STUDIA UBB CHEMIA, LVIII, 2, 2013 (p. 105 – 114)
(RECOMMENDED CITATION)

ELECTROCHEMICAL BEHAVIOR OF THE HEMIN MODIFIED GRAPHITE ELECTRODE FOR H_2O_2 DETECTION

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ABSTRACT. Aiming to detect amperometrically H_2O_2, iron (**III**) protoporphyrin IX (hemin; Hm) was immobilized by simple adsorption on the surface of a graphite (G) electrode. The electrochemical behavior of the G/Hm modified electrode was investigated by using cyclic voltammetry (CV) and square-wave voltammetry (SWV) under different experimental conditions (scan rate or frequency and pH). The catalytic current measured at G/Hm was found to depend linearly on the H_2O_2 concentration from ~0.01 mM up to 0.04 mM H_2O_2 (R/N = 0.987 / 4) and from ~0.01 mM up to 0.08 mM H_2O_2 (R/N = 0.973/14) for CV and SWV measurements, respectively. For both methods the detection limit was ~10 μM, while the sensitivity was much higher for SWV [(276 ± 12) mA/M] than for CV [(13.5 ± 1.6) mA/M].

Keywords: *iron(III) protoporphyrin (IX), hydrogen peroxide, cyclic voltammetry, square wave voltammetry, electrocatalysis.*

INTRODUCTION

As defined by IUPAC, a chemically modified electrode (CME) “is an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic, or polymeric film of a chemical modifier and that by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film” [1]. Usually, the chemically modified electrodes can be obtained by one of the following approaches: (i) chemisorption, (ii) covalent bonding, (iii) polymer film coating, and (iv) entrapment in a conductive material. Among these, the simplest way exploiting the

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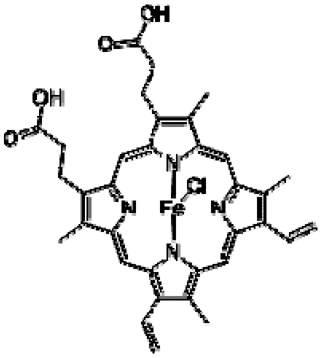
adsorption of the modifier on the electrode surface, generates a stable film. Usually, this approach yields monolayer modified electrodes with high electrocatalytic activity.



Scheme 1

Hemin (iron protoporphyrin IX; Hm) is one of the most important biological substances. It corresponds to the active center of several families of heme proteins, for example C-type cytocromes, peroxidase and oxygen–carrying proteins such as hemoglobin (Hb) and myoglobin (Mb) [2]. Hemin dissolved in an aqueous solution, adsorbed on the electrode surface or incorporated within a polymeric film immobilized on the electrode surface, maintains its electrochemical activity [3].

Hemin, due to its peculiar chemical structure (Scheme 1), is strongly adsorbed on the carbonaceous electrode materials, especially on pyrolytic graphite (PG) [4, 5]. The so obtained modified electrodes were used for the amperometric detection of H_2O_2 [6], superoxide [7], nitritc oxide [8], 4-aminophenol [9] and tryptophan or its derivates [10].

In this work we report on the preparation, by simple adsorption, of a hemin modified graphite electrode. The electrochemical behavior of G/Hm was examined by using two complementary electrochemical methods: cyclic voltammetry (CV) and square-wave voltammetry (SWV). Finally, the G/Hm modified electrode was used for the amperometric detection of H_2O_2 and the electroanalytical and kinetic parameters were estimated.

RESULTS AND DISCUSSION

Electrochemical behavior of G/Hm electrode

In figure 1 are showed the CV and SWV responses, recorded at G/Hm modified electrodes. In both cases, stable and well-defined peaks pairs corresponding to the immobilized hemin were observed. Also, the CV measurements show that the peak potentials shift progressively towards higher absolute values when the scan rate increases, suggesting a quasi-reversible electrochemical process.

The cyclic voltammogram recorded at 50 mV/s (Figure 1A) was used to evaluate the formal standard potential [$E^{0'} = (E_{pa} + E_{pc})/2$, where E_{pa} and E_{pc} stand for the anodic and cathodic peak potentials, respectively] of the hemin redox couple. The calculated value (-0.33 V *vs.* Ag/AgCl,KCl_{sat}) was found in good agreement with that already reported (-0.34 V *vs.* Ag/AgCl,KCl_{3M}) for hemin [11]. Additionally, the peak potentials separation ($\Delta E_p = E_{p,a} – E_{p,c}$ = 0.034 V, at 50 mV/s), confirms that the investigated redox process corresponds to a quasi-reversible one [8]. Additionally, the I_{pa}/I_{pc} ratio is very close to 1 (0.955, at 50 mV/s). Finally, it can be concluded that the adsorbed hemin exhibits the characteristic features for a quasi-reversible redox couple [12].

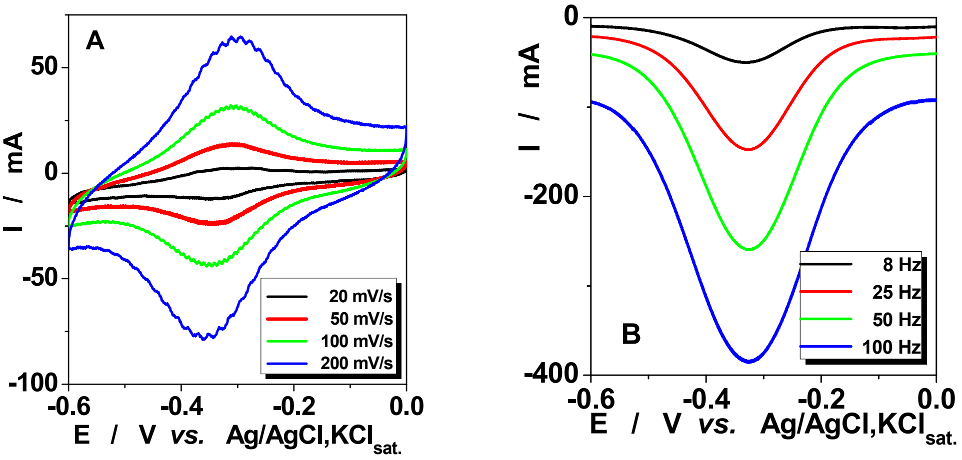


Figure 1. Cyclic (**A**) and square-wave (**B**) voltammograms recorded at G/Hm modified electrode. Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer (pH 7.0); starting potential, -0.6 V *vs.* Ag/AgCl,$KCl_{sat.}$ (A) and 0 V *vs.* Ag/AgCl,KCl_{sat} (B); SWV amplitude, 20 mV; deaerated solution (Ar)

As expected for a surface confined redox couple [13], in the investigated range of potential scan rate (20 – 200 mV/s), the anodic (I_{ap}) and cathodic (I_{cp}) peak currents depend linearly on the potential scan rate (v) (Figure 2A).

Within the limits of the experimental error, this conclusion was confirmed by the slope values obtained from the log(I_p) - log(v) dependencies (Table 1).

Table 1. The slopes of the log(I_p) *vs.* log(v) dependencies for G/Hm electrode.

Redox process	Slope	R / No. of exp. points
anodic	1.18 ± 0.05	0.990 / 6
cathodic	0.82 ± 0.02	0.997 / 7

For CV measurements, this parameter is considered a relevant criterion helping to distinguish between the adsorbed (slope ~1) or dissolved (slope ~0.5) redox couples. Furthermore, it is worth to mention that SWV measurements confirm that hemin behaves as a redox couple strongly immobilized on the graphite surface. Thus, the I_{cp} depends linearly on the square root of the applied frequency (*f*) (Figure 2B).

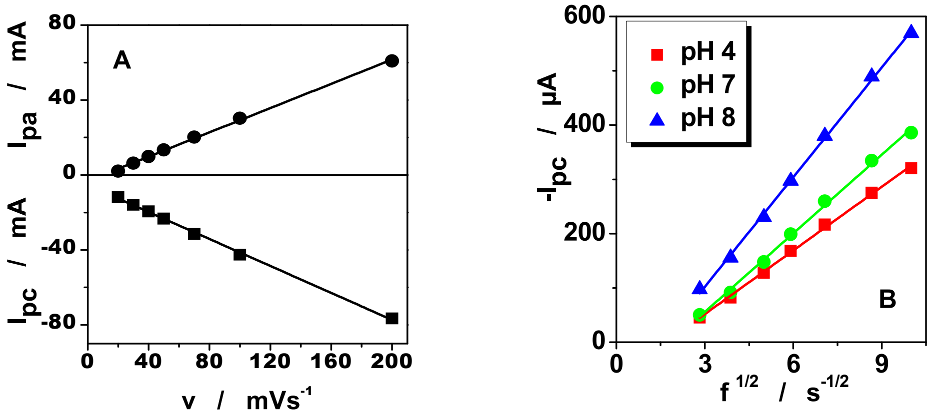


Figure 2. The I_p *vs.* potential scan rate (**A**) and I_{pc} *vs.* $v^{½}$ (**B**) dependencies recorded at G/Hm modified electrode. Experimental conditions: see figure 1.

Both methods (CV and SWV) used to investigate the electrochemical behavior of the G/Hm modified electrode showed that, when the pH of the supporting electrolyte increases, a negative shift of the recorded cyclic voltammograms can be clearly observed (Figure 3).

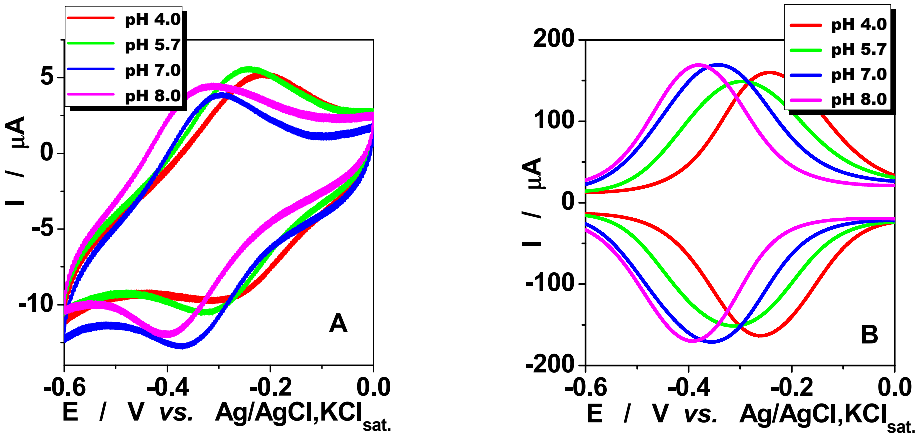


Figure 3. pH influence on the electrochemical response of G/Hm modified electrode recorded using CV (**A**) and SWV (**B**) measurements. Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer; starting potential, -0.6 V *vs.* Ag/AgCl,KCl$_{sat}$ (A) and 0 V *vs.* Ag/AgCl,KCl$_{sat}$ (B); scan rate, 20 mV/s (A); frequency 25 Hz (B); amplitude, 20 mV (B); deaerated solution (Ar).

The $E^{0'}$ values, estimated from the CV and SWV measurements illustrated in figures 3A and 3B, respectively, were found to depend linearly on the pH of supporting electrolyte (results not shown). However, in the pH range from 4 up to 8, the values of the slopes calculated for these dependencies [CV: (-0.041 ± 0.005) V/pH, with R = 0.962 and n = 4; SWV: (-0.033 ± 0.001) V/pH, with R = 0.99 and n = 4], do not agree with the theoretical value (0.059 V/pH) expected for a redox process involving the transfer of $1e^-/1H^+$. This peculiar behavior was already observed for hemin [14, 15] and was attributed to the protonation states of the *trans* ligands of the heme iron, combined with the protonation of the amino acids surrounding the heme or the protonation of the water molecule coordinated to the iron atom [16, 17, 18].

The stability of the modified electrode is a very important characteristic because it provides information on the electrode life-time, a decisive parameter for its future applications. For this reason, the short-time stability of G/Hm was evaluated by continuous cycling of the electrode potential when it was in contact with the supporting electrolyte (0.1 M PB, pH 7). Figure 4A shows qualitatively that for both cathodic and anodic peak currents no significant variation in time can be observed after 12 repetitive full potential scans.

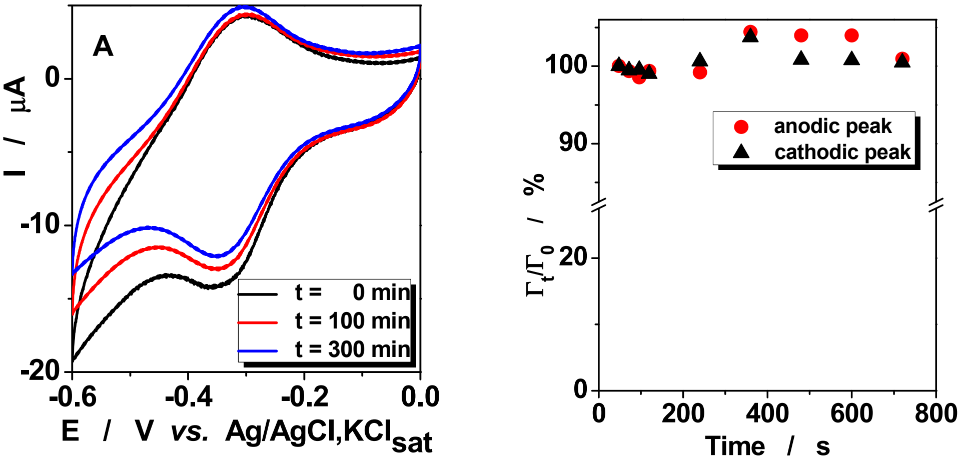


Figure 4. Repetitive CV measurements performed at G/Hm modified electrode (**A**) and the time dependence of the relative surface coverage (Γ_t/Γ_0) (**B**). Experimental conditions: supporting electrolyte, 0.1 M PB (pH 7.0); starting potential, -0.6 V *vs.* Ag/AgCl,KCl$_{sat.}$; scan rate, 50 mV/s; deaerated solution (Ar)

The variation in time of the electrode surface coverage [$\Gamma(\mathrm{mol/cm^2}) = Q/(nFA)$, where Q(Coulomb) is the amount of charge corresponding to the cathodic or anodic under-peak area, estimated after the background current correction; n(=1) is the number of electrons transferred during the redox process, which generates the voltammetric peak; F (Coulomb) is the Faraday's constant; A($\mathrm{cm^2}$) is the electrode geometric area] confirms that hemin is strongly adsorbed on the graphite surface (Figure 4B).

The tables 2-4 summarized the data concerning the variation in time of the surface coverage (Table 2) and the peak parameters (Tables 3 and 4) of the voltammetric response recorded at G/Hm modified electrode. Between measurements the G/Hm electrodes were stored at 4°C, in a water saturated atmosphere. All data prove the high stability of the G/Hm modified electrode. This behavior was related to the hemin insolubility under acidic and neutral conditions [19], associated with the strong π-π interaction between hemin and graphite [20].

Table 2. Short time stability of G/Hm modified electrode.

Time(s)	**Γ_c (nmol/cm^2)**	**Γ_a (nmol/cm^2)**	**$\Delta\Gamma_c$ (%)**	**$\Delta\Gamma_a$ (%)**
48	76.2	129	-	-
240	76.6	128	0.53	0.08
480	76.8	135	0.79	4.65
720	76.5	130	0.39	0.08

$\Delta\Gamma_c = 100(\Gamma_{c,t} - \Gamma_{c,t=48})/\Gamma_{c,t=48}$; $\Delta\Gamma_a = 100(\Gamma_{a,t} - \Gamma_{a,t=48})/\Gamma_{a,t=48}$

Table 3. Short time variation of the peak parameters for the voltammetric response of G/Hm modified electrode.

Time (s)	**E_{pc} (V*)**	**E_{pa} (V*)**	**ΔE_p (V)**	**$E^{0'}$ (V*)**	**I_{ap}/I_{cp}**
48	-0.346	-0.306	0.040	0.020	1.38
240	-0.348	-0.306	0.042	0.021	1.36
480	-0.348	-0.306	0.042	0.021	1.38
720	-0.348	-0.306	0.042	0.021	1.37

*E_{pc}, E_{pc} and $E^{0'}$ were measured *vs.* the Ag/AgCl,$\mathrm{KCl_{sat}}$ reference electrode

Table 4. Long time variation of the peak parameters for the voltammetric response of G/Hm modified electrode.

ΔI_{ap} (%)	**ΔI_{cp} (%)**	**$\Delta\Gamma_c$ (%)**	**$\Delta\Gamma_a$ (%)**	**ΔE_p (mV)**	**I_{ap}/I_{cp}**
7.6	2.4	6.5	9.3	23	1.15

where: $\Delta I_{cp} = 100(I_{cp,\,t=305} - I_{cp,\,t=0})/I_{cp,\,t=0}$; $\Delta I_{ap} = 100(I_{ap,\,t=305} - I_{ap,\,t=0})/I_{ap,\,t=0}$

$\Delta\Gamma_c = 100(\Gamma_{c,t=305} - \Gamma_{c,t=0})/\Gamma_{c,t=0}$; $\Delta\Gamma_a = 100(\Gamma_{a,t=305} - \Gamma_{a,t=0})/\Gamma_{a,t=0}$

Electrocatalytic behavior of G/Hm modified electrode

Taking into account that H_2O_2 is a product of the biochemical reactions catalyzed by oxidases, its detection is of considerable importance in clinical, food, pharmaceutical and environmental analysis [2, 21]. In this context, the electrocatalytic behavior of the G/Hm modified electrode was investigated for H_2O_2 electrocatalytic reduction by using CV and SWV measurements (Figure 6). As can be seen from figure 6 and table 6, the SWV measurements provided better analytical and kinetic parameters than those obtained from CV measurements. Probably, this difference is due to the higher resolution of SWV technique, associated with the easier and better correction for the background current in the case of SWV measurements.

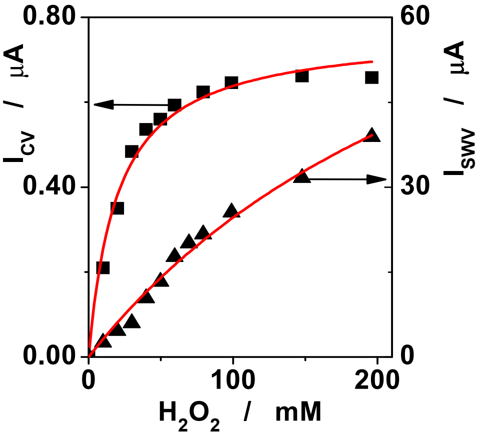


Figure 6. Calibration curve of the G/Hm modified electrode for H_2O_2 detection using CV and SWV measurements. Experimental conditions: supporting electrolyte, 0.1 M PB (pH 7.0); starting potential, -0.6 V *vs.* Ag/AgCl,KCl$_{sat.}$(CV), 0 mV *vs.* Ag/AgCl,KCl$_{sat.}$ (SWV); scan rate, 20 mV/s; amplitude, 20 mV; frequency, 25 Hz; deaerated solution (Ar).

Table 5. Analytical and kinetic parameters of G/Hm modified electrode.

Method	**CV**	**SWV**
Parameters	Michaelis-Menten fitting	
I_{max} (μA)	0.77 ± 0.02	101.4 ± 16.1
K_M^{app} (mM)	20.2 ± 2.5	311.6 ± 68.3
Sensitivity (mA/M)	0.038	0.325
R / N	0.9836 / 11	0.9861 / 12
	Linear fitting	
Slope (A/mM)	16.0 ± 1.3	276.3 ± 11.9
Detection limit /mM	0.011	0.012
Linear range (mM)	10 - 40	10 - 80
R / N	R = 0.979 / n = 5	R = 0.993 / n = 9

***Sensitivity = I_{max}/K_M^{app}**

CONCLUSIONS

This work describes a simple and reproducible way to prepare a modified electrode for H_2O_2 detection, based on the hemin adsorption on the graphite surface. The electrochemical characterization of the G/Hm electrode, performed by using CV and SWV measurements, allowed establishing the influence of the experimental conditions (scan rate, pH and duration of use) on the electrode performances. Additionally, it was confirmed that the immobilized hemin is involved in a quasi-reversible $1e^-/1H^+$ redox process, with the features of a surface confined species. The electrocatalytic behavior of G/Hm modified electrode for H_2O_2 electroreduction recommends it as a promising transducer for sensors and biosensors construction.

EXPERIMENTAL SECTION

Reagents

Hemin, iron (III) protoporphyrin (IX) chloride (Hm), tris(hydroxymethyl) aminomethane (TRIS) and H_2O_2 (30%) were purchased from Fluka, Sigma and Merck, respectively. A stock solution of 5 mM Hm was prepared by dissolving the appropriate amount of salt in 0.05 M TRIS chloride buffer (pH 10.5). The 0.1 M phosphate buffer solution (PB, pH 8.0) was prepared from 0.05 M KH_2PO_4 and 0.05 M K_2HPO_4 (Sigma). The pH of PB was adjusted by using HCl and NaOH (Reactivul-Bucharest). Deionized water was used for preparing all solutions.

Equipments

All electrochemical measurements were carried out using a computer controlled voltammetric analyzer (Autolab-PGSTAT 10 EcoChemie, The Netherlands).

A standard single-compartment three electrode cell was equipped with a Pt counter electrode, a Ag/AgCl,KCl_{sat} reference electrode (Radiometer, France), and the working electrode made of spectral graphite (Ringsdorff-Werke Gmbh, Bonn-Bad Godesberg, Germany).

In order to remove the dissolved oxygen, highly purified argon gas was purged into the working solution for at least 15 minutes prior to the experiment. Additionally, the argon flow was kept over the solution during all time of measurements. All experiments were performed at room temperature (25 ± 2 °C).

A combined glass electrode connected to a digital pH meter (Hanna Instruments HI 1230) was used for the pH measurements.

Preparation of G/Hm modified electrode

Before Hm deposition, the graphite disc electrode (3 mm diameter) was polished by using wet emery paper (320 and P1200C grit), until a smooth surface was obtained. Then, the electrodes were ultrasonicated for 2 minutes. The cleanness of the graphite electrode surface was validated by performing CV measurements in 0.1 M PB (pH 7), between -0.6 and 0 V *vs.* Ag/ AgCl,KCl_{sat} and using a scan rate of 20 mV/s.

The G/Hm modified electrode was prepared by dropping 5 μL of 5 mM Hm solution on the graphite surface. Further, the electrode was dried in air by keeping it during the night. Between measurements, the modified electrodes were at 4 °C.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from ID_PCCE_ 129/2008 (NANOBIOFUN) grant.

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