

ELECTROCHEMICAL PHOSPHATE DETECTION IN OLIGOTROPHIC SEAWATER WITH A STAND-ALONE PLASTIC ELECTRODE

Federico Figueredo¹, Federico Girolametti², Silvia Illuminati², Cristina Truzzi²,
Anna Annibaldi², Sabina Susmel^{1*}

¹Department of Agricultural, Food, Environmental and Animal Sciences-Di4A, University of Udine,
Via del Cotonificio 108, 33100 Udine (Italy), phone +39 0432558823,
e-mail: sabina.susmel@uniud.it

²Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona (Italy)

Abstract – Nitrogen, phosphate and silicon are the main nutrient limiting and stimulating the planktonic primary production. Phosphate can vary from nanomolar to micromolar levels in ocean waters. However, oligotrophic systems are characterized for having nanomolar concentrations of phosphate, nitrogen and silicon. The northern Adriatic Sea is a particular water system, in which the levels of nutrients are commonly low or unbalanced with an abundance of nitrogen inflow both from rivers and water runoff being probably behind several eutrophication events previously reported. Laboratory methods and classical analytical equipment can be used to detect nutrient levels (in particular phosphate) with high precision and sensitivity, however, they are expensive, time cost and lack in portability. So there is a great need to develop analytical systems or new methods to detect nitrate, phosphate and silicon in oligotrophic seawaters as early diagnosis and in-field of the quality of the sea water. In general, phosphate detection can be done with the classical molybdenum-blue method. However, the method cannot be used in oligotrophic seawater samples due to its low sensitivity and high interference problems. Electrochemical methods are often proposed for in field detection to overcome some of the drawbacks as turbidity of the sample and optical interferences. In this study, we present a new method, characterized by the advantage of both simplicity and low cost, based on the application of a plastic conductive electrode containing a molybdenum reagent embedded. The original non-conventional electrode was characterized by surface electron microscopy, energy dispersive X-ray analysis and electrochemical methods. The sensitivity obtained for phosphate detection was high enough to detect this nutrient in oligotrophic seawater systems.

Introduction

The northern Adriatic is the slope region from the Venice-Trieste shoreline to the line connecting Ancona, on the Italian side, of Zadar, on the Croatian coast [1]. It is a semi-enclosed basin with limited water circulation and smoothly flow current being strongly influenced by fresh-water discharge from rivers (as Po in Veneto and Isonzo in Gulf of Trieste). Particularly, phosphate, the available form of phosphorous (P) is essential to all life forms, however, in high concentration induces eutrophication (10 ppb of phosphate is a concentration high enough) [2]. Excessive nutrients inflow of phosphorous as well as nitrogen, is typically consequence of industrial, agricultural or domestic waste runoff [3]. With the aim of environmental protection, water policies set limits for nutrients including phosphate

(i.e. 2 ppm in treated urban wastewater) [2]. However, oligotrophic conditions are now noticed in the northern Adriatic. In fact, in central and southern Adriatic a low nutrient concentration on shallow waters has been detected, in the northern area the nutrient profiles are characterized by high surface concentrations of nitrates, vertically decreasing down to a depth of about 5–10 m, while an opposite trend of the salinity distribution indicates the influence of the Po river inputs. Despite this large river input, the northern Adriatic basin has been proved to be phosphorus limited. The cyclonic circulation effect driving nitrate rich waters southward determines a horizontal gradient of this nutrient, with high levels decreasing from west to east. This pattern is not present for phosphate, which show increasing levels in the centre of the basin, with high coastal depletion due to an intense phytoplankton activity [4].

For these reasons within the framework of the European project “AdSWiM, Managed use of treated urban wastewater for the quality of the Adriatic Sea”, a study on nutrients level and ratio is in progress.

In the analytical field, electrochemical sensors [5, 6] and biosensors are proposed since they are less affected by interferences, can be miniaturized and adapted for on-site detection. The standardized method for DIP detection is based on the colour change of a phosphomolybdate complex, commonly known as molybdenum-blue method. This method is not practical for in field detection since the reaction between the molybdate anions (from a molybdate ion stock solution) and the DIP is accomplished in solution and involve an incubation step for at least 30 min. In particular, for surface seawater samples, molybdenum-blue method is not sensitive enough. In this study, as a first approach for the electrochemical DIP sensor development, a plastic electrode based on graphite, PVC [7] and molybdate anions was prepared. Before the electrode preparation, Northern Adriatic surface waters were characterized and the nutrient levels were quantified during an entire season by classical instrumental approaches. Once the nutrient level baseline was determined, the electrode was prepared and optimized to be used as a phosphate sensor.

Material and methods

Spectrophotometric determinations were done to determine the level nitrate, nitrite, ammonium, phosphate, silicate and dissolved organic phosphate (DOP) in different sites of northern Adriatic (Fig. 1). Nutrients were analysed with a UV-VIS spectrophotometer (Systea EASYCHEM Plus) using standardized method to detect nitrite (EPA Method # 354.1), nitrate (EPA Method # 354.1), ammonia (APHA Standard Methods for the Examination of Water and Wastewater 4500 NH₃ G Automated Phenate Method), silicon (APHA Standard Methods for the Examination of Water and Wastewater 4500 SiO₂) and orthophosphate (ISO 15923).

Tetrabutylammonium octamolybdate (TBA₄Mo₈O₂₆) is a molybdate polyoxometal derivative soluble in non-aqueous solvents that can be synthesized starting from a commercial salt of molybdate. The synthesis procedure was based on the protocol published by Klemperer (1990) [8]. Briefly, 1 g of Na₂MoO₄·2H₂O was dissolved in 2 mL of distilled water. Then, 1 mL of HCl 6M was added when it was stirring in a 10 mL baker. After 10 min, 2 mL of a solution containing 0.78 g of tetrabutylammonium bromide was added and a white precipitate was obtained. The solution was collected in a porosity filter with suction and washed with 20 mL of distilled water, 20 mL of ethanol, 20 mL of acetone and 20 mL of

diethyl ether. The air-dried product was then dissolved in 40 mL of acetonitrile and cooled at -20°C . After 24 h a colorless crystalline powder was obtained.

The plastic electrode containing octamolybdate anions (Mo-PE), was prepared following our previously reported procedure [7] with minor modifications. Briefly, 200 μL of acetonitrile is used to dissolve 10 mg of $\text{TBA}_4\text{Mo}_8\text{O}_{26}$. The $\text{TBA}_4\text{Mo}_8\text{O}_{26}$ solution is mixed with 10 mL THF and then is drop into a 25 mL baker containing 380 mg of synthetic graphite, 50 mg of PVC and 50 μL of BEA. After stirring for 30 min at room temperature, the homogeneous slurry is transferred into a glass tray (6 x 6 cm) and allowed to dry overnight under the hood at room temperature. A double sided tape was used to stick the upper side of the obtained conductive material with a PET film (150 μm) and a paper punch was used to cut the material and obtain the Mo-PE. Electrodes prepared with 10 mg of $\text{TBA}_4\text{Mo}_8\text{O}_{26}$ was named as Mo-PE.

All electrochemical measurements were carried out with Gamry Reference 3000 potentiostat/galvanostat (USA). A graphite bar and Ag/AgCl/KCl (3M) electrodes were used as counter and reference electrodes, respectively.

Results

Preliminary spectrophotometric data collected on samples from April to October of 2019 show nutrients levels (min-max) as follow: N-NO_3 $3.8 \div 107$ ppb; N-NO_2 $5.6 \div 10$ ppb; N-NH_4 $+ 0.7 \div 12$ ppb; DIP $0.2 \div 1.9$ ppb; DOP $12 \div 47$ ppb and Si-SiO_2 $3.2 \div 27.5$ ppb. On the sea side nutrients levels are very low, with no statistically differences between the two sampling points close to Natura 2000 site of Marano's Lagoon and outfall in seawater (Fig. 1).

The conductive plastic material was prepared as a thin film of about 300 μm and the presence of octamolybdate was appreciated with SEM techniques. In Figure 2 it is possible



Figure 1 - Map of the sampling area in Marano's Lagoon (Adriatic Sea, Italy).

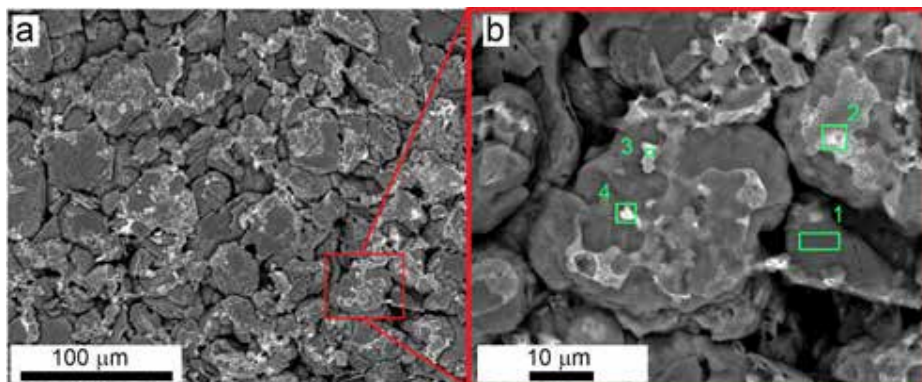


Figure 2 - (a) SEM picture showing the surface of the Mo-PE electrode. (b) High magnification SEM picture taken from the red square area marked in picture “a”, green squares show places where EDS analysis was done.

Table 1 - Results of EDS analysis performed at the Mo-PE electrode surface. The analysis was conducted in the sites marked with green squares in Figure 2b.

Spectrum label	Site 1	Site 2	Site 3	Site 4
C	100 %	79.77 %	90.97 %	93.44 %
O	0 %	13.4 %	5.12 %	2.64 %
Mo	0 %	5.33 %	3.01 %	2.43 %
Cl	0 %	1.51 %	0.90 %	1.49 %

to observe that the molybdate is dispersed over the electrode surface as a discontinuous film and after exploring several preparations, it was confirmed that no crystal was evident but just an amorphous dispersion. The energy dispersive X-ray analysis (EDS) confirmed that the film detected over the plastic surface contains molybdenum and oxygen, also by comparison with places without coverage where only carbon was detected. (Figure 2b and Table 1).

The Mo-PE film (Figure 3a) was cut to obtain the electrode to use as working electrode in a three electrode electrochemical cell. Cyclic voltammetry was conducted in solutions containing different concentrations of sulphuric acid (0.1, 1 and 5 M), NaCl 0.6 M and 100 ppb of phosphate. As can be seen in Figure 3b, the concentration of sulphuric acid is important since the reactivity of octamolybdate towards phosphate increase with the concentration of acid. When sulphuric acid concentration of 5 M was employed, three oxidation peaks and two reduction peaks were observed. However, just one oxidation peak appeared for concentrations of sulphuric acid of 1 M and no peaks were appreciated for concentrations of 0.1 M [8, 9]. Later, the effect of other acids as HCl was evaluated but in contrast with sulphuric acid, at higher concentrations (5 M) no defined peaks were detected (Figure 3c) [9]. Cyclic voltammetry experiments at different scan rates were performed (Figure 3d) to study the mechanism behind the electrochemical reaction taking place at the surface of the electrode. The curves obtained in solutions containing sulphuric acid 5 M, NaCl 0.6 M and phosphate 100 ppb show that there is not a direct relationship between peak signal and the

scan rate which suggest that the electrochemical reaction is independent of the diffusion of any component of the solution employed such as protons or phosphate, among others.

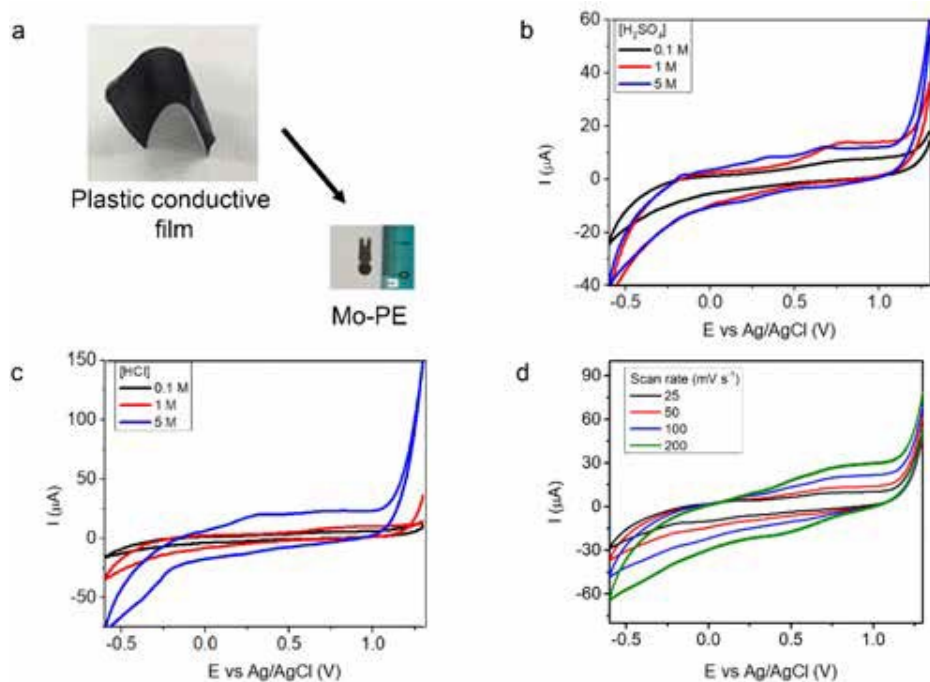


Figure 3 - Electrochemical characterization of Mo-PE electrodes. (a) Digital picture of the plastic conductive film used to prepare the Mo-PE electrode. (b) Cyclic voltammograms (50 mV/s) conducted in solutions containing H_2SO_4 0.1 M (black lines), 1 M (red lines) or 5 M (blue lines). (c) Cyclic voltammograms (50 mV/s) conducted in solutions containing HCl 0.1 M (black lines), 1 M (red lines) or 5 M (blue lines). (d) Cyclic voltammograms conducted in solutions containing H_2SO_4 5M at (black lines) 25 mV s^{-1} , (red lines) 50 mV s^{-1} , (blue lines) 100 mV s^{-1} and (green lines) 200 mV s^{-1} . All solutions employed contain NaCl 0.6 M (35 g/L) and 100 ppb of H_2PO_4^- .

To explore the sensitivity of the electrode towards phosphate, we decided to employ sulphuric acid 5 M and prepare solutions with different amounts of phosphate. As can be seen in the Figure 4a, when the orthophosphate concentration increase, reduction and oxidation peaks increase too. In particular, we obtained a good response for the reduction peaks obtained at around 0 V. In the Figure 4b the results of the analysis of this peak for three electrodes in the range of phosphate concentration studied, are reported. The lineal range obtained for the reduction peak at 0V is 0.1-1 ppb (Peak current = $4.82 [\text{H}_2\text{PO}_4^-] + 9.59$, $R^2 = 0.980$) and the limit of detection (LOD) calculated (3σ) is 0.16 ppb.

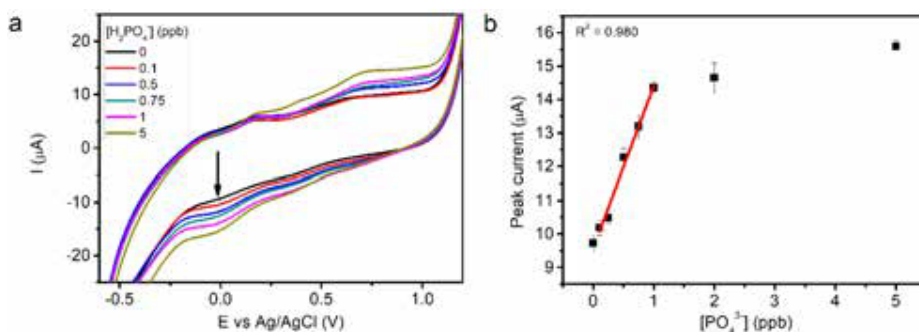


Figure 4 - (a) Cyclic voltammetry performed with different concentrations of phosphate (b) Analytical curve showing the detection range and the lineal range of detection.

Discussion

The nutrient analysis performed between April and October of 2019 show that the Northern Adriatic behave as an oligotrophic system. Lazzari et al. (2016) detected orthophosphate concentrations in northern Adriatic below 2 ppb which is close to those values obtained at the Mediterranean Sea [10]. The interesting fact is that the amount of nitrate is very high in comparison with Mediterranean Sea, which means that a possible eutrophication process can happen if the orthophosphate concentration increase since this last one is the most important limiting nutrient for this oligotrophic system. Among all the nutrients, inorganic phosphate levels are very low (0.2-1.9 ppb). The molybdenum-blue method is the gold standard method for in field detection but for oligotrophic systems some problems arises such as low sensitivity, low kinetic of complex formation and interference from sample turbidity. In this sense, there is an important need to develop new methods for in field detection comprising high sensitivity, fast response and low cost.

The proposed technology developed in this study is based on an electrochemical detection system employing the molybdenum chemistry. For these, a plastic electrode containing a non-aqueous soluble molybdenum salt (octamolybdate) [11] was constructed and tested with simulated oligotrophic seawater samples. After the electrode was prepared, the presence of octamolybdate was identified as a discontinuous film covering the surface of the electrode (Figure 2). Since the main hypothesis behind this study is that the octamolybdate at the surface of the electrode can react with soluble phosphate in the presence of protons to form phosphomolybdate at the interface electrode-water, we performed electrochemical analysis to analyze the effect of the acid. The results obtained suggest that the sulphuric acid is better than chlorhydric. Apparently, the reaction is favorable with sulphuric acid concentrations of 5 M, evidenced in the cyclic voltammogram by the presence of oxidation and reduction peaks (Figure 3b). The voltammogram shape suggest that the reaction produce the phosphomolybdate complex and the formation is accomplished in less than 5 min. In addition, the reaction happens at the surface of the electrode and is not enhanced by the diffusion of any solution component as can be seen in the Figure 3d.

The electrochemical reduction peak signal obtained at 0 V increases when phosphate is added to the sample at ppb levels. These results were confirmed through cyclic

voltammetry showing the possibility to use this technology to detect and quantify DIP in seawater at short incubation times (less than 5 min) with high sensitivity. The LOD obtained is low enough to detect directly the phosphate present in Northern Adriatic surface samples, or similar oligotrophic systems.

Conclusion

A new electrochemical method for phosphate detection was developed. The measurements were done in seawater simulated samples taking into account the phosphate levels detected in the Northern Adriatic surface samples. The method developed is fast and sensitive. The low cost fabrication of the electrodes as well the portability of the technology opens the possibility to use this method to monitor the phosphate levels in oligotrophic seawater samples. However, interferences studies and real seawater sample analysis need to be studied in detail to ensure a practical use of the electrode.

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