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Talanta 63 (2004) 567–574
Talanta
www.elsevier.com/locate/talanta

Investigation of amperometric detection of phosphate Application in seawater and cyanobacterial biofilm samples

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Received 4 July 2003; received in revised form 30 October 2003; accepted 21 November 2003

Available online 1 February 2004

Abstract

Detection of phosphate using amperometry was investigated. The phosphomolybdate complex, formed by addition of nitric acid, ammonium molybdate and phosphate, was reduced at a carbon paste electrode polarised at +0.3 V (versus Ag/AgCl). The major characteristics observed were simplicity of the equipment, a limited consumption of reagents and a low detection limit (0.3 μmol l^{-1}), with a linear range between 1 and 20 μmol l^{-1}. The interference of silicate was completely eliminated using an appropriate concentration of nitric acid and ammonium molybdate.

The amperometric detection of orthophosphate in seawater using the batch injection analysis (BIA) technique was reported.

Moreover, a carbon paste microelectrode was constructed. Its use allows the analysis of small volume of samples with little dilution in supporting electrolyte. This method was applied to the determination of orthophosphate in cyanobacterial biofilms collected from Roman catacombs. There was a good statistical correlation between results obtained with the proposed method and the standard spectrophotometric method.

Keywords: Amperometry; BIA; Orthophosphate; Seawater; Carbon paste microelectrode; Cyanobacteria

1. Introduction

Phosphate is one of the most important electrolytes and an essential component of all living organisms [1], organic phosphate forming a part of ATP, nucleic acids and of the macromolecular cell membrane structure of most organisms.

Phosphate plays a crucial role also in the environment, being responsible for the phenomenon of eutrophication, water pollution caused by excessive plant nutrients. This process can be greatly accelerated by human activities that increase nutrient loading rates to water, and especially by phosphorus (P) inputs [2,3]. Phosphorus, in fact, is an essential element for plant and animal growth and its input has long been recognised as necessary to maintain profitable crop and animal production.

Therefore, P is often the limiting element, and its control is of prime importance in reducing the accelerated eutrophication of fresh waters. As an example, lake water concentrations of P above 0.02 ppm generally accelerate eutrophication. These values are one order of magnitude lower than P concentrations in soil, which are critical for plant growth (0.2–0.3 ppm), emphasising the disparity between critical lake and soil P concentrations and the importance of controlling P losses to limit eutrophication.

Another field where the P control is assuming an increasing importance is the protection of the cultural heritage. It was hypothesised that phosphate plays a major role in bio-deterioration of archaeological sites caused by cyanobacterial biofilms. In fact, a great variety of microrganisms colonising stones can be found both on and beneath surfaces in indoor or outdoor environments. These microrganisms develop not as single colonies but they are components of complex systems called microbial mats or biofilms. In particular, in archaeological hypogea, the abundance of nutrients in the lithic substrata, the input of compounds

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doi:10.1016/j.talanta.2003.11.040

from circulating air and percolating waters, and the high humidity combined with the presence of artificial illumination, provide a suitable niche for those photosynthetic microrganisms that can make use of the spectral emission of lamps [4]. Cyanobacteria are photosynthetic microrganisms that, thanks to their peculiar ability to adapt to extremely low photon flux densities and to acclimate to a variety of spectral emissions, are the major organisms responsible for biofilm formation on any rock surface (i.e. mortar, bricks, marble, frescoes, stuccoes, mosaics, etc.) exposed to light, causing aesthetic, physical and chemical damage [4]. At the same time, the availability of organic matter, produced via cyanobacterial photosynthesis and N_2 fixation, supports the growth of heterotrophic microrganisms (bacteria and fungi, associated in the biofilm), which are able to use the organic matter produced by phototrophs for releasing acidic organic compounds and solubilise the minerals of the substratum [5]. So, macronutrients such as nitrogen and phosphorus may be mobilised from the substratum and metabolised or stored in the cell [6]. Generally, the 0.6% of the dry mass of the cells of cyanobacteria consist of phosphorus, that is generally taken up as orthophosphate [7], the most stable form of inorganic phosphate. Inorganic phosphate is involved in almost all high energy transfer reactions and plays an essential role in the metabolism of calcium.

Spectrophotometric methods, based on colorimetric detection, have been widely applied for the determination of orthophosphate [8,9]. Basically, this method involves the treatment of the sample with an acidic molybdate solution to produce a phosphomolybdate complex which is further reduced by ascorbic acid to an intensely coloured product. As a routine analytical method, this chemistry is carried out in a flow injection analysis (FIA) mani-fold [8,10]. This method requires a careful selection of experimental conditions and can not be applied to turbid samples.

FIA and spectrophotometric techniques, commonly used for the measurement of phosphate in the laboratory [10], are not suitable for on-site testing and monitoring. It is highly desirable to be able to transfer these measurements from the central laboratory to “in situ analysis”. The portable nature and the excellent sensitivity of electrochemical techniques make them very attractive for monitoring in the field.

Amperometric procedures have been reported for the determination of phosphate as phosphomolybdate complex [11,12]. Phosphate has been determined also by using voltammetric methods at carbon paste electrode [13], gold microdisk electrode [14] and glassy carbon electrode [15]; the voltammetric signals obtained, measured the reduction of the phosphomolybdate complex and the detection limit was about a few micromoles per litre.

A voltammetric procedure was also given for determination of phosphate as molybdovanadophosphate at glassy carbon electrode [16]. In this case, phosphate can be determined at 1 $\mu mol\,l^{-1}$. Best sensitivities were obtained for phosphate analysis by FIA, the detection limit at a signal to noise level of 2:1 is 0.02 $\mu mol\,l^{-1}$ orthophosphate [11]. However, in this work, the use of organic solvents is required.

Electrochemical biosensors for determination of inorganic phosphate were also reported [17–21]. These sensors usually require more than one enzyme (i.e. pyruvate oxidase [17], xantine oxidase and nucleoside phosphorylase [18,21], phosphorylase A, phosphoglucomutase and glucose 6-phosphate dehydrogenase [19], maltose phosphorylase, acid phosphatase, glucose oxidase and mutarotase [20]) and a careful control of the activity and the stability of the enzymes used.

The measurement of electroactive species using the batch injection analysis (BIA) technique is particularly attractive for monitoring environmental pollution problems [22]. This technique has been applied to various types of electroactive species, including trace heavy metal ions at the nanomolar level [23]. BIA is a newly developed analytical technique, which involves the injection of small volumes of an analyte in solution. The response to the injection is similar to FIA, except that in BIA technique there is no continuously flowing carrier solution. A selected volume of sample is injected directly over the centre of a disk electrode immersed in electrolyte held at an appropriate applied potential. At common delivery rates of 25–75 $\mu l\,s^{-1}$, it takes 1.3–4 s to dispense the sample. The current rapidly reaches its maximum value directly related to the concentration of species, and then tails off after the injection.

The aim of this study was to develop a simple, rapid and selective method for determination of orthophosphate in real samples. The uses of BIA and carbon paste microelectrode are highlighted. In the first part of this work batch measurements are reported, followed by BIA, including application to sea water samples, and measurements of orthophosphate in cyanobacterial biofilms collected from Roman Catacomb.

2. Experimental

2.1. Apparatus and equipment

Amperometric batch measurements were carried out with BAS LC-3D electrochemical detector (Bioanalytical Systems) and a Yokogawa 3025 X-Y recorder, under constant stirring conditions (300 rpm). Cyclic voltammetry experiments were performed with an Autolab PGSTAT10 System (Ecochimie). The three-electrode system consists of a working electrode (carbon paste or glassy carbon), a SCE reference electrode or Ag/AgCl (3 $mol\,l^{-1}$ KCl) and a platinum auxiliary electrode.

A large open wall-jet cell modified for BIA was used as described previously [24]. Samples were injected by a programmable electronic micropipette. Spectrophotometric measurements were carried out with 6100 JENWAY spectrophotometer.

Tungsten wire (diameter = 0.1 mm) and silver wire (diameter = 0.5 mm) were from Goodfellow (Cambridge, UK). Borosilicate glass capillaries (i.d. = 0.84 mm) were from World Precision Instruments Inc. (Berlin, Germany).

2.2. Reagents

Ammonium molybdate, sodium meta-silicate and sodium dihydrogen phosphate used were of analytical grade. Aqueous solutions were prepared with doubly distilled water. Graphite (1–2 μm) was from Aldrich (USA).

2.3. Procedures

Glassware was washed with 20% hydrochloric acid then rinsed with distilled water. All experiments were carried out at room temperature (23±1 °C). Each individual experiment was performed at least thrice, and then results were averaged.

2.3.1. Glassy carbon electrode

Glassy carbon (BAS, 3 mm diameter) electrode was polished with alumina powder, starting with 5 μm particle size and then with finer grades down to 1 μm. The electrode was then rinsed with distilled water and sonicated for 10 min.

2.3.2. Carbon paste preparation

Graphite powder was cleaned with acetone, rinsed with water, and then activated with aqua regia for 30 min. After this treatment, the particles were washed with distilled water until a neutral pH was reached and then dried at 400 °C for 4 h [25]. The Carbon paste electrode was prepared mixing 1.6 g of graphite powder and 0.4 g of mineral oil using a mortar and pestle.

2.3.3. Carbon paste macroelectrode

The paste was then incorporated into the electrode cavity (laboratory made, 2 mm diameter) and was polished on smooth paper. Electrical contact was made by means of stainless steel wire.

2.3.4. Carbon paste microelectrode

The carbon paste microelectrode was also assembled (Fig. 1). The carbon paste was packed into 20 μm tip of glass capillary tubes. Glass capillaries were pulled by using a Narishige pp-83 puller (Japan). Electrical contact was established via a tungsten wire (diameter = 100 μm) embedded into the carbon paste. Tungsten was chosen because of its greater hardness in comparison with a silver wire of similar diameter, allowing a better electric contact with the paste in the capillary. As reference electrode, a silver wire (diameter = 500 μm) was oxidised at constant potential for 20–30 min in a saturated potassium chloride solution, then introduced into another capillary tube. Both the prepared carbon paste microelectrode and the Ag/AgCl reference electrode were placed into a stainless steel tube used as counter electrode.

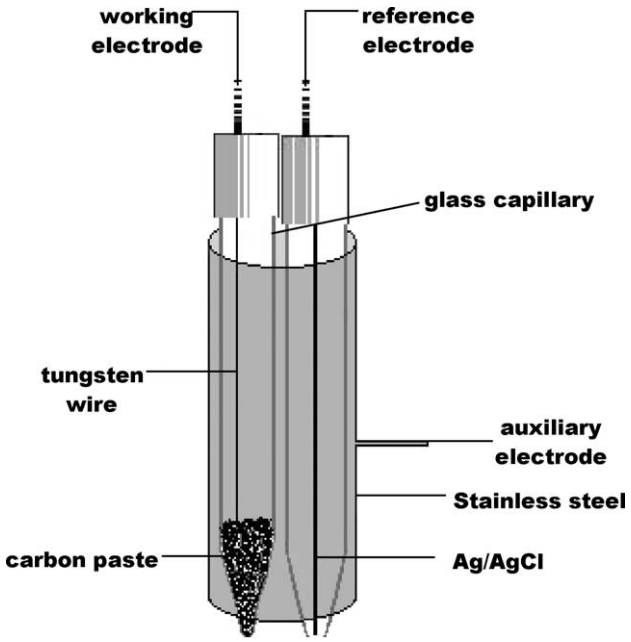

Fig. 1. Scheme of the carbon paste microelectrode.

2.3.5. Batch measurements

Batch measurements were performed using a 10 ml cell volume. A few microliters of phosphate stock solutions 0.01 or 0.001 mol l^{-1} were injected into the cell supporting electrolyte containing nitric acid and ammonium molybdate, reaching final concentrations between 1 and 100 μmol l^{-1}. Following each injection, a steady-state current was obtained and recorded. A 3 ml cell volume was used in the case of batch measurements with micro-electrode.

2.3.6. Batch injection analysis (BIA)

For BIA, the cell of volume 40 ml [24] was filled with 0.2 mol l^{-1} nitric acid as electrolyte. This cell was made of plexiglas. The working disk electrode was inserted through a hole drilled in the bottom of the cell. The cell cover contained four holes, one hole was placed directly above the centre of and perpendicularly to a disk electrode and served for accommodating the programmable pipette (Eppendorf, Research Pro). Two other holes were used to support the counter and the reference electrode [24].

Injection were performed using a programmable electronic micropipette, allowing injections in the range from 5 to 100 μl. BIA experiments were done by injecting 70 μl of analyte volume. In this case, the response was recorded as a peak current using BAS LC-3D electrochemical detector (Bioanalytical Systems) and a Yokogawa 3025 X-Y recorder.

2.3.7. Sea water analysis

Seawater samples were collected from the coast of Mohammedia City (Morocco) and filtered through membrane filters of 0.45 μm pore size and then analysed by amperometric batch injection (BIA) and standard spectrophotometric method [8].

2.3.8. Cyanobacteria biofilms analysis

Cyanobacterial biofilm samples were collected from two Roman hypogea, St. Callistus and Domitilla (Roma, Italy). The dried samples were homogenised with a mortar and a pestle. Therefore, 100 mg of the sample was put in bottles and 3 ml of the (0.2 mol l^{-1} nitric acid/0.1 mol l^{-1} KCl) solution was added and stirred for 1 min using a vortex and centrifuged for 10 min at 10,000 rpm. 60 μl of supernatant liquid was added to a 3 ml cell solution containing 0.2 mol l^{-1} nitric acid/0.1 mol l^{-1} KCl/1 mmol l^{-1} of ammonium molybdate. Another aliquot of 100 μl of supernatant was also analysed using standard spectrophotometric method. This method is based on reduction of phosphomolybdic acid by ascorbic acid to intensely coloured molybdenum blue [8].

3. Results and discussion

3.1. Batch analysis

3.1.1. Selection of electrode material and applied potential

Fig. 2 shows typical cyclic voltammograms of molybdate and phosphomolybdate at glassy carbon and carbon paste electrodes in aqueous solutions containing 0.2 mol l^{-1} nitric acid and 2 mmol l^{-1} molybdate in the absence and in the presence of 0.1 mmol l^{-1} phosphate. A low current response was obtained when cyclic voltammetry was carried out with solution containing only nitric acid and molybdate. The addition of phosphate gives rise to a high current with the two electrodes tested. The best response in terms of voltammogram shape and low background signal was obtained with the carbon paste electrode (CPE). Therefore, the CPE was selected for the rest of the work.

Two consecutive quasi-reversible reductions are observed in the potential range studied. The first reduction peak (0.32 V versus SCE) is smaller than the second is (0.17 V versus SCE). The reduction peak at 0.1–0.2 V was large but difficult to reproduce due to an apparent adsorption phenomenon [11]. In order to choose the best potential to be applied for amperometric determination of phosphate, a hydrodynamic voltammogram was recorded in the potential range 0.5–0.1 V versus Ag/AgCl (Fig. 3). Despite of the high current response at 0.1–0.2 V, we observed high noise of molybdate reduction and baseline drift at these applied potentials (0.1–0.2 V). Since the current response was stable at +0.3 V versus Ag/AgCl, this potential was adopted for further study.

3.1.2. Effect of acid solution

The current reduction at fixed potential (+0.3 V) of 0.43 μA obtained with 0.1 mmol l^{-1} phosphate in the presence of 1 mmol l^{-1} molybdate did not show a significant change when the concentration of nitric acid was changed from 0.1 to 0.4 mol l^{-1}. A similar response was also observed when 0.2 mol l^{-1} of hydrochloric, sulphuric or nitric acid was used. For further experiments, 0.2 mol l^{-1} nitric acid was employed (results not shown).

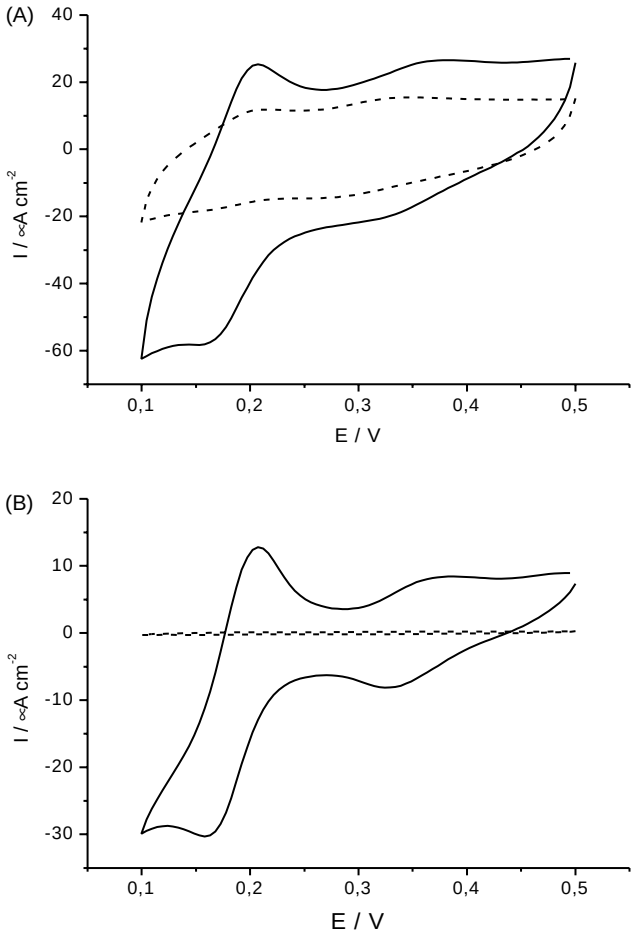

Fig. 2. Cyclic voltammograms recorded on two different electrodes: (A) glassy carbon; (B) carbon paste ($d = 2$ mm). (- - -) 0.2 mol l^{-1} HNO_3 and 2 mmol l^{-1} ammonium molybdate; (—) 0.2 mol l^{-1} HNO_3, 2 mmol l^{-1} ammonium molybdate and 0.1 mmol l^{-1} phosphate.

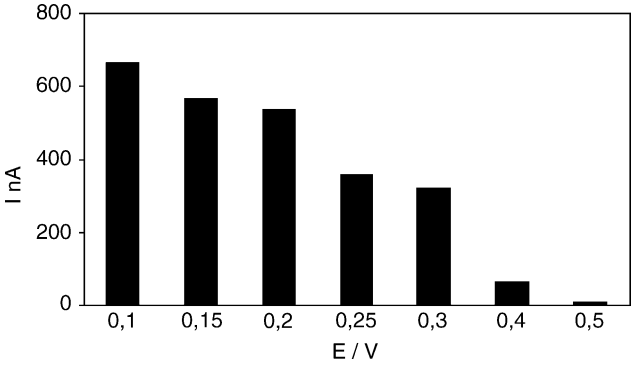

Fig. 3. Hydrodynamic voltammogram recorded on a carbon paste electrode (2 mm diameter) in solution containing 0.2 mol l^{-1} HNO_3, 0.5 mmol l^{-1} ammonium molybdate and 0.1 mmol l^{-1} phosphate, reference electrode was an Ag/AgCl electrode.

3.1.3. Interference study

The major problem in the determination of phosphate is due to the interference of silicate, as reported in several papers [9,15,26,27]. In fact silicate, if present in the sample, can form similar blue complexes with molybdate.

Zhang et al. [27] showed that the interference of silicate can be minimised by the selection of an appropriate $[H^+]$/[molybdate] ratio. Therefore, keeping a fixed concentration of nitric acid, the concentration of molybdate was varied from 0.5 to 10 mmol l^{-1} and the current responses to 0.1 mmol l^{-1} phosphate and to 0.1 mmol l^{-1} silicate were recorded separately at 0.3 V (Figs. 4A and B). As shown in Fig. 4A, the response of silicate was zeroed when the concentration of molybdate was lower than 2 mmol l^{-1}. Fig. 4B shows the response of 0.1 mmol l^{-1} phosphate at different concentrations of molybdate. The maximum response was obtained at 2 mmol l^{-1} molybdate. In the same Fig. 4B, it can be seen that at low levels of phosphate concentration (1–20 μmol l^{-1}), currents of similar magnitude were obtained over the range 0.5–2 mmol l^{-1} of molybdate concentration. Since phosphate concentration was very low, the use of 0.5 mmol l^{-1} molybdate concentration is highly sufficient for the complexation of the all phosphate in this range (1–20 μmol l^{-1}). Moreover, the background and the noise were very low at low molybdate concentration (0.5–1 mmol l^{-1}). Therefore, the concentration of molybdate used in the remaining work was not higher than 1 mmol l^{-1}.

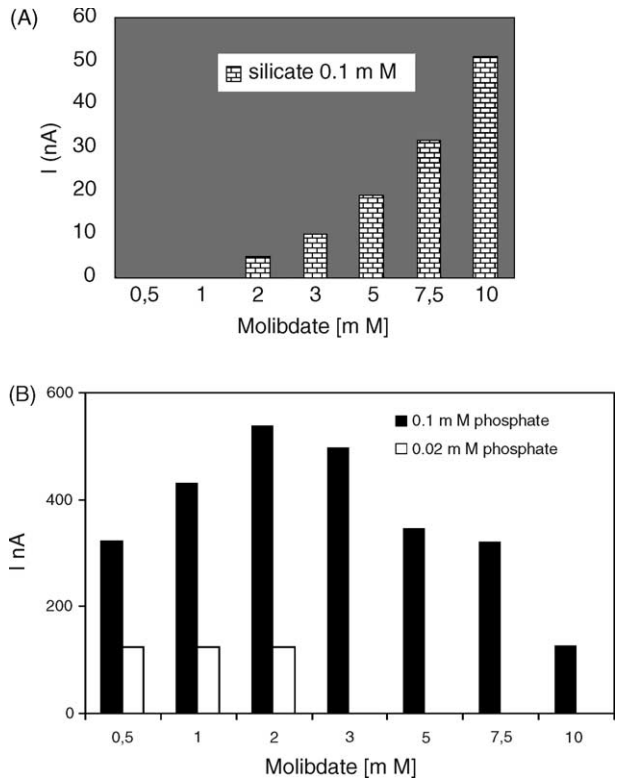

Fig. 4. Interference of silicate on response of a phosphate. Carbon paste macroelectrode, electrolyte: 0.2 mol l^{-1} nitric acid and ammonium molybdate at different concentrations, Applied potential: 0.3 V/Ag/AgCl. (A) 0.1 mmol l^{-1} silicate; (B) 0.02 and 0.1 mmol l^{-1} phosphate.

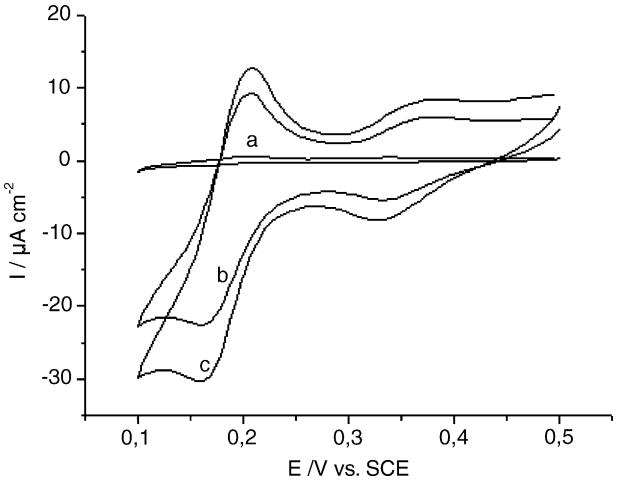

Fig. 5. Cyclic voltammograms at CPE, scan rate: 50 mV/s, electrolyte as in Fig. 3, the reference electrode was a SCE electrode. (a) 0.1 mmol l^{-1} silicate; (b) 0.06 mmol l^{-1} phosphate and (c) 0.1 mmol l^{-1} phosphate.

This better selectivity was also confirmed by cyclic voltammetry (Fig. 5). In all, the range of potential studied (0.5–0.1 V), no response to silicate was observed (Fig. 5a), while, in the presence of phosphate, two well-defined peaks were recorded (curves b and c in Fig. 5) in agreement with Fig. 2.

3.1.4. Calibration plot

Six orthophosphate standards covering the range 1–100 μmol l^{-1} and a blank were analysed, the concentration of molybdate used is 0.5 mmol l^{-1} of molybdate and 0.2 mol l^{-1} of nitric acid were employed. The working electrode was a carbon paste electrode hold at +300 mV versus Ag/AgCl. The response of phosphate was linear in the range 1–20 μmol l^{-1}. The relative standard deviation (R.S.D.) of the slope of six electrodes was equal to 6% and the coefficient of correlation was equal to 0.999. The calculated detection limit (S/N = 3) was equal to 0.3 μmol l^{-1}.

3.1.5. The effect of the organic solvent

It has been reported that the use of aqueous/organic mixtures increases the response of phosphate [11,12,15,16]. We have observed that the use of the electrolyte in water/methanol (70/30, v/v) mixtures increases the current significantly only if the phosphate concentration is higher than 20 μmol l^{-1} (Fig. 6). Because of the low concentrations expected for real samples, this upper limit was considered sufficient, thus our proposed method does not require the use of an organic solvent, making the analysis simples.

3.2. Batch injection analysis (BIA)

3.2.1. Analytical performances

The previous data were obtained in batch conditions. However, we observed that during repeated measurements,

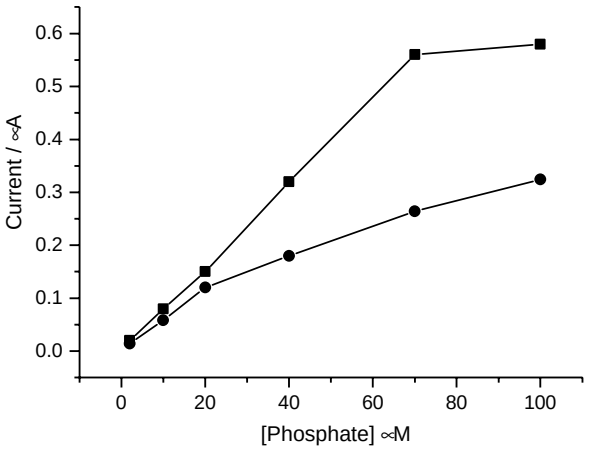

Fig. 6. Reduction current vs. phosphate concentration in batch measurements, electrolyte as in Fig. 3, $E = 0.3$ V vs. Ag/AgCl: (■) 30% MeOH (●) without MeOH.

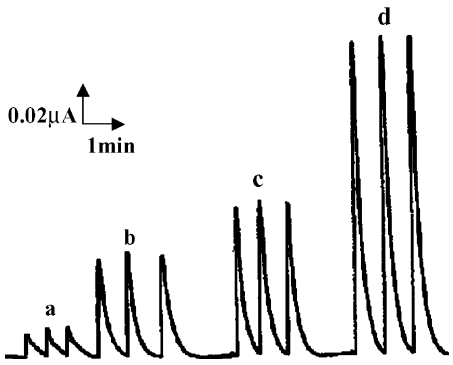

Fig. 7. Typical BIA sensor response to four successive injections of analyte solutions with and without addition of phosphate: (a) 0.2 mol l^{-1} HNO_3, 1 mmol l^{-1} ammonium molybdate; (b) with addition of 5 μmol l^{-1} phosphate; (c) 10 μmol l^{-1} phosphate and (d) 20 μmol l^{-1} phosphate. Injection volume: 70 μl, CPE hold at 0.3 V vs. Ag/AgCl (KCl 3 M).

the signal for the reduction of molybdate or phosphomolybdate was not stable. Indeed, after 20–30 min of contact between the CPE and molybdate or phosphomolybdate solutions, the fluctuation of the current signal increased. It became impossible to carry out next experiments without renewal or washing the electrode surface by dipping in stirred solution of 1 M sodium hydroxide for 5 min in order to eliminate adsorbed phosphomolybdic complex. It was observed that after six measurements performed without washing the electrode surface, the sensitivity decreased of about 20%. Using BIA, the molybdate or phosphomolybdate solution was prepared outside the cell and injected directly over the electrode immersed in a stirred solution. A transient response was recorded, since the contact with the electrode was only for a few seconds. Any possible fouling or adsorption phenomena at the electrode surface were, therefore, avoided.

Before any measurement, standards of phosphate or sample solutions were diluted two times with a solution containing 0.4 mol l^{-1} nitric acid and 2 mmol l^{-1} molybdate.

To check the interference by silicate, an acidic molybdate solution containing 0.1 mmol l^{-1} silicate was immediately injected into the cell solution and gave no response. However, if the mixed silicate/molybdate solution was left more than 5 min, the response of silicate appeared indicating the slow formation of molybdosilicate complexes. This observation was in agreement with the work of Hodgan and Pletcher [28]. The interference of silicate was successfully avoided by adding oxalate at 0.25% as masking agent to the mixed molybdate/nitric acid solutions in agreement with the work of Galhardo and Masini [29]. Under these conditions, the linear range was 1–20 μmol l^{-1} (31–620 mg l^{-1}), the regression equation was $y = 3.70 + 4.43x$, where y was the current in nA and x was the concentration of phosphate in μmol l^{-1}. The coefficient of correlation was equal to 0.998 and the R.S.D. of the slope of six calibration curves was 4%. The detection limit (S/N = 3) was equal to 1 μmol l^{-1}. An example of successive transient currents obtained following injection at fixed potential is shown in Fig. 7 in the absence and in the presence of increasing concentrations of phosphate. The response time (including baseline recovery) was 42 s, allowing a sample injection rate of 85 h^{-1}.

3.2.2. Sea water analysis

This method was applied to different seawater samples where possible interference due to the complexity of the matrix could appear. Recovery studies were carried out on samples to which known amounts of phosphate had been added and the seawater samples chosen did not contained phosphate. The results listed in Table 1 indicate that the recovery was from 70 to 74%. The low recoveries observed were due mainly to the presence of high concentration of salts in seawater. In fact, it was experimentally observed that the presence of NaCl in the supporting electrolyte slightly decreases the sensitivity of the proposed method. Indeed, the slope of the calibration curve decreased to 78% when phosphate standards were prepared in 0.6 mol l^{-1} sodium chloride solution which is the typical concentration in seawater (results not shown). To overcome this problem, the standard addition method was adopted for the analysis of real samples. Under these conditions, eight samples were analysed and results were compared with values obtained using the standard spectrophotometric method [8]. The concentration of phosphate found was low but in the range of the electrochemical method, while two samples were below the spectrophotometric detection limit (Table 2). For the

Table 1
Recoveries of spiked phosphate in sea water samples

Sample no.	Phosphate added (μmol l^{-1})	Phosphate found (μmol l^{-1})	Recovery (%)
1	5.0	3.5	70
2	10.0	7.2	72
3	12.5	9.2	74
4	15.0	11.0	73

Table 2
Comparison of spectrophotometric and amperometric methods for determination of phosphate in seawater samples

Sample N°	Phosphate concentration (mg/l)		
	Amperometric-BIA method, A	Spectrophotometric method, B	Relative error, ((A − B)/B) × 100
1	0.15	0.13	15.4
2	0.13	0.12	8.3
3	0.07	–	–
4	0.05	–	–
5	0.12	0.16	−24.1
6	0.25	0.26	−3.8
7	0.29	0.31	−6.4
8	0.36	0.38	−5.3

samples containing concentration of phosphate higher than 0.2 mg l^{-1}, both methods (electrochemical and spectrophotometric) showed similar response within the relative errors 4–6%. However, for the samples containing low concentration of phosphate (<0.2 mg l^{-1}), the relative errors increased up to 24% probably because the spectrophotometric method is not so sensitive at this level of phosphate concentration. It should be noted here that using a light path of 1 cm and wave length of 880 nm, a linearity range of 0.12–1.2 mg l^{-1} (3.9×10^{-3} to 3.8×10^{-2} μM) of phosphate was observed, indicating that the spectrophotometric method was not as sensitive as the electrochemical BIA method.

3.3. Batch measurements using carbon paste microelectrode

3.3.1. Analytical performances

The analytical performances of carbon paste microelectrode were evaluated using the above optimised parameters (+0.3 V, 1 mmol l^{-1} molybdate and 0.2 mol l^{-1} nitric acid) obtained under batch measurements. Four successive calibration curves were performed in the range 0.5–100 μmol l^{-1} and showed an R.S.D. equal to 4%. A good linearity was observed in the range 0.5–20 μmol l^{-1} with detection limit equal to 0.5 μmol l^{-1} (results not shown). The equations were: $y = 0.09 + 0.10x$; $r^2 = 0.9903$, where y is the current in nA, x the phosphate concentration in μmol l^{-1} and r^2 is the correlation coefficient. After a period of 35 s, the measured amperometric current of reduction reached the steady-state.

3.3.2. Determination of phosphate in cyanobacteria biofilms

The proposed method has been applied to different samples of cyanobacteria biofilms collected from Roman Catacombs. In order to study the effect of matrix complexity on phosphate determination, recovery of five samples was determined (Table 3). Amounts of orthophosphate, varying from 0.2 to 1.0 μmol l^{-1}, were added to 1 ml supernatant of centrifuged cyanobacteria. Values ranged from 88 to 117% with an average equal to 99 ± 7% indicating a good recovery obtained. Similarly, using standard spectrophotometric method, values of recovery ranged from 90 to 107% with an average equal to 96 ± 6% were obtained (results not shown).

Table 3
Recoveries of spiked phosphate in samples of cyanobacteria biofilms

Samples	Phosphate added (μmol)	Phosphate found (μmol)	Recovery (%)
CD12e[a]	0	0.094	–
	0.2	0.294	100
	0.4	0.476	96
	0.6	0.652	93
	0.8	0.913	102
	1.0	1.155	106
CD13d[a]	0	0.553	–
	0.2	0.767	107
	0.4	0.960	102
	0.6	1.126	96
	0.8	1.352	102
	1.0	1.629	108
CSC13[b]	0	0.267	–
	0.2	0.495	114
	0.4	0.647	96
	0.6	0.840	96
	0.8	1.004	92
	1.0	1.169	90
CSC16c[b]	0	0.082	–
	0.2	0.317	117
	0.4	0.508	106
	0.6	0.641	93
	0.8	0.810	91
	1.0	0.958	88
CSC16e[b]	0	0.067	–
	0.2	0.260	97
	0.4	0.467	100
	0.6	0.640	97
	0.8	0.849	98
	1.0	1.059	99

Roman hypogea, [a]Domitilla and [b]St. Callistus (Roma, Italy).

Fig. 8 showed preliminary experiments on phosphate detection in cyanobacteria biofilms performed both with the

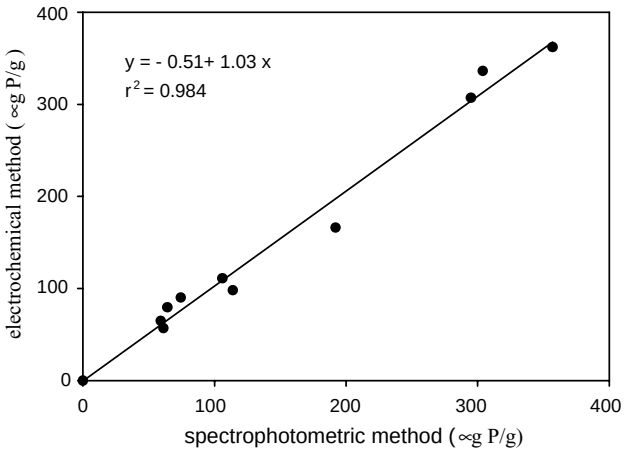

Fig. 8. Comparison between standard spectrophotometric method and the proposed method.

standard spectrophotometric and amperometric methods. The found phosphate in 10 samples was between 57 and 362 μg phosphorus g^{-1} of dry cyanobacteria. The slope = 1.03; intercept = $-0.51\,\mu g\,g^{-1}$, $r^2 = 0.984$ and $n = 10$. The slope of the regression line was not significantly different from 1, or the intercept significantly different from 0, indicating an absence of a constant and proportional bias. The correlation coefficient indicated a good agreement between the two methods.

The principle of construction and use of an amperometric sensor for phosphate detection has been successfully demonstrated. This method is cost effective, rapid, and easy to operate, is applicable for the measurement of phosphate in the μmol l^{-1} range. It showed also a good selectivity, a low detection limit of $0.3\,\mu mol\,l^{-1}$ and is applicable satisfactorily to the determination of phosphate in contaminated seawaters and in cyanobacteria biofilm.

The method based on BIA showed great advantages for rapid in situ measurements. The feasibility of fabricating a carbon paste microelectrode is highlighted in this work.

Acknowledgements

The authors gratefully acknowledge the EU (EVBD-2000-00505 and EVK4-CT2000-00028) and IFS (A/2912-1 and A/2780-1) for financial support of this work.

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