

Development of Electrochemical Microsensors for Phosphate Detection in Aquatic Environments Using a Gold Electrode in a Microfluidic System

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Abstract: Phosphates are key nutrients in aquatic ecosystems (Jiao et al., 2012), but excess concentrations cause eutrophication, degrading water quality and biodiversity. Monitoring phosphate at low levels and in situ is thus essential for environmental protection. This study presents electrochemical microsensors integrated into a microfluidic system for sensitive phosphate detection using Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV), and Differential Pulse Voltammetry (DPV). Two strategies were developed: (1) copper-modified gold electrodes monitoring inhibition of the Hydrogen Evolution Reaction (HER), leading to a limit of detection of about 4.5 ppm; and (2) molybdate based formation of electroactive phosphomolybdate complexes, (Jońca et al., 2011), (Li et al., 2016), enabling quantification with limits of detection of 2 ppm using CV and 0.2 ppm using DPV. This microfluidic platform enable rapid analysis, low reagent use, and operation in complex matrices, making them suitable for on-site environmental monitoring.

Keywords: electrochemical microsensor, microfluidic system, phosphate detection

1. INTRODUCTION

Monitoring nutrient levels in aquatic environments is a critical challenge for ecosystem preservation and sustainable water resource management. Among these nutrients, phosphate plays a key role in biological productivity (Fauzan et al., 2026) However, its excessive accumulation is a primary cause of eutrophication, leading to harmful algal blooms, oxygen depletion, and significant degradation of water quality and biodiversity (Worsfold et al., 2005), (Comber et al., 2013). Consequently, the development of reliable methods for phosphate detection at low concentrations is essential for environmental monitoring and public health protection. Traditional analytical methods for phosphate determination are largely based on the formation of phosphomolybdate complexes in acidic media, followed by spectrophotometric detection (Shyla et al., 2011). The widely used molybdenum blue method, first described by (Murphy & Riley, 1962) remains a reference technique due to its sensitivity and robustness. However, such methods typically require multiple reagents, careful sample preparation, and relatively long analysis times, limiting their applicability for rapid, on-site measurements (Hu et al., 2016), (Privett et al., 2010).

Electrochemical techniques have recently emerged as attractive alternatives due to their high sensitivity, fast response, low cost, and compatibility with miniaturized and portable devices. Their integration with microfluidic systems further enhances performance by reducing reagent consumption and enabling automated, *in situ* analysis (Ben-Aissa et al., 2023).

In this work, we present the development of electrochemical microsensors integrated into a microfluidic platform for phosphate detection. Based in two strategies: (i) an indirect method based on the inhibition of the Hydrogen Evolution Reaction (HER) at copper modified gold electrodes, and (ii) a direct method based on the detection of phosphomolybdate complexes in acidic media. These approaches were evaluated in terms of sensitivity, detection limit, selectivity, and applicability to complex aqueous matrices, with the aim of enabling rapid and field deployable phosphate monitoring (Kolliopoulos et al., 2015), (Jońca et al., 2011).

2. EXPERIMENTAL

2.1. Chemicals and reagents

Sulfuric acid (95–97%), sodium chloride (99%), sodium nitrite (99%), disodium hydrogen phosphate (99.5%), and copper(II) sulfate (99%) were purchased from Fluka. Sodium molybdate dihydrate was supplied by Carlo Erba. All aqueous solutions were prepared using purified water (resistivity

18.2 M Ω ·cm, pH = 6.3) obtained from a Milli-Q purification system (Millipore). Before use, all solutions were deoxygenated by purging with nitrogen gas for 5–10 min. To minimize metal contamination, electrochemical cells were thoroughly rinsed with a 10% (v/v) hydrochloric acid solution followed by Milli-Q water. Gold disk electrodes (2 mm diameter) were commercially obtained and used as received.

2.2. Electrode preparation

The gold electrode (Au) was initially polished using SiC paper (Struers, P4000 grit). After each polishing step, the electrode was thoroughly rinsed with ultrapure water and stored in water until use. Prior to each measurement, the gold electrode was cleaned electrochemically by either chronoamperometry or cyclic voltammetry. In the chronoamperometric cleaning procedure, constant potentials were sequentially applied to oxidize and subsequently reduce the electrode surface, thereby removing surface contaminants. Specifically, a potential of +1.0 V vs. Ag/AgCl was applied for 1 min in 1 M of H₂SO₄ to oxidize impurities and form a gold oxide layer, followed by -0.2 V vs. Ag/AgCl for 1 min to reduce the oxide back to metallic gold. After cleaning, the electrode was rinsed with the electrolyte solution (1 M H₂SO₄) prior to subsequent measurements.

2.3. Electrochemical measurements

Electrochemical measurements were carried out using a Bio-Logic potentiostat (VSP) equipped with a conventional three-electrode cell. A gold disk electrode (2 mm diameter) served as the working electrode, a platinum wire (0.5 mm diameter) was used as the counter electrode, and a silver/silver chloride electrode (Ag/AgCl, 0.1 M KCl) served as the reference electrode.

3. RESULTS AND DISCUSSION

3.1- Phosphate sensing on copper modified gold macroelectrode

Firstly, copper deposition on gold electrodes was monitored by chronoamperometry, applying a constant potential of -0.4 V (vs Ag/AgCl) for 60 seconds in a solution containing CuSO₄ (10 mM) and Na₂SO₄ (100 mM) at pH \approx 5 (H₂SO₄ medium). Then, phosphate detection tests were carried out in acidic medium (H₂SO₄, pH 2.5) containing various concentrations of Na₂HPO₄. Linear Scan Voltammetry (LSV) measurements were performed within a potential window ranging from -0.7 V to -1.5 V (vs Ag/AgCl), at a scan rate of 50 mV·s⁻¹.

In the absence of phosphate, the LSV shows a well-defined cathodic peak around -1.15 V, corresponding to the HER catalyzed by the copper layer (Figure 1.a). The progressive addition of phosphate induces an anodic shift of the reduction potential, accompanied by a decrease in the HER peak current, reflecting a kinetic inhibition (Figure 1.a). This behaviour is attributed to the formation of phosphate/copper complexes at the electrode surface, which reduce the availability of metallic copper for proton reduction. Consequently, the decrease in HER current serves as an indirect electrochemical signal for phosphate detection.

In this phosphate concentration range, the reduction response exhibits linear behaviour (Figure 1.b) with a correlation coefficient of $r^2 = 0.98$. Using this line of best fit, a limit of detection (LOD) around 4.5 ppm, can be calculated (3σ method). This strategy can ensure the phosphate detection by measuring the drop of the electrocatalytic HER current peak.

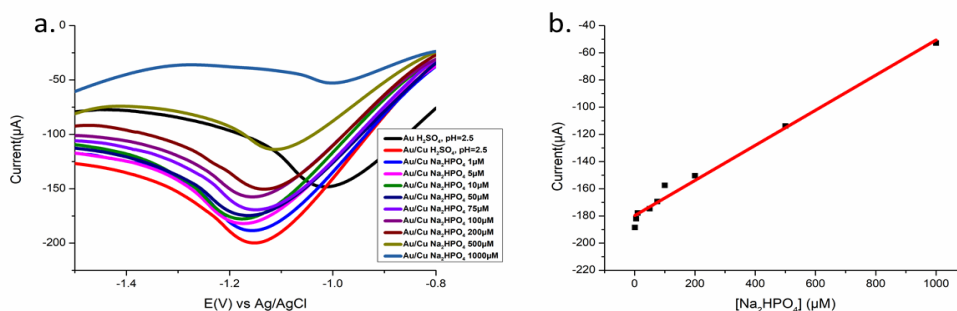


Figure 1: a. Linear Scan voltammetric responses using an Au/Cu modified electrode without and with different phosphate concentrations (1–1000 μmolL^{-1}). Note that a new Au is used for each addition, scan rate: 50 mVs^{-1} vs Ag/AgCl. b. Calibration curve obtained with reduction peak current (concentration from 1 to 1000 μmolL^{-1}).

3.2- Evaluation of phosphate and nitrate co-detection with copper modified gold electrode

The reliability of the phosphate detection method based on the HER response was evaluated in the presence of nitrate ions (NO_3^-), a common anionic species found in natural aquatic environments that may interfere with electrochemical measurements. The tested concentrations were selected to reflect typical environmental conditions: nitrates are generally present at trace levels ($<0.1 \text{ mg/L}$), but their concentration can increase to several mg/L in agricultural areas due to fertilizer runoff.

To assess the nitrate effect, a first series of experiments (Figure 2.a) was conducted in which the phosphate concentration was gradually increased from $1 \mu\text{mol/L}$ to $1000 \mu\text{mol/L}$, while the nitrate concentration was maintained constant at 2 mmol/L . Under these conditions, a well-defined nitrate reduction peak was observed at approximately -0.5 V vs Ag/AgCl. The addition of phosphate at different concentrations preserved its characteristic inhibitory effect on the HER signal. A clear calibration curve was obtained, with a detection limit of approximately 0.15 ppm (Figure 2.b), demonstrating that in the presence of nitrate, a synergistic effect is observed, likely due to combined surface interactions and competitive electrochemical processes, leading to an enhanced sensitivity and improved limit of detection, while maintaining the phosphate-induced HER inhibition mechanism. In parallel, nitrate was also successfully detected over a range of concentrations in the presence of phosphate, yielding a limit of detection (LOD) of approximately 0.4 ppm . These results indicate that simultaneous detection of phosphate and nitrate is possible using this strategy, with phosphate quantified through HER inhibition and nitrate monitored via its reduction peak at -0.5 V .

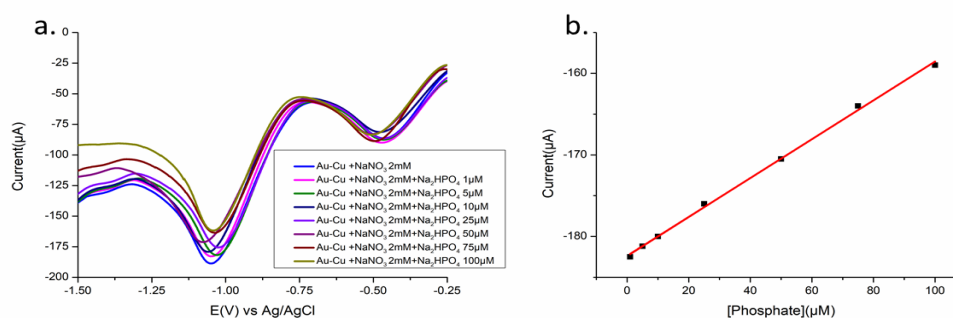
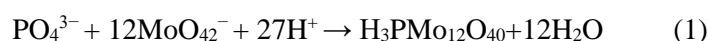


Figure 2: a. Linear Scan voltammetric responses using an Au-Cu modified electrode with 2 mmolL^{-1} of nitrate in the solution without and with different phosphate concentrations ($1-100 \mu\text{molL}^{-1}$). Note that a new Au-Cu is used for each addition, scan rate: 50 mVs^{-1} vs Ag/AgCl. b. Calibration curve obtained with Hydrogen reduction peak current (concentration from 1 to $100 \mu\text{molL}^{-1}$).

3.3- Phosphate sensing using molybdenum in solution (Mo)

a- Phosphate detection with gold (Au) macroelectrodes:

Phosphate is not an electroactive substance; therefore, the electrochemical detection of phosphate is based on the formation of a complex formed by its reaction with molybdate in an acidic medium to form a Keggin anion ($\text{PMo}_{12}\text{O}_{40}^{3-}$) according to Reaction (1). In order to ensure that all the phosphates present are involved in the complexation reaction, the concentration of molybdate used in this work is 0.1 M , which is in large excess over the reaction stoichiometry ($\sim 10 \mu\text{M}$) (Jońca et al., 2011). The pH is adjusted to 1.0 with sulfuric acid.



Gold electrodes were used for cyclic voltammetry analysis in the solution containing different concentration of phosphate (Figure 3.a) and a calibration plot for the oxidation analytical peak was obtained (Figure 3.b). After each phosphate addition, a waiting time of 5 minutes was applied before starting the electrochemical measurements to ensure the complete reaction between molybdenum (Mo) and phosphate (Ph) for the formation of Ph-Mo complex. It is clear that all the peaks increase with increasing phosphate concentration (Figure 3.a), providing an indirect electrochemical methodology.

In the investigated concentration range, the oxidation response at +0.27 V (vs Ag/AgCl) exhibits a linear behaviour with a correlation coefficient of $r^2 = 0.98$ (Figure 3.b). From this calibration curve, a limit of detection (LOD) of 2 ppm was calculated using the 3σ method.

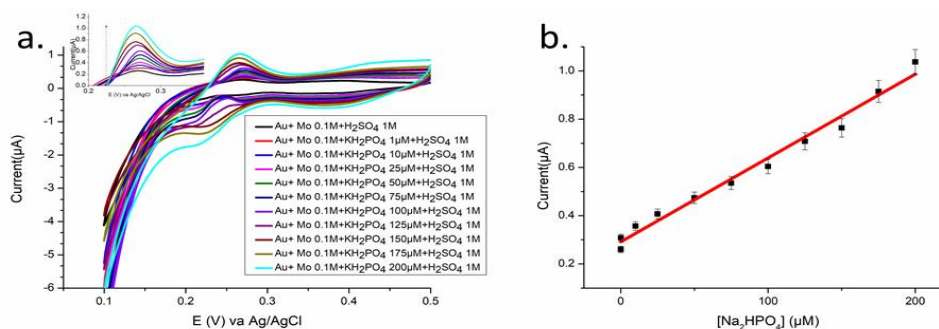


Figure 3: a: Cyclic voltammetric responses using an Au electrode following additions of phosphorus ($1\text{--}200\ \mu\text{mol}\cdot\text{L}^{-1}$) in solution (H_2SO_4 1M) containing $0.1\ \text{mol}\cdot\text{L}^{-1}$ ammonium molybdate tetrahydrate. Note that a new Au and a new solution was used for each addition. Scan rate: $50\ \text{mV}\ \text{s}^{-1}$. b: Calibration curve for phosphate detection obtained with current measured at 0.27 V (vs Ag/AgCl), (concentration from 1 to $200\ \mu\text{mol}\cdot\text{L}^{-1}$).

In order to reproduce these results and improve sensitivity, and therefore the detection limit, three electrochemical techniques were studied: Cyclic Voltammetry (CV), Linear Sweep Voltammetry (LSV), and Differential Pulse Voltammetry (DPV), which is a reference method for improving the signal-to-noise ratio. For each technique, three replicates were performed to ensure reproducibility. The detection limit was calculated for each method, and the results show that DPV provided the best performance, with a limit of detection of approximately 0.2 ppm.

b- Phosphate detection with gold (Au) microelectrodes in a microfluidic cell:

These works first focused on the detection of phosphate on gold macroelectrodes, using a molybdenum-based redox system. Phosphate concentrations in environmental waters are typically targeted at $\text{sub-}\mu\text{g}\cdot\text{L}^{-1}$ levels (around $0.1\ \text{mg}\cdot\text{L}^{-1}$) under the EU Water Framework Directive to prevent eutrophication. In order to improve sensitivity, widen the dynamic range, and reduce reagent consumption with respect to this environmental target, the system was then transposed to gold microelectrodes. The protocol was finally implemented on a microfluidic platform equipped with eight gold microelectrodes ($50\ \mu\text{m}$ width). Molybdenum in solution ($0.1\ \text{mol}\cdot\text{L}^{-1}$ in 1 M H_2SO_4) exhibits reduction peak at around $-0.625\ \text{V}$ vs Pt on the gold microelectrodes in the microfluidic cell, which is used to monitor the detection. Upon addition of phosphate (KH_2PO_4), a molybdenum–phosphate complex forms in solution, resulting in a progressive increase of the reduction peak current (Figure 4.a). This response is linear over the investigated concentration range, with a correlation coefficient $r^2 = 0.99$, enabling the determination of a limit of detection (LOD) of about 0.3 ppm using the 3σ method (Figure 4.b). This result confirms both the reliability of the detection protocol and the robustness of the method on microelectrodes.

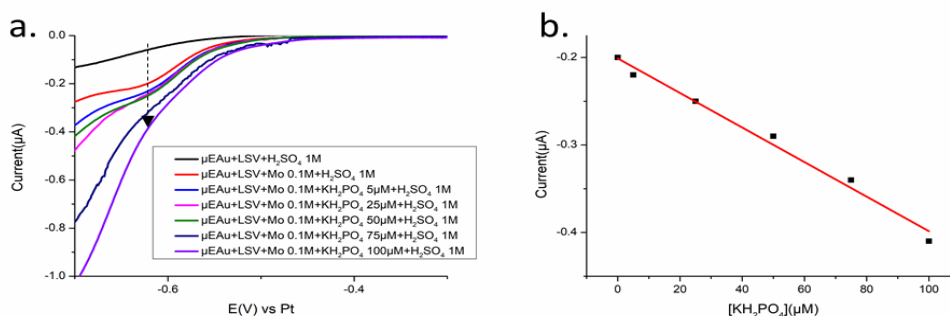


Figure 4: a: LSV of the gold microelectrode following additions of phosphorus ($5\text{--}100\ \mu\text{mol}\cdot\text{L}^{-1}$) in solution (H_2SO_4 1M) containing $0.1\ \text{mol}\cdot\text{L}^{-1}$ ammonium molybdate tetrahydrate, between $-0.5\ \text{V}$ and $-0.7\ \text{V}$ vs Pt. Note that a new Au and a new solution was used for each addition. Scan rate: $50\ \text{mV}\ \text{s}^{-1}$. B: Calibration curve for phosphate detection obtained with current measured at $-0.625\ \text{V}$ (vs Pt), (concentration from 5 to $100\ \mu\text{mol}\cdot\text{L}^{-1}$).

Notably, within the same microfluidic system, phosphate detection was also successfully demonstrated based on the HER inhibition protocol.

4. CONCLUSION

Two electrochemical strategies were developed for phosphate detection: copper modified electrodes based on Hydrogen Evolution Reaction (HER) inhibition, with a Limit Of Detection (LOD) of 4.5 ppm, and phosphomolybdate complex formation, with an LOD of 0.2 ppm, both exhibiting sustained operational stability and reliable performance over time. The integration of these methods into a microfluidic platform enables rapid analysis with low sample and reagent consumption, making them well suited for field applications. In addition, the co-detection of nitrate alongside phosphate was successfully demonstrated with good selectivity.

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