

# Electropolymerized Architecture Entrapping a Trilacunary Keggin-Type Polyoxometalate for Assembling a Glucose Biosensor

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## Abstract

A new design to obtain an amperometric glucose biosensor based on glucose oxidase (GOx) immobilization on an electropolymerized matrix [poly (1,8 DAN)] entrapping a trilacunary Keggin type polyoxometalate (Fe<sub>4</sub>POM) deposited on a graphite electrode was proposed. Some experimental evidences suggest that the modified conducting polymeric matrix consists in a multilayer architecture, formed from positively charged conductive poly (1,8 DAN) alternating with the negatively charged (Fe<sub>4</sub>POM). At an applied potential of  $-0.04$  V (vs. Ag/AgCl, KCl<sub>sat</sub>), the batch amperometric response to glucose of the G/Fe<sub>4</sub>POM-poly(1,8 DAN)/GOx modified electrode is characterized by a sensitivity of  $19.8 \pm 2.4 \mu\text{A M}^{-1} \text{cm}^{-2}$ , a linear range between 2.5 to 20 mM glucose, a response time less than 40 s and a detection limit of 1.2 mM glucose (for S/N = 3).

**Keywords:** Heteropolyoxometalates, Conducting polymer, Glucose, Amperometric biosensor

## 1. Introduction

The catalytic redox activity of polyoxometalates has attracted much attention in recent years. Heteropolyanions and especially their metal-substituted derivatives have some very useful and interesting properties. Such features include the high stability of most of their redox states, the possibility to tune their redox potential by changing the heteroions and/or the addends ions without affecting their structure, the variability of the transition metal cations which can be incorporated into the heteropolymetalates structure, and the possibility of multiple electron transfer. These properties make heteropolyanions attractive as redox catalysts (mediator) for indirect electrochemical processes [1].

The electrocatalysis is very important for the development of both preparative electrolysis and electrochemical sensors. A large number of organic and inorganic compounds and metal complexes including biomolecules have been successfully used as electrocatalysts. However, the number of highly selective and long-time stable redox catalysts is still limited. Therefore, heteropolyanions and their transition metal-substituted derivatives have the potential to fill this gap.

The polyoxometalates can be attached onto the electrode by using an interaction between the polyoxometalates and the electrode surface. There are three methods commonly used to immobilize them. The first method is the adsorption of polyoxometalate on the electrodes by dip coating [1, 2]. The second is the electrodeposition of polyoxometalates on the electrode surface from a polyoxometalate solution

under constant potential [1]. The third method is to entrap polyoxometalates into polymers deposited onto the electrode surface. This entrapment can be carried out in a stable way by two methods [1, 3, 4]. First, the polyoxometalate is incorporated by electrostatic interactions into a polymer (two step method). Such a polymer has been already prepared on the electrode surface, for example by spin coating or electropolymerization. Then, the polymer-coated electrodes are soaked in the solution containing the electrocatalysts. Second, polyoxometalates are immobilized during the electropolymerization procedure (one step method), in other words the oxidative polymerization is carried out in the presence of the polyoxometalates. The last method seems to be very promising and it is a simple way to assemble modified electrode and/or sensors, because:

- The interaction between polymers and the POM is stronger than the interaction between the polymers and the small counter ions of the electrolyte, therefore POMs are efficiently immobilized in the polymer matrix.
- Generally, the electrochemical behavior of the heteropolyanions incorporated in the films is the same as that of the heteropolyanions in solution.
- A better electrochemical control of the catalysts concentration can be performed.

Few investigations on the electrochemical behavior of free [5] or immobilized [6] Fe<sub>4</sub>-POM have been reported, as well of its electrocatalytic activity vs. H<sub>2</sub>O<sub>2</sub>, and no data have been published about its use in assembling biosensors.

In this article, we report on the electrochemical and electrocatalytic behavior of  $\text{Fe}_4\text{POM}$  immobilized on graphite electrodes in a conducting polymer such as poly(1,8 DAN). In order to optimize the construction of G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN)-modified electrode, its voltammetric response stability was investigated for different conditions of electropolymerization. Using the G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN) ability to detect amperometrically  $\text{H}_2\text{O}_2$ , at a low value of the applied potential ( $-0.04$  V vs. Ag/AgCl,  $\text{KCl}_{\text{sat}}$ ), for the first time to our knowledge a glucose amperometric biosensor, assembled by glucose oxidase (GOx) immobilization onto an electropolymerized matrix entrapping a polyoxometalate ( $\text{Fe}_4\text{POM}$ ) is described. Finally, the resulting glucose amperometric biosensor has been fully characterized in terms of detection limit, linear range, response time and stability.

## 2. Experimental

### 2.1. Reagents

The trilacunary Keggin type  $\text{Na}_6[\text{H}_4\text{Fe}_4(\text{PMo}_9\text{O}_{34})_2(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$  ( $\text{Fe}_4\text{POM}$ ) polyoxometalate was prepared by a previously published procedure [5]. The monomer 1,8-diaminonaphthalene (1,8 DAN) was purchased from Fluka Buchs, Switzerland and hydrogen peroxide (30% w/v solution) was from J. T. Baker. A 0.4 M  $\text{Na}_2\text{SO}_4$  (pH 2.4) (Fluka Buchs, Switzerland) and 0.2 M NaCl (Carlo Erba, Italy) solutions were used as the supporting electrolytes. A phosphate buffer solution (pH 6.5) was prepared from a 0.1  $\text{KH}_2\text{PO}_4$  (Fluka Buchs, Switzerland) solution by adjusting the pH with  $\text{H}_3\text{PO}_4$ .

Glucose oxidase (GOx) (E.C. 1.1.3.1, type VII, purified from *Aspergillus niger*; Sigma Chemical Co, St. Louis, Mo, USA), D(+) glucose (Carlo Erba, Italy), albumin from bovine serum (BSA) (Fluka Buchs, Switzerland) and 25% aqueous solution of glutaraldehyde (GA) (Sigma Chemical Co, St. Louis, Mo, USA) were used for assembling the biosensor.

All chemicals were analytical grade and were used without further purification. Solutions were prepared with distilled-deionized water. For pH adjustments, pellets of NaOH and  $\text{H}_2\text{SO}_4$  or HCl solution (Carlo Erba, Italy) have been used. The buffer solutions were kept refrigerated to minimize bacterial growth. All stock solutions were prepared daily.

### 2.2. Apparatus

Voltammetric measurement and electropolymerization of 1,8 DAN were carried out on an AMEL 433 trace analyzer (AMEL, Milan, Italy).

Batch amperometric measurements were performed using a potentiostat AMEL Model 559 (AMEL, Milan, Italy) coupled to an  $X-t$  recorder (LKB Bromma 2210

Recorder, AMEL, Milan, Italy). A magnetic stirrer and bar provide the convective transport.

All measurements were done using a conventional single-compartment three electrode system consisting of a platinum wire counter electrode, an Ag/AgCl,  $\text{KCl}_{\text{sat}}$  reference electrode and a graphite (2.5 mm diameter) (Ringsdorff-Werke, GmbH, Bonn-Bad Godesberg, Germany) working electrode. All experiments were done at room temperature.

### 2.3. Preparation of Modified Electrode

Before the 1,8 DAN electropolymerization, the graphite electrode was cleaned first with diamond paper, followed by successively polishing with 1.0, 0.3, 0.05  $\mu\text{m}$   $\alpha\text{-Al}_2\text{O}_3$  paste (Buehler, Evanston, IL, USA) and then sonicating in distilled water to remove any residual alumina.

The film was grown from an unstirred deaerated (15 min. with nitrogen) solution containing 5 mM 1,8 DAN and 1 mM  $\text{Fe}_4\text{POM}$  in 0.2 M NaCl (pH 1) by cycling the potential between  $-0.15$  and 1.3 V (vs. Ag/AgCl,  $\text{KCl}_{\text{sat}}$ ) at different scan rates (2–50 mV/s). The G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN) modified electrodes were thoroughly rinsed after preparation and stored at  $+4^\circ\text{C}$  to dry, when not in use.

### 2.4. Preparation of Glucose Biosensor

A typical G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN)/GOx glucose biosensor was prepared as follows. 200  $\mu\text{L}$  of a phosphate buffer solution (pH 6.5) containing 2 mg BSA and 500 U GOx were mixed with 2  $\mu\text{L}$  of 1% glutaraldehyde solution. 4  $\mu\text{L}$  of the resulting solution were deposited onto the G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN) modified electrode surface (avoiding air bubble formation), carefully spread out to cover completely the working surface and air-dried at room temperature. When not in use the enzyme electrodes were stored at  $+4^\circ\text{C}$ , to dry.

### 2.5. Electrochemical Measurements

The study of the immobilizing system and the electrochemical characterization of the modified G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN) electrode were done by cyclic voltammetry, performed at different potential scan rates, using as supporting electrolyte a 0.2 M NaCl solution (pH 1.1) and a 0.4 M  $\text{Na}_2\text{SO}_4$  (pH 2.4) solution, respectively.

The amperometric responses of the G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN) and G/ $\text{Fe}_4\text{POM}$ -poly(1,8 DAN)/GOx-modified electrodes to  $\text{H}_2\text{O}_2$  and glucose, respectively, were investigated in a stirred, air-saturated 0.1 M phosphate buffer solution (pH 6.5), by applying a potential of  $-0.04$  V (vs. Ag/AgCl,  $\text{KCl}_{\text{sat}}$ ). The background current was allowed to decay to a steady value, before aliquots of  $\text{H}_2\text{O}_2$  or glucose standard solutions were added and the steady-state reduction currents were recorded.

### 3. Results and Discussion

#### 3.1. Fe<sub>4</sub>POM -Poly(1,8 DAN) Modified Graphite Electrode

In the last few years, the electrochemical polymerization of 1,8 DAN has been studied extensively in non-aqueous solution because of its greater hydrophobicity [7, 8–12]. In these experimental conditions, at gold or platinum electrodes, 1,8 DAN is irreversibly electrooxidized forming a conducting polymer film. The process is accompanied by a gradual increasing potential shift of the oxidation peak, toward more positive values [8, 10].

Cyclic voltammetric measurements performed at graphite electrodes in the monomer presence (Fig. 1A) and after its removal (Fig. 1B) proved that the 1,8 DAN electropolymerization process can be achieved even in an aqueous solution. To be mentioned that, in order to increase the monomer solubility, low pH values have to be used.

As can be seen in Figure 1C, the simply presence of the Fe<sub>4</sub>POM in the 1,8 DAN solution, during the electrode potential cycling between  $-0.2$  and  $+1.3$  V (vs. Ag/AgCl, KCl<sub>sat</sub>), offered an easy way to obtain a POM-modified graphite electrode [G/Fe<sub>4</sub>POM-poly(1,8 DAN)].

It is noteworthy that the growth of the polymer film, proved by the gradual decrease of the A0 peak (assigned to

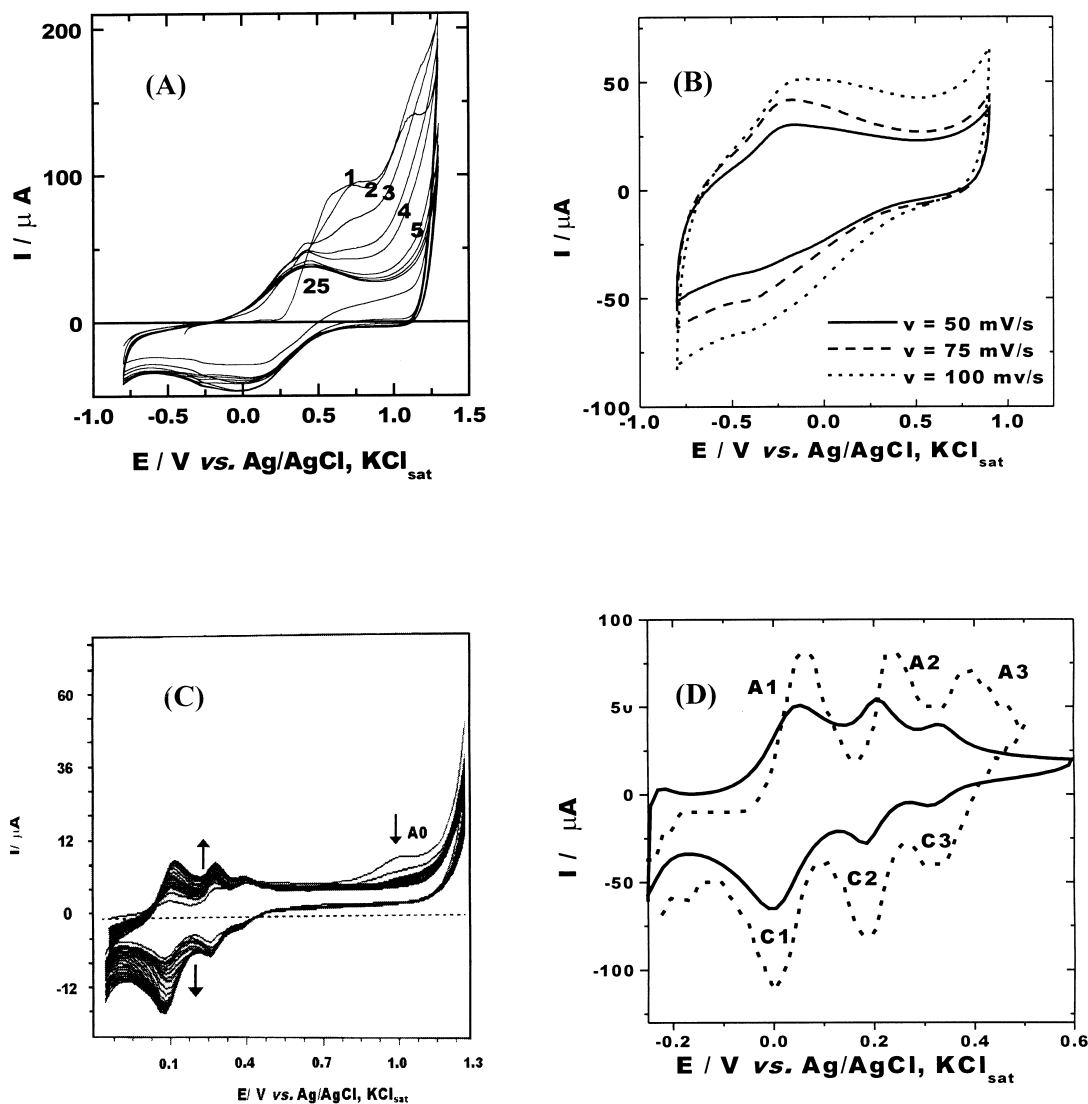


Fig. 1. A) 1,8 DAN electropolymerization by cyclic voltammetry performed at a graphite electrode. Experimental conditions: starting potential,  $-0.8$  V (vs. Ag/AgCl, KCl<sub>sat</sub>); potential scan rate, 25 mV/s; electropolymerization solution, 5 mM 1,8 DAN + 0.2 M NaCl (pH 1.1), deaerated with nitrogen. B) Voltammetric response of 1,8 DAN modified graphite electrode at different scan rate values. Experimental conditions: starting potential  $-0.8$  V (vs. Ag/AgCl, KCl<sub>sat</sub>); supporting electrolyte 0.1 M KNO<sub>3</sub> (pH 5). C) 1,8 DAN electropolymerization by cyclic voltammetry performed at a graphite electrode in the presence of Fe<sub>4</sub>POM. Experimental conditions: starting potential,  $-0.2$  V (vs. Ag/AgCl, KCl<sub>sat</sub>); potential scan rate, 10 mV/s; electropolymerization solution, 5 mM 1,8 DAN + 1 mM Fe<sub>4</sub>POM + 0.2 M NaCl (pH 1.1), deaerated with nitrogen. D) Voltammetric response of the Fe<sub>4</sub>POM-poly(1,8 DAN) modified graphite electrode (—) and of the free Fe<sub>4</sub>POM (---). Experimental conditions: starting potential,  $-0.2$  V (vs. Ag/AgCl, KCl<sub>sat</sub>); potential scan rate, 50 mV/s; supporting electrolyte, 0.4 M Na<sub>2</sub>SO<sub>4</sub>, (pH 2.5).

the monomer), is followed by the POM accumulation inside the polymer matrix (Fig. 1C). The augmentation of POM content inside the polymer film is confirmed by the progressively increase of A1, A2 and A3 peak intensities. Therefore, taking into account the conductivity of the poly(1,8 DAN) film [3, 8–10, 13], it is very probable that the POM-modified polymer matrix grows via a consecutive deposition of a bilayer structure formed from a layer of a conductive polymer and a layer of Fe<sub>4</sub>POM. The process is favored by the electrostatic interactions supposed to exist between the positively charged polymeric matrix (due to the low pH value during the electropolymerization) and the POM polyanion. Recently, similar interactions were used to obtain multilayer structures based on different POMs (i.e., Keggin-type SiMo<sub>11</sub>VO<sub>40</sub><sup>5-</sup> [14], Dawson-tye HPA [15]) and various electrically charged polymers (i.e., quarternized poly(4-vinylpyridine) complexed with osmium bis(2,2'-bipyridine) chloride (QPVP-Os) [14], polypyrrole, poly(*N*-methylpyrrole, poly(4-vinylpyridine) (PVP) [15])

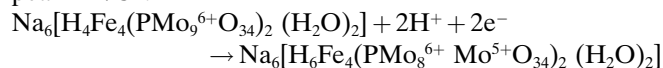
The voltammetric response as well as the stability of the free diffusing Fe<sub>4</sub>POM exhibited an optimum at pH 2.5 [6, 7]. Consequently, this pH value was used for all further electrochemical measurements performed for G/Fe<sub>4</sub>POM-poly(1,8 DAN)-modified electrode investigation.

As expected, the G/Fe<sub>4</sub>POM-poly(1,8 DAN) voltammetric response exhibited the three peaks corresponding to Fe<sub>4</sub>POM, but their current intensities are smaller than that those observed for the free Fe<sub>4</sub>POM (Fig. 1D). The negative shift of the peak potentials, observed for A1 to A3 pair of peaks in the presence of the poly(1,8 DAN) matrix, proves the existence of strong interactions between the negatively charged redox couple and the cationic sites of the polymer, as already reported for other similar redox polymeric matrix [16, 17].

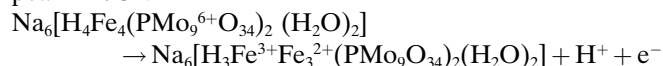
The peak potential splitting was found different from zero for all A1 to A3 pair of peaks (A1: 45 mV; A2: 20 mV; A3: 20 mV), indicating the existence of interactions between the surface confined redox couples, participating to a quasi-reversible electron transfer process [18, 19].

All experimental data were in agreement with the conclusions of our previous study, concerning the pH effect on the electrochemical behavior of free Fe<sub>4</sub>POM [6]. Thus, the following reactions were assigned for the redox processes corresponding to A1 to A3 peaks:

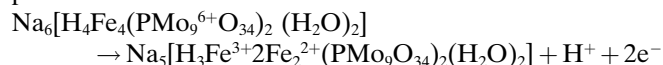
peak A1/C1:



peak A2/C2:



peak A3/C3:



In order to optimize the voltammetric response of G/Fe<sub>4</sub>POM-poly(1,8 DAN), the influence of the potential scan

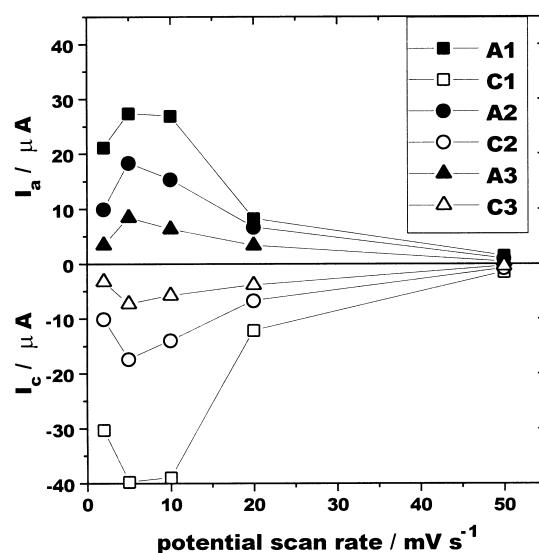


Fig. 2. The influence of the potential scan rate during the electropolymerization process on the voltammetric response of G/Fe<sub>4</sub>POM-poly(1,8 DAN) modified electrodes. Experimental conditions: for electropolymerization as in Figure 1C; for voltammetric response as in Figure 1D.

rate on the electropolymerization process was studied in the scan rate range from 2 up to 50 mV/s. A comparison between the A1 – A3 peaks current, measured for modified electrodes obtained in the same experimental conditions excepting the potential scan rate used during the electropolymerization (Fig. 2), pointed out that, with no exception, the maximum response was observed for scan rates close to 5–10 mV/s.

A long time stability test for G/Fe<sub>4</sub>POM-poly(1,8 DAN)-modified electrodes, obtained at different electropolymerization scan rate, showed that the electrode prepared at the scan rate of 10 mV/s exhibited the best behavior. Thus, after 25 days of dry storage at 4 °C, the modified electrode retained up to 50% from its initial response (Fig. 3). In all other cases, after 2–3 days of storage at 4 °C, a dramatically diminishing of the signal was observed (results not shown).

The influence of the potential scan rate on the peak current intensity for the G/Fe<sub>4</sub>POM-poly(1,8 DAN)-modified electrode (Table 1) allowed to formulate the following conclusions: i) irrespective the investigated peak, a devia-

Table 1. Influence of the potential scan rate on the voltammetric response of G/Fe<sub>4</sub>POM-poly(1,8 DAN).

Scan rate range (mV/s)	Slope of the log I vs. log v dependence Correlation coefficient (R)/ no. of experimental points (n)		
	A1/C1	A2/C2	A3/C3
5–50	0.51	0.85	0.85
	0.9989/5	0.9997/5	0.9998/5
50–500	0.67	0.72	0.82
	0.9996/5	0.9988/5	0.9994/5

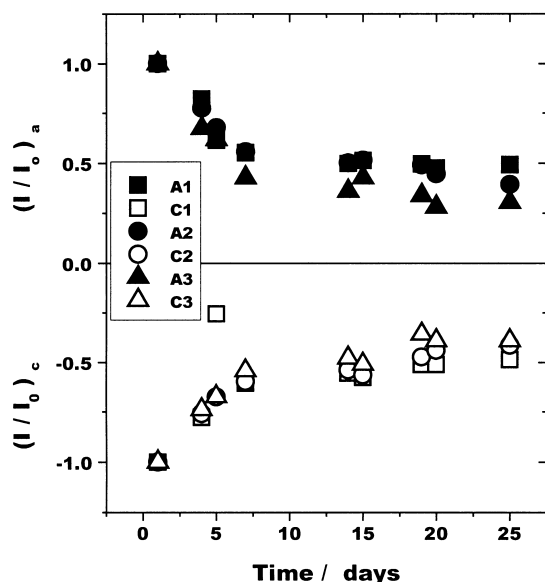
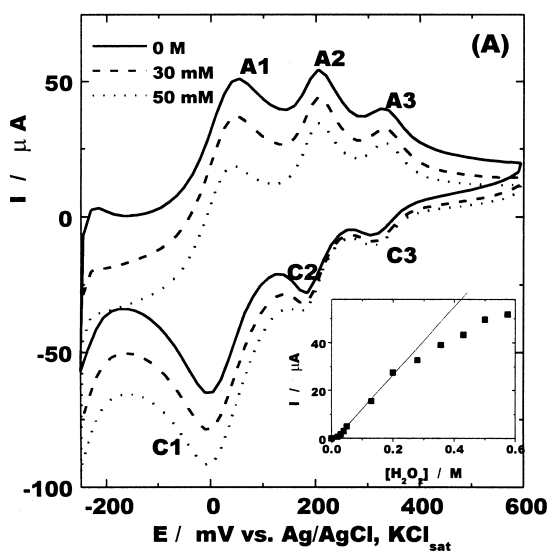


Fig. 3. Long time stability of the G/Fe<sub>4</sub>OM-poly(1,8 DAN) modified electrode. Experimental conditions: for electropolymerization as in Figure 1C; for voltammetric response as in Figure 1D; between measurements the electrode was stored at 4 °C.

tion from the expected value for the rate exponent, corresponding for a surface confined redox couple, was observed; ii) the deviation increased with the potential scan rate increase; iii) the A1/C1 peak showed a particular scan rate dependence, pointing rather to a diffusion controlled behavior. A possible explanation should take into consideration that in the charge transfer process the diffusion contribution increases with the potential scan rate increase.



This is a well-known phenomenon noticed for the multilayer redox architecture [19].

### 3.2. G/Fe<sub>4</sub>POM-Poly(1,8 DAN) as Amperometric Sensor for H<sub>2</sub>O<sub>2</sub>

Heteropolyoxometalates substituted with transition metals represent an attractive class of catalysts because of their ability to facilitate many useful reactions. Their immobilization on the surface of different conventional electrode materials gave rise to a large variety of modified electrodes, exhibiting an electrocatalytic activity depending on the nature of the transitional metal included into the heteropolyoxometalates structure. Thus, for H<sub>2</sub>O<sub>2</sub> amperometric detection were proposed modified electrodes based on: H<sub>2</sub>SiW<sub>12</sub>O<sub>40</sub> [20], Na<sub>12</sub>[Fe<sub>4</sub>(H<sub>2</sub>O)<sub>2</sub>(P<sub>2</sub>W<sub>15</sub>O<sub>56</sub>)<sub>2</sub>] · 58H<sub>2</sub>O [21], K<sub>4</sub>[PFeW<sub>11</sub>O<sub>39</sub>] [22], [H<sub>3</sub>(PMo<sub>12</sub>O<sub>40</sub>) · xH<sub>2</sub>O] [23].

Taking into account the well-known ability of Fe atoms to catalyze the H<sub>2</sub>O<sub>2</sub> reduction (i.e., Na<sub>12</sub>[Fe<sub>4</sub>(H<sub>2</sub>O)<sub>2</sub>(P<sub>2</sub>W<sub>15</sub>O<sub>56</sub>)<sub>2</sub>] · 58H<sub>2</sub>O [21], K<sub>4</sub>[PFeW<sub>11</sub>O<sub>39</sub>] [22]), cyclic voltammetry measurements at the G/Fe<sub>4</sub>POM-poly(1,8 DAN) – modified electrode, in absence and in presence of different concentrations of H<sub>2</sub>O<sub>2</sub>, were performed. As can be seen from Figure 4A a substantial electrocatalytic effect was identified. This effect is especially marked for A1/C1 peak pair, showing a linear range between 0.02 to 0.2 M H<sub>2</sub>O<sub>2</sub> (Fig. 4A, inset) and is characterized by a formal standard potential ( $\epsilon^{\circ} = 0.022$  V) well placed in the optimum potential range for amperometric detection [24]. The corresponding electrocatalytic efficiency (estimated as the ratio between the increase of the catalytic peak current in the presence of H<sub>2</sub>O<sub>2</sub> and the value of peak current in the

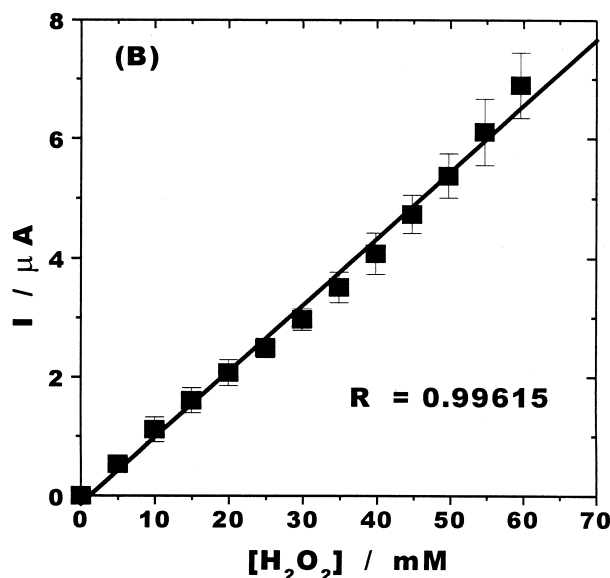


Fig. 4. A) Electrocatalytic activity of the G/Fe<sub>4</sub>POM-poly(1,8 DAN) modified electrode towards H<sub>2</sub>O<sub>2</sub> reduction. Insert: voltammetric calibration plot for H<sub>2</sub>O<sub>2</sub>. B) Amperometric calibration plot for H<sub>2</sub>O<sub>2</sub>. Error bars stand for standard deviation for three successive measurements. Experimental conditions: A) as in Figure 1D; B) applied potential –0.04 V (vs. Ag/AgCl, KCl<sub>sat</sub>); supporting electrolyte. 0.4 M Na<sub>2</sub>SO<sub>4</sub> (pH 2.5); stirred solution.

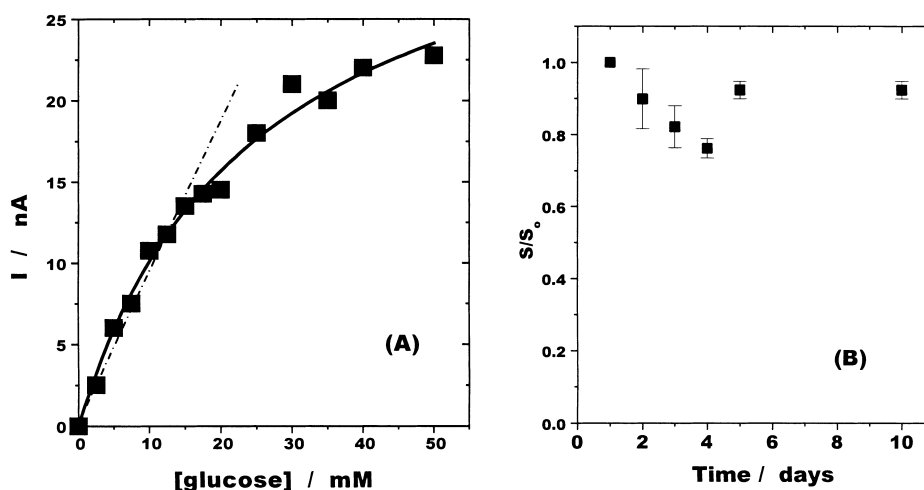


Fig. 5. A) Amperometric calibration plot for glucose at G/Fe<sub>4</sub>POM-poly(1,8 DAN)/GOx bioclectrode. Solid curve is the Michaelis-Menten fitting and dashed line is the linear range fitting of the experimental data. B) Long time stability of the G/Fe<sub>4</sub>POM-poly(1,8 DAN)/GOx biosensor expressed as time evolution of the relative sensitivity. Error bars represent the standard deviation for three consecutive measurements. Experimental conditions: applied potential,  $-0.04$  V (vs. Ag/AgCl, KCl<sub>sat</sub>); supporting electrolyte, 0.1 phosphate buffer (pH 6.5), stirred aerated solution.

absence of H<sub>2</sub>O<sub>2</sub>) was found to be 88.4% for 0.28 M H<sub>2</sub>O<sub>2</sub> (at  $-0.04$  V vs. Ag/AgCl, KCl<sub>sat</sub>, pH 2.4), value which is much greater than that reported in literature of 17.4% for G/PVA/PFeW<sub>11</sub> or 5.5% for G/PPy/PFeW<sub>11</sub> (in the same conditions: pH 2,  $-0.040$  V vs. SCE) [22].

The batch amperometric calibration for H<sub>2</sub>O<sub>2</sub> performed at G/Fe<sub>4</sub>POM-poly(1,8 DAN)-modified electrode (Fig. 4B), recorded at an applied potential of  $-0.04$  V (vs. Ag/AgCl, KCl<sub>sat</sub>), showed a linear range up to 50 mM, with a sensitivity of 0.11 mA/M. The response time, estimated as  $t_{95\%}$ , was less than 1 min. A detection limit of 2 mM H<sub>2</sub>O<sub>2</sub> was found (signal/noise ratio of 3). In the investigated range of H<sub>2</sub>O<sub>2</sub> concentrations the average relative standard deviation was estimated as  $\sim 10\%$ .

### 3.3. G/Fe<sub>4</sub>POM-Poly(1,8 DAN)/GOx as Amperometric Sensor for Glucose

Because the GOx stability is strongly affected by the experimental conditions required during the electropolymerization process especially by the low value of pH, the enzyme immobilization was performed on the G/Fe<sub>4</sub>POM-poly(1,8 DAN)-modified electrode already obtained. Thus, the enzyme active matrix was prepared using the conventional cross-linking method based on glutaraldehyde (GA) and bovine serum albumin (BSA) [25–28]. The enzyme layer showed a good adhesion on the polymer matrix, probably due to the formation of some covalent bonds between GOx and the polymer layer (i.e., via the NH<sub>2</sub> groups existing in the poly(1,8 DAN)).

The catalytic current, observed in the presence of glucose (Fig. 5A), varied linearly with its concentration in the range between 2.5 and 20 mM ( $I$  (nA) = 0.979 + 0.835 [glucose/mM];  $R = 0.999$ ,  $n = 8$ ), values which include the usual blood

glucose concentration for biomedical applications (i.e., 2.2–22 mM [29, 30]). The apparent Michaelis Menten constant ( $K_m' = 24.5 \pm 5.8$  mM) and the maximum current intensity ( $I_{max} = 34.8 \pm 4$  nA), calculated by Hanes-Woolf's linearization ( $R = 0.986$ ,  $n = 12$ ) [31], allowed to estimate the overall biosensor sensitivity as  $20.1 \pm 2.4 \mu\text{A M}^{-1} \text{cm}^{-2}$ .

The relative low value of the biosensor sensitivity (in comparison with values of 0.36–0.81 mA M<sup>-1</sup> cm<sup>-2</sup> [32]) could be due to the hindered diffusion of glucose and/or H<sub>2</sub>O<sub>2</sub> as well as to the decrease of the enzyme activity induced by the crosslinking process.

The response time to reach 95% of the steady state signal was almost independent on the substrate concentration and its average value was less than 40 s. The detection limit of glucose was found to be 1.2 mM (for S/N = 3).

Data from Figure 5B, showing the long time stability of the glucose biosensor, proved that in the first week the biosensor relative sensitivity diminished no more than 10% of its original value. Moreover, at the end of the second week, the biosensor relative sensitivity reached a stable value representing more than 90% from its initial value. Between measurements, the biosensor was stored at 4 °C.

## 4. Conclusions

A new design to obtain a modified electrode for H<sub>2</sub>O<sub>2</sub> and glucose detection, based on the mediator (Fe<sub>4</sub>POM) entrapping in an electropolymerized matrix of poly(1,8 DAN), was proposed. Some experimental evidences suggest that the modified polymeric matrix consists in a multilayer architecture, formed from positively charged conductive polymer alternating with the negatively charged polyoxometalate.

An optimization study concerning the modified electrode preparation was performed in order to realize an efficient, interference free electrochemical interface for glucose amperometric detection. Finally, the GOx immobilization on the modified electrode surface using the GA-BSA crosslinking method resulted in a robust biosensing system for glucose detection at low applied potential, with improved analytical parameters. Despite the low value of the applied potential, considered as a condition for the absence of „electrochemical interference“ [16], the H<sub>2</sub>O<sub>2</sub> produced during the biochemical process as well as the presence of transitional metals in high oxidation states represent potential sources of „chemical interference“. Consequently, the deposition of a supplementary diffusion barrier [33] on the top of the enzymatic matrix is expected to improve the selectivity of glucose detection at G/Fe<sub>4</sub>POM-poly(1,8 DAN)/GOx electrode.

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