In Situ Phosphate Monitoring in Seawater: Today and Tomorrow

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Abstract. Phosphorus is an important macronutrient and the accurate determination of phosphorous species (namely phosphate) in environmental matrices such as natural waters and soils is essential for understanding the biogeochemical cycling of this element, studying its role in ecosystem health and monitoring the compliance with legislation. This paper is focused on phosphate determination in seawater. Thus, the sources, occurrence and importance of phosphate together with several aspects regarding the analysis and terminology used in the determination of this element in the ocean are briefly described. Existing and future *in situ* analytical techniques for the determination of phosphate in seawater are presented. Today's *in situ* phosphate monitoring is dominated by different spectrophotometrical analyzers. Thus, a description of the basis, advantages and disadvantages of the different existing analyzers is provided. It seems that these techniques may be replaced in the near future by electrochemical sensors which provide excellent possibilities for phosphate determination with high precision, long lifetime, low detection limit and good reproducibility. Additionally, electrochemistry allows going further in miniaturization, provides a decrease in energy requirements and avoidance of additional reagents. Recently developed electrochemical methods for phosphate determination will lead to the first *in situ* autonomous sensor (ANESIS) which will fulfill all these expectations.

Keywords: Phosphate, nutrients analyzers, open ocean, sensor, electrochemistry, spectrophotometry.

1 Introduction

With 50 times more carbon dioxide than in the atmosphere, the oceans contain the largest reservoir of carbon actively circulating in the biosphere. In the long term, the ocean plays a dominant role in the natural regulation of CO_2 in the atmosphere via both physical and biological carbon pumps and thus exerts a powerful influence on

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the climate [1]. A comprehensive and quantitative understanding of the way the ocean carbon cycle functions is fundamental to predict the consequences of rising levels of carbon dioxide and other greenhouse gases in the atmosphere. Phosphate is one of the main components of the biological carbon pump and its avaibility can impact primary production rates in the ocean as well as species distribution and ecosystem structure [2]. Thus, the availability of phosphates in marine systems can strongly influence the marine carbon cycle and the sequestation of atmospheric carbon dioxide. It is then critical to monitor properly phosphate concentration in the ocean, if we are to understand climate regulation.

1.1 Chemistry of Phosphate

From a chemical point of view, phosphates are the salts of phosphoric acid. In dilute aqueous solution, phosphate exists in four forms. In strongly-basic conditions, the orthophosphate ion (PO_4^{3-}) dominates, whereas in weakly-basic conditions, the hydrogen phosphate ion (HPO_4^{2-}) is prevalent. In weakly-acid conditions, the dihydrogen phosphate ion ($H_2PO_4^-$) is the most common. In strongly-acid conditions, aqueous phosphoric acid (H_3PO_4) is the main form. More precisely, considering the following three equilibrium reactions:

$$H_2PO_4 \Leftrightarrow H^+ + H_2PO_4^- \tag{1}$$

$$H_2PO_4^- \Leftrightarrow H^+ + HPO_4^{2-} \tag{2}$$

$$HPO_4^{2-} \Leftrightarrow H^+ + PO_4^{3-} \tag{3}$$

the corresponding constants at 25°C (in mol/L) are:

$$K_{a1} = \frac{\left[H^+\right]\left[H_2PO_4^-\right]}{\left[H_3PO_4\right]} \approx 7.5 \cdot 10^{-3},\ pK_{a1} = 2.12 \tag{4}$$

$$K_{a2} = \frac{\left[H^+\right]\left[HPO_4^{2-}\right]}{\left[H_2PO_4^-\right]} \approx 6.2 \cdot 10^{-8},\ pK_2 = 7.21 \tag{5}$$

$$K_{a3} = \frac{\left[H^+\right]\left[PO_4^{3-}\right]}{\left[HPO_4^{2-}\right]} \approx 2.14 \cdot 10^{-13},\ pK_{a3} = 12.67 \tag{6}$$

1.2 The Cycle of Phosphate in the Ocean

The marine phosphorus cycle is presented on Fig. 1 [2] with a special attention to different phosphorus fluxes. The phosphorus content on both oceans and lands is very

small. The main reservoir of phosphorus on land is rocks formed in ancient geological epochs. These rocks gradually ventilate and release phosphorus compounds into marine ecosystems. Continental weathering is the primary source of phosphorus to the oceanic phosphorus cycle [3]. Most of this phosphorus is delivered via rivers with a smaller portion delivered via dust deposition [4]. In recent times, anthropogenic sources of phosphorus have become a large fraction of the phosphorus delivered to the marine environment, effectively doubling the pre-anthropogenic flux. An excess of phosphate causes uncontrolled growth of algae leading to eutrophication or over fertilization of receiving waters [5]. The rapid growth of aquatic vegetation can cause the death and decay of vegetation and aquatic life due to the decrease in dissolved oxygen levels [6, 7]. The main anthropogenic sources of phosphorus are detergents and sewage. The primary sink for phosphorus in the marine environment is the loss to sediments. Much of the particulate flux from rivers is lost to sediments on the continental shelves, and a smaller portion is lost to deep-sea sediments [8]. In surface waters, certain phosphate forms (PO_4^{3-}) are taken up by phytoplankton during photosynthesis. Subsequently, the phosphate travels up through the food chain to zooplankton, fish and other top marine organisms [9]. Much of organic-P is converted back to PO_4^{3-} in surface waters as phytoplankton die but some of it finds its way to the deep ocean (via downwelling and sinking of organic matter) where it is remineralized back to inorganic-P. It may return to the surface waters (via upwelling) or be bound by the widely prevalent cations (Al^{3+}, Ca^{2+}, Fe^{2+}, Fe^{3+}) and stored as minerals for a long time in the rocks and sediments. In that case, only geological processes can bring them back to cycle.

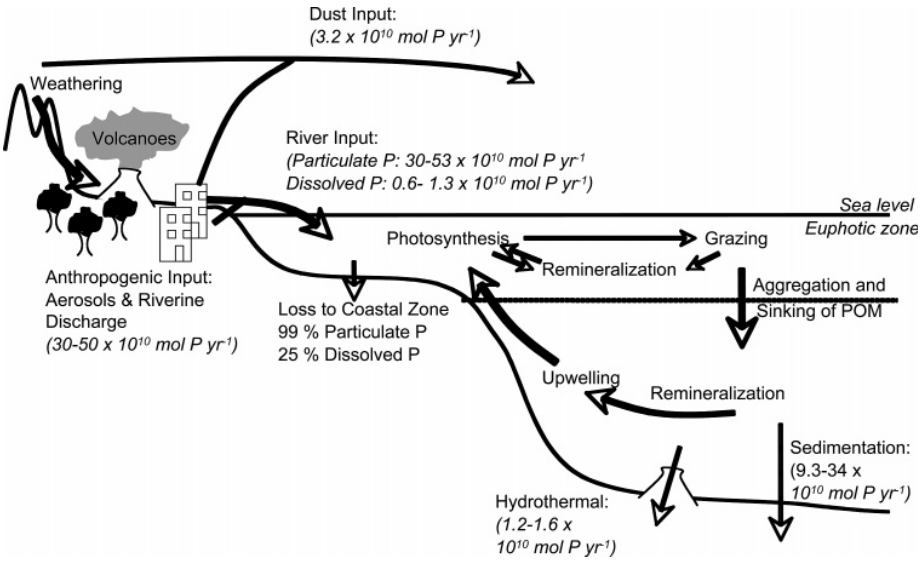

Fig. 1. The marine phosphorus cycle [2]

Vertical sections for phosphate concentrations in sea water are presented on Fig. 2 for the Atlantic, Pacific and Indian Oceans.

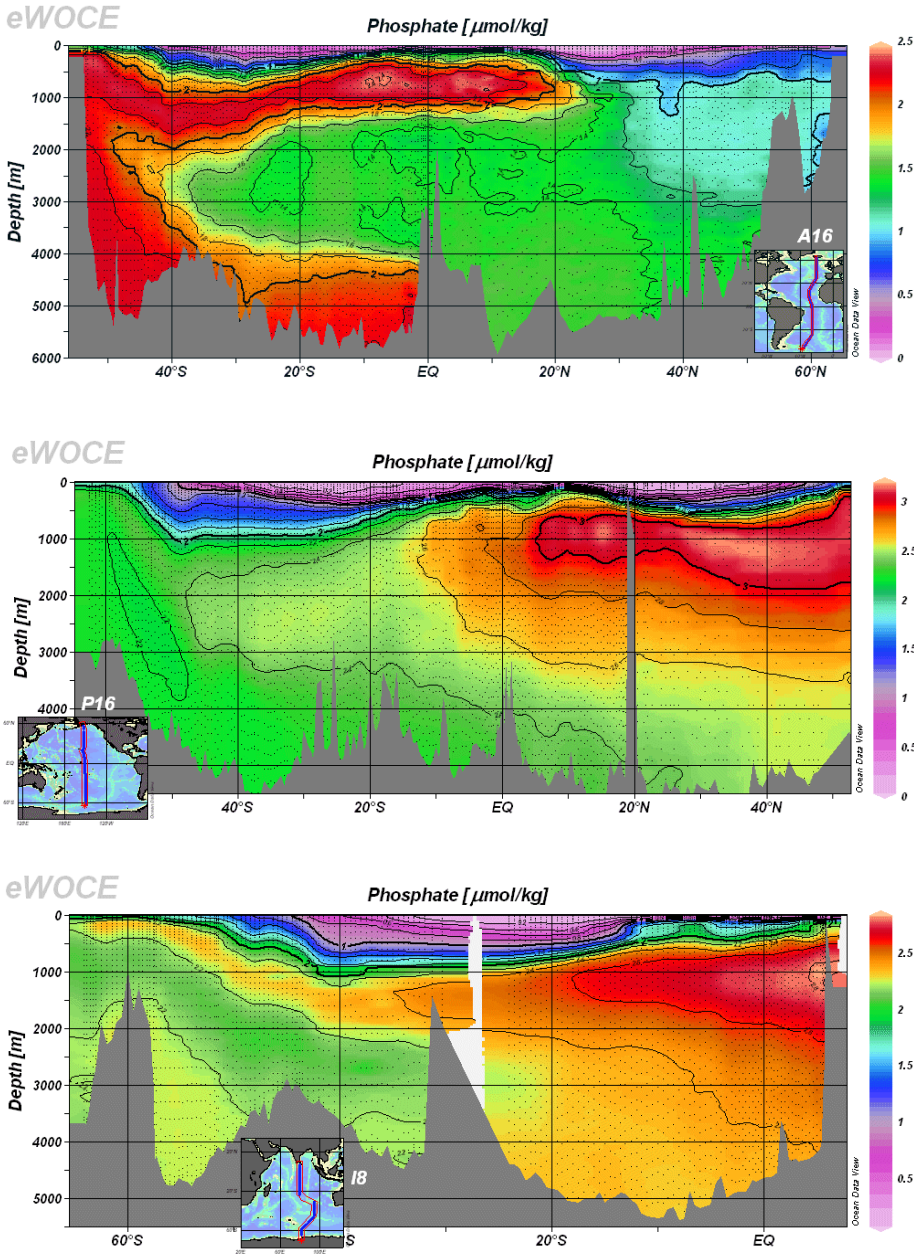

Fig. 2. Vertical section of phosphate concentrations obtained during the WOCE A16, P16 and Indian Ocean 18 expeditions [10]

As phytoplankton and other organisms die, PO_4^{3-} is regenerated in the water column. A maximum regeneration occurs near 1000 m, which is the same depth as the oxygen minimum layer. The maximum values of phosphate concentration in the Atlantic are about 2.5 μM, while in the Pacific values around 3.25 μM occur. The higher values observed in the Pacific (and Indian) oceans as compared to the Atlantic are due to the age of the water masses (older in the Pacific thus accumulating more oxidized plant material). Generally, knowledge of the phosphate concentration in the ocean surface allows us to deduce information on the biological activity in the ocean. Secondly, phosphate is one of the chemical tracers (along with temperature, salinity, and other nutrients concentration) which allows us to analyse water mass mixing and determine the origin of the water masses.

Phosphate concentration and ratio of nitrate over phosphate concentration (NO_3^- /PO_4^{3-}) can inform us about the biogeochemical processes in the ocean where microbial processes coexist: denitrification, nitrification, anammox, nitrogen fixation [11]. These parameters were used to explain unexpected phosphate profiles obtained during the Pelagico 1011-12-BIC OLAYA cruise aboard R/V Josa Olaya Balandra off Peru during austral spring 2010 (November-December 2010) when an intense upwelling occurred whithin this Oxygen Minimum Zone region. At depths ranging from 150-200m a relative minimum in phosphate concentrations was observed. Two cases were noticed: phosphate minimum associated with nitrate minimum and phosphate minimum associated with nitrate maximum. The first case is characteristic of the predominance of denitrification/anammox processes (which consume nitrate over nitrification/remineralization) associated with N_2-fixation (phosphate consumption). The second would be indicative of predominance of remineralization/ nitrification processes (production of nitrate over denitrification/anammox) associated also with N_2-fixation (phosphate consumption, but no nitrate consumption) [12, 13].

1.3 Sensors and Platforms for Long Term Monitoring of the Ocean

Much of what we currently know about the phosphate (and other nutrients) content in the ocean comes from very expensive and time consuming ship-based sampling. An alternative is to obtain autonomous observations supported by spectrophotometric analyzers based on wet chemistry but their use for phosphate analysis is not very much widespread [14]. The development of multi-disciplinary oceanic observatories for long-term monitoring will help to increase the observing capacities in the constantly changing marine environment. This monitoring requires an *in situ* miniaturized autonomous instrumentation able to achieve excellent figures of merit: long lifetime, high precision, low detection limit, fast response time, good reproducibility, resistance to biofouling and high pressure, provision of stable long-term operation, and low energy consumption. Within the past years, sensor technologies for oxygen, chlorophyll, particles and nitrate have been refined [15]. These sensors are capable of deployment on long-endurance missions on autonomous platforms such as profiling floats and gliders.

The ARGO profiling floats are good example of autonomous platforms. ARGOs drift at a fixed parking depth (1000 or 2000m for instance below sea level) for 9 days and then they come up to the surface. On their way up, they measure temperature and salinity (and sometimes nitrate and oxygen concentrations) through the water column. Once they reach the surface, they transmit recorded data to land via satellite and then the cycle is repeated (Fig. 3). The buoyancy of an ARGO float is controlled to make it rise or sink. This is done by pumping oil between internal and external bladders. There is about 3000 of these floats in the ocean [16] equipped with typical physical sensors whereas the same floats equipped with biogeochemical sensors are in number of about 300. The major challenge will be to increase the number of biogeochemical sensors adapted on ARGO floats and other autonomous platforms.

Many sensors detect analytes directly, without addition of liquid reagents. Among them, the most often used are oxygen and pH sensors. Clark-type sensors are essential to study primary production and metabolism of organisms. They are also essential for oxygen measurement of special marine ecosystems like those found in the Oxygen Minimum Zones. There are many examples of applications of these sensors on CTD profilers or floats. pH sensors are essential for measurements of growing acidification of the oceans and its impact on the carbon pump and life of marine organisms [17]. However, there are many species undetectable by direct measurement (phosphate, silicate, etc.). Analyzers have been developed to measure *in situ* these elements but these methods require a set of elements for measuring samples where the detection is done after a chemical reaction (pump, valves, sensors, electronics, etc.).

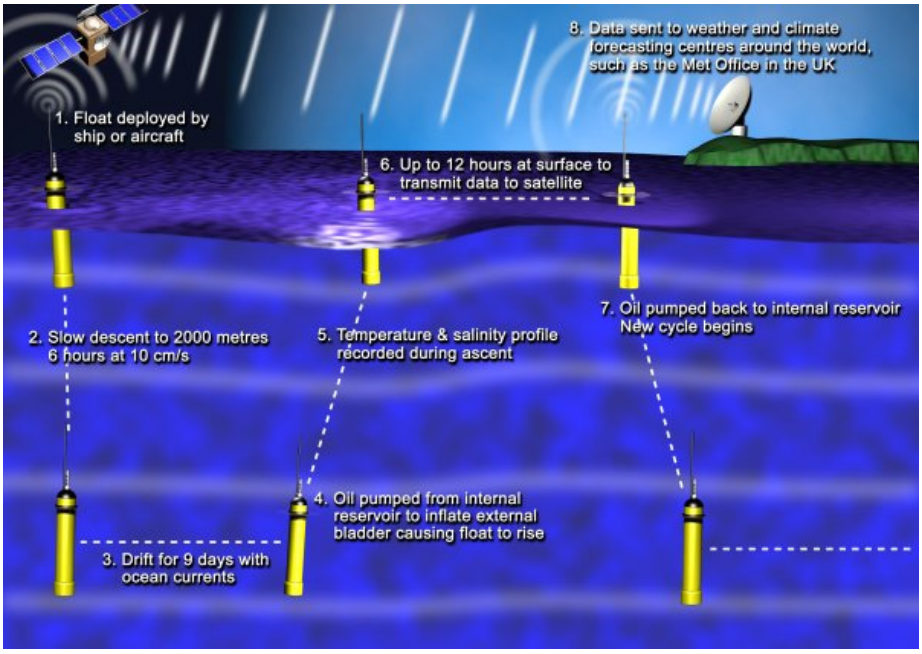

Fig. 3. Operational cycle of an ARGO float [18]

Within a large number of analytical methods, electrochemistry provides promising reagentless methods to go further in miniaturization, decrease in response time and energy requirements. In aquatic systems, electrochemical methods are used routinely for monitoring of pH by potentiometry, dissolved oxygen by amperometry [19] trace metals and speciation by voltammetry [20, 21], conductivity and therefore salinity by impedimetry. Electrochemistry offers also a wide range of possibilities for achieving an excellent phosphate determination in seawater but no autonomous electrochemical sensor for *in situ* phosphate determination exists nowadays. Generally, the use of electrochemical *in situ* sensors in the ocean is not well developed but the opportunities provided by some teams show an increasing interest in this research field.

2 Today's In Situ Analyzers for Phosphate Monitoring in Seawater

Phosphate analysis in the ocean is still based generally on traditional shipboard sampling techniques with subsequent analysis either on board or in a shore-based facility. The *in situ* analyzers use wet chemistry like ANAIS or NAS 3-X. They are able to work about 1-2 months without human intervention and are capable for simultaneous determination of phosphate, silicate, nitrate and nitrite. Such analyzers provide also good accuracy but require addition of reagents and release waste to the ocean. The drawback of these techniques is also their large size and weight.

2.1 Principles of Spectrophotometric Phosphate Measurements

The analysis is performed using the method of Murphy and Riley [22] adapted by Strickland and Parsons [23]. Molybdic acid is formed by conversion of ammonium heptamolybdate in acidic medium (reaction 7). The phosphate in reaction with molybdic acid forms a phosphomolybdate complex which absorbs yellow in the UV (reaction 8):

$$(NH_4)_6Mo_7O_{24}, 4H_2O + 6H^+ \rightarrow 7(MoO_3, H_2O) + 6NH_4^+ \quad (7)$$

$$PO_4^{3-} + 12MoO_3 + 3H^+ \rightarrow H_3PMo_{12}O_{40} \quad (8)$$

$$PMo^{VI}{}_{12}O_{40}{}^{3-} + 4e \rightarrow PMo^{V}{}_4Mo^{VI}{}_8O_{40}{}^{7-} \quad (9)$$

The phosphomolybdate complex (the oxidation state of molybdenum is VI) is reduced using ascorbic acid (reaction 9). This gives a blue complex with molybdenum in an oxidation state of V and VI. This complex has an absorption maximum at 882 nm. The measurement is also possible at 715 nm but with a loss of sensitivity. The concentration can be found by measurements of absorbance which is directly proportional to the concentration as described by Lambert-Beer's law:

$$I = I_0 \, e^{-\alpha \, l \, C} \quad (10)$$

$$A_\lambda = -\log_{10}\frac{I}{I_0} = \varepsilon_\lambda\, l\, C \tag{11}$$

With I_0-intensity of excitation light, I-intensity of light output, I / I_0-transmittance of the solution, A-absorbance or optical density at a wavelength λ, ε-molar extinction coefficient (in mol^{-1} cm^{-1}); l-optical path length traversed in the solution, it corresponds to the thickness of the cell used (cm); C-molar concentration of the solution (mol L^{-1}), α-absorption coefficient (m^2 mol^{-1} or m^3 mol^{-1} cm^{-1}).

The absorption measurements required the use of spectrophotometers. Today, spectrophotometers with simple measurements using cuvettes are still performed but methods coupled with flow-injection apparatus are preferable.

Arsenate and silicate form the same kind of complex which can be a source of interferences. However, normally arsenate concentrations in the open ocean are very small as compared to phosphate and do not exceed 0.03 μM in seawater. Thus, the influence of arsenate on phosphate detection is negligible. From the other hand, silicate concentration is much higher in the ocean and without special treatment it can cause a significant interference. To avoid silicate interferences, the ratio of protons over molybdates H^+/Mo and pH of solution should be in the range of 60-80 and 0.4-0.9, respectively [24].

Some fractions of particulate phosphorus (included in phytoplankton or minerals) react like dissolved phosphate. For this reason, the procedure of analysis of rich coastal waters must be preceded with the removal of particles before analysis, by filtration or centrifugation [25]. Another problem during phosphate sampling is associated with adsorption of phosphate on plastic and glass bottles (especially in brackish waters) and contamination in contact with the skin [25].

2.2 ANAIS – Autonomous Nutrient Analyzer In Situ

The spectrophotometrical *in situ* analyzer ANAIS developed in our team allowed simultaneous measurement of nitrate, phosphate and silicate. This instrument was suitable for the autonomous vehicle YOYO, profiler developed by the LODYC (Laboratory of Dynamic Oceanography and Climatology, now LOCEAN laboratory) in the framework of the European MAST III "YOYO 2001: The Ocean Odyssey". The YOYO vehicle was profiling along a cable stretched between 1000 meters and the surface with a range of 400 up-down cycles with the same principles of motion used by the ARGO floats (change of buoyancy, see Fig 3). This vehicle was also equipped with several other bio-optical, biological, physical and chemical sensors. This instrument was autonomous and capable of *in situ* measurements in pressure between 0 and 1000 m depth [26, 27].

The ANAIS analyzer represented on Fig. 4 is a set of:

1) Three chemical sensors (nitrate, silicate, phosphate) which contain:

   - A manifold where the chemical reaction takes place at a constant temperature maintained between 20 ° and 25 ° C by a heating resistor integrated into the manifold,

- A colorimeter, integrated into the manifold with characteristic wavelength for the studied analyte,
- Two clamping plates sealing the manifold and the fixing of pumps (pumps solenoids Lee Co.).

2) A container in which the three sensors mentioned above are fixed.
3) A set of bags for storing reagents and standard solutions.
4) An electronic card for the sensors' control and data storage (1 card for each salt), receiving control commands transmitted by a central brain outside (located within the YOYO body) and data transmission to the same central brain (one card called master card).

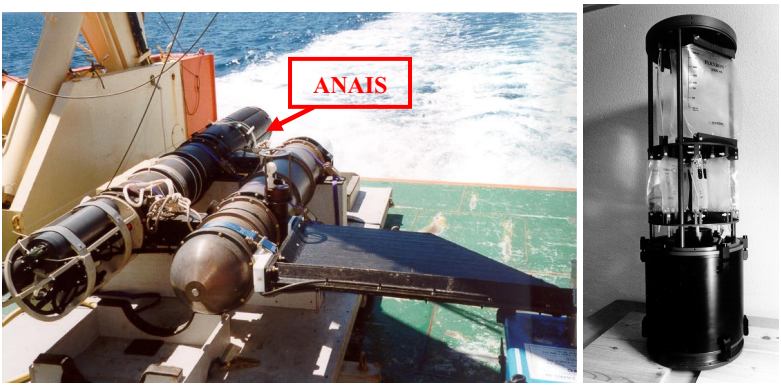

Fig. 4. ANAIS applied on YOYO vehicle (left), and the container with reagent bags and spectrophotometer (right)

The device offers an analytical measurement reproducibility of about 1%, and very good calibration results similar to those obtained with a Technicon colorimetric chain (conventional laboratory equipment).

A series of tests under real conditions, as part of the YOYO 2001 EC project milestones, was performed with ANAIS along offshore Blanes (Spain) and offshore Banyuls sur Mer in the Mediterranean Sea from 1998-2001 [28]. The results show that the standard deviation on ANAIS results is much lower than that achieved on the Niskin samples. This analyzer (only nitrate) was tested in 2003 off the shore of Argentina as part of the CLIVAR programme. The scientific objectives were articulated along two axes: 1) to monitor the Malvinas Current transport at 41 °S with a line of current meters on moorings under a track of the satellite altimeter JASON, 2) to study variations and characteristics of Antarctic Intermediate Water in the Argentine Basin [28, 29].

2.3 Other Spectrophotometric Analyzers

Many adaptations of classic colorimetric methods for the measurement of phosphate (silicate and nitrate, nitrite) have been developed for *in situ* analysis. Many of these

instruments are now commercially available (Fig. 5). The basic principle is an assembly of pumps, valves, manifolds and colorimetric detectors that can be combined to measure multiple analytes simultaneously. The calibration has to be done regulary and thus, bags with standards are needed. Characterization of some phosphate analyzers is presented in Table 1. The characterization is based on a few parameters like: range of measured chemicals, detection limit for phosphates, size and weight, and application of domain analyzer.

Both commercial and experimental analyzers are characterized by a good precision (1-3%) and high sensitivity. Detection limit is in the range of 10 to 100 nM. The analyzers can measure simultaneously all salts (phosphate, nitrate, nitrite, silicate). Some of them have a possibility of additional measurement of iron or sulphur (CHEMINI, SubChemPak Analyzer). Cycle-PO_4 analyzer is dedicated to phosphate measurements. Most of the analyzers are not resistant to high pressure (except ANAIS and CHEMINI) and can not go deeper than 10-200 m. Most of the analyzers can work autonomously for a few months, but require addition of reagents which can be unstable (ascorbic acid). They are usually large and heavy.

Although some analyzers were deployed in natural waters, there are not many examples of phosphate monitoring using these analyzers. Nitrate is the most commonly measured nutrient. For example, the *SubChemPak Analyzer* was installed on a winch-deployable, electronic profiling package. The profiling package also included a Sea Bird Electronics Model 25 Sea Logger CTD with modular sensors for the measurement of conductivity, temperature, pressure, dissolved oxygen, pH, chlorophyll fluorescence, and light transmission and irradiance.

Fig. 5. Commercially available *in situ* nutrients analyzers: Wet Labs Cycle-PO_4 (left) [30], Systea Deep-sea Nutrient Analyzer – DPA (center) [31], Systea Water *In situ* Analyzer – WIZ (right) [32]

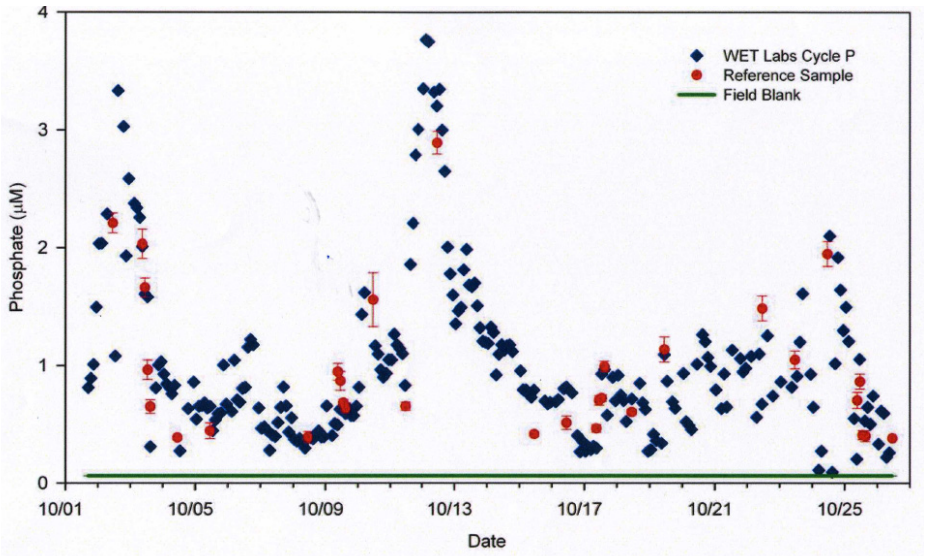

Fig. 6. Time series comparison of WET Labs Cycle-PO_4 measured phosphate concentrations against laboratory measured reference samples and field trip blanks for the Clinton River, MI moored deployment test [30]

The Wet Labs Cycle-PO_4 was successfully used at three different fixed mooring stations. The field tests were performed during 4 weeks at a fixed depth in coastal freshwaters (Clinton River), brackish waters (Chesapeake Bay), and seawater (Resurrection Bay). Phosphate concentrations at Chesapeake Bay were found in the range of 0.039-0.092 µmol L^{-1}, despite a significant contribution of river water to the site. Phosphate concentrations were higher at the Resurrection Bay (even 0.701 µmol L^{-1}) and the highest in the Clinton River (0.09-3.77 µmol L^{-1}) (Fig. 6). The same analyzer was used on CHARM (**CHA**nnel Island **R**elocatable **M**ooring) mooring at Hawai Ocean Time Series (HOTS) station in February-March 2004 at depth of 22 m. The phosphate there varied from 0.1 to 0.9 µmol L^{-1}.

3 Electrochemical Sensors – The Future of Phosphate Monitoring?

As an alternative to spectrophotometry, methods based on electrochemistry have also been proposed. Amperometric procedures have been reported for the determination of phosphate as phosphomolybdate complex [38-40]. Phosphate has been also determined by using voltammetric methods with carbon paste electrode [41], gold microdisk electrode [42] and glassy carbon electrode [43]. Different modifications were proposed to increase the sensitivity or eliminate the interferences from other coexisting molecules. Applications of these methods are proposed but only

Table 1. In situ analyzers for phosphate detection in seawater, detection limits only for phosphate

Name	***Laboratory/ Society***	***Detected chemicals***	***Detection limit***	***Size, mm***	***Weight in air, kg***	***Depth max. m***	***Applications***	***Literature***
ANAIS (Autonomous Nutrient Analyzer In Situ	*LEGOS*	*$Si(OH)_4$, PO_4^{3-}, NO_3^-+NO_2^-,*	*100 nmol L^{-1}*	*320(d)x1000*	*50*	*1000*	*Open ocean, coastal waters*	*[26, 27]*
NAS – 3X MicroLab EcoLab	*EnviroTech LLC*	*$Si(OH)_4$, PO_4^{3-}, NO_3^-+ NO_2^-,*	*50 nmol L^{-1}*	*246(d)x799*	*27*	*250*	*Bays, coastal waters*	*[33]*
DPA/NPA (Deep – sea/ Nutrient Probe Analyer	*Systea*	*$Si(OH)_4$, PO_4^{3-}, NO_3^-+NO_2^-,*	*1 ppb (10 nmol L^{-1})*	*320(d)1220*	*36*	*10-4500*	*Open ocean, coastal waters*	*[31)*
APP 4004	*Me Grisard Gmbh*	*$Si(OH)_4$, PO_4^{3-}, NO_3^-+NO_2^-,*	*np*	*-*	*8*	*7*	*Bays, coastal waters*	*[34]*
SubChemPak Analyzer	*SubChemSystems, Inc. /Wet Labs*	*$Si(OH)_4$, PO_4^{3-}, NO_3^-+NO_2^-, Fe*	*20-50 nmol L^{-1}*	*12(d)x635*	*6*	*200*	*Bays, costal waters*	*[35]*
CHEMINI (Chemical Miniutarized Analyzer)	*IFREMER*	*$Si(OH)_4$, PO_4^{3-}, Fe, H_2S, NO_3^-+ NO_2*	*np*	*120x140+ 140(d)x264*	*4*	*6000*	*Coastal waters, hydrothermal vents*	*[36, 37]*
Cycle – PO_4	*Wet Labs*	*PO_4^{3-},*	*75 nmol L^{-1}*	*560x180*	*6.8*	*200*	*Bays, coastal waters*	*[30]*

a few of them were used for natural analysis of water in laboratory conditions [39, 40, 44]. In spite of many advantages of voltammetric or amperometric methods, some serious problems may appear. In particular, electrode surface fouling by absorbing compounds, or oxidation by oxygen are the most important issues. Additionally, these electrochemical methods still require addition of acid and molybdate ions in order to convert electroinactive phosphate species into electroactive phosphomolybdate complex.

The potentiometric analysis of phosphate requires application of ion-selective electrodes (ISE). In these conditions, the water sample does not have to be pretreated. Numerous membranes are being used [45-49]. Some of them are dedicated to environmental water analysis but so far no *in situ* application of potentiometric sensors in seawater is described. Several issues affect potentiometric methods: the electrode response is often slow, interferences from other species are very common, and miniaturization is tricky especially because the potential is unstable when the electrode approaches micrometric dimensions. Finally, electrodes require very often calibration, sometimes before and after the measurement of interest.

Some teams work on electrochemical phosphate biosensors [50], but so far no *in situ* application is described in the literature. Their development is complicated and requires use of enzymes which are sensitive to the environment changes (temperature, ionic force) [51] and to the presence of heavy metals and pesticides which may inhibit enzyme activity [52].

3.1 ANESIS – Autonomous Nutrients Electrochemical Sensor In Situ

Recently, a totally new electrochemical method was developed in our group for determination of silicate [53, 54] and phosphate [55, 56] without addition of any liquid reagents. The method is based on the oxidation of molybdenum in seawater in order to form silico- or phosphomolybdate complex electrochemically detectable either by means of voltammetry or amperometry. Both methods were tested *in situ* offshore Peru (phosphate) and Drake Passage (silicate) (Fig. 7 and 8).

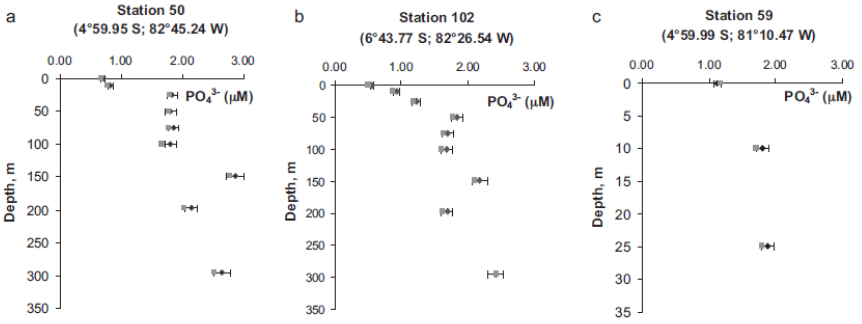

Fig. 7. Comparison of both methods, amperometry (black) and colorimetry (grey) at CTD stations 50 (a), 102 (b), 59 (c). Station 59 is situated very close to the coast and stations 50 and 102 more off-shore [55].

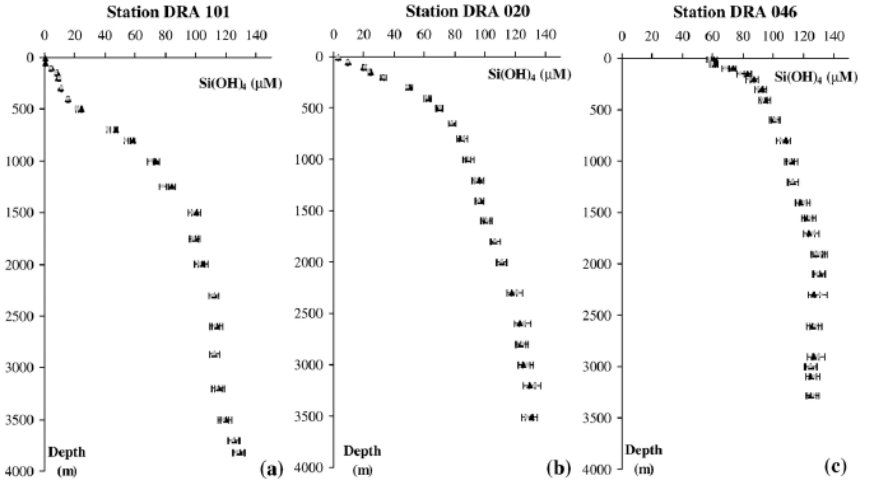

Fig. 8. Comparison of the two methods, voltammetry in black and colorimetry in grey at stations (a) DRA 101, (b) DRA 020 and (c) DRA 046 [53]

The results show excellent agreement with colorimetric classical analysis [53, 55]. The deviation between both methods is 4.9% for phosphate and 3% for silicate concentrations higher than 10 μM. The description below corresponds, however, only to the phosphate sensor.

The electrochemical determination of the non electroactive phosphate is based on the formation of a complex with molybdate. The complex is formed by the reaction with K_2MoO_4 in an acidic solution to form a Keggin anion [55, 56].

$$PO_4^{3-} + 12\ MoO_4^{2-} + 27\ H^+ \rightarrow H_3P(Mo_{12}O_{40}) + 12\ H_2O \quad (12)$$

In order to eliminate the addition of molybdate and protons, a reagentless method was developed. Molybdate salts and protons are produced as the product of molybdenum oxidation in the reaction cell due to the following reaction [55-57].

$$Mo + 4\ H_2O \rightarrow MoO_4^{2-} + 8\ H^+ + 6e^- \quad (13)$$

The created phosphomolybdate complex can then be detected on a gold working electrode by means of amperometry with the detection limit of 0.12 μM. The method described above has an obvious problem of cross interference in the ocean samples which contain both silicate and phosphate. A few methods to avoid these interferences are tested using differences in electrochemistry of the two complexes, influence of pH on complex formation and differences in kinetics of complex formation by choosing an appropriate ratio of protons over molybdates of 70 [55]. The latter one was used during the Pelagico 1011-12 cruise aboard the R/V OLAYA offshore Peru. The results show great potential in the developed method for phosphate detection but it still required extra addition of acid to achieve an appropriate ratio of protons over molybdates (70). According to reaction (13), the ratio of protons over molybdate is 8 during the oxidation of molybdenum. At the beginning, we achieved an appropriate

ratio of 70 by addition of acid. Recently, we developed a method where the desired ratio of protons over molybdate is achieved without addition of any reagents. This method is based on the use of specific membrane technology and a special design of the electrochemical cell made by GIS laboratory (Groupe d'Intrumentation Scientifique, Observatoire Midi-Pyrenées, Toulouse) [56]. The cell consists of 3 compartments. In the first one (1 mL), a primary molybdenum electrode is oxidized

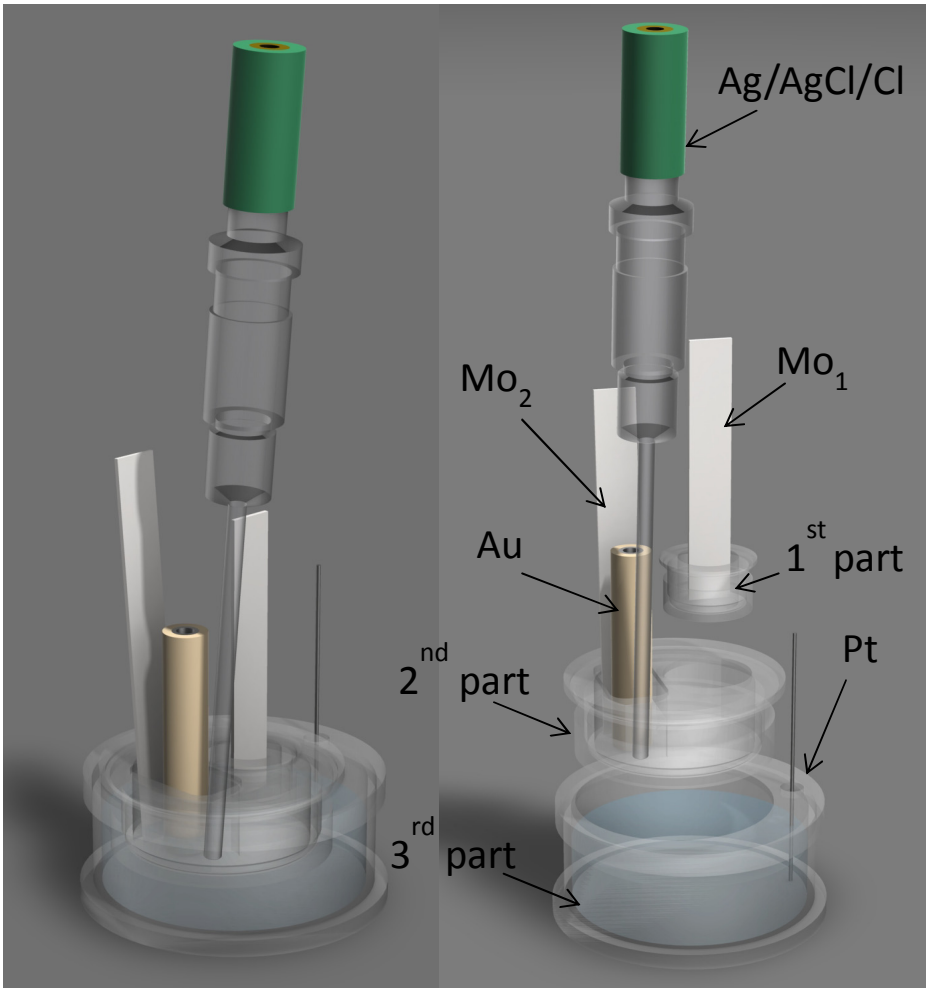

Fig. 9. Schematics of the electrochemical cell with application of membrane technology (Ag/AgCl/Cl-reference electrode, Au-gold working electrode, Pt-platinum counter electrode, Mo_1-first molybdenum electrode, Mo_2-second molybdenum electrode, 1st part-first compartment of the cell with the proton exchange membrane, 2nd part-second compartment of the cell with the non-proton exchange membrane, 3rd part-third compartment of the cell, simulating the open ocean) [56]

and thanks to a proton exchange membrane only protons can pass through to the second compartment and thus acidify the media to pH 1. In the second compartment (5 mL), a secondary molybdenum electrode is oxidized during a short time to produce 1.5 mM of molybdates achieving a ratio of protons over molybdates of 70. The third compartment is in contact with previous parts of the cell by a non-proton exchange membrane where a platinum cathode is immersed to avoid the reduction of protons formed during the two previous oxidations of molybdenum (Fig. 9).

The complex is detectable in the second compartment by means of amperometry or differential pulse voltammetry and the detection time is about 15 min. The detection time can be much shorter by decreasing the volume of the cell. The detection limit is 0.11 μM for amperometry and 0.19 μM for differential pulse voltammetry. The reproducibility tests show average precisions of 5.7% (amperometry) and 3.8% (differential pulse voltammetry). Results show also quite good deviation with an average of 4.3% (amperometry) and 5.0% (differential pulse voltammetry) as compared with colorimetric measurements [56].

For the future phosphate sensor development, it will be necessary to continue monitoring the signal stability during a longer time (a few months) and to establish a protocol for *in situ* cleaning of the gold electrode. Next steps will be to deal with development of the mechanical and electronic parts of the sensor. Indeed, the measurement must be performed in a closed volume implying to sample accurately the sea water. At the same time, a calibrationless method should be developed to make the sensor fully autonomous [58]. The sensor will be characterized by a low power consumption and short response time due to the reduced volume combined with application of a large membrane surface. Miniaturization is also possible thanks to advances in microsystem technology (MST) [57]. MST can be used to miniaturize both spectrophotometric and electrochemical sensors for phosphate detection but for the moment this technology is used mostly for reagent-based protocols [60]. Currently, a close collaboration with LAAS laboratory (Laboratoire d'Analyse et d'Architecture des Systèmes, Toulouse) will allow us to develop a "lab-on-a-chip" microdevices for phosphate and silicate electrochemical detection using silicon and polymer technologies [61]. The adaptation of the sensor on autonomous vehicles profiling the water column (ARGO, AUV) would be the next step. Finally fixed eulerian moorings can allow the acquisition of long data time series at a fixed station. Indeed, within the AMOP project (Activities of research dedicated to the Minimum Oxygen Zone in the eastern Pacific), a shallow (200m) mooring will be deployed next January 2013 offshore Callao, Peru, along 12°S at the IMARPE historical station N°5 for a 3 years period. It is planned that our phosphate sensor will be adapted on the mooring line as soon as possible.

4 Summary

Monitoring of oceanic biogeochemical cycles at proper spatial and temporal scales requires miniaturized *in situ* sensors with excellent figures of merit. Development of the marine sensors is even more complicated due to harsh and difficult access to the

oceanic environment. In spite of the large number of requirements, some very good sensors actually exist and new ones are currently being developed.

Phosphate monitoring is based on wet chemical analyzers. Analyzers have good accuracy, reliability and can measure a few analytes at the same time. However, phosphate detection is based on addition of liquid reagents to the sample and thus analyzers contain reagents and standards bags which make them large and heavy. There is also the issue of stability of the reagents and of high energy consumption of the analyzers. Actually, many teams work on electrochemical sensors for phosphate detection. They are few voltammetric and amperometric sensors for phosphate detection but none of them has been applied *in situ.* Moreover, they still require addition of liquid reagents to transform electroinactive phosphate species into an active phosphomolybdate complex.

Recently, a new electrochemical possibility to detect phosphate is developed in our team. The method is based on oxidation of molybdenum in order to convert phosphate ions into an electroactive phosphomolybdate complex. The method requires application of an electrochemical cell with use of membrane technology. The method is still under investigation but already, the method was validated for natural seawater samples and the results show excellent comparison with classical colorimetric method. If needed, the sensor can be optimized for other applications such as natural freshwaters or waste waters.

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