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Glassy carbon electrode modified with hybrid films containing inorganic molybdate anions trapped in organic matrices of chitosan and ionic liquid for the amperometric sensing of phosphate at neutral pH

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ABSTRACT

This work reports an amperometric method for phosphate analysis based on the use of a surface modified glassy carbon electrode (GC). In one configuration of the electrode the surface is modified with ammonium heptamolybdate incorporated in chitosan matrix. A second configuration of the surface modified electrode is formed by introducing, on top of the chitosan film, an additional layer of molybdate dissolved in ionic liquid, 1-Ethyl -3- methylimidazolium tetrafluoroborate. The molybdate layers are then coated with a film of PSS + PEDOT (Poly styrenesulphonate + Poly (3, 4-ethylene dioxythiophene)). The chitosan matrix provides the acidic conditions required for the surface molybdate to react with phosphate forming electroactive phosphomolybdate which allows amperometric determination of phosphate. The reduction of ammonium phosphomolybdate is monitored at - 0.140 V vs. NCE (Hg/Hg₂Cl₂/1N KCl) at pH = 7.2 in tris buffer. The linear range of detection for phosphate lies between 19 and 100 μ M with the first configuration electrode. The limit of determination could be extended to a lower range of 0.79 to 32 μ M using the second configuration electrode. This method is found to be free from interference from anions like arsenate, nitrate and chloride. The method is validated by determining phosphate concentration in a commercial fertilizer sample. This method is highly

selective, sensitive, enzyme less and requires no other additional reagents. Additionally it works at neutral pH , hence would be applicable for phosphate analysis in biological samples. Unlike ICP-OES analysis which requires highly sophisticated and costly instrumentation, the proposed method involves simple and cheaper instrumentation requirements and is suitable for field measurements.

Keywords:

Ammonium molybdate

Phosphate sensor

Chitosan

Chronoamperometry

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1. Introduction

Molecules containing phosphate group are of special interest because of their ubiquitous presence in life. The presence of inorganic phosphate derived from fertilizers, similarly to nitrates, leads to an excessive growth (eutrophication) of aquatic plants and algae that disrupts aquatic life cycles. Sodium and potassium organo-phosphate compounds are among the most used pesticides in many intensive agricultural activities that are often found in ground waters, leading to severe health problems. In biological systems, dihydrogen phosphate (H_2PO_4^-) controls the balance of many biological processes, and imbalances in dihydrogen phosphate can cause some diseases [1]. Another area where the phosphate control is assuming an increasing importance is the protection of the cultural heritage as phosphate plays a major role in the bio-deterioration of archaeological sites caused by cyanobacterial bio films [2]. For these reasons, H_2PO_4^- recognition is of importance. The analytical range of phosphorus is from 0.2 to 10 mg/L in natural and waste waters and from 0.2 to 50 mg/kg in soil. Maximum permissible concentration of phosphate in river water is $0.32 \mu\text{M}$ and ranges from 0.0143 to 0.143 mM in wastewater. For diagnostic purposes, the concentration of phosphate ion in human saliva is a variable, ranging from 5 to 14 mM. It is in the range of 0.81 to 1.45 mM PO_4^{3-} in adult human serum [3]. Various detection strategies for phosphate have been developed, which include phosphate ion selective electrodes, amperometry, chromatography, flurometry, colorimetry, spectroscopy and enzymatic biosensors [4-17]. Most manual and automated methods of phosphorus determination are based on the reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid. This is then reduced to an intensely coloured blue compound containing mixed oxidation states of Mo(VI) and Mo(V) and determined spectrophotometrically. Ascorbic acid and tin (II) chloride are some of the reducing agents used for spectrophotometric detection. As a routine analytical method, the procedure is carried out in

an automated continuous flow assembly [18]. Spectrophotometric analyses often involve selective extraction into an organic solvent or addition of organic molecules such as tartrate, oxalate, mannitol, acetone or butanone. These added organic compounds are variously said to complex the molybdenum (VI) and thus prevent or slow down the formation of the phosphomolybdate (Keggin anion). Their addition stabilizes the Keggin anion to different extents, influence the ratio of the isomers formed, change the extinction coefficient of the molybdenum blue or destroy the colour of the reduced species. Furthermore, the effect of their addition could vary, depending upon the order in which they are added. Furthermore this method suffers from interference arising from arsenates, silicates and other anions [18]. Thus none of these methods is entirely and universally satisfactory. Recently, extensive work has also been devoted to design new sensors for recognition of phosphate ions in water at biological pH values using various receptors to bind dihydrogen phosphate ions[19-22]. In one of these methods, fluorescence sensing of phosphate anions via intramolecular excimer formation in a pyrophosphate-induced self-assembly of a pyrene-functionalized guanidinium receptor was reported [19]. In another method zinc complex of 2,6-Bis(bis(2-pyridylmethyl)aminomethyl)-4-methylphenol (H-bpmp) was used along with pyrocatechol violet dye for the spectrophotometric determination of phosphate anions in aqueous systems at neutral pH [20]. However there is no suitable protocol available for the electrochemical interrogation of phosphate at neutral pH apart from enzyme based sensing of phosphate [8]. The reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate, the keggin anion, is known for a long time. There are papers which discuss amperometric determination of phosphate based on the electrochemical reduction of phosphomolybdates in solution. These papers report formation of ammonium phosphomolybdate in the test solution which is then electrochemically monitored[23-

26,18,2]. In one of the reports, the authors have used an electrode modified with ammonium phosphomolybdate by electrochemical cycling [23]. However, the analysis procedure required the addition of phosphomolybdate in nitric acid which was used as the test solution. The method is thus not suitable for application in biological systems because it requires acidic environment. In the present work we make use of the same reaction of phosphate with ammonium molybdate. However, we trap the molybdate anions in chitosan matrix and demonstrate that the interfacial acidity associated with the chitosan film is sufficient for the reaction between molybdate and phosphate. Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The amino group in chitosan has a pKa value of ~ 6.5 , thus, chitosan is positively charged and soluble in acidic to neutral solution with charge density dependent on pH. This makes chitosan a bio adhesive which readily binds to negatively charged species. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable. To our knowledge no attempt has so far been made to modify the electrode surface with molybdate anions for the determination of phosphate at neutral pH. The analysis of phosphate is carried out amperometrically via electroreduction of ammonium phosphomolybdate at a fixed potential. The advantage of our method is selectivity, stability and relative environmental insensitivity of electro active labels compared with fluorophores that can quench or photo bleach. Furthermore, our method does not involve the addition of any other reagent. Compared to spectrophotometric method, the electrochemical detection offers the advantage of selectivity and hence the effect of interference is nil. The redox potentials of the probable interferents, molybdosilicate and molybdarsenate are quite different from molybdophosphates.¹⁸ In this paper we report two new effective strategies for the surface modification of glassy carbon electrode by ammonium

heptamolybdate (Scheme 1). In the first configuration glassy carbon electrode is modified with a layer of chitosan containing ammonium heptamolybdate (Electrode configuration 1). In the second configuration an additional layer of ammonium molybdate is added with the help of the ionic liquid, 1-Ethyl -3- methylimidazolium tetrafluoroborate (Electrode Configuration 2). Our sensing protocol is reagent less, enzyme less and selective.

2. Experimental Section

2.1. Chemicals and Reagents

The following chemicals were used as received:

Chitosan powder (gift sample from Bio Everest, India), Acetic acid (Fischer), Ammonium heptamolybdate, (AR, SD fine chem. Ltd), H_2SO_4 (Merck), Poly(3,4-ethylene dioxythiophene)-poly(styrene sulfonate) (PSS-PEDOT; 2.2-2.6% in H_2O , High conductivity grade Sigma Aldrich), Tris (hydroxyl methyl)-amino methane (Amersham Biosciences), Sodium dihydrogen phosphate (Merck), 1-Ethyl -3- methylimidazolium tetrafluoroborate (Merck), Parry's super phosphate containing 16% phosphate-(Coromandel International Limited, Ranipet, Vellore district)

2.2. Preparation of stock solutions for electrode modification

The following stock solutions were prepared and used for the electrode modification.

50 mg of chitosan was weighed and dissolved in 3 ml of 1:1 Acetic acid by stirring continuously for about 4 hrs to get a clear solution.

Molybdate solution was prepared by dissolving 0.3 g of Ammonium heptamolybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 4 ml of 0.05M H_2SO_4 and stirring the solution for 2 hr. A solution of

higher concentration of molybdate was also prepared by dissolving 0.5g of Ammonium heptamolybdate.

50 μ l of the polyelectrolyte solution PSS-PEDOT was diluted to 150 μ l with distilled water.

The ionic liquid solution was prepared by dissolving either 20 mg or 50 mg of Ammonium heptamolybdate in 50 μ l of 1-Ethyl -3- methylimidazolium tetrafluoroborate.

Neutral tris buffer (pH7.2) was prepared from 0.1 M Tris (hydroxymethyl) – amino methane. The pH was adjusted with 0.1M HCl.

A stock solution of the fertilizer sample (10mM phosphate) was prepared by dissolving 0.6097 g of the fertilizer in 100 ml water.

2.3. *Modification of glassy carbon electrode*

Configuration 1

A glassy carbon (GC) electrode ($\Phi=3$ mm) was initially cleaned with the help of alumina slurry and emery papers and subjected to sonication in distilled water. The surface purity was checked by recording the voltammogram of 1mM ferrocyanide in 0.05M sulphuric acid which exhibited reversible features. Modification of electrode was carried out by drop casting 5 μ l chitosan solution on the surface of the glassy carbon electrode and the film was allowed to dry for 1 hr at the room temperature (~ 30 $^{\circ}$ C). The chitosan layer modified electrode was then immersed in the molybdate solution (containing either 0.3g or 0.5 g of ammonium molybdate) for 12 hrs. After that the electrode was gently rinsed with 0.05 M H₂SO₄ and then 5 μ l PSS-PEDOT solution was spread on the molybdate + chitosan modified GC surface by drop casting. The film was allowed to dry for 20 minutes.

Configuration 2

The electrode configuration 2 was prepared by further functionalising the configuration 1 electrode. This was performed by drop casting 5 μl of the ionic liquid solution containing molybdate ions on the first layer (Configuration 1). The layer was allowed to dry at room temperature for about 24 hrs. Finally 5 μl of PSS-PEDOT solution was spread on the molybdate + ionic liquid film by drop casting. The film was allowed to dry for 20 minutes.

Molybdate anion loading levels on glassy carbon electrode

The following stock solutions were used to load different concentrations of molybdate anions on glassy carbon electrode.

- (a) Lower level loading of molybdate: A solution consisting of 0.3 g ammonium heptamolybdate in 0.05M sulphuric acid for modification in the chitosan layer and 20 mg of ammonium molybdate in 50 μl of the ionic liquid for the ionic liquid layer.
- (b) Higher level loading of molybdate: A solution consisting of 0.5 g of ammonium heptamolybdate in 0.05M sulphuric acid for modification in the chitosan layer and 50 mg of ammonium heptamolybdate in 50 μl ionic liquid for the ionic liquid layer

2.4. Electrochemical measurements

Cyclic voltammetry and Chrono amperometry were carried out using the PGSTAT 302N (Autolab; Ecochemie) electrochemical system. Normal calomel electrode ($\text{Hg}/\text{Hg}_2\text{Cl}_2$, 0.1 M KCl) and Pt foil was used as reference and counter electrode respectively. Deaerated tris buffer

(pH=7.0) was used as a supporting electrolyte. For the real sample analysis commercially available phosphate fertilizer (parry's super phosphate, P= 16%, Coromandel International Limited) was used to determine the phosphate concentration.

2.5. Surface Characterisation

The surface electron micrographs (SEM images) were recorded using Zeiss Neon 40EsB. X-ray photoelectron spectroscopic studies were carried out using Multilab 2000 model, Thermo Scientific, UK. A source of Al K α having a binding energy of 1486.4eV was used for the study.

3. Results and Discussion

3.1. Surface characterisation of modified glassy carbon electrode

Chitosan is a well-known bio sorbent for ionic species. Several studies have been carried out on its sorption capability, using native chitin or chitosan or more sophisticated forms resulting from a grafting of specific functional groups [27-30]. In this work we take advantage of the bonding properties of chitosan for trapping molybdate anions. The chitosan based films with trapped molybdate anions were prepared by two different strategies as explained in the experimental section. We probed into the morphology of the modified films through scanning electron micrographs.

The scanning electron micrographs showing the different stages of modification are presented in **fig 1A-C**. As the incorporation of molybdate anions is dictated by electrostatic interactions between cationic chitosan film and the molybdate anions, it was anticipated that the electrostatic interactions may lead to some directional or ordering influence on the morphology. However, the observed morphology of the films does not reveal any organised structure. The film (A) which contains molybdate anions trapped in chitosan matrix is not rigidly packed and

hence the adsorption of molybdate ions is expected to be more facile. One factor limiting the uptake of anions in chitosan matrix is the low porosity of crude chitosan resulting in poor accessibility of internal sorption sites. The sorption may therefore be mostly limited only to the external layers of the sorbent particle. The observed morphology of the films formed in our work should not have such limitations. The addition of PSS-PEDOT forms a patchy film (B) over the chitosan film. The PSS-PEDOT films stabilises the molybdate anions and the film becomes more stable by incorporation of this additional layer. The ionic liquid incorporated molybdate anions formed on the GC surface as a third layer increases the molybdate concentration and the morphology of the layer becomes granular in nature. (C)

3.2. *Electrochemical characterisation of the modified electrodes*

Fig. 2 represents the cyclic voltammograms (CV) of the molybdate in chitosan matrix on GC electrode (Configuration 1) at various scan rates. Three reduction peaks c_1 , c_2 and c_3 are observed. The peak heights increase with scan rate. At 50 mV/s the peaks are located respectively at 0.056V, -0.094 V and -0.266 V. The peak c_1 is not very well defined. The corresponding anodic peaks are also not well defined. The observed CV behaviour of molybdate in the chitosan matrix is similar to that on bare carbon electrode in 0.5M H_2SO_4 except that the anodic peaks of the bare electrode are better defined in the acidic solution. (Supplementary data **Fig. S1**). The difference in behaviour could be explained by the fact that molybdate concentration in the chitosan matrix is low and it is in the solid phase. The CV (**Fig. 3**) of the configuration 2 electrode, which contains a higher concentration of molybdate than the configuration 1 electrode is much better defined. However, in **fig. 3** only two well defined cathodic peaks are obtained. The peak c_1 which appears at 0.056 V in the case of configuration 1

is not clearly seen for the configuration 2 electrode. This is probably related to the increased thickness and/or the presence of the ionic liquid in the film of the configuration 2 electrode. The cathodic peaks occur at slightly more negative potentials than for the configuration 1 electrode.

It is difficult to assign the electron transfer reactions associated with the different peaks observed in the cyclic voltammograms because the speciation chemistry of molybdate is very complex and pH dependent. Apart from the major polyanions, $\text{Mo}_7\text{O}_{24}^{6-}$ other species such as $\text{H}_2\text{Mo}_7\text{O}_{24}^{4-}$, $\text{Mo}_8\text{O}_{26}^{4-}$ can exist in the film [31]. But at $\text{pH} \leq 2$ hexavalent molybdenum exists primarily as monomeric species. The reduction of molybdate (VI) anions in aqueous acidic media is known in the literature to involve 4 electrons and occurs in either three steps [31,32] ($1\text{e}, 1\text{H}^+$; $1\text{e}, 1\text{H}^+$; $2\text{e}, 2\text{H}^+$) or in two steps [2] ($2\text{e}, 2\text{H}^+$; $2\text{e}, 2\text{H}^+$). The three step reduction involves two single electron transfer steps and one two electron transfer step. Each electron transfer is associated with the addition of a proton. The two step reduction involves two electrons in each step together with addition of two protons. The different stages of reduction correspond to different forms of Mo(VI). The fact that the molybdate in the chitosan matrix behaves similarly to that in aqueous solutions indicates that chitosan as used in our work provides the required acidic environment for the electrochemical reactions to occur.

3.3. Effect of addition of phosphate on the CV behaviour of the molybdate modified electrodes

The effect of adding increasing amounts of phosphate in the electrolyte on the CV behaviour of electrodes of both the configurations was investigated. The **fig. 4A** and **4B** show the resultant cyclic voltammograms when increasing amounts of phosphate are added to the solution. The CV's obtained for both the electrode configurations are similar except that the peak current variations are higher and the peaks are better defined for the configuration 2 electrode. The CV's of Configuration 2 electrode are better defined and the current response is higher even at much

lower phosphate concentrations (an order of magnitude lower). This obviously is related to the higher concentration of molybdenum on the configuration 2 electrode which interacts with phosphate forming molybdophosphate electroactive species. The important point to note here is that the peak current of all the cathodic peaks increase with increase in phosphate concentration in the electrolyte (**Fig. 4A and 4B**). This observation could form the basis of amperometric determination of phosphate in solutions.

Based on the literature reports [23], the reactions which could possibly be occurring at the chitosan matrix are



3.4. XPS analysis of the modified electrode

XPS analysis has been carried out to see whether the sensing mechanism is based on the reactions given in equations 1 and 2. Two main peaks (i and ii) corresponding to Mo 3d appear (**fig. 5A**) as doublet (Mo 3d_{5/2} and Mo 3d_{3/2}) before reacting with phosphate. The binding energy of the peaks (232.5 eV and 235.6 eV) correspond to Mo⁶⁺ state [33,34]. The XPS figure corresponding to electrode configuration 1 treated with H₂PO₄⁻ and after reduction at -0.140V, exhibit two broad peaks with some peaks overlapped in between. (Figure B) After deconvolution four peaks were resolved which correspond to mixed oxidation states of Mo. The binding energy of the peaks namely at 231.7 eV (Mo⁵⁺), 233.09 eV (Mo⁴⁺), 234.9 eV (Mo⁶⁺) and 236. eV (Mo⁶⁺) matches with that of Mo⁵⁺, Mo⁴⁺, Mo⁶⁺ [33]. A symmetric doublet (figure A) was obtained before

reaction with phosphate from Mo3d level spectra which is due to spin-orbit coupling of Mo 3d_{5/2} and 3d_{3/2}. But an asymmetric or broader peaks (**fig. 5B**) appeared in Mo 3d level spectra after reaction with phosphate and subsequent electro reduction. It confirms that the surface of the molybdenum composite contains different oxidation states of Molybdenum [35,36]. The observations are in conformity with the reaction presented in the equations 1 and 2 and as per literature reports [23].

3.5. Amperometric analysis of phosphate

As mentioned earlier, the results shown in Figure 4 indicate that the peak currents of all the cathodic peaks vary with phosphate ion concentration. Any of the peaks of **Fig. 4** could be used for amperometric analysis of phosphate. We have chosen the cathodic peak c_2 as the signature peak for this purpose and carried out amperometric measurements by holding the potential of the electrode at -0.140V vs NCE in the Tris Buffer solution at pH 7.2. The resulting currents were measured while standard additions of phosphate solutions were made. Experiments with electrodes of both the configurations were carried out. The results for the configuration 1 electrode are shown in **Fig. 6A**. The standard additions of the phosphate solutions used in the experiment are noted in the figure. The results are used to construct the calibration graph (**Fig. 6B**)

After five standard additions of phosphate aliquots of the test fertilizer samples were added into the reaction cell. The corresponding current changes, recorded in Figure 6A as X_1 , X_2 refer to the additions of the fertilizer sample. The linear range of the calibration graph lies between 19 μM to 95 μM . The sensitivity of detection is 0.044 $\mu\text{A}/\mu\text{M}$. The concentrations of the fertilizer samples as determined by fitting the respective current data on the calibration graph are also noted in Figure 6B. The results obtained were within 5% of the actual values.

The **Fig. 7** shows the results of amperometric experiments carried out with the configuration 2 electrode at -0.140 V. Phosphate solutions of concentrations ten times lower than those used for the configuration 1 electrode were used in the experiment. The results are used to construct the calibration graph (**Fig. 7B**). The linear range of detection in this case is found to lie between 0.79 μM and 3.96 μM . The sensitivity of detection is 0.79 $\mu\text{A}/\mu\text{M}$. The electrode configuration 2 could reliably measure phosphate concentration in the range 0.79 μM to 31.5 μM .

Effect of molybdate loading on phosphate analysis

The results discussed above indicate that by using of two layers of molybdate in the modified matrix (Configuration 2), higher sensitivity and very low detection range for phosphate analysis could be achieved. This observation was further investigated by increasing the amount of molybdate in the chitosan and in the ionic liquid layer as mentioned in the experimental section. For the higher level of loading, the solution containing 0.5g instead of 0.3 g ammonium molybdate was used for the modification of the electrode in the first layer. The amperometric curve for configuration 2 electrode with the higher loading of molybdate is shown in figure 8. The calibration curve derived from the current changes is presented in the inset of **fig. 8**.

The sensitivity of detection is increased to 3.05 $\mu\text{A}/\mu\text{M}$. The increased level of molybdate loading also changes the phosphate detection range. The detection range is 6.4 μM to 51 μM as compared to 2.0 μM to 27 μM obtained with the configuration 2 electrode which has the lower level of molybdate loading. (Figure S2, Supplementary data)

3.6. Effect of interfering ions

The **Fig. 8** also includes the data for the effect of interfering ions such as arsenate, nitrate and chloride on the amperometric determination of phosphate. These ions usually interfere with phosphate analysis by conventional methods like spectrophotometry. The experiment was carried

out using the electrode of configuration 2 loaded from the molybdate solution of the higher concentration (0.5g) After the third standard addition of phosphate, equal quantities of 15 μM solutions of chloride, nitrate and arsenate were added in sequence, one at a time and their influence on the current response was noted. As can be seen from the results in Figure 8 the added ions had no effect on the current indicating that the addition of the interfering ions had no effect on phosphate analysis. The experiment was continued with further standard additions of phosphate. The variation of current with respect to concentration is presented in the inset of the **fig. 8**. In the case of silicate ions, interference was observed to an extent of 24%. Shifting the detection potentials to less negative values is likely to solve this problem and it will be addressed in our future studies.(Figure S3 supplementary data.)

The influence of the matrix effect on the determination of phosphate present in the fertilizer was evaluated by cyclic voltammetry and amperometry. The phosphate present in the fertilizer, irrespective of the other components present therein, could give rise to a well defined response when subjected to cyclic voltammetry or amperometry using the configuration 2 electrode with the higher loading of molybdate. The experimental observations are presented in the supporting information (**Figs S4 and S5**).

The amperometric experiments were repeated four times with each configuration of the electrode for reproducibility and stability. The experiments were highly reproducible the current changes for each addition was compared for all the experiments. The mean deviation of the current values was within 10%

4. Conclusion

A new amperometric method for analytical determination of phosphate utilising a surface modified glassy carbon electrode (GC) has been developed. Two strategies for surface modification of the electrode by ammonium heptamolybdate have been investigated. One of these involves loading of heptamolybdate anions on to a positively charged layer of chitosan on the surface of the electrode. The second involves loading of an additional layer of molybdate dissolved in the ionic liquid, 1-Ethyl -3- methylimidazolium tetrafluoroborate. The stability of the surface film is further improved by an outer layer of PSS + PEDOT (Poly styrenesulphonate + Poly (3, 4-ethylene dioxythiophene)). The molybdate ions in the surface modified layer react with phosphate forming electroactive phosphomolybdate species. This forms the basis of amperometric determination of phosphate at low over potentials (\sim 140 mV vs. NCE). It is found that the amperometric method using molybdate surface modified electrode could be reliably used to determine very low concentrations of phosphate with good sensitivity. The sensitivity of detection achieved in the presence of the ionic liquid is $3.06\mu\text{A}/\mu\text{M}$. Unlike other commonly used procedures the method works at neutral pH. Furthermore the method does not suffer from interference from arsenate, nitrate and chloride, which are known to limit application of other methods of phosphate analysis. The method described in this work offers a selective, sensitive, enzyme less and reagentless analytical determination of phosphate in biological systems and suitable for onsite measurements where we cannot afford to carry costly equipments like ICP-OES.

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Supplementary data

The solution phase cyclic voltammetric response for ammonium molybdate in sulphuric acid, Amperometric analysis of phosphate using configuration 2, cyclic voltammetric and Amperometric analysis for different fertilizer concentrations and amperometric response for interferents.

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Scheme 1

Schematic representation of glassy carbon electrode modification strategies

(A) Electrode configuration 1 (B) Electrode configuration 2, PEDOT= Poly (3, 4-ethylene dioxothiophene); PSS= Poly styrenesulphonate;

Figure captions:

Fig. 1. SEM images of modified GC electrode: (A) Chitosan +molybdate anions (B) Chitosan +molybdate anions/ PSS+PEDOT (C) chitosan+molybdate anions/PSS+PEDOT/Ionic liquid + molybdate anions

Fig. 2. Cyclic voltammograms of ammonium molybdate modified glassy carbon electrode (configuration 1) in Tris buffer (pH=7.2) at different scan rates

Fig. 3. Cyclic voltammograms showing the redox response of the molybdate modified GC electrode (configuration2) at different scan rates in Trsi buffer, pH=7.2

Fig. 4. Cyclic voltammograms showing the effect of addition of different concentration of phosphate (A) Electrode configuration 1 (B) Electrode configuration 2, Scan rate of 50mV/s ; Supporting electrolyte = Tris buffer, pH=7.2

Fig. 5. XPS of Mo 3d level spectra A) molybdate modified GC electrode, configuration 1 B) molybdate modified GC electrode, configuration 1, treated with H_2PO_4^- after reduction at -0.140 V Vs $\text{Hg}/\text{Hg}_2\text{Cl}_2$ (1N KCl) fitted by deconvolution technique

Fig. 6. Amperometric analysis of sodium dihydrogen phosphate using configuration 1 electrode at -0.140 V in Tris buffer (pH 7.2). (A) The amperometry data for various standard additions of phosphate are represented by their concentrations in the figure. X_1 , X_2 , correspond to the additions of the super phosphate fertilizer sample (B) Calibration graph, current versus H_2PO_4^- concentration corresponding to the data in Figure 6A

Fig. 7. Amperometric analysis of sodium dihydrogen phosphate using configuration 2 electrode at -0.140 V in Tris buffer (pH 7.2). (A) The amperometry data for various standard additions of phosphate. (B) Calibration graph, current versus H_2PO_4^- concentration corresponding to the data in Figure (7A)

Fig. 8. Amperometric curve for different additions of phosphate for Configuration 2 electrode with a higher loading of molybdate anions. The inset shows the variation of current for different standard additions of phosphate. The effect of addition of interfering ions (As(V) , Cl^- , NO_3^-) on amperometric curve after 3rd addition of phosphate is included; Detection potential = -0.120 V

Scheme 1

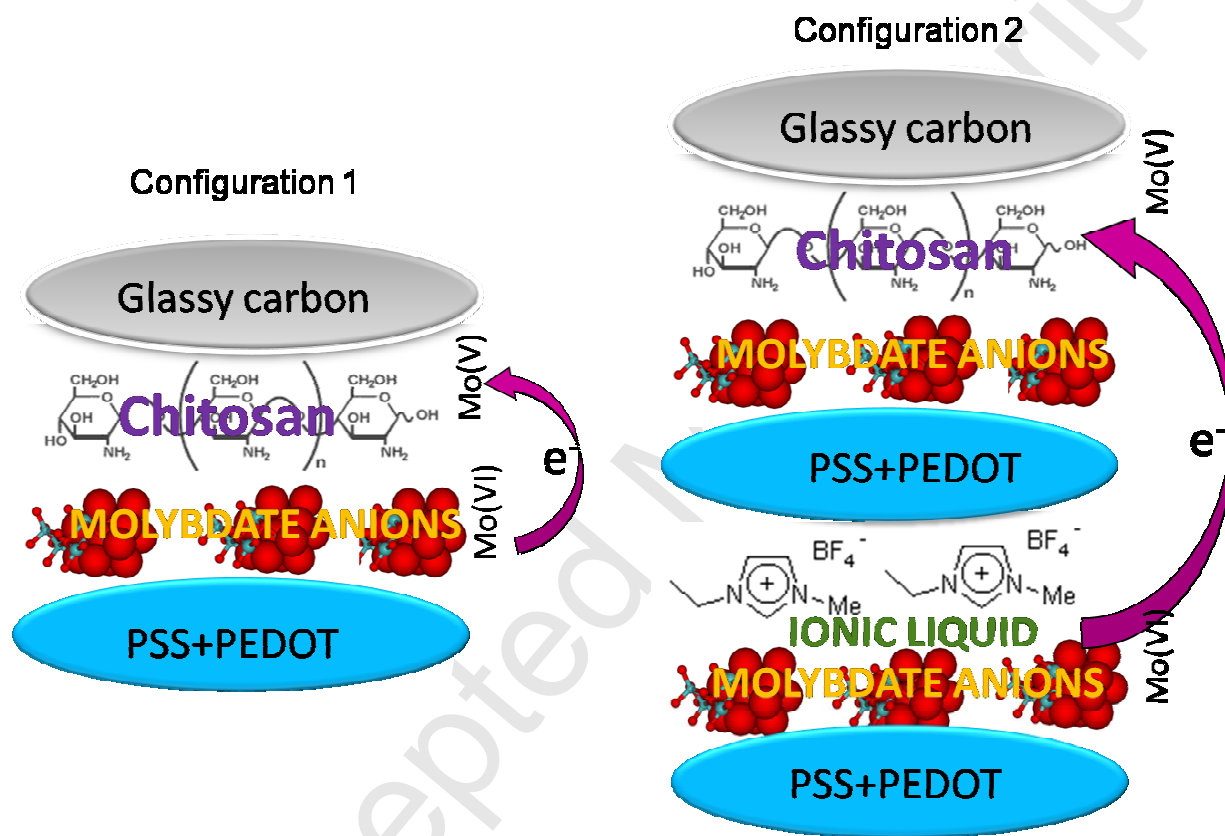


Figure 1

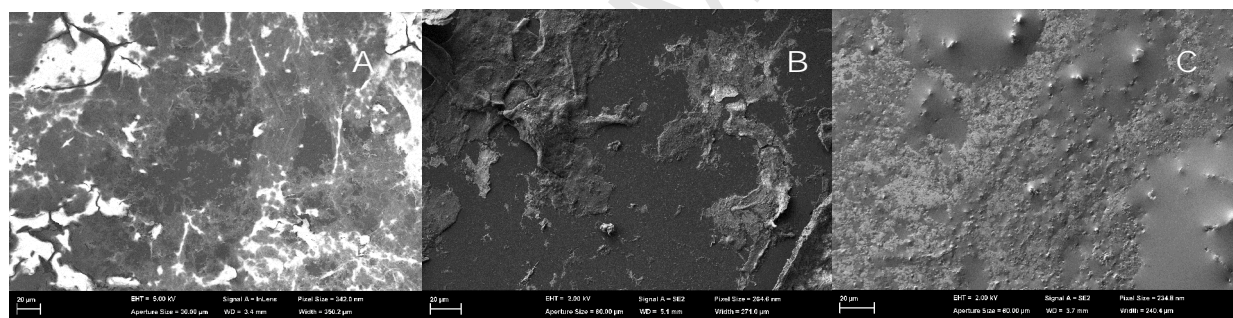


Figure 2

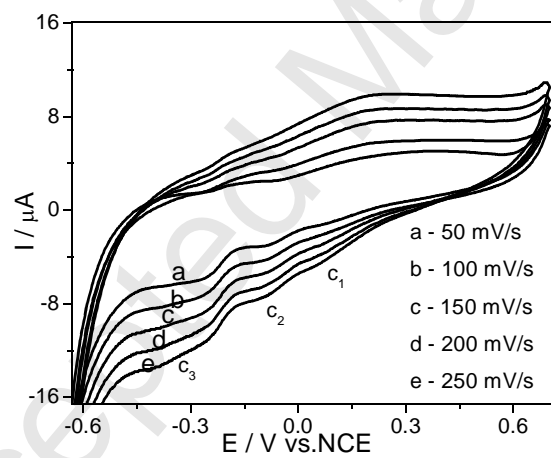


Figure 3

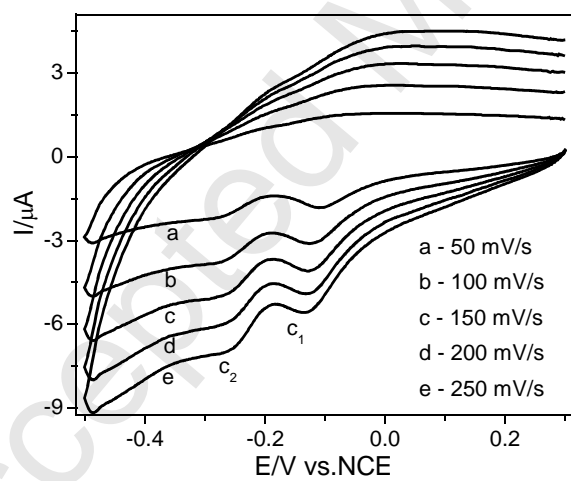


Figure 4

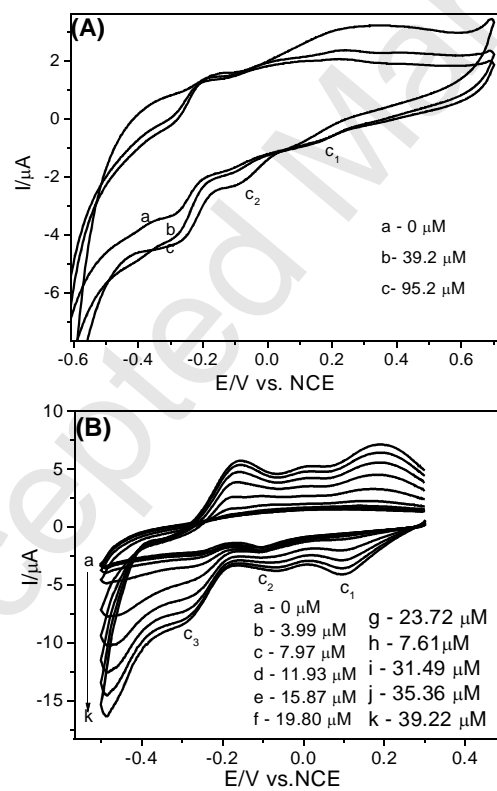


Figure 5

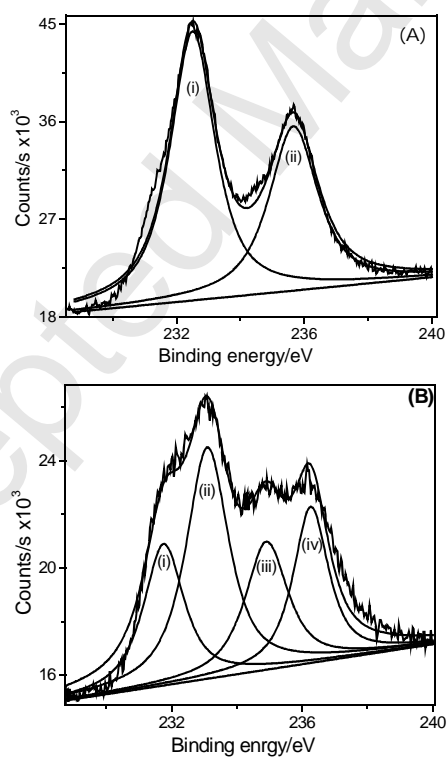


Figure 6

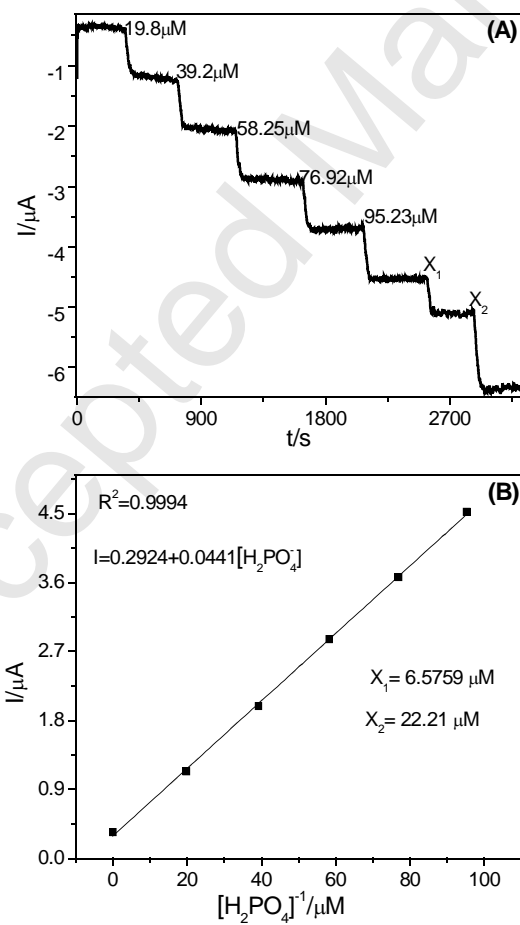


Figure 7

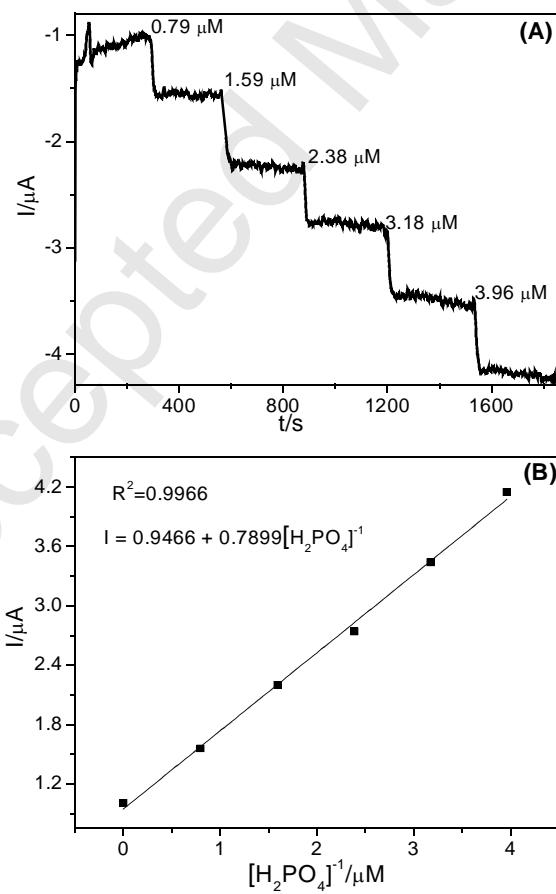
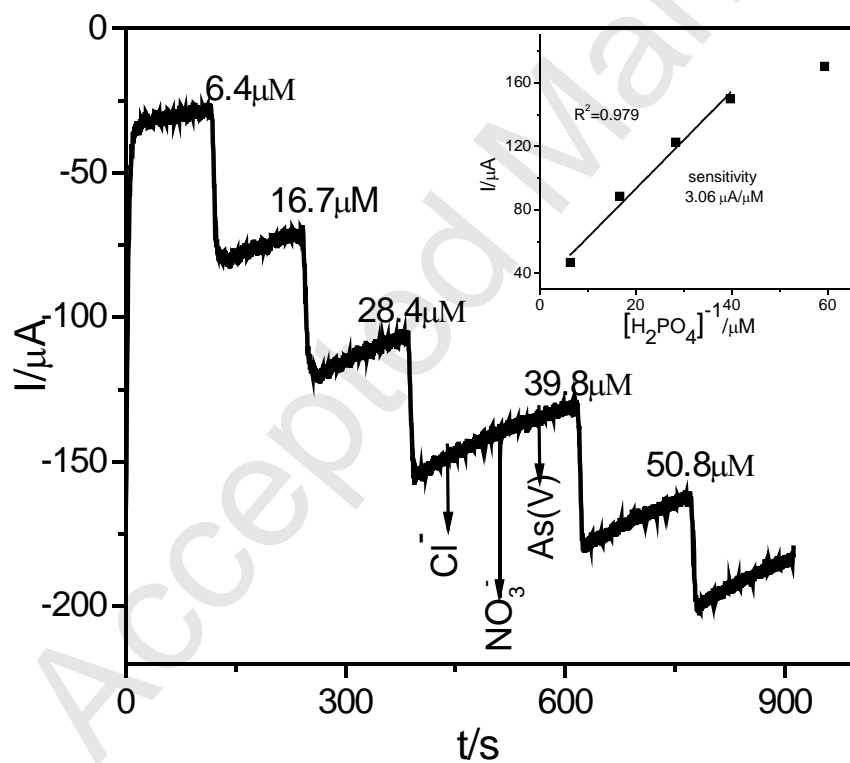


Figure 8



Graphical abstract

Glassy carbon electrode modified with hybrid films containing inorganic molybdate anions trapped in organic matrices of chitosan and ionic liquid for the amperometric sensing of phosphate at neutral pH

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