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Dedicated to Professor Valer Fărcăşan at his 85th anniversary

ELECTROANALYTICAL CHARACTERIZATION OF A Co(II) -PHTALOCYANINE MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT. An effective strategy for circumventing problems of high applied potential on metal electrodes for detecting species of medical or environmental importance is the use of metallophthalocyanines (e.g. Co(II)- phthalocyanine, CoPC) as electrocatalyst. A carbon paste matrix modified with CoPC was used to obtain an amperometric transducer, working at an applied potential lowered with 0.6 V than the corresponding unmodified carbon paste electrode. The electroanalytical parameters estimated from the amperometric calibration curves have values much higher than in the case of unmodified electrode.

Keywords: amperometric transducer, glucose, thiocholine, carbon paste electrode, cobalt phthalocyanine.

INTRODUCTION

Carbon paste (CP) is a mixture of graphite powder and an organic liquid, which is immiscible in aqueous solutions. One of the most important composite electrodes is based on CP (CPEs) and was proposed by Adams in 1958 [1]. CPEs are characterized by: (i) exceptionally low background currents, (ii) a wide operating potential range, (iii) convenient modification procedure, (iv) renewability of the surface, (v) miniaturization possibility and (vi) low cost [2]. The pasting liquid serves not only to maintain a firm electrode shape and to fill up the crevices between the graphite particles, but also insulates the graphite from the contacting aqueous solution and acts as a medium for the entrapment of different compounds.

The use of electrochemical methods for glucose or thiocholine analysis has been limited by the high potentials required for their oxidation at conventional electrodes. As a consequence, CPEs have been widely applied as a substitute for noble metal electrodes.

Remarkable progresses in lowering significantly the oxidation potential are obtained by modification of CPEs with electrocatalysts from the metallophthalocyanines class (e.g. Co(II)-phthalocyanines, CoPC) [3]. Phthalocyanines are a class of benzoporphyrins that have strong pigmenting power, forming a family of dyes [4].

The electrochemistry of phthalocyanines has received a great deal of attention due to good catalytic behavior [5]. Devices using CoPC-modified CPEs have been investigated, mainly with regard to their catalytic oxygen reduction activity or the possibility of decreasing the effective oxidation potential for a variety of organic compounds: cysteine [6], thioglycolic acid [7], secondary alcohols [8], hydrazine [9].

The aim of this work is to investigate by electrochemical methods (i.e. cyclic voltammetry, rotating disc electrode and amperometry), the electrochemical and electroanalytical behavior of CoPC modified carbon paste electrodes, in view to obtain an amperometric transducer for glucose or thiocholine detection, operated at a low potential value.

EXPERIMENTAL

Chemicals

Glucose oxidase (Gox) (E.C 1.1.3.4., type VII, isolated from Aspergillus niger, 88,2 U/mg), acetylcholinesterase (AChE) (E.C. 3.1.1.7, type V-S, from *Electric eel*, 970 U/mg solid), acetylthiocholine chloride (ASChCl) were purchased from Sigma (St. Louis, Mo, USA). Cobalt phtalocyanine, graphite powder (99.9% purity) and paraffin oil were obtained from Fluka (Buchs, Switzerland). The supporting electrolyte was a 1/15 M phosphate buffer (pH 8) prepared by mixing the necessary volumes of $Na_2HPO_4.12H_2O$ and KH_2PO_4 solutions.

The glucose obtained from Reactivul (Bucuresti, Romania) was used to prepare stock solution, which are let to mutarotate at room temperature overnight before use.

The thiocholine solution was freshly prepared just before using, by dissolving the appropriate amounts of enzyme (AChE) and substrate (ASChCl) salts into the phosphate buffer (pH 8) solution.

All other chemicals were of analytical grade and were used without further purification. All solutions were prepared with distilled water.

Preparation of the CoPC/Gox/CP bioelectrodes for glucose detection

Unmodified carbon pastes were prepared by mixing 3 g of graphite powder with 2.2. ml of paraffin oil. The CoPC-modified carbon paste was prepared by thoroughly mixing 0.15 g of unmodified carbon paste with 0.01 g CoPC (7%). The CoPC/Gox/CPE was prepared by mixing 0.16 g of unmodified carbon paste with 0.01 g CoPC (7%) and 0.035 g Gox (≈ 3100 U Gox).

The CPEs were fabricated by pressing the obtained unmodified carbon paste mixture into the end of a plastic syringe having a geometric surface area of 0.03 cm^2. Its inner end was connected to a silver wire. The tip of the syringe was filled with CoPC-modified paste or with CoPC+Gox, in view to obtain the CoPC/CP or CoPC/Gox/CP electrodes, respectively. Before use the surface of all CPEs was smoothed manually on a clean filter paper.

Preparation of the CoPC/CP electrodes for thiocholine detection

Unmodified carbon paste was prepared by mixing 0.15 g of graphite powder with 0.06 ml of paraffin oil. The CoPC-modified carbon paste was prepared by thoroughly mixing 0.15 g of unmodified carbon paste with 0.015 g

CoPC (10 %). The CPEs were fabricated by pressing the obtained unmodified carbon paste mixture into the end of a plastic syringe having a geometric surface aria of 0.07 cm^2. Its inner end was connected to a silver wire. The tip of the syringe was filled with CoPC-modified carbon paste, in view to obtain the CoPC/CPE. Before use, the modified and unmodified CPEs were smoothed manually on a clean filter paper.

Electrochemical measurements

Cyclic voltammetry experiments were carried out using a typical undivided three-electrode cell equipped with a SCE or an Ag|AgCl, KCl_{sat} as reference electrode and a platinum plate as counter electrode. As working electrodes unmodified CPEs and CoPC/CPEs were employed.

Data for the electrochemical oxido-reduction process at unmodified and modified electrodes were collected using a custom-made computer controlled cyclic voltammetry set-up, including a low current potentiostat (Polarograph LP7e, Praha, Czech Republic) and a data acquisition interface (AT MOI-16F, National Instruments, USA). LabView 3.1 software was used to monitor the cyclic voltammetry measurements. All experiments were conducted at ambient temperatures (20 ± 2℃). When necessary, the electrolyte solution was deaerated by argon bubbling. The rotating disc electrode (PINE - Instruments Company, USA) measurements were made using the custom made installation described above.

Batch amperometric measurements with the unmodified and modified electrodes were performed in a thermostated (30° C) and stirred solution of 1/15 M phosphate buffer (different pH), by addition of increasing volumes of substrate solution. A stable potential (values specified in figures) between the working electrode and the reference electrode was maintained with a polarograph (type OH-105, Radelkis, Budapest, Hungary).

RESULTS AND DISCUSSION

1. Electrochemical behavior of the CoPC/Gox/CP bioelectrodes for glucose detection

It is well-know that Gox catalyses the oxidation of glucose to gluconolactone and this reaction is used in the fabrication of amperometric biosensors. On the basis of the structural feature of the electrode matrix, two possible catalytic mechanisms may be considered [5]:

Mechanism I. The CoPC behaves as a mediator for the electron transport from the enzyme redox center to the electrode, following the reactions:

$$\text{Glucose}_{(sol)} + \text{Gox}_{(m)}(ox) \rightarrow \text{gluconic acid}_{(sol)} + \text{Gox}_{(m)}(red) \quad (1)$$

$$\text{Gox}_{(m)}(red) + \text{CoPC}_{(m)}(ox) \rightarrow \text{Gox}_{(m)}(ox) + \text{CoPC}_{(m)}(red) \quad (2)$$

$$\text{CoPC}_{(m)}(red) \rightarrow \text{CoPC}_{(m)}(ox) + e^- \quad (3)$$

where: sol means solution and m means electrode matrix.

Mechanism II. The enzyme catalyses the oxidation of the substrate in the presence of oxygen, and the CoPC catalyses the oxidation of H_2O_2, which is the product of the enzyme-substrate reaction, following the reactions 4 - 5:

$$\text{glucose}_{(sol)} + O_{2\,(sol)} \xrightarrow{\text{Gox(m)}} \text{gluconic acid}_{(sol)} + H_2O_{2\,(m)} \quad (4)$$

$$\text{CoPC}_{(m)}(ox) + H_2O_{2\,(m)} \rightarrow 2H^+ + O_2 + \text{CoPC}_{(m)}\,(red) \quad (5)$$

$$\text{CoPC}_{(m)}(red) \rightarrow \text{CoPC}_{(m)}(ox) + e^- \quad (6)$$

In both mechanisms the electrode reaction involves the electrochemical oxidation of the CoPC from the electrode matrix (equations 3 and 6). However, the main difference between mechanism I and mechanism II is that oxygen is involved in the enzyme reaction of mechanism II and the reaction takes place only in the presence of O_2. On the other hand, mechanism I will not be affected by oxygen and in the mechanism II, the CoPC should have a good catalytic behavior towards H_2O_2.

The cyclic voltamogramms for a CoPC/CPE immersed both in phosphate buffer blank solution and in the same solution in the presence of H_2O_2 are shown in figure 1A. It can be seen that H_2O_2 can be oxidized at ≈ 0.7 V *vs.* SCE on CoPC/CPE, while on bare CPE it is oxidized at ≈ +1.3 V *vs*. SCE (results not shown). The oxidation potential of H_2O_2 shifted negatively with almost 0.6 V suggesting that the CoPC is a good electrochemical catalyst for the H_2O_2 oxidation.

The cyclic voltamogramms of the CoPC/Gox/CP bioelectrode in the absence and the presence of glucose is presented in figure 1B. The addition of glucose induces the appearance of a peak placed, at ≈ 0.7 V *vs.* SCE, which confirms that the detection of glucose is based on the action of CoPC as electrocatalyst of H_2O_2 oxidation (mechanism II).

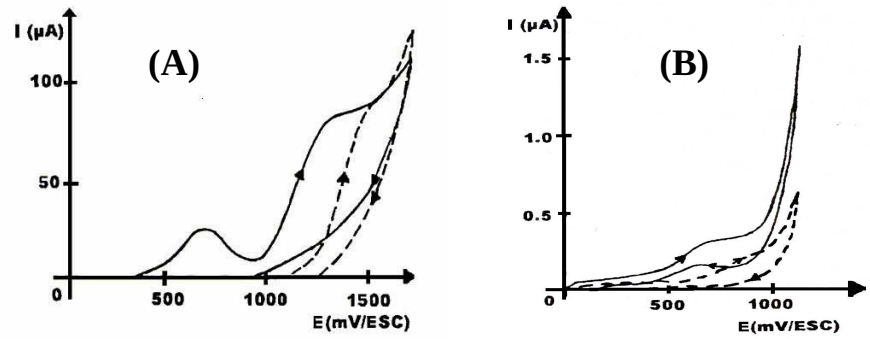

Figure 1. Cyclic voltammetry on CoPC/CP (**A**) and CoPC/Gox/CP (**B**) electrodes in the absence and presence of 83 mM H_2O_2 (**A**) and 11.4 mM glucose (**B**). Experimental conditions: phosphate buffer 1/15 M, pH 7; scan rate, 25 mVs^{-1}; room temperature.

A large excess of enzyme in the electrode configuration decreases the effect of pH variations on the biosensor functionality. Therefore, the pH profiles in the linear measuring range under diffusion control should be substantially less sharp than those for the respective enzyme in solution. Generally, the optimum pH of immobilized Gox is shifted with 0.9 pH toward more alkaline pHs than in the case of the dissolved enzyme [10]. This is due to the formation of gluconic acid within the enzyme matrix, which causes a local pH decrease, resulting in a shift of the optimum pH to higher values than in solution. In this context, the optimum pH of an unmodified Gox/CPE is placed at pH 8 (figure 2), and the presence of CoPC shifts the optimum pH from pH 8 to pH 7 (results not shown).

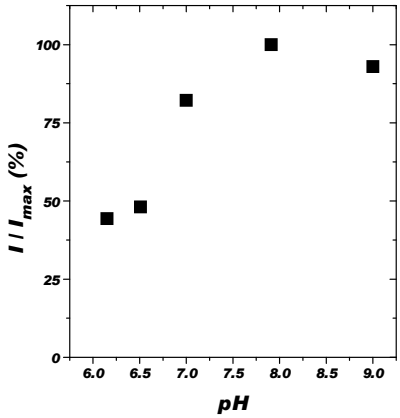

Figure 2. Influence of pH on the electrochemical response of Gox/CPEs. Experimental conditions: phosphate buffer 1/15 M; substrate concentration, 0.6 mM glucose; applied potential, 0.7 V *vs*. SCE; continuos stirring; room temperature.

Electroanalytical parameters of the CoPC/Gox/CP bioelectrode are presented in table 1. As observed, the presence of CoPC enhance the analytical performance (sensitivity and detection limit) of the realized device.

2. Electrochemical behavior of the CoPC/CPEs for thiocholine detection

The thiocholine is an intermediary obtained in the hydrolyse reaction of acetylthiocholine in the presence of acetylcholinesterase [11, 12]:

$$\text{acetylthiocholine} + H_2O \xrightarrow{\text{AChE}} \text{thiocholine} + \text{acetic acid} \quad (7)$$

$$2\ \text{thiocholine} \xrightarrow{\text{oxidation}} \text{dithio-bis-choline} + 2H^+ + 2e^- \quad (8)$$

This reaction pathway is used to evaluate the environmental pollution by indirect measurement of the inhibited acetylcholinesterase activity induced by organophosphorous pesticides.

Oxidation of thiocholine on CoPC/CPE is believed to be a two-step electrocatalytic process. This is initiated by the electrochemical oxidation of the central metal from CoPC species, followed by the chemical oxidation of thiocholine and the regeneration of the original CoPC complex, as described in the following reactions [13, 14]:

$$Co(II)PC \xrightarrow{\text{electrode}} Co(III)PC + e- \quad (9)$$

$$2\ Co(III)PC + 2\ R\text{-}SH \rightarrow 2\ Co(II)PC + RSSR + 2H^{+} \quad (10)$$

where: R-SH is the thiocholine and RSSR is the dithio-bis-choline.

In this context, cyclic voltametry measurements performed on CPE and on CoPC/CPEs in contact with both phosphate buffer (pH 8) and phosphate buffer containing enzymatically prepared thiocholine are presented in figure 3. As expected, the presence of CoPC in the electrode matrix induces the shift of thiocholine oxidation peak potential with 0.6 V (from ≈ 0.8 V *vs*. Ag/AgCl, KCl_{sat} on CPE, to ≈ 0.2 V *vs*. Ag/AgCl, KCl_{sat} on CoPC/CPE). Because the process is an irreversible one, no cathodic response was observed on the reverse scan. Also, an increase of the current intensity is observed in the presence of CoPC. These facts confirm the electrocatalytical effect of the CoPC in the electrode matrix.

For the first time, about our knowledge, the RDE measurements of thiocholine oxidation on CoPC/CPE served for the estimation of the kinetic current ($0.12*10^{-4}$ A) and of the diffusion coefficient ($0.67*10^{-6}$ cm^2*s^{-1}) using the classically Koutecky-Levich plot (figure 4), obtained under mixed control (charge transfer and mass transport).

As in the case of glucose detection, the analytical parameters of the CoPC/CPE used for the detection of thicholine were enhanced, proving the electrocatalytical properties of the studied CoPC (table 1).

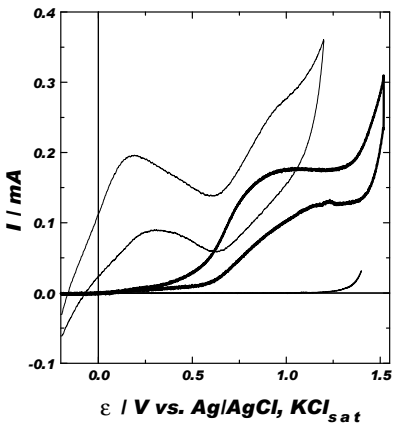

Figure 3. Voltammetric response of 0.03 M SCh at CPE (▬▬) and at CoPC/CPE (- - - - - -) in comparison with buffer solution (——). Experimental conditions: phosphate buffer 1/15 M, pH 8; scan rate 5 mVs^{-1}.

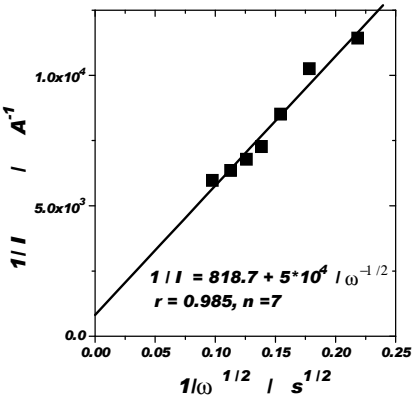

Figure 4. Koutecky-Levich plot for thiocholine oxidation on a CoPC/CPE. Experimental conditions: phosphate buffer 1/15 M, pH 8; thiocholine concentration, 7.15 mM; measurement potential, 0.2 V *vs*. Ag/AgCl, KCl_{sat}.

Table 1.
Analytical parameters of the investigated amperometric transducers.

Analyte*	Working electrode Applied potential	Sensitivity R	Linear range	Detection limit / mM
Glucose	CPE 1.3 V *vs*. SCE	0.14 μA/mM 0.999	0-1.4 mM	0.05
	CoPC/CPE 0.7 V *vs*. SCE	3.02 μA/mM 0.998	0-1.4 mM	0.03
Thiocholine	CPE 0.8-1 V *vs*. Ag/AgCl, KCl_{sat}	3.53 mA/M 0.991	0 - 0.05 M	1
	CoPC/CPE 0.2 V *vs*. Ag/AgCl, KCl_{sat}	6.65 mA/M 0.989	0 - 0.03 M	0.45

* detection at pH 8

CONCLUSIONS

The use of carbon paste as substitute for noble metal electrodes offers several distinct advantage in solid electrode voltammetry. The presented results clearly prove that carbon paste electrodes can be widely applied in their modified form. Good selective amperometric devices for glucose and thiocholine detection were obtained by using CoPC as electrocatalyst. In both cases, for glucose and thiocholine detection, the presence of CoPC improve the electroanalytical parameters, by increasing the sensitivity and decreasing the limit of detection. This type of devices open the way of developing the area of biosensors by realization of enzyme based amperometric electrodes.

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REFERENCES

[1] Q. Chi, W. Gopel, T. Ruzgas, L. Gorton, P. Heiduschka, *Electroanal*. **1997**, 9(5), 357.
[2] J. Kulys, L. Gorton, E. Dominguez, J. Emneus, H. Jarskog, *J. Electroanal. Chem*., **1994**, 372, 49.
[3] M. Sekota, T. Nyokong, *Electroanal*. **1997**, 9(6), 1257.
[4] I. Rosen-Margalit, A. Bettelheim, J. Rishpon, *Anal. Chim. Acta*., **1993**, 281, 327.
[5] Z. Sun, H. Tachikawa, *Anal. Chem*., **1992**, 64, 1112-1117.
[6] M. K. Halbert, R. P. Baldwin, *Anal. Chem*., **1985**, 57, 591.
[7] S. Shahrokhian, J. Yazdani, *Electrochim. Acta,* **2003**, 48(28), 4143.
[8] V. B. Sharma, S. L. Jain, B. Sain, *Tetrahedron Letts*., **2003**, 44(2), 383.
[9] X. Li, S. Zhang, C. Sun, *J. Electroanal. Chem*., **2003**, 553, 139.

[10] H.-L. Schmidt, W. Schuhmann, F. W. Scheller, F. Schubert, “Specific features of biosensors, in “Sensors. A comprehensive survey” (edited by W. Gopel, J. Hesse, J. N. Zemal), vol. 3 “Chemical and Biochemical Sensors”, Part II (edited by W. Gopel, T.A Jones, M. Kleitz, I. Lundstrom, T. Seiyama), VCH, Weiheim, **1992**, p. 772.

[11] I. C. Hartley, J. P. Hart, *Anal. Prooced. Anal. Commun*, **1994**, 31, 333.

[12] G. L. Turdean, I.C. Popescu, L. Oniciu, D. R. Thevenot, *J. Enzy. Inhib. Med. Chem*., **2002**, 17(2), *107-115*.

[13] J. P. Hart, I. C. Hartley, *Analyst*, **1994**, 119, 259.

[14] G. L. Turdean, C. S. Mosneag, I. C. Popescu, *ACH-Models in Chemistry*, **2000**, 137(4), 519.