

*Full Paper*

## **Potentiometric Sensor for Determination of Cysteine Based on Graphene Oxide-Lead Porphyrin Composite**

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**Abstract-** Combination of the exceptional properties of graphene oxide with the selectivity of lead porphyrin leads to fabricate a novel cysteine-selective sensor. The electrode made of 30% PVC, 53% NPOE, 2% NaTPB and 15% GO-PP was found to show the most favorable behavior. The sensor shows a Nernstian response ( $58.8 \text{ mV decade}^{-1}$ ) in the concentration window of  $5.0 \times 10^{-7}$ - $5.0 \times 10^{-1}$  M with detection limit of  $8.0 \times 10^{-8}$  M. The graphene oxide-lead porphyrin composite based sensors displayed a fast and selective response towards cysteine which was successfully applied to the determination of cysteine in real sample (human serum).

**Keywords-** Graphene Oxide Composite, Cysteine, Selective Electrode, Lead Porphyrin, Potentiometry

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### **1. INTRODUCTION**

Cysteine is one of the most important aminoacids. It plays an important role in biological systems and has been widely used in the medicine and food chemistry [1]. Disorders of cysteine metabolism include cystinosis, an autosomal recessive disease produced by a defect in lysosomal transport and cystinuria, a common inheritable disorder of amino acid transport. Altered levels of cysteine have been implicated in hyperhomocysteinemia and in a number of

pathological conditions, including Alzheimer's and Parkinson's disease [2–4]. As a consequence, cysteine detection is of great importance for industrial, health care, and fundamental research applications.

Since the start of the graphene revolution in 2004, it has captured increasing attention and has shown great promise in many applications [5-10], arising from its unique physicochemical properties [11-15]. The special properties of graphene may provide insight to fabricate novel potentiometric sensors for virtual applications. The excellent conductivity and small band gap are favorable for conducting electrons [15]. Chemically functionalized graphene can be readily mixed with polymers in solution to form a stable dispersion and yield novel types of electrically conductive nanocomposites [16-18].

On the other hand, potentiometric sensors, prepared by coating polymer films containing electroactive species on various substrates such as graphite or glassy-carbon and metal-wire electrodes, with no internal electrolyte solution have been shown to be very effective for a wide variety of inorganic and organic anions and cations [36-38]. In this paper, graphene oxide-lead porphyrin composite (GO-PP) was prepared and coated on platinum wire. Metalloporphyrins which have a lewis acidic metal as the coordinating site have become attractive carriers; therefore the combination of the advantages of metalloporphyrin and graphene oxide could make it possible to fabricate a novel sensor. The proposed electrode used for cysteine sensing.

## 2. EXPERIMENTAL

### 2.1. Materials

Reagent grade o-nitrophenyl octyl ether (NPOE), dibutyl phthalate (DBP), sodium tetraphenyl borate (NaTPB), hexadecyltrimethylammonium bromide (HTAB), tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and high relative molecular weight PVC (all from fluka) were used as received. Nature flake graphite was purchased from Sigma–Aldrich. Pb(II) 5,4-(aminophenyl)-10,15,20-triphenyl porphyrin [PP] was synthesized and purified as described elsewhere [10-21]. All other chemicals were of analytical grade.

### 2.2. Equipments

XRD patterns of the solid products were obtained in the  $2\theta$  range of  $5-80^\circ$  using a PW-1840 diffractometer from Philips Co. with Cu-K $\alpha$  radiation ( $\lambda=1.54178 \text{ \AA}$ ). The morphology of the nanosorbent was determined from transmission electron microscopy (TEM) using a Zeiss 900 TEM at a voltage of 80 kV. FTIR spectra were obtained using a Vertex 70 FT-IR spectrophotometer from Bruker Co. A Corning ion analyzer 250 pH/mV meter was used for potential measurements at  $25.0\pm 0.1^\circ\text{C}$ .

### 2.3. Preparation of the sensing element

Synthesis of GO-PP was carried out in two stages: GO was synthesized with the Hummers method [22]. For the synthesis of GO-PP the Xu et al. method [23] was followed. Briefly, graphene oxide was refluxed in  $\text{SOCl}_2$  in the presence of DMF under argon atmosphere. At the end of the reaction, excess  $\text{SOCl}_2$  and solvent were removed and in the presence of triethylamine, the above product was allowed to react with PP in DMF under argon. After the reaction, the solution was cooled to room temperature, and then poured into ether to precipitate the product. The product was isolated by filtration and the excess PP and other impurities were removed through washing cycles. The GO-PP was then washed with a small quantity of water to remove triethylamine, and finally dried under vacuum. A scheme of the process is shown in Fig. 1.

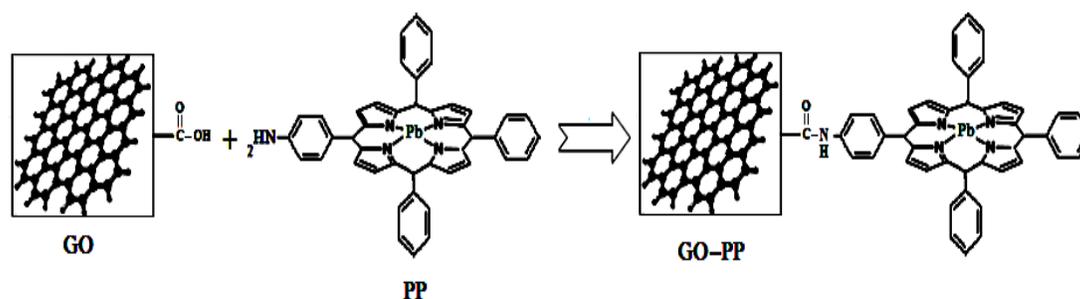


Fig. 1. Schematic procedure of the GO-PP preparation

### 2.4. Fabrication of the electrode

Membrane solution were prepared by thoroughly dissolving 30 mg powdered PVC, 15 mg ionophore GO-PP, 2 mg NaTPB and 53 mg plasticizer NPOE in 5 mL of THF. The resulting mixture was transferred into a glass dish of 2 cm diameter. Electrodes were obtained by dipping each platinum wire into this mixture, until a thin film coating was formed. This film was allowed to dry for 12 h in air. The electrode was finally conditioned for 12 h in a  $10^{-2}$  M of cysteine solution.

### 2.5. Emf measurement

All emf measurements were carried out with the following assembly:

Pt wire / membrane /sample solution /Hg-Hg<sub>2</sub>Cl<sub>2</sub>, KCl (satd.)

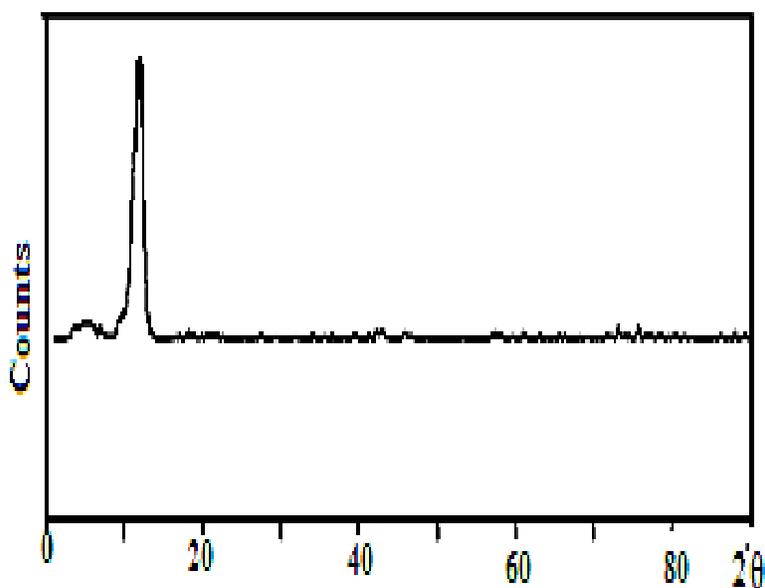
The emf observations were made relative to a double-junction saturated calomel electrode (SCE, Philips) with the chamber was filled with an ammonium nitrate solution. All

measuring solutions were buffered to the appropriate value using phosphate buffer solutions (0.01 M).

### 3. RESULTS AND DISCUSSION

#### 3.1. Structural characterization

The X-ray diffraction (XRD) pattern of GO are shown in Fig. 2. As can be seen a wide peak is observed for GO at  $12.0^\circ$  corresponding to a basal spacing of  $d_{001}=7.33 \text{ \AA}$ . [24, 25] because of the presence of intercalated  $\text{H}_2\text{O}$  molecules and various oxide groups (Fig. 2) [26].

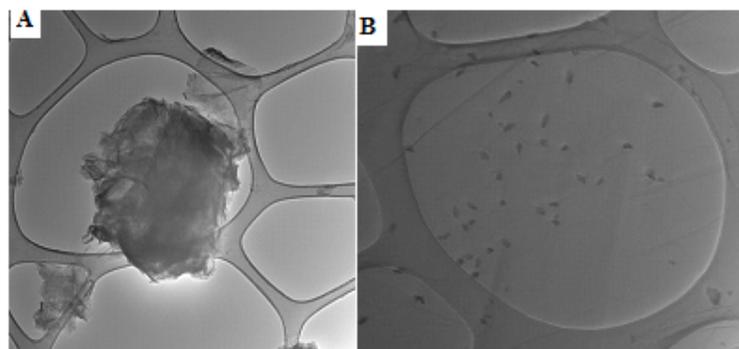


**Fig. 2.** XRD pattern of GO

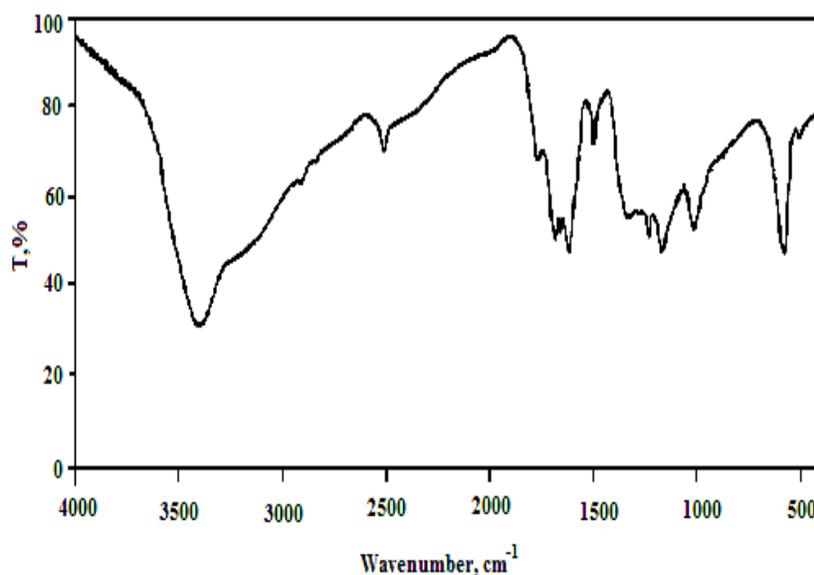
The morphology of the GO sheets and GO-PP complex was investigated by TEM. As shown in Fig. 3, the PP were successfully positioned over the entire surface of GO to form GOM nanocomposite.

In order to understand the interaction between PP and GO, FTIR spectrum was recorded (Fig. 4). It is seen that the GO sheets carried adsorbed water molecules and structural OH groups (a strong absorption band at  $3410 \text{ cm}^{-1}$  due to O-H stretching vibrations), C=O (the C=O stretching vibrations from carbonyl and carboxylic groups at  $1734 \text{ cm}^{-1}$ ), and C-O (C-OH stretching vibrations at  $1200 \text{ cm}^{-1}$  or O - C -O stretching vibrations at  $1050 \text{ cm}^{-1}$ ) groups, indicating the attachment of oxo-groups on GO sheets after the chemical oxidation of flake graphite. The spectrum also showed a band around  $1520 \text{ cm}^{-1}$  corresponding to the C=C stretching vibrations of carbon-carbon bonds in the aromatic ring. The band at  $1689 \text{ cm}^{-1}$  corresponds to the C=O characteristic stretching band of the amide group and the stretching

band of the amide C–N peak appeared at  $1260\text{ cm}^{-1}$ . These results clearly prove that the Pb(II)-porphyrin complex covalently attached to the carboxylic groups of GO through the formation of a stable amide bond.



**Fig. 3.** TEM images of (A) the pristine GO sheet and (B) the GO-PP complex. Scale bar: 200 nm



**Fig. 4.** FT-IR spectra of GO-PP

### 3.2. Effect of membrane Composition

As the potentiometric response and linear dynamic range of the electrode depends on the membrane composition, different electrodes based on the GO-PP for cysteine were prepared and the results are summarized in Table 1.

As seen the sensitivity of electrode response increases with increasing sensing agent (GO-PP) content until a value of 15% is reached (1-3). Further addition of sensing agent will, however, result in diminished response of the electrode (4).

The potentiometric response of the membrane ion-selective electrodes is greatly influenced by the polarity of the membrane medium, [27-29]. The influence of the nature of plasticizer on the cysteine response was studied on electrodes containing two types of plasticizers having different dielectric constants, namely, DBP and NPOE. As shown in Table 1 (3 and 5), NPOE with the highest dielectric constant in the series resulted in the best sensitivity of the potential responses.

The response properties of anion-selective electrodes based on ion carriers are strongly affected by the ionic sites lipophilic ionic sites [30]. So, the effect of additives on the potentiometric response of GO-PP-based electrode was investigated by varying the nature and amount of ionic additives (3, 6 and 7). Table 1 shows that the addition of HTAB as a cationic additive worsens the slope and dynamic range of the calibration graph (compare 3 and 7). On the other hand, addition of NaTPB to the membrane (6) improved the response of the electrode. These results revealed that GO-PP in the membrane acts as a positively charged carrier.

Thus, the membrane M<sub>6</sub> with the optimized composition of PVC: NPOE: GO-PP:NaTPB percent ratio of 30:53: 15:2 was selected for the preparation of membrane electrode for cysteine .

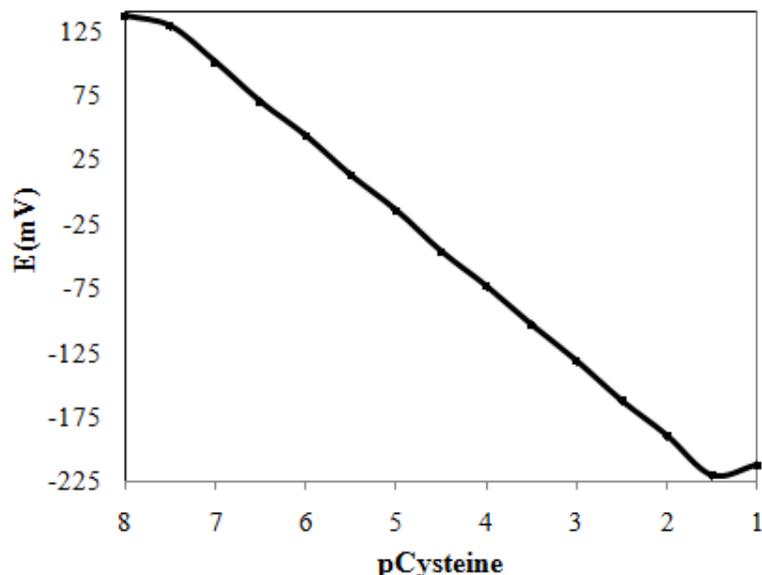
**Table 1.** Optimization of the membrane composition

Membrane No.	Composition (%)				Working range (M)	Slope (mVdecade <sup>-1</sup> )
	PVC	Plasticizer	Ionic site	GO-PP		
1	30	65,NPOE	-	5	$1.0 \times 10^{-3}$ - $5.0 \times 10^{-5}$	-33.0
2	30	60,NPOE	-	10	$1.0 \times 10^{-1}$ - $1.0 \times 10^{-5}$	-44.1
3	30	55,NPOE	-	15	$1.0 \times 10^{-2}$ - $1.0 \times 10^{-5}$	-50.5
4	30	50,NPOE	-	20	$1.0 \times 10^{-2}$ - $5.0 \times 10^{-5}$	-49.5
5	30	55,DBP	-	15	$1.0 \times 10^{-2}$ - $1.0 \times 10^{-5}$	-40.2
6	30	53,NPOE	2, NaTPB	15	$5.0 \times 10^{-1}$ - $5.0 \times 10^{-7}$	-58.8
7	30	52,NPOE	3, HTAB	15	$1.0 \times 10^{-1}$ - $5.0 \times 10^{-5}$	-47.1

### 3.3. Calibration curve

The EMF response of the proposed cysteine sensor based on GO-PP, prepared under optimal membrane ingredients, indicates a linear range from  $5.0 \times 10^{-1}$ - $5.0 \times 10^{-7}$  M (Fig. 5). The slope of calibration curve was  $-58.8 \pm 0.5$  mV per decade. The limit of detection, as

determined from the intersection of the two extrapolated segments of the calibration graph, was  $8.0 \times 10^{-8}$  M.



**Fig. 5.** Potential response of cysteine selective electrode based on GO-PP in buffered solution of cysteine (pH 8.5)

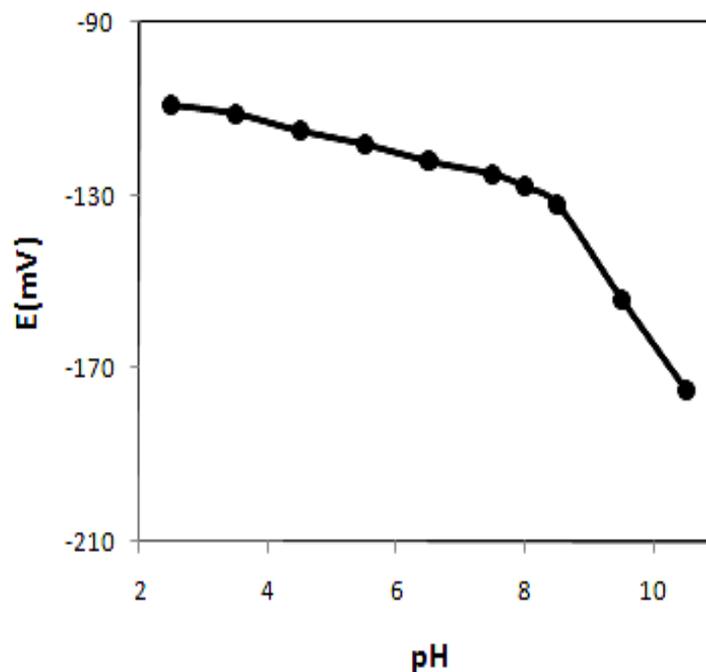
### 3.4. Effect of pH

The influence of pH of the test solution ( $1.0 \times 10^{-3}$  M cysteine) on the potential response of the membrane sensor was tested in the pH range 2.5–10.5 and the results are shown in Fig. 6. In the acidic region the electrode response is deteriorated as a result of partial demetallation of the metalloporphyrin complex during the contact with the low pH test solution [31–32]. For cysteine, the  $pK_a$  values of carboxyl, thiol and amino groups are 1.7, 8.3, and 10.8, respectively [33]. Therefore, cysteine exists mainly as a zwitterion at neutral pH and also does not contribute to the potential response of the membrane electrode. As the pH increases, the equilibrium amount of the thiolate anion of cysteine increases and this form mainly contributes to the response of the electrode. At higher pHs (higher than 8.5), interference from hydroxide ions becomes serious and can affect the response of the electrode. So pH 8.5 was selected for subsequent experiments.

### 3.5. Response time

Dynamic response time is the required time for the electrode to achieve values within  $\pm 1$  mV of the final equilibrium potential, after successive immersions in the sample solutions [34–37]. It is obtained by variation the cysteine concentration in a series of solutions

from  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-2}$  M and recording the time and potential changes. The sensor was able to quickly (about 20 s) reach its equilibrium response in the whole concentration range.



**Fig. 6.** Effect of pH of the test solution on the potential response of the cysteine-selective electrode

### 3.6. Life time

The electrode's lifetime was estimated based on the change in the slope and linear dynamic range with time. After 8 weeks a very slight gradual decrease in slope was observed (i.e., from 58.8 to 56.0 mV/decade) which shows the long-term stability of this kind of sensor.

### 3.7. Selectivity

Selectivity, which describes an ion-selective electrode's specificity toward the target ion in the presence of interfering ions, is the most important characteristic of these devices. The potentiometric selectivity coefficients of the cysteine sensor were evaluated by the matched potential method (MPM) [38-40]. The resulting values of the selectivity coefficients are shown in Table 2. Note that all selectivity coefficients are about  $10^{-4}$ , suggesting were interferences negligible in the performance of the electrode assembly.

**Table 2.** Selectivity coefficients of various interfering compounds for cysteine sensor

Interfering ion	$K_{MPM}$
glycine	-4.6
L-valine	-4.9
L-lysine	-4.2
L-arginine	-3.8
L-histidine	-4.2
Phosphate	-4.8
Nitrate	-4.7
Sulfate	-4.8
Thiocyanate	-3.2
Chloride	-4.4

### 3.8. Real sample analysis

As a practical use, the proposed method was also used to detect cysteine in human serum. The concentration of cysteine in human serum was found to be  $178 \pm 1.3 \mu\text{M}$  by the proposed sensor, which is in satisfactory agreement with that determined through an amino acid autoanalyzer ( $175 \pm 2.3 \mu\text{M}$ ).

## 4. CONCLUSION

In the present work, GO sheets were functionalized by Pb-porphyrin and used as selective sensing element in construction of a coated wire electrode for the measurement of cysteine. The sensor demonstrated advanced performances with a fast response time of 20 s, a low detection limit of  $8.0 \times 10^{-8}$  M and potential responses across the range of  $5.0 \times 10^{-7}$ – $5.0 \times 10^{-1}$  M. The sensor was successfully applied to the determination of cysteine in real sample (human serum).

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