The Glucose Sensor Based on Copper Complex Mediated Reduction of Hydrogen Peroxide

by Ján Labuda* and Monika Hudáková

Department of Analytical Chemistry, Slovak Technical University, SK-81237 Bratislava, Slovakia

Key words: glucose, hydrogen peroxide, biosensor, chemically modified electrode, copper complex, redox mediator

An effective strategy for the elimination of interferences in the detection of hydrogen peroxide for oxidase-type first-generation biosensor is described. Amperometric sensor for glucose was prepared by immobilizing bis(bathophenanthroline)-copper complex onto the glucose oxidase doped paste electrode. The copper complex bound via an electrostatic bond between sulfo-groups of the ligand and the protonated trioctylamine used as pasting liquid offers an electrocatalytic action towards enzymatically produced H₂O₂. At an applied potential of -0.2 V vs. SCE the dynamic response within 10⁻³ mol l⁻¹ concentration range was obtained.

Opisano efektywną strategię eliminowania zakłóceń przy detekcji nadtlenku wodoru za pomocą bioczujnika pierwszej generacji typu oksydazowego. Amperometryczny czujnik glukozy został przygotowany przez unieruchomienie batofenantrolinowego kompleksu miedzi na elektrodzie pastowej domieszkowanej oksydazą glukozową. Kompleks miedzi, elektrostatycznie związany między dwiema grupami sulfonowymi ligandu, oraz protonowana trioctyloamina, zastosowana jako wypełniacz pasty, wykazują działanie elektrokatalityczne w stosunku do enzymatycznie wytworzono H₂O₂. Przy zastosowanym potencjale -0.2 V względem NEK uzyskano dynamiczny sygnał przy stężeniu na poziomie 10⁻³ mol l⁻¹.
Despite the progress in mediated and conducting salt enzyme electrodes the hydrogen peroxide detecting system remains a common approach for the biosensors based on oxidases [1,2]. As the usual monitoring of hydrogen peroxide in the positive potential region of 0.5 to 0.6 V often suffers from interferences, a mediated electroreduction of \( \text{H}_2\text{O}_2 \) is of interest. Electrocatalytic metal-dispersed carbon composite electrodes were proposed for this purpose [3,4]. A copper heptacyanoferrate(II) complex [5] and bis(1,10-phenanthroline)–copper complex [6] offer also electrocatalytic activity at the \( \text{H}_2\text{O}_2 \) reduction. In both cases the cathodic response occurs at a potential more negative than that of the Cu(II) reduction and it is strongly influenced by the dissolved oxygen signal. Recently, an amperometric sensor based on glassy carbon electrode modified by the mixed ligand copper complex with 1,10-phenanthroline and bathophenanthroline attached to a thin film of the anion exchanger Tosflex has been described for the determination of peroxides [7]. The copper bathophenanthroline complex seems to be an effective catalyst, particularly in neutral and week acidic media.

In the previous work we have reported a simple and effective immobilization of hexacyanoferrate anion onto the surface of carbon paste electrode (CPE) with tris-octylamine (TOA) as a pasting liquid [8]. The copper(II) bis(bathophenanthroline) complex, CuL<sub>2</sub>, could be similarly attached to the electrode surface from acidic solution via an electrostatic bond between the sulfo group of the ligand and the protonized amino group of the TOA (Fig. 1). The aim of the present study was to investigate the function of the CuL<sub>2</sub>–TOA–CPE for the detection of \( \text{H}_2\text{O}_2 \) alone and that liberated in the oxidase/O<sub>2</sub> type biosensing. Both, the copper complex surface modified electrode utilizing the glucose oxidase (GOD) in solution as well as the biosensor containing the immobilized redox mediator and enzyme have been tested.

![Figure 1. Scheme of the attachment of the bis(bathophenanthroline)–copper complex](image-url)
EXPERIMENTAL

Apparatus and reagents

Measurements were performed with a Polarographic Analyzer PA4 (Laboratorní prístroje, Prague) in a cell equipped with a modified CPE, a saturated calomel reference electrode (SCE) and platinum-auxiliary electrode. Solutions were prepared from analytical-reagent grade chemicals (Lachema, Brno) and deionized, double-distilled water. Bathophenanthroline was purchased from Lachema, Brno, glucose oxidase was obtained from Fluka.

Preparation of the electrodes

The CPE was prepared by stirring the spectroscopic graphite powder (Elektrokarbon, Topolčany) with an appropriate amount of TOA in a mortar until a homogeneous paste was obtained. The paste was packed in the end of polyethylene conic tube (3 mm d.) provided with a platinum contact. The modification of electrode was done by the immersion of TOA–CPE into unstirred 5×10⁻³ mol l⁻¹ copper sulfate/1×10⁻² mol l⁻¹ bathophenanthroline in 0.02 mol l⁻¹ HCl/ethanol (1:1) mixture at the open circuit. After 15 min the electrode was taken out, rinsed with water and soaked for 1.5 in buffer solution of pH 5.5. For the preparation of the mediator biosensor, dry glucose oxidase was first mixed with the graphite powder (5 mg GOD per 100 mg of graphite). The paste was packed into the plastic tube that had been previously filled with the carbon paste without GOD leaving 2 mm deep well at the base of the tube. Then the surface of biosensor was modified with CuL₂ as described above.

Procedures

All experiments were performed in 0.1 mol l⁻¹ phosphate buffer with 0.1 mol l⁻¹ KCl at room temperature (22°C). For the measurement with the CuL₂–TOA–CPE, glucose solution of pH 7.1 containing 5 mg GOD was previously equilibrated with air for 24 h. The solution (5 ml) was added to the cell and purged with pure nitrogen for 8 min. For the biosensor response measurement, a non-deaerated glucose solution of pH 5.5 was used.

CVs were recorded at the scan rate of 5 mV s⁻¹. Constant potential measurements were carried out at the detection potential of -0.25 V vs. SCE. The modified electrode was periodically restored in the CuL₂ accumulation solution and equilibrated in the buffer solution.

RESULTS AND DISCUSSION

Cyclic voltammetry for CME

In order to ensure the solubility of bathophenanthroline and the protonization of TOA, the 0.01 mol l⁻¹ concentration of HCl in the aqueous/ethanolic accumulation solution was necessary. Figure 2 shows CVs for loading the TOA–CPE by bis(bathophenanthroline) copper complex, CuL₂. Hence, the amine can fulfill the role of pasting liquid as well as the carrier of the redox mediator forming a tris-octylammonium/bathophenanthroline ion-pair associate. The coverage of mediator sites can be varied by changing either the mediator concentration in solution or the preconcentration time (Fig. 2, inset). Potential scan during the modification has no significant influence on the coverage.
The signal of a freshly prepared CuL$_2$--TOA--CPE slowly diminishes in buffer solution without the copper complex and after about 1.5 h reaches a relatively stable value depending on pH. Partially soluble CuL$_2$ species diffuse away from the electrode surface into the bulk solution particularly in acidic medium. The steady-state current vs. pH dependence exhibits a maximum for pH 5.5 (Fig. 3) with a response representing about 60% of the initial value. From the cathodic charge above the base line current at 50 mV s$^{-1}$, the copper complex loading $\Gamma = 3 \times 10^{-8}$ mol cm$^{-2}$ was determined after 15 min preconcentration in the HCl/ethanolic medium and the equilibration at pH 5.5. The reproducibility of the preparation of the modified electrode is characterized by RSD = 6% ($n = 6$). The electrode response decreases in both acidic and weak alkaline media due to the unstability of the CuL$_2$ complex at low pH [3] and the deprotonation of TOA, respectively.

The Cu(I) complex is stable in the presence of dissolved oxygen. The electrochemical reaction of the mediator redox couple for the CuL$_2$--TOA--CPE in solution of pH 5.5 and 7.1 is quasi-reversible as indicated by an increase of the anodic to
cathodic peak potential difference, $\Delta E_p$, from 40 to 200 mV for the scan rate 5 and 200 mV s$^{-1}$, respectively. It is a difference to the CuL$_2$-Tosflex modified GCE which exhibited a quite irreversible reduction of the bis(bathophenanthroline) copper complex [7]. Hence, in the case of TOA–CPE it is not necessary to employ the mixed ligand copper complex to ensure the reversibility of redox process. The peak current vs. scan rate plot is linear for the scan rate from 2 to 200 mV s$^{-1}$, indicating the reaction of surface attached redox center.

![Figure 3](image_url). Plots of the CuL$_2$–TOA–CPE cathodic peak current on pH. Conditions: 0.1 mol l$^{-1}$ phosphate buffer with 0.1 mol l$^{-1}$ KCl, scan rate 50 mV s$^{-1}$

**Electrocatalysis**

In Figure 4 CVs for the reduction of hydrogen peroxide at the CuL$_2$–TOA–CPE can be seen. The reduction proceeds at the redox conversion potential of the mediator. In aerobic conditions the catalytic peak of H$_2$O$_2$ is followed by the reduction peak of oxygen. The peaks interfere at pH higher than 7.5 and the deaeration of solution by purging with an inert gas is necessary to avoid this interference. Another possibility for the elimination of overlapping H$_2$O$_2$ and O$_2$ peaks is to decrease pH to about 5.5 where the peaks are well resolved. This observation is in accordance with the results published by Wang et al. [3].

The generation of cathodic current of CuL$_2$ can be expressed by the following scheme:

\[
\begin{align*}
\text{Cu(II)L}_2 + e^- & = \text{Cu(I)L}_2 \\
2\text{Cu(I)L}_2 + \text{H}_2\text{O}_2 + 2\text{H}^+ & = 2\text{Cu(II)L}_2 + 2\text{H}_2\text{O}^-
\end{align*}
\]

The catalytic reaction, however, does not seem to be very facile, as indicated by the persistence of the mediator anodic peak. The CV experiments yield a linear calibration graph in the 10$^{-3}$ mol l$^{-1}$ H$_2$O$_2$ concentration region with the sensitivity of 2â×10$^{-3}$ A mol$^{-1}$ l and the detection limit (3$\sigma$) of 1â×10$^{-3}$ mol l$^{-1}$. 
Figure 4. Electrocatalytic reduction of H$_2$O$_2$ at the CuL$_2$–TOA–CPE. Cyclic voltammograms for the solution without and with increasing concentration of H$_2$O$_2$ (inset shows the dependence of the cathodic peak current on the H$_2$O$_2$ concentration). Conditions: 0.1 mol l$^{-1}$ phosphate buffer pH 5.5, scan rate 5 mV s$^{-1}$

Figure 5 shows a constant potential experiment with an amperometric detection in stirred solution. The electrode offers a rapid rise (to 10 s) in current in the presence of H$_2$O$_2$. The steady-state current was measured for the consecutive additions of H$_2$O$_2$ (5 $\mu$l 1 mol l$^{-1}$ solution) within the concentration range from 1×10$^{-3}$ to 1.7×10$^{-2}$ mol l$^{-1}$. The initial sensitivity is 2×10$^{-3}$ A mol$^{-1}$ l$. A decrease in the current change indicates a saturation of the catalytic cycle at high H$_2$O$_2$ concentration.

The repeatability of the amperometric signal was proved alternating the measurement in the absence and the presence of H$_2$O$_2$. For 1.7×10$^{-2}$ mol l$^{-1}$ H$_2$O$_2$ the repeatability of the electrode response is given by RSD = 2% (n = 9).

**Determination of glucose**

The mediator surface modified CPE was applied to biosensing in the buffer solution of pH 7.1 containing glucose, GOD and oxygen, previously allowed to equilibrate for 24 h. The detection is based on the enzymatically derived H$_2$O$_2$

$$\text{substrate} + O_2 \rightarrow \text{oxidized substrate} + H_2O_2$$
The glucose sensor...

Figure 5. The steady-state current of H$_2$O$_2$ measured at the CuL$_2$–TOA–CPE for the stepwise additions of H$_2$O$_2$ (5 μl 1 mol l$^{-1}$ solution) within the concentration range 1×10$^{-3}$ to 1.7×10$^{-2}$ mol l$^{-1}$. Conditions: 0.1 mol l$^{-1}$ phosphate buffer pH 5.5, a constant potential of −0.2 V vs. SCE, stirred solution.

A drastic change in the CV recorded after deaeration is seen in Fig. 6. Using the amperometric detection scheme, a linear calibration graph ($R = 0.999$) was obtained within the 5×10$^{-4}$ to 8×10$^{-3}$ mol l$^{-1}$ glucose concentration range with the sensitivity of 2×10$^{-3}$ A mol$^{-1}$ l and the detection limit (3σ) of 5×10$^{-4}$ mol l$^{-1}$.

Figure 6. Cyclic voltammograms obtained with the CuL$_2$–TOA–CPE in the absence (1) and in the presence (2) of 4×10$^{-3}$ mol l$^{-1}$ glucose. Conditions: 0.1 mol l$^{-1}$ phosphate buffer pH 7.1, glucose oxidase in solution (24 h for equilibration in air), scan rate 5 mV s$^{-1}$

These results have prompted us to the preparation of biosensor with GOD immobilized in CP and CuL$_2$ at the electrode surface. The retention of GOD activity in the carbon paste environment is known from the literature [9, 10]. In the absence of glucose, the CuL$_2$–GOD–TOA–CPE assembly shows the CV identical with that obtained for CuL$_2$–TOA–CPE. Upon addition of glucose to non-deaerated buffer solution of pH 5.5, an increase in the reduction current and lack of increase in the oxidation current was observed similarly as in Fig. 6. It confirms again the catalytic
reduction of $H_2O_2$ from the enzyme and oxygen dependent oxidation of glucose. The biosensor exhibits a rapid response (within ten seconds) and a dynamic range within the mmol l$^{-1}$ concentration (Fig. 7) which is comparable with other amperometric biosensors. However, the working time of the biosensor was strongly limited by the stability of the CPE modification with CuL$_2$ to several hours only when operated at 25°C.

![Graph](image)

Figure 7. The steady-state current response of the CuL$_2$-GOD-TOA-CPE to glucose concentration. Conditions: 0.1 mol l$^{-1}$ phosphate buffer pH 5.5, other conditions as in Fig. 5

In conclusion, the results presented here show the viability of CuL$_2$ modified electrode for the indirect determination of glucose. The oxidase and oxygen dependent generation of $H_2O_2$ may be used for the selective determination of other important substrates such as lactate, glutamate etc. The CuL$_2$-GOD/O$_2$ biosensor exhibits suitable overall kinetics. The CuL$_2$ redox mediator can substitute very effectively peroxidase at the reduction of the detection potential of $H_2O_2$. Comparing to ruthenium, rhodium and iridium mediators [4, 5] a more negative detection potential can be applied. Thus, anodic current interferences from ascorbic acid and other species will be avoid. At the same time, the sensor signal is not overlapped by the reduction of oxygen.

The simple CuL$_2$-TOA-CPE may be employed in the case when a device with an enzyme reactor is used. The easy electrode preparation and renewal can be realized also in the flowing stream. The oxidase doped and oxidase coated mediator electrodes represent other possibilities for the realization of the detection scheme. Leaching of CuL$_2$ from the electrode is a serious problem. A more stable attachment of the copper complex (within a polymeric film) or coating the detector e.g. by oxidase/cellulose acetate layer, could improve both the response and the working time of sensor. Further study concerning the response and stability of the sensor is now in progress.

Acknowledgement

This study was supported by EC project PL960522 and Grant Agency of the Slovak Republic.
REFERENCES


Received September 1996
Accepted March 1997