Is the poly (methylene blue)-modified glassy carbon electrode an adequate electrode for the simple detection of thiols and amino acid-based molecules?

Maria Inês Costa Marinho¹, Murilo Feitosa Cabral *, Luiz Henrique Mazo

Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, CEP 13560-970, São Carlos, SP, Brazil

**A R T I C L E  I N F O**

Article history:
Received 15 June 2012
Received in revised form 22 August 2012
Accepted 25 August 2012
Available online 7 September 2012

Keywords:
Poly (methylene blue)
Glyphosate
l-Cysteine
N-Acetyl cysteine
Chemically modified electrode

**A B S T R A C T**

This paper describes the preparation, characterization, and use of poly (methylene blue) (PMB)-modified glassy carbon electrodes (GCE) (GCE–PMB) in the detection of the thiols l-cysteine (l-CySH) and N-acetyl cysteine (Acy), and the herbicide glyphosate (GLYP) in pH 5.3 aqueous solution. The polymer film prepared by electropolymerization showed different characteristics such as robustness, stability, and redox properties satisfactorily. The surface coverage concentration (I) of PMB was found to be 7.90 × 10⁻⁹ mol cm⁻². Moreover, we observed strong adhesion of the polymer film to the electrode surface. The results using GCE–PMB as a sensor indicated that this modified electrode exhibited electrocatalytic activity toward the detection of thiols and glyphosate in 0.1 mol L⁻¹ KCl (pH 5.3). Meanwhile, strong adsorption of the analytes on the GCE–PMB electrodes was also observed. Otherwise, using a low concentration (1 × 10⁻⁶ mol L⁻¹) of l-cysteine and N-acetyl cysteine and 8.9 × 10⁻⁶ mol L⁻¹ of glyphosate, separately, it was possible to observe a well-defined electrochemical response, thus providing an opportunity to further understand the applicability of PMB as a sensor for amino acid-based molecules.

© 2012 Elsevier B.V. All rights reserved.

**1. Introduction**

The development of sensors with important features such as high sensitivity, selectivity, and stability is an area of growth and interest in analytical chemistry. One way to develop a sensor with these characteristics is the modification of solid electrodes, which allows the development of various types of sensors [1–3].

The development of chemically modified electrodes (CMEs) is important, especially regarding the analysis of compounds of interest, such as pharmaceuticals and herbicides. This is possible due to various functions attributed to the CMEs, such as selective interactions [4], electrocatalysis of redox reactions with slow electron transfer on the electrode substrate [4,5], selectivity [4], and the development of biosensors and immunosensors [5–8], as well as stability, reproducibility, and applicability [9,10].

Conducting polymers (CPs) have a wide application in electroanalytical studies because of the versatility of electrochemical polymerization in obtaining polymer films. The synthesis of CPs by electrochemical methods—electropolymerization, in particular—involves the anodic oxidation of a monomer dissolved in an electrolyte by applying an appropriate external potential, usually the oxidation potential of monomers to form cation-radical species [10]. The electroactive polymer film was deposited on the electrode substrate (i.e., glassy carbon or platinum) in its oxidized state, and the positive charges along the structural skeleton of the polymer were compensated for by anions in the supporting electrolyte [10,11].

Surface-modified electrodes based on the electropolymerization of several phenazines, phenoxazines and phenothiazines have been reported in the literature [12]. In the electropolymerization of the methylene blue (MB), a phenothiazine dye in the azines group, growth rates increase as the pH increased, indicating the basic solutions to be optimal media for polymerization. These unique properties of MB electropolymerization and the structure of the monomer molecule allowed one to hypothesize that poly (methylene blue) (PMB) is a representative of a new group of electroactive polymers [7,10,13].

The electrocatalytic activity of PMB in the presence of biomolecules and different inorganic compounds has been reported in previous studies [5–7,14,15]. The PMB film promotes the decreased overpotential of the electrochemical oxidation reaction of the molecule to a greater extent compared to a conventional electrode. The applications reported for PMB include its bioelectrochemistry activity that allows its use as a mediator in the oxidation of compounds as NADH [5,7,11] and as a sensor for hemoglobin [14].

In this work, CMEs with PMB were applied to detect the thiols l-cysteine (l-CySH) and N-acetyl cysteine (Acy) as well as the herbicide glyphosate (GLYP). It is worth noting that these compounds have a common characteristic—an amino acid feature. However,
the oxidation of these compounds in metal (i.e. platinum, gold or mercury) and carbon electrodes has a poor electrochemical response, and it occurs at high overpotentials [16–19]. In addition, the detection of these compounds has limitations such as low selectivity and reproducibility. Electron transfer mediators are used predominately to enhance the sensitivity of the electrode response to analytes that tend to exhibit slow electrode kinetics on the bare, unmodified electrode substrate [19].

Therefore, in the present paper, we propose a simple and efficient method for the electrochemical detection of some compounds with specific features employing poly (methylene blue)-modified glassy carbon electrode (GCE–PMB).

2. Experimental

2.1. Reagents and supporting electrolyte solutions

All reagents used in this work were of analytical grade, and all solutions were prepared in water purified by the Barnstead NanoPure system (resistivity $\geq 18$ M$\Omega$ cm).

The MB monomer was purchased from Aldrich (Germany). The supporting electrolyte solution used for the electropolymerization of MB consisted of 0.05 mol L$^{-1}$ phosphate buffer and 0.1 mol L$^{-1}$ sodium nitrate (pH 8.0). The concentration of the dye dissolved in this solution was always $2.5 \times 10^{-4}$ mol L$^{-1}$.

Solutions of l-CySH (Chromate Chemicals LTDA), Acy (Chromate Chemicals LTDA), and GLYP (Milenia Agrociências) were prepared fresh.

2.2. Electrochemical Instrumentation

The electrochemical measurements were carried out on a computer-controlled potentiostat Autolab$^\text{PC}$ PGSTAT30 with GPES software (Eco Chemie B.V.; The Netherlands).

A conventional three-electrode cell assembly consisting of a GCE (diameter 4.8 mm, sealed in a PTFE tube) or GCE modified with the PMB film as working electrode, an Ag/AgCl 3.0 mol L$^{-1}$ KCl as the reference electrode, and a platinum wire as the counter electrode were used in all electrochemical measurements.

2.3. Preparation of PMB-modified electrodes

The GCE was cleaned with a detailed electrochemical pre-treatment and mechanical polishing prior to electrochemical polymerization. The GCE was polished with 1 µm, 0.3 µm and 0.05 µm alumina (Al$_2$O$_3$) slurries. Then, to remove the alumina particles, the electrode was subjected to an ultrasonic agitation in ethanol for 3 min and in water for 2 min. The electrochemical pre-treatment of the electrode was carried out, first by applying a fixed potential of +900 mV versus Ag/AgCl for 240 s [20], followed by potential cycling between −0.4 and +1.0 V at a scan rate of 50 mV s$^{-1}$ until a stable voltammogram was obtained. The same electrolyte solution was used for pre-treatment and the electropolymerization of the dye monomers. Different experimental variables such as the scan rate, concentration of the MB solution, supporting electrolyte, buffer solution and pH were chosen according to previous studies [7,10]. The PMB was prepared as a film on the electrode substrate by cyclic voltammetry (CV) from the supporting electrolyte solution described above, containing $2.5 \times 10^{-4}$ mol L$^{-1}$ monomer, at a scan rate of 50 mV s$^{-1}$. The potential was cycled between −0.4 and +1.2 V versus Ag/AgCl for 30 cycles. After this step the polymer-modified electrode was washed with supporting electrolyte and kept in refrigerator at 4 °C for 24 h [21]. After the electrochemical measurements, the modified electrode was kept in the refrigerator.

In order to evaluate the electrochemical response of the modified electrode in the presence of l-CySH, Acy and GLYP, the electrolyte solution was changed to 0.1 mol L$^{-1}$ KCl (pH 5.3) and the analyte solutions were prepared using the same solution with 1.0 $\times$ 10$^{-3}$ mol L$^{-1}$ l-CySH, Acy and GLYP, separately. Using the same electrolyte in the preparation of the polymer film did not result in any electrochemical response. This might be attributed to the occurrence of chloride ion doping of the PMB film, as previously described by Simões et al. [3].

3. Results and discussion

3.1. Electropolymerization of the MB and electrochemical characterization of the GCE–PMB

The process of electropolymerization of MB for 30 cycles (Fig. 1) presented quasi-reversible electrochemical behavior. However, for a greater number of cycles, this behavior becomes more irreversible and there was no significant increase in peak current ($I_p$). These results are included as Supplementary material.

In Fig. 1, the voltammetric profile of the polymer-modified electrode has two redox peaks in two regions of different potential, one of which is related to the oxidation of the MB monomer (in the region of negative potential) and the other is related to the formation of the polymer (in the region of positive potential). In the voltammetric profile, a peak was also observed in the potential region around 1.2 V that corresponds to the region of formation of cation-radical species [10].

The surface coverage of the polymer film on the electrode surface can be estimated from the surface coverage concentration ($\Gamma$) of PMB [4]. For this, the $\Gamma$ value was calculated from the value of the charge ($Q$) involved in the electropolymerization process according to the relationship described below:

$$\Gamma = \frac{Q}{nFA}$$

where $A$ is the geometric area of the GCE (0.18 cm$^2$).

The Q value was obtained by integration of the voltammetric peak of PMB in the range of potential between the start and end of their training process (−0.2 to +0.5 V) with a scan rate 50 mV s$^{-1}$.

$$Q = \frac{13,752 \times 10^{-6} \times (A) \times (mV)}{50 \times (mV \times s^{-1})} = 275 \mu C$$

Therefore, the value of Q was related to the Faraday constant, which is 96485.34 C mol$^{-1}$, and provided a value of $2.85 \times 10^{-9}$ mol. However, we have to consider that, in the electro-oxidation...
reaction of MB, there are two electrons involved [7]. Therefore, the value of the charge involved in the electropolymerization process corresponds to \(1.43 \times 10^{-9}\) mol. Thus, the \(I\) value in Eq. (1) is:

\[
I = \frac{1.43 \times 10^{-9}}{0.18} = 7.90 \times 10^{-9} \text{ mol cm}^{-2}
\]

The value of \(I\) obtained in this work \((7.90 \times 10^{-9} \text{ mol cm}^{-2})\) revealed good surface coverage of the PMB film on the surface of the electrode substrate. Therefore, we could assume good physical and chemical stability during the electrochemical measurements.

From the calculated value of \(I\), it was also possible to obtain information on the number of monolayers of electroactive redox species and the thickness of the polymer layer. The number of monolayers was obtained by the equation described below [21]:

\[
\frac{I}{I_{\text{mono}}} = \frac{7.90 \times 10^{-9} \text{ mol cm}^{-2}}{2.0 \times 10^{-9} \text{ mol cm}^{-2}} = 39
\]

The value of film thickness can be estimated from the equation below [12]:

\[
d = v \times \Gamma
\]

where \(v\) and \(d\) represent the molecular volume of the MB in the polymer \((400 \text{ cm}^3 \text{ mol}^{-1})\) and film thickness, respectively [12,15]:

\[
d = 400 \text{ (cm}^3 \text{ mol}^{-1}) \times 7.90 \times 10^{-9} \text{ (mol cm}^{-2}) = 32 \text{ nm}
\]

The calculated result for the estimation of the PMB film thickness showed that the GCE is covered by a thin layer of this film. Previous results using thicker films (about 50 monolayers) of PMB in alkaline media showed that the response became irreversible. These data were confirmed by means of spectroelectrochemical measurements [12].

3.2. Effect of solution pH on the electroactivity of the PMB film

Fig. 2 shows the voltammetric profiles of the polymer-modified electrode in a solution of electrolyte \((0.05 \text{ mol L}^{-1}\) phosphate buffer containing \(0.1 \text{ mol L}^{-1}\) NaNO\(_3\)) to study the electrochemical behavior of the GCE–PMB in relation to the pH of the media. The pH range evaluated in this work was from 5.6 to 9.0.

In Fig. 2a, the voltammetric profiles show a quasi-reversible process with anodic and cathodic peaks, with a potential value that shifts to negative values as the pH increases. We clearly observed that as proton concentration decreases, the voltammetric peaks became broader and the cathodic peak split. It is worth noting that the film electroactivity diminishes with pH, especially at pH 9.0 when the electrode does not bleach to the same extent when reduced as it does at pH values of 5.6 and 7.0. This may be related to a redox process that is inhibited at higher pH values. Fig. 2b was constructed from the values of \(I_p\) and the pH, and there is a decrease in \(I_p\) with increasing pH.

Regarding the study of the polymeric film behavior in acidic conditions around pH 3.0, it was observed that the film loses its electroactivity in electrochemical measurements (data not shown). Thus, the pH must be controlled because it affects the sensitivity and conductivity of the PMB in electrochemical measurements.
3.3. Electroanalytical response of the GCE–PMB

3.3.1. l-Cysteine and N-acetyl cysteine analytes

Fig. 3a shows the voltammetric profile of the GCE–PMB containing 1.0 × 10⁻³ mol L⁻¹ l-CySH in 0.1 mol L⁻¹ KCl (pH 5.3) and in the absence l-CySH. The electrochemical oxidation of l-CySH and Acy on GCE results in very poor electrochemical responses and high overpotentials [16–19], thus demonstrating the necessity of using electrodes that allow the electroanalytical detection of this compound electrochemically under more favorable conditions such as low overpotentials and high sensitivity reactions.

The electrocatalytic effect of the PMB film in the oxidation reaction of l-CySH can be observed in the voltammetric profile in Fig. 3b, by the increase in \( I_p \) (at a given potential of ~0.2 V) in relation to the electrochemical response obtained with the GCE under the same experimental conditions.

Theoretically, the electrocatalytic effect can depend on the interaction between the electroactive species and the electrode surface, as well as some electronic and geometric factors [23]. These terms concern the density current observed during the electrochemical experiment (in our case, the oxidation of l-CySH). We compared two electrodes for the same reaction at a given potential ~0.2 V, and a current peak of ~17 µA (or 98 µA cm⁻², considering a geometric area of 0.18 cm² for the glassy carbon electrode - electrode substrate) could be observed for the GCE–PMB, while a current peak of ~1.13 µA (or 6.28 µA cm⁻², under the same consideration for the GCE–PMB) could be observed for the GCE. This current (or density current) was higher than the current observed for the oxidation of l-CySH on the GCE. The higher current density may be the result of a better catalyst, leading to improved affinity between the analyte and the electrode surface. Further understanding of the interaction between thiols and glyphosate with poly(methylene blue) was obtained by computational studies (see Fig. 7).

The electroactivity of the polymer film was also assessed for the detection of Acy (Supplementary material). The applicability of GCE–PMB was also found for this thiol under the same experimental conditions described for l-CySH; the results showed an electrochemical response similar to that obtained for l-CySH. This might be explained by an interaction between PMB and these compounds via a common group, such as the amino group. Thus, we observed the response of the polymer with Acy in the same region of the potential of l-CySH (0.2 and 0.0 V) and with the same current intensity.

![Fig. 4](image_url) Fig. 4. (a) Voltammetric profiles of GCE–PMB in a solution containing 1.0 × 10⁻³ mol L⁻¹ l-CySH in 0.1 mol L⁻¹ KCl; (––) GCE–PMB; Scan rate: (,), 20, (,), 50, (,), 70, (,), 100, (,), 150, (,), 200, (,), 250, and (,) 300 mV s⁻¹. (b) Plot of peak current vs. square root of scan rate.

![Fig. 5](image_url) Fig. 5. Voltammetric profiles of the GCE–PMB: (–) in 0.1 mol L⁻¹ KCl, pH 5.3; (—) in a solution containing 1.0 × 10⁻³ mol L⁻¹ glyphosate in 0.1 mol L⁻¹ KCl, pH 5.3; scan rate 50 mV s⁻¹.

3.3. Electroanalytical response of the GCE–PMB

3.3.1. l-Cysteine and N-acetyl cysteine analytes

Fig. 3a shows the voltammetric profile of the GCE–PMB containing 1.0 × 10⁻³ mol L⁻¹ l-CySH in 0.1 mol L⁻¹ KCl (pH 5.3) and in the absence l-CySH. The electrochemical oxidation of l-CySH and Acy on GCE results in very poor electrochemical responses and high overpotentials [16–19], thus demonstrating the necessity of using electrodes that allow the electroanalytical detection of this compound electrochemically under more favorable conditions such as low overpotentials and high sensitivity reactions.

The electrocatalytic effect of the PMB film in the oxidation reaction of l-CySH can be observed in the voltammetric profile in

![Fig. 6](image_url) Fig. 6. (a) Voltammetric profiles of the GCE–PMB in a solution containing 1.0 × 10⁻³ mol L⁻¹ glyphosate in 0.1 mol L⁻¹ KCl, pH 5.3; (—) GCE–PMB; scan rate (,), 20, (,), 50, (,), 70, (,), 90, (,), 100, (,), 150, (,), 200, (,) 250, and (,) 300 mV s⁻¹. (b) Plot of peak current vs. square root of scan rate.
3.3.2. Dependence on scan rate

The scan rate was varied from 20 to 300 mV s\(^{-1}\) to assess the nature of the transport of electroactive material to the electrode surface. Fig. 4a shows the cyclic voltammograms at different scan rates in a solution containing 1.0 \(\times\) 10\(^{-3}\) mol L\(^{-1}\) \(\tau\)-CySH in 0.1 mol L\(^{-1}\) KCl (pH 5.3). The voltammetric profiles of the GCE–PMB at different scan rates have shown that the anodic and cathodic peak currents of the film redox couples increases linearly with the increase of scan rates up to 300 mV s\(^{-1}\).

In addition, a study of the scan rate for \(\alpha\)-Cy was also performed and yielded a response similar to that of \(\tau\)-CySH, thus indicating that the process involved in the determining step of the reaction is mass transport to the electrode surface, governed by a diffusion process.

Fig. 4b was constructed from the values of peak current and scan rate. It appears that a linear relationship between peak current and the square root of scan rate (\(r = 0.999\)) exists. This result indicates that the oxidation reaction of \(\tau\)-CySH using GCE–PMB is a process in which the determining step of the reaction is mass transport to the electrode surface, governed by a diffusion process.
is the same, i.e. mass transport to the electrode surface, and is governed by a diffusion process. These results are included as Supplementary material.

3.3.3. Glyphosate

Fig. 5 shows the voltammetric profiles for the GCE–PMB in the presence and absence of GLYP at a concentration of $1.0 \times 10^{-3}$ mol L$^{-1}$. The electrolyte solution was a solution of 0.1 mol L$^{-1}$ KCl (pH 5.3).

In the voltammetric profile, we observed the presence of currents of anodic and cathodic peaks around 0.2 and 0 V (vs. Ag/AgCl), respectively. Interestingly, the voltammetric response of GCE–PMB for GLYP occurred in the same region of the potential obtained for the thiols L-CySH and Acy. Thus, a likely reaction mechanism to justify this result would be the interaction of the active sites of the polymer with a group of GLYP molecule, possibly the amino group as seen in the case of thiols.

The solution pH was measured before and after the electrochemical measurement at 5.3 and 3.2, respectively, and a noted a change in pH of the media. This indicates that the doping process of the PMB occurred and can be attributed to sorption of the glyphosate (acidic pesticide) on the polymer. On the other hand, the glyphosate has affinity for this polymer, and the sorption process increases the concentration of the acidic pesticide on the polymer–solution interface and decreases the local pH, providing proton exchange for the PMB doping.

The doping process of the polyaniline in contact with the acidic pesticides such as glyphosate has already been reported [3]. The results indicated different sorption behavior among pesticides and also some selectivity for the two polymer materials studied.

3.3.4. Dependence on scan rate

The scan rate varied from 20 to 300 mV s$^{-1}$ to assess the nature of the transport of electroactive material to the electrode surface. Fig. 6a shows the cyclic voltammograms at different scan rates in a solution containing $1.0 \times 10^{-3}$ mol L$^{-1}$ GLYP in 0.1 mol L$^{-1}$ KCl (pH 5.3).

The cyclic voltammograms of the GCE–PMB at different scan rates have shown that the anodic and cathodic peak currents of the film redox couples increases linearly with the increase of scan rates up to 300 mV s$^{-1}$. Fig. 6b was constructed from the peak current and scan rate values. It appears that a linear relationship between peak current and scan rate values. It appears that a linear relationship between current density and scan rate values. It appears that a linear relationship between current density and scan rate values.

To the best of our knowledge, no reports have been published involving an interaction with a polymer-modified electrode for GLYP. However, the detection of GLYP was possible using copper-based electrodes by means of chromatography [24] and electrochemical methods [25,26].

Fig. 7a–c depicts the results regarding the lowest concentration detectable by the GCE–PMB electrode in the presence of Cys, Acy and GLYP. For thiols, the concentration was $1.0 \times 10^{-4}$ mol L$^{-1}$ and for GLYP, a good signal was observed with $8.9 \times 10^{-4}$ mol L$^{-1}$. Since these analytes showed a strong interaction with PMB, more detailed electroanalytical studies should be performed in order to promote efficient regeneration of the electroactive surface of the GCE–PMB electrode. Moreover, in terms of disposable electrochemical devices, this kind of electrode should be selected for the detection of amino acids, particularly glyphosate at low concentrations, as advised by regulatory environmental agencies.

On the other hand, aiming to obtain further insight into the origin of the electrochemical signal observed for Cys, Acy and GLYP on GCE–PMB electrodes, computational studies were performed (Fig. 7d–f). Additional information about the computational studies is provided in the Supplementary material. Using a schematic drawing proposed by Karyakin et al. [7,10] for the structure of PMB and schematic drawings for cysteine, n-acetyl-cysteine and glyphosate, it was possible to suggest the type of interaction between these analytes and PMB. Glyphosate is shown with its phosphorus atom and carbonyl group centered in the PMB structure. Otherwise, the thiols are laterally placed with their carbonyl groups close to the nitrogen atom in the central ring of the PMB (monomer) structure.

These observations provide support for the proposition described by several authors [5,21,27,28] that a complex between PMB and the analyte is formed during the electro-oxidation of thiols or glyphosate.

4. Conclusions

The present work reports that PMB modified GCE prepared by the electropolymerization method and presents favorable electroanalytical characteristics for application as a strong adhesion to the surface of GCE and stability in the electrochemical measurements. The study with the GCE–PMB showed that it can be used as a sensor for the detection of the thiols (L-CySH and Acy) and GLYP. In particular, cyclic voltammetry studies showed the potential of the GCE–PMB on direct electrochemical detection of thiols at concentrations from $1 \times 10^{-4}$ mol L$^{-1}$ and the herbicide GLYP at concentrations from $8.9 \times 10^{-6}$ mol L$^{-1}$. The study of variation of scan rate for thiols and GLYP showed a linear relationship between the square root of scan rate and peak current, indicating that the process involved in the determining step of the reaction of GCE–PMB is controlled by mass transport and the whole electrochemical process is governed by diffusion.

Acknowledgments

The authors wish to thank CAPES (M.S. scholarship and M.F.C. PNPD/CAPES 0316083) and FINEP for financial support for this research.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jelechem.2012.08.023.

References