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Original scientific paper

Electropolymerized poly(methylene blue)-modified graphite electrode for phosphate detection

Ana Lucia Ferreira de Barros✉, Leandro Marques Samyn, Vernan Lima and Rajendran Suresh Babu

Laboratory of Experimental and Applied Physics (LaFEA), Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/RJ), Av. Maracanã, 229, 20270-110, Rio de Janeiro, Brazil

Corresponding Author: ✉*ana.barros@cefet-rj.br*; Tel.: +55-21-2566-3179

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Abstract

A graphite electrode modified with an electropolymerized poly(methylene blue) (PMB) film was prepared and evaluated as an electroanalytical platform for phosphate detection in aqueous media. The PMB layer was deposited on graphite by potentiodynamic electro-polymerization via cyclic voltammetry and characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy, scanning electron microscopy and Fourier-transform infrared spectroscopy. Compared to the bare graphite electrode, the modified surface exhibited improved interfacial electrochemical properties and an increased electroactive surface area. The electroanalytical response toward phosphate was investigated using differential pulse voltammetry (DPV), chronoamperometry and CV, revealing a linear response in the concentration range from 50 to 475 μM, as determined from DPV and chronoamperometric measurements. The modified electrode showed good reproducibility, low interference from common inorganic ions, and satisfactory performance in real water samples, with recovery values close to 100 %. These results demonstrate that the PMB-modified graphite electrode constitutes a simple and reliable electroanalytical approach for phosphate determination in environmental samples.

Keywords

Electroanalysis; indirect sensing; electrochemical sensor; redox-active polymer; environmental monitoring

Introduction

The presence of phosphate in natural and treated water systems plays a crucial role in environmental, agricultural, and biological processes. However, excessive phosphate concentrations are directly linked to eutrophication, harmful algal blooms, and the resulting deterioration of water quality and aquatic ecosystems [1,2]. Owing to these environmental impacts, the development of sensitive, selective, and cost-effective analytical methods for phosphate monitoring remains a topic of considerable scientific and technological interest.

Phosphorus occurs in aquatic environments in both inorganic and organic forms, each contributing differently to biogeochemical cycles and ecosystem dynamics [3]. Among these species, inorganic orthophosphates are the most bioavailable at near-neutral pH and therefore represent the most relevant targets for environmental monitoring [2,4-6]. The main anthropogenic sources of phosphate contamination include agricultural runoff from fertilized soils, as well as domestic and industrial effluents discharged into surface waters [2].

Conventional analytical techniques for phosphate determination, such as spectrophotometry, ion chromatography, and colorimetric assays, are well established and widely standardized [3,4]. Nevertheless, these methods often require extensive sample preparation, chemical reagents, and laboratory-based instrumentation, which limit their applicability for rapid, on-site, and continuous monitoring [5-10]. To overcome these limitations, autonomous and in situ analytical platforms, including microfluidic and lab-on-a-chip systems, have been developed for long-term phosphate monitoring in natural waters [6-10].

In this context, electrochemical sensors have emerged as attractive alternatives owing to their simplicity, low operational cost, fast response, reduced reagent consumption, and compatibility with miniaturized and portable systems [11-15]. Despite these advantages, the electrochemical determination of phosphate remains challenging because phosphate ions are not intrinsically electroactive within conventional potential windows. Consequently, most electrochemical sensing strategies rely on phosphate ions effectively interacting with the poly(methylene blue) (PMB) film, where phosphate modulates interfacial charge-transfer processes or selectively interacts with functional materials immobilized at the electrode surface [11,12,15-17]. Reagentless and screen-printed electrochemical platforms have also been proposed to further simplify phosphate analysis and reduce operational complexity [18].

Carbon-based materials such as graphite, graphene, and carbon nanotubes are particularly attractive substrates for electrochemical sensors owing to their wide potential window, low background current, chemical stability, and ease of surface modification [19,20]. Among surface modification strategies, electro-polymerization has been widely employed to fabricate functional polymer films on carbon electrodes, offering strong adhesion, controllable thickness, and excellent reproducibility. Polymer-modified electrodes based on amino acids, dyes, and aromatic monomers have demonstrated remarkable improvements in electroanalytical performance for a wide range of analytes, including pharmaceuticals, biomolecules, and inorganic species [19-22].

In particular, polymer films derived from redox-active molecules have attracted significant attention for their ability to mediate electron transfer, enhance electroactive surface area, and promote selective interactions with target species. Numerous studies have reported polymer-modified carbon electrodes exhibiting wide linear ranges, low detection limits, and good resistance to surface fouling, even in complex matrices [21,22]. From a molecular perspective, selective phosphate recognition has also been explored using synthetic anion receptors, highlighting the importance of electrostatic and coordination interactions in phosphate-sensing mechanisms [23].

Organic dyes constitute an important class of redox-active compounds for electrode surface modification owing to their rich electrochemical behaviour and chemical stability. Electro-polymerization of such dyes yields electroactive polymeric films with enhanced charge-transfer properties. Dye-based polymer films have been successfully employed for the electrochemical detection of nitrogenous bases and pharmaceutical compounds, demonstrating the versatility of this approach in sensor development [24,25].

Methylene blue is a well-known redox dye that exhibits reversible electrochemical behaviour and strong affinity for carbon-based substrates. Upon electro-polymerization, PMB forms a stable

electroactive film containing positively charged redox centres that interact favourably with anionic species such as phosphate, similar to other electropolymerized dye- and amino-acid-based films reported on carbon electrodes [18,22-25]. Despite these promising properties, the use of electropolymerized PMB films for phosphate sensing has received relatively little attention, particularly for low-cost graphite electrodes, with most studies focusing on alternative polymeric systems or different target analytes [20-26].

In this work, we report the fabrication and electrochemical characterization of a graphite electrode modified with an electropolymerized poly(methylene blue) film for phosphate detection in aqueous media. The PMB layer was formed directly on the electrode surface *via* potentiodynamic electropolymerization using cyclic voltammetry. The interfacial and electrochemical properties of the modified electrode were systematically investigated using cyclic voltammetry, electrochemical impedance spectroscopy, scanning electron microscopy, and FTIR spectroscopy. It should be emphasized that phosphate buffer solution is employed solely as a supporting electrolyte to maintain constant pH and ionic strength. The analytical signal does not reflect the absolute phosphate concentration of the buffer, but rather the incremental response of the PMB-modified electrode to successive additions of free phosphate species. These additions induce measurable changes in interfacial charge-transfer processes at the redox-active polymer film, enabling indirect phosphate sensing under controlled background conditions.

The analytical performance was evaluated by differential pulse voltammetry and chronoamperometry, with emphasis on sensitivity, selectivity, reproducibility, and applicability to real water samples. The proposed system exhibited a linear response in the phosphate concentration range of 50 to 475 µM, demonstrating its potential as a simple, reagent-free, and reliable electroanalytical platform for phosphate monitoring in environmental matrices.

Experimental

Materials and instrumentation

Graphite rods (3 mm diameter, 99.99 % purity), methylene blue (MB, ≥99 % purity), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), sodium nitrate ($NaNO_3$), and all other reagents were of analytical grade and purchased from Sigma-Aldrich (USA). All aqueous solutions were prepared using double-distilled water. The use of analytical-grade reagents and high-purity graphite ensured reproducibility and minimized potential interferences during electrochemical measurements.

A 0.1 M phosphate buffer solution (pH 7.0) was prepared by using KH_2PO_4 as the primary buffering species. The pH was adjusted to 7.0 by controlled addition of 0.1 M NaOH under continuous stirring. Sodium nitrate (0.1 M) was then added as a supporting electrolyte to maintain a constant ionic strength. The final solution was ultrasonicated for 30 min to ensure complete dissolution and homogeneity. The pH was verified prior to use. This buffer solution was employed as the supporting electrolyte in all electrochemical measurements.

Electrochemical experiments were carried out at room temperature using an Ivium CompactStat potentiostat, Netherlands, in a conventional three-electrode cell configuration. The poly(methylene blue)-modified graphite electrode (PMB/G), with a geometric surface area of 0.071 cm^2, was used as the working electrode. A platinum wire served as the counter electrode, and an Ag/AgCl (3.0 M KCl) electrode was used as the reference electrode.

Fourier-transform infrared (FTIR) spectra were recorded using a Thermo Nicolet iS10 spectrometer to confirm successful electro-polymerization of methylene blue on the electrode surface.

Surface morphology was examined by scanning electron microscopy (SEM) using a Thermo Scientific Apreo microscope. Solution pH values were measured with a SIMPLA-PH-140 digital pH meter (Lab Solutions, São Paulo, Brazil).

Although phosphate buffer solution (0.1 M PBS) was employed as the supporting electrolyte to ensure constant pH and ionic strength, the electrochemical response of the PMB-modified electrode is governed by incremental additions of free phosphate species. The analytical signal reflects relative changes in interfacial charge-transfer processes rather than the absolute phosphate content of the buffer.

For phosphate-sensing experiments, standard phosphate solutions were prepared by successive additions of potassium dihydrogen phosphate (KH_2PO_4) to the electrochemical cell containing phosphate buffer solution (PBS, pH 7.0).

Electrochemical impedance spectroscopy (EIS) measurements were performed in the frequency range from 100 kHz to 0.1 Hz, using a sinusoidal AC voltage perturbation of 10 mV, superimposed on a DC potential of -0.30 V *vs.* Ag/AgCl. All electrochemical techniques, including cyclic voltammetry, differential pulse voltammetry, chronoamperometry, and electrochemical impedance spectroscopy, were performed under identical experimental conditions to allow a reliable comparison between bare and modified electrodes.

Electrode modification

Prior to modification, graphite electrodes were mechanically polished to obtain a smooth, mirror-like surface, thoroughly rinsed with distilled water, and dried at room temperature. Electro-polymerization of methylene blue was carried out directly on the graphite surface by cyclic voltammetry in a three-electrode electrochemical system.

The polymeric film was deposited by cycling the potential between -0.6 V and +1.0 V *vs.* Ag/AgCl at a scan rate of 50 mV s^{-1} for 30 consecutive cycles in an aqueous solution containing 1.0 mM methylene blue. The number of electro-polymerization cycles was selected to ensure the formation of a stable and homogeneous polymeric film while preserving efficient electron-transfer properties at the electrode interface. After electro-polymerization, the modified electrodes were rinsed repeatedly with double-distilled water to remove physically adsorbed or unreacted monomer species and then dried at room temperature prior to electrochemical characterization and sensing experiments.

Figure 1 summarizes the experimental workflow employed in this study.

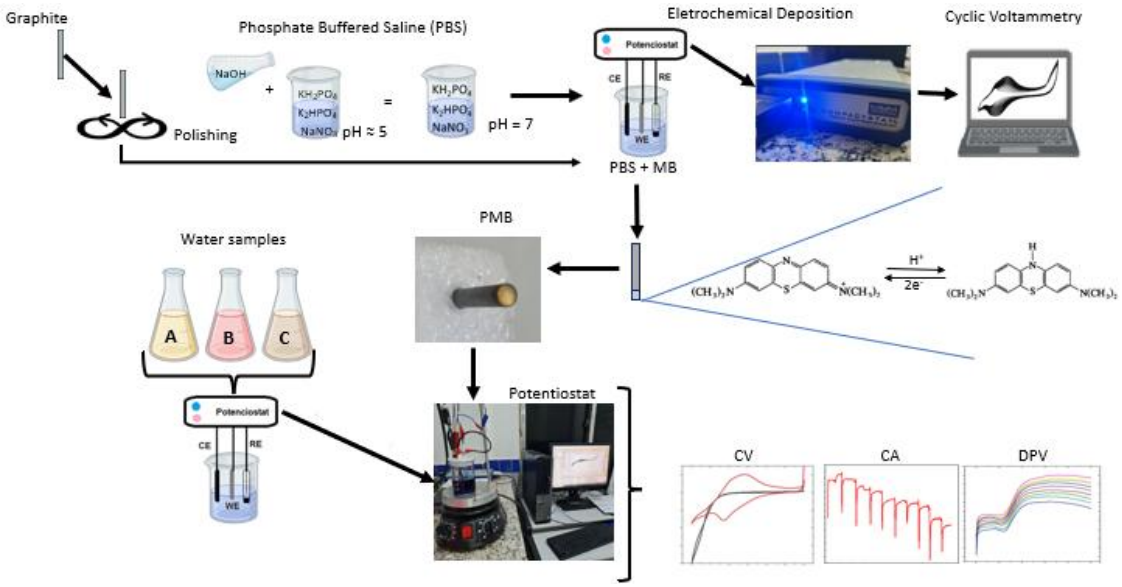

Figure 1. *Schematic diagram of the PMB-modified graphite electrode preparation, electro-polymerization of methylene blue on graphite, and electrochemical detection of phosphate in real water samples (A-C)*

The schematic illustrates the preparation of the graphite electrode, the electro-polymerization of methylene blue to obtain the PMB-modified surface, and the subsequent electrochemical characterization. The figure also outlines the electroanalytical procedures used for phosphate detection and the application of the proposed electrode to real water samples (samples A-C) using the standard addition method. This representation provides an overview of the main experimental steps involved in the fabrication, characterization, and practical application of the PMB-modified graphite electrode. This experimental protocol was designed to ensure good reproducibility of the electrode modification process and consistency among independent measurements.

Results and discussion

Electro-polymerization of methylene blue and surface characterization

The electro-polymerization of methylene blue on the graphite electrode surface was done by cyclic voltammetry. As shown in Figure 2(a), the voltammograms recorded during successive potential cycles display a well-defined anodic peak near 0.0 V and a corresponding cathodic peak at approximately -0.30 V *vs.* Ag/AgCl, which are characteristic of the redox couple of methylene blue and its polymeric form. The progressive increase in both anodic and cathodic peak currents with increasing cycle number indicates the gradual formation and growth of the PMB film on the electrode surface, confirming successful electro-polymerization.

A comparison between the first and the thirtieth voltammetric cycles, highlighted in Figure 2(b), provides further insight into the evolution of the polymer layer. In addition to the significant enhancement of the peak currents, the appearance and intensification of distinct anodic (A1, A2) and cathodic (C1, C2) redox features suggest the establishment of a stable redox-active PMB film. Only the first and the thirtieth cycles are displayed. These changes are associated with the accumulation of electroactive sites and an increase in the effective electroactive surface area as the polymer layer develops.

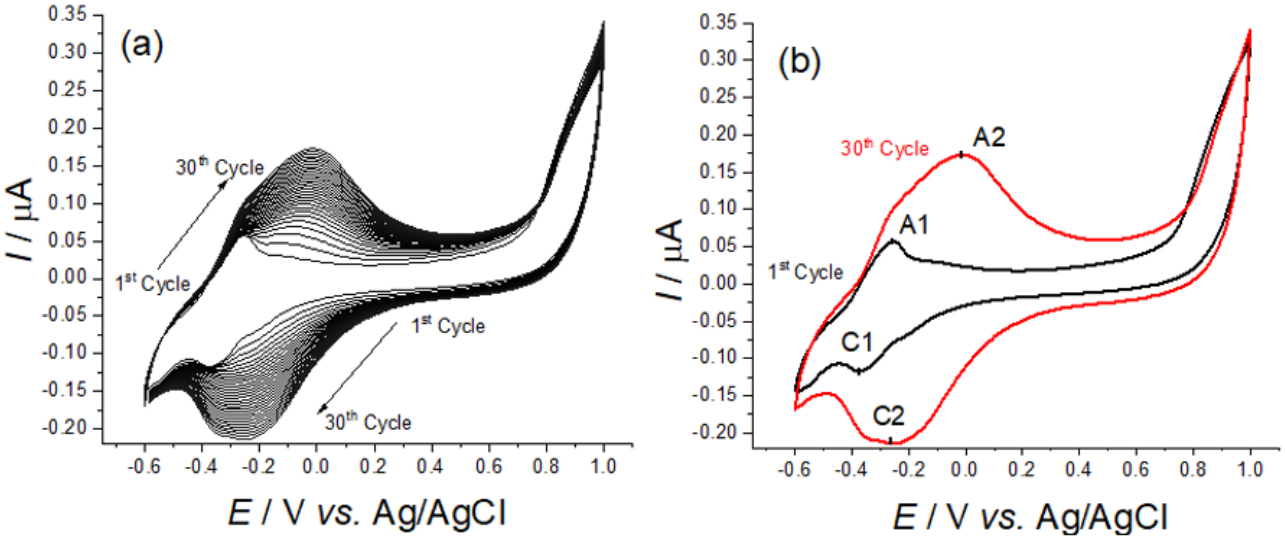

Figure 2. *(a) Cyclic voltammograms recorded during potentiodynamic electro-polymerization of methylene blue on the graphite electrode by successive potential cycling in an aqueous solution containing 1.0 mM methylene blue. (b) Representative cyclic voltammograms highlighting the main anodic (A1, A2) and cathodic (C1, C2) redox processes associated with the formation of the poly(methylene blue) film*

This electrochemical behaviour is in good agreement with previous studies on the electro-polymerization of phenothiazine-based dyes and supports the formation of a robust and electrochemically active PMB coating suitable for subsequent sensing applications [26-29].

The progressive increase in peak currents observed during electro-polymerization can be further correlated with the continuous increase in electroactive surface coverage and the formation of a conjugated polymeric network. As the number of cycles increases, the growing PMB film provides a higher density of redox-active sites, facilitating electron hopping within the polymer backbone and improving charge transport across the electrode-electrolyte interface. This behaviour is characteristic of surface-confined redox polymers and indicates that the electropolymerized layer remains electrochemically accessible rather than forming an insulating film.

Moreover, the preservation of the voltammetric profile and the absence of significant peak broadening or distortion after multiple cycles suggest that the PMB film exhibits good electrochemical stability and adhesion to the graphite substrate, which are essential requirements for its application in repeated sensing measurements.

Morphological characterization by scanning electron microscopy

Morphological characterization by scanning electron microscopy (SEM) further confirms the successful surface modification of the graphite electrode. As shown in Figure 3a, the bare graphite electrode exhibits a relatively smooth and compact surface, with no discernible micro- or nanostructured features. In contrast, the PMB-modified electrode (Figure 3b) shows a homogeneous granular morphology uniformly distributed across the surface, consistent with the formation of a continuous polymeric film during electro-polymerization. Higher-magnification images reveal nanoscale agglomerates, indicating the formation of a textured, porous surface.

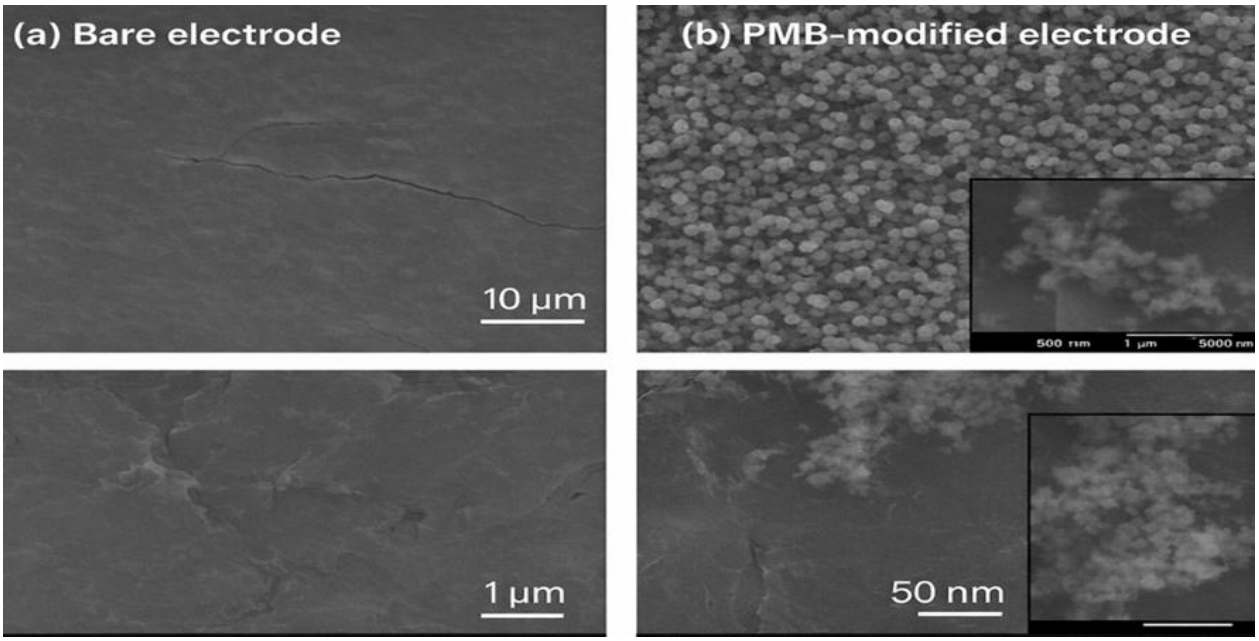

Figure 3. *SEM images of the (a) bare and (b) PMB-modified graphite electrodes at different magnifications*

These pronounced morphological differences between the bare and modified electrodes confirm the effective electro-polymerization of methylene blue on the graphite surface. The formation of a uniform and nanostructured PMB layer is consistent with the electrochemical results discussed above and supports the enhanced interfacial properties and electroanalytical performance observed for phosphate detection.

The porous and granular morphology observed for the PMB-modified electrode is expected to favour mass transport of phosphate ions toward the electroactive sites, thereby increasing the effective contact area between the analyte and the polymeric film. In this context, such nano-

structured surfaces are frequently associated with enhanced sensitivity in polymer-modified carbon electrodes, as they promote both adsorption phenomena and efficient interfacial charge-transfer processes.

Differential pulse voltammetric response toward phosphate

Differential pulse voltammetry (DPV) was employed to investigate the electroanalytical response of a poly(methylene blue) (PMB) modified graphite electrode for phosphate detection. As illustrated in Figure 4(a), successive additions of inorganic orthophosphate, introduced as potassium dihydrogen phosphate (KH_2PO_4), in the concentration range from 0.056 to 2.2 mmol L^{-1} resulted in a progressive increase in the cathodic peak current when measurements were carried out in 0.1 mol L^{-1} phosphate buffer solution (PBS), pH 7.0. Although phosphate additions were performed up to 2.2 mmol L^{-1}, linear behaviour was observed between 0.05 and 0.475 mmol L^{-1}. This systematic current enhancement indicates that phosphate ions effectively interact with the PMB film, modulating its redox behaviour and enabling indirect electrochemical sensing.

The corresponding calibration curve (Figure 4(b)), constructed from cathodic peak current values, shows a linear dependence on phosphate concentration over the investigated range, with a correlation coefficient of R^2 = 0.9889. The analytical response can be described by the linear regression Equation (1):

$$I = aC + b \quad (1)$$

where I represent the cathodic peak current, C is the phosphate concentration, and a and b correspond to the slope and intercept, respectively.

The limit of detection (LOD), estimated based on a signal-to-noise ratio of 3, was determined to be 0.15 μmol L^{-1}. Although this value is not among the lowest reported for phosphate sensors, it is suitable for environmentally relevant concentration levels and highlights the advantages of the proposed platform, namely its simple electrode fabrication, reagent-free operation, and reliable analytical performance.

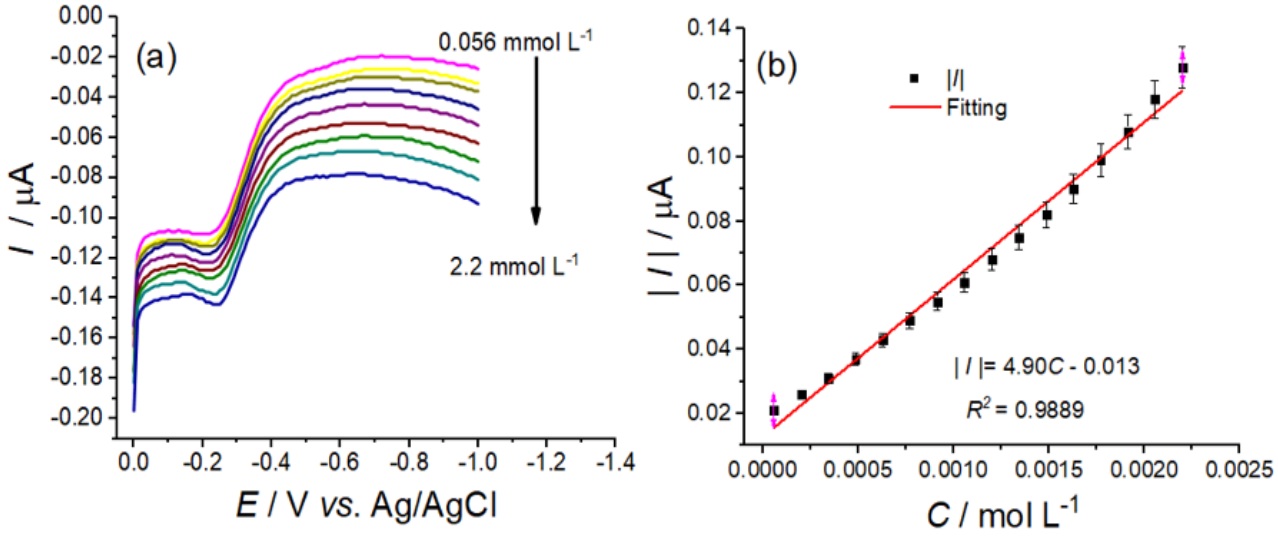

Figure 4. *(a) DPV responses at the PMB-modified graphite electrode for increasing phosphate concentrations and (b) calibration curve obtained from the absolute current versus concentration*

Considering that phosphate ions are not intrinsically electroactive within the investigated potential window, the observed DPV response is attributed to an indirect sensing mechanism. In this mechanism, phosphate ions interact electrostatically with the positively charged redox centers of the PMB film, leading to modulation of the polymer's redox equilibrium and enhancement of the

cathodic current. Similar indirect sensing mechanisms based on redox-active polymer films have been reported for anionic species in aqueous media and are consistent with the preserved voltammetric shape observed upon phosphate addition. Although the detection limit is not among the lowest reported, it should be emphasized that the proposed system operates without additional reagents, enzymes, or complex surface functionalization steps. Therefore, the achieved LOD represents a favourable compromise between analytical performance, operational simplicity, and cost-effectiveness, particularly for routine environmental monitoring applications.

It is important to emphasize that although the supporting electrolyte contains phosphate species at relatively high concentration (0.1 M), the electrochemical response of the PMB-modified electrode is sensitive to incremental additions of phosphate due to interfacial interactions between phosphate anions and the positively charged redox sites of the polymeric film. The sensing mechanism is therefore not based on the absolute phosphate concentration in bulk solution, but rather on changes in the interfacial equilibrium and charge-transfer dynamics induced by additional phosphate species. Consequently, small micromolar concentration variations can be reliably detected despite the presence of background phosphate in the buffer.

Based on the combined electrochemical, morphological, and spectroscopic results, the proposed indirect phosphate sensing mechanism is schematically illustrated in Figure 5. Phosphate ions interact electrostatically with the positively charged redox centres of the poly(methylene blue) film, modulating interfacial charge-transfer processes without undergoing direct electrochemical transformation.

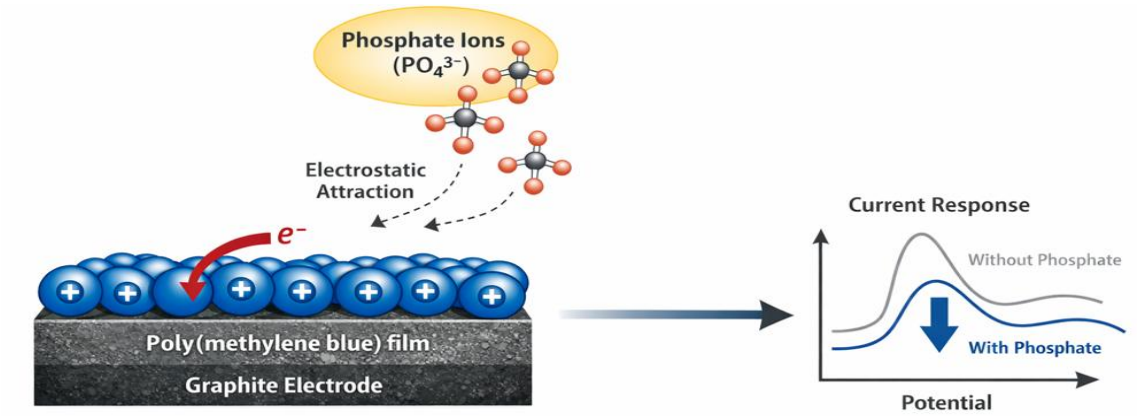

Figure 5. *Schematic illustration of the proposed indirect phosphate sensing mechanism at the poly(methylene blue)-modified graphite electrode, highlighting the electrostatic interaction between phosphate ions and the redox-active polymer film and the resulting modulation of the electrochemical response*

Chronoamperometric response

The dynamic electrochemical behaviour of the PMB-modified graphite electrode was evaluated by chronoamperometry. Measurements were conducted in 0.1 M PBS, pH 7.0, under constant stirring at 300 rpm, with the applied potential set at -0.30 V *vs.* Ag/AgCl. As shown in Figure 6a, successive additions of phosphate induced rapid increases in cathodic current, followed by well-defined and stable steady-state responses.

The fast current stabilization observed after each addition indicates efficient charge-transfer processes at the PMB-modified surface and demonstrates the suitability of the electrode for time-resolved amperometric measurements. The steady-state currents (I_{ss}) extracted from the chrono-amperometric traces were used to construct the calibration curve shown in Figure 6(b). A linear relationship between current and phosphate concentration was obtained over the investigated range, with a high correlation coefficient, confirming the good reproducibility and stability of the

amperometric response. These results further support the applicability of the PMB-modified electrode as a reliable platform for phosphate detection in aqueous media.

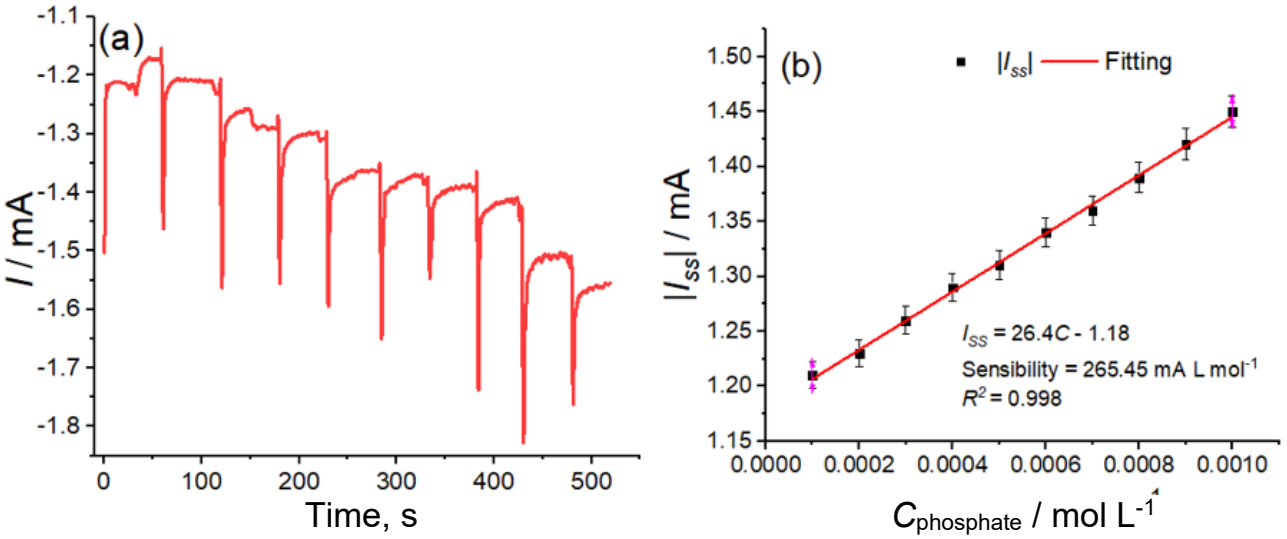

Figure 6. *(a) Chronoamperometric response and (b) calibration curve for phosphate detection using the PMB-modified graphite electrode in 0.1M PBS, pH 7.0*

The agreement between the linear responses obtained by DPV and chronoamperometry indicates that the phosphate-induced modulation of the PMB redox activity is consistent across different electrochemical techniques. This consistency reinforces the robustness of the sensing mechanism and confirms that the analytical signal is governed by interfacial processes rather than transient or non-reproducible effects.

Cyclic voltammetric behaviour in the presence of added phosphate

The electrochemical behaviour of the bare and poly(methylene blue)-modified graphite electrodes toward phosphate was investigated by cyclic voltammetry. As shown in Figure 7(a), the bare graphite electrode exhibits negligible faradaic current in 0.1 M phosphate buffer solution (PBS, pH 7.0), indicating limited electroactivity of the unmodified surface within the investigated potential window. In contrast, the PMB-modified electrode shows a well-defined redox peak centered at -0.30 V versus Ag/AgCl, associated with the redox transitions of the polymeric methylene blue film in the presence of inorganic orthophosphate.

Upon successive additions of inorganic orthophosphate (introduced as KH_2PO_4) in the concentration range from 50 to 475 μM, a gradual increase in the cathodic peak current is observed, while the overall voltammetric profile of the PMB film remains essentially unchanged. This behaviour indicates that phosphate ions interact with the redox-active polymer layer without disrupting its electrochemical integrity. The preservation of the voltammetric shape supports an indirect sensing mechanism, in which phosphate modulates the interfacial charge-transfer processes of the PMB film rather than undergoing direct electrochemical oxidation or reduction.

The quantitative capability of the cyclic voltammetric response was further evaluated by correlating the absolute cathodic peak current (I_{CP}) with phosphate concentration. As shown in Figure 7(b), a linear relationship is obtained over the investigated concentration range, with an excellent correlation coefficient, demonstrating the reproducibility of the voltammetric response and confirming the suitability of cyclic voltammetry for monitoring phosphate-induced changes at the PMB-modified electrode.

The absence of additional redox peaks or significant shifts in peak potentials upon phosphate addition further supports the indirect nature of the sensing mechanism. The interaction between phosphate and the PMB film alters charge-transfer kinetics without inducing chemical transformation of the analyte, thereby maintaining electrode stability during repeated measurements.

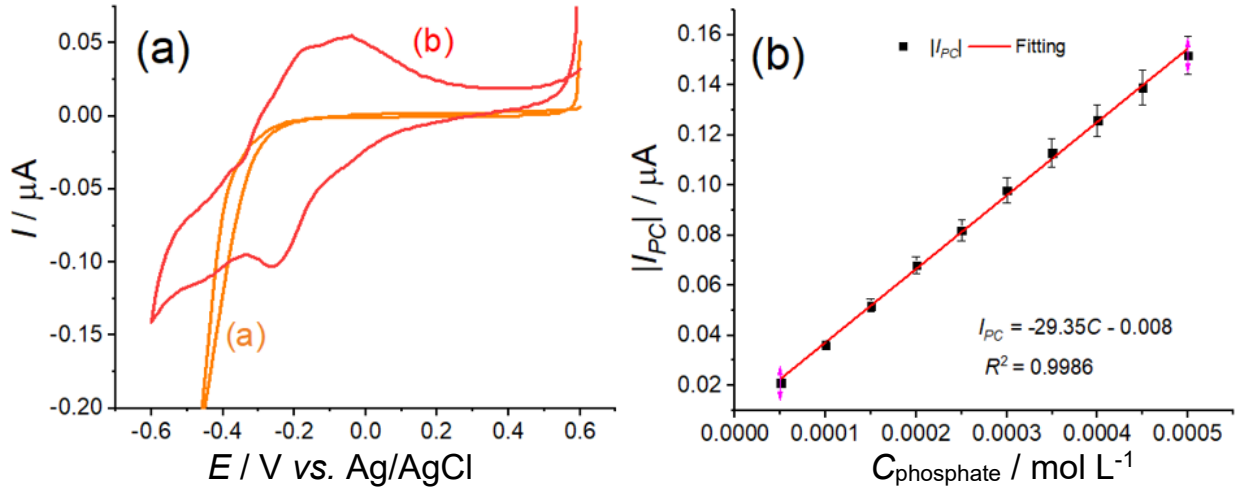

Figure 7. *Electrochemical response of the bare and PMB-modified graphite electrodes, toward added phosphate: (a) cyclic voltammograms recorded in 0.1 M PBS, pH 7.0 (a - bare electrode, b - PMB electrode) and (b) calibration plot of the absolute cathodic peak current as a function of inorganic orthophosphate concentration (150 mM of added KH_2PO_4)*

Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) was employed to investigate the interfacial properties of the bare and poly(methylene blue)-modified graphite electrodes. All EIS measurements were performed in 0.1 M PBS, pH 7.0, at a DC bias potential of -0.30 V *vs.* Ag/AgCl, corresponding to the redox potential of the PMB film.

As shown in Figure 8(a), the Nyquist plot of the bare graphite electrode exhibits a large, depressed semicircle in the high- to mid-frequency region. The charge-transfer resistance (R_{ct}), estimated from the semicircle diameter along the real impedance axis (Z'), is approximately 600 Ω, indicating sluggish interfacial electron-transfer kinetics at the unmodified graphite surface.

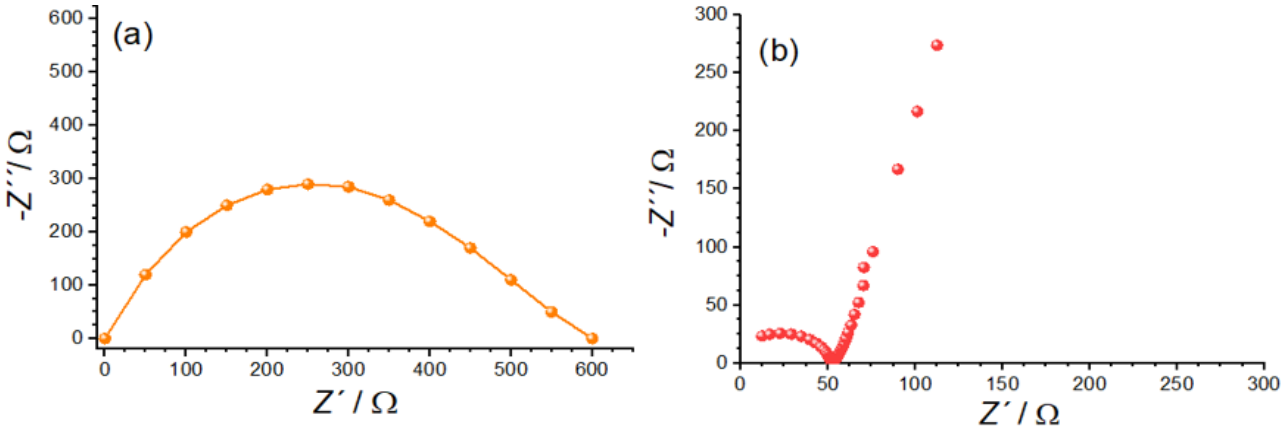

Figure 8. *Nyquist plots obtained from EIS measurements of (a) bare graphite electrode and (b) PMB-modified graphite electrode recorded in 0.1 M PBS, pH 7.0, at -0.30 V vs. Ag/AgCl*

In contrast, the Nyquist plot of the PMB-modified graphite electrode (Figure 8(b)) displays a markedly smaller semicircle, with an estimated $R_{ct} \approx 40$ to 50 Ω, evidencing a substantial reduction in charge-transfer resistance after electropolymerization. This nearly one-order-of-magnitude

decrease in R_{ct} demonstrates that the PMB film enhances charge-transfer processes rather than acting as a blocking layer. The depressed semicircle suggests non-ideal capacitive behaviour, commonly associated with surface heterogeneity and distributed relaxation times in polymer-modified electrodes.

At lower frequencies in Figure 8(b), the impedance response becomes an inclined line, indicative of mass-transport contributions typically observed in polymer-modified electrodes. This feature can be attributed to ion transport within the redox-active polymeric matrix and/or across the electrode-electrolyte interface.

Overall, the EIS results confirm that electropolymerization of methylene blue significantly improves interfacial electron-transfer kinetics while introducing additional low-frequency transport phenomena. The PMB film therefore acts as an efficient electron-transfer mediator, directly contributing to the enhanced electroanalytical performance observed in voltammetric and amperometric measurements.

FTIR characterization

FTIR spectroscopy was used to confirm the successful electropolymerization of methylene blue on the graphite electrode surface. As shown in Figure 9(a), the bare graphite electrode exhibits a largely featureless spectrum in the investigated region, with only weak and broad contributions that can be attributed to surface-adsorbed moisture and minor oxygenated functionalities typically present on carbon materials. In contrast, the spectrum of the PMB-modified electrode (Figure 9(b)) displays distinct absorption bands characteristic of the polymerized methylene blue structure. The broad band cantered around 3190 cm^{-1} is attributed to N-H and O-H stretching vibrations, while bands in the 1650 to 1600 cm^{-1} region correspond to C=N stretching and aromatic C=C vibrational modes. In addition, the absorption band observed near 1255 cm^{-1} is associated with C-N stretching vibrations of the polymeric framework. The appearance of these bands relative to the bare graphite confirms the formation of a poly(methylene blue) film on the electrode surface and indicates the incorporation of redox-active functional groups relevant to the electrochemical response toward phosphate.

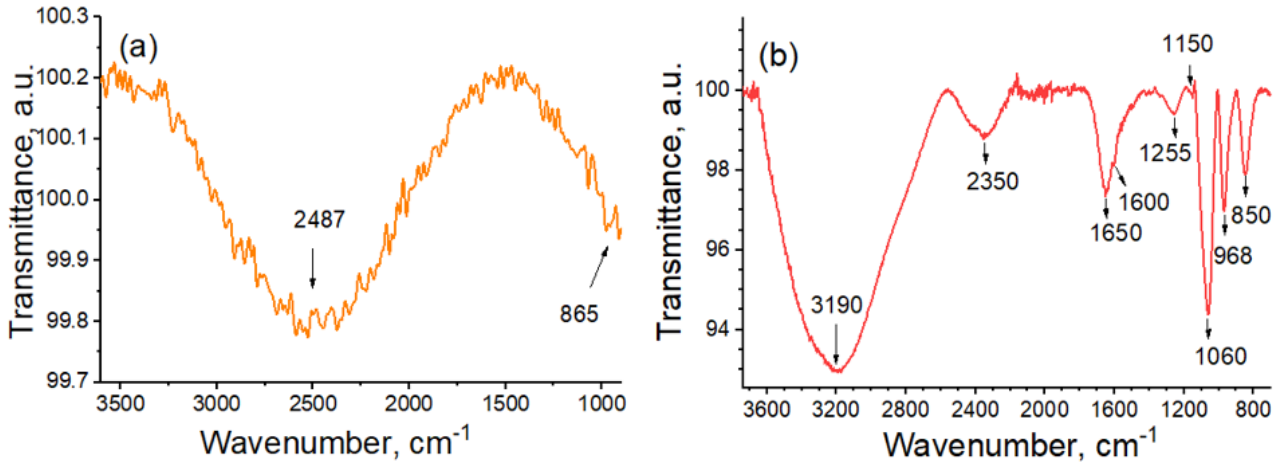

Figure 9. *FTIR spectra of (a) bare graphite electrode and (b) PMB-modified graphite electrode*

In addition to the main absorption bands discussed above, the FTIR spectrum of the PMB-modified electrode (Figure 9(b)) exhibits additional features at approximately 2350, 1150, 1060, 968 and 850 cm^{-1}. The band near 2350 cm^{-1} is attributed to atmospheric CO_2 and is commonly observed in FTIR measurements performed under ambient conditions. The absorptions in the 1150 to 1060 cm^{-1} region can be assigned to C-N and C-S stretching modes and coupled vibrations involving the aromatic framework of the phenothiazine-based polymer. Bands observed at 968 and 850 cm^{-1} are associated

with out-of-plane bending modes of aromatic rings, which are characteristic of conjugated polymeric structures. Together, these spectral features further support the successful formation of a poly(methylene blue) film on the graphite electrode surface.

The presence of nitrogen- and sulphur-containing functional groups in the PMB film is particularly relevant for phosphate sensing, as these sites may contribute to electrostatic and hydrogen-bonding interactions with phosphate species in solution, thereby reinforcing the proposed indirect detection mechanism.

Analysis of real water samples

The analytical applicability of the proposed PMB-modified graphite electrode was evaluated using real water samples collected from different locations in the city of Rio de Janeiro. Phosphate determination was carried out using the standard addition method to compensate for potential matrix effects. Recovery values ranged from 99.6 to 106.4 %, with relative standard deviations below 5 %, indicating satisfactory accuracy and precision.

Representative DPV responses obtained for increasing phosphate concentrations in the three water samples are presented in Figure 10. In all cases, successive additions of phosphate result in a systematic increase in the cathodic peak current, while the overall voltammetric profile is preserved.

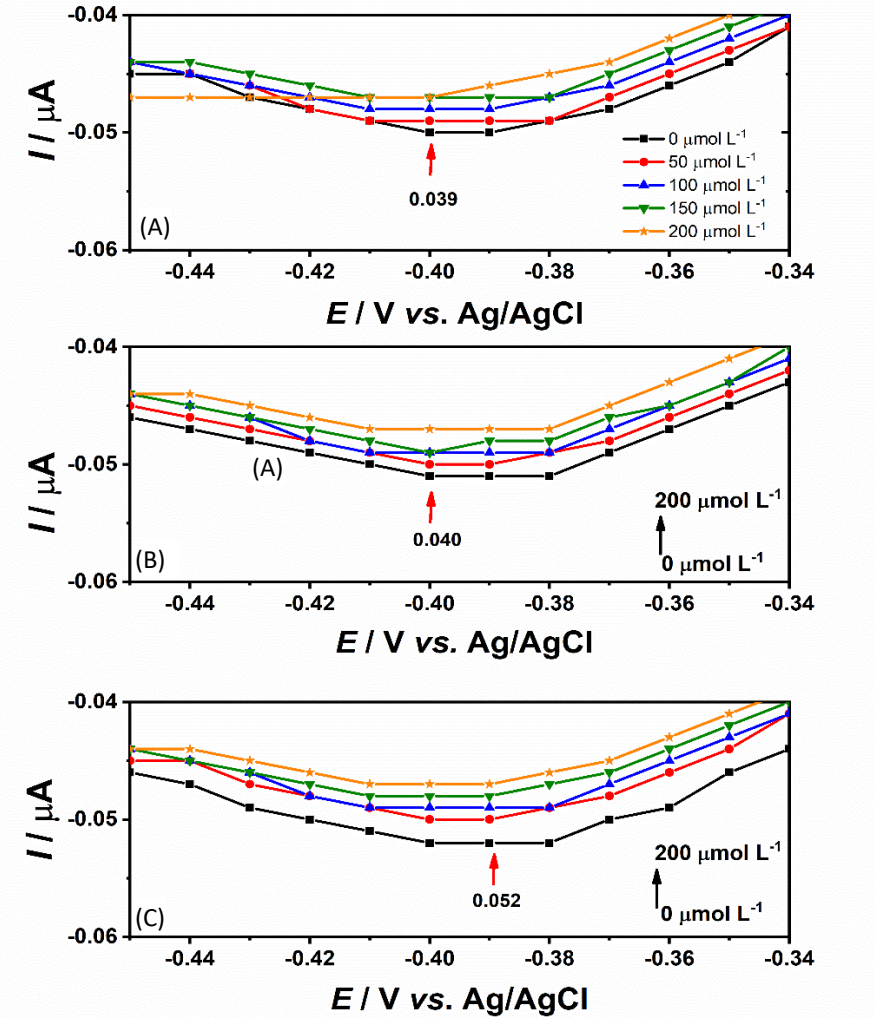

Figure 10. *DPV responses of the PMB-modified graphite electrode in three different real water samples (A to C) upon successive additions of phosphate up to 200 µmol L^{-1}*

This behaviour indicates that the PMB-modified electrode maintains a stable and reproducible electroanalytical response even in complex aqueous matrices. The consistent current increments

observed for samples (A) to (C) confirm the suitability of the proposed platform for phosphate determination in real environmental water samples. For a broader assessment of the analytical performance of the proposed PMB-modified graphite electrode, a comparison with selected phosphate sensors reported in the literature is presented in Table 1. The comparison includes different electrode materials, analytical techniques, linear concentration ranges, limits of detection, and sample matrices.

Although the proposed PMB-modified electrode does not achieve the lowest detection limit among the reported platforms, its analytical performance is comparable to that of several electrochemical sensors reported in the literature. The main advantages of the proposed system lie in its simple fabrication procedure, low-cost materials, reagent-free operation, and satisfactory performance in real water samples. The satisfactory recovery values obtained for real water samples demonstrate that the PMB-modified electrode is resilient to common matrix effects typically encountered in environmental waters. This performance highlights the potential of the proposed sensor for practical applications beyond controlled laboratory conditions.

Table 1. *Analytical performance of the PMB-modified graphite electrode compared with selected phosphate sensors reported in the literature*

Electrode material modification	Analytical technique	Linear range, mmol L^{-1}	LOD, μmol L^{-1}	Sample matrix	Ref.
PMB-modified graphite electrode	DPV, CA	0.05 to 0.475	0.15	Environmental water	This work
Activated nickel electrode	CV	0.01 to 1.00	3.00	Aqueous solutions	[12]
Paper-based screen-printed electrode	Amperometry	0.02 to 1.00	5.00	Water samples	[26]
PVC membrane fluorescent sensor	Fluorescence	0.001 to 0.100	0.80	Aqueous solutions	[16]
Poly (9-aminofluorene)-modified electrode	CV	0.01 to 0.50	2.00	Aqueous solutions	[17]
Pyruvate oxidase biosensor	Amperometry	0.005 to 0.500	1.00	Wastewater	[14]

Conclusions

This work reports the successful fabrication of a poly(methylene blue)-modified graphite electrode obtained by electro-polymerization and its application as an electroanalytical platform for phosphate detection in aqueous media. The formation of the PMB film resulted in a stable, homogeneous, and redox-active surface, leading to improved electrochemical behaviour compared with the bare graphite electrode, as evidenced by cyclic voltammetry and electrochemical impedance spectroscopy.

The use of an electropolymerized redox-active dye film on a low-cost graphite substrate represents a straightforward and effective strategy for indirect phosphate sensing, distinguishing the proposed platform from more complex enzyme-based or reagent-dependent approaches reported in the literature.

The PMB-modified electrode exhibited a reproducible and stable electroanalytical response toward phosphate through an indirect detection mechanism. A linear response was obtained in the concentration range from 0.05 to 0.475 μmol L^{-1}, with a detection limit suitable for environmentally relevant phosphate levels. Complementary SEM and FTIR analyses confirmed the successful surface modification and the presence of characteristic functional groups associated with electropolymerized methylene blue.

The combined electrochemical and spectroscopic results support an indirect sensing mechanism based on the interaction between phosphate ions and the positively charged redox centres of the

PMB film, which modulates interfacial charge-transfer processes without compromising the electrochemical integrity of the polymer layer.

The applicability of the proposed platform was further demonstrated by phosphate determination in real water samples, yielding satisfactory recovery values and relative standard deviations below 5 %, even in complex aqueous matrices. Although not aimed at achieving ultralow detection limits, the PMB/graphite electrode offers operational simplicity, low cost, good reproducibility, and adequate analytical performance, making it a reliable option for routine phosphate analysis in environmental water samples.

Future studies may focus on optimizing the polymer film thickness, exploring alternative electropolymerization conditions, and extending the approach to other environmentally relevant anions. In addition, integrating the proposed electrode into portable or miniaturized electrochemical devices could further expand its applicability for in situ, real-time water quality monitoring.

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