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Rapid and Portable Electrochemical Quantification of Phosphorus

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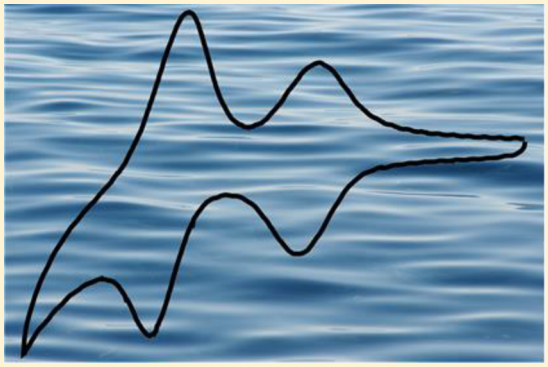
Supporting Information

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 the Environmental Protection Agency (EPA) for the detection of total phosphorus is colorimetric and based upon the color 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 the measurements, all forms of phosphorus are converted into orthophosphates via sample digestion (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 from 0.5 to 20 μg L^{-1} in ideal pH 1 solutions utilizing 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 a reaction time of 5 min, which is essential in the standard colorimetric approach, is not required in the new proposed electrochemically adapted protocol. The proposed 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 and provides a portable device for the rapid electrochemical detection of dissolved phosphorus using screen-printed electrodes.



Nutrients phosphorus and nitrogen play critical roles for living cells but their excess leads to nutrient pollution or overenrichment,[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 significant 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 hydrolyzed into orthophosphates. The low limits of the phosphorus concentrations in natural waters can be several micrograms per liter,[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 utilized only to identify serious eutrophication problems.[2] The measurement of low concentrations (less than 10 μg L^{-1}) of phosphorus in natural water makes the monitoring of phosphorus challenging. As described by the EPA, there are

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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 color 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 analyzed 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] see (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 colored a-keggin anion, ammonium phosphomolybdate. The reaction is as follows:[22,23]

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

The concentration of phosphate is proportional to the blue color 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 of the Supporting Information. Such approaches have utilized 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 the 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 SECTION

All chemicals used were obtained from Sigma-Aldrich. The phosphate standard solutions were prepared with predried (105 °C for 1 h) 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 canal water samples were adjusted to pH 1 with 5.5 M sulfuric acid[15] in accordance with the optical method in order to avoid possible interference of silicate.[12,28] All solutions were prepared with deionized 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 graphite electrodes (denoted as SPEs) were fabricated in-house with appropriate stencil designs using a microDEK 1760RS screen-printing machine (DEK, Weymouth, U.K.). Note that this screen-printed electrode design has been previously reported[37−41] "as is" without electrode pretreatment or modification in various electroanalytical endeavors. For fabrication of the SPEs, first a graphite ink formulation (product code: C2000802P2; Gwent Electronic Materials Ltd.) utilized 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 μm thickness) flexible film. The graphite ink layer was cured in a fan oven at 60 degrees for 30 min. Next, a silver/silver chloride reference electrode was included by screen-printing Ag/AgCl paste (product code: C2040308P2; Gwent Electronic Materials Ltd., U.K.) onto the polyester substrates, which was subsequently cured once more in a fan oven at 60 degrees for 30 min. Finally, a dielectric paste (product code: D2070423P5; Gwent Electronic Materials Ltd.) 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 min, the SPEs are ready to be used. These electrodes have been characterized 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 preclude useful measurements from being obtained.

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

The persulfate digestion for the detection of dissolved phosphorus (orthophosphates, organic, and hydrolyzable

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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 min 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. Sodium bisulphite, 5.2 g, was added and the solution was mixed and placed in a 95 °C water bath for 30 min.[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 a guard column and Dionex IonPac AS18 as a separation column.

The inductively coupled plasma optical emission spectrometry (ICP-OES) for the detection of dissolved phosphorus in the real samples was performed with 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 toward 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 +0.27 V and at +0.13 V are observed,

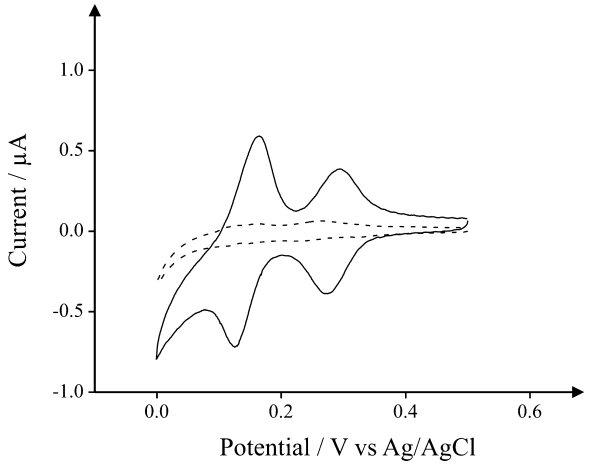

Figure 1. Cyclic voltammetry of SPE upon solution containing 640 mg L^{-1} ammonium molybdate tetrahydrate at pH 1 with and without the addition of 20 μg L^{-1} phosphorus. Dashed line: without phosphorus. Solid line: with 20 μg L^{-1} phosphorus. Scan rate: 50 mV s^{-1} vs Ag/AgCl.

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 +0.16 V and at +0.30 V 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 utilizing SPEs. Figures S2−S4 of the Supporting Information

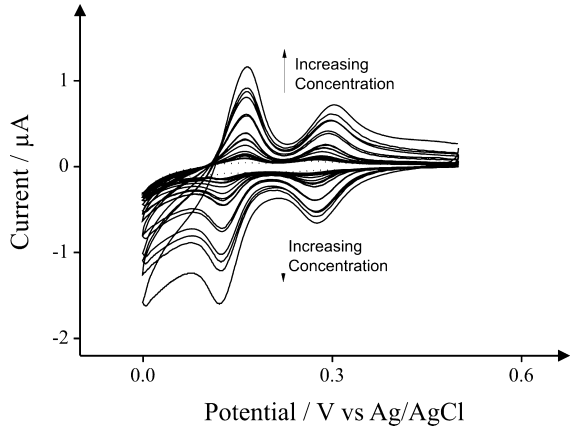

Figure 2. Typical cyclic voltammetric responses using a SPE following additions of phosphorus (0.5−100 μg L^{-1}) into an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. Note that a new SPE is used for each addition. Dotted line: without phosphorus (blank). Scan rate: 50 mVs^{-1} vs Ag/AgCl.

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 +0.16 V (see Figure S2 of the Supporting Information) ($I_P/\mu A = 2.76 \times 10^{-2}\ \mu A/(\mu g\ L^{-1}) + 5.21 \times 10^{-2}$

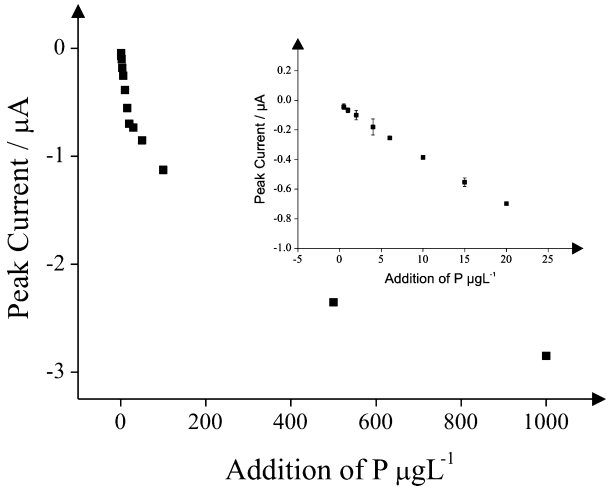

Figure 3. A typical calibration plot corresponding to additions of phosphorus (0.5−1000 μg L^{-1}) into an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. The data is from the analysis of the analytical reduction peak observed at +0.13 V. Inset: zoom of the calibration plot for additions of phosphorus (0.5−20 μg L^{-1}). Error bars in the inset arise from three measurements. A different SPE has been used each time.

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μA; R^2 = 0.9938 N = 3), for using the oxidation peak at +0.30 V (see Figure S3 of the Supporting Information) ($I_P/\mu A = 4.2 \times 10^{-3}\ \mu A/(\mu g\ L^{-1}) + 12.12 \times 10^{-2}\ \mu A$; R^2 = 0.7544 N = 3), for using the reduction peak at +0.27 V (see Figure S4 of the Supporting Information) ($I_P/\mu A = -4.8 \times 10^{-3} \mu A/(\mu g\ L^{-1}) -13.16 \times 10^{-2}\ \mu A$; R^2 = 0.6898 N = 3) and for using the reduction peak at +0.13 V (see Figure 3) ($I_P/\mu A = -3.4 \times 10^{-2}\ \mu A/(\mu g\ L^{-1}) -3.84 \times 10^{-2}\ \mu A$; R^2 = 0.9979 N = 3). The reduction peak at +0.13 V 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 from 0–20 μL^{-1}, using this line of best fit. The limit of detection (3σ) for phosphorus when utilizing new SPE for each addition was determined to be 0.3 $\mu g\ L^{-1}$.

In the EPA-recommended optical method, the time of reaction is crucial, and this is the reason why a minimum time of 5 min 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 +0.13 V in relation with the reaction time is shown in Figure S5 of the Supporting Information. It is clear from the figure that the reaction time does not 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^-, NO_2^-, NO_3^-, and HCO_3^- have been studied, and the results are depicted in Figure 4. It can be concluded that the ions,

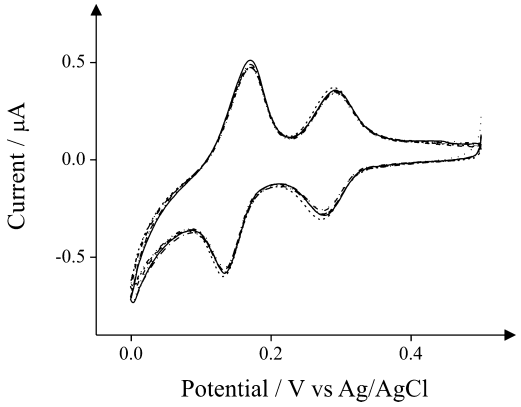

Figure 4. Typical cyclic voltammetric responses in the absence (solid line) and presence of 100 $\mu g\ L^{-1}$ of the ions Cl^- (dashed line), HCO_3^- (dotted line), NO_3^- (dashed-dotted line), and NO_2^- (dashed-dotted-dotted line) recorded in an ideal solution (pH 1) containing 640 mg L^{-1} ammonium molybdate tetrahydrate and 20 $\mu g\ L^{-1}$ phosphorus. Scan Rate: 50 mV s^{-1} vs Ag/AgCl.

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 deionized water (see Experimental Section). Cyclic voltammetric responses using SPE resulting from additions of phosphorus (1–20 $\mu g\ L^{-1}$) 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 of Figure 5 reveals a linear response for the sensing of orthophosphates using the reduction peak at

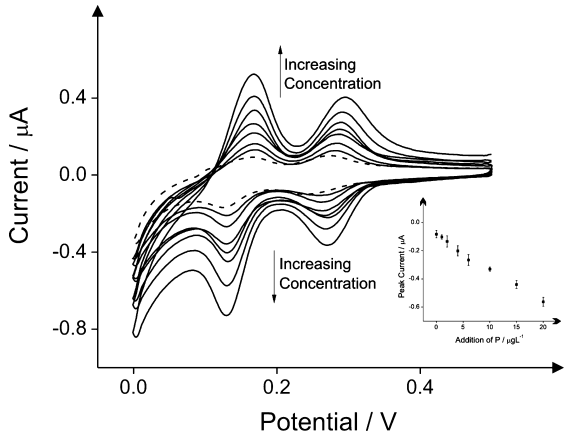

Figure 5. Cyclic voltammetric responses using SPEs following additions of phosphorus (1–20 $\mu g\ L^{-1}$) into a canal water (diluted 1:4) sample (adjusted to pH 1 with H_2SO_4) containing 640 mg L^{-1} ammonium molybdate tetrahydrate. A new SPE is used for each addition. Dashed line: without phosphorus. Analytical reduction peak at +0.13 V is analyzed to provide the data presented in the inset figure. A new SPE is used for each addition. Error bars (N = 3); scan rate: 50 mV s^{-1} vs Ag/AgCl.

+0.13 V ($I_P/\mu A = -2.37 \times 10^{-2}\ \mu A/(\mu g\ L^{-1}) + 9.33 \times 10^{-2}\ \mu A$; R^2 = 0.9923 N = 3). The initial concentration of orthophosphates of the diluted (1:4) canal water can be determined at 3.9 $\mu g\ L^{-1}$ as phosphorus. Consequently, the concentration of orthophosphates in the canal water was initially $4 \times 3.9 = 15.6\ \mu g\ L^{-1}$ as phosphorus. This value was confirmed by ion chromatography (Figure S6 of the Supporting Information), where orthophosphates were independently determined to be 15 $\mu g\ L^{-1}$ 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 $\mu g\ L^{-1}$) 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 of Figure 6 reveal a linear response for the sensing of orthophosphates using the reduction peak at +0.13 V was achieved ($I_P/\mu A = -1.11 \times 10^{-2}\ \mu A/(\mu g\ L^{-1}) -8.87 \times 10^{-2}\ \mu A$; R^2 = 0.9925 N = 3). The initial concentration of dissolved phosphorus of the diluted (1:100) canal water is determined to correspond to 8.0 $\mu g\ L^{-1}$. Consequently, the concentration of dissolved phosphorus in the canal water was initially $100 \times 8.0 = 800\ \mu g\ L^{-1}$ phosphorus. This value was confirmed by ICP-OES (Figure S7 of the Supporting Information), where dissolved phosphorus was determined to correspond to 885 $\mu g\ L^{-1}$ with a relative standard deviation of 10.1%. Reasonable agreement between the proposed electrochemical method and the traditional ICP-OES suggests that 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 of the Supporting Information. Presently this device has the potential to be used in the field, and we present a convenient

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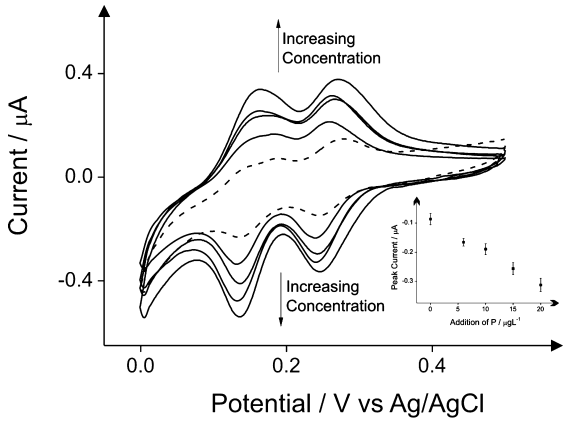

Figure 6. Cyclic voltammetric responses using SPE as a result of additions of phosphorus (1−20 μg L^{-1}) into a digested canal water (diluted 1:100) sample (using persulfate; see Experimental Section). The pH of the canal water sample was adjusted to pH 1 with H_2SO_4. 640 mg L^{-1} ammonium molybdate 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 +0.13 V is analyzed to provide the data presented in the inset figure. A new SPE is used for each addition. Error bars (N = 3); Scan rate: 50 mV s^{-1} vs Ag/AgCl.

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 pretreatment and the need of the sulfuric acid and other reagents such as ammonium persulfate makes the incorporation of these during the screen-printed fabrication 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 utilizes cost-effective screen-printed sensors providing an appealing alternative to the existing 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 EPA colorimetric method, but additionally it exhibits lower detection limits than the EPA approach and 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".

ASSOCIATED CONTENT

Supporting Information

Table summarizing 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 phosphorus determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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