Rapid and Portable Electrochemical Quantification of Phosphorus

Athanasios V. Kolliopoulos, Dimitrios K. Kampouris and Craig E. Banks*

Faculty of Science and Engineering, School of Chemistry and the Environment,
Division of Chemistry and Environmental Science, Manchester Metropolitan University,
Chester Street, Manchester M1 5GD, Lancs, UK

*To whom correspondence should be addressed.

Email: c.banks@mmu.ac.uk; Tel: ++(0)1612471196; Fax: ++(0)1612476831

Website: www.craigbanksresearch.com

Abstract

Phosphorus is one of the key indicators of eutrophication levels in natural waters where it exists mainly as dissolved phosphorus. Various analytical protocols exist to provide an offsite analysis and a point of site analysis is required. The current standard method recommended by Environmental Protection Agency (EPA) for the detection of total phosphorus is colorimetric based upon the colour of a phosphomolybdate complex formed as a result of the reaction between orthophosphates and molybdates ions where ascorbic acid and antimony potassium tartrate are added and serve as reducing agents. Prior to measurements all forms of phosphorus are converted into orthophosphates by digesting (heating and acidifying). The work presented here details an electrochemical adaptation of this EPA recommended colorimetric approach for the measurement of dissolved phosphorus in water samples using screen-printed graphite macroelectrodes for the first time.

This novel indirect electrochemical sensing protocol allows the determination of orthophosphates over the range 0.5 to 20 μg L^{-1} in ideal pH 1 solutions utilising cyclic voltammetry with a limit of detection (3σ) found to correspond to 0.3 μg L^{-1} of phosphorus. The reaction time and influence of foreign ions (potential interferents) upon this electroanalytical protocol was also investigated where it was found that unlike the standard colourimetric method, where a reaction time of 5 minutes is required before the colourimetric measurement is not necessary in the electrochemical adapted protocol. The electrochemical method was independently validated through the quantification of orthophosphates and total dissolved phosphorus in polluted water samples (canal water samples) with ion chromatography and ICP-OES respectively.

This novel electrochemical protocol exhibits advantages over the established EPA recommended colorimetric determination for total phosphorus with lower detection limits and shorter experimental times. Additionally this electrochemical adaptation allows the determination of dissolved phosphorus without the use of ascorbic acid and antimony potassium tartrate as reducing agents (as used in the colorimetric method). The potential portability of this protocol is demonstrated in the development of the PhosQuant electrochemical device provides a portable device for the rapid electrochemical detection of dissolved phosphorus using screen-printed electrodes.

Keywords: Phosphorus, Screen-printed graphite macroelectrode, Electroanalysis, Molybdate, Canal water, Sensors.

Introduction

The nutrients phosphorus and nitrogen play critical roles for living cells but their excess leads to nutrient pollution or over-enrichment [1] which is one of the most extended, expensive and challenging environmental problems.[2] High levels of nutrients is the main cause of eutrophication where growth of algal and plankton increase to a substantial extent (known as algal bloom).[3] Eutrophication results in reduction or elimination of dissolved oxygen which is crucial for fish and other aquatic life. Some algal blooms produce toxins [4-7] which are harmful for humans if they come in contact with this polluted water, consume tainted shellfish [5], or drink contaminated water.[8,9] The biogeochemical cycle of phosphorus is vast but very slow [10] and human activities have caused significantly acceleration of the natural phosphorus cycle. The mining of phosphorus-rich rock to produce plant fertilizers is the major intervention of human activity in the phosphorus cycle.[11] The use of phosphorus in agriculture can contaminate natural water such as lakes and sea via rivers. The use of phosphorus within domestic detergents is another source of phosphorus in natural water through sewage disposal. [11]

Phosphorus can exist in natural water in three broad classes: orthophosphates, condensed phosphates (pyro-, meta-, and poly-) and organic phosphorus.[3] However, soluble phosphorus in natural water mainly consists of orthophosphates. Other forms of soluble phosphates like organic phosphates and polyphosphates are eventually hydrolysed into orthophosphates. The low limits of the phosphorus concentrations in natural waters can be several micrograms per litre [12] and additionally it is the key indicator of eutrophication level in natural waters.[13,14]

As small differences of phosphorus concentrations can have a pronounced impact on streams, less sensitive methods should be utilised only to identify serious eutrophication problems.[2] The measurement of low concentrations (less than 10 $\mu g\ L^{-1}$) of phosphorus in natural water makes the monitoring of phosphorus challenging. As described by the EPA, there are four main tests for phosphorus: [15]

1. *Orthophosphate test.* This approach measures dissolved or/and suspended orthophosphate since the sample is not filtered. The EPA-approved method for measuring orthophosphate involves a colorimetric approach where ammonium molybdate reacts with orthophosphate with ascorbic acid to form a blue compound. The intensity of the blue colour is directly proportional to the amount of orthophosphate in the water.
2. The *total phosphorus test* measures all the forms of phosphorus in the sample (orthophosphate, condensed phosphate, and organic phosphate). This is accomplished by

digesting (heating and acidifying) the sample to convert all the other forms to orthophosphate. The resulting orthophosphate is measured by the colorimetric test described in (1) above. Since the sample is not filtered, the procedure measures both dissolved and suspended orthophosphate.

3. The *dissolved phosphorus test* measures that fraction of the total phosphorus which is in solution in the water (rather than those attached to suspended particles). In this approach, the sample is first filtered then analysed the filtered sample for total phosphorus.

4. *Insoluble phosphorus* is calculated by subtracting the dissolved phosphorus result from the total phosphorus result.

There are chromatographic [16] fluorescence [17] and spectrophotometric methods for the determination of total phosphorus;[18-20] (2) above. The EPA recommended current standard method for the detection of phosphorus, as described above in (1) is colorimetric.[21] In this approach, ammonium molybdate, ascorbic acid and antimony potassium tartrate are all added in unison to acidified samples in order to form the blue coloured a-keggin anion, ammonium phosphomolybdate. The reaction is as follows:[22,23]

$$7PO_4^{3-} + 12Mo_7O_{24}^{6-} + 72H^+ \Rightarrow 7PMo_{12}O_{40}^{3-} + 36H_2O \tag{1}$$

The concentration of phosphate is proportional to the blue colour of the sample and is determined spectrophotometrically. This method suffers from refractive index errors and turbidity interference.[12,24] For this reason electrochemical methods have been explored and reported for phosphate detection. A variety of electrode substrates and analytical methods have been reported for the determination of phosphates as it is shown in Figure S1. Such approaches have utilised metal electrodes, ion selective membranes enzyme based electrodes, [25-27] gold and glassy carbon modified electrodes, [28] modified carbon paste electrodes, [27,29] cobalt wire ion selective electrodes, [30,31] lead ion selective electrodes, [32] and cobalt phthalocyanide modified screen-printed electrodes as amperometric sensors. [33,34]

Consequently, in this work, the established EPA recommended colorimetric protocol by using molybdate ions as complexing agent is electrochemically adapted alleviating the need for the use of ascorbic acid and antimony potassium tartrate as reducing agents.[35,36] The reduction of the phosphomolybdate ion is accomplished electrochemically. The present electro-analytical protocol maintains the high sensitivity and selectivity of the standard colorimetric method and additionally reaches lower detection limits, minimizes the

experimental time and simplifies the procedure using fewer reagents. This indirect electroanalytical protocol is found to exhibit a limit of detection (3σ) of 0.3 μg L^{-1} of phosphorus over the range 0.5 to 20 μg L^{-1} and is demonstrated to be successfully applied into canal water samples and is independently validated by ion chromatography and inductively coupled plasma optical emission spectrometry (ICP-OES) for the determination of orthophosphates and dissolved phosphorus respectively.

Experimental

All chemicals used were obtained by Sigma Aldrich. The phosphate standard solutions were prepared with pre-dried (105°C for one hour) potassium dihydrogen phosphate. [15] The molybdate stock solution was a 0.8% w/v ammonium molybdate tetrahydrate solution. Interfering solutions were made with potassium chloride, sodium nitrite, sodium nitrate and sodium bicarbonate. All the solutions including the sample from canal water were adjusted to pH 1 with 5.5 M sulphuric acid [15] in accordance with the optical method in order to avoid possible interference of silicate.[12,28] All solutions were prepared with deionised water of resistivity 18.2 MΩ cm. All glassware was washed in 15.8 M nitric acid prior to use. All voltammetric measurements were carried out using Ivium Compactstat potentiostat (The Netherlands). The screen-printed three electrode configuration have a geometric graphite working electrode area of 3 mm diameter in addition to on-board pseudo Ag/AgCl reference and graphite counter electrodes.

Measurements were conducted using screen-printed three electrode configurations. Screen-printed carbon-based electrodes (denoted as SPEs) were fabricated in-house with appropriate stencil designs using a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). Note that this screen-printed electrode design has been previously reported [37-41] "as is" without electrode pre-treatment or modification in various electroanalytical endeavours. For fabrication of the SPEs, first a carbon ink formulation (Product Code: C2000802P2; Gwent Electronic Materials Ltd, UK) utilised for the efficient connection of all three electrodes and the electrode material for both the working and counter electrodes was screen-printed onto a polyester (Autostat, 250 micron thickness) flexible film. The carbon ink layer was cured in a fan oven at 60 degrees for 30 minutes. Next a silver/silver chloride reference electrode was included by screen-printing Ag/AgCl paste (Product Code: C2040308P2; Gwent Electronic Materials Ltd, UK) onto the polyester substrates which was subsequently cured once more in a fan oven at 60 degrees for 30 minutes. Finally, a dielectric paste (Product Code: D2070423P5; Gwent Electronic Materials Ltd, UK) was then printed onto the polyester substrate to cover the connections and define the active electrode areas including that of the working electrode (3 mm diameter). After curing at 60 degrees for 30 minutes the SPEs are ready to be used. These electrodes have been characterised electrochemically in a prior paper and have heterogeneous rate constants of 1.08×10^{-3} cm s^{-1}.[42] It is important to note that in all cases that a new SPE was used for each additions/measurement. As such any potential adsorbing interferents/organics will not precluded useful measurements to be obtained.

Inland water samples were used as real samples in this study. Inland waters is a term to defines any area of water not categorised as 'sea' - *e.g.* canals, tidal and non-tidal rivers, lakes, and some estuarial waters. Canals are inland waterways used for navigation, crop irrigation, water supply, or drainage. The canal water utilised was obtained in a plastic container from Manchester's city centre. The sample was filtered with a 0.45μm filter from Millipore and then stored at room temperature and use within a day of sampling.

The persulfate digestion for the detection of dissolved phosphorus (orthophosphates, organic and hydrolysable phosphorus) took place according the EPA's standard colorimetric method where 50 mL of 100 times diluted filtered canal water were transferred into a 125 mL Erlenmeyer flask and 1 mL of 5.5 M sulfuric acid was added. After the addition of 0.4 g ammonium persulfate, the sample was mixed and boiled gently for 30-40 minutes until a final volume of about 10 mL is reached. It was left in order to reach the room temperature, diluted to 40 mL and filtered. 5.2g of sodium bisulphite was added and the solution was mixed and placed in a 95°C water bath for 30 minutes.[15] The final solution was cooled and diluted to 50 mL. The amount of dissolved phosphorus was determined following this procedure according to the colorimetric method used for orthophosphates.

The ion chromatography for the detection of orthophosphates in real sample performed by Dionex, ICS-2000 Ion Chromatography System accompanied by Dionex IonPac AG18 as guard column and Dionex IonPac AS18 as separation column.

The inductively coupled plasma optical emission spectrometry (ICP-OES) for the detection of dissolved phosphorus in real sample performed by Thermo Scientific DUO iCAP 6300 ICP Spectrometer.

In accordance to the EPA's standard colorimetric method, the concentrations of total orthophosphates and dissolved phosphorus in this paper are reported as phosphorus P which is approximately three times lower than the concentration in phosphates. Such an approach is commonly used in the literature.

Results and Discussion

The colorimetric method for the determination of orthophosphates requires antimony potassium tartrate and ascorbic acid as reducing agents. The proposed electrochemical adaptation of this method avoids the use of these reducing agents since the product of the complexing reaction ammonium phosphomolybdate can be electrochemically interrogated.

The voltammetric response of the SPEs towards 640 mg L^{-1} ammonium molybdate tetrahydrate at pH 1 with and without the addition of 20 µg L^{-1} phosphorus is shown in figure 1. Two reduction peaks at +0.27V and at +0.13V are observed which are likely due to electrochemical reduction of Mo(VI)→Mo(IV) and Mo(IV)→Mo(II) respectively [12]. The two corresponding oxidation peaks observed at +0.16V and at +0.30V are likely due to the electrochemical oxidation of Mo(II)→Mo(IV) and Mo(IV)→Mo(VI) respectively. Note that all 4 peaks increase with increasing concentration of phosphate providing an indirect electrochemical methodology.

The voltammetric responses observed from additions of phosphate into an ideal solution (pH 1) are depicted in figure 2 utilising SPEs. Figures S2-S4 and figure 3 depict the calibration plots for all the four oxidation and reduction analytical peaks. The linear responses for the sensing of phosphorus are as follows: for using the oxidation peak at +0.16V (see figure S2) ($I_P/\mu A = 2.76 \times 10^{-2}\ \mu A/mgL + 5.21 \times 10^{-2}\ \mu A;\ R^2 = 0.9938\ N = 3$), for using the oxidation peak at + 0.30V (see figure S3) ($I_P/\mu A = 4.2 \times 10^{-3}\ \mu A/mgL + 12.12 \times 10^{-2}\ \mu A;\ R^2 = 0.7544\ N = 3$), for using the reduction peak at + 0.27V (see figure S4) ($I_P/\mu A = -4.8 \times 10^{-3}\ \mu A/mgL - 13.16 \times 10^{-2}\ \mu A;\ R^2 = 0.6898\ N = 3$) and for using the reduction peak at + 0.13V (see figure 3) ($I_P/\mu A = -3.4 \times 10^{-2}\ \mu A/mgL - 3.84 \times 10^{-2}\ \mu A;\ R^2 = 0.9979\ N = 3$). The reduction peak at +0.13V was chosen as the analytical peak since it is found to exhibit the best linear relationship with the concentration of phosphorus upon additions over the range 0-20 µL^{-1}; using this line of best fit. The limit of detection (3σ) for phosphorus when utilising new SPE for each addition was determined to be 0.3 µg L^{-1}.

In the EPA recommended optical method, the time of reaction is crucial and this is the reason why a minimum time of 5 minutes is suggested following the addition of all the chemicals in order to determine the concentration of phosphorus spectrophotometrically. The peak current of the reduction peak at + 0.13V in relation with the reaction time is shown in figure S5. It is clear from the figure that the reaction time doesn't affect the voltammetric signature/analytical signal. This is because the electrochemical protocol does not require reducing agents as mentioned above.

Next, potential interferences were explored. Interference from ions that might be found in water samples such as Cl^{-1}, $NO2^{-}$, NO_3^- and HCO_3^- have been studied and the results are depicted in figure 4. It can be concluded that the ions, which have been studied in concentrations five times higher than concentration of phosphorus, do not interfere to the proposed electrochemical method.

Attention was focused on determining the concentration of orthophosphates (as phosphorus) via standard addition techniques into filtered canal water (diluted 1:4) with deionised water (see experimental). Cyclic voltammetric responses using SPE resulting from additions of phosphorus (1 – 20 μgL^{-1}) into the canal water sample (acidified to pH 1 with H_2SO_4) containing 640 mg L^{-1} ammonium molybdate tetrahydrate are shown in figure 5. Analysis of the data presented in the inset figure of figure 5 reveals a linear response for the sensing of orthophosphates using the reduction peak at +0.13 V ($I_P/\mu A = -2.37 \times 10^{-2}\ \mu A/mgL +9.33 \times 10^{-2}\ \mu A;\ R^2 = 0.9923\ \ N = 3$). The initial concentration of orthophosphates of the diluted (1:4) canal water can be determined at 3.9 μgL^{-1} as phosphorus. Consequently, the concentration of orthophosphates in the canal water was initially 4 x 3.9 = 15.6 µg L^{-1} as phosphorus. This value was confirmed by ion chromatography (Figure S6) where orthophosphates were independently determined to be 15 µg L^{-1} as phosphorus.

The total amount of dissolved phosphorus in the canal water sample was determined by using the standard addition technique in diluted canal water (1:100 with deionized water) after persulfate digestion (procedure detailed in the experimental section) in order to convert all the forms of phosphorus into orthophosphates. Cyclic voltammetric responses using SPEs following additions of phosphorus (1 – 20 μgL^{-1}) in the digested sample (adjusted to pH 1 with H_2SO_4) containing 640 mg L^{-1} ammonium molybdate tetrahydrate are shown in figure 6. Analysis of the voltammetric responses shown in the inset figure of figure 6 reveals a linear response for the sensing of orthophosphates using the reduction peak at +0.13 V was achieved ($I_P/\mu A = -1.11 \times 10^{-2}\ \mu A/mgL -8.87 \times 10^{-2}\ \mu A;\ R^2 = 0.9925\ \ N = 3$). The initial concentration of dissolved phosphorus of the diluted (1:100) canal water can be determined at 8.0 µg L^{-1}. Consequently the concentration of dissolved phosphorus in the canal water was initially 100 x 8.0 = 800 µg L^{-1} as phosphorus. This value was confirmed by ICP-OES (Figure S7) where dissolved phosphorus was determined to correspond to 885 µg L^{-1} with relative standard deviation 10.1%. Reasonable agreement between the proposed electrochemical method and the traditional ICP-OES suggests the electroanalytical protocol has merit.

Finally, the potential portability of the analytical protocol proposed for the detection of phosphorus using screen-printed graphite electrodes was demonstrated successfully by using the PhosQuant Device. This novel device is presented in figure S8. Presently this device has the potential to be used in the field and we present a convenient and cost-effective approach to do this. There would be advantages if the phosphomolybdate complex could be incorporated into the bulk of the sensor to simplify the overall analytical protocol. One must not forget that the sample still needs to pre-treated and the need of the sulphuric acid and other reagent such as ammonium persulfate make the incorporation of the necessary reagents during the screen-printed process more complicated and is part of future work.

Conclusions

The sensing of dissolved phosphorus in ideal and real samples (canal water) using screen-printed graphite electrodes has been reported using an indirect protocol for the first time. The proposed electroanalytical procedure is simple and utilises cost effective screen-printed sensors provide an appealing alternative to the existed standard colorimetric method which is traditionally used for the sensing of dissolved phosphorus. The screen-printed graphite electrodes are demonstrated to allow for the low level sensing of dissolved phosphorus in canal water samples, in addition to analysis under ideal conditions. The proposed electroanalytical protocol is as sensitive and selective as the established standard colorimetric method but additionally it exhibits lower detection limits, requires less experimental time and overcomes refractive index errors and turbidity interferences and also eliminates the need for ascorbic acid and antimony potassium tartrate. Such sensors provide a potential solution to the common problem of the transition of laboratory-based analytical procedures to real world applications in the ‘field’ combining the low-cost benefits of carbon based materials with ease of mass production and facile use of screen-printed sensors. The potential portability of this protocol is exhibited in the development of the PhosQuant which is a portable device for the rapid electrochemical detection of phosphorus using screen-printed electrodes in the ‘field’.

Electronic Supporting Information. The ESI contains extra information related to the main paper which include a table summarising amperometric and voltammetric methods for the detection of phosphates applied to its analysis in water samples along with calibration plots using the novel electrochemically adapted protocol for phosphorous determination. This material is available free of charge via the Internet at http://pubs.acs.org."

Figure 1

Cyclic voltammetry of SPE upon solution containing 640 mg L^{-1} ammonium molybdate tetrahydrate at pH 1 with and without the addition of 20 μgL^{-1} phosphorus. Dashed line: without phosphorus. Solid line: with 20 μg L^{-1} phosphorus. Scan rate: 50mVs^{-1} *vs* Ag/AgCl.

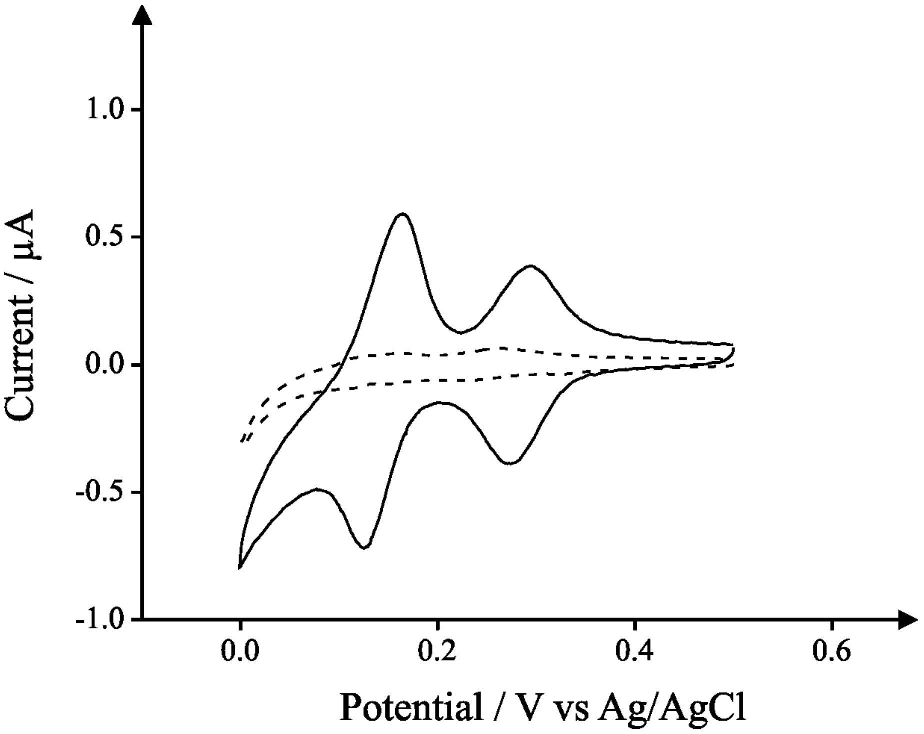


Figure 2

Typical cyclic voltammetric responses using a SPE following additions of phosphorus (0.5 – 100 µgL^{-1}) into an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. Note that a new SPE is used for each addition. Dotted line: without phosphorus (blank). Scan rate: 50 mVs^{-1} *vs*. Ag/AgCl.

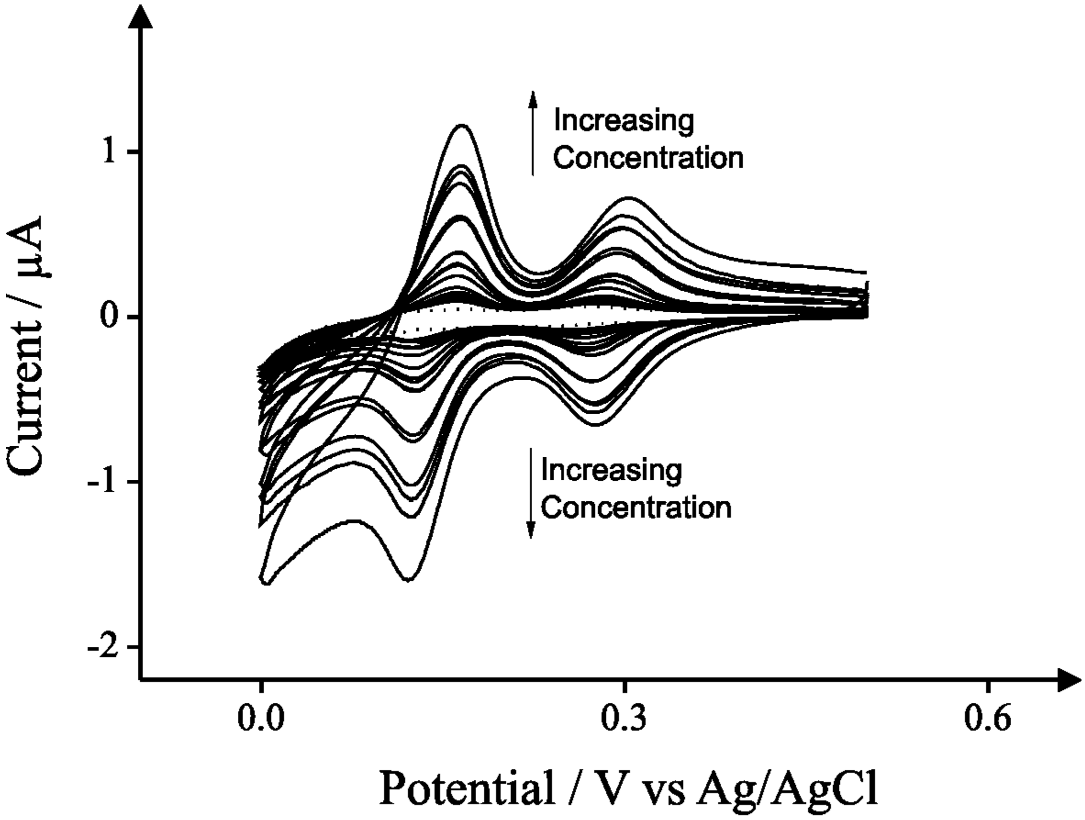


Figure 3

A typical calibration plot corresponding to additions of phosphorus (0.5 – 1000 ugL^{-1}) into an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. The data is from the analysis of the analytical reduction peak observed at +0.13 V. Inset: zoom of the Calibration Plot for additions of phosphorus (0.5 – 20 µgL^{-1}). Error Bars in the inset arise from three measurements. Different electrode has been used each time.

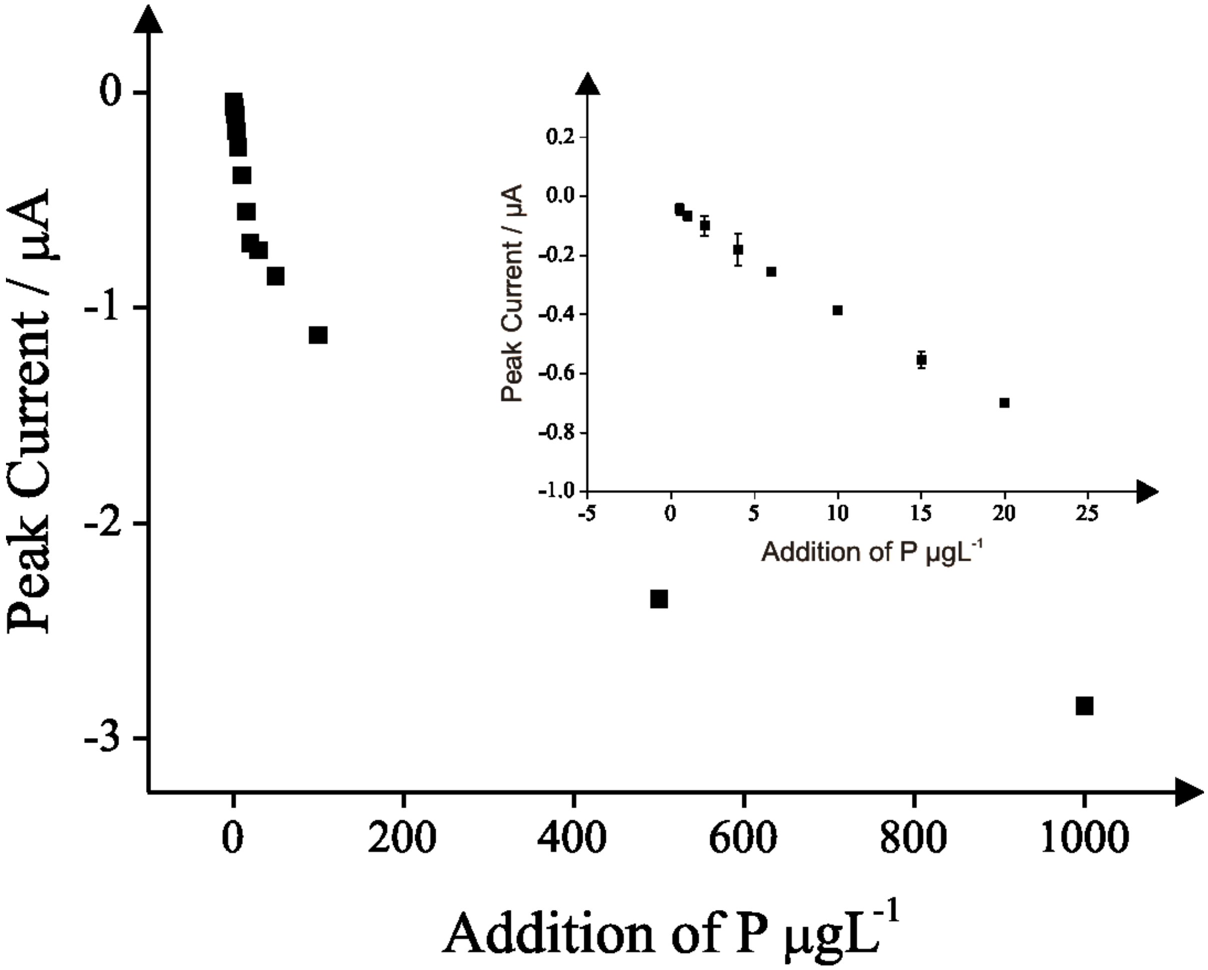


Figure 4

Typical cyclic voltammetric responses in the absence (solid line) and presence of 100 μgL^{-1} of the ions Cl^{-1} (dashed line), HCO_3^- (dotted line) NO_3^- (dashed-dotted line) and NO_2^- (dashed-dotted-dotted line) recorded in an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate and 20 μgL^{-1} phosphorus. Scan Rate: 50 mVs^{-1} *vs* Ag/AgCl.

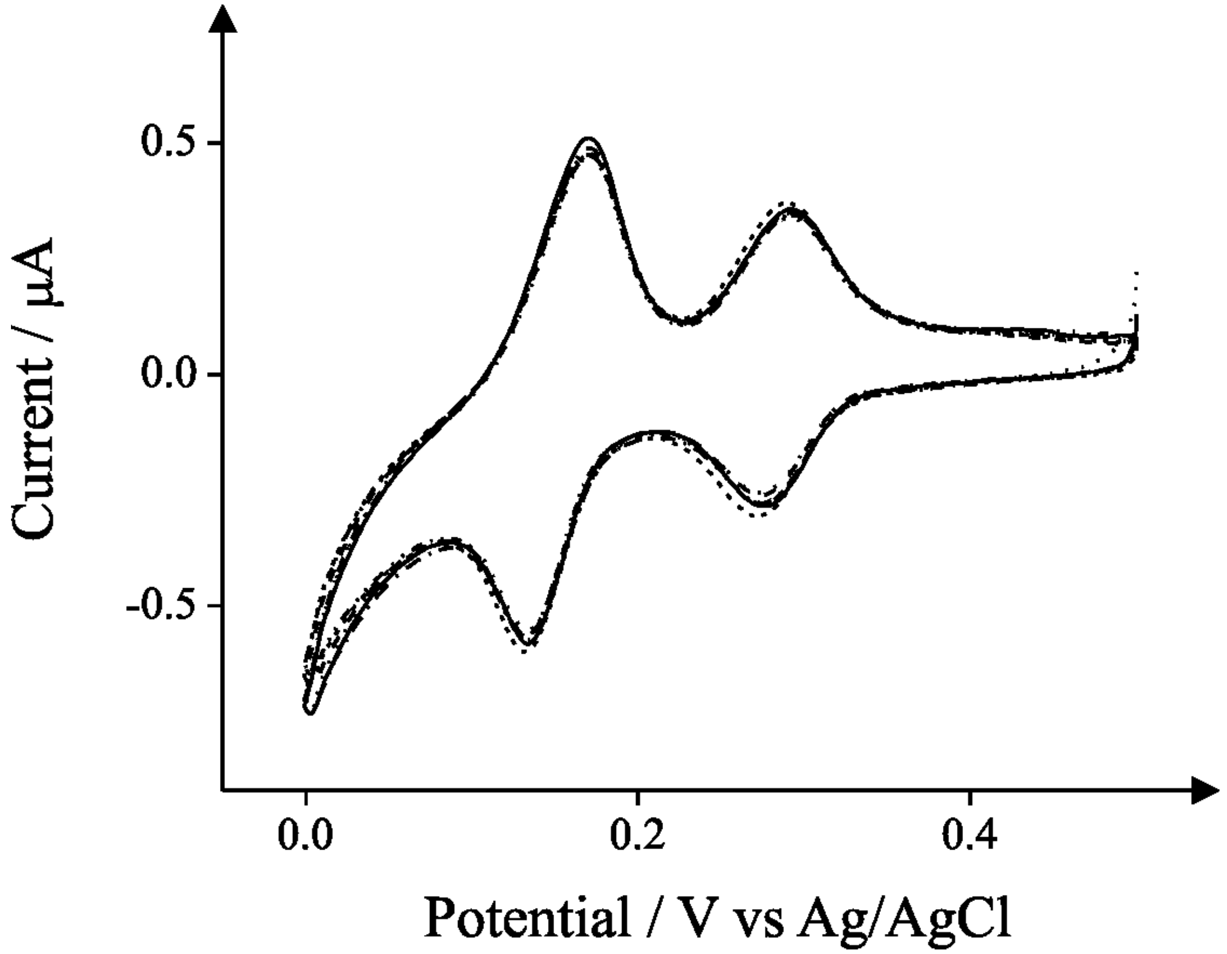


Figure 5

Cyclic voltammetric responses using SPEs following additions of phosphorus (1 – 20 μg L^{-1}) into a canal water (diluted 1:4) sample (adjusted to pH 1 with H_2SO_4) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. A new SPE is used for each addition. Dashed line: without phosphorus. Analytical reduction peak at + 0.13V is analysed to provide the data presented in the inset figure. A new SPE is used for each addition. Error bars ($N = 3$); Scan rate: 50 mVs^{-1} *vs*. Ag/AgCl.

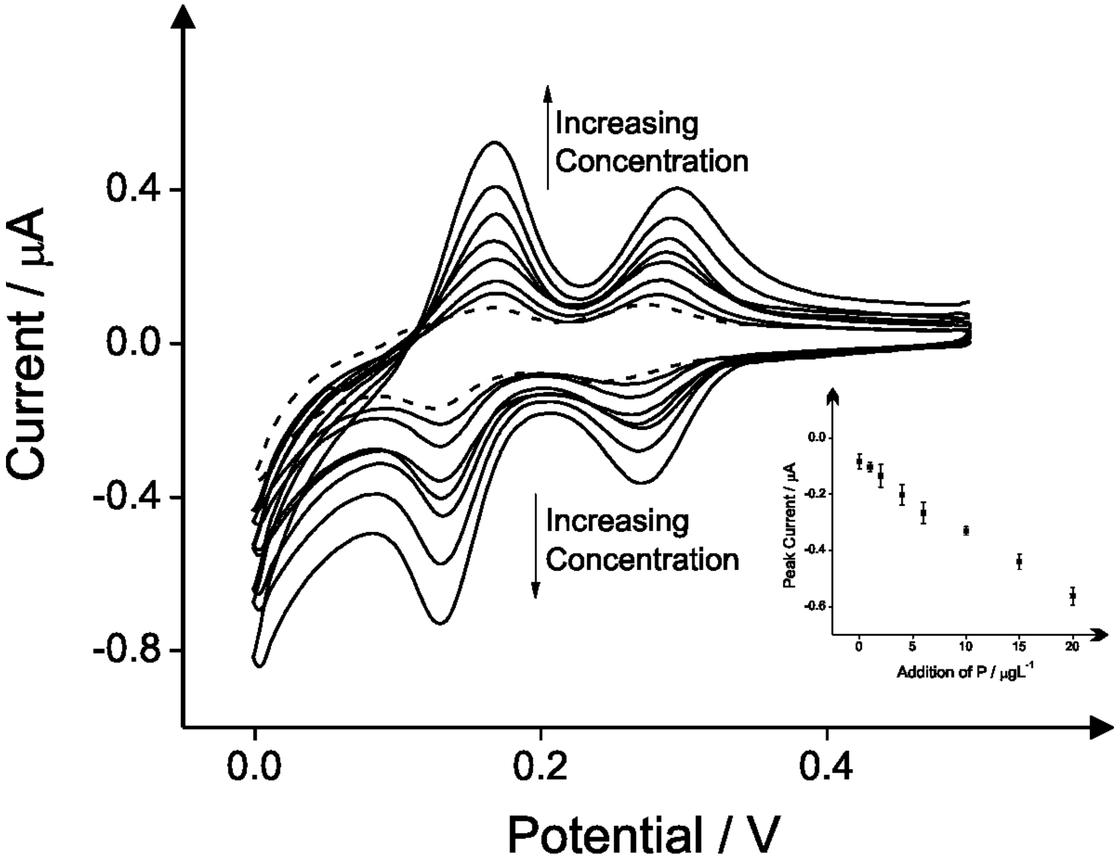
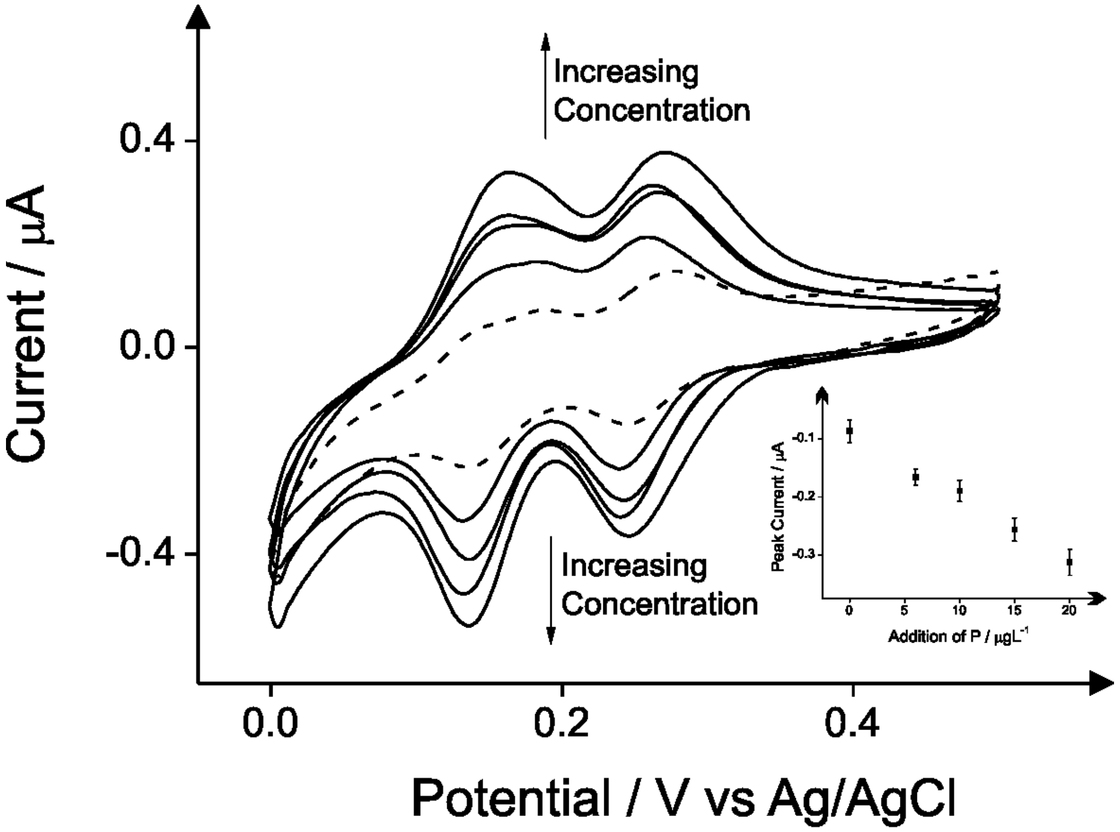


Figure 6

Cyclic voltammetric responses using SPE as a result of additions of phosphorus (1 – 20 µg L^{-1}) into a digested canal water (diluted 1:100) sample (using persulfate; see experimental). The pH of the canal water sample was adjusted to pH 1 with H_2SO_4. 640 mg L^{-1} ammonium molybdate tetrahydrate was added in the solution prior to electrochemical measurements. A new SPE is used for each addition. Dashed line: without phosphorus. Analytical reduction peak at + 0.13V is analysed to provide the data presented in the inset figure. A new SPE is used for each addition. Error bars (N = 3); Scan rate: 50 mVs^{-1} *vs.* Ag/AgCl.



References

(1) Lee, K.-S.; Short, F. T.; Burdick, D. M.: Development of a nutrient pollution indicator using the seagrass, Zostera marina, along nutrient gradients in three New England estuaries. *Aquatic Botany* **2004**, *78*, 197-216.

(2) EPA; Environmental; Protection; Agency: *Working in Partnership with States to Address Phosphorus and Nitrogen Pollution through Use of a Framework for State Nutrient Reductions* **2011**, Washington, D.C.

(3) Korostynska, O.; Mason, A.; Al-Shamma'a, A.: *International Journal on smart Sensing and Intelligent Systems* **2012**, *5*, 149-176.

(4) Smith, E. A.; Blanchard, P. B.; Bargu, S.: Education and public outreach concerning freshwater harmful algal blooms in Southern Louisiana. *Harmful Algae* **2014**, *35*, 38-45.

(5) Turki, S.; Dhib, A.; Fertouna-Bellakhal, M.; Frossard, V.; Balti, N.; Kharrat, R.; Aleya, L.: Harmful algal blooms (HABs) associated with phycotoxins in shellfish: What can be learned from five years of monitoring in Bizerte Lagoon (Southern Mediterranean Sea)? *Ecological Engineering* **2014**, *67*, 39-47.

(6) Branch, G. M.; Bustamante, R. H.; Robinson, T. B.: Impacts of a 'black tide' harmful algal bloom on rocky-shore intertidal communities on the West Coast of South Africa. *Harmful Algae* **2013**, *24*, 54-64.

(7) Seoane, S.; Puente, A.; Guinda, X.; Juanes, J. A.: Bloom forming and toxic phytoplankton in transitional and coastal waters of Cantabria region coast (Southeastern Bay of Biscay, Spain). *Marine Pollution Bulletin* **2012**, *64*, 2860-2866.

(8) Fleming, L. E.; Rivero, C.; Burns, J.; Williams, C.; Bean, J. A.; Shea, K. A.; Stinn, J.: Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. *Harmful Algae* **2002**, *1*, 157-168.

(9) Weirich, C. A.; Miller, T. R.: Freshwater Harmful Algal Blooms: Toxins and Children's Health. *Current Problems in Pediatric and Adolescent Health Care* **2014**, *44*, 2-24.

(10) Liu, Y.; Chen, J.: Phosphorus Cycle. In *Reference Module in Earth Systems and Environmental Sciences*; Elsevier, 2014.

(11) Caraco, N. F.: Disturbance of the phosphorus cycle: A case of indirect effects of human activity. *Trends in Ecology & Evolution* **1993**, *8*, 51-54.

(12) Bai, Y.; Tong, J.; Wang, J.; Bian, C.; Xia, S.: Electrochemical microsensor based on gold nanoparticles modified electrode for total phosphorus determinations in water. In *IET Nanobiotechnology*; Institution of Engineering and Technology, 2014; Vol. 8; pp 31-36.

(13) EU: *Directive 2000/60/EC* **2000**, Establishing a framework for Community action in the field of water policy. Council Directive, Oct23, 2000.

(14) (MEP): *Ministry of Environmental protection of of the People's Republic of China*, 'Environmental quality report of the first half of 2012 in key river water environments' Announcement No.52, 2012.

(15) E.P.A.: *Determination of Phosphorus by Semi-Automated Colorimetry* **1993**, Environmental Monitoring Systems Laboratory Office of Research and Development U.S. Environmental Protection Agency.

(16) Ruiz-Calero, V.; Galceran, M. T.: Ion chromatographic separations of phosphorus species: a review. *Talanta* **2005**, *66*, 376-410.

(17) Kröckel, L.; Lehmann, H.; Wieduwilt, T.; Schmidt, M. A.: Fluorescence

detection for phosphate monitoring using reverse injection analysis. *Talanta* **2014**, *125*, 107-113.

(18) Li, Y.-S.; Muo, Y.; Xie, H.-M.: Simultaneous determination of silicate and phosphate in boiler water at power plants based on series flow cells by using flow injection spectrophotometry. *Analytica Chimica Acta* **2002**, *455*, 315-325.

(19) Nakatani, N.; Kozaki, D.; Masuda, W.; Nakagoshi, N.; Hasebe, K.; Mori, M.; Tanaka, K.: Simultaneous spectrophotometric determination of phosphate and silicate ions in river water by using ion-exclusion chromatographic separation and post-column derivatization. *Analytica Chimica Acta* **2008**, *619*, 110-114.

(20) Neves, M. S. A. C.; Souto, M. R. S.; Tóth, I. V.; Victal, S. M. A.; Drumond, M. C.; Rangel, A. O. S. S.: Spectrophotometric flow system using vanadomolybdophosphate detection chemistry and a liquid waveguide capillary cell for the determination of phosphate with improved sensitivity in surface and ground water samples. *Talanta* **2008**, *77*, 527-532.

(21) Murphy, J.; Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **1962**, *27*, 31-36.

(22) Warwick, C.; Guerreiro, A.; Soares, A.: Sensing and analysis of soluble phosphates in environmental samples: A review. *Biosensors and Bioelectronics* **2013**, *41*, 1-11.

(23) Guanghan, L.; Xiaogang, W.; Yanhua, L.; Shenlai, Y.: Studies on 1:12 phosphomolybdic heteropoly anion film modified carbon paste electrode. *Talanta* **1999**, *49*, 511-515.

(24) Udnan, Y.; McKelvie, I. D.; Grace, M. R.; Jakmunee, J.; Grudpan, K.: Evaluation of on-line preconcentration and flow-injection amperometry for phosphate determination in fresh and marine waters. *Talanta* **2005**, *66*, 461-466.

(25) Akyilmaz, E.; Yorganci, E.: Construction of an amperometric pyruvate oxidase enzyme electrode for determination of pyruvate and phosphate. *Electrochimica Acta* **2007**, *52*, 7972-7977.

(26) d'Urso, E. M.; Coulet, P. R.: Phosphate-sensitive enzyme electrode: a potential sensor for environment control. *Analytica Chimica Acta* **1990**, *239*, 1-5.

(27) Xue, Y.; Zheng, X.; Li, G.: Determination of phosphate in water by means of a new electrochemiluminescence technique based on the combination of liquid–liquid extraction with benzene-modified carbon paste electrode. *Talanta* **2007**, *72*, 450-456.

(28) Jońca, J.; León Fernández, V.; Thouron, D.; Paulmier, A.; Graco, M.; Garçon, V.: Phosphate determination in seawater: Toward an autonomous electrochemical method. *Talanta* **2011**, *87*, 161-167.

(29) Ejhieh, A. N.; Masoudipour, N.: Application of a new potentiometric method for determination of phosphate based on a surfactant-modified zeolite carbon-paste electrode (SMZ-CPE). *Analytica Chimica Acta* **2010**, *658*, 68-74.

(30) De Marco, R.; Phan, C.: Determination of phosphate in hydroponic nutrient solutions using flow injection potentiometry and a cobalt-wire phosphate ion-selective electrode. *Talanta* **2003**, *60*, 1215-1221.

(31) Chen, Z.; Grierson, P.; Adams, M. A.: Direct determination of phosphate in soil extracts by potentiometric flow injection using a cobalt wire electrode. *Analytica Chimica Acta* **1998**, *363*, 191-197.

(32) Hara, H.; Kusu, S.: Continous-flow determination of phosphate using a lead ion-selective electrode. *Analytica Chimica Acta* **1992**, *261*, 411-417.

(33) Gilbert, L.; Jenkins, A. T. A.; Browning, S.; Hart, J. P.: Development of an amperometric, screen-printed, single-enzyme phosphate ion biosensor and its application to the analysis of biomedical and environmental samples. *Sensors and Actuators B: Chemical* **2011**, *160*, 1322-1327.

(34) Gilbert, L.; Jenkins, A. T. A.; Browning, S.; Hart, J. P.: Development of an amperometric assay for phosphate ions in urine based on a chemically modified screen-printed carbon electrode. *Analytical Biochemistry* **2009**, *393*, 242-247.

(35) Backheet, E. Y.; Emara, K. M.; Askal, H. F.; Saleh, G. A.: Selective spectrophotometric method for the determination of ascorbic acid in pharmaceutical preparations and fresh fruit juices. *Analyst* **1991**, *116*, 861-865.

(36) Uprety, M. C.; Revis, B.: Elevated temperature studies on stability of ascorbic acid in certain fruit juice and aqueous vehicles. *Journal of Pharmaceutical Sciences* **1964**, *53*, 1248-1251.

(37) Hallam, P. M.; Kampouris, D. K.; Kadara, R. O.; Banks, C. E.: Graphite screen printed electrodes for the electrochemical sensing of chromium(vi). *Analyst* **2010**, *135*, 1947-1952.

(38) Choudry, N. A.; Kampouris, D. K.; Kadara, R. O.; Banks, C. E.: Disposable highly ordered pyrolytic graphite-like electrodes: Tailoring the electrochemical reactivity of screen printed electrodes. *Electrochemistry Communications* **2010**, *12*, 6-9.

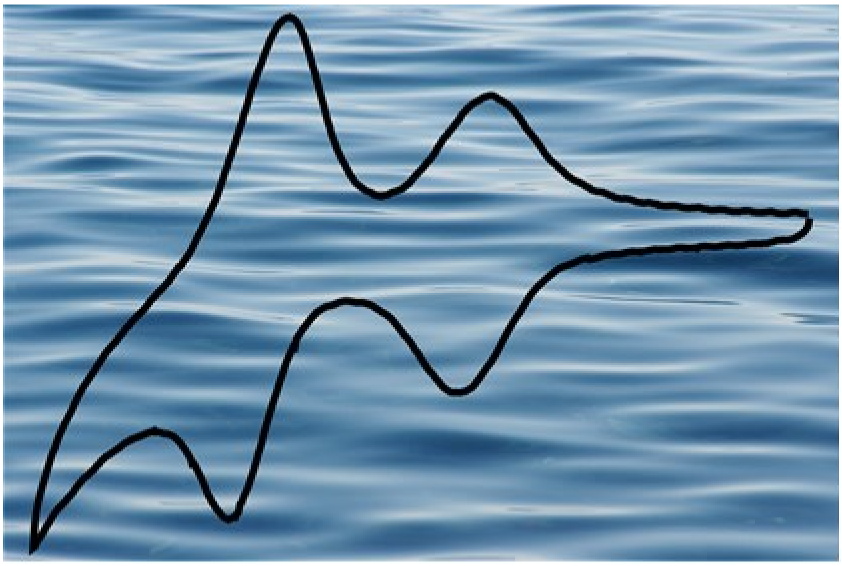
(39) Khairy, M.; Kampouris, D. K.; Kadara, R. O.; Banks, C. E.: Gold Nanoparticle Modified Screen Printed Electrodes for the Trace Sensing of Arsenic(III) in the Presence of Copper(II). *Electroanalysis* **2010**, *22*, 2496-2501.

(40) Kolliopoulos, A. V.; Metters, J. P.; Banks, C. E.: Electroanalytical sensing of selenium(iv) utilising screen printed graphite macro electrodes. *Analytical Methods* **2013**, *5*, 851-856.

(41) Kolliopoulos, A. V.; Metters, J. P.; Banks, C. E.: Screen printed graphite electrochemical sensors for the voltammetric determination of antimony(iii). *Analytical Methods* **2013**, *5*, 3490-3496.

(42) Smith, J. P.; Metters, J. P.; Kampouris, D. K.; Lledo-Fernandez, C.; Sutcliffe, O. B.; Banks, C. E.: Forensic electrochemistry: the electroanalytical sensing of Rohypnol[registered sign] (flunitrazepam) using screen-printed graphite electrodes without recourse for electrode or sample pre-treatment. *Analyst* **2013**, *138*, 6185-6191.

For TOC only



For TOC only