitoring

The EU has requested Member States to reach Good Environmental Status by 2020. Noise has been identified as a pressure and a Descriptor (D11) has been defined for Underwater Noise. The descriptor requires monitoring of underwater noise. The MSFD requires noise monitoring up to 10kHz with specific data analysis on the 1/3rd octave bands centred respectively at 63Hz and 125Hz.

We find that systems that are on the market are designed specifically to meet particular needs, such as: a) detection of marine mammals for noise mitigation purposes, and, b) behavioural studies on marine mammals.

These are all bespoke systems with proprietary software and their ruggedness and durability has been tested in a limited number of field deployments. This does not guarantee the long term survival of the systems, and their success for multiple deployments.

The one thing they seem to indicate is that some degree of onboard pre-processing of acoustic data is desirable in order to limit storage and transmission needs. Also, the advantage of pre-processing is to ensure that demands, both for data transmission but also power consumption are kept to a minimum.

4.1.8.1 Commercially available systems

IC-Listen [OCE14]

The IC listen project is fairly innovative: four different products are offered to cover very low (nominally 1Hz) to high (200kHz) frequencies. All hydrophones come in an all-in-one packaging with batteries, storage and processing hardware. They are a portable solution that fits the traditionally distinct pieces namely hydrophone-preamplifier-filters-AD/DA converter-data storage into one package which is small and portable. Additionally they have a data input possibility for GPS or other data synchronization input. The setup and the access to data and settings during operation are achieved via web, which may prove very useful for operational changes that need to be carried out at short notice. In order to be fit for mobile monitoring solutions IC listen need to expand battery capacities and data storage (currently limited to 32GB). Also, it is unclear at this stage if IC records raw data (in wav or similar formats) and with which affective accuracy (24bits are declared). A further unknown is the possibility of calibrating the hydrophone in the field and if and how this can be done from the factory. To date the product is too young to be suitable for mobile prototype testing, and prices are unclear. However, it is certainly a very interesting tool to be monitored in the future.

Decimus [PAM14]

A system which is available on the market and that has been extensively tested is the pambuoy). The evolution to it is called Decimus, and has come very recently to the market. In substance, this is an autonomous sound recording-detection system that has been designed and built by the University of St Andrews in Scotland for the purpose of detecting and studying marine mammals for a variety of purposes, such as passive acoustic monitoring (PAM) as required with the oil and gas production industry. Currently a version is being adapted to be deployed of a surface unmanned service vehicle. Decimus is autonomous and bespoke-built and relies on proprietary hardware and software and is TM by SMRU Ltd and the University of St Andrews.

At the present stage it appears as if Decimus' operational target is different than that of the MSFD and that the fact that it is bespoke built limits its application.

DTAG-DMON [USN14]

Originally built at Woods Hole Oceanographic Institute for the Office of Naval Research, the DTAG-DMON family were very promising as autonomous and clever engineered sound recording devices with a number of additional capabilities that were developed for monitoring marine mammals

behaviour being deployed on board the animal. However, currently they are the property of the US Navy and are not made available for the wider market.

4.1.8.2 Noise Monitoring References

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{PAM14} Pambuoy Decimus www.pambuoy.co.uk Accessed 14 Aug 2014

[USN14] DTAG-DMON <http://www.onr.navy.mil/reports/fy11/mbhurst.pdf> Accessed 14 Aug 2014

4.1.9 Dissolved Oxygen

There are four common methods for measuring dissolved oxygen in natural waters. The first and most accurate is the Winkler titration method, against which all others are compared to and tested for accuracy. However, this Winkler involves the use of hazardous chemicals and requires a skilled analyst. Other methods that may be used include the Clarke type polarographic sensor which is the most common followed by the optical dissolved oxygen sensor and the galvanic oxygen sensor.

- Azide-WINKLER Titration on collected samples.
- Polarographic sensor – Clark Cell electrodes – *In situ* measurement.
- Optical sensor – *In situ* measurement.
- Galvanic sensor – *In situ* measurement.

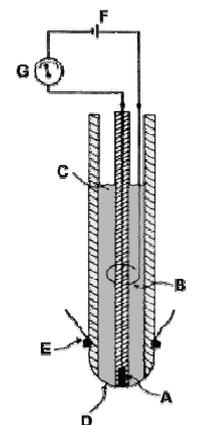
Each technology/method has its advantages and disadvantages and it is briefly introduced below.

4.1.9.1 Winkler Titration

The Winkler titration procedure [Win88, Ril76, Car66, Gol83] is the first recognized method for determination of oxygen concentrations in natural waters. The technique is an iodometric titration where aqueous samples are treated with manganous-sulfate, potassium hydroxide, and potassium iodide to form manganous-hydroxide, $Mn(OH)_2$. Oxygen in the sample reacts with the Mn(II) species giving Mn(III). The Mn(III) is inherently unstable and will further react with another O_2 molecule to form the Mn(IV) species. In order to fix the reaction, acidification is used to convert $MnO(OH)_2$ into manganic sulfate which acts as an oxidizing agent to release free iodine, I_2 . This iodine is stoichiometrically equivalent to the dissolved oxygen in the sample and is titrated with sodium thiosulfate or phenylarsine oxide to its starch indicator endpoint. The Winkler method is subject to numerous interferences such as the presence of nitrite ion, ferrous and ferric iron, suspended solids, and organic matter. The method is prone to over reporting Dissolved Oxygen concentrations in anoxic and under reporting Dissolved Oxygen concentrations in hyperoxic environments as the aqueous sample and Winkler reagents are exposed to air during the procedure.

4.1.9.2 Polarographic oxygen sensors – Clark cell electrodes

Membrane covered amperometric detectors are commonly used for the measurement of oxygen in natural waters, with most designs following principles described in a fundamental patent awarded to H. A. Clark [Cla59] "Clark cell" designs have a thin organic membrane covering a two-electrode cell, separating the cell and electrolyte solution from the test solution, and keeping a thin layer of electrolyte in direct contact with the cathode. Oxygen diffuses through the membrane and is reduced on the cathode surface. The reduction occurs because the cathode is held at a sufficiently negative voltage to reduce the oxygen, with careful consideration to keep the bias voltage sufficiently large to reduce the oxygen but not so high as to reduce other species. The dissolved oxygen in a given sample is calculated by measuring the cathodic current and sample temperature. A relative measure of dissolved oxygen compared to a fully saturated sample is determined using the cathodic current, temperature, barometric pressure, and salinity. In a Clark cell electrode design, the greater the oxygen partial pressure, the greater the rate of oxygen diffusion through the membrane. To maintain



A = Pt-Kathode,
 B = Ag/AgCl-Anode,
 C = KCl-electrolyte
 D = Teflon membrane –
 E = sensor body, F battery,
 G = Galvanometer.

the accuracy and precision of dissolved oxygen measurements, it is necessary to maintain a sufficient flow of water across the sensor to avoid depletion of oxygen at the membrane surface. This is due to the consumption of oxygen at the cathode and the diffusion dependence of oxygen through the membrane, often referred to as the stirring effect. Other interferences include organic growth or decay that can add or remove oxygen from the water prior to transfer of oxygen through the membrane.

In addition, contamination from oils and other polymers can lead to a decrease in diffusion rates, changing the calibration function of the electrode. Some materials used in commercial Clark cell electrodes are susceptible to poisoning by contaminants, which leads to a decreased response. Over time, membranes in Clark cells deteriorate to the point of needing replacement, the chemical composition of the electrolyte changes, and the electrodes are consumed to the point of limited response to oxygen exposure.

Full ocean (7000 dbar) polarographic oxygen sensors¹

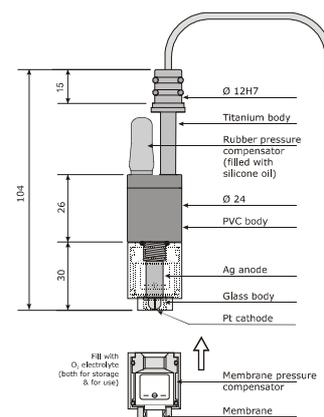
In 1980, Idronaut (Italy) added the following improvements to the basic Clark oxygen sensor by:

- Using the microcathode technique where a very small diameter platinum wire is embedded in a glass body, allowing very low oxygen consumption and so minimizing the need to stir or pump the sample;
- Perfectly sealing the platinum wire in a special glass, which guarantees a zero current output when the sensor is exposed to an anoxic environment, thus avoiding the zero point calibration required by the traditional polarographic oxygen sensors;
- Adding a membrane pressure compensator on its replaceable membrane cap to avoid stressing the measuring membrane and so to achieve 700 bar operation;
- Leaving the oxygen sensor always polarized even when its electronic amplifier is off to avoid the usual drift after switching on the CTD equipped with oxygen sensor.

This made available a very accurate and reliable full ocean oxygen sensor, widely used in the world, where more than 5000 units were produced in the last 30 years.

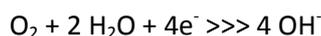
The Idronaut oxygen sensor (from the Ocean Seven 316Plus CTD Operator's Manual)

"The oxygen sensor is of the polarographic type and consists of two half-cells, the anode and the cathode. The anode is a silver tube inside the sensor, which encircles a glass body where a thin platinum wire is sealed. The cathode is the small surface area of the platinum wire exposed at the tip of the sensor where the glass body is rounded. A cap with a gas-permeable replaceable membrane containing the electrolyte screws onto the sensor. The special electrolyte inside of the cap allows the diffusion current to flow between the anode and the cathode which is proportional to the amount of oxygen diffusing through the membrane. The membrane is shielded from accidental bumps by a protective ring. The anode acts as a reference cell, providing a constant potential with respect to the cathode. The cathode, where oxygen is consumed or reduced, is separated from the sample to be analyzed by a thin layer of electrolyte and a special composite membrane. The electrolyte permits the chemical reaction to occur whereas the membrane constitutes a barrier against ions and other substances. By applying a polarizing voltage to the half-cells, the sensor develops a current proportional to the concentration of oxygen in the sample in front of the cathode. Oxygen from the sample is drawn across the membrane, at the sensor tip, in the area of the cathode. The applied polarization voltage is such that



¹ www.idronaut.it

the sensor only responds to oxygen. The sensor is insensitive to nitrogen, nitrous oxide, carbon dioxide and other gases. In order to avoid stray ground current leaks, in case of membrane leaks, the anode is kept at ground potential while the cathode is polarized at a fixed negative voltage. The oxygen sensor limits stirring effects on the measurement and reads at least 97% of the true value, even with a stagnant aqueous sample. This is because the very small cathode area and special cathode geometry, associated with a unique composite membrane, minimize the consumption of the oxygen contained in the sample in contact with the membrane. The function of this sensor depends on the reduction of oxygen at the cathode, as expressed by the formula:



The developed electrons represent the measuring current and are supplied by the silver/silver chloride anode.”

In 2002 SeaBird Electronics introduced on the market the full ocean SBE 43 dissolved oxygen sensor. Since the SBE pumping system is already available to pump the SBE conductivity cell, no pump is to be added when user requires this additional sensor together with the SBE basic CTD.

This oxygen sensor, despite being less stable than the optical one, is still successfully and widely used, and well accepted by the scientific community.

The Sea-Bird Electronics oxygen sensor for 7000 m max depth (from SBE’s brochure)²

“The SBE 43 sets the oxygen measurement standard for oceanographic research. The sensor is a complete redesign of the Clark polarographic membrane type in which careful choices of materials, geometry, and sensor chemistry are combined with superior electronics interfacing and calibration methodology to yield major gains in performance.



Calibration stability is improved by an order of magnitude, and the sensor requires less frequent calibration. Calibration drift is caused primarily by membrane fouling from ocean contaminants, and less so by chemical processes inside the sensor. If the membrane is kept clean, the sensor’s improved chemical stability yields demonstrated calibration drift rates of less than 0.5% over 1000 hours of operation (*on time*).

Temperature response is dramatically improved. The chemical and physical processes that underlay the oxygen measurement are very sensitive to temperature. Accurate characterization of the internal sensor temperatures that control these processes, especially when water temperature is changing rapidly, is a key accomplishment of this design. Not only does the SBE 43 sensor measure temperature in the right place: the temperature equilibration time of the entire sensor head has been reduced to a few seconds, so it tracks the changing water temperature much more faithfully.

Hysteresis is largely eliminated in the upper ocean (1000 meters) due to improved temperature response. Residual mismatch between up and down casts in this part of the ocean is due to sensor alignment, correctable in post-processing. At higher pressures, changes occur in gas-permeable Teflon membranes that affect their permeability characteristics. These changes have long time constants, depend on the sensor’s time-pressure history, and result in hysteresis at depths greater than 1000 meters. These effects are predictable and are also correctable in post-processing. The resultant SBE 43 measurement resolves oxygen features more precisely, reducing the ambiguity about locking measured sensor values to bottle Winklers.

Continuous polarization eliminates stabilization wait-time after power-up. The sensor is always ready for immediate use. Earlier sensors required several minutes to *polarize* following power-up. During that time, sensor readings were inaccurate. In the SBE 43, micropower electronics and an internal, five-year, board-mounted battery eliminate power-up delay.

² www.seabird.com

Poisoning in hydrogen sulfide environments was a phenomenon common to early oxygen sensor designs that used silver as the cathode element. The SBE 43 uses a noble metal (gold) as the cathode and silver as the anode, and shows no degradation of signal or calibration when used for profiling in hydrogen sulfide environments.

Signal resolution is increased by on-board temperature compensation, and a CTD channel is made available for other purposes because there is no *temperature* output signal. Even when oxygen concentration is constant, the normal range of ocean temperatures causes the output of earlier sensors to vary by a factor of two. The SBE 43's internal temperature compensation eliminates this variation, allowing the sensor to pre-amplify the signal proportionately; resolution with existing CTD systems is correspondingly increased.

Effective plumbing strategies allow for longer moored deployments. Plumbing isolates the SBE 43 from continuous exposure to the external environment, allowing trapped water to go anoxic, minimizing electrolyte consumption between samples. The black plenum and installing black tubing block light, reducing in-situ algal growth".

Conclusions on the polarographic oxygen sensors (advantages and disadvantages)

The advantage of the polarographic sensor is that it is a mature, well-known and accepted measurement technique and that cleaning and maintenance are easy. Furthermore, re-calibration of the sensor is very easy and can be carried out in air without needing any special equipment. In addition, the polarographic electrode, thanks to the fast response time $< 3s$, is ideal for the real-time water profiling.

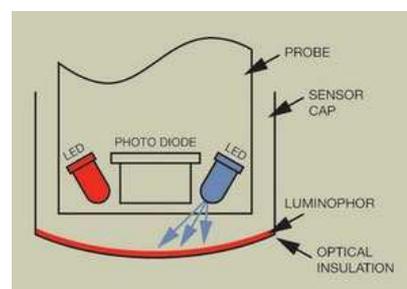
Its main great limitation is that it requires frequent, but a very simple calibration in air and its drift is unpredictable with time.

4.1.9.3 Optical Oxygen sensors

Optical oxygen sensors work according to the principle of dynamic fluorescence quenching. The sensors contain fluorescent dye that is excited by light of a certain wavelength. Depending on the amount of oxygen molecules present, the luminescence response of the optical sensor varies. A polymer optical fiber transmits the excitation light of the sensor and at the same time also transmits the fluorescence response of the sensor to the measurement device. The optical fiber can, but does not have to be in contact with the sensor material which enables non-invasive measurement through transparent or semi-transparent vessel walls.

The oxygen sensitive dye is immobilized in a polymer matrix. This polymer can be applied to carrier material and used as sensor spots or sensor foil. It can also be coated directly onto the optical fiber. Oxygen quenching luminophores have been studied from at least 1939 when Kautsky described quenching of luminescence by oxygen [Kau39]. More recently, as optical sources, detectors, and data processing have become more advanced, the application of luminophores to the measurement of oxygen concentrations in liquids has resulted in bench-top instruments and optodes, with significant advances made in the 1990's³. Recent advances in blue light-emitting diodes and low-powered high-speed electronics have enabled the miniaturization of oxygen sensitive optodes to the point of field-deployable units. The sensors do not consume oxygen and are stable over long deployment periods. As shown in the above figure, the luminescent dissolved oxygen sensor's active optical components consist of a pair of blue and red light-emitting diodes (LEDs) and a silicon photodetector.

The sensor cap has a coating of a platinum based luminophor that is excited by the light from the blue LED. The luminophor is coated on the outside with a carbon black polystyrene layer for optical insulation, providing excellent protection against photobleaching from external light sources when the sensor cap is attached to the sensor. The blue excitation LED is sinusoidally modulated at a frequency related to the luminophor's luminescence lifetime and the upper and lower lifetimes of analytical interest. The measured parameter of interest from the optode is the phase delay



(essentially a time delay) between the exciting blue LED signal and the detected red emission from the luminophor, with the phase delay inversely related to the amount of dissolved oxygen near the luminophor, typically oxygen in the water of interest. This phase-modulation technique is used to measure the lifetime of the oxygen-dependent quenching of luminescence.

The use of the phase-modulation technique means that intensity fluctuations of the blue LED or bleaching effects of the luminophor have no discernable impact on the lifetime measurement throughout the life of the sensor.

The first full ocean (7000 dbar) optical oxygen sensor on the market since 2004 was the Aanderaa oxygen Optode, as per the below description and because of the PreSens invention and development.

The Aanderaa oxygen optode (from Aanderaa-Xylem's brochure)³

“The oxygen optode is designed to measure absolute oxygen concentration and % saturation. The optode can be used from streams to deep sea, from fish farms to waste water and from polar ice areas to hydrothermal vents. The lifetime-based luminescence quenching principle offers the following benefits:

- Response time <8 sec (63%).
- High accuracy.
- Not stirring sensitive (it consumes no oxygen).
- Lower fouling sensitivity.
- Measures absolute oxygen concentrations without repeated calibrations.
- Better long-term stability.
- Hot water monitoring.
- Less affected by pressure.
- Not sensitive to H₂S.
- Not freezing sensitive.



Since oxygen is involved in most of the biological and chemical processes in aquatic environments, it is one of the most important parameters to be measured. Oxygen can also be used as a tracer in oceanographic studies. For environmental reasons it is critical to monitor oxygen in areas where the supply of oxygen is limited compared to demand, e.g.:

- In shallow coastal areas with significant algae blooms.
- In fjords or other areas with limited exchange of water.
- Around fish farms.
- In areas interesting for dumping of mine or dredging waste.

The Aanderaa oxygen optodes are based on the ability of selected substances to act as dynamic fluorescence quenchers. The fluorescent indicator is a special platinum porphyrin complex embedded in a gas permeable foil that is exposed to the surrounding water. A black optical isolation coating protects the complex from sunlight and fluorescent particles in the water. This sensing foil is attached to a window providing optical access for the measuring system from inside a watertight housing. The foil is excited by modulated blue light, and the phase of a returned red light is measured. By linearizing and temperature compensating, with an incorporated temperature sensor, the absolute O₂ concentration can be determined.

Advantages

- Optical measurement principle.
- Lifetime-based luminescence quenching principle.

³ www.aanderaa.com

- Long time stability.
- More than one year without recalibration.
- Low maintenance needs.
- User friendly.
- Smart sensor technology - provides calibrated data directly.
- Use with SmartGuard, RCM 9 and RDCP.
- Use as stand-alone sensor.
- Output format: SR10, RS232, Analog 0-5V/4-20mA. “

The Sea-Bird Electronics SBE 63 optical dissolved oxygen sensor (from SBE’s brochure)⁴

“Due to the increasing demands of science, Sea-Bird developed an individually calibrated, high-accuracy, optical oxygen sensor to assist in critical hypoxia and ocean stoichiometric oxygen chemistry research. With this new sensor, a myriad of moored and float-based platforms can contribute significantly in these driving areas of importance. The SBE 63 sets the oxygen measurement standard for oceanographic research. Careful choices of materials and geometry are combined with superior electronics and calibration methodology to yield significant gains in performance.



Each SBE 63 is calibrated individually in a temperature-controlled bath. Bath temperatures are varied at each of 4 oxygen values, providing a comprehensive 24-point calibration. Two reference sensors in each bath are standardized against Winkler titrations. Response time tests are conducted on each sensor, using gas. Salinity and pressure impacts on sensor response are each checked at two separate points.

The SBE 63 is designed for use in a CTD's pumped flow path, providing optimum correlation with CTD measurements. The elapsed time between the CTD and associated oxygen measurement is easily quantified, and corrected for in post-processing. The plumbing's black tubing blocks light, reducing in-situ algal growth.”

JFE-Advantech manufactures two kinds of optical dissolved oxygen sensors:

The Rinko II - IID (from JFE-Advantech’s brochure)⁵

Rinko II is a digital output version with the fast optical DO sensor. The instrument can be easily integrated on platforms, because it works with communications by RS-232C or RS-485 and an external power (12 to 24V DC).

Rinko IID has not only a temperature sensor but also a depth sensor. Their models can monitor DO in real time, if you have a personal computer and an external power.



The Rinko III (from JFE-Advantech’s brochure)

Rinko III is an analog version with the fast optical DO sensor and a temperature sensor. With an external 12 DCV power, the instrument seamlessly outputs the analog data (0 to 5V) of the sensors.

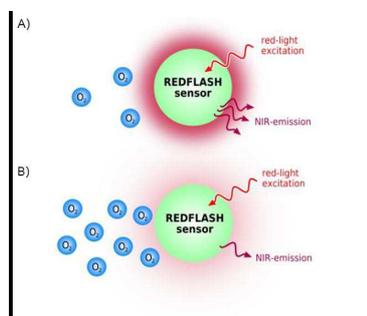
Rinko III can be easily integrated on various platforms with an Impulse cable. By the fast response, the instrument provides high accurate DO data without restricting profile speeds.

More recently, the Optical Sensor has been improved with the “REDFLASH” technology (from **PyroScience GmbH**)⁶

⁴ www.seabird.com

⁵ www.jfe-advantech.co.jp

“The new REDFLASH technology is based on the unique oxygen-sensitive REDFLASH dyes. In contrast to common techniques using blue-light excitation, the REDFLASH dyes are excitable with orange-red light and show an oxygen-dependent luminescence in the near infrared (NIR). The REDFLASH technology impresses by its high precision, high reliability, low power consumption, low cross-sensitivity, and fast response times. The orange-red light excitation significantly reduces interferences caused by auto-fluoresced samples. Further, the NIR detection technology significantly reduces interference with ambient light, known from the old blue-light techniques. The new REDFLASH technology is based on the unique oxygen-sensitive REDFLASH indicator showing excellent brightness. The measuring principle is based on the quenching of the REDFLASH indicator luminescence caused by collision between oxygen molecules and the REDFLASH indicator immobilized on the sensor tip or surface. The REDFLASH indicators are excitable with red light (more precisely: orange-red at a wavelength of 610-630 nm) and show an oxygen-dependent luminescence in the near infrared (NIR, 760-790 nm). *Principle:* Red light excited REDFLASH indicators show luminescence in the near infrared (NIR), which decreases with increasing oxygen (quenching effect). A) high NIR emission at low oxygen and B) low NIR at high oxygen. The measuring principle is based on a sinusoidally modulated red excitation light. This results in a phase-shifted sinusoidally modulated emission in the NIR. The FireSting O2 measures this phase shift (termed “dphi” in the software). The phase shift is then converted into oxygen units based on the Stern-Vollmer-Theory. The red light excitation significantly reduces interferences caused by autofluorescence and reduces stress in biological systems. The REDFLASH indicators show much higher luminescence brightness than other optical sensor working with blue light excitation. Further, due to the excellent luminescence brightness of the REDFLASH indicator, the actual sensor matrix can be now prepared much thinner, leading to fast response times of the oxygen sensors.”



Optical oxygen sensor calibration

The optical quenching of the luminophore is strongly temperature dependent. It is important to measure the temperature with high precision (repeatability to a measured temperature) and to closely monitor the temperature of the luminophore sensor cap during the measurement cycle. When calibrating the instrument, it is critical that the luminophore sensor cap be in thermal equilibrium with the water of interest and with the temperature probe measuring the temperature of the water of interest.

For example, when using water-saturated-air for calibration, it is necessary that the luminophore sensor cap and the temperature probe both be completely out of the water and in temperature equilibrium with the water-saturated-air for the calibration of 100% saturation for the probe. Similarly, when using air saturated-water for calibration, it is necessary that the luminophore sensor cap and the temperature probe both be fully immersed in the water and in temperature equilibrium with the air-saturated-water for the calibration of 100% saturation for the probe.

The Sea & Sun fast optical oxygen sensor (from Sea & Sun Technology's brochure)

“The measuring principle is based on red light excitation. Indicators showing luminescence in the near infrared (NIR), which decreases with increasing oxygen (quenching effect). The red light excitation significantly reduces interferences caused by autofluorescence and reduces stress in biological systems.

The sensor is equipped with an own temperature sensor for internal calculation and linearization. Due to its analog output it can easily be connected to SST multiparameter probes as well as to third party equipment.

⁶ www.pyroscience.com



Technical Specifications

- excitation wavelength: 620 nm
- detection wavelength: 760 nm
- max. sample rate: 2 samples / second
- internal resolution: 14 bit
- low power consumption
- analog Output: 0–2.5 VDC
- operational depths down to 6000 m
- titanium housing.”

Conclusions on the optical oxygen sensors (advantages and disadvantages)

Optical oxygen sensors present the advantage, compared to commonly used polarographic electrodes, that no electrolyte solution is necessary and oxygen is not consumed in the measurement process. The optical sensors presents excellent long-term stability, are not influenced by the flow rate of samples, do not consume oxygen and are stable over long deployment periods.

On the contrary, optical oxygen sensors have a rather slow response time, typically with $t_{0.63}$ of less than 60 seconds, often less than 30 seconds for changes as high as 8 mg/l, and have important dependency on the temperature that must be accurately measured to correct the reading. Furthermore, calibration is cumbersome and must be carried out in the laboratory (mainly the manufacturer’s calibration laboratory), using special equipment. Another disadvantage is that as the luminophore cannot last more than three months/one year after the beginning of its use, it must be routinely replaced by sending back the sensor to the manufacturer or by locally replacing the membrane cap (if present) and recalibrating the sensor by following the rather complex manufacturer instructions. “

The main and key disadvantage of the optical sensors is that any accidental abrasion of the foil surface, which is rather delicate, requires the return of the optical sensor to the manufacturer, if the sensor is not provided with a sort of “field replacement membrane cap”.

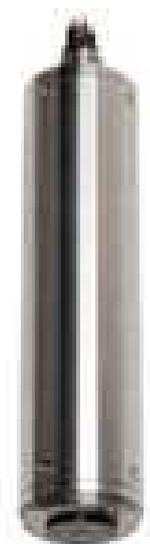
In any case, the cost of the “membrane cap” or of the luminophore foil is much more expensive than any common Teflon (or similar material) membrane used on the polarographic oxygen sensor.

4.1.9.4 Galvanic Sensor

The OxyGuard International OxyGuard 505 oxygen galvanic sensor (from OxyGuard International’s brochure)⁷

“The OxyGuard 505 Probers a combined dissolved oxygen sensor and transmitter with galvanic isolation. It is designed to be fitted to a sonde or sensor array for measurements in the sea, lakes or similar. Two types are available. When fitted with an Ocean sensor the OxyGuard 505 can be used at depths up to 2000 m, and when fitted with a Profile sensor it can be used for making profiling measurements where it is moved through the water. The Profile sensor can, however, only be used at depths up to 150 m. The OxyGuard 505 Probe has separate output signal and power supply. It therefore has two connections for the output of 0-5V and two for the supply. It can be powered by supplies between 8.5 and 24 VDC. The probe can be supplied with a cable, with a cable and connector or be fitted with a connector to suit user specifications.

The output signal is directly proportional to the oxygen partial pressure sensed and is compensated for the temperature characteristics of the membrane. As standard the output signal of 0-5 V corresponds to 0-200% saturation dissolved oxygen.”



⁷ www.aanderaa.com

Conclusions on the galvanic oxygen sensors (advantages and disadvantages)

Advantages

The advantage of the galvanic sensor is that it is a mature, well-known and accepted measurement technique and that cleaning and maintenance are easy. Furthermore, re-calibration of the sensor is very easy and can be carried out in air without needing any special equipment.

Disadvantages

Measurements are influenced by temperature, pressure and barometric pressure variations respect to the sensor calibration conditions. Thus sensor needs dedicated algorithms to compensate these influences. Other limitation is that it can only operate at 2000 m depth and that it requires frequent calibration in air and its drift is unpredictable with passing of time.

4.1.9.5 Dissolved Oxygen References

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[Gol83] Golterman, H.L. 1983. The Winkler determination. In Polarographic oxygen sensors. Edited by E. Gnaiger and H. Forstner. Springer- Verlag, New York. pp. 346–351.

[Cla59] Polarographic DO₂ sensors: Clark US Patent 2,913,3866, “Electrochemical device for chemical analysis” November 1959.

[Kau39] Kautsky, H. Quenching of luminiscence by oxygen. Trans. Faraday Soc., (1939), 25, pp. 216-219.

4.1.10 Temperature

Oceanic pressure and temperature data have been measured with a variety of different platforms and instruments at various depths over the post-industrial period. Today most measurements come from ships, fixed and drifting buoys, unmanned vehicles and satellites. In this report we will broadly review some of the most important temperature and pressure sensors and detection devices used in monitoring those properties of sea water.

Table 9. Ocean state variables, their typical ranges and mean values in the ocean, and the accuracy to which they are measured (or estimated) in the deep ocean (they can be lower at surface or in coastal regions where the natural variability is much higher).

Variable	Ocean Range	Ocean Mean	Required Accuracy
Temperature	-2°C to 40°C	3.5°C	±0.002°C
Pressure	0 dbar to 11000 dbar	1850 dbar	< ±3 dbar

Temperature, pressure, and salinity are the three most important properties of a seawater parcel since they determine other physical characteristics of the seawater such as density, specific heat, sound velocity, solubility of other substances, etc. Density in particular is an important property in ocean science because it is the main driver for ocean dynamics. For instance, horizontal density gradients as small as 0.001kg/m³/km will result in spatial variations in pressure generating current velocities of some cm/sn. [Paw13] Temperature has been both the earliest and wide most measured property of sea water, according to the classical oceanographic literature *e.g.* [SJF42]. In addition to its role on the ocean dynamics through its participation in the water density, temperature is involved in many oceanic processes associated to heat and mass budgets. In particular, it is a key factor on air-sea exchanges, including the water cycle and oceanic heat storage and release. Additionally, it is a

relevant parameter for most of the water chemical properties since solubility of many substances, such as CO₂, depends on temperature. The importance of temperature monitoring is reflected in its designation as an essential climate variable of the global climate observing system.

As a consequence the monitoring of the different physical properties of sea water is crucial to understand and predict oceanic behavior. In the following table it is possible to observe the different ranges and mean values of temperature and pressure observed for sea water.

4.1.10.1 Temperature Measurement

As previously described water temperature is one of the most important physical characteristics of seawater. Temperature is used to determine the density through the International Equation of State for Sea water [UNESCO81] and to calculate salinity through electrical conductivity (see the corresponding report). In the following pages we will review the different devices that have been used for the measuring of temperature.

Reversing thermometers

The first thermometers were based on the thermal expansion rate of substances, such as mercury. Its use for manual temperature readings has been widely spread and applied to the air, water and other environmental conditions. Thermometer however must be read by human eyes and *in situ*, while in contact with the body whose temperature is being measured. Both conditions prevented use of thermometers as autonomous temperature sensors. By the end of 19th century (1874) the so-called *reversing thermometers* were developed by Negretti & Zambra in London [SJF42] to overcome the second problem (Fig. 4.1.54).

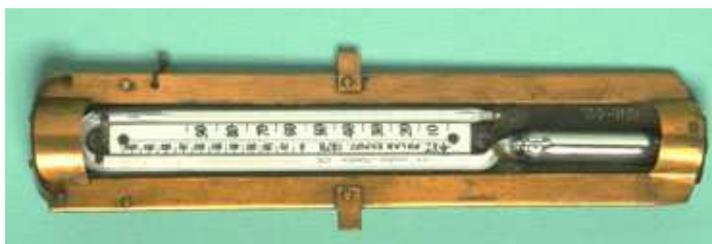


Figure 4.1.54. Self-registering thermometer by Negretti & Zambra

Such thermometers were designed to keep the temperature reading after they were reversed. In addition, there is also an *unprotected* version of those thermometers, leaving the bulb and glass column exposed to the environmental pressure thus giving a pseudo-temperature reading. The difference between protected and unprotected thermometer readings

was used to estimate the pressure where thermometers were reversed (see [SJF42] for details). High precision reversing thermometers, properly calibrated, could reach accuracies from 0.02°C to 0.001°C, according to the temperature ranges for which they were produced. Reversing thermometers have been used in all oceanographic expeditions during almost the whole 20th century to give the first precise worldwide global distributions of ocean temperature, based on discrete measurements in space and time (*e.g.* the Atlantic Ocean atlas published on occasion of the International Geophysical Year 1957-58 [Fug60]).

Thermograph

Mechanical temperature recording sensors, *thermographs*, have also been used in fixed positions, typically at coastal stations, to obtain continuous time-dependent temperature records. The method consisted on a stylus or a pen attached to a temperature sensitive coil, writing on a slow rotating paper drum. Thermographs have also been used attached to an inflow water of a ship so that the time-dependent temperature record could be translated into the space record along the ship's track.



Figure 4.1.55. A bathythermograph and a vertical temperature profile obtained with it

During the '30s, the *bathythermograph* was the first attempt for a mechanical automatic recording sensor of both temperature and pressure [Spi38]. Its mechanism consisted of a stylus attached both to a long coil and to a membrane covering a small oil tank. Expansion or contraction of the long coil due to temperature changes controlled the horizontal displacement of the stylus while the membrane, under the environmental pressure, controlled its vertical displacement. The stylus against a glass slide covered by a smoke coating thus produced a graph of temperature vs depth when the bathythermograph was lowered into the ocean. After rinsing and fixing the graph on the smoked glass it could be read using a calibrated mesh to obtain a vertical profile of temperature (Fig 4.1.55)

Electronic temperature measurements

By the mid 20th century, with the onset of electronics, electrical properties of materials began to be exploited for temperature measurement. The first attempts were based on the well-known property of changes in resistance of any material as its temperature changes. The rate of change would depend on the material and also the temperature because in most cases the relationship is far from being linear. In any case, a good instantaneous measurement of the resistance could be converted to a temperature value provided a conversion formula, according to a known resistance versus temperature relationship after a calibration process. The quality of the temperature measurement, then, depends on several factors, assuming a good calibration:

- (i) the stability of the electronics of the circuit.
- (ii) the bias caused by heating effect of the current on the resistor.
- (iii) the precision of the resistometer in relation to the amplitude of the resistance variation at the environmental temperature range.

In addition to the mentioned conditions there are other crucial factors influencing the quality of the temperature measure, such as the response time to a change or the stability and shape of the resistance versus temperature function, which is related to the minimum number of points required for a calibration. The ideal situation would be a material showing wide amplitude with nearly linear response at the -3° to 33°C temperature interval. Platinum (Pt) was one of the best materials for this purpose: it is the most stable, has a wide response range and is almost linear, but because of its slow response time, high frequency variability of temperature cannot be solved with such sensors.

Thermocouples

Early electronic temperature measurements used Pt resistance sensors but they had the problem of self-heating and instability of the rest of the circuit components, giving low accuracy of the readings. To avoid some of these problems other electrical properties of materials have also been used. One of them is the relation between the voltage generated by a bimetallic union and temperature, called *thermocouple*. There is no self-heating since no current is needed but has other problems such as, drifts and low precision because the water temperatures range is small as compared with the typical total range of most of thermocouples. Currently thermocouples are by far the most widely used type of sensor in industry. They are very rugged and can be used from sub-zero temperatures to temperatures well over 2000°C .

A thermocouple is formed by joining two different metal alloy wires at a point called a junction. This point is called the measuring or “hot junction”. The thermocouple lead ends are usually attached to a temperature indicator or controller. This connection point is called the reference or “cold junction”. When there is a temperature difference the measuring junction is heated, a small DC voltage is generated in the thermocouple wires. The controller converts this millivolt signal into the temperature reading. Among the different types of thermocouples certain combinations of metal alloys have become standards. In the following table you can find some of the most used.

Table 10. Thermocouple Standard Types

Thermocouple Standard Type	Metal content in Positive Leg.	Metal content in Negative Leg	Temperature Range	Tolerances	
				Standard	Special
B	70.4% Platinum (Pt) 29.6% Rhodium (Rh)	93.3% Platinum (Pt) 6.1% Rhodium (Rh)	870-1700°C	±0.5%	-
E	90% Nickel (Ni) 10% Chromium (Cr)	55% Copper (Cu) 45% Nickel (Ni)	0-900°C	±1.7°C or ±0.5°C	±1.0°C or ±0.4%
J	99.5 % Iron (Fe)	55% Copper (Cu) 45% Nickel (Ni)	0-750°C	±2.2°C or ±0.75%	±1.1°C or ±0.4%
K	90% Nickel (Ni) 10% Chromium (Cr)	95% Nickel (Ni) 5% Various Elements	0-1250°C	±2.2°C or ±0.75%	±1.1°C or ±0.4%
N	84.4% Nickel (Ni) 14.2% Chromium (Cr) 1.4% Silicon (Si)	95.5% Nickel (Ni) 4.5% Silicon (Si)	0-1250°C	±2.2°C or ±0.75%	±1.1°C or ±0.4%
R	87% Platinum (Pt) 13% Rhodium (Rh)	100% Platinum (Pt)	0-1450°C	±2.2°C or ±0.75%	±1.1°C or ±0.4%
S	90% Platinum (Pt) 10% Rhodium (Rh)	100% Platinum (Pt)	0-1450°C	±2.2°C or ±0.75%	±1.1°C or ±0.4%
T	100% Copper (Cu)	55% Copper (Cu) 45% Nickel (Ni)	-200-350°C	±1.0°C or ±0.75%	±0.5°C or ±0.4%
C*	95% Tungsten (W) 5% Rhenium (Re)	74% Tungsten (W) 26% Rhenium (Re)	0-2135°C	±4.5°C or ±1.0%	-
D*	97% Tungsten (W) 3% Rhenium (Re)	75% Tungsten (W) 25% Rhenium (Re)	0-2135°C	±4.5°C or ±1.0%	-
G*	100% Tungsten (W)	74% Tungsten (W) 26% Rhenium (Re)	0-2135°C	±4.5°C or ±1.0%	-

Other temperature sensors widely used are the resistance temperature detectors or RTDs.

Resistance Temperature Detector (RTD)

Metallic RTDs are precision temperature sensors used in industrial and laboratory applications. RTD are typically more accurate than thermocouples and maintain that accuracy over a longer period of time. They are usually used up to 650°C.

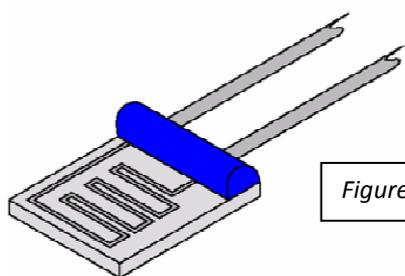


Figure 4.1.56. Thin Film RTD

In a RTD sensor that are based on metals, resistance changes linearly with temperature and a controller measures the value of the resistance and transform it into temperature. Unlike on a thermocouple there is no electrical signal generated by a RTD.

Table 11. RTD Types

Element type	Temperature Range	Base Resistance	TCR ($\Omega / \Omega / ^\circ\text{C}$)
Platinum DIN	-200 600 °C	100 Ω at 0°C	0.00385
Platinum JIS	-200 600 °C	100 Ω at 0°C	0.003916
Copper	-100 260 °C	10 Ω at 25°C	0.00427
Nickel	-100 205 °C	120 Ω at 0°C	0.00672

Finally the role of temperature modifying the current across a diode or any other, more or less complicated, integrated circuit has also been used as sensor and is being used in controlling internal temperatures as well as environmental thermometers in many electronic instruments. All these properties have been used at certain steps of the electronics development during the last 60 years. However, since the stability and precision of the electronic components has become much higher, these methodologies have been progressively abandoned in towards the use of the *thermistors*.

Thermistor

Thermistors are also temperature-sensitive resistors, generally composed of semiconductor materials, which are the most sensitive sensors in terms of the rate of resistance change per unit of temperature. As a consequence, thermistors are currently the most used non-remote sea water temperature monitoring devices.

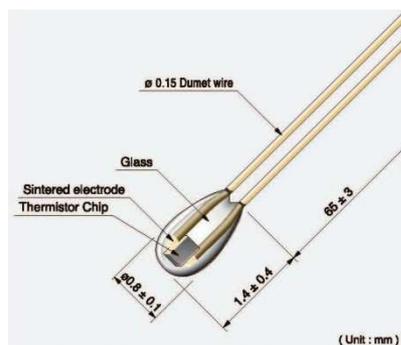


Figure 4.1.57. Thermistor

It has to be noted that most thermistors have a negative relationship, that is, their resistance decreases with increasing temperature. The advantage of thermistors, in addition to its potential precision, is the fast response time. The disadvantages are the highly non-linear relationship of resistance versus temperature and its relatively low stability. The first problem requires a high number of calibration points. The second is being improved with new materials.

Thermistors exhibit a very large resistance change for a small temperature change (usually 3-5% vs. the 0.4% of RTDs) this makes them very sensitive to small temperature changes. Their

typical working ranges are from -100 to 300°C. Currently there are no standards for thermistors.

Remote temperature monitoring sensors

Thus, far we have explored contact temperature sensors, where the sensor must physically touch a material before it can sense its temperature. However, temperature can also be measured from the long-wave emitting energy of a body according to the Stefan-Boltzmann law [SBL14]. This method is used in remote sensed temperatures from an aircraft or a satellite. The main limitation of the method is that since long wave radiations does not penetrate into a water body, readings of infrared are limited to the few microns of the skin of the oceans. Another problem related to remote sensed temperatures is caused by clouds, vapor or dust interfering net radiation from the ocean. In contrast, infrared remote sensed temperatures may cover simultaneously a wide area of the ocean surface enabling simultaneous information over large areas.

Commercial Marine Temperature Sensors

The SBE3 series from Sea-bird Electronics contains a range of sensors intended for environmental, marine or industrial monitoring. These include thermistors and digital reversing thermometers in a range of housings. Sensors are analog or digital with multiple communications options and long battery lifetimes.

One of the latest products on offer is the SBE 39plus [SBE14], is a high-accuracy temperature (pressure optional) recorder with internal battery pack and non-volatile memory for deployment at depths up to 10,500 meters. The 39plus is intended for moorings or other long-term, fixed-site

applications, as well as deployments on nets, towed vehicles, or ROVs. Calibration coefficients are stored in EEPROM, allowing output in decimal or XML ASCII engineering units (raw output available). It has an internal USB connector for fast upload and a RS-232 serial interface with optional external connector



Figure 4.1.58. SBE 39plus from Seabird Electronics

IDRONAUT Temperature sensor

The temperature sensor consists of a platinum resistance thermometer (type Pt 100 ohms at 0°C), fitted on a thin stainless steel housing, able to withstand up to 700 bar. The sensor has a very low response time (50 ms) and a high stability of reading with ageing. The drift of reading is less than 0.0003 °C per year.

Table 12. Temperature Sensor Comparison Chart. Extracted from [Wat95]

Sensor	Advantages	Disadvantages
Thermocouple	<ul style="list-style-type: none"> • Simple, Rugged • High temperature operation • Low cost • No resistance lead wire problems • Point temperature sensing • Fastest response to temperature changes 	<ul style="list-style-type: none"> • Least stable, least repeatable • Low sensitivity to small temperature changes • Extension wire must be of the same thermocouple type • Wire may pick up radiated electrical noise if not shielded • Lowest accuracy
RTD	<ul style="list-style-type: none"> • Most stable over time • Most accurate • Most repeatable temperature measurement • Very resistant to contamination/corrosion of the RTD element 	<ul style="list-style-type: none"> • High cost • Slowest response time • Low sensitivity to small temperature changes • Sensitive to vibration (strains the platinum element wire) • Decalibration if used beyond sensor's temperature ratings • Somewhat fragile
Thermistor	<ul style="list-style-type: none"> • High sensitivity to small temperature changes • Temperature measurements become more stable with use • Copper or nickel extension wires can be used 	<ul style="list-style-type: none"> • Limited temperature range • Fragile • Some initial accuracy "drift" • Decalibration if used beyond the sensor's temperature ratings • Lack of standards for replacement
Infrared	<ul style="list-style-type: none"> • No contact with the product required • Response times as fast or faster than thermocouples • No corrosion or oxidation to affect sensor accuracy • Good stability over time • High repeatability 	<ul style="list-style-type: none"> • High initial cost • More complex - support electronics required • Emissivity variations affect temperature measurement accuracy • Field of view and spot size may restrict sensor application • Measuring accuracy affected by dust, smoke, background radiation, etc.

4.1.10.2 Temperature References

- [SBE14] http://www.seabird.com/products/spec_sheets/39plusdata.htm (2014) Accessed on 14 Aug, 2014
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- [Wat95] (1995) *Temperature Sensors B-4*; Watlow Educations Series ed. <https://www.watlow.com/literature/education.cfm>.
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[UNE81] UNESCO (1981) The Practical Salinity Scale 1978 and the International Equation of State of Seawater 1980. Unesco technical papers in marine science 36, 25 p.

[Fug60] Fuglister, F. (1960) Atlantic Ocean Atlas of Temperature and Salinity Profiles and Data from the International Geophysical Year of 1957-1958. Woods Hole Oceanographic Institution, 209 p.

4.1.11 Conductivity (Salinity)

Conductivity is an intrinsic property of any given material indicating its ability to transfer electric charge. Seawater, due to the presence of dissolved salts, is a good electrolyte, in which the electric charge is maintained by the presence of positive and negative ions resulting from the dissociated sea salts. Seawater conductivity increases with temperature and with the salt content, i.e. salinity.

The conductance, Λ , expressed in Siemens (S), is equal to the reciprocal of the resistance, R expressed in Ohms (Ω), i.e.:

$$\Lambda = \frac{1}{R} \quad (1)$$

Since cell geometry affects conductivity values, the specific conductivity, χ , expressed in Siemens per cm ($S\text{ cm}^{-1}$), as absolute measurement value, is defined as the conductivity of a measurement surface of a length = 1 cm and an area = 1 cm^2 . In practice, it is given by:

$$\chi = c \cdot A \quad (2)$$

where: $c = l/A$ is the measurement "cell constant", with l = the distance between the electrodes (cm) and A = the electrode area (cm^2).

Oceanographic sensors measure the conductance, i.e. the voltage produced in response to the flow of a known electrical current. From the measured conductance, conductivity is estimated by the means of the "cell constant", that reflects the ratio of length and cross-sectional area of the sampled water volume in which the electrical current actually flows. The first conductive salinometer was developed by the International Ice Patrol [WSS30]. Since then the estimation of salinity by the measurement of conductivity gradually substituted early salinity estimation methods by titration.

The requirements for modern oceanographic instruments must meet the following specifications [UNE88]:

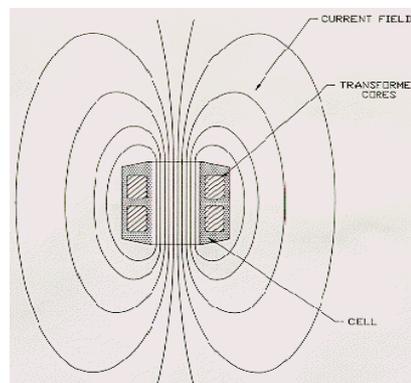
- Range: 1-65 mS/cm,
- Accuracy: 0.005 mS/cm
- Resolution: 0.001 mS/cm
- Stability/month: 0.003 mS/cm

Today, the CTD (Conductivity, Temperature, Depth) or multiparameter probes for profiling or monitoring seawater use two main kinds of conductivity sensors: i) Inductive sensors; ii) Platinum Poly-electrode sensors. Both alternative technologies for the measurement of conductivity are offered on the oceanographic instrumentation market and there is a competition between the manufacturers in order to satisfy customers' requirements to meet these specifications.

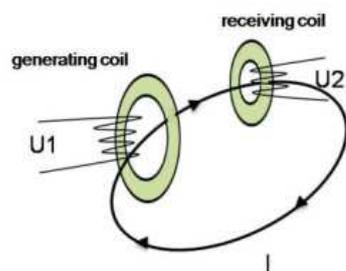
Apart from the inductive and poly-electrode conductivity sensors, there are research institutes, in particular around Europe, which are studying an alternative design like micro-fabrications (sensor on chip) to develop innovative low-cost miniaturized conductivity sensors. However, to date, nothing seems to be coming out from the laboratory and/or soon adopted in commercialized CTDs.

4.1.11.1 Inductive Conductivity sensors

A toroidal inductive measurement of conductivity may be made by passing an alternating current (AC) through a toroidal generating coil as it induces a current in the electrolyte solution. The induced solution current induces, in turn, a current in a second (receiving) toroidal coil. The amount of current induced in the receiving coil is proportional to the solution conductivity.



Different types of inductive conductivity sensors exist: the single transformer, the double transformer and the double transformer with an additional loop. All existing inductive cell types have external fields. Sensors that have external fields are prone to shift their calibration, if nearby objects such as guards, struts, sensor housings -- or marine growth -- distort the external field. Antifoulant-bearing materials placed close enough to be effective also distort the external field, and in a way that change as the antifouling material leaches out, as it must.



Ironically, the calibration shifts resulting from placing antifoulant materials on the outside of an external field cell also prevent the protection of its internal "hard parts".

With inductive conductivity sensors, the electrical current flows in closed paths through the "hole" in the transformer cores. The magnitude of this current (and therefore the output signal from the sensor) depends on the field density along the path. In the area away from the hole, the paths are widely separated and the resistance is low. In the "hole" through the cell, the paths converge and the resistance is higher; the area immediately exterior to the hole will also contribute significantly to the total resistance. But typically about 20% of the resistance occurs outside the relatively well-defined hole itself.

The main advantage of the inductive conductivity sensor is that it is mechanically strong and so it is largely used especially for monitoring. Moreover, it does not require any cleaning (or replatinization) of the platinum electrodes because the electrodes themselves are not present! In addition, the inductive coils are protected by a plastic film.

The disadvantages are the following:

- The magnetic field irradiated from the sensor is spread over a large water sample area, and so any geometrical or mechanical change of the measuring area alters the calibration. This is often referred to as the *proximity effect*.
- To achieve an accurate calibration, a rather large calibration tank (filled with well-stirred seawater) is needed if proximity effects are to be avoided.
- Since the sensor construction cannot be miniaturised, the sensor cannot be installed in confined spaces unless it is calibrated *in situ*.
- The electrical signal generated by this sensor is very small and particularly affected by the other physical variables (temperature and pressure). Hence, a rather complex associated electronics is needed. Since this kind of electronics basically requires a rather significant amount of current, this sensor cannot be used where very little power is available. The sensor requires temperature and pressure compensations; the latter is rather difficult to be obtained as the pressure error to be quantified for each specific sensor needs a big volume pressure simulator. The cost for manufacturing this simulator is very high if very high pressures are required (150 - 1000 bar) and a lot of time for testing is needed.

There are four main manufacturers of inductive conductivity sensors: Aanderaa, RBR, RDI/Teledyne and Valeport⁸.

4.1.11.2 The platinum polyelectrode sensors

Polyelectrode sensors, provided with (2), 3, 4, 5 and 7 electrodes are currently present on the market to carry out profiles and/or monitoring: fresh, sea and ocean waters.

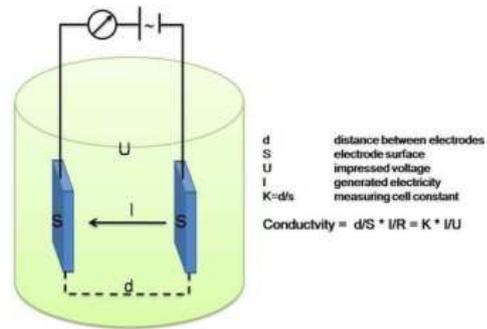
The two-electrode conductivity sensor

Traditional conductivity cells, which have existed since the late 19th century, are based on two planar electrodes facing each other at a given distance. The best performance is obtained by using platinum

⁸ www.valeport.co.uk, www.rbr-global.com, www.rdinstruments.com, www.aanderaa.com



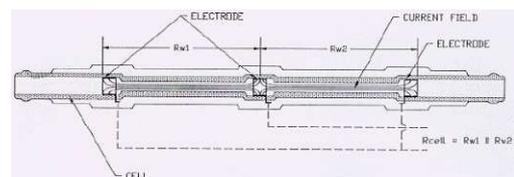
as the electrode material. Other possible materials to produce "low cost" electrodes are carbon, nickel and stainless steel. An AC square wave or sinusoidal voltage is applied to the two electrodes, which generates a current when the cell is immersed in the measuring solution. Amplification and record of this current allows determining the resistance of the media in accordance with Ohm's Law and finally the specific conductivity by combining eqs. 1 and 2 above presented. This is the most common conductivity sensor, as it has been used in the laboratory and in the field probably for 100 years. The two electrodes are usually sealed and supported inside a glass tube which acts as the measuring cell. This avoids/limits the induction of the electrical fields outside the measuring area. The two electrodes are to be electrochemically covered with a black sponge platinum deposit, to avoid the polarisation effects created by the measuring current circulating through the water sample. Due to the electrolytic effect, the measuring current is the cause of H₂ and O₂ gases formation by the two electrodes which are, at the same time, the measuring and the excitation electrodes. The limitations of this simple sensor are several, especially as far as the measurement or rather high conductivity of marine waters is concerned. Since the sensor needs the black platinum deposit, it cannot be mechanically cleaned as this action may partially remove the black sponge, thus altering the calibration dramatically. Furthermore, the black platinum porosity favours the adsorption of organic, colloidal and/or particulate matters which may be present in the measuring media. Therefore, the black platinum layer must be removed and plated again, quite frequently, depending on the application type. Most end-users are unsuccessful in accomplishing this operation, so a cell with contaminated electrodes must be discarded or returned to the manufacturer for maintenance. The two-electrode sensor requires specific AC frequency excitation, depending on the measuring range. Especially at very high conductivity levels (sea water), the frequency to obtain the best linearity can be up to 10 KHz and this presents some electronic difficulties.



In conclusion, this two-electrode conductivity sensor is not used any more in the modern CTDs

The SBE three-electrode conductivity sensor⁹

Evolution of the two-electrode sensor is the three-electrode sensor (See the SBE three-electrode schematics below). The three-electrode sensor does not generate any external field because its outer electrodes are connected together and no voltage creates any external electrical current. In practice, it is a "two-electrode" cell in which the electrode resistances are in series with (and indistinguishable from) the cell resistance proper. Because the electrode resistances are low and the cell resistance high, errors resulting from changes in the electrode resistances are very small. On the other hand, the sample volume entirely determined by the cell's "hard parts" is immune to proximity errors. This sensor is probably the most used for oceanographic measurements, as it is installed in all the SBE CTDs. However, it must be used in clean waters only, as the three electrodes are provided with black platinum coating which, if contaminated, requires the return of the sensor to the factory for replatinization. The small internal diameter of the measuring cell makes it mandatory to install a cumbersome and power-consuming submersible pump for sampling and flushing and, to prevent the recessed conductivity cell from getting clogged, SBE must use Tributyltin (TBT) as an antifouling, which is a heavy chemical poison



(TBT is toxic to fish and other aquatic life and are accumulated by these species. At an international level, the use of TBTs in marine situations is gradually being phased out as the potentially harmful

⁹ www.seabird.com

effects they may have on aquatic life are recognised. TBTs are listed as substances for priority action under the Helsinki and OSPAR Conventions which protect the marine environments of the Baltic Sea and north-east Atlantic Ocean respectively).

The four-electrode conductivity sensor

Another important improvement on the basic two-electrode conductivity sensor, made in the fifties, was the addition of another two electrodes, to get the four-electrode cells. The cell design is usually based on 4-ring electrodes, embedded in a glass support, at a given spacing distance. In this cell, the AC excitation voltage is applied to the two outer electrodes and the current generated, when the cell is immersed in the water, causes a drop of voltage between the two inner electrodes. Measurement of this voltage is achieved by high-impedance input amplifiers which prevent any current flow at the two measuring inner electrodes and, by the way, the electrode polarization and thus the need for the black platinum coating. Thanks to the advantages linked to the 4-electrode cell design and measurement principle, important improvements in conductivity measurements have been achieved with respect to the basic two-electrode sensor.

The four-electrode technique allows several kinds of layouts. We herewith show only the simplest one.

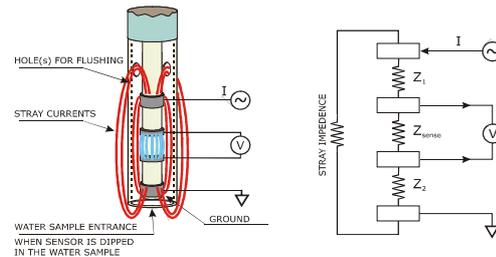


Figure 4.1.59. Four electrode conductivity sensor

The above picture shows the layout and the schematic of an on-line 4-electrode sensor where the excitation electrode also generates external stray currents which affect the current flow. This design, however, is used for laboratory applications only, as it cannot well withstand high pressures. The limitation of this 4-ring electrode design is that the cell requires one (or more) hole, to allow the sample flushing inside the cell. This hole causes part of the current flowing from the innermost electrode (to which is applied the excitation voltage) and the outermost (which represents the ground) to flow through the hole thus reaching the ground electrode from the outside of the cell. This generates a measuring error when the cell is placed in a small container, e.g. a beaker, as its wall alters the external current flow depending on the distance between the cell (and in particular its hole) and the wall itself. In practice, the sensor suffers from a sort of “wall effect” where, if the hole diameter for venting the cell is very small, the error potentially generated is small, e.g. 1%, but the flushing of the cell becomes rather difficult and, if the hole is rather big, the flushing of the cell is good but the error due to the “wall effect” becomes important, even 10% of the reading.

The AML OCEANOGRAPHIC full ocean four-electrode conductivity cell¹⁰



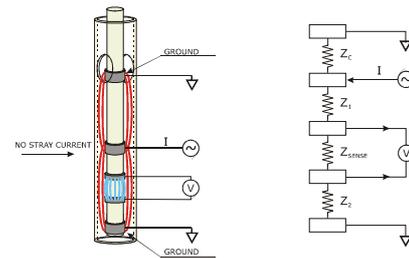
Figure 4.1.60. AML Oceanographic Four electrode conductivity sensor (a) New Style and (b) Old Style

AML OCEANOGRAPHIC does not provide any detail about characteristics of this cell, apart from accuracy which, with its associated electronics, is 0.01 mS/cm.

¹⁰ www.amloceanographic.com

The Idronaut five-electrode conductivity sensor¹¹

To overcome the limitation of the 4-ring sensors, the 5-electrode cell, has been developed by Idronaut (patent applied for it) few years ago. By adding a fifth outer electrode near the excitation voltage electrode and grounded together with the other external ground electrode. In this way, no stray currents are present and the “wall effect” no longer exists. Another important advantage is that if, for any reason an electrical current is circulating in the solution to be measured, this cannot influence or alter the internal measurement as, because of the two external grounded electrodes, this cell is completely shielded. Another innovation of this sensor is the removable (glass) “cuvette” which allows very easy cleaning of the 5-ring platinum electrodes.

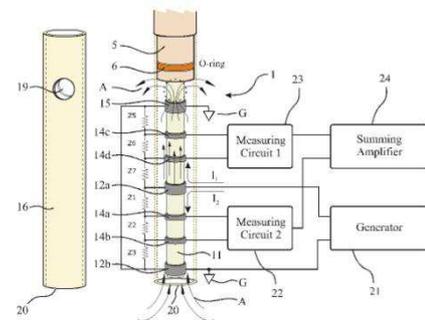


This sensor does not require the black platinum coating of the five electrodes but, due to its complete glass construction, it is rather fragile and so is only used for laboratory applications.

The Idronaut seven-electrode conductivity sensor

CTDs, based on a high-performance seven-electrode conductivity cell, have been present on the market for more than 30 years. This type of conductivity sensor is a flow-through cell and consists of a double-cell differential measuring system using seven platinum rings fused and deposited in the slots of a quartz tube.

The central ring is excited with an alternate current flowing to both the outermost rings. The two adjacent pairs of rings sense, differentially, the relative drop in voltage due to the electrical conductivity of the measured water. The outermost pair of rings is grounded to shield the measuring cell from any outside electrical interference and so this sensor does not suffer from any proximity effect.



The cell is mounted in a cylindrical plastic body which guarantees thermal insulation and which is filled with silicone oil and provided with a rubber bellows to achieve pressure compensation, gaining full ocean deployment.

The conductivity sensor can well operate even in polluted waters since its seven platinum electrodes do not need to be coated with platinum black sponge, which can be easily contaminated and so requires to be replatinized by the factory. The measuring cell can be easily cleaned in the field if any foreign material is deposited onto the platinum rings, causing a decrease in the electric signal, to obtain the original calibration. The cleaning is simply accomplished with cotton buds and liquid soap, without requiring any particular precaution.

The only drawback of this sensor is that its construction is rather complex and time consuming and so its manufacturing cost is rather high.

Conclusions on the poly-electrode conductivity sensors

The conductivity sensors equipped with 3, 4, 5 and 7 electrodes, are good conductivity sensors (based on electrodes and inductive) for clean oceanic waters. However, for monitoring polluted waters and/or harbour areas, only the non-platinized (without black platinum coating) electrodes are those which can be used more successfully, as they can be mechanically cleaned (even with hydrochloric acid) when contaminated.

¹¹ www.idronaut.it

4.1.11.3 Conductivity References

[UNE88] UNESCO, 1988: The acquisition, calibration, and analysis of CTD data. A report of SCOR Working Group 51, UNESCO technical papers in marine Science, 54.

[WSS30] Wenner, F., E.H. Smith, and F.M. Soule, 1930: Apparatus for the determination aboard ship of the salinity of sea water by the electrical conductivity method. Bureau of Standards Journal of Research, 5, 711-732.

4.1.12 Pressure

According to its definition, pressure is the normal force per unit area exerted by water (or air in the atmosphere) on both sides of the unit area. Usually, ocean pressure is usually reported in decibars where 1 dbar = 0.1 bar. The force due to pressure arises when there is a difference in pressure between two points. In the ocean, pressure increases with increasing depth and the pressure at a given depth depends on the mass of water lying above that depth. A pressure change of 1 dbar occurs over a depth change of slightly less than 1 m. Pressure in the ocean thus varies from near zero (surface) to 10,000 dbar (deepest). Pressure is usually measured in conjunction with other seawater properties such as temperature, salinity, and current speeds. The properties are often presented as a function of pressure rather than depth. Horizontal pressure gradients drive the horizontal flows in the ocean. For large-scale currents (of horizontal scale greater than a kilometer), the horizontal flows are much stronger than their associated vertical flows and are usually geostrophic. The horizontal pressure differences that drive the ocean currents are on the order of one decibar over hundreds or thousands of kilometers. Horizontal variations in mass distribution create the horizontal variation in pressure in the ocean. Pressure is usually measured with an electronic instrument called a transducer. The accuracy and precision of pressure measurements is high enough that other properties such as temperature, salinity, current speeds, and so forth can be displayed as a function of pressure. However, the accuracy, about 3 dbar at depth, is not sufficient to measure the horizontal pressure gradients. Therefore other methods, such as the geostrophic method, or direct velocity measurements, must be used to determine the actual flow. Pressure sensors are also employed on the measurement of the sea level (or even swell). When used for this purpose, sensors must be placed at very low depth at a geodetically well defined point. The precision of these instruments usually reaches the millimeter and require a simultaneous measurement of the atmospheric pressure. Numerous pressure sensor technologies are available on the market. Only a small number fit these criteria. This section presents two of the sensors that are often encountered in oceanography.

Piezoresistive pressure sensors

Piezoresistive sensors use strain gauges that generate an electrical signal when deformed. Thus, the pressure changes suffered by a system can be monitored by measuring the electrical signals generated by the pressure mediated deformation of the strain gauge. Common technology types are Silicon (Monocrystalline), Polysilicon Thin Film, Bonded Metal Foil, Thick Film, and Sputtered Thin Film. Generally, the strain gauges are connected to form a Wheatstone bridge circuit to maximize the output of the sensor and to reduce sensitivity to errors. This is the most commonly employed sensing technology for general purpose pressure measurement. Generally, these technologies are suited to measure absolute, gauge, vacuum, and differential pressures.



Piezoelectric pressure sensors



Piezoelectric pressure sensors make use of the piezoelectric effect (generation of electricity by material deformation, strain,) in certain materials such as quartz to measure the strain upon the sensing mechanism due to pressure. In order

to improve the metrological characteristics of the sensors, certain manufacturers have made oscillators from them. By inserting quartz crystal electrodes into an oscillating electrical circuit, it is possible to move the frequency of the circuit if the blade is subject to compression. This technology is commonly employed for the measurement of highly dynamic pressures.

4.1.12.1 Examples of real oceanic monitoring devices

In order to respond to recommendations dictated by WOCE HPO, oceanographers [FM91] must have appropriate instruments at their disposal that are fit for in situ use. Their performance must be close to the limits that can be reached with the most advanced laboratory instruments. Conductivity–temperature–depth (CTD) profilers have been created to achieve such limits. Today, CTDs are regularly used when measuring the profile of water columns. The carrier ship comes to a stop, the CTD descends vertically into the water with the help of a winch and temperature, conductivity and pressure are continually measured at a rate allowing the correct sampling of crossed water layers during phases of descent and ascent.

SEABIRD PRESSURE sensor

Strain Gauge: the SBE 29 [SBE14] pressure sensor, used on the SBE 25 SEALOGGER CTD, is a modular sensor consisting of a mechanical strain-gauge pressure transducer with thermistor temperature compensation. The SBE 29 measures absolute pressure in one of seven full-scale pressure ranges from 20 to 7000 meters depth. The sensor elements and their interface electronics are modular and self-contained, providing easy installation, service, and calibration. An internal pressure sensor is mounted inside foam insulation near the bottom of the card file. A properly operating sensor exhibits a square wave frequency of 32 - 40 kHz at this point.

IDRONAUT Pressure sensors [IDR14]

PA-10: The Series PA-10 pressure transducers cover all pressure ranges from 100 mbar to 1000 bar. They have been produced for over 20 years. These pressure transducers are in use world-wide in a variety of different applications. Main fields of application are: Level technology, pneumatics, hydraulics, avionics. A high-sensitivity piezoresistive silicon chip is used for pressure sensing. The chip is protected against ambient influences by a stainless steel housing sealed with a concentrically corrugated diaphragm. The housing is filled with silicone oil so as to ensure the transfer of the pressure from the diaphragm to the sensing component. All metal parts in contact with the pressure media are made of stainless steel 316L. The fully welded housing is vacuum-tight. Each pressure transducer is subjected to comprehensive tests as to its pressure response and temperature characteristics.

Highly precise pressure sensor: This high-precision 0,01 %FS pressure transmitter is based on the stable, floating piezoresistive transducer and the newly developed XEMICS microprocessor with an integrated 16-bit A/D converter. Temperature dependency and nonlinearity of the sensor are mathematically compensated by the interfacing electronics. The output rate is 400 Hz. Accuracy and Precision: “Accuracy” is an absolute term, “Precision” a relative term. Deadweight testers are primary standards for pressure, where the pressure is defined by the primary values of mass, length and time. Higher class primary standards in national laboratories indicate the uncertainty of their pressure references with 70 to 90 ppm or close to 0.01%. Commercial deadweight testers used to calibrate the transmitters indicate an uncertainty or accuracy of 0.025 %. Below these levels, expression “Precision” is the ability of a pressure transmitter to be at each pressure point within 0.01 %FS relative to these commercial standards.

Disadvantages of current pressure sensors:

The sensor technologies described earlier allow the accuracy required by oceanographic measurements to be achieved. When dynamic measurements are taken, however, errors can appear because of:

Problems with hysteresis. As the instrument rapidly increases in pressure, then returns to the surface, the load cell of the sensor does not instantly revert to its initial form.

Problems in temperature lags. Sensors used being compensated in temperature, this compensation can happen with lags or advances on the true temperature of the sensor as the instrument crosses zones of high thermal gradients, which then leads to errors in the measured and corrected pressure.

4.1.12.2 Pressure References

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[IDR14] Idronaut Pressure Sensors

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[SBE14] Seabird SBE-29 Pressure Transducer

http://www.seabird.com/products/spec_sheets/29data.htm Accessed 01 Aug 2014

4.1.13 pH

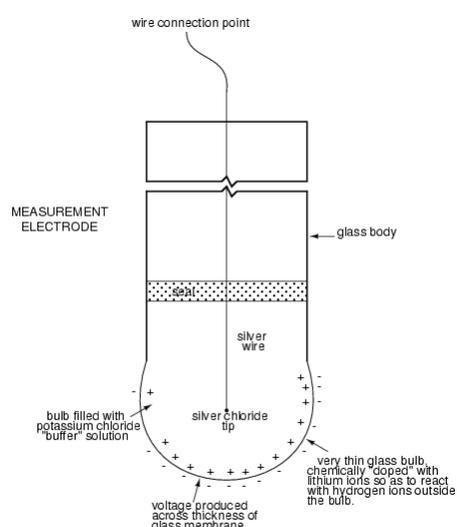
Currently, the lack of accurate *in situ* pH measurements, apart from very few exceptions, limits our understanding of natural pH variability. In order to predict future pH changes in the oceans, quality high temporal resolution pH data are needed.

Ocean acidification is a pressing environmental problem that has gained widespread recognition as an early manifestation of coastal climate change. Observed changes in the marine carbonate system include a rise in the oceanic partial pressure of CO₂ (pCO₂), decrease in pH, and decrease in carbonate ion concentration due to the absorption of anthropogenic CO₂. Very little is known about the inherent variability in pCO₂ in near shore marine waters or the relationships between ocean acidification, pCO₂, and pH values in estuaries.

The hydrogen ion is a ubiquitous species encountered in most chemical reactions [KAG07]. It is quantified in terms of pH –the negative logarithm of its activity:

$$\text{pH} = -\log a\text{H}^+ \quad (1)$$

The pH sensors are widely used in chemical and biological applications such as environmental monitoring (water quality), blood pH measurements and laboratory pH measurements amongst others. The earliest method of pH measurement was by means of chemical indicators, e.g. litmus paper that changes its color in accordance to a solution's pH. For example, when litmus is added to a basic solution it turns blue, while when added to an acidic solution the resultant color is red. Since many chemical processes are based on pH, almost all aqua samples have their pH tested at some point. The most common systems for pH sensing are based upon either amperometric or potentiometric devices. The most popular potentiometric approach utilizes a glass electrode because of its high selectivity for hydrogen ions in a solution, reliability and straight forward operation. Ion selective membranes, ion selective field effect transistors, two terminal microsensors, fibre optic and fluorescent sensor, metal oxide and conductometric pH-sensing devices have also been developed [KAG07]. However, these types of devices can often suffer from instability or drift and, therefore, require constant re-calibration.



4.1.13.1 The pH glass electrode

A pH electrode is a potentiometric or electrochemical sensor that has a voltage output. A potentiometric sensor consists of two electrochemical cells or electrodes: the measuring sensor and the Ag/AgCl reference sensor. The active part of a pH measuring electrode is the sensor glass membrane. The glass membrane can be manufactured in different shapes, depending on the application and is constructed from a special composition glass which senses the hydrogen ion concentration. This glass is mostly amorphous silicon dioxide (SiO₂), with embedded oxides of alkali metals, mainly Na. It is made to be as thin as possible, about 0.1 mm thick. To keep its electrical impedance as low as possible, the pH measuring electrode, which is sensitive to the hydrogen ion, develops a potential (voltage) directly related to the hydrogen ion concentration of the solution. The reference electrode provides a stable potential against which the measuring electrode is referred. When immersed in the solution, the reference electrode potential does not change with the changing hydrogen ion concentration. A solution in the reference electrode makes the electrical contact with the sample solution and the measuring electrode through a junction, completing the circuit. Output of the pH measuring electrode changes with temperature (even though the process remains at a constant pH), according to the Nerst equation, so a temperature sensor is necessary to correct it.

4.1.13.2 Commercially available Marine pH Sensors

The SBE pH sensor [SBE14]

“The SBE 18 pH Sensor uses a pressure-balanced glass-electrode /Ag/AgCl-reference pH probe to provide *in-situ* measurements at depths up to 1200 meters (3900 ft). The replaceable pH probe is permanently sealed and is supplied with a soaker bottle attachment that prevents the reference electrode from drying out during storage. The sensor and associated interface electronics is a modular, self-contained package that is easy to install, service, and calibrate.

The SBE 18 is intended for use as an add-on auxiliary sensor for profiling CTDs. Power / signal interface cables and mounting hardware are available separately. The SBE 18's interface circuits buffer and offset the differential glass-electrode/reference potential to produce a high-level pH-dependent output voltage. Computation of pH in engineering units is typically done using a specific software. Sea-Bird calibrates the pH sensor against precision buffer solutions (4, 7, and 10 pH ± 0.02 pH). These calibration results are tabulated on a certificate furnished with each sensor.” This combined pH/reference sensor, in practice, is the “traditional” 12 mm diameter pH electrode commonly used in the laboratories.

The ADM pH sensor [ADM14]

“The pH sensor for depths of up to 6,000 m consists of a pre-amplifier covered by a titanium housing and a separate pH and a reference electrode. This complete sensor has been developed for the external interfacing to CTD probe systems and can be used for monitoring or profiling. Differing from



Figure 4.1.61. ADM pH Sensor The AMT pH-combined sensor for submersible probe

the standard single tube design, a separate reference electrode is used with a silver/silver chloride cell in a chloride gel.

Contact with the unknown solution is made through a small hole in the tip of the sensor. This minimises and stabilises the potential

between electrolyte and sample.” The pH and separate reference sensors installed are made by Idronaut.

The problem:

Measuring pH in sea water with glass electrodes demands a special sensor construction to avoid mistakes caused by the high ionic strength of the sea water.

Troubles may also occur when using conventional reference electrodes with ceramic diaphragm because of the high and variable junction potentials which are developed at pressures. This was the reason, that up to now pH electrodes have been offered only for depths of up to 1,500 m. Besides, sometimes it was a little bit inconvenient to use two channels of the probe system for pH measurement - one for the glass electrode and another one for the reference electrode.

The solution - a new combined electrode for submersible probe systems



Figure 4.1.62. The AMT pH-combined sensor for submersible probe system

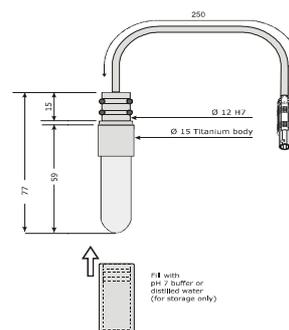
The pH-combined sensor consists of a reference electrode and a pH sensitive glass electrode in one housing to save one free channel of the probe system. To realize accurate measurements in the deep sea too, a double diaphragm for the reference electrode was used to avoid problems

with the signal stability during pressure changes. The interface reference electrode/sample is realized in this case by means of a hole diaphragm. Inside the reference electrode follows a KCl containing gel with a special built-in second diaphragm containing the Ag/AgCl-reference system in a potassium chloride solution. The pH-combined sensor for the *in-situ* determination of pH is available as deep sea version (up to 600 bar) without integrated temperature sensor.”

The limitation of this approach is that, since the pH and reference electrodes are combined, there is not too much space for the KCl gel of the reference electrode and so this sensor can have a limited lifetime for monitoring.

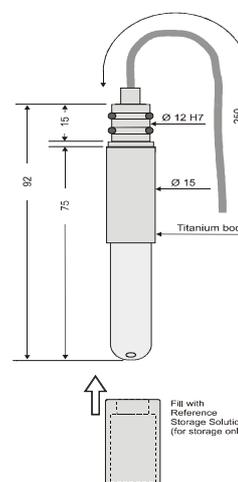
The combined pH/reference sensor

The pH and reference electrodes are usually built together into one device called “the combined pH sensor for laboratory application”. In the past decades, these combined pH sensors have been modified and installed in CTD multiparameter probes in the attempt to get valuable ocean data. One company (Idronaut), more than 30 years ago, decided to go back and use again the original separate pH and reference electrodes and an optional and innovative NaCl gel reference sensor, designed for long-term monitoring in the oceans. See the below descriptions.



The Idronaut pH sensor [IDR14a]

The measurement of pH in seawater demands high accuracy since seawater has a high ionic strength and is weakly buffered. The pH range in the oceans is particularly restricted and, only in very special cases, the observed values are outside the range of 7.8 and 8.4 pH and, in some seas, the range extends from 6.5 to 9.0 pH. Some problems have always arisen from the use of traditional reference sensors with porous diaphragms, when measuring the pH in seawater, in particular at pressures in excess of a few bars, due to the high and variable junction potentials that are generated. The Idronaut pH sensor has a glass body and a very low-impedance “blue” pH sensitive glass tip, which can withstand pressures up 700 bar. The sensor head is made of titanium. During all periods of inactivity, the glass tip must be fitted with a white plastic hydrating cap filled with the pH 7 Buffer Solution, or simply with clean water. This is to prevent the pH-sensitive glass from dehydration, which slows down the sensor response.



The Idronaut reference sensor [IDR14b]

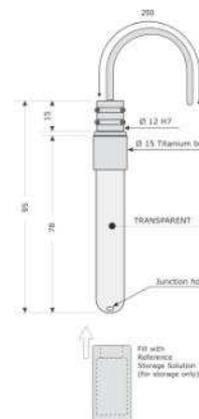
The Idronaut reference sensor is in contact with the unknown solution by means of a small hole in the glass tip. This minimizes and stabilizes the junction potential between the inner gel electrolyte and the liquid to be

measured. The reference sensor is a Silver/Silver Chloride cell in a saturated potassium chloride solid gel and the sensor head is made of titanium. The glass body of the sensor is fitted with a plastic hydrating cap filled with the Idronaut Reference Sensor Storage Solution based on 3-mol KCl (or NaCl) or, if not available, even with KCl saturated solution to avoid drying of the gel when not in use. Idronaut has been providing these pH and reference sensors to ADM, AML, RBR, Valeport, others for a long time, as declared in their web sites.

The Idronaut NaCl reference sensor [IDR14b]

Several years ago, Idronaut developed an innovative NaCl gel reference electrode to minimize the diffusion potential at its junction, which causes systematic pH errors on seawater measurements and related calibration.

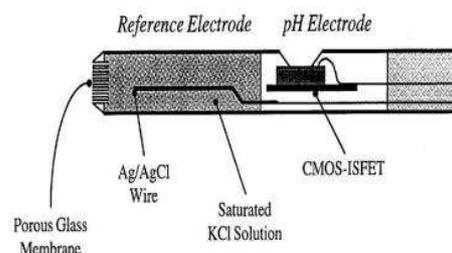
When a reference electrode is immersed in a calibration or in a measuring solution, some liquid junction potential is generated by any salt flowing from the inside of the reference electrode into the solution and/or vice versa, as below described. If the reference electrode is based, as in most cases, on a KCl saturated or a 3-mol solution or gel and is immersed in a seawater sample, KCl flows from the reference electrode into seawater and NaCl flows into the reference electrode through its junction (ceramic, fibre or simply a small hole, when a solid gel electrolyte is placed inside the reference electrode), due to the high difference in concentrations of both metals between the internal electrolyte of the reference electrode and the seawater media. In such a case, the junction potential, which is caused by the cations flow, is the greatest as both potentials are summed, their flow being opposite. Therefore, in order to obtain accurate pH measurements, the calibration buffer and the measuring solution must have, in particular, very similar salt composition so that the junction potential generated is practically identical and becomes nullified during the pH zero calibration phase.



In this case of seawater measurements, some additional advantages are obtained by using a NaCl reference electrode instead of the “traditional” KCl reference electrode. In fact, the NaCl flow outside is small being minimal the difference of concentration of NaCl of the two solutions and, in addition, in one way only both during the calibration and the measuring phases; the residual junction potential is nullified during the pH zero calibration phase. Another important advantage of using the NaCl reference electrode for seawater determination is that it will last much longer than any other high quality KCl reference electrode as its internal NaCl salt will not be “stripped” during prolonged measurements and continuous seawater monitoring, and NaCl seawater salt will not contaminate the inner AgCl half-cell with the passing of time, thus causing a prolonged drift of up to about 30 mV when KCl is completely replaced by NaCl. A particular construction is adopted as one or two thin glass concentric tubes will be installed inside the NaCl gel electrode so as to avoid that the inner Ag-NaCl half-cell soon becomes contaminated because of the near junction hole. This mechanical approach, which to the best of our knowledge is innovative, makes it possible to obtain similar performance of a very long and cumbersome reference electrode in small dimensions.

The ISFET pH sensor

ISFET (Ion Sensitive Field Effect Transistor) is a 10-year mature technology applied to industrial and laboratory applications that is now attracting interest for the environmental pH monitoring too. This type of sensor, based on the Ion sensitive field effect transistor, is combined with integrated reference sensor. The ISFETs are sensible to the sample temperature and therefore a thermal compensation is mandatory to achieve results comparable to the glass pH electrode. The advantages of ISFET include robustness, stability and



precision, which make it suitable for ocean pH measurement at low pressure. A novel sensor based on the ISFET technology is the SEAFET pH sensor, developed by Dr Kenneth Johnson of the Monterey Bay Aquarium Research Institute (MBARI) and Dr Todd Martz of the Scripps Institution of Oceanography, University of California San Diego and presently commercialized by a USA company, Satlantic [SAT14].

SeaFET Ocean pH-Sensor

The SeaFET Ocean pH Sensor is designed to accurately measure pH in both marine and freshwater environments. Satlantic has collaborated with MBARI and Scripps to make the instrument available to the scientific community. The SeaFET utilizes Ion Sensitive Field Effect Transistor (ISFET) technology which has been used extensively for industrial, clinical and environmental pH monitoring. The ISFET offers unsurpassed robustness, stability and precision that together, represents a substantial improvement in performance over traditional pH sensors. With data logging, scheduling and internal battery pack, the SeaFET provides flexible deployment options. The SeaFET can be deployed autonomously or integrated with existing sensors and systems. The sensing element of the SeaFET™ is an ion sensitive field effect transistor (ISFET) which is potentially



measured against a reference electrode bearing a liquid junction (internal reference) and against a solid state reference electrode, without a liquid junction (external reference).



SP100-SM QUIMA pH sensor [SEN14]

SensorLab was established in 2011, and QUIMA group prototypes gave birth to the first commercial submarine sensor, the SP100-SM. The experience acquired with this sensor led to the new SP101-SM, packing several improvements over the previous generation of sensors. These improvements included a 40 percent reduction in the sensor power consumption, thanks to a redesigned higher efficiency electronic controller, and a new low-power LED light source. The corrosion resistance has also been improved with the addition of an epoxy coating, plus polyurethane finishing on top of the 6060 hard anodized aluminum

housing.

The SP101-SM is a rugged and extremely stable pH sensor for shallow waters, designed for oceanographic research. The design of the SP101-SM allowed the same accuracy obtained on lab-scale, but autonomously deployed in the ocean, allowed long-term measurements without human intervention.

4.1.13.3 pH References

[ADM14] ADM Sensors

<http://www.adm-elektronik.com/senors.html> Accessed 01 August 2014

[IDR14a] Idronaut pH Sensor

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[IDR14b] Idronaut Reference Sensors

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[SEN14] SensorLab SP101-SM

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4.1.14 Partial Pressure Of Carbon Dioxide (pCO₂) measurements

Measuring dissolved gases is always a challenging task. Taking samples for lab analysis is complex and any interaction with the sample will change the concentration, especially while working with

pressurised samples from different depths. Therefore, reliable sensors for in-situ measurements need to be used. In addition, not every sensor is suitable for every application. A “cheap” sensor used for industrial boundary value detection will most of the time not meet the requirements (in terms of resolution and accuracy) for a scientific application. Furthermore, Sensors for salt water are always exposed to extreme conditions and special engineering for reliable and long lasting solutions is required. For autonomous application the power consumption of a sensor is always a limiting factor and need to be as low as possible.

The partial pressure of CO₂ in water is a parameter which is hardly directly measurable. Methods to determine the pCO₂ of water directly are high pressure liquid chromatography (HPLC), mass spectrometer analyses and measurements based on colorimetric methods. There are different methods to determine the pCO₂ in water and seawater indirectly. The first one is to measure the pCO₂ value of gas which is in equilibrium with a water sample. The second one is to measure the pCO₂ concentration as a change in pH using an electrode. Both methods are based on an equilibration measurement. The third method is based on a colorimetric analysis. The fourth indirect method is to estimate the pCO₂ from the sea surface temperature.

- HPLC – *Laboratory* measurement.
- Colorimetric analysis – *In situ* measurement.
- Severinghaus electrode – *In situ* measurement.
- pCO₂ gas analyser – *In situ* measurement.
- pCO₂ estimation using sea surface temperature – correlation method.

Each technology/method has its advantages and disadvantages which are briefly introduced below.

In addition, it is important to point out that most of the available systems deliver data which need to be corrected by different parameters, such as water temperature (for flow-through systems at the water intake and at the sensor), salinity and pressure. Each parameter needs to be measured with high accuracy at the right place in parallel to the pCO₂ measurement.

Currently available instrumentation using these methods are available from the following suppliers: Battelle, Franatech, General Oceanics, IDRONAUT, NKE instrumentation, OxyGuard International, SubCtech, Sunburst and Turner.

4.1.14.1 High pressure liquid chromatography (HPLC)

The HPLC is used to identify and quantify each component of a sample. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length.

This method is used in laboratories and needs a lot of know-how from the person handling the sample.

4.1.14.2 Colorimetric analysis

The colorimetric analysis uses a water sample which is prepared with a reagent and different wavelength to determine the concentration of pCO₂. This method determines a quantitative, physical relationship between colour intensity and concentration of the solved matter.¹²

The advantages of this method are the high accuracy and the long-time stability. The use of reagents is a disadvantage, especially when the system is difficult to reach and the reagent needs to be replaced often. The disposal of a sample contaminated with some reagent is always an issue which needs to be considered when used on an autonomous system.

Systems working with this method are from **NKE** and **Sunburst**.

¹² <http://www.spektrum.de/lexikon/biologie/kolorimetrie/36710> accessed on 23 July 2014

The nke instrumentation Carioca system¹³

CARIOCA is an automated drifting buoy that measures the partial pressure of dissolved CO₂ in seawater in order to quantify air/ocean exchanges. *p*CO₂ is measured in liquid phase by colorimetry on a reagent using a 3-wavelength spectrophotometer with a 4 µatm accuracy.

The buoy is autonomous, data is sent via the ARGOS satellite system, which also controls the positioning. The buoy's autonomy is one year based on an hourly measurement rate.

Measuring campaigns have been carried out in the Mediterranean, Greenland Sea, Equatorial Pacific and the Antarctic. The CARIOCA project is part of the EUREKA EU 819 programme.

The Sunburst Sensors SAMI-CO2¹⁴

- Measures the partial pressure of carbon dioxide *p*CO₂ in water from 150-700 µatm (other ranges available by request)
- Uses a highly precise and stable colorimetric reagent method
- Provide researchers with valuable *in-situ* time series data
- Deployable to depths up to 600 meters
- Can be deployed in the ocean or in freshwater
- Long-term deployments - can run for more than a year taking hourly measurements
- Can support up to 3 external instruments such as PAR, dissolved oxygen, chlorophyll fluorometer, or CTD
- Can support inductive modems or external loggers if required.
- Biofouling Package available for deployments in productive environments

¹³ <http://www.nke-instrumentation.com/products/profilers/surface-drifting-buoys/carioca.html?m=0> accessed on 23 July 2014

¹⁴ <http://www.sunburstensors.com/products/oceanographic-carbon-dioxide-sensor.html> accessed on 23 July 2014

Table 13: SAMI-CO2 Performance Parameters

Typical calibration range*	150-700 μatm
Deployment duration	~10,000 measurements
Response time	~5 minutes
Accuracy**	+/- 3 μatm
Precision	<1 μatm
Long term drift	<1 μatm units over 6 months
Thermistor accuracy, precision	0.1° C, +/- 0.01° C
Dimensions (housing length, diameter)	55 cm, 15.2 cm
Weight in air/seawater	7.6 kg / 1.1 kg

4.1.14.3 pCO₂ Gas Analyser

To get the $p\text{CO}_2$ value of water samples (continuously or from a single sample) for gas analysis, they need to be in equilibrium with a carrier gas and the $p\text{CO}_2$ of the gas is measured.

Equilibration

To reach equilibrium between a gas and water four different kinds of equilibrators are used (Figure and Figure). The aim of the first three types is to get a maximum surface (phase boundary) between the gas and the water for an optimal exchange. The fourth equilibrator is using a membrane to separate the water and the gas but still enables an exchange through it.

Shower type (a): The water is spread in small water drops into a chamber filled with carrier gas.

Bubble Weiss type (b): The carrier gas is bubbled through a frit into the water (in many small bubbles).

Laminar flow type (c): Both types of equilibrators mentioned above have a curved surface as phase boundary. However, the “Laminar flow type” equilibrator has a constant flow of water and of carrier gas. These flows are in contact, but the direction of the flow is in the opposite direction, the phase boundary is flat. [Koe95]

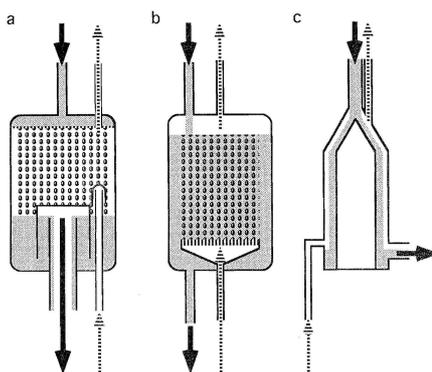


Figure 4.1.63. Schematic representation of the most common equilibrator set ups: a) Shower type, b) Bubble Weiss type, c) Laminar flow type.

Flat-Membrane Equilibrator: The water and the gas are separated by a semi-permeable membrane which allows CO₂ as well as H₂O molecules to pass through and allows therefore an equilibration. The materials for these membranes are silicon and silicon-composites. The thickness of the membrane has an influence of the mechanical and chemical properties in terms of exchange rate and pressure resistance. [HCT04]

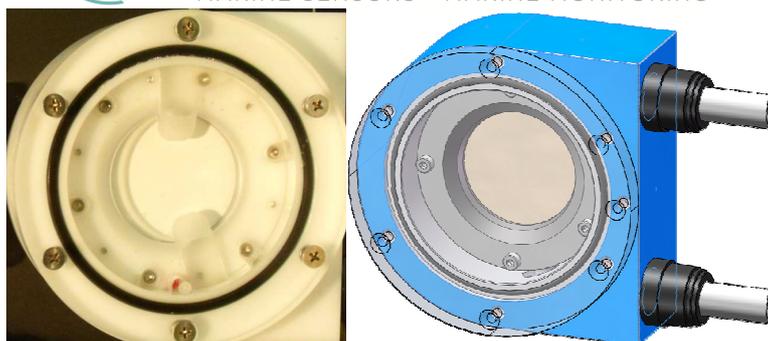


Figure 4.1.63. Picture of flat-membrane equilibrator for flow-through application (SubCtech).

The advantages/disadvantages are listed in Table X

Table 14. Advantages/Disadvantages of equilibrators

Equilibrator	A	B	C	d
Size	Big	Big	Big	Small
Suitable for flow-through systems	Yes	Yes	Yes	Yes
Suitable for in-situ under water measurements	No	No	No	Yes
Effected by sediments	Yes, might cause damage to the analyser	Yes, might cause damage to the analyser	Yes, might cause damage to the analyser	No influence on measurement quality
Effected by biofouling	Yes	Yes	Yes	Strong biofouling might slow down the transfer through the membrane

The gas used for the analyses is either exchanged every time after measured (open cycle system) or reused all the time (closed cycle system). The closed cycle has an advantage for small changes because of the pre-equilibration; however, for bigger changes an open system equilibrates faster. [Koe95, HCT04]

Analysis of the gas

The equilibrated gas can be either analysed with a gas-phase chromatograph or detectors based on infrared measurements.

Gas-phase chromatograph: The CO₂ is reduced with hydrogen to methane, which is detected. For this method the volume of the sample has to be known and the time to process a single sample is long. To take a sample for this analyses special equipment and knowledge is needed. [Koe95]

Infrared: The infrared method is one of the commonly used methods and based on the absorption of energy by the CO₂ inside the gas phase. The analyser correlates the detected, not absorbed, rays with the values from a reference channel [GKR03]. This method is applied by analysers from LI-COR Biosciences®.

Cavity-ring-down-Spectroscopy: This method is a highly sensitive optical spectroscopic technique that enables measurement of absolute optical extinction by gashouse samples that scatter and absorb light. A typical CRDS setup consists of a laser that is used to illuminate a high-finesse optical cavity, which in its simplest form consists of two highly reflective mirrors. When the laser is in resonance with a cavity mode, intensity builds up in the cavity due to constructive interference. The laser is then turned off in order to allow the measurement of the exponentially decaying light intensity leaking from the cavity. During this decay, light is reflected back and forth thousands of

times between the mirrors giving an effective path length for the extinction on the order of a few kilometers.

If something that absorbs light is placed in the cavity, the amount of light decreases faster—it makes fewer bounces before it is all gone. A CRDS setup measures how long it takes for the light to decay to $1/e$ of its initial intensity, and this "ringdown time" can be used to calculate the concentration of the absorbing substance in the gas mixture in the cavity.¹⁵ These methods are applied in systems from Los Gatos and Picarro, Inc.. However, currently they are not implemented in the available commercial salt-water sensors.

The infrared and cavity-ring-down-spectroscopy have a lot of advantages in terms of reliable results and the quick response time. Most of the time the analyser is connected directly to the equilibrator and the gas is pumped through the analyser and no manual sample taking is needed (reduction of contamination risks). However, there are although some disadvantages in terms of size, weight and price for the analyser used for scientific applications. Especially cavity-ring-down- spectroscopy analyser have a high power consumption (250W). Infrared and cavity-ring-down-spectroscopy analyser are initially designed high resolution atmospheric measurements. When lower resolution and data quality is needed (e.g. for aquaculture and leak detection) less expensive industrial sensors (e.g. for smoke detection) are implemented (e.g. from Edinburgh Inc.). The internally used analyser mainly determines the accuracy and precision of the whole sensor.

The General Oceanics $p\text{CO}_2$ Analyser¹⁶

General Oceanics supplies complex, autonomous, flow-through $p\text{CO}_2$ measurement systems for scientific research since 1999. The actual version is Model 8050. The Analyser (normally Li-COR analyser, infrared method) is not included and need to be bought and installed separately by the owner of the system. For this system a shower type equilibrator is used. The resolution and accuracy is depending on the analyser bought and the calibration gases (calibration is recommended several times a day).



Figure 4.1.64. Flow-through system by General Oceanics

Specifications:

- Wet Box: Length 24 inch (61 cm) Width 24 inch (61 cm) Depth 10 inch (25.4 cm)
- Dry Box: Length 24 inch (61 cm) Width 24 inch (61 cm) Depth 10 inch (25.4 cm)
- Satellite Box: Length 14 inch (35.6cm) Width 10.5 inch (26.7cm) Depth 8 inch (20.4cm)
- Note: The User must supply the following items:
 1. Main water intake pump and plumbing (interfaces to the wet box), see pump and through hull intake plumbing option above.
 2. Calibration gases and connecting lines.
 3. CO_2 analyser (Licor 6262 or 7000 recommended)

The Model 8050 $p\text{CO}_2$, Measuring System is an autonomous analytical system for measuring carbon dioxide in oceanic surface water. The system is modeled after instruments previously built by Craig Neill/University of Bergen. The system may be used on a ship underway or, on a variety of at -sea platforms. The present $p\text{CO}_2$ system operates fully automatically and consists of:

- An equilibrator that balances the CO_2 in seawater with a headspace gas that is analyzed

¹⁵ <https://www.tu-braunschweig.de/pci/forschung/gericke/forschung/crds> accessed on 23 July 2014

¹⁶ <http://www.generaloceanics.com/product.php?productid=1462&cat=69&page=1#tabs> accessed on 23 July 2014

- An infrared analyser (User Supplied) to quantify the CO₂ concentration in the equilibrator headspace and marine air
- A network of valves and pumps that select, control, and monitor flows of seawater, equilibrator headspace, marine air, and CO₂ gas standards
- An on deck enclosure that provides a GPS signal, atmospheric pressure measurement and satellite data transfer.
- An integrated notebook computer, computer interface, and software to control valves and pumps and to log data (pressures, temperatures, flows, analyser response, date, time and position).

The system consists of three enclosures: the dry box, the wet box, and the satellite deck box. The dry box contains a Li-COR CO₂ analyser (user supplied), electrically actuated valves to control the gas flow through the analyser, a gas flow sensor, computer and interface with solid-state relays. There are inlets for up to eight gases. Each inlet has a needle valve for adjusting the flow rate. A three-way solenoid valve directs the gas exiting the analyser towards a vent or towards the equilibrator (when analyzing the equilibrator headspace). The dry box utilizes an RS485 module and cables to communicate with the other two boxes.”

The SubCtech pCO₂ Analyser¹⁷

Since 2007 the existing flow-through systems were analyzed and weaknesses and strengths determined. The following issues were identified and addressed by SubCtech and successfully realized.

- Reducing the size
- Reducing the complexity
- Reducing the maintenance
- Lowering the operation cost
- Increase the robustness
- Reliable autonomous application
- High precision CO₂-analyser already included
- High quality after sale support
- Installation and training support

For flow-through as well as for in-situ measurements sensors were developed. The used analyser are from the LI-840x (for water measurements) and Li-7200x (for air measurements) from Li-COR® Biosciences and already included. Other analysers like CO₂ analysers (e.g. Picarro) can be included instead of Li-COR on request. The equilibrator used is a flat-membrane type, which allows the system to be quite small.

- LI-COR® Biosciences LI-840x CO₂/H₂O sensor fully included: Highest accuracy due to automatic temperature, pressure and H₂O compensation - dual-wavelength NDIR detector
- Patented robust Flat-Membrane-Equilibrator cassette – lifetime 10+ years, easy to handle
- Hand-held, easy to operate and maintain system with intuitive overall design. Low maintenance costs! No special setup required. Just plug’n’play.

Figure 4.1.65. pCO₂ analyzer by SubCtech



¹⁷ http://subctech.eu/empemco2_monitoring/pco2_analyzer/ Accessed on 14 August 2014

- Robust, versatile and compact housing for offshore and laboratory applications. No need for special filters. Just provide water up to 20 l/min – max. 6 Bar (best: 5-10 l/min – 1 Bar)
- High time series stability due to obligatory standard offset zeroing. Optional auto- or manual span gas calibration or reference gas measurements supported.
- SmartDI[®] (Smart Data Interface) Datalogger concept: easy instrument integration for e.g. Aanderaa Optode; TriOS, Turner or WET Labs Fluorometer; Seabird or Sea&Sun CTD and Thermosalinograph; SYSTEA nutrient analyser any may other.
- Optionally connection of external devices through RS-485 bus, e.g. meteorology sensors or sea- water pumps. A full water supply is supported.
- Real-time data interface NMEA-0183: simple ASCII protocol, configurable to your needs.
- 12/24V dc or 110/230V ac power supply
- Data logging on 2GB CF (Compact-Flash) Card. Automatic report of interferences and initiation of diagnostic routines. Redundant data and configuration files.
- Optional GPS geo reference for all data and position event control

The Battelle pCO₂ Analyser¹⁸

In 2009 Battelle (USA) was chosen by NOAA's (National Oceanic and Atmospheric Administration) Pacific Marine Environmental Lab (PMEL) to meet the requirements for a sensor to detect air-sea CO₂ fluxes.

The self-contained, modular design has the ability to be deployed on a wide variety of platforms. It is designed to operate unattended for more than 12 months at a time. Currently, more than a dozen systems are placed on a variety of buoys around the world. NOAA is using this system to develop a global array of moored observation systems to determine air-sea flux in support of the Global Ocean Observing System (GOOS). This new analytical instrument measures the partial pressure of carbon dioxide (pCO₂) in the ocean and atmosphere, which is needed to understand the global ocean uptake of atmospheric CO₂. The need for ocean carbon measurements is becoming increasingly important because high levels of carbon dioxide in the oceans have led to ocean acidification in surface waters, which could have significant effects on a variety of marine organisms and ecosystems. The system has a measurement range of 100 to 600 parts per million, with an extended range available upon request. Incredibly, the system also has a precision accuracy to about one part per million. Self powered by batteries, the system contains compact flash memory storage and an Iridium satellite communications link.

The Franatech pCO₂ Analyser¹⁹

In 2012 Franatech (Germany) developed a flow-through sensor as well as an in-situ analyser for process control in aquaculture, long-term monitoring in hydroelectricity reservoirs and for attachment to a Ferrybox system.



Figure 4.1.66. In-Situ pCO₂ sensor by Franatech

Specifications of in-situ CO₂-sensor

- Weight of sensor alone: 2.3 kg
- Range: 0-50 mg/l

¹⁸ <http://www.battelle.org/media/press-releases/battelle%27s-field-tested-autonomous-pco2-monitoring-system-is-now-commercially-available> accessed on 23 July 2014

¹⁹ <http://www.franatech.com/co2sensor.html> accessed on 23 July 2014

- Operation temperature: +2°C to +40°C
- Storage temperature: -10°C to +50°C, < 85% humidity
- Water tight: IP68 5bar
- Output: 4-20mA
- Power supply: 110 / 230 VAC (50 / 60 Hz)
- Current drain: 200mA"

The OxyGuard International pCO₂ Analyser

This sensor is normally used in aquaculture applications (industrial) to monitor the CO₂ concentration in water and to avoid fish dying.

Principle of Operation

The probe measures the carbon dioxide content of the water directly by detecting the carbon dioxide partial pressure in the water. It is important to note that it is NOT based on a pH measurement - it does NOT detect any change in pH that carbon dioxide causes. Neither does it withdraw a sample for analysis - it detects the free dissolved CO₂ - the carbon dioxide partial pressure - in the water.

The instrument's range is 0-50 mg/l. Before use the system is calibrated using mixtures of water and calibration chemicals in a special calibration beaker with stirrer.

Specification

Size: Probe: 40 mm dia. x 140 mm. Cable length 7 m.

Meter: 120 x 120 x 58 mm

Calibration beaker: 65 mm dia. x 260 mm.

Weight: Probe with cable and transmitter 1.0 kg.

Calibration accessories: 1.0 kg.

Supply: 230 VAC (115 VAC or 24 V DC on request)

Range: 0 - 50 mg/l dissolved carbon dioxide.

Operating conditions: 3-35°C

Accuracy: Depends on calibration. Practical accuracy up to +/- 1 mg/l.

Response time: Typically 5 minutes at 20°C depending on flow velocity past probe.

In still water up to 15 minutes.

Flow requirements: The instrument does not consume carbon dioxide for its measurement, but a certain flow is necessary to ensure that the sensing element of the probe is in equilibrium with the surrounding water and to avoid "spot" measurements.

Output: 4 - 20 mA current loop."²⁰

The Turner pCO₂ Analyser²¹

Recently Turner (USA) released an in-situ pCO₂ sensor whose different measurement ranges and applicable up to 600m.

"C-sense™ probes are compact, lightweight sensors for measurement of the partial pressure of gas in liquids. Designed for applications involving immersion in water, oil, or water and oil mixtures, the sensors combine an oil-resistant interface with a compact, temperature-compensated non-dispersive

Figure 4.1.67. OxyGuard CO₂ analyser



²⁰ <http://www.oxyguard.dk/images/stories/pdf/g02%20oxyguard%20co2%20brochure%20gb%200912.pdf> accessed on 23 July 2014

²¹ <http://www.turnerdesigns.com/products/submersible-fluorometer/c-sense-in-situ-pco2-sensors> accessed on 23 July 2014

infrared (NDIR) detector. Designed for integration, C-sense™ enables $p\text{CO}_2$ monitoring at a significantly lower price than traditional $p\text{CO}_2$ sensors.

Product Highlights:

- Available in 3 measurement ranges:
 - 1000ppm
 - 2000ppm
 - 4000ppm
- Accuracy 3% of full scale
- Submersible to 600m
- Easily integrated: 4-pin analog output
- Small size: <2" x 8" and <1lb
- Low power consumption: 80mA @ 6V DC
- Water temperature: -2 to 35°C
- Ambient Temperature -20 to 50°C
- Weight: 430grams"



Figure 4.1.68. C-sense sensor by Turner

4.1.14.4 Severinghaus electrode

The Severinghaus electrode was invented by Dr. Severinghaus in 1953.

This glass electrode is surrounded by a film of bicarbonate solution and covered by a thin plastic carbon dioxide permeable membrane. An equilibration of CO_2 through the membrane between the bicarbonate solution and the water occurs. The changed CO_2 concentration leads to a change in the pH of the bicarbonate solution (see below), which is measured and correlated to a certain $p\text{CO}_2$ value.²²

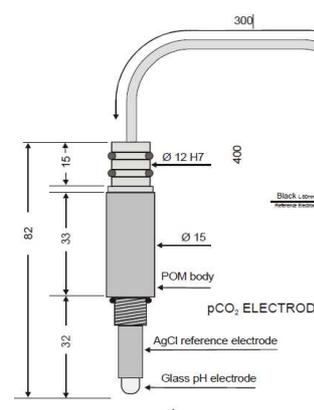


The advantage of the Severinghaus electrode is the very small size and the relatively low price. Disadvantages are long response times, high calibration need (due to drifts) and limited detection boundaries (especially for low values).

The Idronaut $p\text{CO}_2$ Electrode²³

Idronaut supplies a $p\text{CO}_2$ electrode. Based on the Severinghaus electrode

The $p\text{CO}_2$ electrode consists of a measuring half-cell and a reference half-cell both part of one common or combination electrode. The measuring half-cell with the pH sensitive glass at the tip is sealed within the glass electrode's body. Inside the electrode, the constant pH buffer and Ag/AgCl electrode are located. The reference electrode (Ag/AgCl wire) is a band on the exterior of the electrode. The $p\text{CO}_2$ electrode inserts into the membrane cap containing $p\text{CO}_2$ electrolyte, allows the reference electrode to make electrical contact with the measuring electrode. The components of the electrode are described below.



The outer silastic membrane

²² <http://www.chemie.de/lexikon/Severinghaus-Elektrode.html> accessed on 23 July 2014

²³ <http://www.idronaut.it/cms/view/products/sensors/pcosub2sub/s333> accessed on 23 July 2014

The gas permeable outer membrane of the Idronaut PCO₂ electrode is a 150 μ silastic film. This membrane acts as a selective barrier in the sense that it is permeable to gas (CO₂) but not to ions. Thus, ions of the sample do not diffuse across the membrane to contribute to the pH change of the pCO₂ measuring system. The silastic membrane represents a break-through in the improvement of the response time of the pCO₂ systems.

The inner nylon spacer

Since diffusion rates are slow relative to the desired response time, the measuring pH glass electrode system must be placed as close as is practical to the gas permeable membrane. However, the pCO₂ electrode requires a thin film of aqueous pCO₂ electrolyte between the glass tip and the reference band of the electrode. A nylon mesh spacer covers the tip of the electrode and maintains a layer of electrolyte between the glass tip and the silastic membrane.”

4.1.14.5 pCO₂ estimation using sea surface temperature

This method is an alternative to the punctual measurement of pCO₂. Because of the high variability of the value in ocean water there are some difficulties to get an area-wide and continuous profile. Therefore Lafèvre and Taylor [LT02] used the strong correlation between pCO₂ and sea surface temperature. The authors estimated the pCO₂ value from the sea surface temperature at different locations of the South and North Atlantic gyres. Different regression lines were used and the algorithm includes although latitude, longitude and atmospheric pressure. The area wide data are collected using satellites.

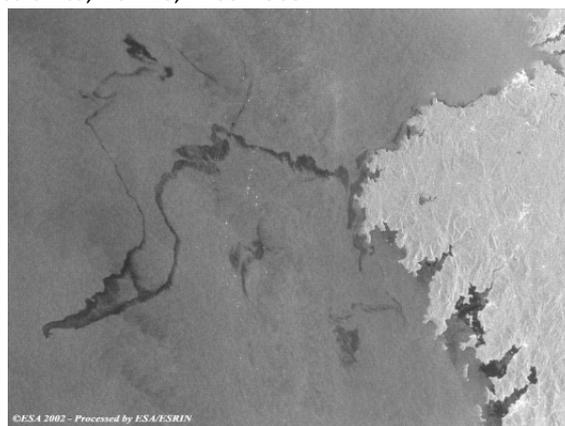
Advantage of this measurement is that the values covering a great area, the price for the data is relatively low in comparison for the amount of data and the collection takes place nearly maintenance free. The disadvantages are, good weather is needed for accurate measurements, only the surface value for pCO₂ can be calculated, no profiling and the satellite might not be at the right position to the right time.

Data from those satellites are made available to the public by different organizations, for example PMEL²⁴

4.1.14.6 pCO₂ References

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- [LT02] Lafèvre, N., and Taylor, A., (2002). “Estimating pCO₂ from sea surface temperatures in the Atlantic gyres,” *Deep-Sea Research I*, Vol. 49, P. 539-554.

Figure 4.1.69. pCO₂ electrode from Idronaut



²⁴ <http://www.pmel.noaa.gov/co2/story/Surface+CO2+Flux+maps>

4.1.15 Hydrocarbons

4.1.15.1 Introduction

Oil spill detection is crucial to fight against one of the most important source of marine pollution. The environmental damage associated to the hydrocarbons coming from oil spills is particularly evident in case of big oil spills either from tanker accidents (e.g., Prestige, Erika, Exxon Valdez, etc) or from the increase of deep and offshore oil and gas rigs. Additionally illegal actions related with cleaning tanks and the lack of regulations of gray waters from cruise ships is a latent source of hydrocarbons pollution at sea.

Detection at sea has been focused basically on remote sensing from radars on board satellites (SAR) or from airborne mounted systems that can also use multispectral radiometers to classify substances released at sea. This is one of the routine tasks performed by coastal guards aircraft surveys to monitor oil spills. Recently attempts to detect and classify oil spills from MODIS satellite imagery [Ala11] and from ship borne LIF/LIDAR [Yar11] has been designed to detect and classify oil spills in marine water. In the case of radar systems the parameter is the surface roughness. It is known that oil spills modify the surface tension properties of sea waters and thus the Bragg backscattering resonance signal that is recovered by radars. Typically the detection of oil spills appears as a distinct backscatter area in a radar image (Fig. 1).

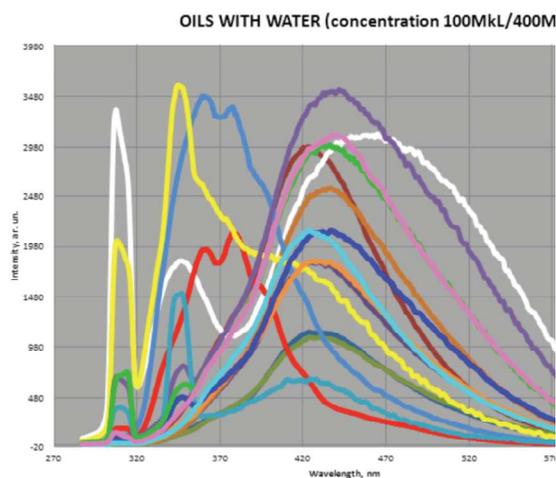


Figure 4.1.70. SAR image of the Prestige accident in 2002, NW of Spain. The oil spill is clearly seen revealing the path followed by the tanker up to the sinking point.

Figure 4.1.71. Spectral of fluorescence of different hydrocarbons (taken from [Yar11])

Because sea surface roughness changes in response to wind, sometimes the backscatter signal cannot be uniquely associated to an oil spill often making it difficult to separate low wind areas from real oil spills. For example, the black regions at the southern coastal areas in Fig. 1 are due to a wind screening effect of the local orography not to the presence of oil.

In-situ detection of oil spill is much more difficult. The monitoring systems are based on the fluorescence spectral signature of hydrocarbon molecules. Oils are excited in the ultraviolet wavelengths (300-400 nm) and their response (reflectance) at visible wavelengths from 400-600 nm whose signals are modified by additives and the refinement process (Kar05, Yar11) (Fig. 2).

4.1.15.2 Instruments

In-situ fluorometers have been manufactured in a similar way like the typical chlorophyll-*a* fluorometer but working on the oil spectral

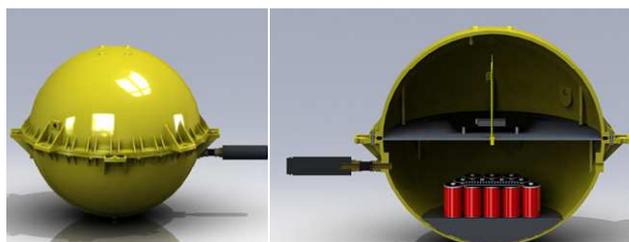


Figure 4.1.72. Scheme of a drifter carrying an *in situ* fluorometer.

range. Natural waters are excited through a specific ultraviolet wavelength and a band pass emission filters allow the instrument to read a broad range of dissolved compounds [LC10].

To our knowledge, there is only one manufacturer, Turner Designs, which provides this kind of instrument, CYCLOPS-7 (C-7), that can be easily integrated in several platforms of observation. In particular there is one suitable for drifting buoys (Fig. 4.1.72). These buoys, called Oil drifters, have been used during field experiments in the frame of the TOSCA project (Tracking Oil Spills and Coastal Awareness Network²⁵). The scheme consisted of a C-7 sensor mounted in the hull of a surface drifter with ARGOS telemetry. The fluorometer was attached externally to the spherical buoy, slightly under the buoyancy line to keep it close to the surface. Inside the sphere, the electronics consisted of a single board with conditioning circuitry to manage data from the sensors, which facilitated the



Figure 4.1.73. Real prototype of Oil drifter used in a field experiment. The side view (top right) shows the configuration of the 5- m fluorometer.

production and testing of the system. The core of this PCB was an 8-bit micro controller Microchip with a Harvard architecture and RISC instruction set. Such boards are known to be widely open for connections to facilitate any future expansion with capacity to manage external instruments with RS232 output and converters up to 24 bits for the data processing of self-developed sensors.

The electronic control was made by a Microchip 16F876A microcontroller that managed data acquisition with a 10 bit A/D converter, with a compression algorithm, and sent

the information to a SEIMAC X-CAT transmitter. A sequence of 8 voltage data was acquired every 10 minutes. Each voltage value was stored in two bytes in a 18-byte transmitting packet. The remaining two bytes were informative about the humidity inside the buoy to inform on the working conditions of the circuitry.

The system's primary power supply was formed by a compact pack of lithium thionyl chloride batteries which is able to provide the highest energy density among the currently available technologies. The power system also integrated "super capacitors" in order to supply the high current pulses (> 1 A) required by satellite transmitters without overloading the batteries thus ensuring a 2-year minimum operation time of the system without maintenance.

4.1.15.3 Results

An Oil drifter manufactured as described was tested in a field experiment (Balearic Experiment), in the Mediterranean near the Baleric Islands (Figure 4.1.74). The unit was attached to a WOCE standard drifter, equipped with ARGOS telemetry, driven by a holey sock centered at 10 m depth. The main properties of the C-7 fluorometer used, according to the calibration solutions are shown in the table below

²⁵ <http://www.tosca-med.eu>

Table 15. Properties of C-7 fluorometer

TYPE	Detection limit	Dynamic Range	Excitation	Emission
OIL-CRUDE	0.2 ppb ²⁶	0-2700 ppb	325/120 nm	410-600 nm
OIL-FINE	10 ppb ²⁷ 10 ppm ²⁸	>10.000 ppb >100 ppm	290 nm	350/55 nm

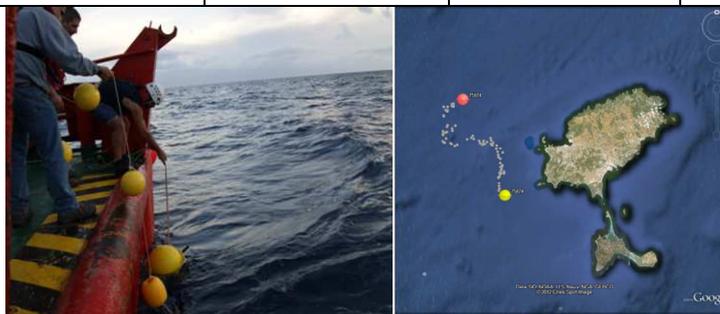
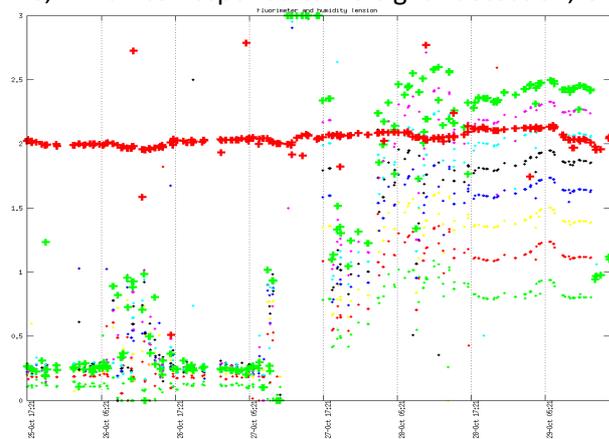


Figure 4.1.74. Left: Deployment of the Oil drifter from a Research Vessel. Right: 4-day trajectory of the oil drifter during the Balearic Experiment.

The drifter was recovered 4 days after its deployment. During that period the fluorometer was programmed to analyze 8 equal bands within the operating wavelength range. In Fig. 6 the time series of the signal transmitted (in volts) for each band during the experiment is represented along with the humidity inside the sphere (for control). Two different periods, separated in October 27th at 5 am, can be clearly seen in Figure 4.1.75. During the first period, all the bands are following a base line, which corresponds to no signal detection, except for a short event, during October 26th where some real signal was detected. The second period, after October 27th the mean voltage fell to 0 V and immediately saturated to 3 V, which is a clear indication that the fluorometer was loosed and the connector was in direct contact with the sea water, probably due to the heavy sea conditions during such period. The system thus was able to monitor the presence of an oil spill *but the Oil drifter was not durable enough to resist heavy weather situations.*



used for *in-situ* water monitoring for oil spills. It is a submersible fluorometer that can be deployed in

Figure 4.1.75. Time series of the 8 bands (in different colored dots) during the drifter trajectory. Green thick crosses are the mean voltage of all the combined bands. Red crosses represent the internal humidity sensor properly scaled.

any manned or unmanned platforms. Provided a robust fixation and adequate installation it can be working up to two years without maintenance on drifting buoys. This is especially relevant since such buoys can be released on an oil spill and track the evolution both in concentration, evolution and degradation. A new model C-3, also submersible and with the same optics as C-7, is now also available from the same manufacturer. This model incorporates a built-in depth sensor and can collect depth and position of every fluorometric reading.

²⁶ PTSA (1, 3, 6, 8-Pyrenetetrasulfonic Acid Tetrasodium Salt)

²⁷ 1, 5- Naphthalene Disulfonic Disodium Salt

²⁸ BTEX (Benzene, Toluene, Ethyl benzene, Xylenes)

Although *in-situ* fluorimeters offer a unique opportunity for monitoring the fate of oil spills, there are still some open problems inherent to these kind of measurements, especially at the surface, where oils tend to stay for a long time. In addition to the stress due to wind, waves and swell, there can be other problems such as interferences with the solar light or other buoyant substances. The alternative, only suitable for oceanographic vessels, is the direct analysis of water collected.

4.1.15.5 Hydrocarbons References

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4.1.16 Microspectrometers

The market drivers for microspectrometers are small, size and weight, low power consumption, with the possibility to be integrated into a portable device, and reduced maintenance costs. The main drawbacks of current microspectrometers are their limited wavelength range and limited sensitivity. The drivers are cost and time savings (on-site, in-field, on-the-go), new opportunities, regulations and rapidly decreasing costs of calculation power, enabling complex data analysis. Figure 4.1.76. shows various application fields for microspectrometers. Microspectrometers covering the VIS - VNIR range, 400 – 800(1100) nm, are manufactured by a number of companies: Avantes, Ocean Optics, BVtech, Bayspec, Hamamatsu, TRIOS etc. The price of an OEM spectrometer module is in the range 1000 to 15000 € depending on the spectral resolution and signal-to-noise ratio. According to their technology, there are mainly three types of microspectrometers: grating, Fourier Transform Infrared Spectrometer (FTIR) and Fabry-Perot [TLJ99]. More recently, there are a resonator-array spectrometer, another microspectrometer technology consisting of a 1-D array of small microdonut resonators [Xia11], and a MEMS (Micro-Electro-Mechanical System) grating spectrometer [Sch14], where the miniature grating elements is a MEMS. An example of a fixed grating microspectrometer is the device developed by Hamamatsu [Ham14], whose dimensions are as small as 27.6 x 13 x 16.8 mm and its weight only 9g. Such devices can measure light in the wavelength range from 350 nm to 1040 nm with a spectral resolution of 14 nm and 20 nm, depending on the type of image sensor chosen.

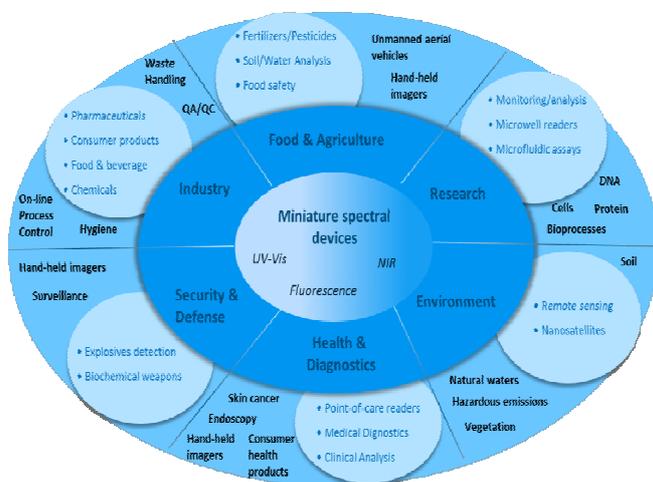


Figure 4.1.76. Application fields for microspectrometers [Ant10]

4.1.16.1 References

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4.2 Novel Sensors

4.2.1 Polymer pH Sensors

We will refer to a comprehensive review on the application of polymers in various sensor devices and more specifically, review of various methods used for pH measurement [KAG07].

Namely, by introduction of functional groups, polymers can be designed to selectively swell and shrink, resulting in changing mass and elasticity, as a function of analyte concentration. The ion-exchange properties of conducting polymers are of special interest for potentiometric- sensor development [KAG07]. Conducting polymers are ideally suited for sensor applications because they not only exhibit high conductivity and electroactivity but they could also be used as a general matrix and can be further modified with other compounds in order to change selectivity. Compared to conductive polymers, nonconductive polymers usually have a high selective response and a high impedance, which is important for eliminating interference by other electroactive species.

4.2.1.1 Review on Existing pH Sensors based on Polymers

a) Optical and Fluorescent pH-Sensors

Optical methods can be used to measure the concentration or the activity of hydrogen ions. The basic concept of the optical methods of pH measurement relies on the fact that the incident beam of light is passed through a light guide to the active end of the sensor where it interacts with the chemical indicator, which alters the beam's intensity, usually by absorption or by fluorescence [KAG07]. The modified optical signal is guided to the detector. Several fiber-optic pH sensors have been proposed, which base their working principle on the fluorescence or the absorption of an appropriate chromophore [Wol02]. Remote sensing can be achieved since the optical signal can be carried over long distances. Most of the reported fiber optic pH sensors exploit indicator dyes, which when immobilized on part of the optical fiber, cause pH sensitive changes in the absorption spectrum of the test solution. Interaction with the analyte leads to a change in the optical properties of the reagent phase, which is probed and detected through the fiber optic. A major issue with this approach is the sensitivity of the device, which is directly related to the thickness of the pH sensitive layer.

Microspheres of polyacrylamide containing bound phenol red and smaller polystyrene microspheres for light scattering were packed in an envelope of cellulosic dialysis tubing at the end of a pair of plastic optical fibres [PGF80]. The probe measured pH over the physiological pH range of 7.0 to 7.4 with 0.01 pH resolution and this flexible construction was about 0.4 mm in diameter. However, these methods are often more difficult to implement and may lead to loss of dye sensitivity or result in poor fluorescence properties. Non-covalent immobilization techniques for pH sensors have involved methods of entrapment behind semi-permeable membranes and adsorption within polymeric supports [PGF80]. As a drawback, coatings based upon non-covalent methods of dye immobilization suffer from leachability.

Polyaniline (PANI), which belongs to the group of organic conducting polymers, has an excellent pH sensing properties. The use of PANI receives attention due to its high conductivity, ease of synthesis,

and its stability under ambient conditions. PANI based sensor can measure pH in the range from 2.15 to 12.54 based on PANI film deposited onto ITO glass employing constant-potential (0.80 V) electropolymerization. pH-dependent spectral variations of PANI films were explained by their transformation from the protonated to the unprotonated form.

A novel method for pH measurements between pH 7.5 and 10.4 with a precision of 0.2 pH unit was also reported [TWG03]. The method combines Raman spectroscopy and the automated sequential injection analysis system (SIA) and makes use of the acid-base properties of a commercially available water dispersion of PANI nanoparticles with a mean particle size of 46 nm. The pH measurements were conducted with the 633-nm laser excitation wavelength by calculating the difference between the Raman intensities of the primary and reference wavenumbers, where the pH-sensitive CH=CH stretching band at 1422 cm⁻¹ and C-H in-plane bending band of the quinoid form at 1163 cm⁻¹ were chosen as the primary wavenumbers [TWG03].

The nano-pH sensor based on dual-fluorophore-doped nanoparticles that offers the advantages of adequate sensitivity, accuracy and rapid detection of pH was proposed by Gao et al [GTD07]. Additionally, this sensor showed excellent stability, high reproducibility and a fast response time. The development of a pH-sensitive fluorescence nano-sensor by co-doping the indicator dye and reference dye in silica nanoparticles is considered due to their biocompatibility.

b) Electrodes modified with pH-sensitive polymers

Characteristic pH chemical sensors based on polymer-film-coated electrodes include electropolymerization of monomers such as pyrrole, aniline, thiophene, or benzene derivatives. The measurements of pH using the mentioned conductive polymers had poor reliability due to defects and pinholes present in the films structure. For example, Platinum electrodes, modified by a coating of a thin L-PEI film, resulting from the anodic oxidation of pure ethylenediamine, exhibited a linear, reversible, and stable in time potential response sensitive to pH changes in aqueous media. The assembly of the electrode surface coated with electropolymerized ethylenediamine acted as a transducer of the electrode potential versus the pH value in aqueous solutions. A possible mechanism by which the linear polyethylenimine responds to pH changes could be due to the affinity of the numerous amino groups to the protons in solution [HLH01]. The reaction of H⁺ with amino groups creates local charge density excess at the electrode surface. The potentiometric response can be considered as behaviour controlled by a surface reaction, which takes place on the PEI film. Protonation and deprotonation of superficial amino groups of the PEI is symbolically described in Eq. (2) [KAG07]:



c) Potentiometric pH sensors

The ion-selective electrodes (ISEs) used for potentiometric pH-sensors, as well as their optical counterparts, ion-selective bulk optodes, have the unique capability of sensing free ion activities instead of the total concentration. Bulk optodes belong to a newer class of sensors and are usually based on the competitive or cooperative extraction of the analyte ion with protons between the polymeric and aqueous phase. This two-phase sensing mechanism has advantages in reaching a lower detection limit dictated by the thermodynamics ion extraction, and may lead to the mass production of monodisperse ion sensing microbeads that can be flexibly coupled with analytical flow cytometry or optical-fiber based microsensor arrays. The unique ion-sensing capabilities of ISEs and bulk optodes make them very useful for environmental monitoring. Various polymers have been considered suitable for potentiometric pH sensors [LHL05]. Potentiometric pH sensors based on linear polyethylenimine (L-PEI) and linear polypropylenimine (L-

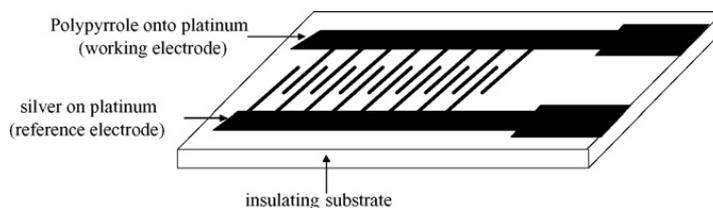


Figure 4.2.1. Schematic drawing of interdigitated microarray electrodes

PPI) (two synthetic enzymes and biocompatible polymers) films were prepared by electropolymerization of three different monomers: ethylenediamine (EDA), 1,3-diaminopropane (1,3-DAP) and diethylenetriamine (DETA). These polymers are considered good candidates for pH biosensors due to their strong bonding to the electrode surfaces during the electropolymerization step.

Characteristic type of potentiometric pH miniaturized sensors based on electrosynthesized polypyrrole films is shown in Fig. 4.2.1. These pH sensors have two interdigitated microelectrodes, fabricated by photolithography. One electrode of the sensor is coated by a polypyrrole film, while the other one is coated by a silver film used as reference electrode, as shown schematically in Fig. 4.2.1. Four sensors with various film thicknesses were tested for pH response, where the samples have film thicknesses of: PPy1 – 150 nm, PPy3 – 400 nm, PPy10 – 1750 nm and PPy15 – 2400 nm. The potentiometric response of this sensor is linear to pH changes for the pH range between 2 and 11. The long-term stability was assessed and during 30 days no deterioration in the sensor performance was registered.

d) Miniaturized pH sensors

Advances in the semiconductor industry combined with novel recently developed polymer materials enable engineers to design various sensors with optimised performance parameters. Thong et al [TGS06] reported a novel design of silicon piezoresistive pH sensors, based on a pH sensitive hydrogel, namely poly(vinyl alcohol)–poly(acrylic acid). The general sensor principle is as follows: swelling due to pH value changes causes bending in a flexible plate. In the case of capacitive transducers, a change in capacitance will be detected. For resistance bridge sensors, due to membrane deflection, the corresponding membrane stress will change the bridge resistance and consequently the output voltage.

Patterns of polypyrrole (PPy) have been fabricated on flexible poly(tetrafluoroethylene) (PTFE) films using a combination of micro-contact printing, electroless deposition of copper and electropolymerisation of pyrrole [PBP07]. A patterned elastomeric stamp was used to deliver a nitrogencontaining silane coupling agent to an argon plasma-pretreated PTFE surface. The surface was subsequently activated by PdCl₂ and immersed in an electroless copper plating bath allowing selective metallisation. Electropolymerisation of pyrrole was performed on copper-patterned PTFE resulting in the formation of micrometer-scale PPy structures. Potentiometric measurements have demonstrated that PPy-patterned PTFE has potential application as a pH sensor.

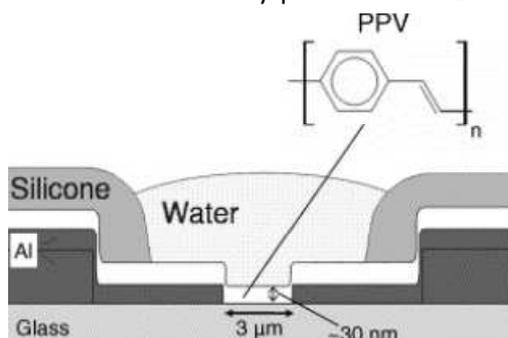


Figure 4.2.2. Schematic drawing of the pH sensitive device showing the aluminium contacts (dark grey) below a thin (30 nm) layer of PPV (white).

Miniaturized pH biosensors were developed using a photolithography process to promote commercial applications. This method allowed the patterning of the metal electrodes on an oxidized silicon surface. A sputtering technique was chosen to coat the patterned electrodes with platinum. Then, one electrode was coated with a polymeric film while the other one was coated with silver to be used as reference electrode. Interdigitated array electrodes were patterned in order to increase the exchange surface between the polymer coated electrode and the solution being analysed. The polymer films coated on the platinum electrodes showed linear potentiometric responses to pH changes from pH 3 to pH 10. Resulting electrodes exhibited both reversibility and stability versus time.

Among the group of conjugated polymers, poly(*p*-phenylene-vinylene) (PPV) and derivatives are some of the most promising candidates for organic electronics applications. The delocalized π -electrons from the phenyl-rings and conjugated carbon chains are responsible for the semiconducting properties of PPV. It is generally believed that for

polymers the primary photoexcitation results in the creation of excitonic states, rather than in the direct creation of free charge carriers as is the case for inorganic semiconductors. Fig. 4.2.2 shows a schematic drawing of a pH-sensitive device that uses the photoconductive properties of a thin PPV layer reported by Pistor et al [PCP07]. No device passivation was necessary since the applied voltages were below the threshold for electrolysis.

4.2.1.2 pH Sensors References

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4.2.2 Nitrate-Nitrite Sensors

Potential technologies and chemical analysis systems which may be employed to achieve cost-effective sensors for nitrate and nitrite measurement are discussed in this report. Furthermore, some systems which allow for the simultaneous monitoring and determination of nitrate, nitrite and other parameters are also investigated. Table 16 below summarizes the discussed technologies; the method of operation and the advantages and disadvantages associated with each technique are highlighted.



Table 16 Summary of technology with advantages and disadvantages

Technology / Technique	Method of Operation	Advantages	Disadvantages
LOC measurement of Nitrite and Nitrate based on colorimetry	Field deployable platform, developed by Beaton <i>et al.</i> (2012), for automated in situ colorimetric nitrate and nitrite analysis using Griess assay, based on microfluidics. For nitrite analysis, absorption is determined in two sequential measurement cells. For nitrate analysis, fluid is combined with imidazole buffer, then passed through cadmium tube, following mixing with Griess reagent, absorption is then determined within the measurement cells.	<ul style="list-style-type: none"> • Suitable for deployment in waters of varying salinity. • Detect nitrate and nitrite up to 21.7 ppm as NO_3^- • LOD of 0.0016 ppm for NO_3^- and 0.000092 ppm for NO_2^-. • Small size and low power consumption, suitable for integration in oceanographic platforms. • Minimal cost 	<ul style="list-style-type: none"> • Use of cadmium to facilitate reduction
Determining nitrate using Griess method utilizing zinc reduction	Sensitive reagent-injection flow analysis method for spectrophotometric determination of nitrate in marine and fresh water, developed by Ellis <i>et al.</i> (2011). Nitrate is reduced in micro column containing zinc granules at pH 6.5. Nitrite formed is reacted with Griess reagent and resulting azo compound is quantified.	<ul style="list-style-type: none"> • Zinc column offers a promising means for reduction of nitrate due to its simplicity, low cost, lack of toxicity, excellent reproducibility and high nitrate reduction efficiency. 	<ul style="list-style-type: none"> • Reactivation of the column in situ with HCl or EDTA solution did not prove satisfactory, and installation of a fresh column was performed after a day of use.
Technology / Technique	Method of Operation	Advantages	Disadvantages
Ion-selective nitrate LOC sensor	Nanobead packing technology applied to fabricate self-assembled nanobead hetero columns in a polymer chip cartridge, to achieve a potentiometric nitrate LOC sensor. This work has been carried out by Jang <i>et al.</i> (2013). A self assembled nBH column is manufactured to hold liquid ion exchange membrane and electrolyte. Analyte detection is then achieved by potentiometry.	<ul style="list-style-type: none"> • New electrochemical sensing platform with high sensitivity and ion selectivity. 	<ul style="list-style-type: none"> • Linearity is only observed within the NO_3^- conc. range of 10^{-5} to 10^{-1}. • The holding capacity of LIX membrane solution and electrolyte in the pores is shown to be different, an issue which needs to be overcome.
Microfluidic nitrate selective sensor based on doped-polyppyrrrole nanowires	Aravamudhan and Bhansali (2008) achieved selective chemical recognition towards nitrate by electrochemical nitrate doping of polypyrrrole	<ul style="list-style-type: none"> • Polypyrrrole nanowire electrodes exhibited a detection limit of $4.5 \pm 1 \mu\text{M}$ within standard seawater samples. • Linear response within the nitrate range of 0.14 ppm to 14 ppm nitrate. 	<ul style="list-style-type: none"> • Potential for presence of residual nitrates in system. • In the presence of high levels of interfering ions the peak current response can decrease by 15-20%.



Technology / Technique	Method of Operation	Advantages	Disadvantages
<i>Microfluidic platform for nitrite monitoring using photoresponsive microvalve</i>	Czugala <i>et al.</i> (2013) have developed a wireless, portable, integrated microfluidic analytical platform for point of care monitoring of nitrite in water. The Griess assay is implemented. A biomimetic photo-switchable microvalve based on phosphonium ionogel functionalized with spiropyran is contained within the microfluidic device. The microvalve is actuated by illumination with a light emitting diode. A paired emitter-detector diode (PEDD) detector is used to determine nitrite concentration.	<ul style="list-style-type: none"> Platform only requires 2.3 μL of Griess reagent per assay, resulting in low cost associated with reagent use, power consumption and waste disposal. The photo-switchable ionogel microvalve is low cost to produce, and the light intensity used to control the microvalve is $\sim 1 \text{ mW cm}^{-2}$, whereas the power consumption of typical miniature solenoid valves is up to 500 mW. The LED used to produce the light for controlling of the microvalve costs approximately €0.80. The PEDD detector used is low cost and highly sensitive. 	<ul style="list-style-type: none"> Short term reusability of ionogel microvalve. Shrinking mechanism of gel results in release of protons into external solution; this does not affect this method, but may have an effect on other assays, such as enzyme based methods.
<i>Simultaneous determination of nutrients using Lab-on-a-disc technology</i>	Platform based on centrifugal microfluidics for the simultaneous determination of NO_2^- , NO_3^- , NH_4^+ , PO_4^{2-} , and silicate in water developed by Hwang <i>et al.</i> (2013). See section 4.1 for reaction mechanisms. All processes were integrated and automatically performed on a rotating disc device. Liquid transfer was controlled by laser irradiation on ferrowax-based microvalves.	<ul style="list-style-type: none"> Total analysis time for simultaneous evaluation of five nutrients was 7.5 min. Requires only 100 μL sample volume for each nutrient. See table 3 for performance characteristics. Low cost Small opportunity for human error. Potential for on-site analysis of seawater. 	<ul style="list-style-type: none"> Limited number of samples can be processed simultaneously on one disc with current design. The optical path length, limited to the thickness of the disc, can limit the devices sensitivity for colorimetric detection.

4.2.2.1 INTRODUCTION

In aquatic environments, an excess of nitrates may lead to eutrophication of the ecosystem due to over enrichment with nutrients. Although the nitrate ion is a chemical species of the basic nitrogen cycle and is commonly found in a diversity of natural processes, nitrate contamination is most frequently associated with anthropogenic activities at the ground surface, such as the fertilisation of agricultural crops, animal wastes, chemical synthesis as precursors of a large variety of chemicals and in the food industry as preservatives [JZK11]. Nitrite (NO_2^-) is both a nutrient and an excretion product of phytoplankton, and is important with respect to the global nitrogen and carbon cycles, with concomitant effects on climate [SFO10]. It should also be noted that NO_2^- present in water systems is readily converted to nitrates by nitrifying bacteria, thus potentially contributing to eutrophication [KWW13].

In general, current commercially available systems to measure nitrate and nitrite concentrations have limited deployment capabilities and scenarios, mainly due to their large physical size, high



power consumption and excessive reagent usage. Thus it is not typically feasible or cost-effective to use these macro-scale systems for distributed sensor networks that continually monitor nutrient levels in aquatic environments [BSF11]. Microfluidic technology permits the miniaturization of chemical analytical methods which are traditionally undertaken using benchtop equipment in the laboratory environment. When applied to environmental monitoring, such as monitoring within aquatic environments, these 'lab-on-chip' (LOC) systems may allow for cost-effective high performance chemical analysis methods to be performed in situ over distributed sensor networks with large numbers of measurement nodes [BCT12]. Portable monitoring systems which are electrochemical sensing-based also appear well suited to in situ analysis and complement standard analytical methods for a number of environmental monitoring applications [JZK11]. These types of portable systems could save tremendous amounts of time, reagent and sample, and may facilitate accurate assessments of marine water quality.

4.2.2.2 LOC MEASUREMENT OF NITRATE AND NITRITE BASED ON COLORIMETRY

[BCT12] have developed a field-deployable platform for automated in situ colorimetric nitrite and nitrate analysis using the Griess assay (diazotization with sulphanilamide and subsequent coupling with N-(1-naphthyl) - ethylenediamine dihydrochloride (NED) to form an intensely colored azo dye). The system which was developed is highly configurable and has low power consumption (1.5 W). The system without reagents and power supply is approximately 100 mm in diameter and 200 mm in length. The use of on-chip sequential absorption cells that use tinted polymethyl methacrylate (PMMA) to exclude background light results in a high sensitivity system with a dynamic range (0.025 to 350 μM) suitable for deployment in a variety of natural waters including estuarine environments and waters of varying salinity.

Fabrication

The fluidic path diagram of the microfluidic platform developed is depicted in figure 4.2.3 below and figure 4.2.4 illustrates a CAD (computer-aided design) drawing of the microfluidic chip.

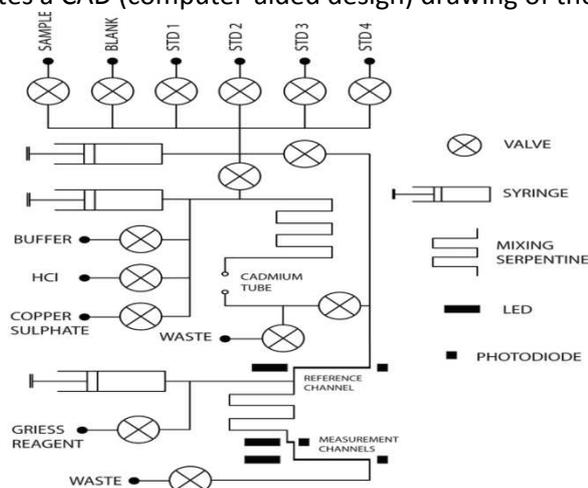


Figure 4.2.3. Fluidic path diagram indicating syringes, valves and absorption cells [BCT12].

The microfluidic platform is based on a circular block of PMMA with a diameter of 100 mm. The microchip is machined in 5.0 mm thick tinted PMMA by micro-milling. The chip incorporates a fluidic manifold that permits selection of one of four standards of nitrate or nitrite, the sample and a blank. 15 micro inert solenoid valves are mounted directly to the chip and these facilitate fluid control. A custom designed pumping architecture drives fluid through 2.78 mm internal diameter titanium syringes, syringe 1 for sample/standard, syringe 2 for the buffer solution and syringe 3 for the Griess reagent. The plungers are driven by a stepper motor based linear actuator and Hall-effect sensors are used for syringe pump feedback and control. Green 525 nm LEDs and photodiodes are fixed directly to the chip using optical adhesive [SFO10]. The chip contains three absorption cells: a 25 mm reference cell, a 25 mm measurement cell for concentrations below 30 μM , and a 2.5 mm measurement cell for concentrations above 30 μM . In terms of housing, the system was housed in a darkened water-tight acrylic tube terminated with acetal plastic end-caps and sealed with o-rings.

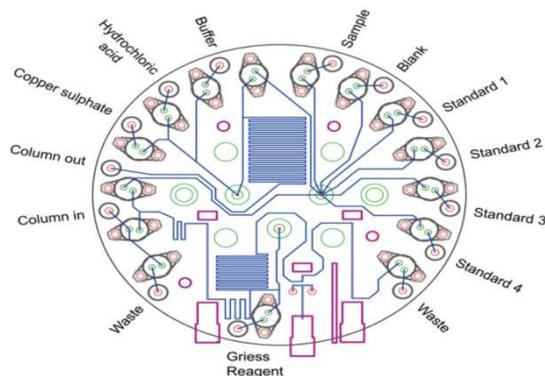


Figure 4.2.4. CAD drawing of the microfluidic chip with fluidic connections labelled [BCT12].

Measurement Method

For nitrite analysis, fluid is routed through a reference cell then mixed with Griess reagent before passing through a 0.25 m long serpentine mixing channel. Absorption is then determined in the two sequential measurement cells, which are separated by a milled groove to prevent crosstalk. For nitrate analysis, fluid is combined with imidazole buffer, passed through a 0.46 m serpentine mixer then through an off-chip cadmium tube to facilitate reduction before passing through the reference cell and mixing with the Griess reagent, absorption is then determined within the measurement cells. It can be noted that the cadmium tube can be conditioned periodically to recover reduction efficiency by passing 5 mM copper sulfate solution, 6% HCl, and then copper sulfate solution again.

System Performance

The system can detect nitrate and nitrite up to 350 μM or 21.7 ppm as NO_3^- . The system also expressed a limit of detection (LOD) of 0.025 μM (0.0016 ppm) for nitrate and 0.02 μM (0.00092 ppm) for nitrite. The LOD which is observed is superior to other in situ nitrate systems, for example, superior to those based on UV absorption. The small size and lower power consumption make this system suitable for integration into a range of oceanographic platforms including autonomous underwater vehicles (AUVs) and buoy systems. The device was deployed in an estuarine system and was able to track changes in the nitrate - salinity relationship of estuarine water due to increased river flow after a period of high rainfall. This system demonstrates the potential of LOC nutrient analysis for inclusion in large-scale networks for ocean observation and monitoring of other natural water systems [BCT12].

4.2.2.3 NITRATE DETERMINATION USING GRIESS METHOD UTILIZING ZINC REDUCTION

[EHG11] successfully developed a sensitive reagent-injection flow analysis method for the spectrophotometric determination of nitrate in marine, estuarine and fresh water samples. The method is based on the reduction of nitrate in a micro column containing zinc granules at pH 6.5. The nitrite formed is reacted with Griess reagent, and the resulting azo compound is quantified spectrophotometrically at 520 nm. Typically, nitrate determination is achieved by the reduction of nitrate to nitrite using a column of copperized cadmium granules or a Cd tubular reductor. The use of cadmium may be seen as undesirable due to the potential occupational health issues and

the production of toxic waste [GFM04]. Therefore, the use of an environmentally friendly reductant is highly desirable. Historically, copperized zinc columns have successfully been employed within flow injection methods for the determination of nitrate in water samples [CP91], using similar reduction conditions to that employed in the Cd reduction method.

The method employed by [EHG11] for online nitrate determination using the Griess method, in which reduction is achieved through the use of zinc, was successfully deployed in extended field trials aboard a catamaran. The reliability of the method was assessed through the analyses of certified reference materials and recovery experiments, and a satisfactory reliability was demonstrated.

This successful application of zinc as the reduction agent suggests that zinc may potentially be employed within lab on chip measurement systems for nitrate determination. This could potentially eliminate the use of cadmium and in turn the associated toxic waste, and lead to a reduced environmental impact.

4.2.2.4 MICROFLUIDIC PLATFORM MONITORING NITRITE USING PHOTORESPONSIVE MICROVALVE

A wireless, portable, fully integrated microfluidic analytical platform for the monitoring and determination of nitrite anions in water has been developed by [CFO13]. The research group reports the design, fabrication and testing of a wireless portable, integrated microfluidic analytical platform for point-of-care monitoring and quantitative determination of nitrite in freshwater, but the system may find application in a marine setting. The Griess assay is implemented for determining nitrite within a PMMA microfluidic device. The platform integrates optical fluid processing and detection, enabling monitoring of the kinetics of the Griess reaction and the detection of nutrient levels.

It can be noted that liquid handling and manipulation are key factors inhibiting network deployments for applications involving liquid-phase measurements such as water quality monitoring, due to the cost and power demand of conventional valves. Stimuli responsive materials, actuated by light irradiation, can significantly facilitate liquid movement within microfluidic devices and this has been demonstrated by a number of research groups who have reported the use of flow valves based on thermoresponsive poly (N-isopropylacrylamide) or pNIPAAm polymer gels [STM06].

For fluid control within the system developed by [CFO13], the microfluidic device contains a biomimetic photo-switchable microvalve based on a phosphonium ionogel functionalized with spiropyran. The microvalve is simply actuated by illumination with a light emitting diode, allowing for lower energy consumption compared to other microvalve systems. In addition, the nitrite concentration is determined by a highly sensitive, low cost wireless paired emitter-detector diode (PEDD), ensuring cost-effective fabrication and functioning of the platform. The basic principle of this detection mode lies in the employment of two linear light emitting diodes, one operating in normal mode as a source and the other in reverse bias which serves as a light detector [OBS09].

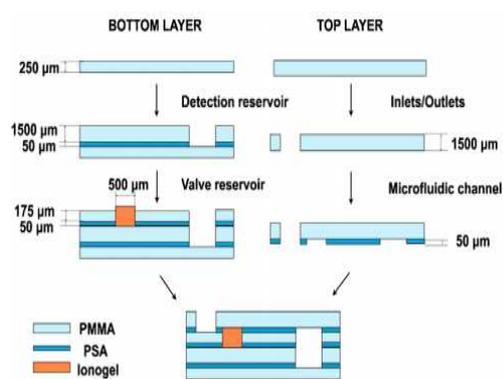


Figure 4.2.5. Schematic of the microfluidic device fabrication procedure [CFO13]

light

Fabrication

The microfluidic device consists of a multi-layer structure made of PMMA and pressure-sensitive adhesive sheets. Using laser ablation, reservoirs and microchannels were machined into the PMMA along with 50 μm and 80 μm thick double-sided, pressure-sensitive adhesive (PSA) layers. Once the appropriate pieces were machined, they were aligned and bonded using a thermal roller laminator. The ionogel valves were photopolymerized in-situ in a circular reservoir for 25 min using a UV irradiation source ($\lambda = 365 \text{ nm}$). Following polymerization, the resulting ionogels were rinsed with deionised water to remove any unpolymerized monomer and excess ionic liquid. Finally, the top PMMA layer was then bonded. The procedure associated with the fabrication of the microfluidic device is illustrated in figure 4.2.5.

After assembly, the upper part of the microchannel (γ -branches) was filled with 1mM HCl aqueous solution and kept for two hours to bring about swelling of the pNIPAAm gels functionalized by spirobenzopyran chromophores (pSPNIPAAm) or the pSPNIPAAm ionogels, thus closing the microvalve. Upon illumination of the ionogel with white light from an LED source the microvalve opens due to shrinking. This process is demonstrated in figure 4.2.6.

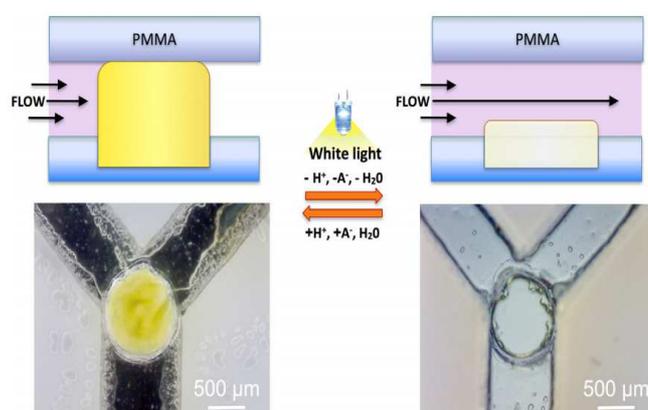


Figure 4.2.6. Photoresponsive microvalves in closed (left) and opened (right) state [CFO13]

The microfluidic device which was constructed consisted of a small structure of 20 x 30 mm² dimension. Round inlets were placed at the top of the Y-shaped channel for the water sample (radius 2.25 mm) and for the Griess reagent (radius 250 μm). The junction of the collecting channels, where the integrated microfluidic microvalve was located, was followed by the mixing part of the channel. The detection chamber, of radius 2.4 mm, was followed by a 1 mm width channel leading to waste, which was connected to a back pressure system. Figure 4.2.7 depicts the microfluidic device.

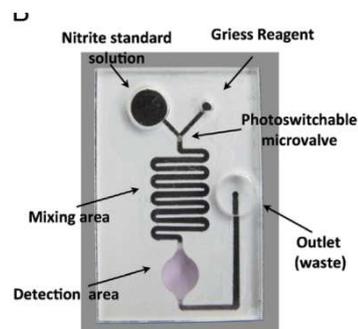


Figure 4.2.7. Fabricated microfluidic device by CO₂ laser ablation [CFO13]

Measurement Method

The sample to reagent ratio which was adopted was 15:1 v/v. Measurements were obtained by introducing 34.5 μL nitrite sample and 2.3 μL Griess reagent into their respective storage reservoirs as demonstrated in figure 4.2.7. Using 25 mbar back pressure from a vacuum pipe connected to the microfluidic device via the outlet, the liquids were moved from the storage reservoirs and allowed to mix through the serpentine microchannel as they travelled towards the detection chamber where concentration measurement occurs. Following the filling of the detection chamber, the intensity of the coloured solution was determined using the PEDD detector.

The PEDD detector achieves colorimetric detection through the use of LEDs, a green LED (540 nm) which is



Figure 4.2.8. Microfluidic device with the white LED for microvalve actuation and the PEDD detector LEDs (green-emitter and red-detector) positioning [CFO13]

the emitter and a red LED (660 nm) which is the detector, arranged in absorbance/transmission mode. This arrangement is portrayed in figure 4.2.8. Light generated by the emitter LED is partly absorbed by the Griess-nitrite complex, which absorbs strongly around 540 nm ($\lambda_{max} = 547$ nm) as demonstrated by [OSL07]. Hence the photon flux reaching the reverse biased detector LED depends upon the concentration of nitrite within the sample. This in turn generates a photocurrent from the reversed biased detector diode, which discharges a pre-set capacitance. The time taken to discharge the capacitance depends upon the photocurrent generated by the detector LED, thus is dependent on the nitrite concentration in the sample.

Cost and Energy Consumption

Reagent Consumption - Typical flow injection analysis systems employing the Griess method for nitrite detection consume relatively large amounts of reagent, approximately 5-20 mL per sample [SFO10], making the technique quite difficult to scale up. In contrast, this particular prototype platform which was developed only requires approximately 2.3 μL of Griess reagent per assay, resulting in reduction in costs associated with reagents, servicing visits, platform size, power consumption and waste disposal.

Microvalve Actuation - The photo-switchable ionogel microvalve used is very low cost to produce in terms of materials, and its fabrication via in situ photopolymerisation opens the possibility of creating complex microfluidic structures incorporating large numbers of valves. In addition, as it is actuated by light, no physical contact is required with the actuating stimulus, and thus the microfluidic system can be sealed from the electronics, and the valve structures subsequently introduced. In terms of energy, the white light intensity used to control the pSPNIPAAm ionogel microvalve in the current arrangement is ~ 1 mW cm^{-2} , whereas the power consumption of typical miniature conventional solenoid valves is up to 500 mW. Moreover, the white LED used is a standard off-the-shelf component resulting in low cost actuation.

Communications

Many microcontroller devices using wireless modules elect for the 2.4 GHz ISM band, however for platforms associated with environmental applications, the 900 MHz radio band offers advantages over the 2.4 GHz band. The 900 MHz radio band, which was used for this platform, is capable of communicating around objects such as trees, the landscape etc., all of which can be attenuated at 2.4 GHz [TV05].

System Performance

The LOD, calculated as the concentration of nitrite which produced an analytical signal three times the standard deviation of the blank, was determined as 34.0 ± 0.1 $\mu\text{g L}^{-1}$ nitrite. The platform which was developed was successfully deployed to determine traces of nitrite in freshwater and exhibited a very good correlation with bench top instrumentation. The technology could potentially be deployed within a marine environment, and although nitrite was selected as the target analyte, the platform could easily be extended to include monitoring of nitrate and the wide range of other important analytes for which effective colorimetric methods exist [CFO13].

4.2.2.5 SIMULTANEOUS DETERMINATION OF NUTRIENTS USING LAB-ON-A-DISC TECHNOLOGY

[HKC13] have developed a novel platform based on centrifugal microfluidics for the simultaneous determination of nitrite, nitrate and nitrite, ammonium, orthophosphate and silicate in water samples. All processes from sample measuring to detection were integrated and automatically performed on a rotating disc device. The transfer of liquid was controlled by laser irradiation on ferrowax-based microvalves. Liquid samples and reagents were pumped by centrifugal force in the rotating disc, and their positions and movements were manipulated via a programmable light from a laser diode. The novel platform which was developed requires only 100 μL of liquid sample for each nutrient, and 10-30 μL of reagents for colorimetric detection. In addition, the automated parallel processes and efficient mixing in the rotating disc allows for a significant reduction in total

analysis time and increased accuracy. Using a seawater certified reference material, validation was performed and it was determined that the platform accurately measured nutrient concentrations within water samples. Furthermore, seawater samples were collected from Chunsu Bay in Korea, and were measured by the lab-on-a-disc and by a commercialised autoanalyser, and it was demonstrated that the results were comparable.

In centrifugal microfluidic devices, centrifugal force that occurs through the rotation of a device acts as a driving force for transporting fluids through microchannels. Through the development of sacrificial active valves, such as laser-irradiated ferrowax microvalves (LIFMs), the development of integrated centrifugal microfluidic platforms, referred to as lab-on-a-disc, has been accelerated. As an example, [XTS10] have developed a centrifugal microfluidic platform for rapid sequential determination of nitrate and nitrite which is well suited for field use, where a disc with 24 identical reaction chambers facilitate the simultaneous mixing of injected water samples and powdered reagents.

Measurement Method

The reaction mechanisms associated with the determination of each of the nutrients are as follows:

Nitrite - NO_2^- is reacted with sulfanilic acid to form an intermediate diazonium salt, which coupled with chromotropic acid produces a pink complex directly proportional to the amount of nitrite present in a simple one-step reaction.

Nitrate and Nitrite - For NO_3^- and NO_2^- , bromine water was added to the sample to oxidise nitrite. In a second step, a cadmium reduction method based on NitriVer6 was applied to the sample to reduce nitrate to nitrite, and the same reaction mechanism was applied to detect the amount of nitrite corresponding with that of the total nitrite and nitrate. By subtracting the amount of nitrite from the measured concentration of nitrate and nitrite, the concentration of nitrate in the sample can be estimated.

Ammonium - Ammonium compounds are combined with chlorine to form monochloramine, which reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is then oxidised by a sodium nitroprusside catalyst to form a blue-coloured compound which morphs to green due to the yellow of the excess reagent.

Orthophosphate - The orthophosphate is reacted with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid is then used to reduce the complex, giving an intense molybdate blue colour.

Silicate - Silicate and phosphate in the sample react with molybdate ions under acidic conditions to form yellow silico/phosphomolybdic acid complexes. The phosphate complexes are destroyed with citric acid, and the amount of silicate can then be determined by measuring the remaining yellow colour.

Following the completion of the reactions on the disc, the absorbance of the solution at wavelengths 507, 655, 880, and 452 nm were measured in order to detect the levels of nitrite and nitrate and nitrite, ammonium, orthophosphate, and silicate respectively, using an optical fibre-coupled spectrophotometer in the stop position.

Fabrication

In terms of the fabrication of the centrifugal microfluidic device, the processes involved have been described in detail by [LLK11]. Microchannels and chambers were cut into a polycarbonate plate in the middle layer of the device using a CNC milling machine. Detection chambers were created by making holes across the middle layer. The depth of the detection chambers was 5 mm, as depth was dictated by the thickness of the middle layer, which determined the optical path length for colorimetric absorbance measurements. The middle layer was sandwiched between two 1 mm

thick polycarbonate plates, and holes were drilled into the top plate for ventilation and sample injection. All layers were bonded together with double-sided adhesive tape.

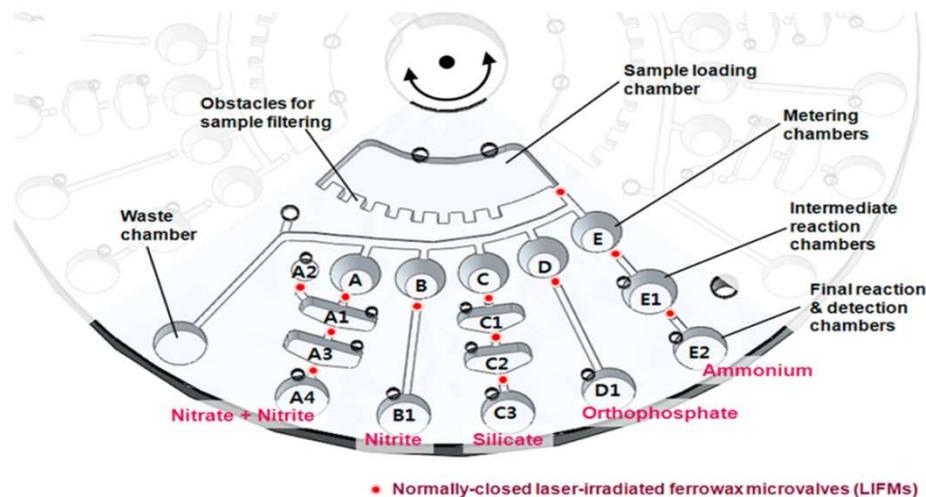


Figure 4.2.9. Schematic representation of lab-on-a-disc device [HKC13]

Table 17. Reagents utilized within centrifugal microfluidic device for determination of nitrite, nitrate, ammonium, orthophosphate and silicate [HKC13]

Target	Chamber	Reagent	Major Ingredient
Nitrate and Nitrite	A1	Bromine Solution	Bromine
	A2	Phenol Solution	Phenol
	A3	NitriVer6	Cadmium
	A4	NitriVer3	Sulfanilic acid Chromotropic acid
Nitrite	B1	NitriVer3	Sulfanilic acid Chromotropic acid
Silicate	C1	Molybdate Reagent	Sodium Molybdate
	C2	Acid Reagent	Sulfamic acid
	C3	Citric Acid Reagent	Citric acid
Orthophosphate	D1	PhosVer3	Sodium Molybdate Ascorbic acid
Ammonium	E1	Cyanurate Reagent	Sodium Dichloroisocyanurate
	E2	Salicylate Reagent	Sodium Salicylate Sodium Nitroferrocyanide

One disc, with 12 cm diameter, includes four compartments with chambers for loading and centrifugal filtering of particulates within water samples. During sample filtering, particulates settle to the bottom as a result of centrifugal force, and due to the presence of sawtoothed obstacles at the bottom wall of the chamber, overflow is prevented. The filtered sample is then aliquoted into five 100 μ L metering chambers, designated A-E. Additional chambers for reaction and detection were located along the radial direction from each metering chamber. The microchannels and chambers present within the lab-on-a-disc device are depicted in figure 4.2.9 below. In addition, the reagents contained in each chamber are listed in table 17 above.

Laser-irradiated ferrowax microvalves were present between each chamber in order to isolate and store reagents and samples without cross-contamination and evaporation. LIFMs were closed with

ferrowax, which is a mixture of paraffin wax and iron oxide nanoparticles which act as nanoheaters and are designed to instantaneously melt with laser irradiation directed on the microvalves.

Only one chamber for both reaction and detection was required for the colorimetric reactions associated with nitrite (chamber B1) and phosphate (D1). When the microvalves are opened, samples in chambers B and D are transferred into chambers B1 and D1 and are mixed with the necessary reagents. For ammonium determination, the sample in chamber E is transferred into chamber E1 along with the Cyanurate reagent and monochloramine is formed. The sample is then transferred into chamber E2 for reaction with the salicylate reagent and colorimetric detection. For silicate, the sample in chamber C is transferred in C1 and C2 chambers in succession where it reacts with molybdate reagent and the acid reagent, respectively, to form a yellow silico-/phosphomolybdic acid complexes. The sample is then transferred into chamber C3 with the citric acid reagent where phosphate complexes are destroyed in order to isolate silicate complexes. Finally for the nitrite and nitrate, the sample is pretreated with highly concentrated bromine water for oxidising all nitrite species to nitrate, as even small amounts of nitrite can interfere with nitrate detection due to similarities in reactions mechanisms [Hac11]. The sample is transferred through three intermediate reaction chambers (A1-A3), which contain bromine, water, phenol solution and NitrVer6 respectively. The sample is then transferred to the A4 chamber for reaction with NitrVer3 to achieve nitrite detection (as occurred in chamber B1) and to facilitate subsequent nitrate calculations.

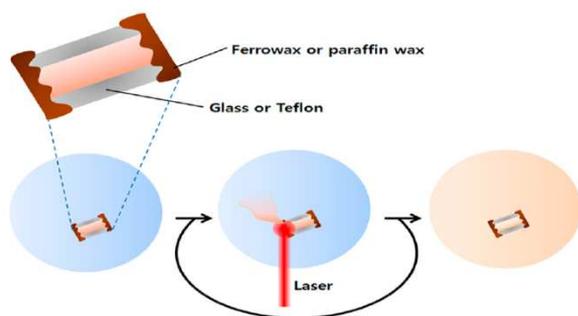


Figure 4.2.10. Container and melting of ferrowax doors resulting in diffusion of chemical [HKC13]

Due to the fact that bromine water is used within the lab-on-a-disc, and bromine water is highly reactive with plastics, [HKC13] also developed a new storage method for highly reactive chemicals. The bromine water was encapsulated in a Teflon or glass tube with thermosensitive doors and ferrowax. The release of the bromine water could then be remotely controlled using laser irradiation. The chemical container is forced to the bottom wall of the device and fixed via centrifugal force during rotation, and the laser source is aligned to melt the wax at preprogrammed positions.

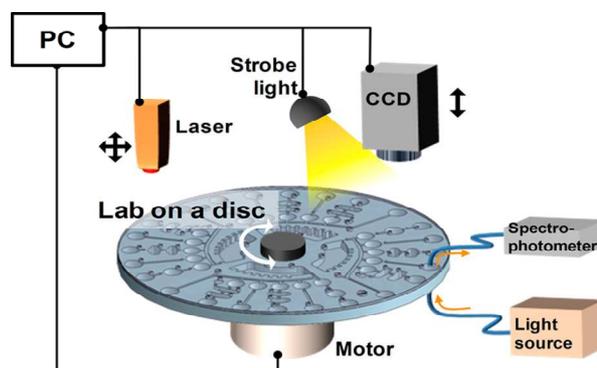


Figure 4.2.11. Design of the lab-on-a-disc device for simultaneous determination of five nutrients [HKC13]

When the wax doors are heated, the ferrowax door melts, and an opening forms through which the bromine water (or potentially another reactive chemical) can diffuse out from the capsule.

Chemical release can then be stopped due to the resolidification of the wax doors. This novel chemical container which can be used for the storage of highly reactive chemicals and its operation is depicted in figure 4.2.10. The full experimental configuration of the lab-on-a-disc is demonstrated in figure 4.2.11. The centrifugal microfluidic device was located on a programmable spinning motor. The closed wax valves could be selectively melted and opened through the use of a high power laser diode, and fluid movement was recorded using a charge-coupled device camera with a strobe light.

System Performance

The total analysis time for the simultaneous evaluation of the five nutrients was 7.5 min, which is 10 times faster in comparison to conventional methods using the same reagents [Hac11]. This faster rate of analysis is due to the fact that nutrients could be analysed simultaneously and because the lab-on-a-chip device allowed for more efficient mixing of fluids on a shaking disc. In addition to this, the lab-on-a-disc device required only 100 μL sample volumes for each nutrient, which is 100 times smaller in comparison to conventional method requiring 10 mL sample volumes. Calibration curves showed exceptional linearity with R^2 values higher than 0.998 for all nutrients tested. Furthermore, lower coefficient of variation values were obtained for all nutrients by using the lab-on-a-disc platform compared to the results obtained through manual handling of the same samples and reagents. The analysis results and performance of the system are summarized in table 18.

Table 18. Performance characteristics for simultaneous determination of nutrients using the lab-on-a-disc system [HKC13]

Nutrient	Nitrite	Nitrate and Nitrite	Ammonium	Orthophosphate	Silicate
<i>Analyte Analysis Time</i>	2 min	5 min 40 s	7 min	2 min	5 min 5 s
<i>Sample Volume</i>	100 μL	100 μL	100 μL	100 μL	100 μL
<i>Reagent Volume</i>	10 μL	20 μL	20 μL	10 μL	30 μL
<i>LOD</i>	0.008 mg of NO_2^- -N/L	0.05 mg of NO_3^- -N/L	0.01 mg of NH_4^+ -N/L	0.008 mg of PO_4^{2-} - P/L	0.19 mg of Si/L
<i>Linear Dynamic Range</i>	0.027 - 10 mg of NO_2^- - N/L	0.07 - 10 mg of NO_3^- -N/L	0.05 - 10 mg of NH_4^+ - N/L	0.024 - 1 mg of PO_4^{2-} -P/L	0.79 - 100 mg of Si/L

In terms of validation, the validation exercise demonstrated that nutrient concentrations measured on the lab-on-a-disc system were comparable with the certified concentrations in the CRM sample. In addition, the concentrations within the field seawater from the Chunsu Bay measured by the lab-on-a-disc were comparable to those measured by conventional benchtop methods. When considering design limitations, as a trade-off to the compact design and easy portability of the lab-on-a-disc, the number of samples that can be processed simultaneously on one disc is limited to the area of the disc. In the future, an autosampler or an autodisc exchanger could potentially be integrated into the lab-on-a-disc system in order to improve process volume.

This centrifugal microfluidic device for the simultaneous determination of nitrite, nitrate and nitrite, ammonium, orthophosphate and silicate in water samples provides new opportunities for on-site monitoring of water bodies. The lab-on-a-disc platform allows for smaller sample volume, shorter analysis time, lower cost, less opportunity for human error and portability. On-site analysis of rivers or seawater would be possible by applying this lab-on-a-disc device into commercialized portable instrumentation associated with blood analysis (e.g. Samsung IVD-A10A Compact Blood Tester) [Sam09]. For diverse fluidic operations in complex multistep processes, this lab-on-a-disc

system uses simpler and more stable configuration than those required for pump-based microfluidic chips. Such configurations would allow for the on-site analysis of river or seawater using portable instrumentation and may deliver to the needs of environmental researchers and monitoring, that is, better, cheaper, faster and lighter technology [HKC13].

4.2.2.6 ION-SELECTIVE NITRATE LOC SENSOR

A novel approach, in which [JZK11] are working on, is the development of the 'smart' membrane, which may be seen as one of the most difficult tasks in the development of ion-selective microelectrodes [JGD08]. [JZK11] are aiming to produce a nitrate LOC sensor. For the development of a potentiometric nitrate LOC sensor, a nanobead packing technique is applied to fabricate self-assembled nanobead hetero (nBH) columns in a polymer chip cartridge.

When making planar nitrate microelectrodes and Ag/AgCl reference microelectrodes using MEMS fabrication technologies, the main issues are how to hold the liquid ion exchange (LIX) membrane to permit ion selectivity and how to contain the internal KCl solution to make a reliable miniature reference electrode. Typical planar miniature solid-state reference electrodes coated by electrodeposition or screen-printing techniques are constructed without any internal electrolyte. Such planar solid-state microelectrodes have resulted in only short-term stability, poor reproducibility, and poor lifetime as demonstrated by [VAG05]. Based on several methods described within literature (e.g. [VAG05]), to overcome potential drift problems, the next most promising method of construction of miniature ion-selective and reference microelectrodes would be a liquid-junction electrode containing LIX and saturated KCl solutions. One way to achieve this liquid-junction electrode is to manufacture a self-assembled nBH column to hold LIX or electrolyte.

Fabrication

For fabrication of the nBH column on a chip, cyclic olefin copolymer (COC) was used. It can be noted that COC may be seen as a favourable substrate material for environmental lab chip applications due to the COC's relatively high solvent resistance [ACB04]. The COC chip which was developed by Jang *et al.* is pretreated with O₂ plasma for 2 min to produce hydrophilicity on the surface of the microchannel. Pretreated open microchannels showed high enough hydrophilicity to drive the silica colloidal suspension to the end of the channel by capillary force.

Once the colloidal silica particles reach the end of the capillary channel, spontaneous three-dimensional packing of the silica beads starts from the end of the microchannel due to the slow evaporation of water. It takes less than 2 min for the bead packaging process to be completed in a micro-volume channel of 0.0125 mm³.

As illustrated in figure 4.2.12, the hydrophilic and hydrophobic nanobeads were packed in a designated region by capillary electrophoresis. As a result, the developed nBH column was composed of a hydrophilic silica bead packed area for electrolyte loading and a hydrophobic polystyrene bead packed area for a LIX membrane which is permeable for target ions but impermeable for interfering species. Analyte detection is then achieved through potentiometry.

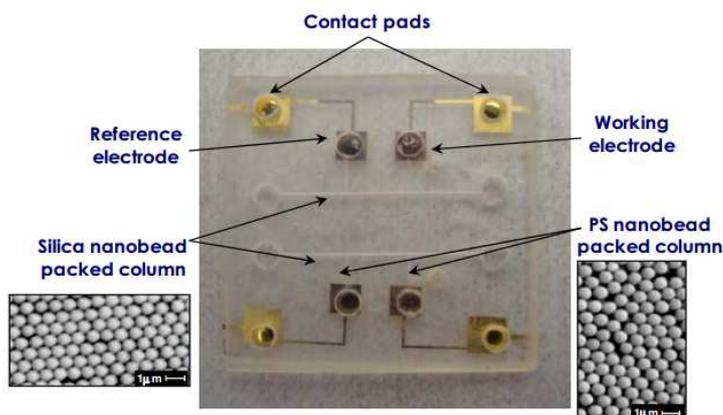


Figure 4.2.12. Photograph of the dual ion-selective lab chip and the scanning electron microscope images of the heterogeneous nanobead packed column [JZK11]

System Performance

To assess the electrochemical detection performance of the ion-selective sensor chip for nitrate, potentiometric responses were measured. The potentiometric response of the nitrate ion-selective sensor chip was linear within the NO_3^- concentration range of 10^{-5} to 10^{-1} . The response time of the nitrate sensor was less than 7s. This range is quite low, however, through further developments and improvements, such as improving the control of pore size and structure of the self-assembled nanobead packed column, a greater range may be achieved. Nonetheless, the nBH column on a lab chip, developed by this research group, provides a new electrochemical sensing platform with high sensitivity and excellent ion selectivity for environmental monitoring and through further research may find use in portable water monitoring [JZK11].

4.2.2.7 MICROFLUIDIC NITRATE SELECTIVE SENSOR BASED ON DOPED-POLYPYRROLE NANOWIRES

[AB08] have developed a nitrate-selective electrochemical sensor using doped-polypyrrole (PPy) nanowires on a microfluidic platform for the analysis of seawater. Typically commercial ion-selective electrodes (ISEs) possess no anion recognition functionalities and respond based on the anions lipophilicity [PBW88]. ISEs typically respond to ions according to the Hofmeister series, which implies that nitrate ion-selective electrodes are more selective to perchlorates and iodides than nitrates. Thus selective interactions must be incorporated between ionophores and nitrate using chemical recognition principles. This would allow for true selective nitrate sensor development, which is not limited by the Hofmeister series.

[AB08] achieved selective chemical recognition towards nitrate by electrochemical nitrate doping of polypyrrole. Lower detection limit and improved sensitivity is achieved by utilizing polypyrrole nanowires, as opposed to conventional polypyrrole films. Nanowires, being one-dimensional structures have a large surface-to-volume ratio and lower diffusion resistance and therefore enhance the capture cross-section. Polypyrrole is a widely studied conducting polymer, due to its high conductivity, ease of preparation, flexibility and stability as an ISE [Mar94]. Moreover, polypyrrole-doped nanowires demonstrate high selectivity and rapid reactivity towards the dopant ion [BKH05].

In its oxidised state, polypyrrole exists as a polyradical cation and at the oxidation stage; nitrate anions are attracted electrostatically into the polypyrrole matrix as dopants or counter ions. This is depicted in figure 4.2.13 below.

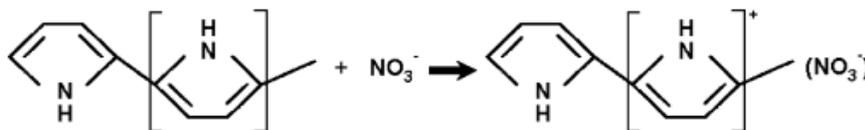


Figure 4.2.13. **Nitrate anions attracted electrostatically into PPy [AB08]**

In the research carried out by Aravamudhan and Bhansali, the selective chemical recognition for nitrate is achieved by electrochemical polymerization (i.e. doping) of a suitable monomer, in this case pyrrole, under controlled conditions to facilitate generation of selective recognition sites in the PPy layers. By polymerizing PPy layers in the presence of sodium nitrate (NaNO_3), pores which are complementary to the size of target nitrate ions are produced. The pore size and charge distribution within the doped-polypyrrole form a host cavity for nitrate, thus achieving greater selectivity over conventional nitrate-selective ISEs [AB08].

Synthesis and Fabrication

Polypyrrole nanowires (PPy-NWs) synthesis - The PPy-NWs were synthesized in a two-compartment cell, as demonstrated by [CZW05]. A nanoporous alumina template was placed between the two compartments, with 0.2 M of pyrrole monomer and 0.2 M of FeCl_3 oxidant on each side. At first, only pyrrole monomer was allowed diffuse through the template. Then the FeCl_3 oxidant was added in the second compartment. The monomer and oxidant diffuse towards each other through the pores of the template, resulting in polymerization of polypyrrole nanowires. Next, the PPy-NWs were released from the template by dissolving the template in warm 45% KOH with periodic agitation and centrifugation at 7000 rpm. The supernatant was then decanted, replaced with clean methanol and ultra sonicated for about 1 min. This procedure of nanowires collection, solvent addition and ultra-sonication was repeated three times to ensure clean PPy-NWs.

Fabrication of electrochemical sensor chip - The fabrication of the electrochemical sensor and nanowires assembly were performed in a similar manner to the method employed by [ARB07]. The polypyrrole nanowires were assembled onto Pt lines using dielectrophoresis. This technique uses a non-uniform electric field to achieve selective assembly of neutral particles in a liquid dielectric medium [BE05]. Interdigitated assembly lines and counter electrode were fabricated in Pt, with Ti as an adhesion layer on 7740 Pyrex substrate (2.5 mm x 2.5 mm). The microfluidic flow paths and reagent chamber were then patterned using 100 μm thick SU-8 epoxy film. The film was then hard baked at 180°C to prevent out-gassing or contamination. The Interdigitated Pt lines which were fabricated had a width of 50 μm and 10 μm spacing. Dilute suspensions of the PPy-NWs in methanol were dispensed on the Interdigitated Pt lines. An alternating electrode voltage of 10-20 V_{rms} at a frequency of 20 kHz was applied to the left Pt lines (represented as 1 in figure 4.2.14) relative to the right grounded Pt lines (represented as 2 in figure 4.2.14) for about 60 s.

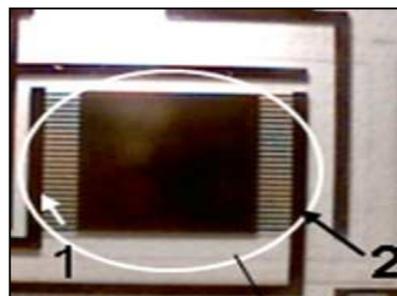


Figure 4.2.14. Picture of cell with Pt assembly lines and counter electrode [AB08]

The assembly was then monitored by studying the series current and later verified by microscopy. The PPy-NWs on the Interdigitated Pt lines then act as the working electrode in electrochemical measurements. As a final step, the PPy-NWs and electrochemical sensor chip is then cleaned to remove any excess nanowires. Figure 4.2.15 below illustrates the electrochemical sensor chip.

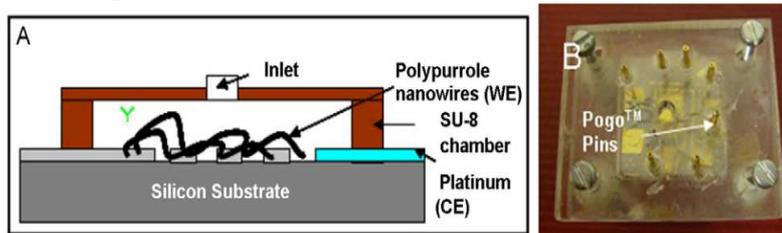


Figure 4.2.15. (A) Illustration of the electrochemical sensor chip; (B) Depiction of the electrochemical cell [AB08]

Electropolymerization of PPy-NWs - The polymerization of polypyrrole-doped with nitrate was performed galvanostatically. The electropolymerization solution was composed of 0.5 M pyrrole and 0.5 M NaNO_3 , which were deoxygenated before use by purging with N_2 . A galvanostat was used to supply a constant current at 2 mA cm^{-2} for 10-20 min. Silver wire and on-chip deposited platinum electrodes were used as reference and counter electrodes, respectively. After the polymerization reaction, the doped PPy-NW electrode was conditioned in 0.1 M NaNO_3 .

System Performance

After successful calibration testing of the PPy-NW electrodes under standard conditions, the electrodes were firstly used to analyse IAPSO standard seawater only to give baseline stability. It was demonstrated that for successive nitrate increments, the peak current also increased linearly, maintaining a stable current response. In the second set of experimentation, the baseline effluent analysed contained IAPSO standard seawater and $50 \mu\text{M}$ nitrate ions with intermittent nitrate spikes injected into the system at certain intervals. After every sudden nitrate spike, the baseline carrier effluent was flown through the sensor. It was noted that even though the sensor responded well to nitrate spikes, the initial baseline current was not obtained. This could be a result of residual nitrate ions present in the system. To resolve this, sensor regeneration by further optimizing the electropolymerization parameters could be performed, and control valves could be integrated into the system.

The selectiveness of the doped PPy-NW electrodes and effect of interfering ions on current response for various anions were also investigated. It was found that for up to 0.2 mM of chloride, sulphate, phosphate and perchlorate ions in the baseline seawater solution, no significant effect was found in the current response. However, at higher levels of interfering ions the peak current response decreased by about 15-20%. To further improve the selectivity of the doped PPy-NW electrodes, techniques such as deposition of solvent polymeric membranes and selective coordination interaction could be investigated [AB08].

By using this electrochemical-doping approach on polypyrrole nanowires, a highly sensitive and selective nitrate sensor was developed, and was demonstrated on a microfluidic platform. The PPy-NW electrodes exhibited a $4.5 \pm 1 \mu\text{M}$ detection limit within standard seawater samples and rapid response to changing nitrate concentrations in a flow-through system. The developed sensor further showed a linear response within the nitrate range of 0.14 ppm to 14 ppm nitrate with a sensitivity of 1.17 - 1.65 $\text{nA}/\mu\text{M}$. The sensor exhibited good selectivity to the nitrate ion, with only 15-20% decrease in current due to interfering ions, and through further investigation may find application within a marine environment. Future investigation and development may be directed towards the effect of electropolymerization parameters on sensor characteristics and control valves and micro-pump assembly to achieve a truly field-deployable sensor.

4.2.2.8 REFERENCES

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4.2.3 New Generation Phosphate Sensors

Chemical analysis systems and techniques which may be utilised to achieve cost-effective sensors for the determination of phosphate are summarised in table 19 below. The associated advantages and disadvantages of each technique are highlighted in the table also.

Table 19. Summarization of novel analysis systems for phosphate determination

Technology / Technique	Method of Operation	Advantages	Disadvantages
<i>Microfluidic phosphate analyser using vanadomolybdate method</i>	The system determines orthophosphate based on the rapid direct reaction of orthophosphate with an acidified vanadomolybdate reagent which results in a yellow colored complex, the intensity of which is proportional to orthophosphate concentration.	<ul style="list-style-type: none"> • System exhibited an LOD of 52 nM and can analyse up to 10 samples per hour. • System may be used in low nutrient oligotrophic waters. • Low reagent volume and power consumption. 	<ul style="list-style-type: none"> • Requires micro-milling and microfabrication
<i>Phosphate LOC sensor</i>	Miniaturized phosphate sensor which uses an on-chip planar cobalt microelectrode and integrated microfluidic channels. In the presence of phosphate in solution, cobalt phosphate is produced on the cobalt surface. Thus, by using the Nernst equation, phosphate concentration can then be determined.	<ul style="list-style-type: none"> • Low cost fabrication. • Potentially suitable for large scale field deployment. • Dynamic range from 5×10^{-5} to 5×10^{-2} M. • LOD of less than 10^{-5} M. • Low volume consumption 	<ul style="list-style-type: none"> • Further development required

Technology / Technique	Method of Operation	Advantages	Disadvantages
<i>Reagentless electrochemical phosphate determination in seawater</i>	A phosphomolybdate complex is formed and then detected by differential pulse voltammetry at a stationary gold electrode using optimized pulse amplitude.	<ul style="list-style-type: none"> • Free from liquid reagents and silicate interferences. • Method allows for the detection of phosphate in the concentration range found in the ocean. 	<ul style="list-style-type: none"> • Further development required in terms of minimizing the system and adaptations to protect from corrosion.
<i>Compact flow analysis system for total phosphorus determination</i>	Portable flow analysis system for in situ determination of total phosphorus. UV photo-reactor and thermal heating used for peroxodisulfate digestion of total phosphorus to orthophosphate, followed by spectrophotometric detection using the molybdenum blue method.	<ul style="list-style-type: none"> • LOD of 1.3 $\mu\text{g P L}^{-1}$. • Linear over the range 0-200 $\mu\text{g P L}^{-1}$. 	<ul style="list-style-type: none"> • Use of 10 W heater and mercury lamp. • Instability of reagents
<i>Fluorescence detection of phosphate using reverse injection analysis</i>	The determination of the phosphate concentration is based on the reaction of molybdate to phosphomolybdate, which quenches the fluorescence of Rhodamine 6G. The decrease in fluorescence intensity is proportional to the phosphate concentration.	<ul style="list-style-type: none"> • System can achieve a sampling frequency of 300 samples per hour. • A measuring range of 0-40 $\mu\text{g L}^{-1} \text{PO}_4^- \text{P}$ was obtained. • LOD of 0.45 $\mu\text{g L}^{-1} \text{PO}_4^- \text{P}$ for seawater. 	<ul style="list-style-type: none"> • Requires further development in order to achieve long term in situ deployment.

4.2.3.1 INTRODUCTION

Phosphorus is an essential nutrient for all living organisms, it plays a key role in biological metabolism and compared to other macronutrients required by biota, phosphorus is the least abundant and typically the first nutrient to limit biological productivity. Phosphorus in aquatic systems occurs in three forms: inorganic phosphorus, particulate organic phosphorus and dissolved organic phosphorus. Aquatic biota require inorganic phosphate, typically in the form of orthophosphate ions (PO_4^{3-}), for nutrition. This is the most significant form of inorganic phosphate and may be referred to as soluble reactive phosphorus (SRP) [Wet01]. Phosphorus occurs naturally in rocks, soil, animal waste and plant material. However in recent times, anthropogenic sources of phosphorus have become a large fraction of the phosphorus delivered to the aquatic environment. Human activities such as agriculture, industrial and municipal waste discharge and water runoff from residential and urban areas result in phosphorus entering aquatic systems. High levels of phosphorus within an aquatic system can lead to excessive phytoplankton growth which can cause undesirable effects such as: a decrease in biodiversity; a decline in ecologically sensitive species; an increase in plant and animal biomass; an increase in turbidity; and anoxic conditions [CCM04]. Consequently, limits for phosphate concentrations in natural waters are being reduced by regulatory organisations. This evolution of water quality policy in Europe and internationally is increasing the need for the accurate determination of phosphate through the

use of sensitive automated analytical systems [LSW13]. This report highlights and evaluates a number of chemical analysis systems and potential techniques which may be utilised to achieve cost-effective sensors for the determination of phosphate in marine environments.

4.2.3.2 MICROFLUIDIC PHOSPHATE ANALYSER IN MARINE WATER USING VANADOMOLYBDATE METHOD

[LSW13] have developed a high performance autonomous analytical system based on the vanadomolybdate method for the determination of SRP or orthophosphate in seawater. The system combines a microfluidic chip manufactured from tinted polymethyl methacrylate (PMMA), a custom syringe pump, embedded electronics and on-board calibration standards. This lab-on-a-chip system was successfully deployed in coastal waters in South West England, and open ocean waters in the North Atlantic. The miniaturized system compared well with a reference bench-operated phosphate auto-analyser and showed no significant differences in terms of analytical results (t-test at 95% confidence level). The optical technology utilised, comprising of tinted PMMA and polished fluidic channels, facilitated an improvement of two orders of magnitude of the limit of detection (LOD) compared to currently available portable systems. The system allowed for high resolution measurements of soluble reactive phosphorus in seawater. It can be noted that the vanadomolybdate method or yellow vanadomolybdophosphoric acid method offers the greatest potential in terms of achieving deployable microfluidic systems for phosphate determination in aquatic environments, examples of successful deployments include [CSM08] and [SCL10].

Fabrication

High power UV-LEDs were used as the light source and photodiodes for the absorbance detection. Both the LEDs and photodiodes were directly glued onto the microfluidic device with an optical adhesive. The microfluidic device was produced by micro-milling of 5.0 mm thick tinted PMMA. Through the use of tinted PMMA the amount of light reaching the detector coming from ambient sources is reduced, and also stray light from the LEDs do not pass through the analyte. A solvent vapour bonding procedure was used to bond the microfluidic manifold and the lid. This technique produces smooth channel surfaces and allows for longer optical cells. The fluidic manifold of the chip included two absorbance cells of 25 mm length, i.e. a reference and a measurement cell. This is illustrated in figure 4.2.16A. Fluid handling was performed using seven micro-inert valves which allowed for switching from the sample inlet to blank or on-board standards stored in 500 mL fluid bags. A stepper motor was used to drive two 200 μL liquid delivery syringes, which were manipulated by two Hall-effect sensors.

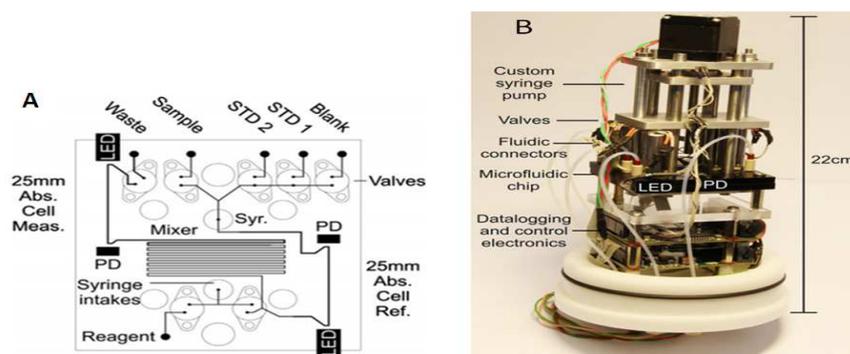


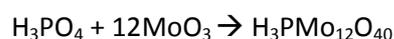
Figure 4.2.16. (A) Schematic of microfluidic chip. (B) Micro-analyser including custom syringe pump, valves, microfluidic chip and electronics [LSW13]

The dead volume of the device was 500 μL . This was minimized as all fluidic connectors, optical alignment grooves, valve mounts and syringe pump mounts were directly milled into the microfluidic device. Seawater was filtered at the inlet using a 0.45 μm pore size polyethersulfone filter unit. The

outputs of the photodiodes and the final data were stored on a 2 GB flash memory card. The entire system was housed in a water-tight acrylic tube and is portrayed in figure 4.2.16B above.

Measurement Method

The determination of orthophosphate here is based on the rapid direct reaction of orthophosphate with an acidified vanadomolybdate reagent which results in a yellow coloured complex. The “yellow method” offers excellent stability of the reagent mixture over time, reported to be over one year, compared with four to six weeks for the classical “blue method” [BD03]. The inorganic phosphorus species in seawater include orthophosphoric acid, dihydrogen phosphate, and hydrogen phosphate and their abundance at equilibrium depends on the pH. Under acidic conditions, orthophosphoric acid is dominant and reacts with molybdate ions to form molybdophosphoric acid, as shown in the reaction below. In the presence of vanadate ions, this complex will form vanadomolybdophosphoric acid with a molar absorptivity of $3.6 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 385 nm [ML05].



The analytical protocol involved a series of four main steps: (1) withdrawal of water sample from the environment; (2) injection of the sample, mixing of the sample and reagent into the on-chip mixer; (3) colour formation and (4) absorbance measurement. Absorbance of the sample blank or standard was determined within the reference optical cell prior to the addition of the reagent. This step is carried out in order to correct for any background absorbance. Mixing is accomplished on the chip by passing blank, standard or sample together with reagent through a 250 mm long serpentine mixer. The reagent and sample were pumped through the chip at a flow rate of $200 \mu\text{L min}^{-1}$, at a ratio of 1:1. The flow was stopped to facilitate colour formation and the associated absorbance was measured in the second optical cell. The reaction time was between 180 and 300s, to allow sufficient time for the reaction to develop colour. In addition, to minimise any potential contamination or carry-over between samples, an extra $320 \mu\text{L}$ sample volume was flushed through channels between samples.

System Performance

This novel automated lab-on-a-chip colorimetric analyser for SRP was successfully deployed in coastal and open ocean environments. The system exhibited an LOD of 52 nM and can analyse up to 10 samples per hour. The measurements undertaken by the novel miniature analyser agreed with those made by a conventional bench-top SRP analyser. The fabrication of the microfluidic device facilitates and permits an extension of the path length of the optical cell which improves the LOD according to the Beer-Lambert law, and allows for the use of the system in low nutrient oligotrophic waters. The reagent volume ($340 \mu\text{L}$ per sample) and power consumption (756 J per sample) of the microfluidic system were orders of magnitude lower than for conventional systems and due to the enhanced stability of the reagent of the “yellow method” allows for long term monitoring of SRP in marine waters [LSW13]. This successfully deployed system highlights the excellent potential the vanadomolybdate method offers in terms of microfluidic platforms.

4.2.3.3 PHOSPHATE LOC SENSOR

Due to their instrumental simplicity, moderate cost and portability, a lot of analytical method development has concentrated on electrochemical sensors. Although in the last decade there has been growing interest in the use of electrochemical sensors in environmental pollution monitoring, their configuration with an internal filling solution is not well suited for miniaturization. Alternatively, the replacement of the liquid internal solution with a solid-state membrane is promising. The use of solid-state membranes has a number of advantages over other conventional methods, including simple structure, high sensing performance and ease of compatibility with other advanced technologies such as MEMS techniques.

Jang *et al.* (2011) have developed a miniaturized phosphate sensor with an on-chip planar cobalt microelectrode and integrated microfluidic channels, which is depicted in figure 4.2.17, using standard MEMS fabrication technology [JZK11]. The proposed sensor is produced very cheaply and is potentially suited for large-scale mass production and disposable usage without cross contamination. Further benefits of the proposed sensor include low volume of analyte consumption and waste generation and rapid sensing time. Eventually, the sensor may be utilised for large-scale field deployment for environmental applications such as marine monitoring. Furthermore, the sensor could be easily integrated into lab-on-a-chip devices, coupled with sample preparation and additional analyses.

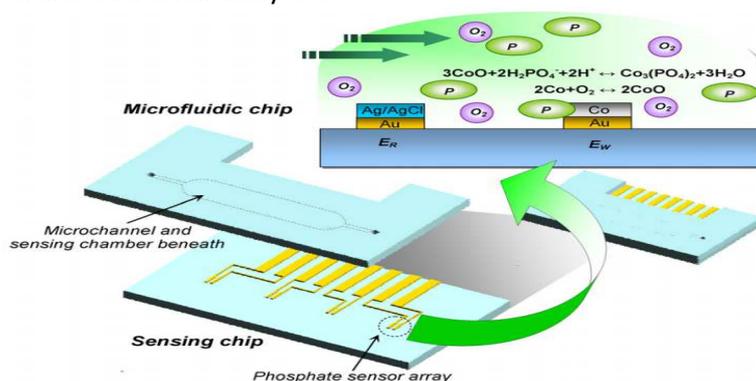
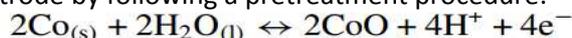


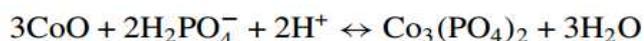
Figure 4.2.17. Illustration and working principle of the on-chip phosphate sensor with planar cobalt electrodes on polymer substrates [JZK11]

Measurement Method

The interaction of the cobalt surface with oxygen is suggested in order to explain the sensing mechanism of the cobalt toward the phosphate ions by a number of authors [DPC98] and [XYL95]. It has been noted that in both acidic and basic medium there is an oxidation reduction on the surface of the cobalt electrode by following a pretreatment procedure:



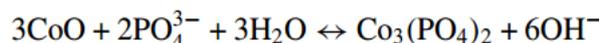
In this way, it is postulated that the CoO layer formed at the electrode surface serves as the sensitive membrane responding towards the phosphate ions according to the host-guest mechanism. Specifically, in the presence of phosphate in the solution, cobalt phosphate is produced on the surface of cobalt by the following three reactions, depending on pH value of the solution. At pH 4 in 25 mM potassium hydrogen phthalate buffer:



At pH 8 in *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulfonic acid (HEPPS) buffer:



At pH 11 in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer:



Thus, through the application of the Nernst equation, the corresponding electrode potential response versus the logarithm of the phosphate concentration can be directly determined.

Fabrication of Phosphate Sensor and Microfluidic Chip on Polymer Substrate

A gold layer of 100 nm and a Co layer of 300 nm were deposited on a 3 inch blank cyclic olefin copolymer (COC) wafer using an e-beam metal evaporator. Au and Co electrodes were patterned by photolithography and etched by Co (0.5% HNO₃) and Au (TFA) etchants. The Ag/AgCl layer was deposited on the reference electrode using electroplating on the Au seed layer. The sensing time of the proposed sensor can be significantly reduced by using the integrated polymer microfluidic

chip. After drilling holes for fluidic interconnection at inlet and outlet, the microfluidic chip was bonded with the sensor chip using a UV adhesive bonding technique at room temperature [ZHJ07], to achieve the final device. The details of the Co working electrode and the Ag/AgCl reference electrode are depicted in figure 4.2.18. Both electrodes have lengths of 1.5 mm, widths of 200 μm , and a spacing of 200 μm .

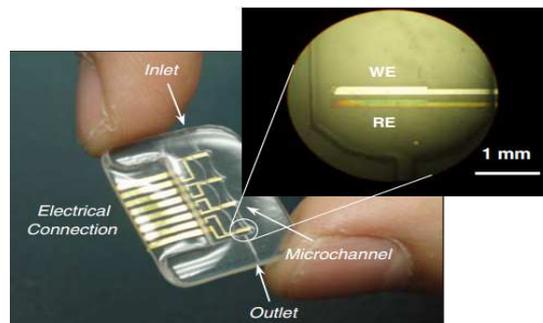


Figure 4.2.18. Fabricated device and microscopic image of on-chip phosphate sensor composed of Co working electrodes and Ag/AgCl reference electrodes [JZK11]

Performance

The feasibility of this electrochemical sensor to monitor inorganic phosphate compounds has been fully demonstrated. By incorporating mass-production microfabrication techniques and high throughput plastic micromachining, the proposed on-chip phosphate sensor with the integrated microfluidic chip could be fabricated at very low cost while maintaining excellent performance. The miniaturized sensing system is suitable for large-scale field deployment and the proposed on-chip microsensor is fully integrated with the polymer microfluidic system and could be developed as multi-analyte polymer lab-on-chips. These Co-wire electrodes show a very good response to inorganic phosphate in a dynamic range from 5×10^{-5} to 5×10^{-2} M with a detection limit less than 10^{-5} M. Furthermore, it can also be noted that this proposed electrode exhibited a high selectivity for phosphate ions with respect to most other anions [JZK11].

4.2.3.4 REAGENTLESS ELECTROCHEMICAL PHOSPHATE DETERMINATION IN SEAWATER

An initial electrochemical method for the determination of phosphate in seawater was developed by [JLT11]. The method was based on the oxidation of molybdenum in order to form molybdates and protons, subsequently creating the phosphomolybdic complex electrochemically detectable by means of amperometry at a rotating gold disk electrode. To avoid silicate interferences, the method required an appropriate ratio of protons over molybdates equal to 70. Since the ratio of protons over molybdates created during molybdenum oxidation is only 8, the method still needed addition of sulphuric acid and thus was not free of additional liquid reagents. In the present, most recent work carried out by [JGB13]; this aspect is solved by modification of the electrochemical cell construction. The method is now totally free from addition of any liquid reagents and gives a possibility to determine phosphate by amperometry in the concentrations range found in the open ocean with a detection limit of 0.11 μM . However this method would not be particularly suitable for miniaturisation. Having energy savings and miniaturisation in mind for in situ sensor development, differential pulse voltammetry at a static gold electrode was also investigated and was successfully applied as phosphate could be determined with a detection limit of 0.19 μM using this method. This proposed method is suited to miniaturization and by minimising the system could find application as an in situ sensor for marine monitoring.

Measurement Method and Fabrication

Electrochemical measurements are carried out with a potentiostat μ -Autolab III, and the reference electrode is an Ag/AgCl/KCl 3 M electrode. Measurements at the stationary electrode are recorded in a three electrode cell with a platinum counter electrode and gold working

electrode. The working electrode is polished with lapping film sheet and electrochemically cleaned in 0.5 mol/L sulphuric acid solution before each measurement. The molybdenum electrode has a surface of around 100 mm². Molybdate is produced by molybdenum anodic oxidation performed at a constant electrolysis current of 50 mA or at constant potential of 2 V. The simultaneous production of molybdate and protons with the appropriate ratio is based on the use of a cell divided in three parts. In the first one (1 mL) a primary molybdenum electrode is oxidised and thanks to a thin 30 µm proton exchange membrane only protons can pass through to the second compartment and thus acidify the medium to pH 1, to avoid interference from silicate. In the second compartment (5 mL), a secondary molybdenum electrode is oxidised during a short time and thus achieving a ratio of protons over molybdates of 70. To avoid the reduction of protons formed during the two previous oxidations of molybdenum, the platinum electrode is placed in the third compartment which is in contact with the former two part of the cell by a non-proton exchange membrane with thickness of 180 µm. During molybdenum oxidation, the platinum electrode acts as a cathode, while during phosphate determination it acts as a counter electrode. Figure 4.2.19 illustrates a schematic of the cell.

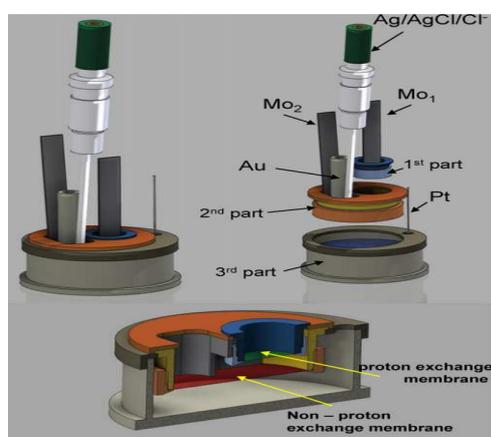


Figure 4.2.19. Depiction of electrochemical cell with application of membrane technology (Ag/AgCl/Cl⁻ - reference electrode, Au working electrode, Pt counter electrode, Mo₁-first molybdenum electrode, Mo₂-second molybdenum electrode, 1st part-first compartment of cell with proton exchange membrane, 2nd part-second compartment of cell with non proton exchange membrane)

The phosphomolybdate complex was detected by differential pulse voltammetry at the stationary gold electrode using optimised pulse amplitude. The detection limit obtained was 0.19 µM. Reproducibility tests also exhibited suitable precision.

Summary of Electrochemical Cell

Through this novel construction of the electrochemical cell and utilization of membrane technology coupled with molybdenum oxidation, electrochemical detection of phosphate within seawater is accomplished. The method is free from addition of any liquid reagents and free from silicate interferences. During molybdenum oxidation in a sample containing phosphate, the phosphomolybdic complex is formed. The complex is then detected by differential pulse voltammetry on a gold electrode. The method allows for the detection of phosphate in the concentration range found in the ocean with a suitable detection limit, reproducibility and accuracy. The electrochemical cell is suitable for miniaturisation and through further developments both in terms of minimising the system and adaptations to protect against corrosion, biofouling etc. the cell may be used as an in situ sensor for the determination of phosphate in seawater [JGB13].

4.2.3.5 COMPACT FLOW ANALYSIS SYSTEM FOR DETERMINATION OF TOTAL PHOSPHORUS

[GEF10] developed a portable flow analysis system for the in situ determination of total phosphorus. The system employed an ultra-violet photo-reactor and thermal heating for peroxodisulfate digestion of total phosphorus to orthophosphate, followed by spectrophotometric detection with a multi-reflective flow cell and low-power light emitting diode using the molybdenum blue method. Reagents were stored under gas pressure and delivered using software controlled miniature solenoid valves. As mentioned the instrument comprised of a UV photo-reactor, constructed from Teflon tubing wound around a medium pressure mercury lamp, as well as a heating unit consisting of an electrically heated Teflon tubing coil. Sample is merged with an acidic peroxodisulfate solution prior to passing through the photo-reactor and heater. Digested sample is filtered through a 0.22 μm in-line hollow-fibre filter before being pumped into a FIA system for the detection of orthophosphate by the molybdenum blue method. Phosphomolybdenum blue is then detected spectrophotometrically. The flow analysis system was successfully deployed aboard the SV Pelican 1 catamaran during a 2 week study of Port Phillip and Western Port Bays in Victoria, Australia.

Instrumentation

A sampler and digestion module was used to perform all sample treatment operations, including digestion, debubbling and filtration. Sample was collected with a peristaltic feed pump. A miniature peristaltic pump was used to pump sample from the feed pump at 2 mL min^{-1} which merged with 2 mL min^{-1} of acidic peroxodisulfate digestion reagent. The sample-digestion reagent stream passed through a UV photo-reactor consisting of a UV lamp ($\lambda_{\text{max}} = 254 \text{ nm}$) wound with 2000 mm of 0.2 mm i.d. Teflon tubing, and then to a 600 mm length of 0.5 mm i.d. Teflon tubing maintained at 80°C by a 10 W heater. The digested sample was filtered using a hollow fibre cross flow filter constructed of a single 100 mm length of micro-porous polypropylene tubing supported internally by a perforated piece of 0.5 mm i.d. Teflon tubing. The hollow fibre was housed in a perspex block (20 mm x 20 mm x 105 mm) inside a chamber with a 2.5 mm i.d. bore, with both ends being sealed by glue. The digested sample was introduced through a port in one end of the block. A length of 0.3 mm i.d. tubing connected to an exit port was used to increase the trans-membrane pressure differential to enhance the flow rate through the membrane. The harsh acidic oxidising conditions of the digested stream effectively prevent any particulate build-up on the surface of the polypropylene tubing. However, the polypropylene tubular membrane only had an operating lifetime of approximately 1 week.

The reagent injection flow analysis system was used for the analysis of the orthophosphate produced by on-line digestion. The sample stream was driven by a peristaltic pump at 1.8 mL min^{-1} and two solenoid valves were utilised for the introduction of the acidic molybdate chromophoric reagent (R1) and the acidic tin (II) chloride-hydrazine reductant (R2) which was held in a reagent storage chamber.

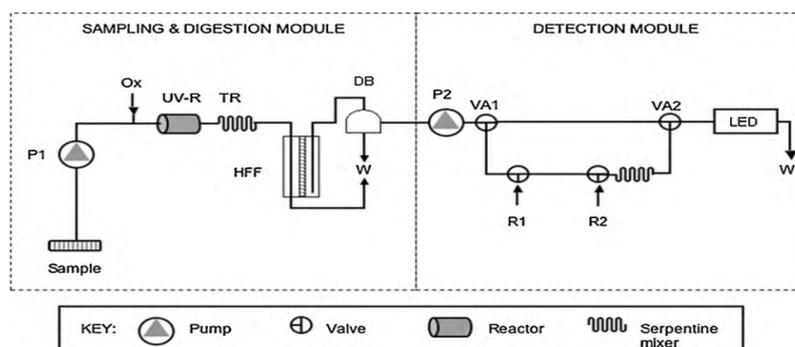


Figure 4.2.20. Illustration of flow analysis system [GEF10]

The sample and reagents were mixed using a 600 mm serpentine coil constructed from 0.5 mm i.d. Teflon tubing threaded through a plastic support plate to form a square grid pattern. A multi-reflective flow cell and a red light emitting diode ($\lambda_{\max} = 660 \text{ nm}$) were used to detect the absorbance of the Phosphomolybdenum blue produced. Automation of the sampler unit and analyser functions was achieved using a measurement computing™ A-D DAQ board, interfaced to a PC running LabView™. Figure 4.2.20 above is a diagrammatic representation of the flow analysis system used for the determination of total phosphorus.

System Performance

The flow system and method displayed excellent sensitivity and linearity over the calibration range $0\text{--}200 \mu\text{g P L}^{-1}$, and achieved a detection limit of $1.3 \mu\text{g P L}^{-1}$. This detection limit is adequate for coastal, marine and highly pristine fresh waters. A RSD of 4.6 % was obtained when measuring a $100 \mu\text{g P L}^{-1}$ phytic acid standard, which indicates that the conversion of phytic acid to orthophosphate is reasonably precise. However, a number of disadvantages are associated with the system when considering long term deployment. For example, the instability of the reagents used in the molybdenum blue method, the use of a 10 W heater and the use of a mercury lamp to facilitate mineralisation of organic phosphorus compounds. Nevertheless, by perhaps altering the materials and reagents used, and through further improvements the system may be utilised to achieve long term deployment in aquatic environments.

4.2.3.6 FLUORESCENCE DETECTION FOR PHOSPHATE USING REVERSE INJECTION ANALYSIS

[KLW14] have developed a compact flow-through fluorescence detector for analyses applications and prove its functionality through experimentation. The detector operates by detecting the diffusely emitted fluorescence in a glass capillary, which is a measure for the concentration of the analyte to be detected. The fluorescence is excited via an axially coupled fibre providing LED light and is collected by a photodiode. Dissolved reactive phosphate is successfully detected using the developed system. The determination of the phosphate concentration is based on the reaction of molybdate to phosphomolybdate, which quenches the fluorescence of Rhodamine 6G. The miniaturised fluorescence detector is compact and is designed for flow analysis. The main features of the device are the large collection of isotropically emitted light, strong suppression of exciting light and low consumption of sample and reagent volumes.

Optical detector for fluorescence measurements and FIA arrangement

Fluorescence detectors for online applications in water must fulfil specific requirements, these include: small spatial footprint, small sample volume, adaption to the fluidic system, high attenuation of the exciting light, and effective collecting of the diffusely emitted fluorescence. The functional principle of the developed fluorescence detector relies on the phosphate induced fluorescence quenching of the dye Rhodamine 6G with higher phosphate concentration leading to a more quenched fluorescence. Rhodamine 6G has maximum absorption and fluorescence at 527 nm and at 556 nm respectively. For fluorescence excitation a blue LED with an emission peak at 470 nm and full-width-half-maximum of 25 nm was used. For suppressing the exciting light an edge filter was used. Only 0.3 % of the LED light passes through the filter.

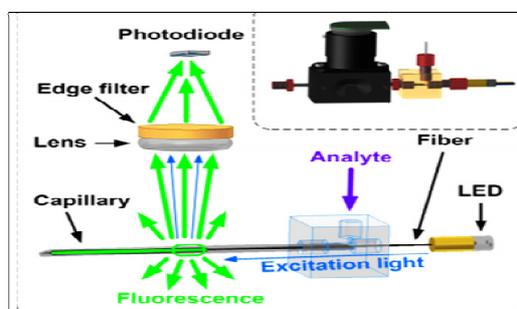


Figure 4.2.21 Fluorescence detector with optical components [KLW14]

The detector was made of a blank cubic formed capillary holder fabricated out of polyoxymethylene, with a central hole for the borosilicate glass capillary. The holder has four openings, one at each side around the capillary. The detection volume is $4.7 \mu\text{L}$. The fluorescence inside

the capillary is excited via a multimode fibre which is stuck into the capillary with its end being precisely positioned close to the location of the detector unit. The multimode fibre is connected to the blue LED. Strong light transfer from LED into glass capillary is ensured by using a 550 μm thick multimode fibre with a numerical aperture of 0.22. The fibre ends outside the detection channel of the capillary holder. The fluorescence light is collected by a photodiode using a high-aperture converging lens. Undesired excitation light is suppressed via an edge filter positioned in front of the photo detector. The developed fluorescence detector and the associated optical components are illustrated in figure 4.2.21; in the upper right corner of the figure is a 3D model of the fluorescence detector.

In terms of the flow injection analysis (FIA) arrangement, reversed FIA is based on injecting the reagents into a sample stream used as carriers. This method is applied when sample material is abundant and is characterized by low reagent consumption and high sample throughput. The developed reversed FIA setup is composed of two main parts: sample acquisition and sample analyzing. The arrangement of the analytical setup consists of a reagent container, a mixing coil and the developed fluorescence detector. Figure 4.2.22 below shows the reversed FIA set up.

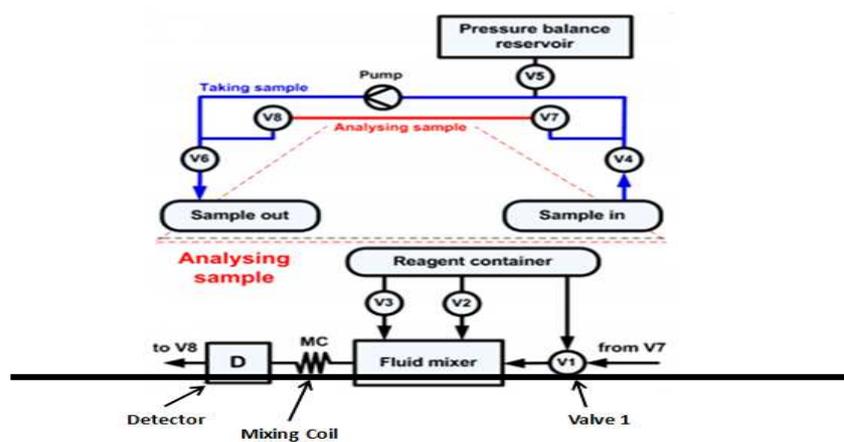


Figure 4.2.22 Constructed R-FIA system with acquisition unit and analysis unit

Measurement method and reagents

The employed method is based on the reaction of orthophosphate with molybdates under acidic conditions producing molybdophosphate, a chemical forming a non-fluorescent complex with Rhodamine 6G [KLW14]. In the developed system this reaction leads to a decrease in the fluorescence intensity by static fluorescence quenching. This quenching concentration dependency is described by the Stern-Volmer relationship

$$I_0/I = 1 + K_s Q$$

I_0 and I being the intensities of fluorescence without and with a quencher, respectively, K_s the Stern-Volmer quenching constant, and Q the quencher concentration. Reagent solution 1 (RS1) consists of Rhodamine 6G (1.5 mg L^{-1}) and the emulsifier p-octylphenoxypolyethoxyethanol (500 mg L^{-1}). Reagent solution 2 (RS2) is made up of molybdates (58 mmol L^{-1}), hydrochloric acid (1 mol L^{-1}), and also p-octylphenoxypolyethoxyethanol. All solutions were filtered, degassed and stored in plastic bottles.

System performance

By combining the optics and the fluidic setup a measuring range of $0\text{-}40 \text{ }\mu\text{g L}^{-1} \text{ PO}_4^- \text{ P}$ with detection limit of $0.22 \text{ }\mu\text{g L}^{-1} \text{ PO}_4^- \text{ P}$ for water and detection limit of $0.45 \text{ }\mu\text{g L}^{-1} \text{ PO}_4^- \text{ P}$ for seawater were obtained. The novel system can achieve a sampling frequency of up to 300 samples per hour

and demonstrated adequate repeatability. [KLW14] have presented a miniaturised, robust and low-cost fluorescence detector for FIA applications. It displays an excellent sensitivity and is highly selective to the chosen analyte. Compared to other published concepts the developed fluorometric sensor and the R-FIA system together have an equal or greater performance in terms of sensitivity, detection limits and sampling rate, and show great potential in terms of in situ analysis.

4.2.3.7 Novel Phosphate References

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4.2.4 Next Generation Ammonia Sensors

Numerous analysis systems and techniques which may be employed to achieve cost-effective sensors for ammonium determination in the marine environment are summarised in table 20 below. The advantages and disadvantages associated with each technology are also highlighted.



Table 20. Summarization of analysis systems for ammonium determination in marine water

Technology / Technique	Method of Operation	Advantages	Disadvantages
<i>Miniature multi-pumping flow analyser for ammonium</i>	Quantification of ammonium is accomplished by fluorescence spectrometry after its reaction with o-phthaldialdehyde and reduction with sulfite (OPA method). Isoindol-1-sulfonate is the reaction product and shows an intense fluorescence at 425 nm using an excitation wavelength at 365 nm. The intensity of the fluorescence obtained can be related to the ammonium concentration.	<ul style="list-style-type: none"> • Method is not dependent on salinity or temperature. • High sensitivity can be achieved. 	<ul style="list-style-type: none"> • A considerable amount of further development is required to allow the system to be used for long term deployment.
<i>Portable fluorometric analyser for ammonium determination</i>	The OPA method is employed in conjunction with a specifically constructed LED photodiode-based fluorescence detector within a portable analyser.	<ul style="list-style-type: none"> • LOD of 10 nM was obtained. • Inexpensive system. • Working range of 0.03 - 10 μM. 	<ul style="list-style-type: none"> • The system was successfully tested in autonomous operation for a period of a month, but not for longer periods of deployment.
<i>Colorimetric determination of ammonium using variation of Berthelot method</i>	Ammonium reacts with hypochlorite and then with salicylic acid and an intense colour is generated. The intensity of the colour is proportional to the ammonium concentration.	<ul style="list-style-type: none"> • Salicylic acid is less toxic and more stable than phenol. • Linearity was demonstrated over the range 0-15 mg/L ammonium. 	<ul style="list-style-type: none"> • Construction of a generation 1 prototype microfluidic system based on the method has only recently begun, so portability has not fully been tested.
Technology / Technique	Method of Operation	Advantages	Disadvantages
<i>Microchip with LED induced fluorescence detection</i>	The OPA method was again employed, and an LED was used as the excitation source. The fluorescence generated was detected using a photomultiplier tube.	<ul style="list-style-type: none"> • A linear range of 0.018-1.8 μg/mL was obtained. • The LOD was 3.6×10^{-4} μg/mL ammonium. • The developed system offers simplicity, low-cost and stability. 	<ul style="list-style-type: none"> • In order to achieve long term deployment using this system, improvements are required in terms of pumping, battery power and communications.
<i>In-situ ammonium analyser</i>	Sample solution is mixed with a base to raise the pH above 10.5 and converts ammonium ions to ammonia gas. The gas then diffuses across a gas permeable membrane into a receiving solution for detection using a conductivity detector.	<ul style="list-style-type: none"> • Matrix effects are eliminated. • A Linear range up to 18 μM ammonium. • LOD of 0.20 μM ammonium. • The method is suited for in-situ instrumentation due to its simple and robust chemistry. 	<ul style="list-style-type: none"> • The intricate design of the diffusion cell may be seen as a disadvantage. • A considerable amount of tubing is required.

4.2.4.1 INTRODUCTION

Ammonia is an important component of the nitrogen cycle and as it is oxidised in the environment by microorganisms (i.e. nitrification), it is a large source of available nitrogen in the environment. The complexity of the nitrogen cycle, various rate determining environmental conditions for nitrification (e.g. pH, temperature), and the physical behaviour of ammonia (e.g. volatilization, adsorption) make determining the fate of ammonia in the environment extremely complex. Ammonia is highly soluble in water and its speciation is affected by a wide variety of environmental parameters including pH, temperature and ionic strength. In aqueous solutions, equilibrium exists between un-ionized (NH_3) and ionized (NH_4^+) ammonia species. Unionized ammonia refers to all forms of ammonia in water with the exception of the ammonium ion (NH_4^+), whereas ionized ammonia refers to the ammonium ion. Ammonia is used in a number of applications in the refrigeration, pulp and paper, mining, food processing and animal husbandry sectors [EC97]. However the principle use of ammonia is the production of nitrogenous fertilisers. Ammonia commonly enters the environment as a result of municipal, industrial, agricultural and natural processes. Natural sources of ammonia include the decomposition or breakdown of organic waste matter, animal waste, the discharge of ammonia by biota, and nitrogen fixation processes [CCM10].

At the pH in seawater, ammonia occurs predominantly in the ammonium form [WCL13]. It can be noted that episodic increases of ammonium in the water column have been directly related to zooplankton or ichthyoplankton excretion. Ammonium released by zooplankton within the mixed layer where exogenous nitrogen is often in short supply, can be a significant recycled nitrogen source supporting phytoplankton production. In polluted waters, high concentrations of ammonium can be toxic to organisms such as fish, crab, shrimp, and sea urchin, especially larvae or juveniles of these species [AZO13]. In fact, ammonium is the nitrogen compound most rapidly cycled in coastal and marine waters with a typical residence time of hours or less [CRJ08]. Measured concentrations from water samples in oligotrophic waters typically average less than $1 \mu\text{M}$ but there may be considerable temporal and spatial variability, especially since the proximal sources of ammonium are predominantly biological [JLB08]. Given the degree of variability accurate measurement of ammonium in natural marine waters requires in situ instrumentation. For long-term deployment such a system should be simple, robust and compact. Although commercial ammonium electrodes are compact they are at present neither stable nor sensitive enough for the measurement of ammonium in marine environments. This report highlights and evaluates numerous analysis systems and potential techniques which may be employed to obtain cost-effective in situ sensors for the determination of ammonium in marine environments.

4.2.4.2 MINIATURE MULTI-PUMPING FLOW ANALYSER FOR AMMONIUM MONITORING IN SEAWATER

Spectrometric determination by the “Berthelot” or “Indophenol-blue” reaction is the classic and widely applied analytical method for the quantification of ammonium in seawater. It is based on the reaction of ammonium with hypochlorite and further with a phenol compound to form the corresponding blue-green indophenol. However the affection of the sample salinity, the required high reaction pH and hypochlorite degradation present important drawbacks. In addition, the insufficient sensitivity for oligotrophic waters unless using long-path detection cells is another drawback worth mentioning [LZM05]. During the last two decades, quantification of ammonium by fluorescence spectrometry after its reaction with o-phthaldialdehyde (OPA) and reduction with sulfite has become commonly accepted. The reaction product isoindol-1-sulfonate shows an intense fluorescence at 425 nm using an excitation wavelength of 365 nm. This method shows negligible dependency on sample salinity and high sensitivity can be achieved. The method is further well-suited for trace analysis in seawater and estuarine waters [MMM06].

[HDC11] have developed a simple, cost-effective miniature flow analyser for ammonium in seawater using solenoid micropumps and fluorescence detection. The work carried out by the group was aimed at the development of a transportable analyser system for ship-board monitoring. The OPA method was adopted on a solenoid micropump system applying a reverse FIA concept, i.e. the reagent was injected into the sample flow which was consequently also used as a carrier. The influence of sample salinity on the method was studied and it was determined that the method was reliable for quantification of ammonium in open ocean seawater. The influence of the samples pH was also studied over a range of 7.3 to 8.4. Between pH 7.6 and 8.3, no significant influence on the methods sensitivity was found. In general, the method showed to be reliable and robust in respect to pH, salinity, temperature and gas content of the sample. This application of the OPA method demonstrates the potential of the method in terms of obtaining portable miniature systems capable of performing ammonium analysis in marine environments.

4.2.4.3 PORTABLE FLUOROMETRIC ANALYSER FOR AMMONIUM DETERMINATION IN MARINE WATER

As mentioned, the spectrophotometric indophenol blue method, which relies upon the Berthelot reaction, is a typical method used to measure ammonium in seawater. However, the traditional method does suffer from drawbacks [AKK97]. It has been demonstrated that a fluorescent method, in which o-phthaldialdehyde (OPA) and sulfite react with ammonium provides high sensitivity and has been successfully used for the determination of ammonium in seawater [WBC05]. In 2008, Amornthammarong *et al.* successfully developed a continuous flow shipboard analyser for underwater measurement of ammonium in seawater which was highly sensitive and had no refractive index interference [AZ08].

In more recent work carried out by the group, a new system called an autonomous batch analyser (ABA) was developed, which was simple, robust and inexpensive [AZO11]. The ABA achieved complete mixing of sample with reagents using a syringe and a sample mixing chamber. The ABA could also autonomously produce a calibration curve by auto-diluting a single stock standard solution and achieved the same accuracy as traditional manual calibration methods.

Most recently, Amornthammarong *et al.* have developed a new, improved portable system for the analysis of ammonium based on the ABA. An improved LED photodiode-based fluorescence detector was designed and constructed to be used in the ABA [AZO13]. This resulted in the system being smaller and provides a higher sensitivity than the previous design. In addition it incorporates pre-filtration to facilitate suspended sediment-laden field samples. The portable analyser was deployed and successfully documented diurnal cycles and the potential transport of ammonium into the coastal ocean. The system produced is compact, has low power demands and is suitable for in situ ammonium measurement.

Measurement Method and Fabrication

In terms of reagents, reagent 1 (R1) is o-phthaldialdehyde at a concentration of 15 mM. Reagent 2 (R2) is 10 mM sulfite in 5 mM HCHO. A 0.1 M NH_4Cl stock solution was also prepared. Acidic traps made of acid-washed silica were used to protect reagents and standard solutions from possible atmospheric ammonia contamination. All components of the instrument were housed in a metal case (12.7 cm x 22.9 cm x 43.2 cm) as depicted in figure 4.2.23A. The power consumption is 40 W. The fluidic system is illustrated in figure 4.2.23B.

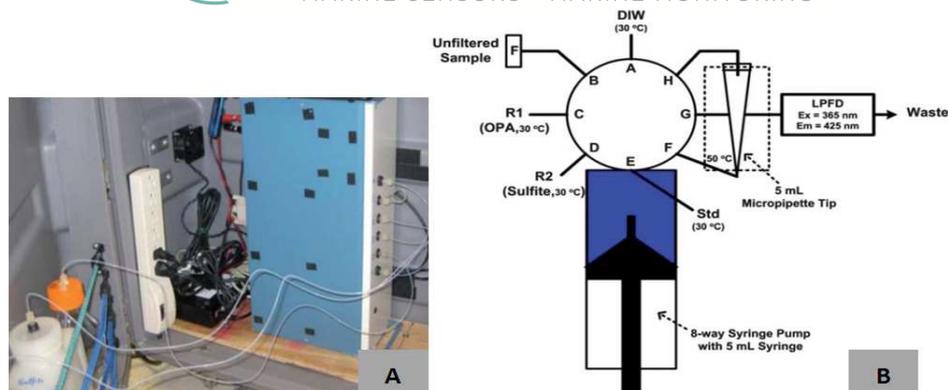


Figure 4.2.23. (A) Components of the portable analyser. (B) Illustration of the portable ammonium analyser and fluidic system [AZO13]

The system consists of one syringe pump equipped with an eight-way distribution valve. The syringe pump is equipped with a 5 mL capacity zero dead volume syringe. In order to completely and rapidly mix the solutions, the syringe itself acts as the primary mixing chamber, and a 5 mL pipette tip as the secondary mixing chamber. Mixing is accomplished in 5 cycles. The mixed solution is held in the syringe for 3 more minutes allowing for 1-sulfonateisoindole to be formed. The syringe pump then pushes the solution into the LED photodiode-based fluorescence detector to obtain a response signal. Finally the system is cleaned using deionised water, which is pumped through the syringe, the pipette tip and the detector several times before the system is ready to take another measurement. The LED photodiode-based fluorescence detector is depicted in figure 4.2.24.

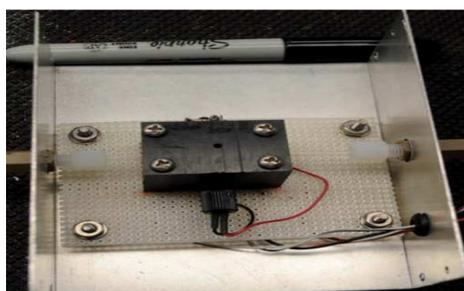


Figure 4.2.24. Depiction of the fluorescence detector [AZO13]

System Performance

A working range of 0.03 - 10 μM and a limit of detection of 10 nM was obtained. The system and reagents exhibited suitable stability at an ammonium level of 5 μM . The system was tested in Biscayne Bay, Florida on the dock of the RSMAS campus, University of Miami over the period of a month, where it was configured with a 15 minute sampling frequency. Seawater samples were also collected at the same site hourly during weekdays and were analysed in the lab by an ammonium analyser. The two methods demonstrated good agreement. The portable analyser was deployed in the waterway south of Lake Mabel and the Port Everglades inlet, Florida and successfully measured ammonium in the surface waters.

The portable analyser which Amornthammarong *et al.* developed for ammonium determination in seawater was successfully tested in autonomous operation [AZ13]. The system is inexpensive and proved to be robust and reliable. An LED photodiode-based fluorescence detector was constructed and achieved higher sensitivity than previous designs. The compact, inexpensive detector instead of the expensive and bulky commercial fluorescence detector enabled the analyser to be portable and suitable for field deployment in marine environments.

4.2.4.4 COLORIMETRIC DETERMINATION OF AMMONIUM USING VARIATION OF BERTHELOT METHOD

[CCP13] have successfully employed a colorimetric method for the determination of ammonium based on a variation of the Berthelot method which utilises salicylic acid instead of phenol, thereby eliminating a toxic and unstable reagent component. Salicylic acid has been shown to be stable for at least 12 months. The intense colour which is generated in the presence of ammonium is easily detected at a wavelength of 630 nm. The method which was developed was tested using solutions from 0-15 mg/L ammonium and the calibration curve which was obtained demonstrated a R^2 value of 0.998, this agreeable calibration curve highlights the potential suitability of this method for integration into field deployable microfluidic sensing platforms and the research group have begun construction of a generation 1 prototype system based on this method [CCP13]. This variation on the Berthelot method may find application on a microfluidic platform which could be utilised for ammonia monitoring within marine environments.

4.2.4.5 INTEGRATED MICROCHIP WITH LED-INDUCED FLUORESCENCE DETECTION

[XUL12] developed a microfluidic device integrated with a fluorescence detection system for on-line determination of ammonium in aqueous samples. A 365 nm LED was used as an excitation source along with a minor band pass filter and were mounted into a polydimethylsiloxane (PDMS) based microchip for the purpose of miniaturization of the entire analytical system. The sample containing ammonium is reacted with o-phthaldialdehyde on-chip with sodium sulfite as the reducing agent to produce the fluorescent isoindole derivative, which emits fluorescence signal at 425 nm when excited at 365 nm. The system which was developed was applied to determine the ammonium concentration in rain and river waters but could also be used for the selective measurement of ammonium in coastal, estuarine or even wastewater [XUL12]. The developed technique has a number of associated benefits such as simplicity, speed, low cost, high sensitivity and selectivity.

Fabrication

The PDMS chip used was fabricated based on the standard soft photolithographic and wet etching techniques as detailed in previous work [GWG10]. The microfluidic chip was designed with a Y-intersection channel for solution injection, mixing and chemical action on-chip. The channel consisted of a 45 mm straight channel designed with "V" grooves for efficient mixing and chemical reaction. The dimension of the Y-intersection channel on-chip was

60 mm in length, 0.6 mm wide and 0.08 mm in depth. The microfluidic chip with the Y-intersection channel for on-chip mixing is depicted in figure 4.2.25, the "V" grooves are also shown.

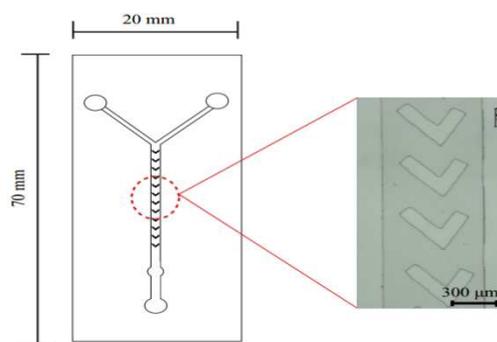


Figure 4.2.25. Schematic of microfluidic chip with Y-intersection channel and "V" grooves [XUL12]

A 365 nm LED together with an optical band pass filter was firstly fixed in an aluminum tube. It was then perpendicularly put onto the detection cell of the PDMS slide. In order for the LED and filter to be firmly fastened onto the PDMS slide, they were inserted into the PDMS before it was fully cured. After the PDMS cured, the PDMS slide together with the LED and the filter was peeled off from the silicon mold, then treated with oxygen plasma and irreversibly bonded with glass substrate. To reduce the scattering light from the channel, a sheet with a 0.8 mm aperture was used to restrict the beam size under the detection cell. An interference filter is used to eliminate the excitation light as much as possible, which may overlap the emission spectrum of the ammonium complex. The integrated instrumental set-up is illustrated in figure 4.2.26. Light from

the LED was firstly filtered by the optical band-pass filter to block stray light of the LED, and then reaches the solution. Under the detection cell, a sheet of silver paper with a 0.8 x 0.8 mm aperture was used to cut off the redundant stray light from the LED and allows fluorescence to go through. The fluorescence emitting from the solution is collected at a photomultiplier tube which is equipped with an emission filter to allow 425 nm fluorescence pass through.

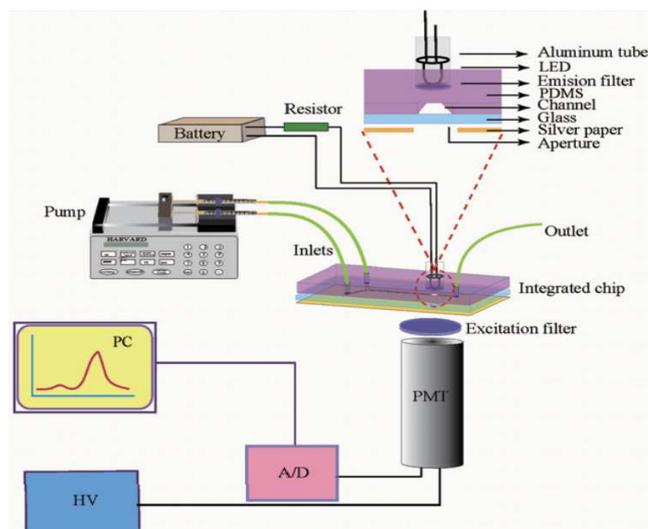


Figure 4.2.26. Schematic of set-up used for ammonium fluorescence determination [XUL12]

Measurement Method

To achieve ammonium determination, the microchip integrated with a LED was put into a dark booth in which the detection window of the photomultiplier tube (PMT) was shielded by a black sheet with a hole aligned with the detection cell of the chip. The sheet acted as a shutter to allow fluorescence of the solution to pass through and block the superfluous light of the LED. The working reagent and standards or samples were delivered into the micro-channels separately by syringe pumps, using two syringes (1.0 mL) connected with PTFE tubes, then mixed and reacted on-chip. At the detection cell, solutions are excited by the LED light, and then fluorescence is generated and detected using the PMT. The change in current of the PMT is monitored and then translated using an analog-to-digital converter.

System Performance

A suitable linear relationship between ammonium concentration and fluorescence intensity was observed with ammonium concentrations ranging from 0.018 - 1.8 $\mu\text{g}/\text{mL}$. The repeatability and limit of detection of the developed method were studied and confirmed that the method was satisfactory for quantitative determination of ammonium in aqueous samples. The detection limit for ammonium was $3.6 \times 10^{-4} \mu\text{g}/\text{mL}$. The proposed microchip-based method was successfully applied to determine NH_4^+ in environmental water samples obtained from a lake. The performance of the developed microchip method was evaluated by analysing the same samples using a fluorescence spectrophotometer. By comparison, the two methods showed a good agreement. The developed method offers simplicity, stability, low-cost, portability and may be seen as a promising alternative for ammonium determination in aquatic ecosystems.

4.2.4.6 IN-SITU AMMONIUM ANALYSER FOR ESTUARINE AND COASTAL WATERS

[PJN09] developed an in situ analyser for measuring ammonium in estuarine, coastal and shelf waters at depths of less than 3 m which is referred to as the NH_4 -Digiscan. This wet chemical analyser uses micro-solenoid pumps to propel sample and reagents, a gas diffusion cell to isolate the analyte from the matrix and a conductivity detector for analyte detection. The system was successfully deployed for 30 days, sampling hourly and demonstrated stable measurements. The

simple chemistry, in situ capability and high resolution sampling minimises the use of toxic reagents, minimises many of the problems plaguing ammonium analyses and assists in capturing the high temporal variability of coastal and other waters.

As previously discussed the orthophthalaldehyde (OPA) method is very sensitive and has an attractive dynamic range; however the reaction may be effected by temperature changes. An alternative approach to ammonium determination uses gas diffusion to separate ammonium from the sample matrix prior to detection [HA92]. Generally, a sample is mixed with a base to raise the pH above 10.5 and convert ammonium ions to ammonia gas. The gas then diffuses across a hydrophobic gas permeable membrane into a receiving solution for detection. This technique is advantageous as the separation is very selective, it makes preconcentration possible and eliminates matrix effects from the sample solution. [HA92] used 50 μM HCl for the receiving solution and detect changes in conductivity as ammonia diffused across the membrane. Ammonia gas reacts with the protons in the acid solution forming ammonium ions at the expense of protons. Since an ammonium ion is less mobile than a proton, the net result is a drop in the receiving solution conductivity. This conductivity change is proportional to the ammonium concentration of the sample. This method is well suited for in situ instrumentation due to its simple and robust chemistry, low detection capabilities and nontoxic reagents. The in situ ammonium analyser, referred to as the NH₄-Digiscan, developed by [PJN09] is based on the chemistry developed by [HA92].

Measurement Method and System

The NH₄-Digiscan ammonium analyser is based on a set of individually controlled micro solenoid diaphragm pumps. This pulsed flow technology is similar to the multi-pumping flow system described by [LDS04]. It offers a number of advantages over traditional flow injection analysis systems driven by peristaltic pumps. These advantages include smaller size, lower power consumption, greater analytical flexibility, reduction in reagent usage, less contamination, improved mixing and better flushing. In situ chemical analysers based on this concept have previously been developed by [CCJ04] where a data logger/controller powered solenoid pumps, which propel reagents and sample through a flow path yielding a chemical reaction. The reaction product is quantitatively measured with a detector, and the resulting data are then processed and stored by the logger. The NH₄-Digiscan is primarily utilised for in situ use at depths of less than 3 m. For in situ applications, the electrical components reside in housings and reagents are contained in bags.

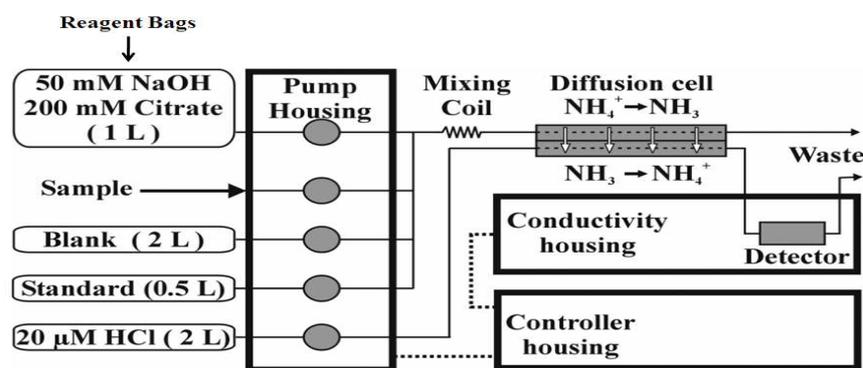


Figure 4.2.27. Schematic representation of the in situ NH₄-Digiscan [PJN09]

200 mM trisodium citrate dehydrate is mixed with sample, standard or blank. This mixture is pumped through 0.5 m mixing coil and then through one side of the gas diffusion cell, where the ammonia gas diffuses across the membrane into a 20 μM HCl receiving solutions on the other side. Following this the volume of

ammonium-enriched HCl in the diffusion cell is pumped through the conductivity cell for

detection. The in situ NH₄-Digiscan is schematically represented in figure 4.2.27. Darkened circles represent solenoid pumps; solid lines represent fluid circuitry, while dotted lines represent electrical connections between the housings.

In terms of the gas diffusion cell, the cell consisted of a 127 x 51 x 0.071 mm strip of military grade Teflon pipe tape sandwiched between two polysulfone blocks. Each block (105 x 40 x 13 mm) contained a fluid track 324 mm long by 1.52 mm wide. The sample side was 0.20 mm deep while the receiving solution was 0.10 mm deep yielding 100 μ L and 50 μ L volumes respectively. The track was serpentine in shape to increase mixing and the mating faces were machined flat to 0.025 mm. The gas diffusion cell is illustrated in figure 4.2.28 below.

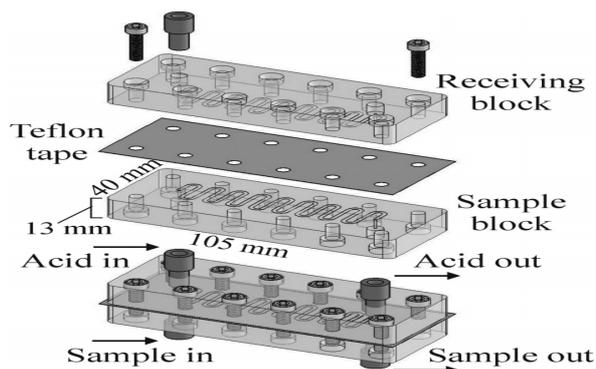


Figure 4.2.28. Diffusion cell depicted in both assembled and disassembled states [PJN09]

In relation to detection and control, conductivity was measured using a conductivity cell and detector board. The conductivity board was modified so the gain was double. For in situ analysis the cell and board were powered by a 12 volt battery pack, and all components were contained within a watertight PVC housing. The analyser is controlled by a Tattletale 5F data logger/controller running a specifically developed program. The software manages the pumping sequences, flow rates, sampling and calibration intervals, data collection, processing and storage, and testing routines. An analysis can be divided into three parts: the flushing sequence, the loading sequence, and the eluting sequence. Each sequence can have a unique flow rate and pumping schedule. The flushing sequence delivers fresh sample, standard or blank into the system, flushes the sample path with blank and then loads the receiving side of the diffusion cell with fresh acid. The loading sequence mixes sample, standard or blank with base and pushes it through the diffusion cell. The eluting sequence then pushes acid through the conductivity cell for detection. A typical in situ analysis utilizing the system for coastal waters uses 1.65 mL of sample, 3.8 mL of reagents and requires less than 7 min. The battery and controller module were packaged in a separate watertight PVC housing.

System Performance

The response of the NH₄-digiscan to a given sample ammonium concentration is dependent upon several factors such as; detector gain, moles of ammonia passing through the diffusion cell, transfer efficiency of ammonia gas across the membrane, volume and concentration of the receiving acid solution in the diffusion cell, and the dispersion of the conductivity signal as it travels from the diffusion cell through the conductivity cell since response was measured as peak height. In terms of stability, sensitivity and precision the analyser was quite stable and showed little drift over 30 days when deployed on the L01 mooring of the Land/Ocean Biogeochemical observatory infrastructure in Elkhorn Slough, California. The operation of the ammonium analyser is quite flexible by design. When the NH₄-digiscan was set up to measure coastal waters and deployed on the L01 mooring, the analyser had a linear range up to 18 μ M and a detection limit of 0.20 μ M. The accuracy of the instrument was tested by collecting duplicate samples and comparing the gas diffusion-conductometric approach to the traditional indophenol blue method

(IPB) method. A total of 160 coastal samples were collected during two 24 hour surveys in Elkhorn Slough, California and 98 offshore samples were collected and analyzed. Suitable agreement between the two techniques was demonstrated. This system offers great potential in terms of in situ analysis of ammonium in the aquatic environment, and through further improvement and developments the system may be utilised for long term deployment in marine environments.

4.2.4.7 REFERENCES

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4.2.5 Heavy Metal Detection In Water Samples

In this report a broad overview of detection of heavy metals, mainly lead, cadmium, zinc, copper and mercury among others, in water samples is reviewed. Since the most of the analytical methods to determine heavy metals are based on the electrochemical techniques using great variety of electrodes, this report is only focused in the use of screen-printed electrodes as transducers of the sensors, given the importance of these electrodes in the development of devices that allow decentralised or in situ measurement of these metals in wastewaters, rivers, lakes or sea water.

Stripping voltammetric techniques are an interesting alternative for the detection of trace levels of heavy metals in high saline matrices. These techniques present a significant sensitivity due to their unique ability to preconcentrate target species during the accumulation step on the working electrode, next to its combination with pulse measurement techniques that generate a highly favourable signal-to background ratio. Mercury-based electrodes, such as mercury film electrodes (MFE), and hanging mercury drop electrodes (HMDE), have traditionally been used in stripping techniques because of their advantages, such as high sensitivity, reproducibility, purity of the surface, high hydrogen overpotential, and possibility of amalgam formation. Therefore, they have been recognized as the most sensitive electrodes for the determination of heavy metals [KBJ07]. In particular, the HMDE has successfully been used for the determination of lead and cadmium in seawater samples [Dui77, GUS84]. More recently, Süren et al. introduced a modification in the system by preceding the differential pulse stripping voltammetry (DPSV) by adsorptive collection of complexes with 8-hydroxyquinoline (oxine) on to the mercury electrode [SYT07]. On the other hand, several studies with MFE are reported [Kho02, CNK07, MLH00, Fis99, MCS04, JJW81]. For example, Khoo and Guo investigated various mercury (I)/(II) salts as modifiers for carbon paste electrodes [Kho02]. In an attempt to work with environmentally friendly metals, a comparative study of the simultaneous determination of heavy metals in highly saline samples by ASV using both mercury and bismuth film electrodes as working electrodes was carried out [CNK07].

The coupling of disposable screen-printed electrodes (SPEs) with stripping techniques presents an attractive alternative to conventional stripping analysis. SPE is related to a mass production technology based on screening of electroconductive and insulating inks onto planar substrates (plastic, ceramic, etc.) at a controlled thickness. The use of stripping analysis based SPEs eliminates the problems related to the use of conventional electrochemical cells: the associated cumbersome handling, the lengthy cleaning procedures and the need for a de-aeration step, using electrochemical techniques less influenced by oxygen interference. Moreover, SPEs related instruments can be portable by including a compact battery achieving a user friendly field-deployable device that allows the direct monitoring of heavy metal traces in an *in situ* control of pollution [Dom07, PLM05, CBS07, PCM99].

Here the different determination of heavy metal using SPEs is reviewed. These will be organized by the types of materials used to modify the working electrodes.

4.2.5.1 Screen printed carbon and metal electrodes

There are very few works related to the use of unmodified screen printed carbon electrodes (SPCEs) in the determination of interesting analytes [Hon03]. Graphite materials are preferred due to their simple technological processing and low-cost. However, most studies of heavy metal determination using SPEs show that mercury, gold, silver, bismuth or other materials modified on the surface of SPEs can improve selectivity or sensitivity [ZAC09, CSC01, LPM06, WLH00, FZO11, CZR09].

Despite it, Honeychurch et al. [HHC00] performed the determination of lead by differential pulse anodic stripping voltammetry (DPASV) using SPCEs. A detection limit of $2.5 \mu\text{g L}^{-1}$ was obtained and the coefficient of variation, determined on one single electrode, was at 2.4% ($n = 5$). The method was used in the determination of lead in water samples. Similarly, trace levels of copper (II) were determined using this procedure in samples of water, which established a detection limit of $8.2 \mu\text{g L}^{-1}$ [HHH02].

Although most SPEs are fabricated with graphite inks, other materials such as gold and silver-based inks are also used in their construction for the analysis and determination of various elements. Thus, Mascini and co-workers performed the determination of lead and other environmentally hazardous metals such as copper, mercury and cadmium on gold-based SPEs using square wave anodic stripping voltammetry (SWASV), which resulted in detection limits of 0.5, 2.0, 0.9 and $1.4 \mu\text{g L}^{-1}$ and RSDs of 7, 12, 4 and 14%, respectively [LPM06]. This method has also been applied to the determination of lead in wastewater and soil extracts by Noh et al. [Noh06]. Equally, SWASV determination of lead has been performed on an silver-based SPE, without chemical modification, which could also be exploited as a disposable lead sensor with a detection limit of $0.46 \mu\text{g L}^{-1}$ [ZYK02].

Commercial gold-based SPEs make routine measurements of mercury in water samples simpler and more attractive because preplating steps are unnecessary. Commercial gold-based SPEs (SPGEs, DropSens, Spain) have been successfully used for the measurement of mercury in ambient water [BSP11].

4.2.5.2 Film-coated SPCEs and others

Hg film-modified SPCEs

In most cases, the working electrode consists of thin mercury film plating applied to the graphite surface of the electrode, which enables electrochemical preconcentration of heavy metals.

Wang pioneered the use of these electrodes by demonstrating the viability of determining lead at ppb levels using stripping voltammetry and potentiometric measurements in urine and water samples [Wan92]. Subsequently, he went on to perform a joint determination of various metals such as cadmium, lead and copper at ppb levels on mercury-coated carbon strip electrodes [Wan94] demonstrating results that were as satisfactory as those obtained on glassy carbon electrodes, and on HMDEs.

Since the research conducted by Wang, other authors have fine-tuned various methods for the determination of metals such as lead, copper, zinc and cadmium among others, which are based on the easy accumulation on mercury films. Likewise, mercury-coated SPCEs form the subject of a number studies by Ashley et al. [Ash94, Ash95, AWM01], and Desmond et al. [DLA96, DLA98] obtained detection limits of 55, 71, 64 and $123 \mu\text{g L}^{-1}$ for zinc, cadmium, lead and copper, respectively, using DPASV and a deposition time of 300 s. Palchetti et al. [PCM99] applied SWASV and potentiometric stripping analysis (PSA) in order to determine copper, lead and cadmium on mercury-coated SPCEs. The detection limits they obtained were $0.4 \mu\text{g L}^{-1}$ for lead, $1 \mu\text{g L}^{-1}$ for cadmium, and $8 \mu\text{g L}^{-1}$ for copper by using SWASV, and $0.6 \mu\text{g L}^{-1}$ for lead, $0.4 \mu\text{g L}^{-1}$ for cadmium and $0.8 \mu\text{g L}^{-1}$ for copper by using PSA.

The mercury-coated screen-printed sensors can be prepared beforehand in the lab for immediate on-site use. In this way, handling, transport and disposal of toxic mercury (II) solutions during decentralized measurements is avoided, as the coating is pre-deposited on the electrode surface [PLM05]. This method combined with SWASV analysis has been successfully applied in the determination of various metals and detection limits of 0.3, 1 and $0.5 \mu\text{g L}^{-1}$ were found for lead, cadmium and copper, respectively.

Modification of Hg-coated SPCEs with crown-ether based membranes also seems to be a convenient and inexpensive technique for trace metal detection. Analytical results showed that these electrodes were simultaneously able to detect $\mu\text{g L}^{-1}$ levels of lead and cadmium with good sensitivity and reproducibility, at different pH values by using linear scan anodic stripping voltammetry (LSASV) [PBA06].

In the work conducted by Choi et al. [CSC01], the working electrode was SPE with phenol resin-based carbon ink containing fine particles of mercury oxide as a built-in mercury precursor. The mercuric oxide particles exposed on the surface were reduced to fine mercury droplets by in situ or pre-cathodic conditioning so that they behaved as heavy metal collectors in the anodic stripping analysis. This sensor was evaluated using lead and cadmium as probe metals.

A thin-film mercury SPE was combined with a micro-well to detect cadmium [PAB07]. The use of a micro-well reduced the volume of the sample to 200 μL without stirring, which minimized the loss of sensitivity. Moreover, this working electrode was used for direct analysis in raw samples of soil solution without pretreatment, which provides a useful method for the in situ determination of heavy metals.

Some researchers have also printed auxiliary electrodes with the working electrode on the same polymer plates [ZAC10]. This screen-printed sensor was ex situ electrodeposited with a small quantity (approximately 5 μg) of mercury at -0.1 V (vs. Ag/AgCl). However, when this sensor was used, an additional commercial reference electrode or counter electrode was needed to compose a 3-electrode system for detection. Therefore, a screen-printed 3- electrode system on the same substrate would be more convenient.

A 3-electrode device with a standard USB port conveniently connected to a PC or laptop computer was designed [ZLS10].

However, as Hg is toxic, its incorporation in sensors poses environmental problems, especially bearing in mind that these SPEs are disposable and, as a consequence, other metal films and even unmodified SPEs are under investigation.

Despite it, as it will be shown below, although bismuth is widely used to modify SPEs for the detection of heavy metals in order to avoid the use of mercury, this approach exhibits shortcomings. For example, acidic or highly alkaline working media may be necessary for in situ plating of bismuth film, because of the Bi(III) hydrolysis in neutral and alkaline media [Eco05]. In contrast, the quasi-noble-metal behavior of mercury allows analyses over a wide pH range [ZAC10].

Because of the high affinity of mercury for metals associated with the analytical performance of stripping voltammetry, low detection limits can be achieved with relatively short analysis times without degassing [BPP07]. Mercury is therefore still used as the modifier for trace metal detection [ZAC10, ZLS10]. However, because of the toxicity of mercury, researchers are actively pursuing ways to reduce the amount that is needed for modification of SPEs.

Thus, microelectrodes provide new tools for environmental analysis, however, the difficulty of recording very low currents using microelectrodes needs to be overcome [Fle99]. Therefore, microelectrode arrays have been consequently developed to obtain a higher current output [DBB08]. Based on femtosecond laser ablation technologies, square arrays of 64 microelectrodes (8×8) were made in a polymer substrate and then screen-printed carbon ink [CZR09]. Because of the small overall surface of $\text{SP}\mu\text{EA}$, only a low quantity of mercury (less than $1\mu\text{g}$) was ex situ deposited, and the devices were sufficient to perform a trace analysis of river water close to a zinc mine.

Bi-coated SPCEs and Bi SPEs

The most widely used modifier of SPEs for electroanalysis is bismuth because of its good analytical performance and its “environment-friendly” characteristics. Since the pioneering report in 2000 [WLH00], bismuth modified SPEs (BiSPEs) have been broadly applied to the field of environmental [SPH10] monitoring. Advantages, such as not requiring the removal of dissolved oxygen during stripping analysis and characterization over a wide negative-potential window [Eco05, SPH10, Kok08], make testing convenient. Moreover, the BiSPEs can improve sensitivity; for example, the detection limits of lead can reach the ppb level or lower [Man09].

Bismuth modified electrodes may be prepared ex situ or in situ via electrodeposition. This approach involves careful preparation of the electrode surface between samples and to eliminate this preparative step, the use of disposable screen printed electrodes as underlying electrode substrates have been reported [Zac09]. Towards simplifying the electrochemical methodology further, bismuth-

powder modified carbon paste electrodes [HSV05] have been reported as well as bismuth nanopowder modified electrodes where the bismuth nanopowder is immobilised with Nafion [LLU20]. Hwang et al. have recently reported the screen printing of a bismuth oxide layer onto a screen printed electrode surface which is then electrochemically reduced *ex situ* in sodium hydroxide to produce a bismuth film.

A true disposable bismuth oxide screen printed electrode was reported by Kadara et al. [Kad08] where bismuth oxide is incorporated into the bulk of the screen printed electrode and was explored towards the chronopotentiometric analysis of lead and cadmium in soil and water samples. It was demonstrated that the simultaneous detection of cadmium and lead was not possible due to the lead stripping response being distorted at highly negative potentials.

Recently Banks and col. have reported on a bulk modified bismuth oxide screen printed electrode [KBJ09, KKK10] and demonstrated its applicability towards the sensing of zinc, cadmium and lead in model systems and applied the disposable sensor for the screening of cadmium and lead in river water. The sensor is greatly simplified over bismuth nanoparticle modified electrodes and bismuth film coated screen printed electrodes but also provides similar detection limits and analytical ranges. Within the examples of bismuth modified electrodes, the literature reports of the sensing of zinc are quite seldom [BKH04, KBN04].

A novel sensor was developed for the one-step sensing of lead [FZO11, TGWE10] by covering a paper disk impregnated with buffer and reagents (including an internal standard of zinc and a plating solution of Bi(III)) on a SPCE. After the sample was added to the surface of the electrode, it could be applied to run a single-step assay for lead detection. The performance of the electrode was satisfactory ($5\text{--}50\mu\text{g L}^{-1}$ lead detected), and only a final, small volume of the sample ($10\mu\text{L}$) was needed to be placed on the surface of the paper for running one assay. In addition, an internal standard with a known concentration was added to improve the accuracy and precision [HLM09, MLL10]. Hg-free screen-printed microband electrodes (μBSPEs), which are easily manufactured with the option to renew the electrode surface, was used to detect lead and a theoretical detection limit of $2.3\mu\text{g L}^{-1}$ was calculated based on a signal-to-noise ratio of three [HBH11].

Gold-coated SPCEs

Gold has also been used to modify SPCEs thereby eliminating the use of toxic elements such as mercury. One such example is the work carried out in 1993 by Wang, which demonstrates the possibility of analysing lead [Wan93a] and mercury [Wan93b] on gold-coated SPCEs to obtain highly reproducible responses for both elements.

Lead has also been evaluated in spiked drinking and tap water samples [MLS03]. The recoveries of lead were 103% (R.S.D.: 2.8%) and 97.9% (R.S.D.: 7.1%), $n = 5$, respectively. Measurements in the presence of typical interferences such as copper, cadmium, zinc, iron, chromium and mercury were reported.

Mercury is also one of the most problematic heavy-metal pollutants in environmental monitoring and its organic form is bioaccumulative and toxic. Moreover, the accumulation of mercury in the human body can cause severe disease, such as kidney and respiratory failure, and damage to the gastrointestinal and nervous systems [Zal00].

Bare gold electrodes or modified gold electrodes are usually chosen for inorganic mercury detection because the high affinity for mercury enhances preconcentration [GZS10]. However, the major drawbacks of the use of a gold electrode are structural changes at the surface caused by amalgam formation. For this reason, time-consuming cleaning treatments are necessary to achieve reproducibility [GAM08].

SPCE, coated with a thin gold film, are used for highly sensitive potentiometric stripping measurements of trace levels of mercury [Wan93b]. Applicability to trace measurements of alkyl mercury and selenium is also demonstrated. Such adaptation of screen-printing technology for the development of reliable sensors for trace mercury should benefit numerous field applications.

Metallic nanoparticle-modified SPEs

The design of new nanoscale materials has acquired ever greater importance in recent years due to their wide-ranging applications in various fields. Among these materials, metallic nanoparticles are of great interest due to their important properties and multiple applications. The bibliography lists numerous methods describing the synthesis of metallic nanoparticles in solution as well as by deposition on solid surfaces. They include chemical synthesis by means of reduction with different reagents [SZD03], UV light or electron-beam irradiation [FYH03] and electrochemical methods [MHL06, DNH04, EOO03, WBS05, YMW03, TLD01, CIK06, PGG09, YGG12]. The latter provides an easy and rapid alternative for the preparation of metallic nanoparticle electrodes within a short period of time. The combination of electrodeposition and the screen-printing process is beginning to allow mass production of electrochemical sensors that possess various catalyst activities. The sensor strips fabricated by this process are promising tools with more sensitive detection rates that are now starting to come on stream. Thus, gold nanostructured SPCEs are demonstrated to be suitable transducers for the determination of lead in water and blood [PGG09] or mercury in water [YGG12] based on the under deposition potential of these metals on gold nanoparticles.

Poly(l-lactide) stabilized gold nanoparticles were also used to modify a disposable SPCE for the detection of arsenic by DPASV. The sensitivity was good enough to detect arsenic at ppb levels and provides a direct and selective detection method for arsenic in natural waters [SMC06].

Dominguez and Arcos [Dom07] have fine-tuned a novel, user friendly and rapid method of incorporating silver nanoparticles onto the surface of SPCEs. This method is based on the direct electrodeposition of these nanoparticles. The modification of SPCEs with silver nanoparticles increases the already well known performance of these kinds of disposable electrodes. In order to demonstrate their practical applications, they were used to analyze antimony, a significant pollutant of priority interest. The silver nanoparticle-modified SPCE developed in this work presents an environmentally friendly method for the analysis of antimony. It brings with it important advantages that include a high degree of sensitivity and selectivity in antimony determination. Moreover, the electrochemical response is not influenced by common interferents in antimony determination, such as bismuth.

Other modified SPEs

SPEs made of carbon, bismuth and carbon nanotubes (CNTs) ink were used for the determination of mercury in tap water and human hair. The addition of Bi and CNTs to the carbon ink to fabricate the SPE, improves the sensitivity separately and especially together [NZL11].

Other carbon nanostructured material that has been employed in the analysis of mercury is carbon black, material with a high number of surface defects. This material has high sensitivity to the measurement of thiol groups, which form a stable complex with mercury. Palleschi et al. developed an amperometric sensor employing SPEs modified with carbon black for the indirect analysis of mercury in drinking water. The sensor responds to the oxidation of thiols and the analytical signal is lower when there is presence of mercury in the sample [AMA11].

Heated SPEs with carbon nanoparticles (SPCNPsE) were employed for heavy metals determination in seawater. The use of heated electrodes increased the mobility of ions achieving a faster deposition of metals and a higher sensitivity [APM11].

The use of carbon nanomaterials in the electrochemical determination of mercury presents some important improvements compared to the lack of modification with this kind of materials. The increased surface area of the working electrode increases the sensitivity and the measurement of low amounts of mercury is achieved. However, where carbon nanomaterials stand out is when accompanying the modification with other selective compounds resulting in a higher power of preconcentration over the electrode and a higher sensitivity. Although graphene has excellent properties for use in electrochemical analysis, the modification of electrodes with only graphene or its derivatives has not been tested for mercury determination. In the published works the

modification of electrodes with graphene has always been accompanied with species that can interact with mercury as gold nanoparticles or species containing functional groups.

Although the use of single nanostructured materials could present advantages to using the macroscopic material, researchers are still looking for new ways to enhance these characteristics with innovative technologies. One such technology is the use of hybrid nanostructured materials. These hybrid systems can have properties that amplify those of the single nanostructured materials. There are several methodologies for modifying electrodes with nanohybrid materials, similar to the modification with single nanomaterials, which depend largely on the kind of working electrode and the materials used. Several nanohybrid materials have been used in different applications of electrochemical analysis, and also in the determination of mercury. SPCEs have also been modified with nanohybrid materials such as graphene/AuNPs and MWCNTs/AuNPs. The SPEs were modified with carbon nanomaterials by means of physical adsorption, and then the AuNPs were generated applying a constant current to a solution of HAuCl_4 . The use of nanohybrid materials improved the sensitivity and lower detection limits than using only AuNPs were obtained. The results showed that the nanohybrid formed by MWCNTs/AuNPs was more adequate for analysis of mercury. Moreover, the reutilization of the SPEs modified with nanohybrids for several measurements of mercury in water samples was achieved [YGG12].

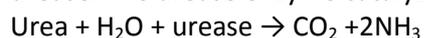
Strip-type preconcentrating/voltammetric sensors, prepared by incorporating a cation exchange resin within screen-printed carbons inks, have been described by Wang and applied to the determination of copper [NWD95]. The device presents good reproducibility (a relative standard deviation of 2%) and a detection limit of $32\mu\text{g L}^{-1}$ with a 10 min accumulation time.

Some researchers have detected mercury using SPEs based on the electrochemical behavior of a Hg-complex. A thiol-probe based on SPEs modified with a film of nanostructured carbon black is highly sensitive to thiocholine and cysteine. After the mercury is added, a non-electroactive complex is formed, which leads to a diminished signal [AMA11]. This sensor is a promising replacement for the traditional gold electrode. The application of polyaniline (PANI) has been investigated for the preconcentration and speciation of inorganic mercury and methyl mercury, $[\text{CH}_3\text{Hg}]^+$, in various water samples [KKR05, GSD04]. Other researchers have modified bare SPCEs with a conducting polymer layer of PANI, polyaniline–poly(2,2'- dithiodianiline) (PANI-PDTDA) [SLM10a] or polyaniline–methylene blue (PANI-MB) [SLM10b], and these SPCE/polymer sensors could be used as alternative transducers for the voltammetric stripping and analysis of inorganic mercury. Moreover, this electrochemical sensor has been utilized for the simultaneous determination of mercury, lead, nickel and cadmium [SHI11, SIH09].

4.2.5.3 Enzyme-modified SPEs

It is a well-known fact that some metallic ions, especially heavy metals, can inhibit the activity of various enzymes. Disposable biosensors based on the principle of inhibition have to date been applied for a wide range of significant analytes, amongst which heavy metals [RBT04a, AMB06, OTW05, RBT04b, TZK05].

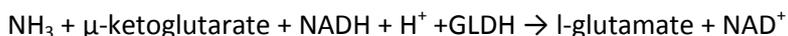
The most widely employed enzyme in the inhibitive detection of heavy metals ions using SPE is urease. The urease enzyme catalyzes the hydrolysis of urea and the reaction produces ammonium:



As a consequence of the ammonium liberation, a variation in the pH value takes place. This change might cause a decrease in the potential of an internal pH-subsensor. Thus, for example, the presence of ruthenium dioxide in the biosensing film causes pH-dependent potentiometric sensitivity [OTW05, TZK05]. The presence of silver and copper causes the heavy metals to inhibit the enzyme which leads to a decrease in enzymatic activity and, as a result, a lower quantity of ammonium is liberated that is recorded as an analytical signal by the sensor [OTW05].

The analysis of copper, mercury and cadmium can be carried out employing a disposable screen-printed biosensor [RBT04a, RBT04b]. Amperometric measurements of urease activity are possible after coupling this enzyme to glutamate dehydrogenase (GLDH), which catalyzes the synthesis of l-

glutamate from μ -ketoglutarate. Both dihydronicotinamide adenine dinucleotide (NADH) and NH_4^+ are required in equimolecular amounts for this reaction to take place:



NADH consumption is monitored by measuring the decrease in its amperometric signal. The urease enzyme is inhibited by the presence of heavy metal ions resulting in decreased ammonia production. This leads to a reduction in the oxidation rate obtained for NADH. The presence of metal ions can be determined by comparing the latter with the NADH oxidation rate in an uninhibited reaction.

4.2.5.4 Simultaneous detection of several different metals

SPEs designed for the simultaneous determination of different metals are more interesting for time and cost reasons. SPEs modified with mercury and bismuth, either in situ [Noh11] or ex situ [PAA11, GAF08], are commonly used to determine lead and cadmium concentrations.

Otherwise, bismuth could be deposited on SPEs in many forms, such as bismuth oxide [HHP08, Kad08, KJB09], bismuth nanoparticles [RMG09], bismuth co-deposited with metal ions in solution [RMG08] and bismuth pre-deposited as a film [SDA10]. As far as we can determine, Bi nanoparticles plated on SPEs using the SWASV technique exhibited the lowest detection limits of lead, cadmium and zinc. When combined with a flow cell, the detection limits could be lower [RMG09].

The normal Au-film-coated SPEs displayed excellent linear behavior. Moreover, the researchers improved the preconcentration technique by modifying the magnetic particles (superparamagnetic iron oxide (Fe_3O_4) nanoparticles [YMS07, WSH09] with thiols to preconcentrate heavy metals from samples whose concentrations were lower than $1\mu\text{g L}^{-1}$ [MIA10].

Because crown-ethers can form complexes by fitting metal cations into the cage of the crown structure, some researchers have modified crown-ether-based membranes on a thin-film of Hg coated SPCE to achieve a selective uptake of lead and cadmium [PBA06].

In addition, the crown-ethers can protect the working surface from interferences during analysis. Without deoxygenation and stirring, this device was successfully used to detect lead and cadmium. Also, this device was utilized in raw samples and in the semicontinuous monitoring of lead and cadmium for 42 h in tap water [BPP07].

Chitosan, poly- $[\mu\text{-}1\text{-}4]\text{-}2\text{-amino-}2\text{-deoxy-d-glucopyranose}$ (CTS), can form stable chelates with many transition-metal ions through hydroxyl and amino groups. CTS and its derivatives have been used for the electrochemical determination of metal ions with glassy carbon electrode (GCE) [MJL07]. Some researchers modified CTS on the surface of SPCEs for the simultaneous determination of lead, copper, cadmium and mercury with preconcentration time of only 30 s [KHH10]. In addition, CTS could be used for the homogeneous dispersion of MWCNTs [SZS10] and the CTS-MWCNTs composite film exhibited significant improvements in tensile modulus and strength [WSZ05]. A small amount of mercury was subsequently deposited in situ, and a random array of mercury nano-droplets formed on the surface of the MWCNTs-CHIT/SPE. This sensor was used for the simultaneous determination of lead, cadmium and copper. Moreover, a hybrid analytical method, which combined electrochemical "adsorption-desorption" with colourimetric analyses, was developed to detect heavy-metal ions [LLL11]. The electrochemical adsorption-desorption procedure, which is more convenient and less time-consuming, was used as a pretreatment process for the colourimetric analysis. The colourimetric approach used for quantitative analysis may avoid errors that arise from the instability of the electroanalytical method.

4.2.5.5 Heavy Metal References

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4.2.6 Microfluidic chip/disc systems

Since the “miniaturised total analysis system” (μ TAS) concept was introduced by Manz *et al.* in 1990 [Man90], it has been recognised that the properties of microfluidic or “lab-on-a-chip” systems make them eminently suitable for the development of compact, autonomous analytical devices for a range of applications including environmental monitoring. The advantages of microfluidics-based *in situ* sensing systems for can be summarised as:

- The small sample sizes used, typically in the sub 100 μ L range, minimise reagent consumption and waste generation.
- The minimisation of fluid volumes reduces the power requirements associated with fluid control/movement, allowing for long deployable lifetimes.
- The small size of the microfluidic manifold facilitates the development of compact and portable analytical systems.
- Fast analysis times result from performing chemical analysis on the μ m scale, where diffusion-based mixing can be an efficient process, allowing high frequency measurements.
- Low-cost sensing devices can be developed by combining microfluidic systems with simple, low cost detectors.

This combination of properties makes microfluidic systems highly attractive as a basis for reagent-based monitoring of chemical species in the aquatic environment. There are however, drawbacks associated with operating at the micro-scale.

- Using μ L-scale sample sizes has implications in terms of adequately representing the complete body of water which is to be measured. This issue is significantly mitigated by the higher measurement frequency which is possible using *in situ* systems.
- Due to the small dimensions of the microfluidic channels, they are susceptible to blockage or interference by fine particulate matter. The use of fine filters at the sampling point can eliminate or minimise this issue, although at the cost of limiting the analytical parameter to the dissolved fraction of the target species.

These issues, and others including measurement interferences caused by bubble formation/trapping within the micro-analytical system, have represented significant barriers to the development and uptake of microfluidics-based in-situ sensing systems. Although numerous micro-analytical systems have been developed and their performances described under laboratory conditions, examples of integrated micro-analytical devices which have been successfully deployed for extended periods under real environmental conditions are much scarcer. Therefore, while the development of sensing nodes for the aquatic environment which are sufficiently reliable and inexpensive to form the basis of extensive wireless sensor networks has been anticipated, realisation of this goal has been more difficult to achieve. The challenges in developing such systems include achieving adequate stability and reliability of the analytical system, minimising the cost and power consumption of the integrated device, robustness under harsh environmental conditions, and fouling due to microorganisms and particulate matter. In developing autonomous environmental monitoring devices, deployable lifetimes on the order of months to a year are desirable. Achieving such lifetimes without elevating the fabrication cost of the integrated system to unviable levels has been described as a key challenge for the analytical science community [Nie10].

4.2.6.1 Examples of miniaturised/microfluidic nutrient analysers.

The area of nutrient detection via integration of colorimetric assays into microfluidic manifolds with light emitting diode (LED) and photodiode (PD) based optical detection systems has seen significant progress. Gardolinski *et al.* [Gar02] developed a miniaturised, LED-based chemical analyser for in-situ monitoring of nitrate. The limit of detection (LOD) with a 20 mm path length flow cell was 2.8 mg L⁻¹ N. The linear range could be adjusted to suit local conditions in the field by changing the flow cell path length. The analyser was tested in shipboard mode for mapping nitrate concentration in the North Sea and in submersible mode to conduct a transect of Tamar Estuary (England).

Doku and Haswell [DH99] developed a micro-flow injection analysis (μ FIA) technique for orthophosphate based on the molybdenum blue reaction. The μ FIA manifold was formed by etching of borosilicate glass, and electroosmotic flow was used both for the mobilization of reagents and for sample injection. A LOD of $0.1 \mu\text{g mL}^{-1}$ was achieved, with a rapid analysis time of 60 s and low sample/reagent volume (total system volume of $0.6 \mu\text{L}$).

Greenway *et al.* [Gre99] produced a similar μ FIA system for the determination of nitrite, based on the Greiss reaction to form an azo dye. Following optimisation of the electro-osmotic flow characteristics, reaction chemistry, and injection time, a LOD of $0.2 \mu\text{mol L}^{-1}$ was achieved. A further progression of this work was to incorporate a cadmium reduction column within the device. This allowed for the analysis of nitrate in water, for which a LOD of $0.51 \mu\text{mol L}^{-1}$ [Pet01] was achieved.

Daridon *et al.* [Dar01] investigated the Berthelot reaction for the determination of ammonia in water utilising a microfluidic device consisting of a silicon chip between two glass plates. The pathlength in this device was $400 \mu\text{m}$, and the integrated system comprised fibre optics coupled to an LED. High aspect ratio channels ($30 \mu\text{m}$ wide \times $220 \mu\text{m}$ deep) were used for sample/reagent mixing in order to achieve efficient diffusional mixing.

Azzarro and Galletta [AG06] described an automatic colorimetric analyser prototype (MicroMAC FAST MP3) for high frequency measurement of nutrients in seawater. This system utilises the Berthelot method for ammonia detection, the sulphanilamide/ethylendiamine method for nitrate detection, and the blue phosphomolybdate method for phosphate detection, and LODs of 5, 2.5 and $2.5 \mu\text{g L}^{-1}$ were reported for N-NH_4 , N-NO_3 and P-PO_4 respectively. The system was based on loop flow analysis technology and was further developed via the WARMER FP6 project [WAR09] to provide a system more suited to deployment on typical monitoring platforms. The resulting multi-nutrient analyser system was trialled in collaboration with YSI Hydrodata at two locations in the United Kingdom (Hannigfield Reservoir and River Blackwater Estuary) [MT09].

A number of other systems for *in-situ* nutrient monitoring have been assessed by the Alliance for Coastal Technologies (ACT) [ACT14] at Chesapeake Biological Laboratory, USA [ACT07], [ACT08a],[ACT08b]. ACT conducts technology evaluations which focus on classes of commercially available instruments in order to provide confirmation that each technology meets the manufacturer's performance specifications or claims under real deployment conditions. The American Ecotech NUT 1000 was originally developed at Monash University and commercialised by Ecotech Pty. Ltd. The system can achieve LODs for reactive phosphate of $< 3 \mu\text{g L}^{-1}$ with a response time of 30 seconds. The high sampling rate is allowed by the use of rapid sequenced reagent injection in combination with a multi-reflection flow cell. Reagent injection also minimises the reagent consumption, allowing over 1000 measurements to be performed with only 20 mL of reagent. In the ACT trials, the NUT 1000 was used for surface mapping on board a research vessel in Monterey Bay, California [ACT08a]. Very good correlation with validation samples was achieved, after correction for a measurement offset of $50 \mu\text{g L}^{-1}$ P-PO_4 , which was attributed to differences in refractive index of natural seawater versus the reagent grade water used for preparation of internal standards and machine calibration. The ACT has also reported on successful deployments of the WET Labs Cycle-P nutrient analyser [ACT08b] and the YSI 9600 Nitrate Monitor [ACT07].

Vuillemin *et al.* [Vui09] described a miniaturised chemical analyser (CHEMINI) for dissolved iron and total sulphide, based on FIA and colorimetric analysis. Designed for deep-sea applications, the system is submersible to a depth of 6000m, with detection limits of $0.3 \mu\text{M}$ and $0.1 \mu\text{M}$ for iron and sulphide respectively. It was used to monitor the chemical environment within mussel beds, over a 6 month deployment period during which 8 measurements per day were performed.

Mowlem and co-workers at the National Oceanography Centre (NOC), Southampton, UK, have developed *in-situ* sensors for a range of chemical parameters [Pat08],[Sie10],[Bey11],[Flo11],[Bea12] including nitrite, nitrate, ammonia, phosphate and iron. The first of this series of microfluidic chemical analysis systems [Patey08] was used to detect nitrate and nitrite with a limit of detection

(LOD) of 0.025 μM for nitrate (0.0016 mg L^{-1} as NO_3^-) and 0.02 μM for nitrite (0.00092 mg L^{-1} as NO_2^-). The device was deployed in an estuarine environment (Southampton Water, UK) to monitor nitrate and nitrite concentrations in waters of varying salinity and was able to track changes in the nitrate-salinity relationship of estuarine waters due to increased river flow after a period of high rainfall. In subsequent work, nanomolar detection limits were achieved for iron [Sie10], ammonium [Bey11], phosphate and nitrate [Flo11]. The NOC is a major player in the development and deployment of microfluidic sensor systems for marine monitoring with a range of deployable platforms including research vessels, gliders and autonomous underwater vehicles (AUVs) for long term, long range, deep oceanographic applications. However, like the CHEMINI system, the level of engineering required to achieve the ruggedisation and high analytical performances required in this environment means that the cost of such systems is likely to be prohibitive in terms of deploying large numbers of devices for routine monitoring applications.

4.2.6.2 Autonomous microfluidic platform for nutrient analysis

Bowden *et al.* [Bow02a],[Bow02b] evaluated the yellow (vanadomolybdophosphoric acid) method as an analytical method for the determination of phosphate in water within a microfluidic device with the aim of producing an automated device with a field-deployable lifetime of one year. In this method ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 7\text{H}_2\text{O}$, is reacted with ammonium metavanadate, NH_4VO_3 , under acidic conditions. The combined reagent and sample containing orthophosphate react to form the vanadomolybdophosphoric acid complex, $(\text{NH}_4)_3\text{PO}_4\text{NH}_4\text{VO}_3\cdot 16\text{MoO}_3$. The resulting solution has a distinct yellow colour arising from the strong absorbance of this complex below 400 nm. It was shown that batches of the reagent could be used for over a year without significant loss in performance [Bow02a]. This method was therefore selected in preference to the molybdenum blue method, due to the greater stability of the reagents used in the analysis, and resulted in an assay with an LOD of 0.2 mg L^{-1} and a dynamic linear range of 0–30 mg L^{-1} .

Following these investigations, an autonomous microfluidic analyser for phosphate was developed, which has been successfully deployed in wastewater, freshwater and estuarine locations. The first-generation prototype for this system consisted of bottles for storing the reagent, calibration solutions and cleaner, a sample port for collecting the water sample to be analysed, and an array of solenoid pumps for pumping the required liquids through the microfluidic chip. The microfluidic chip allowed mixing of the reagent and sample, and also presented the reacted sample to a photodiode and LED for an absorbance measurement. The analysed sample was then pumped to the waste storage container. All of the fluid handling and analytical components were controlled by a microcontroller which also performed the data acquisition and stored the data in a flash memory unit. A GSM modem was used to communicate the data via the SMS protocol to a laptop computer. The first generation phosphate analyser was validated in the laboratory [Sla10] and during deployments in wastewater [Cle09] and estuarine water [Cle10].

A substantial redesign of the phosphate analyser was later carried out in collaboration with an industry partner in order to reduce the component cost of the system (to approx. €200 per unit) and improve manufacturability while maintaining or improving the analytical performance. The major design alterations and improvements implemented during this process are described in detail in [Cle13a]. These include:

- More compact, cylindrical design for better portability and deployability.
- Improved external mounting system.
- Folded sheet metal frame for mounting of internal components.
- Flexible bags for storage of colorimetric reagent, calibration solutions and waste.
- Low-cost dual channel peristaltic pumps.
- Redesign of microfluidic chip.
- Bubble detection and removal protocol.
- Implementation of ZigBee radio based communications.

- Redesign of sample intake/filter module to incorporate antifouling measures.



Figure 4.2.29. (a) Microfluidic chip for phosphate detection. (b) DCU's second-generation phosphate sensor in situ during a freshwater monitoring deployment.

The second-generation phosphate sensor (Fig. 4.2.29b) was deployed at Broadmeadow Water Estuary, Co. Dublin, Ireland [Cle13a]. The sensor performed 124 measurements during the trial, and a number of manual samples were collected for laboratory analysis. These samples were analysed in the laboratory using a Hach-Lange DR890 Portable Colorimeter. An excellent correlation ($R^2 = 0.9706$) was achieved between the sensor output and the laboratory analysis. The second generation phosphate sensor has also been used for wastewater monitoring at Osberstown Wastewater Treatment Plant, Co. Kildare, Ireland. An autosampler (Buhler BL 2000, Hach-Lange Ireland Ltd.) was used to collect validation samples at 7-hour intervals, these were stored within the refrigerated compartment of the autosampler, collected periodically, and analysed in the laboratory using the vanadomolybdophosphoric acid method (yellow method) for phosphate detection and a UV-vis spectrophotometer (UV-1600PC Spectrophotometer, VWR International). Over the course of a 22 day deployment good agreement between the sensor output and the validation dataset was again achieved [Cle13a].

Recent developments of DCU's deployable analyser platform have focussed on implementing colorimetric methods for additional parameters including pH, ammonia, nitrate and nitrite [Clea12],[Cle13b],[Cog13],[Cog14].

4.2.6.3 Disc-based microfluidic systems

DCU has also developed a fully integrated portable centrifugal microfluidic analysis system (CMAS) for on site colorimetric analysis [Czu12a], [Czu12b],[Czu13]. This handheld device with wireless communications capability is based on a reconfigurable low cost optical detection method employing a paired emitter detector diode device (PEDD) which allows a range of centrifugal microfluidic layouts to be implemented. In this kind of system, disc spinning provides the energy for fluid control and movement. Due to the wireless communication, acquisition parameters can be controlled remotely and results can be uploaded in remote locations and displayed in real time. CMAS is coupled via Bluetooth to an Android tablet for data collection, geotagging and Cloud sharing. The portability and wireless communication provide the flexibility needed for on site water monitoring. A centrifugal microfluidic disc was designed for nitrite detection in water samples, and a limit of detection (LOD) of $9.3 \mu\text{g/L}$ was obtained [Czu13].

Other implementations of centrifugal discs for environmental monitoring have been reported. Salin and coworkers developed discs for the detection of aqueous sulphide [KS12], and nitrate and nitrite [Yon10] employing centrifugal microanalysis. Hwang et al. reported [Hwa13] a microfluidic centrifugal disc that is capable of simultaneous determination of several nutrients in water samples (nitrite, nitrate, ammonium, orthophosphate, and silicate). However, in these cases a bench-top fiber-based optics/spectrophotometer system was employed for the optical detection. Therefore,

while these platforms did demonstrate the suitability of centrifugal discs for implementing environmental assays, the devices were not suitable for on-site water quality monitoring.



Figure 4.2.30. CMAS portable system for on-site monitoring of water quality. (a) Loading sample onto the disc. (b) A disc for performing nitrite analysis at a range of concentrations for calibration purposes.

Centrifugal disc based microfluidic systems such as CMAS have significant potential for use as handheld/portable devices for environmental monitoring and for diagnostic/point-of-care biomedical applications [Gorkin10]. However, in terms of deployable, fully autonomous devices for *in situ* environmental monitoring, their usefulness is limited by the relatively high power consumption required in order to achieve the revolution speeds (typically 300 – 3000 rpm) needed to drive fluid motion in the disc.

4.2.6.4 Microfluidic chip/disc systems References

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4.3 Antifouling

4.3.1 Generic aspect

Fouling is the accumulation of unwanted material on solid surfaces to the detriment of function. The fouling material can consist of either living organisms "Biofouling" or a non-living substance (inorganic or organic). Fouling is usually distinguished from other surface-growth phenomena in that it occurs on a surface of a component, system or plant performing a defined and useful function, and that the fouling process impedes or interferes with this function.

Biofouling or biological fouling is the undesirable accumulation of micro-organisms, algae and diatoms, plants, and animals on surfaces, for example ships' hulls, or piping and reservoirs with untreated water. Bacteria can form biofilms or slimes. Thus the organisms can aggregate on surfaces using colloidal hydrogels of water and extracellular polymeric substances (EPS). The biofilm structure is usually complex. Bacterial fouling can occur under either aerobic (with oxygen dissolved in water) or anaerobic (no oxygen) conditions. In practice, aerobic bacteria prefer open systems, when both oxygen and nutrients are constantly delivered, often in warm and sunlit environments.

Anaerobic fouling more often occurs in closed systems when sufficient nutrients are present. Examples may include sulfate-reducing bacteria or sulfur-reducing-bacteria, which produce sulfide and often cause corrosion of ferrous metals (and other alloys). Sulfide-oxidizing bacteria on the other hand, can produce sulfuric acid, and can be involved in corrosion of concrete.

Sensors immersed in natural water are affected by fouling and biofouling. Since the time when long-term monitoring was first carried out in water, strategies to protect the deployed instrument/sensor from fouling have been adopted and most of the time with indeterminable results.

4.3.2 Present solutions

Among others mechanical brushes, chemical substances, copper foil, copper paints and other complex devices have been used to mitigate the fouling/biofouling effects on deployed instrument and sensor packages.

Antifouling treatments

Tributyltin (TBT) is primarily used as an antifoulant paint additive in the marine environment to discourage the growth of marine organisms. TBT by itself is unstable and will break down in the environment unless it is combined with an element such as oxygen. One of the most common TBT compounds is bis(tributyltin) oxide, or TBTO. TBTs are toxic to fish and other aquatic life and are accumulated by these species. TBTs can bind strongly to particles in water bodies and to sediments and can then persist for a considerable time. There is concern that exposure to TBTs can interfere with animal hormones, that they are "endocrine disruptors".

European Directives regulating levels of TBTs include that concerned with pollution of the aquatic environment (76/464/EEC) [EC2014] and they are listed as priority substances for the Water Framework Directive. At an international level, the use of TBTs in marine situations is gradually being phased out as the potentially harmful effects they may have on aquatic life are recognised. In the UK, for example, the use of TBTs is restricted to large boats (of more than 25 metres in length). TBTs are listed as substances for priority action under the Helsinki and OSPAR Conventions which protect the marine environments of the Baltic Sea and north-east Atlantic Ocean respectively.

To emphasize the attention given to the danger of this poisoning treatment, we below show the circular that one of the manufacturers of marine instrumentation disseminated some years ago to their users:

.....





ATTENTION

Return Policy for Instruments with Anti-fouling Treatment

WET Labs cannot accept instruments for servicing or repair that are treated with anti-fouling compound(s). This includes but is not limited to tri-butyl tin (TBT), marine anti-fouling paint, ablative coatings, etc.

Please ensure any anti-fouling treatment has been removed prior to returning instruments to Wet Labs for service or repair.

Mechanical antifouling devices

The Zebra-Tech Hydro-wiper [Zeb14]



Figure 4.3.1.
Hydro-wiper

“The Hydro-Wiper is an innovative mechanical wiper system, with versions available for a wide variety of underwater instruments. Using a regular gentle brushing action, the Hydro-Wiper keeps the sensing window of the instrument clean from unwanted deposits such as mud and bio-fouling.

The Hydro-Wiper reduces the need for costly site visits to manually clean the instrument, maintaining data quality throughout long deployments, even in the harshest environment.

All Hydro-Wipers can be supplied in two formats, either self contained or data logger controlled.”

Turner Design mechanical wiper [TUR14]

“The Mechanical Wiper, installed in the C3 Submersible Fluorometer, is designed to reduce biofouling during extended deployments. It helps maintain consistent readings by reducing bio-fouling. Replaceable brushes available.”



Figure 4.3.2. Turner Designs Mechanical Wiper

WetLabs – Water Quality Monitor (WQM) [Wet14]

The WQM employs active flow control, passive flow prevention, light-blocking, active biocide injection and passive inhibitors to effectively and safely combat internal and external fouling. With fouling minimized, the superior inherent stability of the WQM sensors translates directly to superior long-term data quality.

Figure 4.3.3.
Wetlabs WQM



Anti-Fouling Features

- Pump-controlled flow
- Extensive copper cladding
- BLEACH Injection System (BLIS) protects DO sensor and CTD flow path
- Anti-Foulant (AF) collar for conductivity cell (EPA approved)
- Light-blocked sample chambers
- Bio-wiper protects optical components

Electro-chemical antifouling devices

The Idronaut innovative antifouling system

“The antifouling kit, installed by the measuring sensors of the Ocean Seven 316 probe, is composed of a central titanium electrode which supplies an electrical current towards six external titanium electrodes on which a copper foil (replaceable) surrounding the sensors, can be wrapped. In addition to free chlorine formation, there is copper dissolution in an ionic form by the sensors. The copper foil has a typical endurance of more than one year. The sensors are washed when the probe starts profiling.

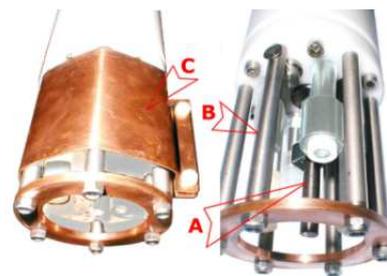


Figure 4.3.4. Idronaut anti-fouling system:
 A) Titanium inner electrode.
 B) no. 6 titanium outside electrodes.
 C) Copper foil (replaceable).

The fouling probably represents the major difficulty during continuous monitoring of waters and, although many efforts in research at worldwide level have been dedicated to this problem during the past decades, a definitive solution is not yet available.

Therefore, a compromise must be made each time according to the specific difficulty encountered. Because our 601 BUOY PROFILER monitoring system is frequently used in inshore waters where fisheries or shellfish beds may be present, we have avoided the use of TBT antifoulants by using a polarised copper foil antifouling system. To the best of our knowledge, this kind of antifouling system does not appear in the scientific literature and no other manufacturer has ever used it until now. So, we intend to apply for a patent on it.

A foil of copper (1 mm thickness) has been placed around the sensor cage in such a way that it can be easily replaced with a new one without damaging the measuring sensors and to also avoid reducing the flow of water to be measured. The presence of this simple device has, on average, increased the time between each cleaning from 1 to 4 weeks when deployed in warm and very productive waters. Passive antifouling systems based on copper lose their activity after a few days because the surface of the copper becomes oxidised and no longer produces sufficient copper ions to remain effective. To avoid passivation of the copper, a titanium central electrode has been installed and a small electrical current is passed between the central titanium electrode and the copper foil. In this way the copper foil, which acts as a flow-measuring chamber, is always clean and so this allows the copper dissolution in an ionic form. This effect has drastically decreased the formation of fouling around the sensors. The current between the titanium electrode and the copper foil is only applied when the probe idles and not carrying out measurements. Vice versa, when the probe starts descending to acquire measurements, the current is turned off and the water to be measured flows between the sensors and flushes out the ionic copper previously dissolved. This improvement considerably increases the “time between cleanings” up to three/four weeks, depending on the water quality.

Optical antifouling devices – UV disinfection

AML UVXchange [AML14]

AML recently launched UV•Xchange - an ultraviolet biofouling control module. Biofouling solution unlike any method currently on the market, UV•Xchange™ prevents biofouling during long-term, in-situ deployments. The module bathes critical surfaces in ultraviolet (UV) light, killing early growth to prevent biofouling, and thus eliminating environmental drift in CTDs and multi-parameter instruments. Comparative studies show UV•Xchange to be as effective as chemical protection methodologies like TBT at eliminating drift due to biofouling.

UV•Xchange offers significant advantages over existing biofouling control technologies. It does not use toxic chemicals, simplifying deployment and maintenance while at the same time eliminating environmental damage. The module has no moving parts and hence greater reliability compared to wipers and plungers. Finally, Each UV-

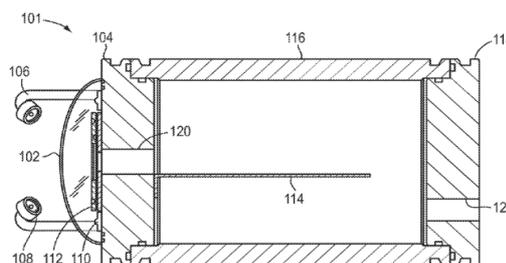


emitting LED can be adjusted independently and positioned as desired, ensuring effective coverage of all critical surfaces, regardless of geometry.

Like all other members of the Xchange suite, UV•Xchange is field-swappable and easily configured to fit the needs of any operation. Installed directly on the end cap of an X•Series instrument, the module can be set to various positions, enabling optimal coverage of any sensors requiring protection. When used in conjunction with AML's C•Xchange conductivity sensor-head, the resulting CTD system has overall drift performance due to biofouling similar to a Sea Bird. UV•Xchange can also protect third party devices: cameras, transducers, sensors, etc. Simply install the UV•Xchange module onto a Micro•X instrument, point, and apply power to protect

Woods Hole Oceanographic Institution, Woods Hole, MA (US) – United States patent No. US 2014/0078584 A1

The present invention relates to systems and methods for reducing fouling of a surface of an optically transparent element with a light source. According to one aspect, the invention is a system including an LED for emitting UV-C radiation, a mount for directing emitted UV-C radiation toward the optically transparent element and control circuitry for driving the LED. The system may be used to remove a desired amount of biofilm.



4.3.2.1 Antifouling – general References

[AML14] AML Oceanographic UV•Xchange: http://www.amloceanographic.com/Products-Services-Hydrographic-Oceanographic/Biofouling-Control_3/UVXchange Accessed 13 Aug 2014

[EC14] Dangerous substances documentation: http://ec.europa.eu/environment/water/water-dangersub/lib_dang_substances.htm Accessed 13 Aug 2014

[TUR14] Turner Designs Mechanical Wiper: http://www.turnerdesigns.com/inline-monitoring?page=shop.product_details&flypage=flypage_default.tpl&category_id=96&product_id=255 Accessed 01 Aug 2014

[WET14] WetLabs WQM: <http://www.wetlabs.com/wqm> Accessed 01 Aug 2014

[ZEB14] Hydro-wipers: http://www.zebra-tech.co.nz/product_type/hydro-wipers/ Accessed 01 Aug 2014

4.3.3 Antifouling strategy for nutrient sensors

Implementation of antifouling protection for the COMMON SENSE nutrient sensing platform will focus on three main areas:

1. Protection of the sensor housing.
2. Protection of the sample intake/filter assembly.
3. Protection of the internal fluidic system and microfluidic chip.

4.3.3.1 Protection of sensor housing

Protection of the sensor housing can be seen as the least critical of the three areas, as fouling of the housing will not have direct or immediate impact on sensor performance. Nevertheless, given the high degree of fouling which can occur on surfaces exposed to the marine environment, this aspect should not be neglected for a number of reasons [Del10]:

- **Ease of use** – it is obviously preferable from a user point of view to have a (relatively) clean or easy to clean instrument at the end of a deployment period, particularly if a post-deployment calibration is to be carried out in the laboratory.
- **Effect on post-deployment calibration** – when a sensor is heavily fouled after deployment, users are likely to clean it using potentially aggressive techniques such as high pressure water jet, brushes or chemical methods. These have potential to affect the instrument and perhaps render the post-deployment calibration invalid.

- **Macro-fouling effects** – extensive fouling by organisms such as barnacles, mussels and macro-algae can affect the chemical composition of the local environment.

Fouling potential of the housing material should be considered when selecting/designing the sensor housing. A variety of commercially available antifouling paints/coatings should also be considered as protective measures.

- **Antifouling paints with active biocides** such as copper compounds, copper oxides and co-biocide chemicals can be used to protect the sensor container. Other biocides are incorporated in antifouling paints in addition to, or in replacement of, copper compounds; these biocides are designated as pesticides, algicides or bactericides and are not used frequently to protect sensors for ocean monitoring.
- **Self-polishing paints** can be effective to protect the sensor container provided that the deployment site has an active water flow. As for conventional biocide-based antifouling paint, self-polishing paints often contain biocides and consequently can disrupt the environment to be monitored by the sensor.
- **Paints based on non-stick coatings** incorporate silicone materials or fluorinated polymers, and can be biocide free. They are known to be effective to protect ship hulls since the movement of the ship creates sufficient shear forces to remove the fouling. In the case of sensor housing protection non-stick coatings can help to inhibit fouling growth if the currents at the site are sufficient as this will help with cleaning.

4.3.3.2 Protection of the sample intake/filter assembly.

DCU's approach to nutrient monitoring is based on mixing a filtered sample with a colorimetric reagent inside a microfluidic chip and using light emitting diode (LED) and photodiode (PD) based optical detectors to measure the colour formed. The measurement is therefore carried out within a protected environment, with only the filter (and associated fittings) directly exposed to the sample; this compares favourably to optical or electrochemical probe systems in which the sensor surface is directly exposed to the bulk sample and is consequently vulnerable to biofouling. The current version of DCU's phosphate sensor uses commercially available copper-containing fitting to hold a membrane filter in place. In addition to being cost-effective and facilitating easy exchange of the membrane filter, the copper content is of benefit in terms of protecting the membrane filter from biofouling. Copper, copper alloys and copper compounds are widely used as biofouling inhibitors for sensors and other devices deployed in aquatic environments [Del10], [SR11]. The filter/intake is located on the bottom of the cylindrical sensor unit. This is a practical placement for deployment purposes, and has the benefit of partly shielding the membrane filter from ambient light. Light favours growth of micro-organisms which can promote the formation of biofilms on surfaces. The membrane filter used is a polyethersulfone (PES) membrane [PAL14]. PES is a hydrophilic polymer with extremely low protein binding, and these membranes are designed for biological sample preparation. As attachment of macromolecules (organic and inorganic) and microorganisms represent the initial stages in the biofouling process [Del10], [SR11], this combination of measures is expected to delay and/or limit biofouling of the filter surface.

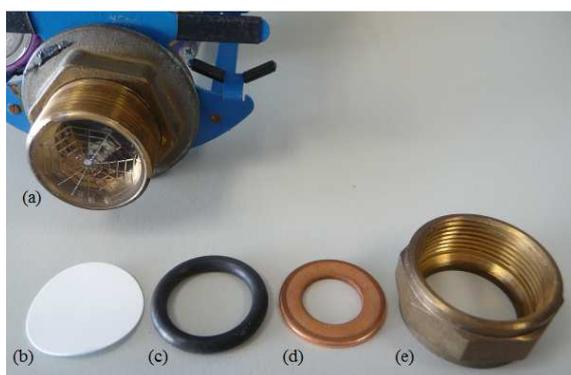


Figure 4.3.5 Components of the sample intake/filter assembly used on DCU's phosphate sensor. (a) PMMA filter backing with "spider web" pattern, (b) polyethersulfone membrane filter, (c) O-ring seal, (d) copper washer, (e) brass screw-on fitting.

The sample intake/filter assembly is shown in Fig. 4.3.5. A “spider-web” pattern is formed on the surface of a PMMA disc by micro-milling. The disc is then fitted into a brass connector where it acts as a backing for the polyethersulfone membrane filter. The spider-web pattern allows a higher proportion of the membrane surface to be used for sample intake, while minimising the dead volume created. The membrane is held in place using an O-ring seal, a copper washer and a brass threaded nut. These copper/brass fittings allow the filter to be easily replaced while also offering a degree of protection from biofouling.

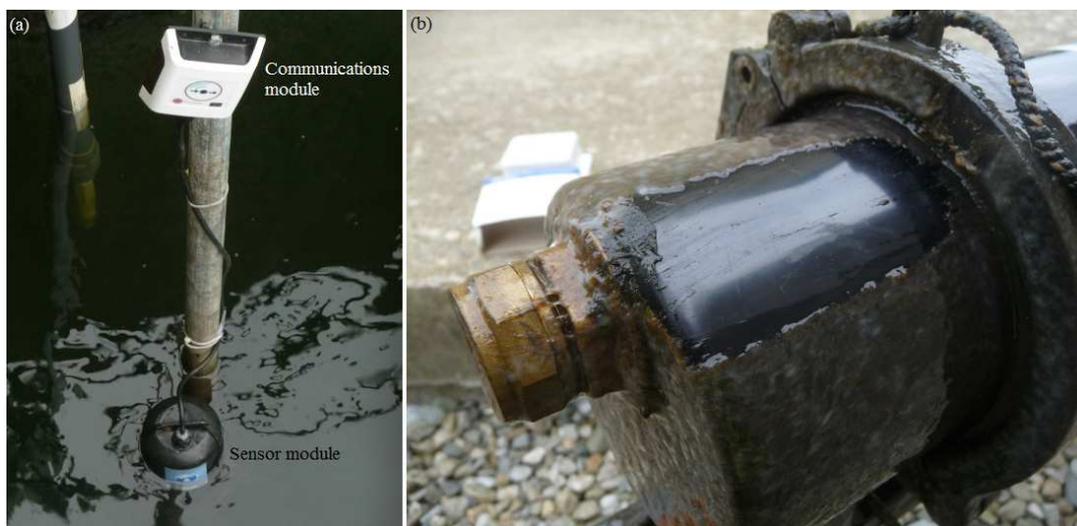


Figure 4.3.6. Second generation phosphate sensor in-situ during wastewater monitoring trial. (b) Biofouling on the sensor housing after approx. 2 weeks immersion in wastewater.

Fig. 4.3.6 and Fig. 4.3.7 show the extent of biofouling which occurred during a phosphate sensor trial in wastewater monitoring application. Fouling of the sensor body was a significant feature of this trial. Fig. 4.3.6(b) shows the partially cleaned sensor module after removal from the effluent tank after approx. 14 days immersion. A fouling layer approximately 1 mm in thickness was observed. The copper-containing filter housing was noticeably less affected than the sensor body. Fig. 4.3.7(a) shows that the membrane filter was also fouled with a thin, adherent layer of material, although there was no discernible effect on the sensor performance. The filter was replaced and the system was replaced in the effluent tank. At the end of the trial (after a further 10 days) the membrane and



Figure 4.3.7. Fouling of membrane filters and filter assembly after approx. 14 days (a) and 10 days (b) immersion in wastewater during a sensor trial at Osberstown Wastewater Treatment Plant.

housing were again examined. At this point the filter holder was heavily fouled as shown in Fig. 4.3.7.(b). A layer of fouling material was again in evidence on the membrane filter itself. However, in this case the material was found to be only loosely adherent to the filter surface, and was readily removed with gentle washing with deionized water.

The qualitative difference between the types of fouling material on the two filters shown in Fig. 4.3.7. highlights the variability of fouling which can occur over a relatively short period of time, presumably due to changes in sample composition and prevailing weather conditions. While no specific effect of fouling on sensor performance was observed in this trial, the rapid build-up of material suggests this could be an issue in longer-term deployments in wastewater and in other sample matrices including seawater. For this reason testing of this design in seawater will be carried out during the COMMON SENSE project, to determine the effects of filter biofouling on (i) sample delivery through the filter membrane and (ii) measured levels of nutrients (as localised uptake of nutrients by biofilms on the filter surface could potentially impact on the measured value).

Additional measures to be considered for protection of the sample intake/filter assembly include the addition of a copper screen outside the filter, and the use of commercially available antifouling coatings on the assembly.

A variety of active strategies have also been implemented for protection of sensors, these include approaches based on vibration, wipers, water jets, ultrasound, UV irradiation, electric pulses, and mechanical systems for immersing the sensor at time of measurement and withdrawing it from the water between measurements. These approaches all entail additional power consumption and are therefore not ideal for use with autonomous *in situ* monitoring systems.

4.3.3.3 Protection of the internal fluidic system and microfluidic chip.

Fouling of the nutrient sensors' internal fluidic components (tubing, pumps, valves) and microfluidic chip by particulate matter or biofilm growth has the potential to effect the system by blocking/interfering with fluid flows or by interfering with the optical measurement (e.g. light scattering by particles between the light source (LED) and detector (PD) could result in spuriously high values for light absorbance). In laboratory testing of the phosphate sensor using real samples, and during field deployments in freshwater, wastewater and estuarine waters [Cle09], [Cle10], [Cle13], this has been observed only in the case of filter failure resulting in ingress of suspended particles.

In the case of the phosphate sensor, an additional protection is automatically conferred by the use of a highly acidic reagent (the colorimetric "yellow reagent" contains 10% HCl by volume, in addition to ammonium molybdate and ammonium metavanadate). This aggressive reagent protects against biofouling of the microfluidic chip and any other internal fluidic components which are exposed to the reagent (pre- or post-reaction with sample). Reagents used for detection of other nutrients may confer similar protection, however consideration should be given to increasing the acidity/basicity of these reagents where feasible.

The biofouling potential of seawater samples should also be considered in the selection of all internal fluidic components. In this context, the tubing delivering sample from the intake/filter point to microfluidic chip (as well as any pumps or valves between these points) may be considered as most vulnerable to fouling, since this section of the fluidic system is exposed to sample without being exposed to the protective properties of the reagent. Testing with seawater samples should be used to test for occurrence of biofouling within tubing and other components, and if necessary anti-fouling measures should be implemented. Viable antifouling methods for this section of the system may include:

- Use of fluidic components with lower susceptibility to biofouling.
- Implementation of a cleaning cycle using a solution with biocidal properties.
- Internal coating of the fluidic components with antifouling layers, e.g. those based on nanomaterials with biocidal/antimicrobial properties [Cha10].
- Localised UV irradiation.

4.3.3.4 Nutrient Sensors Anti-fouling References

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4.3.4 Micro-Plastics

The analytical methods described in Section 4.1.7 (Microplastics) are based on optical measurement technologies, meaning that components such as lenses, mirrors and light sources are critical to ensure reliable results. An important restriction to use optical devices in marine environment is fouling. Equipment's operating in marine environments must be protected to prevent the deposition of materials/substances and organisms (biofouling).

There are different types of anti-fouling treatments and coatings. Currently, the most common method to prevent the occurrence of fouling is the combination of paints and biocides. Among existing options, the most used are TBT-based paints (using tributyltin and derivatives). However, paints based solutions are not applicable to optical measurement systems, so it is necessary to find alternatives for anti-fouling protection[MKD03].

Some alternative anti-fouling methods to avoid the use of paint coatings are based on the application of nanoscale surface treatments that hinder the adhesion of organisms. In particular, the combination of surface plasma treatments with TiO₂ nanoparticles has shown good anti-fouling and photocatalytic properties with high hydrophilicity [YSL12].

Another possible solution is the application of high-transparent epoxy coatings. This solution maintains most of the advantages of paint coatings (easy to apply, proven) with minimal alteration of the optical properties of reading systems. As reported by Kiu Ho Chae *et al.* [CJK06], epoxy polymers containing phosphorylcholine (MPC) groups, show high anti-fouling effect while maintaining good adhesion properties and high transparency at wavelengths of more than 300 nm.

During this project, different possible solutions will be tested to avoid fouling presence in microplastic sensor. Those sensor elements in contact with seawater (fluidic channels, light emitters, light receptors) will be protected against fouling using one of the techniques described above or a combination of different options. It is important to notice that the use of light conducting materials (such as optic fiber) could help to prevent the contact between seawater and the most sensible elements.

4.3.4.1 Micro-Plastics Anti-fouling References

- [CJK06] D. H. Chae, Y. M. Jang, Y. H. Kim, O. Sohn, J. I. Ree; "Anti-fouling epoxy coatings for optical biosensor application based on phosphorylchlorine", Sensors and Actuators (2006): 153-160
- [MKD03] D. Messeguer, S. Kiil, K. Dam-Johansen; "Anti-fouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings", Progress in Organic coatings (2003): 75-104.
- [YSL12] You SJ, Semblante GU, Lu SC, Damodar RA, Wei TC; "Evaluation of the antifouling and photocatalytic properties of poly(vinylidene fluoride) plasma-grafted poly(acrylic acid) membrane with self-assembled TiO₂", Journal of Hazardous Materials (2012): 10-19

4.4 Remote Sensing

4.4.1 Introduction

Remote sensing means obtaining information about objects or areas from a distance, e.g. from aircraft or satellites, typically using the attenuation or reflection of beams of electromagnetic energy. Starting from the 1970s, the use of satellite data for the study of water reservoirs, including seas and oceans, has become increasingly popular, and today satellites are key instruments for the global monitoring of climate. Among others, parameters like sea surface temperature, sea level, sea state, sea ice, surface wind are routinely measured in this way [EUMET14].

In general, remote sensing of water reservoirs involves the selection of a specific band of the electromagnetic spectrum and then comparing the spectral response of reference water with the spectral response of the substance to be measured.

With the development of new, more complex observation systems and algorithms for data analysis, the use of remote sensing for monitoring the state of water through the measurement of a wide range of parameters has become more popular [WJO13]. Modern satellite imaging sensors offer new possibilities, making them suitable for water quality assessment. The use of multispectral and hyperspectral sensors [MHS10] increases the accuracy of the data collected and allow us to monitor a wide number of ecological and hydrological parameters. As a matter of fact, the large number of contiguous spectral bands recorded by hyperspectral sensors increase the probability of finding a unique combination of spectral data that allows the discrimination of the desired observable among the other elements in the image. The current limitation of remote sensing methods includes the need of external data (e.g. atmospheric model) for a correct processing, cloud coverage and limited spatial resolution. Moreover, remote sensing is limited to the surface of the water column, with a penetration depth ranging from nearly zero (reflected UV radiation) to few tens of meters (visible light in the blue-green range); nevertheless the advantages of remote collection of large scale data, to complete and complement in situ measurements that are very demanding in terms of money, time and manpower, are enormous [Chip04].

Well known examples of satellite sensors used for ocean observation are the Coastal Zone Colour Scanner (CZCS), MEdium Resolution Imaging Spectrometer Instrument (MERIS) and Sea viewing Wide Field-of-view Sensor (SeaWiFS), all of which are devoted to studying the colour of the sea. In 2001 the European Space Agency (ESA) launched the hyperspectral sensor Compact High-Resolution Imaging Spectrometer (CHRIS), as part of the Project for the On-Board Autonomy platform system. Moreover, the launch of three new hyperspectral satellite missions, Environmental Mapping and Analysis Program - EnMAP, Hyper-spectral Imager Suite - HISUI and PRecursores IperSpettrale of the application mission - PRISMA, of the German, Japanese and Italian Space Agencies, respectively, are programmed for next year [Cav14].

Water monitoring based on aerial measurements is less diffused than satellite-based data. Nevertheless, hyperspectral spectrometers for aircraft installation have been developed, with a spectral resolution comparable with satellite instruments and a much higher spatial resolution (down to few cm). One of the first imaging spectrometers was the AVIRIS instrument, developed by NASA, working in the 0.4 - 2.5 μ m range with 224 channels, that was developed for geological, hydrological and environmental monitoring purposes [Lee01].

A new generation aerial hyperspectral systems is the AAHIS (Advanced Airborne Hyperspectral Imaging System), which was designed specifically for marine applications, working in the 0.39- 0.84 μ m range with a maximum resolution of 6.5 cm. The AAHIS scanner was originally produced for military purposes [TPS02].

4.4.2 Chlorophyll sensing

Satellite ocean color sensors data, based on the passive measurement of visible light emerging from the ocean surface, are widely used for the detection, mapping and monitoring of phytoplankton

growth. Since the 1970s, a wide number of color sensors and data analysis algorithms have been developed, the advance in sensor technology being driven by the demand for more effective and reliable environmental monitoring of the open and coastal waters. Present challenges include overcoming the severe limitation of these algorithms in coastal waters and refining detection limits in various oceanic and coastal environments [BGD14].

Quantitative information about optically active substances present in water can be in principle obtained from its spectral reflectance spectrum. Spectral bands located in the blue, green, yellow,

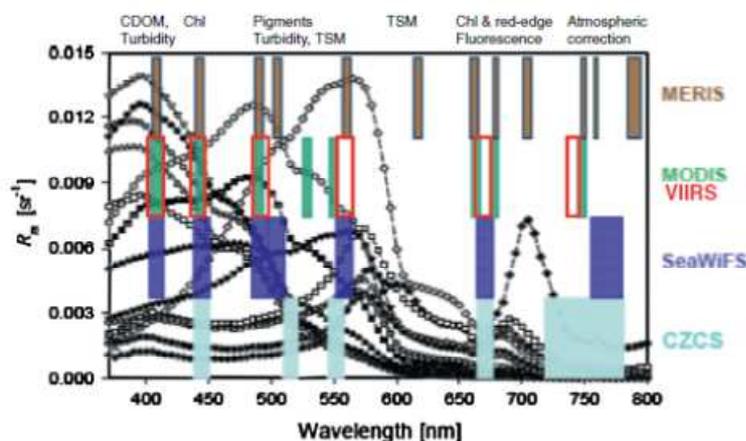


Figure 4.4.1. Spectral band positions for five ocean color sensors of the first (CZCS), second (SeaWiFS), third (MERIS, MODIS) and fourth (VIIRS) generations, compared with some experimental spectra (from reference BGD14). The potential applications for each spectral region are indicated.

red or near-infrared (NIR) in the reflectance spectrum can be combined in many ways to detect algal blooms, measuring essentially the concentration of chlorophyll-a. The reliability of the measured reflectance is disturbed by factors like the variable thickness of the floating algal layer, the presence of suspended matter, bottom reflectance and atmospheric conditions, that requires correction procedures. This reliability is even more questionable when dealing with coastal waters, where other optically active substances affect the water-leaving radiance, so care must be taken in the quantitative interpretation of remotely sensed

data [Kut09].

Most algorithms for Chl-a calculation use empirical blue–green (440–550 nm) spectral bandratios. However, the best results with these methods are obtained in clear, oligotrophic waters, while in coastal waters due to the usually high concentration of concentrations of color dissolved organic matter (CDOM) or gelbstoff, that includes essentially humic and tannic substances, and total suspended matter (TSM), the use of blue–green reflectance and in general of visible wavelengths can be unreliable and must be combined with the analysis of, as an example, red-NIR spectral bands. The use of standard visible light band-ratios has been demonstrated to significantly overestimate Chl-a, with errors as big as 50% in open ocean and 100% in coastal waters [Kom09].

Probably the most promising algorithm tested to overcome this problem is the Fluorescence Line Height (FLH) that exploits the solar induced fluorescence of Chl-a, centred approximately at 685 nm. The FLH is more sensitive than blue-green band-ratio but is still influenced by the presence of CDOM, turbidity and the algorithms that better describe the amount of dissolved Chl-a are concentration-dependent [Xin07].

Summarizing, quantitative measurements of chlorophyll from remote sensing still requires a refinement of calculation methods and data processing, especially in complex coastal water where the high concentration of Chl-a is accompanied by the presence of many other compounds and characterized by high spatial variability. The new generation of ocean color sensors addressed the need for more spectral bands (see Figure 4.4.1), thereby enabling the development of sophisticated schemes for both atmospheric correction and water dissolved substances discrimination.

Moreover, remote sensors covers typically very wide areas, giving an ensemble picture of the region of interest (a gulf, a portion of shoreline, an estuary) in just “one shot”. This is typically exploited to track the position of drifting buoys as they move with ocean currents (GPS).

4.4.2.1 Example of a combined remote sensing – in situ eutrophication study

Satellite images have been used for applications on eutrophication events. The ISAC CNR of Rome, in collaboration with the Area of Research, Development and Remote Sensing of Hydrometeorological Service of ARPA EMR has elaborated MODIS images (Moderate Resolution Imaging Spectroradiometer) with the aim of detecting the presence of mucilaginous aggregates in the Adriatic Sea, with particular attention on the northern and central areas off the monitored coast. The information obtained from the images was to supplement the data collected at sea and, together with the images produced by the numerical forecasting models, allowing information and possibly any predictions on the dynamics of the mucilaginous phenomenon. From the archives of NASA MODIS data were collected and subsequently processed at high resolution (250m and 500m). In parallel the spectral properties of the mucilage with issues related to the quality of the satellite signal and its validation by comparing the data collected in situ by Regional Agencies for Environment have been studied. The correlations between the in situ observations concerning the presence of mucilaginous aggregates and archive images led to the creation of a database to validate the spectral methods for the detection of clusters.

In addition, the CNR ISAC has continued to develop daily data of sea surface temperature (SST) from the AVHRR sensor of NOAA satellites that have been provided to the centers of numerical calculation of the INGV - University of Bologna for assimilation into models to predict the movement.

The MODIS sensor is one of the main instruments on board the Terra and Aqua satellites. The orbit around the planet is planned so that it passes from north to south across the equator in the morning, while Aqua passes from south to north across the equator in the afternoon.

The images acquired by the MODIS Aqua and Terra satellites can be downloaded for free from the NASA website at <http://daac.gsfc.nasa.gov>.

4.4.3 Sea surface salinity

Satellite observation of the ocean in the microwaves frequency field have provided accurate measurements of the sea surface height (altimeters) and surface winds (scatterometers) for about 30 years. Satellite altimetry was a real revolution for large-scale ocean observation, allowing precise sea-level values far from coast to be used in ocean circulation numerical models.

Sea surface salinity (SSS) is a fundamental physical parameter that can in principle be measured remotely. The electromagnetic radiation spontaneously emitted from a body is proportional to its physical temperature and can be measured by a radiometer, retrieving a brightness temperature T_B . The proportionality coefficient (emissivity) between T_B and T for sea water depends on the dielectric constant that depends on conductivity, that is, on salinity [Fon12]. The sensitivity of T_B on salinity shows a maximum at low microwave frequencies [Ber02].

Until recently, remote measurement of salinity has

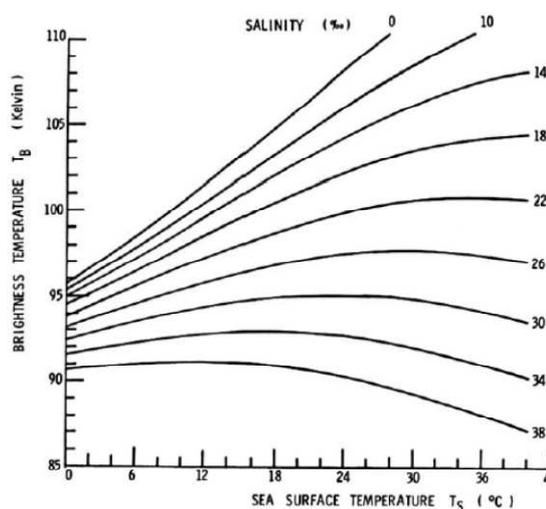


Figure 4.4.2. Variation of brightness temperature with sea surface temperature for different water salinities at L-band and normal incidence. Adapted from [Kle11]

been carried out only with airborne microwave radiometers, with all the limits of this approach (limited aircraft operating range, complex planning, cost). Airborne Scanning Low-Frequency Microwave Radiometers (SLFMRs) have been used on rivers, estuaries, and coastal waters to map their salinity, exploiting the relation among brightness temperature and salinity described above. The Salinity, Temperature, and Roughness Remote Scanner (STARRS), operated by the USA Naval Research Laboratory, is a more modern instrument used to map temperature and salinity. Apart from an L-band radiometer for the brightness temperature and an IR radiometer for physical temperature, STARRS features another microwave radiometer that measures sea surface roughness. This parameter affects the apparent brightness of the sea surface and must be measured to increase the accuracy of salinity calculation. [PWD06].

Measurement of sea surface salinity (SSS) from space, though postulated many years ago, has become accessible only in recent times and given the importance of salinity as a global climate variable, the availability of satellite data is expected to be a major breakthrough for oceanography and climate studies [BNY12]. Some of the results expected from global SSS measurements from satellites include the improved characterization of its spatial and temporal variability on the surface of the oceans, the observations of seasonal salinity changes (ice melting, monsoon) and a better understanding and prediction of events such as El Nino oscillation [SSS11].

Because surface salinity must be measured using long wavelengths (20 – 30 cm), reliable measurements from the space would require a very large antenna, unsuitable for satellite operations. Recently, the problem of antenna size have been overcome with a new interferometric technology, as shown on the European Soil Moisture and Ocean Salinity (SMOS) satellite. Its radiometer, the Microwave Imaging Radiometer using Aperture Synthesis (MIRAS), operates over a range of incidence angles and features 69 mini-antennas mounted on 4 meters long Y-shaped arms: the signals collected by each radiometer are cross-correlated (somewhat as in synthetic aperture imaging) to construct an interferometric image of brightness temperatures over a 1000-km-wide swath (official data from ESA). [Kle11]

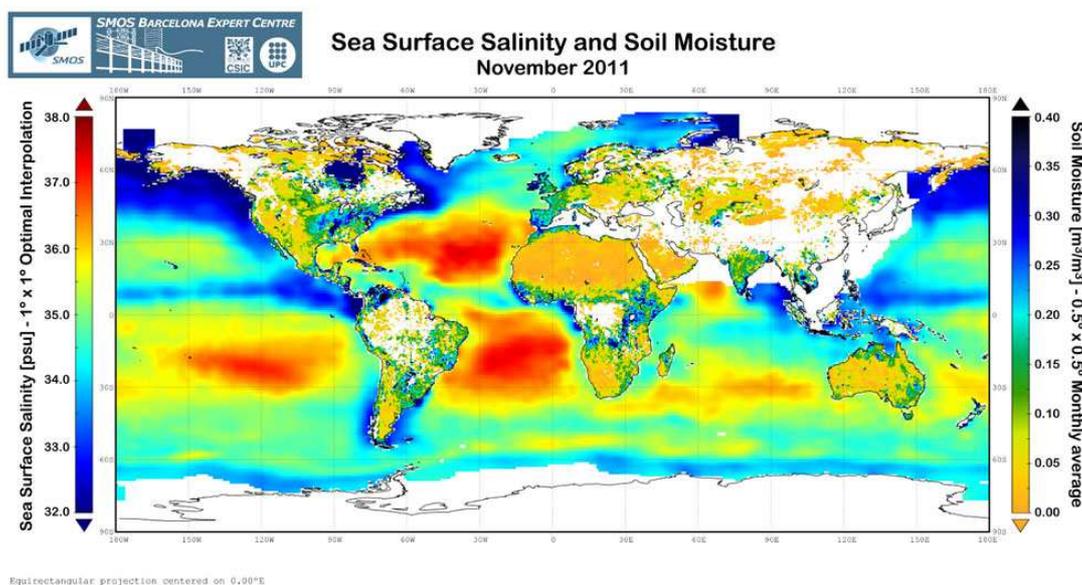


Figure 4.4.3. Combined global SSS and soil moisture map produced with SMOS data (<http://www.smos-bec.icm.csic.es>).

4.4.4 Sea Pollutants

4.4.4.1 Heavy metals

Heavy metals inserted into the water stream by industrial activities can be extremely dangerous for health as they may enter the food chain, accumulate into the body of marine animals and from here end into human alimentation. Both fishery and environmental management would then take advantage from the development of new methods for the estimation of heavy metal concentration in water.

Recently the feasibility of concentration measurement of three heavy metals (copper, lead and zinc) by remote sensing through sea color observation has been demonstrated [Che10].

To simulate satellite acquired data, the water-leaving radiance was measured with a spectrometer in the 340 – 1040 nm range, at the same time water samples were collected for lab analysis of water component, including chlorophyll, CDOM, sediments and metals. The relationships between the concentration of the three heavy metals (Cu, Pb and Zn) and the remote sensing reflectance were analyzed, showing a relatively good correlation with specific bands and an error estimated between 26 and 40%. The algorithms developed from field testing were then applied also to satellite (Landsat) acquired data, with acceptable results [Che12].

4.4.4.2 Marine debris

The NOAA Marine Debris Program (<http://marinedebris.noaa.gov/>) defines marine debris as: “any persistent solid material that is manufactured or processed and directly or indirectly, intentionally or unintentionally, disposed of or abandoned into the marine environment”. Of particular interest due to their abundance and potential danger are plastic debris and abandoned fishing gears/nets.

The common strategy used to predict the position of litter clusters over ocean surface is based on the accurate mapping of surface currents, main gyres and local eddies, that are obtained from the combination of remote observations (both from satellite and aircrafts), and from the tracking of drifting buoys motion. From these data, the motion of floating particles can be simulated and the position where the particles will most likely accumulate can be identified [Mac12].

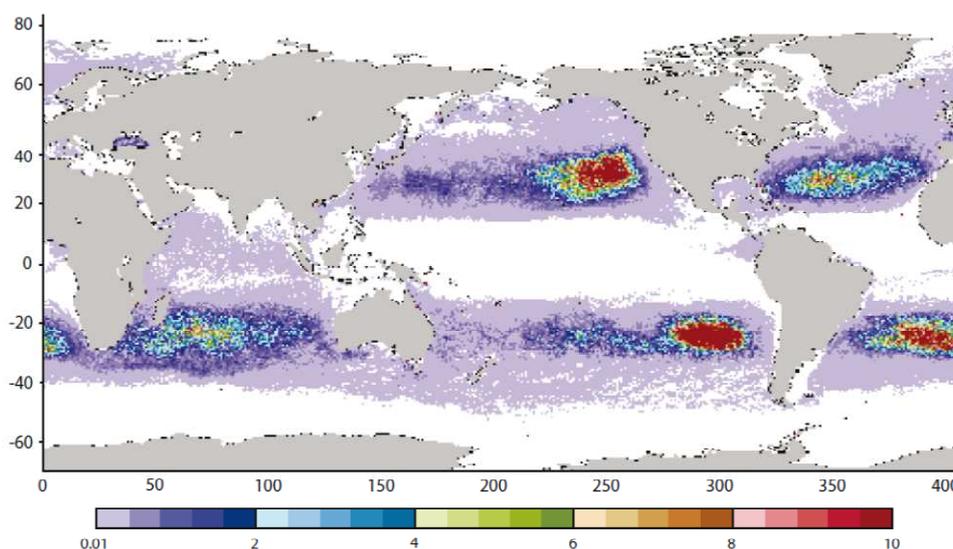


Figure 4.4.4. An example of simulated distribution of marine litter in the ocean, calculated starting from an uniform distribution. Floating plastic finally concentrates in the five subtropical gyres [IPRC08].

Satellite remote sensing has not been used for the direct detection of marine debris, due to the small dimension of the objects to be observed and to the fact that many of them can be suspended under water surface [MCS11]. Airborne observations are on the contrary widely used for this purpose:

apart from visible light observations, a number of techniques can be used to increase the sensitivity, like laser imaging devices (LIDAR) and infrared imagers [PVC12].

4.4.5 Sources

http://www.uvm.edu/rsenr/nr146/general_information/rsterms.pdf

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<http://casde.unl.edu/glossary/>

<https://earth.esa.int>

4.4.6 Remote Sensing References

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4.5 Reliability challenges for inventory infrastructure

Moorings and platforms are structures that allow water scientists to position instruments, collect samples, and take long-term measurements in water bodies. A mooring typically consists of a flexible cable that is tethered to the ocean floor by a weight or anchor and suspended from the sea surface by a buoy. On the contrary a platform has a flat workspace for instruments and people, and can be free-floating, moored to the seafloor, or rigidly fixed in place. Moorings and platforms often accommodate numerous pieces of measuring equipment, allowing scientists to collect several types of data at a site. A typical oceanographic mooring holds a large array of instrumentation including current meters/profilers, temperature gauges, turbidity scanners, salinity/conductivity/pressure meters. Also the wind speed and direction, air temperature, relative humidity at the sea surface are recorded. Together with shipboard and satellite data, the global network of measuring sites provides further understanding of the ocean-atmosphere system and its influence on global climate and biological productivity.

Moorings and platforms are left at their sites to record data for extended periods of time (years) with a periodic servicing (e.g. every 6/12 months). They carry transmitting systems to send data to receiving stations. Moorings often also features global positioning system (GPS) instruments that measure their precise location over time, and self-check systems that collect information about mechanical/electrical failures. An international initiative called the Global Ocean Observing System (GOOS) is a global scientific collaboration that seeks to collect data from moorings and platforms all over the planet, and to compile this data into a global database that would be available to scientists for comparison and analysis.

Moorings and platforms are designed to work while submerged in corrosive seawater and exposed to extreme weather conditions for long periods of time. Their buoys have heavy-duty steel flotation collars, resilient solar panels, safety devices such as beacons and radio transmitters, and insulated, reinforced wells that protect sensitive scientific instruments. Mooring cables, while flexible, are extremely strong. The mooring line can be made up of synthetic fiber rope, steel wire, chain or a combination of the three, based on cost and design requirements.

Concrete reinforced with carbon steel is widely used for structures such as permanent platforms and measuring stations in, or in close proximity to, marine environments. Under normal conditions the natural alkalinity of the cement paste promotes the formation of a protective oxide film around the steel bars, which provides the reinforcing with some protection against corrosion. However, when chloride-ions diffuse through the cementitious cover down to the steel bars in sufficient concentration, the oxide layer is destroyed and corrosion is started in the presence of water and oxygen. Corrosion is manifested in two primary forms: cracking and spalling of cover concrete due to the expansion of corrosion products at the reinforcement, and local pitting of the steel which reduces the cross-sectional area of the bar.

The impact of chloride-induced corrosion can be described by an initiation/propagation model: in the initiation phase, chloride-ions diffuse through the concrete channels and cracks until a critical concentration is reached at the depth of the reinforcement. During the propagation phase, steel

oxidation goes on creating an additional volume of products that induces a pressure over the concrete coverage, resulting in cracks and delamination [TUU82]. Penetration of the cover concrete occurs because chloride-ions are transported through the pores by a variety of different chemo-physical processes. In the parts that are permanently under water, chloride ions diffuse in the water that saturates the pores, while above the high tide line, where the surface of concrete is reached only by splashes, water is transported inside by capillary suction.

The loss of concrete, in combination with the narrowing of steel section, reduces the load bearing ability of the structure and can also compromise its structural integrity. Normally the corrosion rate below water level is limited by low oxygen availability, and conversely lower chloride and moisture content limit the corrosion rate above the high tide level. Corrosion is most severe within the splash and tidal zones where alternate wetting and drying result in high chloride and oxygen content [LCH05].

In order to guarantee the absence of any significant cracking/spalling during service life, the structure must be designed so that the first initiation phase is longer than the foreseen life of the concrete installation. To reduce the penetration of chloride ions into concrete, the beneficial effect of incorporating materials such as fly ash, furnace slug and silica into the cementitious material is widely recognized.

It must be noted that, in the marine environment, concrete surface may be also damaged by sulfate attack, freeze/thaw cycles and erosion due to wave motion. In particular, sulfate dissolved in water are responsible for chemical reactions with the constituent of cement paste that result in extensive cracking, expansion followed by delamination, and bond failure between the aggregate and the cement paste. All these effects weaken the structure and enhance water penetration.

Over the last years, cathodic protection has increasingly been used to provide long-term corrosion control for reinforced concrete structures in marine environments. This protection process can effectively use both sacrificial (galvanic) and impressed current anodes and can be applied to all zones (tidal, splash) affected by corrosion problems.

Impressed current protection utilize an inert anode material, such as titanium, which is forced to slowly oxidize in favor of the steel reinforcement. This is obtained by injecting a current into the steel/anode system. The current must be adjusted depending on the corrosion rate, that changes from one part of the structure to the other. This is a major point to be taken in account for the design of the protection system. Electrical isolation between the anode and steel must be guaranteed to avoid short circuit that hinder the effectiveness of the anodic protection.

Sacrificial or galvanic cathodic protection exploits the different position of metals in the galvanic series and then the higher or lower tendency to oxidation. No auxiliary power supply is required and, as the anode is connected directly to the steel reinforcement, shorting of the anode is not a concern. This method can also be used for prestressed or post tensioned concrete parts. However, depending on which anode is used, the life expectancy of ICCP anodes is typically much greater than sacrificial anodes. For instance the life of a conductive coating system in a marine environment could be less than 10 years, whereas titanium mesh can readily exceed 75 years [Dai09].

4.5.1 Reliability challenges References

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4.6 Communication and positioning systems

Underwater sensor network (USN) technology enables opportunities to; collect oceanographic data e.g. pollution monitoring, nitrogen content in water; investigate environmental issues and coastal surveillance. Moreover, it can be used for underwater warfare including real-time control of unmanned-guided vehicles and submarines. However, USN has several communication challenges due to the large signal attenuation and low-band width of data transmission for both acoustic, optical and radio frequencies in seawater. Additionally, in-deep water only acoustic signals can propagate because radio frequencies attenuate rapidly and optical signals scatter.

Another challenge of underwater sensor nodes is the energy requirements, because it is different from radio based terrestrial sensor node. Therefore, high-energy propulsion and reducing communication energy are not the main areas of concern in USN [ME13]. However, selection of appropriate synchronization protocol, localization techniques, novel medium of communication, energy-harvesting techniques and network architecture is still a big challenge [ME13].

Underwater Wireless Sensor Networks have severe communication challenges due to large acoustic or electromagnetic (EM) signal attenuation in deep water [HDL87]. The propagation of acoustic signals in submarine media is possible with very low frequency signal carriers only, with reduced bandwidth and low transmission rates, thus extending transmission duration and diminishing battery life. Following are some state of the art communication techniques used for USN applications.

4.6.1 Satellite communication:

The marine satellite communication systems are widely used for positioning and tracking of buoys and marine vessels. For that purpose, geostationary satellites are well suited to cover large areas. Specifically for marine communication, there are three geostationary satellites placed above the Atlantic, Pacific, and Indian oceans. These three satellites cover almost the entire ocean areas and establish communication links with vessels and buoys [HDL87]. This fact led to the development of the MARISAT maritime satellite communication system by COMSAT in the early 1970's.

From 2000 onwards, maritime satellite communications using the INMARSAT system employ a second and third generation of satellites and new ship earth stations (SES). The new SES standards are using very small antennas with gains between 0 and 15dBi. At the lower end of SES, there will be no antenna stabilization. INMARSAT provides good communication links to buoys and ships at sea. These links use a VSAT type device to connect to geosynchronous satellites, which in turn link the ship to a land based point of presence to the respective nation's telecommunications system. Following Figure 4.6.1 shows the typical layout of satellite communication system.

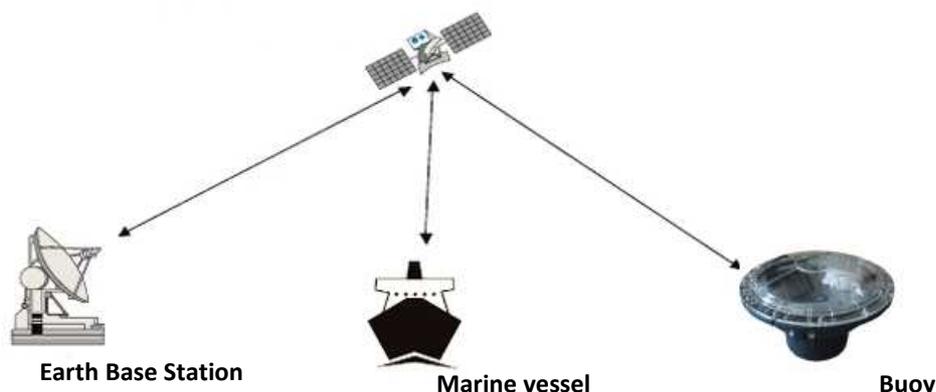


Figure 4.6.1. Block diagram of Satellite Communication

4.6.1.1 GPRS packet based Communication:

There are several off the shelf equipment and products available in the market, based on marine VSAT network, which uses GSM/ GPRS and Iridium communication technologies. GSM on-board

works perfectly with the marine VSAT package. A GSM server will be connected to VSAT, which creates mobile coverage on your vessel even in international waters. The main advantage for GSM/GPRS based packet communication network is that the equipment used for transmission and reception of telemetry and telecommand is economical and off the shelf. There is no special need to install sensitive and expensive equipment on-site.

4.6.2 Radio Communication:

Usually WSNs utilize the unlicensed ISM bands for communications which varies from tens of Mega Hz to few Giga Hz [EMO11] [AMA12] [GGA12]. The main drawback of radio frequencies is the rapid attenuation underwater. However, in [EMO11] [GGA12], the authors proposed a unified model for EM wave propagation in seawater environment and this model accurately calculates the path loss during underwater radio transmission. Following are successful implementation of radio-frequency based USN models specifically for marine applications

4.6.2.1 High frequency (HF):

In [APM05], the author gave reference to the case study of Mica2 motes from University of California. These motes communicated up to 120cm at 433MHz underwater close to the surface. Recent case study has been done in [ABP09], in which performance of EM communication on different wavelengths and sea-water conditions has been presented in detail. Moreover, on comparison of Electromagnetic (EM), optical and acoustic physical layers, EM signal with state-of-art digital signal processing and filtration technique makes preferable choice in shallow coastal water environment.

4.6.2.2 VLF (Very-low Frequencies) & ELF(Extremely low frequencies):

Apart from underwater acoustic telephones, VLF or ELF is the only feasible wireless communication method with submerged vehicles and systems. The ranges of ELF are from 30Hz to 300Hz and VLF is from 3KHz to 30KHz²⁹. The main advantage of low-frequencies transmission is that signals can be received in-deep waters and even in the earth's-crust. However, the VLF transmitter antenna size is too big and practically not possible to transmit from a submerged platform. Therefore, VLF & ELF are preferably used for simplex communication mode. For full duplex communication, different frequencies such as VLF for reception and HF for transmission can be used.

4.6.3 MULE System

MULE (Mobile Ubiquitous LAN Extension) system consists of a vehicle that physically carries a computer with storage between remote locations to effectively create a data communication link³⁰. A data mule is a special case of a sneakernet, where the data is automatically loaded and unloaded when the data mule arrives at its terminal locations. There are several applications in which data MULE systems have been implemented, such as in remote village or in rural area where there is no internet connectivity available. Computers with a disk and Wi-Fi link are attached to transport buses. As bus stops at the bus station in city to pick up passengers, the router on the bus communicates through Wi-Fi with the router installed at Bus-station. In the meantime, email or other required stuff can be downloaded and uploaded, while passengers boarding in bus, as shown in Figure 4.6.2. In wireless sensor network, several studies have been done on MULEs based mobile agent [DDA11]. Thus, MULE system is suitable for low priority, nearly constant and noncritical applications. However, in marine applications, most of the time we need real-time telemetry and telecommand from submerged sensors and floating-platforms. Therefore, this technique is not suitable for such critical applications.

²⁹ <http://submarinecommunications.weebly.com/index.html>. Accessed on 15 Aug, 2014

³⁰ http://en.wikipedia.org/wiki/Data_mule Accessed on 1 Aug 2014

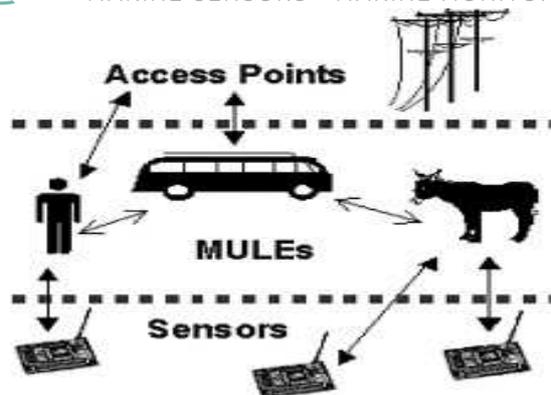


Figure 4.6.2 Data Mule System

4.6.4 Communication and positioning systems References

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4.7 Sensor network operating systems

Operating systems for sensor network nodes are typically less complex than general-purpose operating systems. They more strongly resemble embedded systems, for two reasons:

1. **Specific application.** Sensor networks are typically deployed with a particular application in mind, rather than as a general platform.
2. **Low costs and low power requirements.** Most sensor nodes must be implemented with low-power microcontrollers ensuring that mechanisms such as virtual memory are either unnecessary or too expensive to implement.

It is therefore possible to use embedded operating systems for sensor networks such as:

- **eCos.** (<http://ecos.sourceforge.org/>). Free and open source real-time operating system, intended for embedded systems
- **MicroC/OS.** (<http://micrium.com/rtos/ucosii/overview/>). Real-time multitasking operating system kernel for microprocessors, written mainly in C programming language

However, such operating systems are often designed with real-time properties.

Other sensor network operating systems are:

- **TinyOS** [Lev09]. Perhaps the first operating system specifically designed for wireless sensor networks. It is based on an event-driven programming model instead of multithreading. TinyOS programs are composed of event handlers and tasks with run-to-completion semantics. When an external event occurs, such as an incoming data packet or a sensor reading, TinyOS signals the appropriate event handler to handle the event. Event handlers can post tasks that are scheduled by the TinyOS kernel some time later.
- **RIOT**. Implements a microkernel architecture. It provides multithreading with standard Application Programming Interface (API) and allows for development in C/C++. RIOT supports common Internet of Things (IoT, <http://www.theinternetofthings.eu/>) protocols such as *IPv6 over Low power Wireless Personal Area Networks (6LoWPAN)* [Ham13].
- **Contiki**. (<http://www.contiki-os.org/>). Contiki is an open source operating system for the IoT. Contiki connects tiny low-cost, low-power microcontrollers to the Internet.
- **LiteOS**. (<http://www.liteos.net/>). Newly developed OS for wireless sensor networks, which provides UNIX-like abstraction and support for the C programming language.
- **ERIKA Enterprise**. (<http://erika.tuxfamily.org/drupal/>) Open-source and royalty-free OSEK compliant Kernel [OSK05], a standards body that has produced specifications for an embedded operating system, a communications stack and a network management protocol for automotive embedded systems. As major features, this OS supports multicore, memory protection and kernel fixed priority, adopting C programming language.

Online platforms for collaborative sensor data management

The online platforms for collaborative sensor data management are on-line database services that allow sensor owners to register and connect their devices to feed data into an online database for storage and also allow developers to connect to the database and build their own applications based on that data.

Examples include:

- **Xively** (<https://xively.com/>). Xively offers an IoT platform as a service, business services and partners, that enable businesses to quickly connect products and operations to the Internet.
- **Wikisensing**. The architecture of the Wikisensing system is described in [Sil12] defining the key components of such systems to include APIs and interfaces for online collaborators, a middleware containing the business logic needed for the sensor data management and processing and a storage model suitable for the efficient storage and retrieval of large volumes of data.

Such platforms simplify online collaboration between users over a large variety of data sets ranging from environmental data to information on energy, or all kind of data collected from transports. Other services include: allowing developers to embed real-time graphs & widgets in websites, analyse and process historical data pulled from the data feeds, or send real-time alerts from any datastream to control scripts, devices or environments.

4.7.1 Sensor network operating systems References

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4.8 Miniaturization

Miniaturisation of sensors and sensing systems is a major area of research and development, driven by a number of factors which include:

- Potential for faster, more cost effective, and more convenient analysis.
- Reduced consumption of reagents, sample and power.
- Lower manufacturing costs.
- Ease of transport and shipping.
- Reduced space requirements for laboratory devices.

Demand in a number of emerging/growing application areas is also increasing demand for miniaturised chemical sensors, these include:

- Portable/handheld devices for point-of-care assays.
- Portable and field deployable devices for environmental monitoring.
- Wearable and implantable sensors for monitoring of health and athletic performance.
- The needs of the pharmaceutical industry for microscale, highly parallel drug discovery assays.
- Trends towards compact, portable electronic consumer devices (smartphones, tablets, smart watches, smart glasses etc.) which incorporate sensors.

Analysis in Frost & Sullivan's report on "World Emerging Sensors Markets" [FS11] predicts that in the next seven to ten years miniaturisation of systems is expected to drive innovation; and OEMs will increasingly incorporate smaller sensors to improve performance, reliability, and longevity as well as reduce costs. Sensor materials are also expected to play a key role. Small form factor, less power consumption, higher feature integration, and low costs are some of the trends driving sensors market growth in numerous industries and applications. In addition, supporting electronics and packaging have become more advanced, so much so that sensors have become indispensable in every walk of life.

Ongoing developments in the area of Wireless Networks make the implementation of large-scale deployments (in terms of number of sensing nodes and geographical spread) of environmental sensors increasingly feasible. The challenge in this area is to provide compact, robust and reliable chemical sensors at a cost sufficiently low to enable deployment of large numbers of sensing nodes.

The coming years will see the emergence and deployment of low cost, low power, intelligent wireless sensor networks (WSN) in various applications such as environmental monitoring, agriculture, traffic control, home automation and healthcare. Miniaturisation and modularity of the sensing platforms will be necessary for these and many other uses that require large numbers of dispersed monitoring points [HOP09].

4.8.1 Miniaturisation based on microfluidics

Microfluidic technology has the potential to affect chemical analysis and synthesis in the same way that microchips have revolutionised computers and electronics [Gra02]. Microfluidics is based on devices and processes which handle volumes of liquid on the microliter or nanolitre scale. The silicon, glass or plastics devices have typical overall dimensions of a few millimetres; fluids flow through microbore channels produced by a range of high precision techniques. Fluids circulating in such channels can exhibit dramatically different performance from that in macro-scale tubes. Flow is normally turbulence-free, so layers containing different components can move along together, mixing only by diffusion. Speed is one of the main advantages of microfluidic systems. Chemical

separations such as electrophoresis, for example, are 100 times faster when a system is 10 times smaller. The advantages of, and challenges for, microfluidic systems are summarised in Table 21.

Table 21. Advantages and challenges of microfluidic analytical systems.

Advantages	Challenges
Very low sample and reagent consumption	Microfabrication requirements
Very low power consumption	Interfacing to the macro-world
Small size of analytical system allows for compact portable and <i>in situ</i> devices	Robustness and reliability under environmental conditions
Fast processes / short time of analysis	Representativeness of very small samples
Good reproducibility	Achieving highly sensitive detection using very small samples
Low cost	Scaling fabrication from prototype to production scale

Advantages such as reduced consumption of sample, reagents and power are direct results of the small size of micro-scale analytical systems. Micro-scale engineering also makes it possible to integrate chemistry with mechanics, electronics and optics, and to integrate multiple analytical systems into very small areas. However, in many cases it is necessary to interface the microfluidic chip with conventional-sized peripheral equipment for sampling, pre-treatment and fluid handling. While the development of on-chip pumps and valves for truly integrated fluid control is an active area of research [CFOB12],[GKB10],[BBR10],[OSC11],[BMZ12],[Rerolle13], many of these systems are limited in terms of response times, long-term repeatability, mechanical durability, and chemical compatibility, and are therefore generally limited to single use/disposable or short-term applications. Microfluidics-based analytical systems designed for long-term deployment under environmental conditions have so far chiefly relied on conventional off-chip (though miniature) pumps, valves, filter assemblies etc. Peristaltic pumps and syringe pumps are among the most widely used solutions for fluid control in such systems [ME01], with size, cost and power consumption, as well as pumping characteristics such as flow rate and reproducibility, being among the primary factors influencing the selection of pump for a particular device or application.

Other issues which require consideration when designing a microfluidic analytical system include:

- *Materials and fabrication techniques*

Microfluidic chips are manufactured using a range of materials and fabrication techniques, as summarised in Table 2. Materials selection is based on factors such as chemical compatibility, optical transparency, mechanical properties, and ease of processing. Due to the small size of microfluidic devices, costs of processing typically take precedence over materials costs.

Selection of fabrication technique depends on the material to be used, the volume of chips required, and the required dimensions and aspect ratios of channels and other micro-scale features to be formed.

3D-printing of microfluidic devices has recently been reported [SKY12], [ESG14], representing a potentially flexible and low cost route for fabrication, however this approach has not been widely implemented to date.

- *Scale up issues*

Fabrication techniques typically used by microfluidics researchers allow designs to be iteratively modified with new designs produced rapidly in small numbers (typically < 5). However, due to the specialised equipment, skilled personnel and individual processing steps involved, these techniques are not scalable to production/commercialisation levels. On the other hand, processing techniques suitable for high volume production typically entail significant initial setup costs, leading to high costs per chip in early commercialisation and for niche applications. This gap between prototyping and manufacturing scales can represent a

significant barrier to commercialisation of microfluidic analytical systems, and it is important that a clear route to fabrication of the microfluidic chips to be used in the COMMON SENSE nutrient sensors is developed from initial design phase onwards.

- *Interfacing with the macro world*

All microfluidic systems need to interact with the macro world in order to obtain sample and reagents, remove waste, and communicate the measurement to the user. Fluidic connections in/out of the chip are critical components and can represent a significant cost. While standardisation of these components would be a major advantage for the development and implementation of microfluidic systems, progress in this area has been limited due to the large variety of designs, dimensions and processes used by researchers, developers and manufacturers.

- *Representativeness and sensitivity*

Some issues inevitably arise when using an assay performed on a very small (typically sub 100 μL) analytical sample to represent, for example, a large body of water such as a river, lake or area of ocean. In addition, depending on the detection technique used, the size of the sample may represent a limitation on the measurement sensitivity which can ultimately be achieved [VH14]. It is therefore important to realise that an individual miniaturised device may not compete in terms of precision or accuracy with the “gold standard” laboratory technique. Rather, field deployable micro-analytical devices will have the advantage of measuring *in situ*, at higher frequency, and at a larger number of locations than is achievable using conventional techniques, thereby ultimately increasing the amount of information available. It should also be noted that samples are invariably filtered before entering a microfluidic analytical system, in order to avoid channel blocking and/or interference with the optical measurement by particulate matter, and the analytical measurement is therefore limited to the soluble fraction of a particular analyte, rather than the total.

Table 22. Materials and corresponding processing techniques typically used in the production of microfluidic chips [VH14, RZW13]. (PDMS = polydimethylsiloxane, PMMA = polymethylmethacrylate, PC = polycarbonate).

Materials	Fabrication Technique	Features
Silicon and Glass	Photolithography Wet Etching Dry etching (fused silica only)	High volumes, high setup costs. High precision fabrication processes well developed due to electronics industry. Good mechanical properties and chemical resistance. Silicon not optically transparent.
Elastomers (chiefly PDMS)	Casting	Convenient for prototyping, easy bonding, widely used in research laboratories, low volume, low setup cost. Long term use limited by mechanical properties and porosity/permeability.
Polymers (PMMA, PC)	Micromilling/laser milling Injection moulding Hot embossing	Convenient for prototyping, widely used in research laboratories, low volume. High setup cost, medium batch sizes. Medium setup cost, high batch sizes.

4.8.2 Marine monitoring issues

Aquatic environments in general, and the marine environment in particular, are extremely challenging for *in situ* sensing systems. Sensors deployed at sea face multiple challenges such as:

- Salt water ingress.

- Bio-fouling of equipment.
- Damage from wave/tidal action (if deployed at/near surface).
- High pressures and low temperatures (if deeply submerged).
- Limited power supply.
- Communications range constraints.
- Access limitations (e.g for maintenance purposes).

All of these factors considerably add to the expense of maritime environmental measurement systems, which often renders them unviable as long-term environmental monitoring tools for applications such as environmental research and government agency operated monitoring and management programs. While a number of miniaturised, microfluidics-based analytical systems have been developed and successfully deployed in marine environments, these systems are highly engineered, consequently very expensive, and unlikely to be feasible for deployment in large numbers.

Table 23. Planned improvements to DCUs low-cost nutrient sensing system

Analytical aspects	Ruggedisation aspects
High performance pumping system Miniature diaphragm pump or syringe pump will be implemented in place of low-cost peristaltic pumps.	Electronics All electronic components to be specified for required temperatures.
Improved optical detection system Measures will be taken to improve light path. Approaches under consideration include the use of: <ul style="list-style-type: none"> • Optical waveguides and/or longer optical pathlengths. • Micro-LEDs to localise light source close to microfluidic channel/cuvette. • Optical fibres to precisely direct light in/out of optical cuvette. • Optical filters to isolate wavelengths of interest. 	Housings & Connectors Robust housings, rated to IP68 and specified for submersion to 5m, with appropriate fittings to allow mounting/installation in a variety of deployment scenarios. Any external connectors will be specified to the same rating as the main housing, and to be suitable for use in marine environments.
Detection chemistries Stability of reagents and calibrants tested at relevant temperatures for period of at least 3 months. Assay performance and response time tested at relevant temperatures.	Antifouling measures Antifouling measures will be adopted to protect the sensor housing, sample intake and filtering assembly, and internal fluidic system from biofouling.

DCU's approach in this project is to build on the platform provided by a number of low-cost microfluidic systems for water monitoring which have been previously developed and validated under laboratory and environmental conditions [CCP13], [CMD13], [SCL10], [CMS10] including in freshwater, in estuarine water, and in wastewater. In order to ensure that the required analytical and ruggedisation specifications are achieved, a number of improvements will be implemented as summarised in Table 23.

Due to the more demanding requirements, the cost base of the COMMON SENSE nutrient sensors will be significantly higher than the previous systems. However, by using the low-cost systems as a starting point, and by limiting the deployment depth (to $\leq 3\text{m}$), the cost base of the COMMON SENSE nutrient sensors is projected to be significantly lower than current marine sensing systems.

4.8.3 Compact LED/photodiode-based optical detectors

Portable, robust, accurate methods of analysis are needed to achieve widespread *in situ* environmental monitoring. The miniaturisation of analytical instruments using microfluidics is one strategy to move this concept forward, and the resulting ability to manipulate smaller amounts of sample, combined with the need for fast response times, has placed great demands on the corresponding detection systems [GK07]. The advancement in LED sources and photodetector technologies provide a solution to these issues as they are compact, low power and low cost detectors for incorporating colorimetric analytical methods into remotely deployable devices [SDD02],[Dia04].

Following the trend of miniaturisation, detectors must provide high sensitivity for small detection volumes (sub 100 μL range) and low analyte concentrations, in addition they must be affordable, versatile, reliable, accurate and small in size. To form an optical detector, LEDs can be coupled to a wide variety of detectors such as, photodiode-arrays, photomultiplier, light dependent resistors, phototransistors, photodiodes, and LEDs. The most common detector used in LED based chemical sensors is the photodiode (PD). PDs are extremely versatile and have been employed in various configurations, such as flow through detectors in flow injection analysis (FIA) and separation systems. PDs are popular because of their low cost, rapid response and wide range of response. LED and PD based optical detectors have been primarily used in DCU's deployable nutrient sensors to date [CMD13], [SCL10], [CMS10].

The novel use of an LED as both light source and detector (paired emitter detector diode, or PEDD) for analytical applications was developed by Lau et al. [LBS04], [OLD05], [LBO06]. The emitter LED is forward biased while the detector LED is reverse biased. A simple timer circuit is used to measure the time taken for the photocurrent generated by the emitter LED to discharge the detector LED from 5 V (logic 1) to 1.7 V (logic 0), giving digital output directly without using an analog-digital converter or operation amplifier. This method achieves excellent sensitivity [OLS07]. DCU's handheld disc-based microfluidic system for water quality analysis uses a PEDD as the optical detection module [CGP12].

A distinct advantage of using the PEDD optical sensor in comparison with widely used LED-PD system is that the LED-LED combination is less expensive in both the cost of components and the cost of the signal transduction circuitry. The measuring technique employed by the PEDD device does not require a relatively expensive analog-digital converter as the output seen by the microprocessor is a direct pulse-duration-modulated signal. Additional advantages of the PEDD approach is the size, low power consumption (can operate in microwatts range), detection of low absolute light levels, response to a broad spectral range (247 to >900 nm) and ability to achieve good signal/noise ratio. The PEDD sensor is also versatile and can be configured in a variety of ways to measure transmittance/absorbance or reflectance [OD08].

4.8.4 Design considerations for COMMON SENSE nutrient sensors

The maximum possible extent of miniaturisation of the COMMON SENSE nutrient sensors is limited by a number of factors, including:

- *Fluid control components*

As fully integrated on-chip fluid control elements (pumps/valves) with sufficient long-term reliability are not currently available, the system will rely on miniature conventional components (such as high-performance miniature diaphragm, syringe-based or peristaltic pumps) for this key operation. A number of pumps will be required for each analytical parameter, as a typical colorimetric assay requires inputs for reagent (single- or dual-component), sample, 2 x calibration solutions, and potentially a conditioning/cleaning cycle. The space requirements for the required pump arrays are therefore significant, in comparison to the micro-analytical system itself, despite the small size of individual pumps and their ability to be stacked in compact formats.

Power consumption and compactness will be among the key factors in determining the choice of pumping system, and the small volumes required for each assay will minimise the power consumption per analytical cycle. Dead volume within the fluidic system will also be carefully reduced/eliminated to ensure the minimisation of reagent and power consumption.

- *Power supply*

Achieving the longest possible maintenance-free deployable lifetime is a key consideration for the COMMON SENSE nutrient sensors. Key factors will include the selection of power-efficient components (microcontroller, electronic components, fluid control components, optical detection components), the implementation of low-powered “sleep” modes between analytical cycles, and the minimisation of total volume of fluids moved per analytical cycle.

Identification and implementation of efficient high-capacity batteries will also be of key importance in terms of maximising the available power/battery volume ratio. However, with the goal of maximum deployable lifetime taking precedence over miniaturisation, significant space will be devoted to the power supply.

- *Reagent storage requirements*

The COMMON SENSE nutrient sensors will require storage for reagent(s), calibration solutions, conditioning/cleaning solutions, and waste solutions. Despite the small volume of each solution required for an individual assay, the goal of implementing high-frequency measurements (e.g hourly) over sustained periods (weeks/months) implies that total storage volume requirements used per deployment will be on the litre scale.

The fluid storage system will be designed for compactness; one possible solution is to store the various solutions within flexible reagent bags which will be collectively contained within a rigid container. This will maintain the advantages of bag-based storage (compactness, obviating the need for pressure equalisation systems, and efficient use of space by allowing the gradually expanding waste bag to use the space previously occupied by the gradually contracting reagent/calibrant bags) while providing a single, robust, modular reagent pack to achieve a user-friendly solution to reagent replenishment/waste removal.

Compactness and portability are important design factors for the COMMON SENSE nutrient sensors, and miniaturisation of the analytical system (fluidic system, microfluidic chips, and detector modules) will be prioritised in order to minimise reagent and sample consumption, waste generation, and power consumption.

However, there is limited justification for pursuing miniaturisation of the device as a primary objective in itself. The COMMON SENSE nutrient sensors will ultimately be deployed on/from relatively large deployment platforms and in conjunction with other COMMON SENSE sensor units, implying that the overall deployable platform will be of significant size. Moreover, maintenance-free deployable lifetime will ultimately be of key importance in terms of maximising the sensors’ cost-efficiency and ease of implementation to the end user, and this, in addition to factors such as deployability, physical robustness, and ease of access to key internal components (e.g. for scheduled maintenance purposes) should be prioritised over device miniaturisation.

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4.9 Energy storage and usage

Energy storage plays a very important role especially in distributed sensor network. Autonomous and unmanned instruments at sea require batteries to supply energy to the sensors, microprocessors, storage and transmission of the information. In certain cases, e.g., buoys at fixed points or moorings with surface elements, solar panels or even windmills, able to recharge the batteries, can be installed. However, in most cases batteries cannot be recharged, e.g. drifting buoys, Argo profilers, gliders, etc. In general, the cost of instruments left at sea is relatively high and they are difficult to recover once they are out of power, either because they cannot be located or because retrieving them is more expensive than their cost. In order to save money, it is convenient that such instruments should be working for as long as possible. Battery life may be, then, a crucial weak point for instruments left at sea.

4.9.1 Batteries

There are several types of chemical compositions of rechargeable batteries available in market. Due to limited space, high rechargeable cycles and long backup time, it is still a big challenge to selection appropriate battery technology especially for the Marine sector. In order to meet the growing demand for mobile energy used in marine applications required not only reliability but also cost efficient. However, environmental pressure is high, the temperature is around five degrees Celsius or below, and conditions are unsuitable for many chemical reactions in the deep sea.

Therefore, for underwater (deep sea) applications, the costs are mainly driven by the construction of robust pressure housing, which is the typically made of titanium. Table 24 shows the detailed comparison of different battery technologies [SK11].

Table 24. Comparison between different battery technologies

Battery Type	Nominal Voltage	Capacity mAmph	Efficiency %	Charging Method	Recharge Cycles	Self Discharge % per month
SLA	6	1300	70-92	Trickle	500-800	20
NiCd	1.2	1100	70-90	Trickle	1500	10
NiMH	1.2	2500	66	Trickle	100	20
Li-ion	3.7	740	99.9	Pulse	1200	<10
Li-polymer	3.7	930	99.8	Pulse	500-1000	<10

The battery capacity is dependent upon its mass and cruising range of underwater vehicles is proportional to the mass of the battery. Therefore, for long duration of cruising displacement, we need bulky-battery. In order to resolve such problem completely, batteries are enclosed with oil, to equate environmental pressure [HY11]. This is called oil compensated method and it is applied to various types of batteries. In [HY11], author showed the testing results of oil filled Li-ion, Ag-Zn, Ni-H, Ni-Cd batteries. However, oil-filled Lithium-ion battery showed promising results and it not only sustain under high pressure (up to 3000m in deep sea) but also give 1.3 times more backup time by using oil filled method.

In [WS09], author proposed especially designed “Pressure-tolerant Lithium Polymer Batteries” for marine applications. The main advantages are compact size, maximum discharged rate 30Amps, microcontroller-based electronics package integrated all types of electrical protections, solid-state switch cuts the supply before reaching to that level where fuse would blow, integrated telemetry and tele-command via RS-485 [WS09]. In [SFN13], author also used lithium-ion batteries and listed several advantages of Li-ion batteries over Lead-acid, Nickel cadmium and silver zinc batteries.

In recent survey report [SFN13] on recorders for passive acoustic monitoring of marine mammals stated that around 45 data recorders deployed in deep sea from more than 18 countries including Canada, UK, Russia, Italia etc. for acquiring data of marine mammals. In most of the recorders Lithium-ion and Alkaline battery technology have been used.

In conclusion, Lithium-ion technology showed promising results especially in high-pressure tolerant for marine application. It can be used for energy storage in underwater sensor nodes, because of following advantages:

- High energy density resulting in long duration of cruise time.
- Compacted and sealed housing, no gas generation.
- Maintenance free.
- Highly reliable, long charging and discharging cycles up to 800-1000.
- Economical and plug-in charging.

4.9.1.1 Battery supervisory circuit

Li-ion batteries are sensitive to overvoltage, overcurrent or under-voltage conditions. Therefore an electronic supervisory circuit is required to ensure, the batteries operate smoothly under aforementioned conditions. The circuit which monitor and control the battery terminal voltage and current during charging/ discharging is called the Battery Supervisory Circuit (BSC). Batteries equipped with a BSC are switching off on low tension to prevent damage. It disconnects the load for the time of the defective condition such as an over current or short circuit.

Li-ion batteries can deliver high output current (up to 50A), in contrast to other battery technologies. Li-Ion batteries are chosen especially for the marine industry and work even at very low temperatures much more efficient than other battery technology such as Alkali prime or Ni-Mh batteries.

The Li-ion battery must never be discharged too low, therefore charging and discharging could be easily controlled by BSC module, which enables a safe and gentle charge process. The BSC cuts off when the battery discharges to about 3.0V/cell, by stopping the current flow. If the discharge continues to about 2.70V/cell or lower, the BSC protection circuit puts the battery into a sleep mode. This renders the pack unserviceable and a recharge with most chargers is not possible. To prevent a battery from falling asleep, apply a partial charge before a long storage period.

While charging the housing of the battery system could be left closed because the Li-ion process does not exhausts any gas. Thus, the BSC module can be integrated with the battery pack and ideal for deep-sea or ocean applications. Following are some guidelines for Li-ion batteries:

- Charge at a moderate temperature. Do not charge below freezing.
- Lithium-ion does not need to be fully charged; a partial charge is better.
- Discontinue using charger and/or battery if the battery gets excessively warm.
- Before prolonged storage, apply some charge to bring the pack to about half charge.
- Over-discharged batteries can be “boosted” to life again. Discard pack if the voltage does not rise to a normal level within a minute while on boost.

4.9.1.2 Commercial Example - PowerPacks from SubCtech

SubCtech relies on standard Li-Ion cells for their rechargeable PowerPacks. They provide the highest capacity by low weight and volume. SubCtech produces complete battery packs, the PowerPacks™, in cooperation with manufacturers of high-class and certificated industrial cells. The PowerPacks™ are easy and safe to operate with the matching charging technology SmartCharger™. Charging is easily



Figure 4.9.1. SubCTech Li-Ion PowerPack ¹

controlled by the SmartCharger™ which enables a safe and gentle charge process. While charging the housing of the battery system could be left closed because the Li-Ion process does not exhaust any gas. Thus, the packs can be even charged built in or in water.

Comparing SubCTech Li-Ion PowerPacks™ with other – non-chargeable - battery technologies the Li-Ion batteries are already profitable after the third charging process. It is environmental friendly. Good operating data of the Li-Ion technology

simplify projects and usage.

The Li-Ion PowerPacks™ provide more power under rough conditions than Alkali primary cells or older storage battery technologies - such as very low temperatures, high currents (also peaks) or under difficult offshore conditions. The easy handling is an important characteristic that makes the PowerPacks™ ideal for the on board or under extreme conditions like under ice or in the deep ocean.

Specification

- High-power Li-Ion PowerPack™
- Standard range of capacities 666Wh, 1700Wh up to max. 4kWh
- Voltages typical 14,8V up to 52V
- Currents typ. 7A, optional up to 50A (high-power option)
- Robust Titanium housing with standard ranges 300m, 1000m, 3000m, 6000m
- SubConn BH4F titanium connector or other - depending on requirements
- Simple installation and operating

Options

- High-power output up to 50A
- Battery Management System (→ [SmartBMS™](#))
- Data interface RS-232 or RS-485, galvanic de-coupled
- Data logging on industrial μSD card of up to 32GB
- Control LED
- Redundant construction for high-safety requirements
- Multiple connectors
- Build-in DC/DC converter for your devices
- Design, capacity and mechanical customization to your project
- Manual operation or ISO ROV Switch (→ [ROV Switch](#))

4.9.1.3 Optimisation for high energy pulses

In those cases where batteries cannot be recharged, the duration of the monitoring could be limited by the effective life of the batteries, which can be seriously compromised by high current demand, see Figure 4.9.2.



Milliamp-Hours Capacity

Continuous discharge to 0.8 volts at 21°C

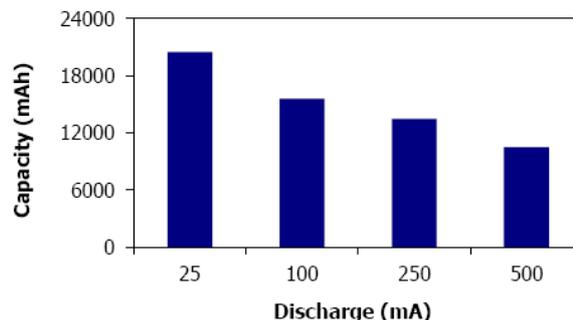


Figure 4.9.2. Capacity of a typical D 1.5 V alkaline cell as function of the intensity of a continuous discharge [Ene13].

Such behaviour can be stressed if the battery, instead of suffering a continuous discharge, is being discharged in short pulses of high current (> 1 A) such as those required for satellite transmissions. The development of solutions for getting maximum sampling for long-time deployments, including satellite transmissions, requires finding a way to prolong the batteries life.

How to save the batteries life in a stressing circuitry

At the Institut de Ciències del Mar (CSIC) a circuitry to power instrumented drifting buoys left at sea was developed [FSJ07]. Combining batteries and other elements such as supercapacitors, a special circuit was designed to satisfy short pulses of high-current demands without stressing the batteries. The suggested technical solution is a battery-capacitor hybrid set, submitting the batteries to a continuous drainage at low current to slowly charge the capacitors that, in turn, will supply the high current short pulse required by the transmissions (Fig. 4.9.3).

Due to high current pulses of 1 A/s, we choose aerogel capacitor class, made of carbon-foam dielectric, for a range of 1 to 50F. In order to provide enough isolation between each battery subset, it is necessary to use Shottky diodes which have a voltage drop less than 0.3 V. Finally, to provide the whole set with the necessary thermal and current protection, PTC resistors with positive abrupt characteristics are used.

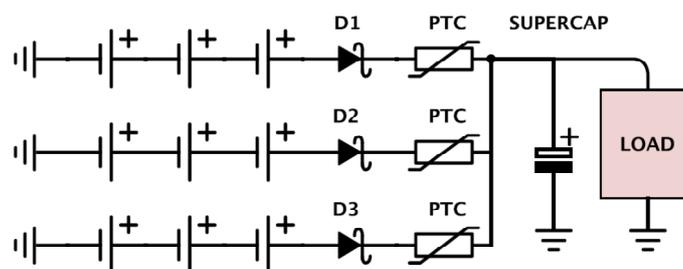


Figure 4.9.3. Scheme of a circuit to avoid charge dropping for moderate current consumption, used to power ICM-CSIC Drifting buoys

More than 50 drifting buoys using the above mechanism have been launched since 2008 [Fer13]. Among those, there is the oldest still working, more than 3 years after they were launched (7 July 2011). 3 of them are the only surviving in 2014 among the 39 launched in 2011 (http://www.locean-ipsl.upmc.fr/smos/drifters/liste_SSS.html)

4.9.2 Fuel cells

An underwater power source is one of the critical elements to perform underwater experiments and sensor data-collection. There is no source available in deep-underwater and typically series of experiments/ operations take place, for longer cruising distance simply relying on rechargeable

batteries are not enough. This is the reason why underwater sustainable sources and storage became a hot research topic.

In [Tad11], the authors proposed a novel concept of second generation fuel-cell system for underwater power source named as HEML (high efficiency multi less) fuel-cell system. The overall efficiency of HEML is over 60% with blower-less, humidifier-less, leakage-less, purge-less and few minute's start-up time. This novel 2nd generation fuel-cell system tested over long cruising range up to 3000 Kilometres with large endurance up to 600 hours in harsh sea environment.

Solid oxide fuel cell (SOFC) uses a hard ceramic compound of metal (such as calcium or zirconium) oxide. SOFC has around 60% efficiency, which is quite high as compared to other types of fuel cells. However, SOFC can be used for low power application up to 100KW. In [Ghe14], author demonstrated Hybrid Solid Oxide Fuel cell (HSOFC)-Battery system up to 37KW for long displacement vessels. This system operated and tested for more than 28,000 hours with peak efficiency and idea for marine applications.

Autonomous unmanned vehicles are operating commercially on HUGIN3000 all over the world. The technology used in HUGIN3000 is based on alkaline aluminium/ hydrogen peroxide semi fuel-cell (AL/HP SFC) [HSF06]. The stack consists of six serially connected cells, which generates around 30VDC. The main advantages of the AL/HP SFC power source are high energy density, low weight in water and normal operations in ambient pressure, that is why it is ideal for deep-water systems (e.g. 3000m) [HSF06].

4.9.3 Energy harvesting

Energy harvesting is the technique of capturing, converting and storing the generated energy that can be used to power-up low energy electronics circuit. There are several techniques can be used for energy harvesting such as solar, piezoelectric, kinetic, inductive, thermoelectric, Pyro electric materials and electromagnetic.

4.9.3.1 Solar energy harvesting:

Solar energy is one of the most important sources of renewable energy and it is gaining market interest because it has the greatest availability compared to other energy sources. In surveys [KG14] [AO09], the amount of solar energy which shines on earth in one day is more than the total amount of energy we require in a whole year. Our earth's crust consists of 70% of water, therefore, for marine deployments, solar energy could be a major source of energy. Solar energy is clean and free of emissions, since it does not produce pollutants or by-products harmful to nature. The conversion of solar energy into electrical energy has many application fields such as residential, vehicular, aircraft, and naval applications are the key fields of solar energy. On the basis of technology, solar energy systems are classified into two main categories.

Active Solar Energy System

In active solar energy systems, semiconductor materials are used to convert electricity directly from Sunlight. This process is known as the photoelectric effect and the device is called a photovoltaic cell. The light falls on the photovoltaic cell is absorbed and converted into electric charges. On the bass of application or deployment, we can classify the active solar energy system into two main groups.

Surface water solar cells:

On the surface of the ocean, there are three main types of materials of photovoltaic cells (PV cells) which can be used, which are mono & poly silicon, amorphous silicon and single crystal gallium arsenide (GaAs) PV cells. GaAs is especially suitable for use in multi-junction and high-efficiency solar applications, as shown in Figure 4.9.4.



Figure 4.9.4. Floating active solar platforms.

Underwater solar cells:

Good quality Gallium indium phosphide (GaInP) cells can be used for underwater energy harvesting operation. This technique showed promising results - even at a maximum depth of 9 meter generates 7watts per square meter. Figure 4.9.5 shows the deployment of underwater solar cells.

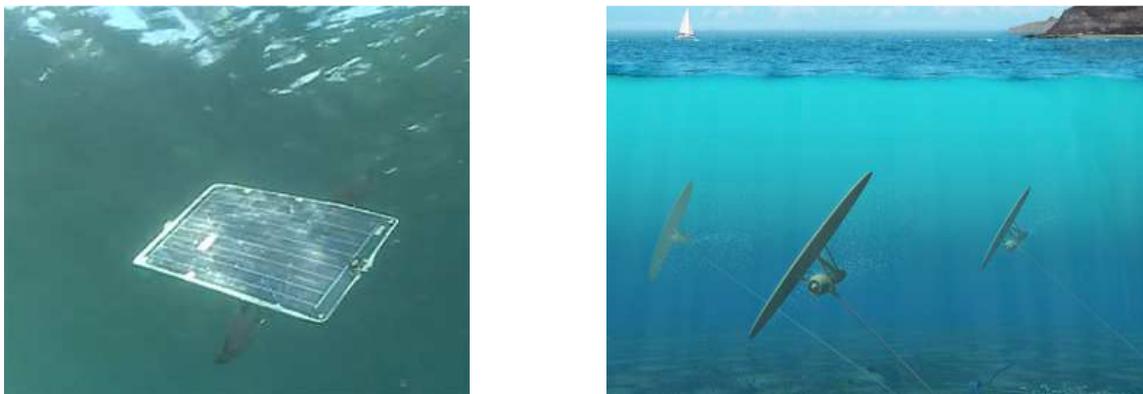


Figure 4.9.5. Deployment of underwater solar panels

Passive Solar Energy System

In a passive solar energy system or concentrated solar energy system, sunlight is converted into heat energy and then used to generate electrical energy by using heat pumps, gas-turbine, steam engine etc. However, there is no direct conversion possible like in PV cells. Figure 4.9.6 shows practically installed passive solar energy system in marine environment.

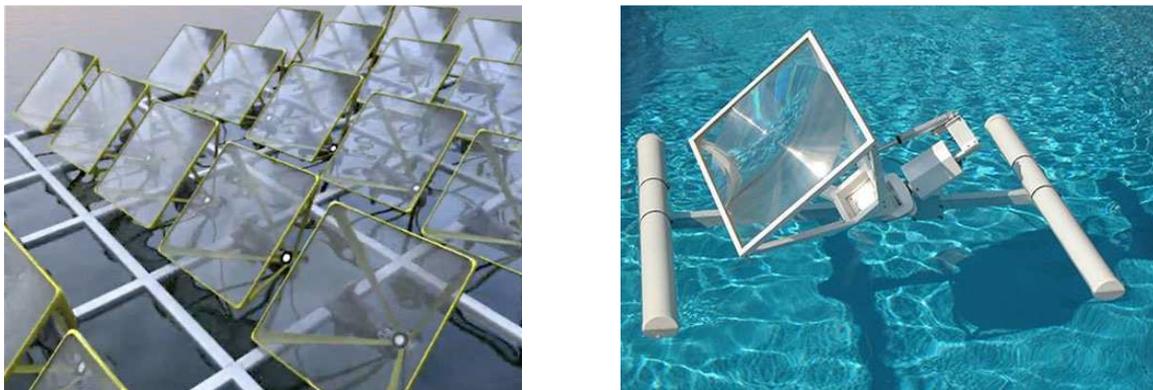


Figure 4.9.6. Passive solar energy system

4.9.3.2 Wave Energy harvesting:

Non-uniform heating around the earth causes winds to blow that generates ocean waves. The motion of the water waves creates kinetic energy which is proportional to the wind speed. It is the cheapest sources of energy and around the world's coastlines are generating estimated 2 to 3 million megawatts [AO09]. There are several ways methods to converts ocean wave energy into electricity. Typically, different types of turbines are used, however, generators and power electronics are also involved in conversion of wave mechanical energy into electrical energy.

Both rotational and linear generators produce variable amplitude and frequency of AC power, which is rectified and converted into DC before transmission, as shown in Figure 4.9.7.

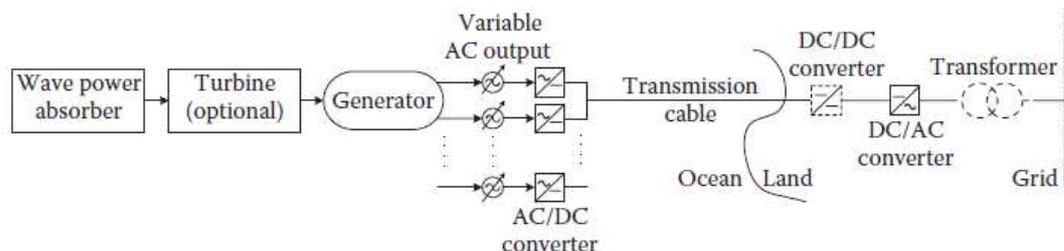


Figure 4.9.7. Block diagram of wave power generation system

Several types of wave energy harvesting technologies have been proposed and practically implemented such as the Salter Cam, a hinged floating system developed in England [SCG75], a pressure activated submerged generator developed by Kayser [IWS76] in Germany, the wave-powered pump developed at Scripp's Institution of Oceanography, and a pneumatic wave converter originally developed by Masuda [Mcc76] in Japan. Generally, there are two types of wave energy generation technologies used, which is dependent upon the distance from the shore. Following are the details regarding wave energy harvesting topologies.

Off-shore Energy Harvesting

Off shore wave energy generating system are located far from shore and they are mostly floating platforms either manoeuvring or fixed to the ocean bottom. Typically, linear generators connected with buoys are being used in off shore energy harvesting.

Dynamics of Fixed Bodies in Water

In this technique, when the waves hit a body fixed to the bottom of the ocean, it generates alternate electricity. The ocean waves force the body to move in a forward and backward direction, which can be seen in 2D diagram of the generator in Figure 4.9.8.

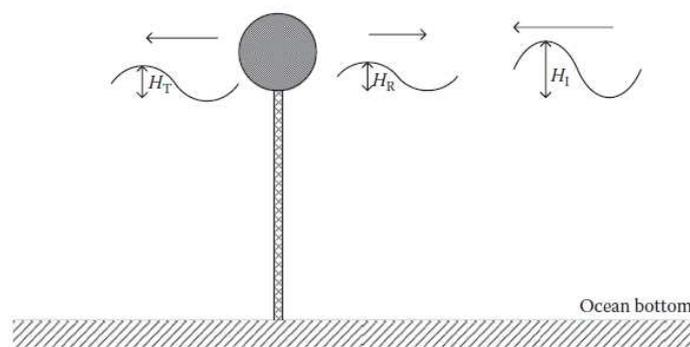


Figure 4.9.8. Dynamics of Fixed Bodies in Water

Dynamics of Floating Bodies in Water

In this technique, the object can float freely on the ocean surface. Typically, the orientation and motion are both quite random i.e. heave, sway, and roll, which can be seen in Figure 4.9.9. Such type



of floating objects can generate electricity, which can be utilized only for on-board operations such as lights or communication equipment installed on buoys.

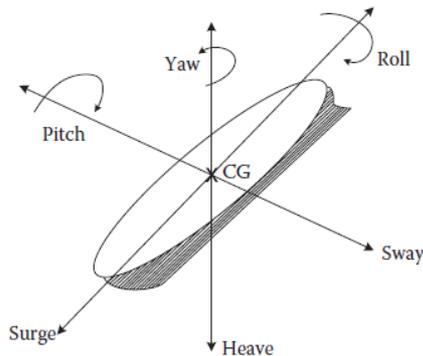


Figure 4.9.9. Dynamics of Fixed Bodies in Water

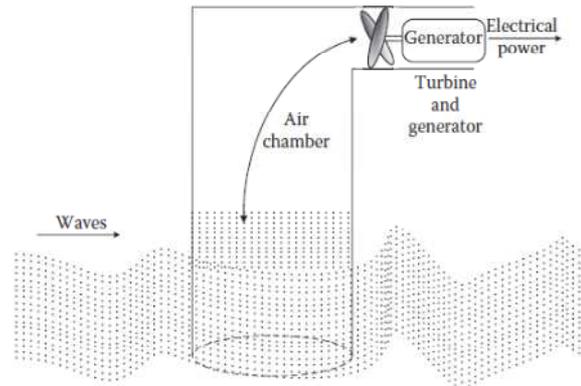


Figure 4.9.10. Illustration diagram for air driven turbines

Air-Driven Turbines

The air driven turbine system can be designed for near-shore and offshore power generation applications. It consists of two parts such as primary part and secondary part. The primary part of the turbine converts wave energy into compressed or pressurized air. In second portion of the turbine, pressurized air drives the electric turbine. However, this technique requires a fully sealed air chamber and the ventilating generator must be mechanically insulated, which adds complexity and high cost of the system. Figure 4.9.10 shows the detail operation of the turbine.

Fixed Stator and Directly Driven PM Linear Generator – Based Buoy Applications

In this technique, fixed linear generators are used with floating buoys. This method is useful for those areas where water level goes up and down significantly as shown in Figure 4.9.11.

The buoys move vertically on a pillar, which is connected to a hull. PMs are mounted on the surface of the hull, and coil windings on the outside of the hull. The pillar and stator are connected on a foundation standing on the seabed of the ocean. The hull with the mounted magnets is called the rotor or the piston of the generator, and they are the moving parts of the generator.

Since the motion is linear, this generator is called a linear generator. The generator AC voltage starts at zero when the buoy is in its lowest position, increases until the buoy reaches its highest position at the top of the wave and descends back to zero as the buoy stops.

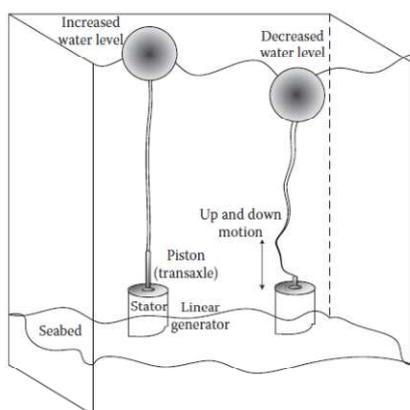


Figure 4.9.11. Illustration diagram of fixed linear generator connected with floating buoys

Salter Cam Method

In the salter cam technique, the motion of the cam is converted from wave into a hydraulic fluid, and then the hydraulic motor is used to convert the pressurized hydraulic fluid into rotational mechanical

energy. Consequently, the rotational mechanical energy is converted to electricity by utilizing electric generators, as shown in Figure 4.9.12.

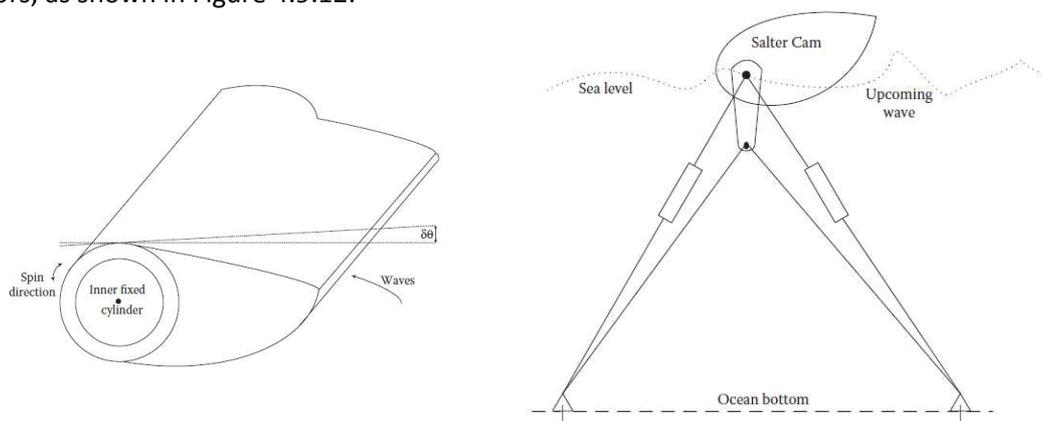


Figure 4.9.12. Diagram of Salter Cam generator

4.9.3.3 Near-shore Energy Harvesting Topologies

Near shore topologies are applied on coastal areas or within the surfing zone of the ocean. The near shore topologies are not so productive like offshore topologies and some of them are error prone. Following are some methods typically used near shore for power generation.

Near-shore Wave Energy Harvesting by the Channel/ Turbine Method

In this technique, wave energy / wave currents can be funnelled into a narrow channel, in order to intensify their power and size. The waves can be channelled into a catch basin and used directly to rotate a turbine, as shown in Figure 4.9.13. This method is expensive and huge infrastructure is required for generation.

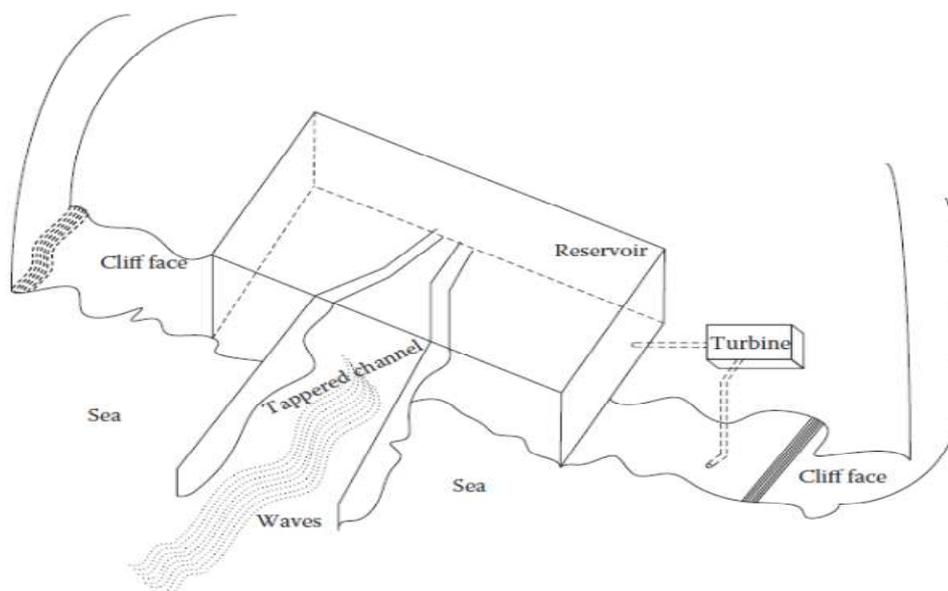


Figure 4.9.13. Wave energy harvesting technique by channel turbine method

Air-Driven Turbines Based on the Near-shore Wave Energy Harvesting Method

Another way to harness wave energy is with an oscillating water column that generates electricity from the wave-driven rise and fall of water in a cylindrical shaft or pipe. The rising and falling water drives air into and out of the top of the shaft, powering an air-driven turbine as shown in Figure 4.9.14.

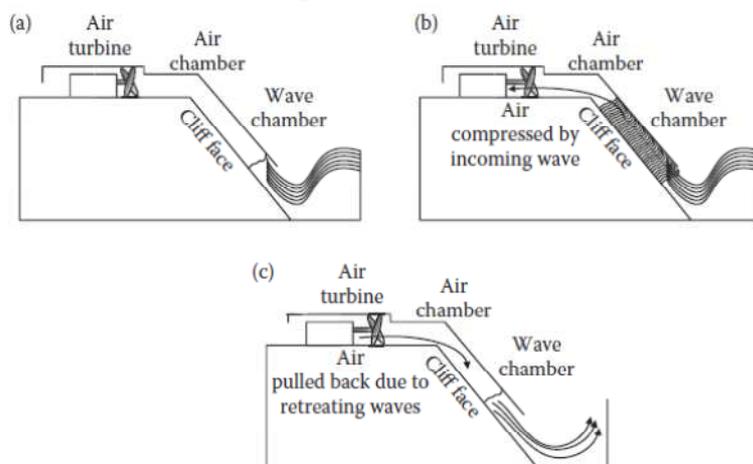


Figure 4.9.14. Wave Energy harvesting technique by air-driven turbine

4.9.3.4 Other Harvesting techniques:

Galvanic Cell

In [RKJ12], the authors proposed energy harvesting technique based on galvanic energy. Two electrodes from dissimilar metal strips with electrolyte, which is river water. The generated energy is enough to power-up on board electronics circuitry.

RF Inductive charging:

This type of energy harvesting technique can be used for those sensor nodes that are close or floating on the surface of water [Ene14].

Piezoelectric transducer:

Fiber-based flexible piezoelectric material can generate power by underwater thrust [ED11]. The average power generated by 10g of piezoelectric material (area: 50.8mm x 25.4mm x 0.017mm) on thrust of 19mN is around 120mW. The generated power is enough to power-up low electronics circuitry.

Smart Solid-State battery:

In order to eliminate bulky chemical batteries, thin-film solid-state rechargeable batteries can delivery up to 6mAh. Solid-State batteries have low profile, minimum footprint, improved electrochemical efficiency, high rechargeable cycles fabricated inside same processor package and available as a separate electronics chip/part [Cym14].

Sweater battery:

In [STK94], the authors proposed conceptual model of battery having energy density of 1008Wh/Kg over a period of 12 months in sub-sea environment. However, this design is too old, but still viable to use.

Salinity gradients:

Energy harvesting by reverse electro-dialysis from anodic alumina nano-pores is recently experimentally done in [KKK13]. The peak power generated is 542nWatts, which is far better results obtain as compared to previous studies.

4.9.4 Energy generator

We can generate significant amount of energy by using tidal generators. These are underwater turbines which can be placed in areas where there are high tidal movements. It converts mechanical energy of the ebbing and surging of ocean tides into electrical energy. Typically, electromagnetic generators are used to convert mechanical energy to electrical energy. However, miniature type of electrostatic generator can be used to power up low power electronics loads.

4.9.4.1 Electromagnetic generator:

This technique converts mechanical field into electrical field by using an oscillating coil in the magnetic field. We can use such type of custom small generator designed for underwater application. Following are the two types of electromagnetic generators which are typically used in the tidal energy harvesting.

- Rotary Magnetic generators
- Linear Magnetic generators

4.9.4.2 Piezo-Ceramic Device:

Ruggedized laminated piezo (RLP) device is used as a power generator, which converts the tidal motion into electrical energy. The main advantage of the RLP device is the simple mechanical design and high volume of power generation with small foot print, as shown in Figure 4.9.15.

4.9.4.3 Electrostatic generator:

Varactor or variable capacitor can be used as a nano-generator. It is initially charged then separates its plates by vibrations; in this way, mechanical energy is transformed into electrical energy.

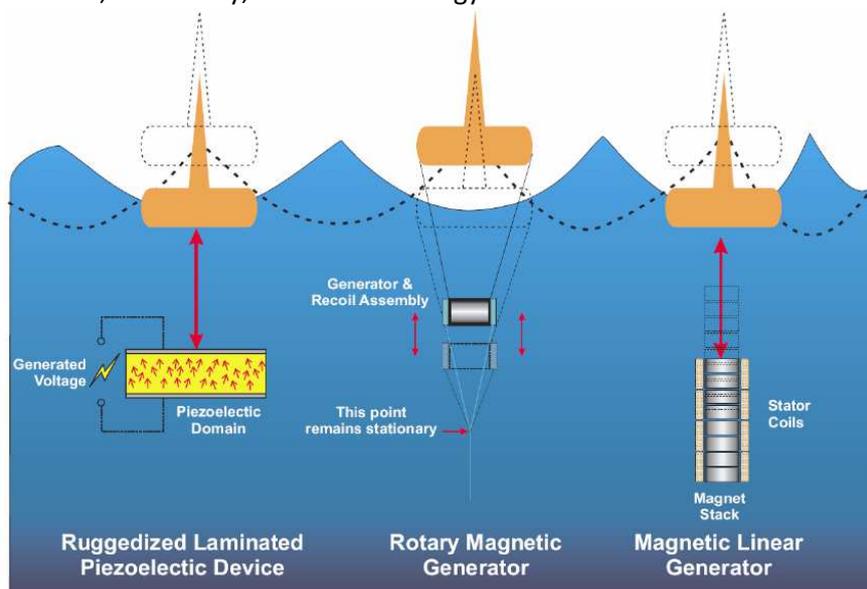


Figure 4.9.15. Illustration diagram of working principles of piezo, rotatory and linear type of generators.

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5 CONCLUSION

The scientific and technological objectives of the COMMON SENSE project are comprehensively targeting a significant number of the OCEAN 2013.2 call requirements - See COMMONSENSE DoW.

These objectives include ; the development of cost-effective sensors suitable for large-scale production and to take advantage of "new generation" technologies such as within the fields of miniaturisation, communication, positioning systems, disposable technologies, and IT tools, software, energy storage and usage. In order for the 'Technology' WP's [WP4-8] to achieve these objectives, they require:

- A comprehensive understanding and an up-to-date state of the art of existing sensors.
- A working basis on "new generation" technologies in order to develop cost-effective sensors suitable for large-scale production.

In consultation with the WP2 task leaders and the other WP Leaders, a comprehensive matrix was generated of topics to be reviewed for this deliverable. The 11 partners who contributed to D2.1, experts in their own fields, have used that domain knowledge to perform an exhaustive review of scientific literature, existing sensors and relevant commercially available sensors. This impressive collection of information provides a solid foundation for the real technical work of the COMMONSENSE to build upon

ACRONYMS

μFIA Micro flow injection analysis

ACT Alliance for Coastal Technologies

AVHRR (Advanced Very High Resolution Radiometer):

A five-channel scanning instrument that quantitatively measures electromagnetic radiation, flown on NOAA environmental satellites. AVHRR remotely determines cloud cover and surface temperature. Visible and infrared detectors observe vegetation, clouds, lakes, shorelines, snow, and ice. TIROS Automatic Picture Transmissions (APT) are derived from this instrument.

API Application Programming Interface

AUV Autonomous underwater vehicles

CHRIS (Compact High Resolution Imaging Spectrometer):

CHRIS is a sensor located on board of the Project for On Board Autonomy (PROBA) satellite. This satellite was launched on October 22nd 2001 by ESA. CHRIS can acquire data up to a 63-band mode with a spatial resolution of 36 meters, in the range between 410nm and 1050nm.

CFP Common Fisheries Policy

CMAS Centrifugal microfluidic analysis system

CZCS (Coastal Zone Color Scanner):

The first spacecraft instrument devoted to measurement of ocean color. Although instruments on other satellites have sensed ocean color, their spectral bands, spatial resolution, and dynamic range were optimized for geographical or meteorological use. In the CZCS, every parameter is optimized for use over water to the exclusion of any other type of sensing. The CZCS flew on the Nimbus-7 spacecraft.

EnMAP (Environmental Mapping and Analysis Program):

EnMAP is a German hyperspectral satellite mission providing high accuracy hyperspectral image data of the Earth surface. The satellite provides a high resolution hyperspectral imager capable of resolving 244 spectral bands from 420 to 2450 nm with a ground resolution of 30 m x 30 m. The envisaged launch of the EnMAP satellite is in 2015.

FIA Flow injection analysis



FTIR (Fourier Transform Infrared):

An analytical technique utilizing an interferometer with moving and stationary mirrors to "transform" the spectral distribution of wavelengths in the IR into a form that can be mathematically converted in a conventional infrared intensity spectrum.

GEOSS Global Earth Observation System of Systems

IR (Infrared):

Electromagnetic radiation with frequency lower than visible, usually having a wavenumber range of 400 to 4000 cm⁻¹.i.e., a wavelength range of 2.5 to 25 μm.

LANDSAT:

Owned and launched by the United States, this is a series of remote sensing satellites that use the visible and infrared parts of the spectrum to record images of the Earth's surface.

LED Light emitting diode

LIDAR (Light Detection and Ranging):

A pulsed laser system used like a radar system where the time of return of reflected light is detected and used to determine the distance of the target.

LOD Limit of detection

MIRAS (Microwave Imaging Radiometer with Aperture Synthesis):

MIRAS is the major instrument on the Soil Moisture and Ocean Salinity satellite (SMOS). MIRAS employs a planar antenna composed of three deployable arms carrying in total 69 receivers which detects radiation in the microwave L-band, both in horizontal and vertical polarizations. The aperture of the detectors, point directly toward the Earth's surface as the satellite orbits. The arrangement and orientation of MIRAS makes the instrument a 2-D interferometric radiometer that generates brightness temperature images, from which geophysical variables are computed.

MERIS (MEDium Resolution Imaging Spectrometer):

MERIS is a programmable, medium-spectral resolution, imaging spectrometer operating in the solar reflective spectral range. Fifteen spectral bands can be selected by ground command.

The instrument scans the Earth's surface by the so called "push-broom" method. Linear CCD arrays provide spatial sampling in the across-track direction, while the satellite's motion provides scanning in the along-track direction. The MERIS is one of the main instruments on board the European Space Agency (ESA)'s Envisat platform

METEOSAT:

(METEOrological SATellite) Europe's geostationary weather satellite, launched by the European Space Agency and now operated by an organization called Eumetsat. METEOSAT transmits at 13691 MHz.

MODIS (Moderate-resolution Imaging Spectroradiometer):

MODIS is a payload scientific instrument launched into Earth orbit by NASA on board the Terra and Aqua satellites. The instruments capture data in 36 spectral bands ranging in wavelength from 0.4 μm to 14.4 μm and at varying spatial resolutions.

MSFD Marine Strategy Framework Directive

NOAA (National Oceanic and Atmospheric Administration)

NOAA was established in 1970 within the U.S. Department of Commerce to ensure the safety of the general public from atmospheric phenomena and to provide the public with an understanding of the Earth's environment and resources. NOAA includes: the National Ocean Service which charts the oceans and waters of the U.S. and manages 265,000 acres of

estuarine reserves; the National Marine Fisheries Service which maintains the world's largest and most complex marine fisheries management system; the NOAA Corps which operates 18 NOAA research and survey ships and flies 15 NOAA aircraft; and the Office of Oceanic and Atmospheric Research which supports experiments, laboratories, and the National Sea Grant College Program, among other efforts. NOAA has two main components: the National Weather Service (NWS), and the National Environmental Satellite, Data, and Information Service (NESDIS).

NOC	National Oceanography Centre
OEM	Original equipment manufacturer
PC	Polycarbonate
PD	Photodiode
PDMS	Polydimethylsiloxane
PEDD	Paired emitter detector diode
PMMA	Polymethylmethacrylate

PRISMA (Hyperspectral Precursor of the Application Mission):

PRISMA is an Earth Observation system with innovative, electro-optical instrumentation that combine a hyperspectral sensor with a medium-resolution panchromatic camera. The advantages of this combination are that in addition to the usual capability of observation based on recognising the geometric characteristics of the scene there are hyperspectral sensors which determine the chemical-physical composition of the objects present on the scene. This offers the scientific community and users many applications in the field of environmental monitoring, resource management, crop classification, pollution control and other things. In addition, there may be other applications in the field of National Security.

SeaWiFS (Sea viewing Wide Field-of-view Sensor):

SeaWiFS was the only scientific instrument on GeoEye's OrbView-2 (AKA SeaStar) satellite, and was a follow-on experiment to the Coastal Zone Color Scanner on Nimbus 7. It stopped collecting data on December 11, 2010. SeaWiFS was designed primarily to monitor ocean chlorophyll-a concentrations from space, however it also collected many other parameters for research and educational purposes. These parameters included reflectance, the diffuse attenuation coefficient, particulate organic carbon concentration (POC), particulate inorganic carbon concentration (PIC), colored dissolved organic matter (CDOM) index and normalized fluorescence line height (NFLH)

SMOS (Soil Moisture and Ocean Salinity):

The SMOS is a satellite which forms part of ESA's Living Planet Programme, intended to provide new insights into Earth's water cycle and climate. The SMOS satellite carries a new type of instrument called Microwave Imaging Radiometer with Aperture Synthesis (MIRAS)

VIIRS (Visible Infrared Imaging Radiometer Suite):

VIIRS is a sensor designed and manufactured by the Raytheon Company on board the Suomi National Polar-orbiting Partnership weather satellite. VIIRS is a scanning radiometer which collects imagery and radiometric measurements of the land, atmosphere, cryosphere, and oceans in the visible and infrared bands of the electromagnetic spectrum. VIIRS data is used to measure cloud and aerosol properties, ocean color, sea and land surface temperature, ice motion and temperature. It extends and improves upon a series of measurements initiated by the Advanced Very High Resolution Radiometer (AVHRR) and the Moderate Resolution Imaging Spectroradiometer (MODIS).