

*Dedicated to prof. dr. I. C. Popescu
on the occasion of his 70th anniversary*

CHARACTERIZATION OF A MODIFIED GRAPHITE ELECTRODE OBTAINED BY HEMIN ELECTROPOLYMERISATION

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ABSTRACT. A new modified electrode (G/polyHm) was obtained by electropolymerisation of iron (III) protoporphyrin (IX) (hemin, Hm) at the surface of a graphite electrode (G), by repetitive scanning the potential between -0.85 and 0 V vs. Ag/AgCl, KCl_{sat}. The cathodic peak revealed by linear sweep voltammetry at the electrode in phosphate buffer, pH 8, corresponds to one electron transfer process occurring at the Hemin(Fe³⁺)/ Hemin(Fe²⁺) metallic center of the immobilized hemin. All the estimated electrochemical parameters are in accordance with the values reported for immobilized hemin. The electrode exhibits electrocatalytic properties towards the H₂O₂ and NO₂⁻ reduction, respectively, proving an enzyme-like behaviour of the immobilised hemin, characterised by hyperbolic Michaelis Menten behaviour, when increasing concentrations of substrate were added, irrespective of the scan rate used.

Keywords: *iron (III) protoporphyrin (IX), linear sweep voltammetry, hydrogen peroxide, nitrite*

INTRODUCTION

Over the past several decades, the greatest interest of the electrochemical investigation techniques is to use a tailor made chemically modified electrode (CME) for the determination of organic and inorganic compounds in sensitive and selective analytical applications [1-3]. According to IUPAC definition [4], the distinguishing feature of a CME is that a generally quite thin ionic or polymeric film of a chemical modifier is bonded to or

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coated on the surface of an electrode made of a conducting or semiconducting material. This endows the electrode, with the specific and/ or desirable properties of the film in a rational, chemically designed manner. CME can be obtained by (1) chemisorption, (2) covalent bonding, (3) polymer film coating, or (4) composite mixture. Polymer film-coated electrodes may be further subdivided by the process type used to apply the film: (i) dip-coating, (ii) solvent evaporation, (iii) spin coating, (iv) electrochemical deposition, (v) electrochemical polymerization, (vi) radiofrequency polymerization, (vii) cross-linking. There are several reasons for the use of polymer modification derived from the relevant polymer-film properties: (a) the stability (consisting in the multi-layer resistance to degradation compared with the monolayers), (b) the permeability of various species to penetrate the polymer matrix, (c) the site population measured by the concentration of some designated chemical sites within the polymer film (e.g., the electrochemical responses of the multiple layers of redox sites are larger) and (d) the conductivity of the polymer film which may transport electrons (propagate charge) by localized site-site hopping (electron self-exchange) or by an electron delocalization mechanism, when highly conjugated polymer chains are present. Due to the lack of sufficient knowledge regarding the structure and properties of polymer films and the morphological changes that they undergo when subjected to various chemical, electrochemical and physical processes the mechanism of mass transport and electron transfer is not completely explained [4, 5].

In order to exert more direct control over the chemical architecture of an electrode surface/interface, research in the development of CMEs prepared by electropolymerization, offers a unique strategy to obtain homogeneous and reproducible immobilization of a redox modifier with various degrees of electrochemical activity onto electrode surface, a strong adherence to electrode surface, and a chemical stability of the film [6-8].

Used in numerous electronic and electrochemical devices, metalloporphyrins derivatives are ubiquitous organometallic compounds (containing Mn [9], Fe [2, 9,10], Ni [11]) having attractive physico-chemical properties due to their ability to vary their oxidation states without changing their molecular structure. The electrochemical activity towards a given reaction is primarily monitored by the metal center coordinated to the porphyrin ring as well as, to some extent, by the substituents of the porphyrin molecule [2].

As all iron derivatives, iron protoporphyrin IX (hemin, Hm) uses as electron source the reversible Hemin(Fe³⁺)/Hemin(Fe²⁺) redox couple and shows strong electrocatalytic properties in the presence of many small molecules such as hydrogen peroxide [10, 12-14], oxygen [12], nitrite [10, 12, 13, 15], nitric oxide [2, 9, 10, 12], l-tyrosine [12], organohalides [9], tryptophan and its derivatives [9], and for superoxide [9].

Literature presents hemin coated electrode obtained either by electropolymerisation of metalloporphyrins derivatives [2, 9] or by various methods mentioned above. In terms of enhanced sensitivity, selectivity and stability of the fabricated electrodes when detecting the same analyte, the observed differences, can be attributed to the differences between the fabrication methods [16].

The aim of this paper is to investigate the electrocatalytic and analytical performances of a hemin thin film obtained by electropolymerization on a graphite electrode (G/polyHm), for the catalytic reduction of hydrogen peroxide and nitrite, using linear sweep voltammetry as investigation technique.

RESULTS AND DISCUSSION

Electrochemical characterization of the G/polyHm electrode

Figure 1 shows a typical cyclic voltammogram of 0.5 mM hemin in 0.05 M TRIS buffer + 0.1M KBr (pH 8) as supporting electrolyte on bare G electrode at a scan rate of 0.05 V s^{-1} . The quasi-reversible redox peak (I_a/I_c) pairs of hemin recorded at ca. -0.43 V vs. Ag/AgCl, KCl_{sat} corresponds to the Hemin(Fe^{3+})/Hemin(Fe^{2+}) redox couple.

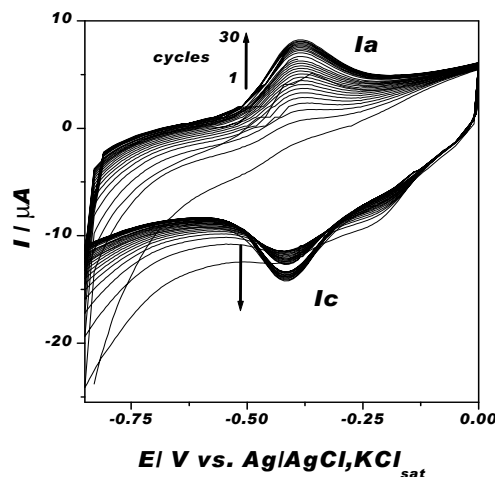


Figure 1. Preparation of G/polyHm modified electrode by cyclic voltammetry. Experimental conditions: 0.5 mM Hm in 0.05 M TRIS buffer + 0.1 M KBr, pH 8; starting potential, -0.85 V vs. Ag/AgCl, KCl_{sat} ; scan rate, 0.05 V s^{-1} ; number of cycles, 30; deaerated solution (with N_2).

Repetitive scanning in the reduction potential window results in the electrodeposition and growth of a hemin-based electroactive film, as evidenced by the gradual increase in the peak currents. The electroactive film obtained by electropolymerization (G/polyHm) appears as a stable, deeply colored thin film.

Similar current increase was reported in literature for the electropolymerization in the oxidative window of hemin derivatives [9, 17].

Comparing the electrochemical behavior of G and G/polyHm in phosphate buffer, at 0.005 V s^{-1} , the last electrode emphasizes a reduction peak corresponding to the single electron transfer into the metallic center Hemin(Fe^{3+})/Hemin(Fe^{2+}) of the immobilized hemin (Figure 2).

The electrochemical parameters of the G/polyHm modified electrode (Figure 2) are $E_{pc} = -0.45 \text{ vs. Ag/AgCl, KCl}_{sat}$, $I_{pc} = 5.76 \mu\text{A}$ at 0.05 V s^{-1} , parameters which are in accordance with those reported in literature for immobilized hemin using other techniques [13-15].

As expected for a surface confined redox couple [18], in the scan rates ranging from $0.005 - 0.100 \text{ V s}^{-1}$ the peak currents (I_{pc}) increase linearly with the scan rate (v) and not with $v^{1/2}$ (Figure 2, inset). The slope of the dependency: $\log I = (1.6 \pm 0.02) + (0.65 \pm 0.01)\log v$, $R^2 = 0.9994$, $n = 5$ points, is considered a relevant criterion helping to distinguish between the adsorbed (slope ~ 1) or dissolved (slope ~ 0.5) redox couples, confirming the slow adsorption of the hemin on the electrode surface.

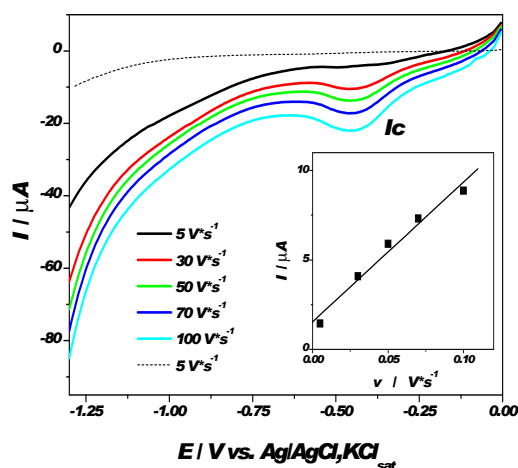


Figure 2. Voltammetric response of G (thin dash line) and G/polyHm (thick lines) electrode at different scan rate. Inset: I vs. v dependence. Experimental conditions: electrolyte, 0.05 M phosphate buffer + 0.1 M KBr, pH 8; starting potential, 0 V vs. $\text{Ag/AgCl, KCl}_{sat}$; deaerated solution (with N_2).

The electrocatalytic behavior of G/polyHm electrode Case of hydrogen peroxide.

It is well-known that hydrogen peroxide undergoes peroxidase-mediated reduction *via* both mediated electrochemical reaction and direct electron transport at a potential close to 0.0 V vs. Ag/AgCl or at the mediator redox potential. As consequence, detection of hydrogen peroxide *via* peroxidase-catalyzed reaction is preferred, and many biosensors involving the participation of hydrogen peroxide have been developed based on this reaction scheme [19]. Detailed investigation on the electrocatalytic reduction of H_2O_2 at the surface of bare G and G/polyHm were made, as shown in Figure 3A and 3B, respectively.

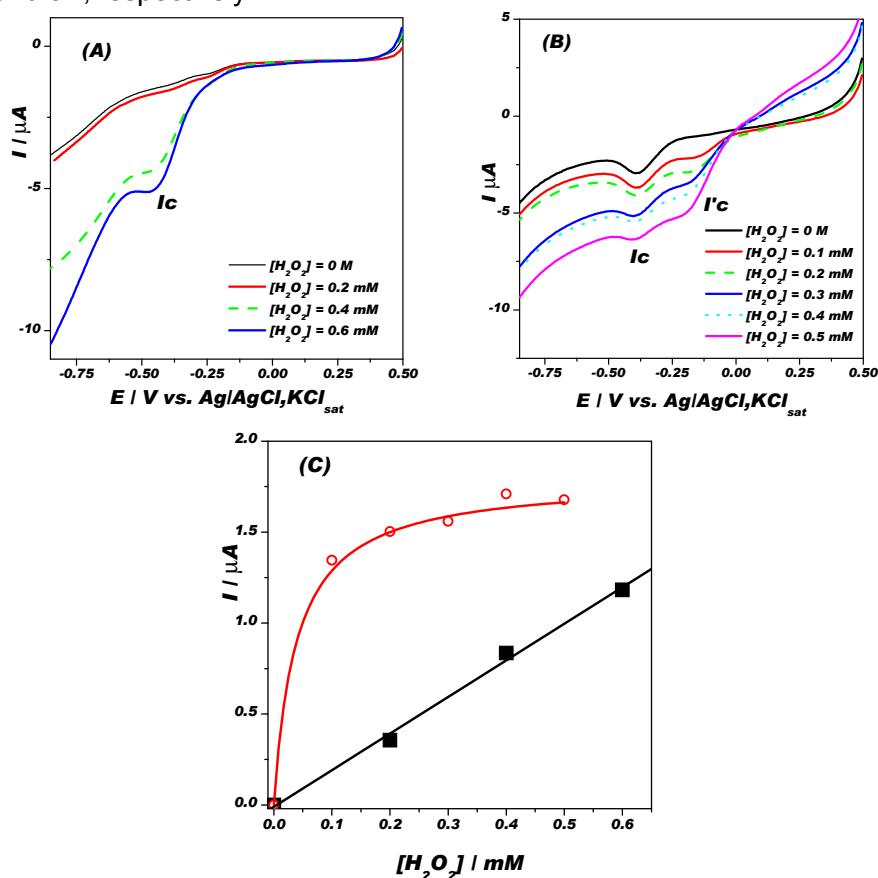
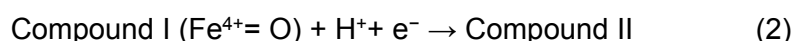


Figure 3. Linear scan voltammograms at G (A, ■) and G/polyHm (B, ●) modified electrode for different concentrations of H_2O_2 and the corresponding calibration curves for peak I_c (C). Experimental conditions: electrolyte, 0.05 M phosphate buffer + 0.1 M KBr, pH 8; scan rate, $0.02 V s^{-1}$; starting potential, +0.5 V vs. $Ag/AgCl, KCl_{sat}$; deaerated solution (with N_2).

As can be seen, the augmentation of the current intensity maybe attributed to the excellent catalytic activity of hemin present on the electrode surface. Comparing with the bare G electrode where the H_2O_2 reduction occurs in one step, at G/polyHm modified electrode a two steps mechanism is observed (peaks I_c, I'_c). The following schemes describe the simplified and possible mechanism for the electrochemical catalytic reaction [20]:



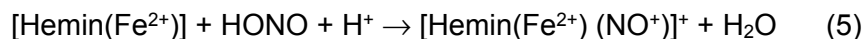
The analytical parameters of the detection of H_2O_2 at bare G electrode were: sensibility (slope of the calibration curve Figure 3C) is 2.15 ± 0.44 mA/M ($R^2 = 0.9897$, $n = 4$), linear range from 0.1 mM to 0.6 mM, with a detection limit of 0.1 mM. Also, for the detection of hydrogen peroxide *via* enzyme-like catalyzed reaction, the response of the G/polyHm modified electrode follows a Michaelis Menten hyperbolic mechanism having the following kinetic parameters: $K_{M,app} = 0.04 \pm 0.01$ mM and $I_{max} = 1.8 \pm 0.2$ μA , ($R^2 = 0.9968$, $\chi^2 = 0.0017$, $n = 6$). In this case, the sensibility (calculated as $I_{max}/K_{M,app}$ ratio) is 45.5 ± 0.2 mA/M, which is higher than the value obtained for the bare G, proving the electrocatalytic behavior of immobilized hemin. As expected the detection limit for G/polyHm modified electrode is lower, 0.05 mM (Figure 3C). The obtained values are also in accordance with the literature data [13, 14].

Case of nitrite

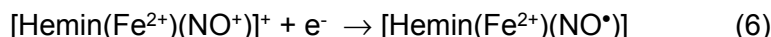
The electrocatalytic reduction of nitrite was also studied at both G bare and G/polyHm electrode. The Figures 4A-B show the cyclic voltammograms in phosphate buffer (pH 8.0) upon addition of increasing concentrations of NaNO_2 . In the reduction potential window, the nitrite is not electroactive, therefore no peak is shown when the hemin is absent (Figure 4A). At low enough scan rate values, in the presence of immobilised Hm (Figure 4B) the reduction mechanism consists of two steps.

In agreement with the literature data, several sequential reduction pathways have been proposed [13, 15, 21, 22]. As example, the first step of the catalytic process involves the formation, in neutral aqueous solution, of an iron-nitrosyl complex as the consequence of the metal-centered $\text{Hemin}(\text{Fe}^{3+})/\text{Hemin}(\text{Fe}^{2+})$ reduction, followed by nitrite binding (peak II_c placed at about -0.7 V vs. Ag/AgCl, KCl_{sat}), according to equations 4-5:





These steps are then followed by the reduction of the iron-nitrosyl adduct (peak IIIc at about -0.9 V vs. Ag/AgCl, KCl_{sat}) according to equation 6:



Also, it is worth to mention that no clear direct evidence regarding the presumed above-cited reduction process and no accurate overall catalytic process were established in literature [15, 21].

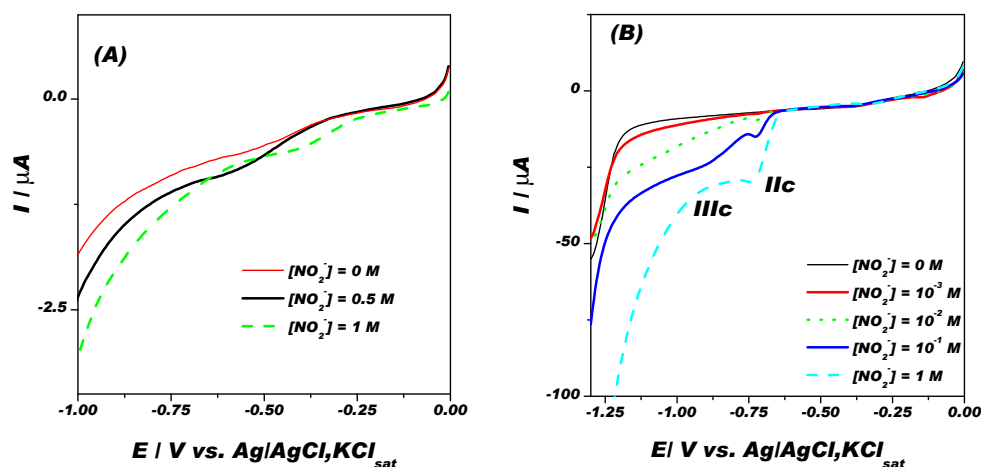


Figure 4. Linear scan voltammograms at G (A) and G/polyHm (B) modified electrode for different concentrations of NO_2^- . Experimental conditions: electrolyte, 0.05 M phosphate buffer + 0.1 M KBr, pH 8; scan rate, 0.02 V s^{-1} ; starting potential, 0 V vs. Ag/AgCl, KCl_{sat} ; deaerated solution (with N_2).

At different scan rates, the behavior of the G/polyHm obeys a Michaelis-Menten hyperbolic equation (Figure 5), having the kinetic parameters summarized in Table 1.

As expected, the sensibility to nitrite detection of the G/polyHm electrode increases with the increase of the scan rate. However, a scan rate of 0.02 V s^{-1} is appropriate to evidence the intermediates of the two reduction steps.

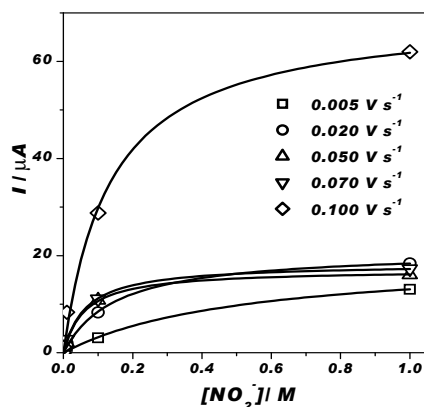


Figure 5. Calibration curve for G/polyHm modified electrode for different scan rate. Experimental conditions: see Figure 4.

Table 1. Kinetic parameters for the detection of NO_2^- at G/polyHm modified electrode. Experimental conditions: see Figure 5.

$v / \text{V s}^{-1}$	$K_{M, \text{app}} / \text{M}$	$I_{\text{max}} / \mu\text{A}$	$S^* / \mu\text{A/M}$	R / n
0.005	0.52 ± 0.180	19.85 ± 2.74	38.30 ± 0.62	0.9961 / 5
0.020	0.15 ± 0.010	21.10 ± 0.63	142.18 ± 0.22	0.9990 / 5
0.050	0.06 ± 0.004	17.15 ± 0.28	281.82 ± 0.28	0.9996 / 5
0.070	0.06 ± 0.003	18.34 ± 0.20	287.16 ± 0.34	0.9998 / 5
0.100	0.13 ± 0.020	70.09 ± 3.29	519.82 ± 0.58	0.9975 / 5

*sensitivity calculated as $I_{\text{max}}/K_{M, \text{app}}$ ratio.

CONCLUSIONS

A new modified electrode (G/polyHm) was obtained by electropolymerisation of iron (III) protoporphyrin (IX) at the surface of a graphite electrode, by continuous scanning in the reduction potential window ranging between -0.85 to 0 V vs. Ag/AgCl, KCl_{sat}. The recorded redox process corresponds to a single electron transfer process into the metallic center Hemin(Fe^{3+})/Hemin(Fe^{2+}) of the immobilized hemin on the new electrode, and was compared with the electrochemical behavior in phosphate buffer pH 8 of bare graphite (this work) or with other modified electrode (in cited literature). Also, the influence of the scan rate on the linear sweep voltammograms recorded at the electrode confirms the adsorption of hemin on the electrode surface (slope of $\log I_{\text{pc}}$ vs. $\log v$ higher than 0.5).

The electrode exhibits electrocatalytic properties towards the H_2O_2 and NO_2^- reduction, respectively, proving an enzyme-like behaviour of the immobilised hemin, characterised by hyperbolic Michaelis Menten behaviour, when increasing concentrations of substrate were added.

EXPERIMENTAL SECTION

Reagents

Hemin, iron (III) protoporphyrin (IX) chloride (Hm), tris (hydroxymethyl) aminomethane (TRIS), sodium nitrite and hydrogen peroxyde (30%) were purchased from Fluka, Sigma–Aldrich GmbH and Merck, respectively. A stock solution of 0.5 mM Hm was prepared by dissolving the appropriate amount of salt in 0.05 M TRIS chloride containing 0.1 M KBr buffer (pH 10.5). The 0.05 M phosphate buffer solution containing 0.1 M KBr (pH 8.0) was prepared from appropriate amounts of KH_2PO_4 and K_2HPO_4 (supplied by Sigma–Aldrich GmbH). The pH of used electrolytes was adjusted by using H_3PO_4 and NaOH (Sigma–Aldrich GmbH). Deionized water was used for preparing all solutions.

Equipments

All electrochemical measurements were carried out using a computer controlled AMEL 433 trace analyzer (AMEL, Milan, Italy).

The standard uncompartament cell was equipped with a Pt counter electrode, a $\text{Ag}/\text{AgCl}, \text{KCl}_{\text{sat}}$ reference electrode (Radiometer, France), and a working electrode made of spectral graphite (Ringsdorff-Werke GmbH, Bonn-Bad Godesberg, Germany) (diameter 0.3 cm). In order to remove the dissolved oxygen, highly purified nitrogen gas was purged into the working solution for at least 15 minutes prior to the experiment. During the measurements, the oxygen-free atmosphere was maintained using a continuous N_2 flow over the solution. All experiments were performed at room temperature (25 ± 2 °C). A combined type pH glass electrode connected to a digital pH meter type AMEL 338 (AMEL, Milan, Italy) was used for the pH measurements.

Preparation of G/poly-Hm modified electrode

Before Hm deposition, the graphite disc electrode was mirror-polished with 1.0, 0.3, 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ paste (Buehler, Evanston, IL, USA). Then, the electrodes were ultrasonicated for 2 minutes. The cleanness of the graphite electrode surface was checked by performing cyclic voltammograms in 0.05 M phosphate buffer + 0.1 M KBr (pH 8), between -0.6 and 0.6 V vs. $\text{Ag}/\text{AgCl}, \text{KCl}_{\text{sat}}$ at 20 mV/s.

The thin film of hemin was electropolymerized on the graphite surface (G/polyHm) from a 0.5 mM hemin dissolved in a mixture of 0.05 M TRIS chloride containing 0.1 M KBr buffer (pH 10.5). The graphite electrode was immersed in the hemin solution and continuously cycled between -0.85 V and 0 V vs. Ag/AgCl, KCl_{sat} in oxygen-free atmosphere, at 0.05 V s⁻¹, for 30 cycles. After each modification, the electrode was thoroughly rinsed with distilled water to remove excesses. Between measurements, the modified electrodes were kept at 4 °C.

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