Studies Regarding the Membranous Support of a Glucose Biosensor Based on Gox

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Abstract
To obtain glucose biosensors based on glucose oxidase (GOx), the enzyme can be immobilized on the sensitive surface of a glass electrode by different techniques: deposition on membranous support (cellophane or other macromolecular material) or entrapment in a matrix. Deposition on membranous support also involves cross-linking with glutaraldehyde or entrapment in silica gel, following the sol-gel procedure.

The aim of this preliminary work was to study the influence of cellophane replacement with a PVA based membranous support on the glucose biosensor performance. The data obtained at pH measurements of buffer solutions with cellophane and PVA membranous supports respectively, show that the PVA based membrane assures superior performances of the biosensor for low glucose concentrations determination (about 10^{-4} M). These results allow the transition to an improved immobilization technique, namely the enzyme entrapment in membranous material.

Keywords: glucose oxidase, glucose biosensor, potentiometric, glass electrode, PVA membrane, glutaraldehyde.

1. Introduction

Electrocatalytic oxidation of sugars is of great interest from several points of view ranging from medical applications of the blood glucose sensing to ecological approaches, such as waste-water treatment in food industry. Many efforts have been made to develop reliable glucose biosensors using different methods [1-4]. Among these methods for glucose determination, potentiometric biosensors have been preferred because of their simplicity, relative low cost and effectiveness. One of the most important issues for the final performances of these biosensors is the enzyme immobilization. Since the functional characteristics of a biosensor depend on the membranous support, searching for materials that provide good environment for the efficient enzyme loading and maintenance of enzyme bioactivity is highly desired [5-7]. Glucose oxidase (GOx) is one of the most used enzymes for glucose biosensors due to its stability and high selectivity to glucose. It catalyzes the oxidation of glucose according to the following reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} H_2O_2 + \text{ gluconic acid}$$

The biosensor with membrane based on glucose oxidase, deposited or immobilized on cellophane in different ways (with glutaraldehyde or with tetrakis (2-hydroxyethyl) orthosilicate, THEOS) was previously studied [8, 9]. Also, attempts to replace the cellophane membranous support with one of biodegradable material have been investigated. Previous studies [10] suggest that, considering its very complex biological and physicochemical properties, the biodegradable membrane is not as compatible as cellophane with the enzymatic glucose assay system. Its use is not justified because the performance of this biosensor is lower, especially regarding the enzymatic reaction rate and sensitivity. The present work aims to study the influence of the replacement of

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the cellophane membranous support with polyvinyl alcohol (PVA) on the functional characteristics of the biosensor. On this purpose, the performances of the two biosensors were compared in the assay of glucose solutions of a large range of concentrations ($0.75\cdot10^{-4}$ M - 10^{-2} M).

2. Materials and methods

Reagents. The checking glucose solutions set

Glucose oxidase (GOx) (EC 1.1.3.4, 215 U/mg) and D-(+)-glucose (anhidrous) were obtained from Fluka. Polyvinyl alcohol (PVA) (90/95 hydrolysis grade 98%), sulphuric acid, acetic acid, methanol, sodium dihydrogenophosphate and disodium hydrogenophosphate were of analytical grade and used without further purification. As membrane, a commercial cellophane was used.

Glucose solutions of different concentrations ($0.75\cdot10^{-4}$ M, 10^{-4} M, $1.25\cdot10^{-4}$ M, 10^{-3} M) were prepared by succesive dillution of a 10^{-2} M glucose solution in 10^{-3} M phosphate buffer, pH 6.9. These five glucose solutions represent the checking solution set. The 10^{-3} M phosphate buffer solution, pH 6.9, was prepared from sodium dihydrogenophosphate, disodium hydrogenophosphate and Na_2SO_4 10^{-1} M, added to maintain the ionic strength of glucose solutions at a constant value. The stock solutions of D-glucose were allowed to mutarotate before use (at room temperature, for 24 h).

Electrolyte solutions, 0.1 M phosphate buffer, pH 7.0, used to equilibrate the biosensors and 10^{-3} M phosphate buffer solution, pH 6.9, for the preparation of the checking solutions, were obtained according to [6, 9].

Bioactive membranes

Bioactive membranes were obtained by deposition of 150 μL enzymatic solution (525 U GOx) on a 5 cm diameter cellophane (*biosensor a*) and on PVA, (*biosensor b*). After the enzyme application on the support, the enzymatic membranes were dried for three hours at 4°C and than were applied on a pH sensor.

The PVA membranous support was obtained by mixing 5 mL PVA solution (10%, containing 6% EtOH abs. v/v) with 1 μL sulphuric acid 1%, 2 mL methanol 50% and 10 μL glutaraldehyde 25%. After homogenization, 5 mL of the mixture were spread in thin film on a plastic surface and then kept, overnight, to dry at room temperature. The membrane was kept in distilled water for 4-5 hours.

Biosensors. Potential measurement cell. Apparatus

To manufacture the biosensor, a pH electrode, ESH 02, from NAPOSENZ Cluj–Napoca was used. The bioactive membranes were fixed on the active surface of the pH sensor with a rubber ring. The biosensor was stored at 4ºC in dry state.

To perform potentiometric measurements, the following electrochemical cell was used (*Figure 1*):

Ag / AgCl / saturated KCl solution / sample / membrane (support + GOx + glass membrane) / 0.1 N HCl internal solution / Ag / AgCl / internal cable

Double junction Ag/AgCl reference electrodes are also from NAPOSENZ, Cluj-Napoca. The experiments were performed with a digital pH-meter, Hanna Instruments - pH 209. The biosensors were equilibrated in a 10^{-1} M phosphate buffer solution, pH 7.0, for approximately one hour before use and for 10 min prior to each determination. All the experiments were performed at room temperature (20 ± 2°C).

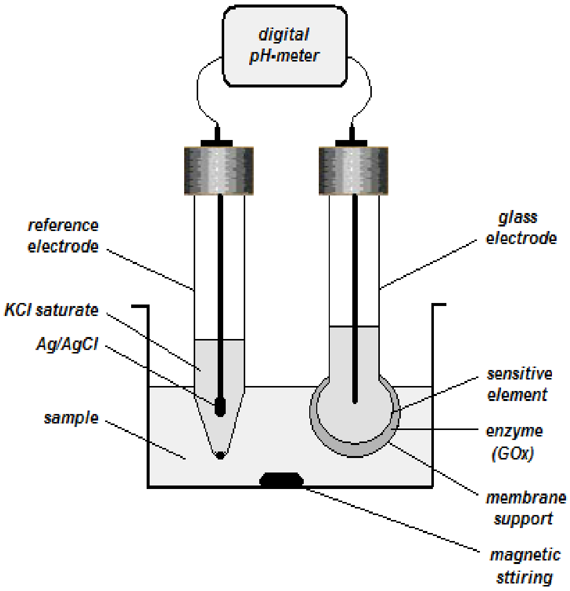

Figure 1. Potentiometric cell for pH measurements

3. Results and discussion

To study the effect of the cellophane replacement with PVA on the functional characteristics of the biosensor, the assays were done during three consecutive days. The enzymatic reaction and the decrease of the pH in time, pH = f(t), were followed up.

The results obtained are presented in *Figure 2* for the *biosensor a*, in the first and third day of assays and in *Figure 3* for the *biosensor b*, also in the first and last day of assays.

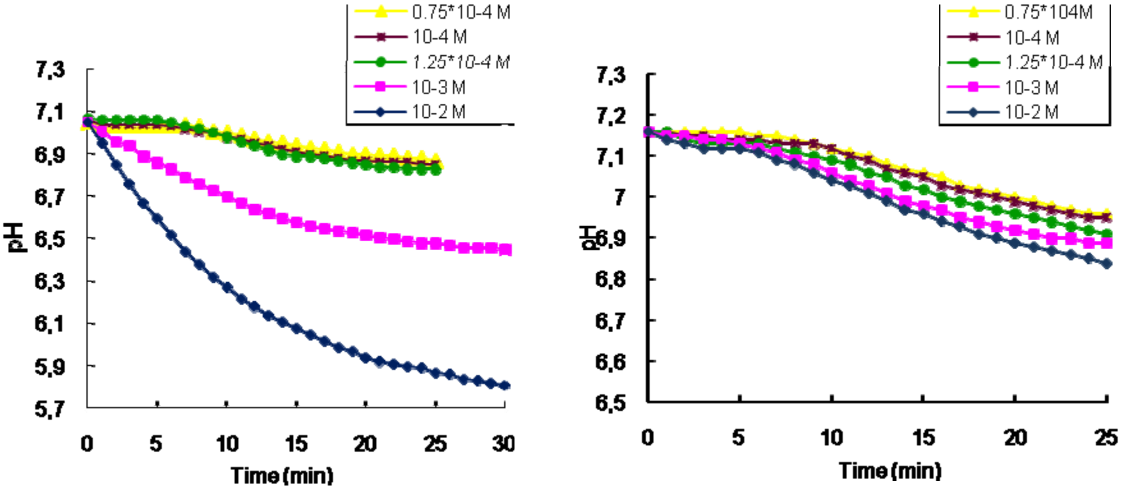

Figure 2. pH = f(t) for the *biosensor a* (*cellophane*), in the first and third day of assays

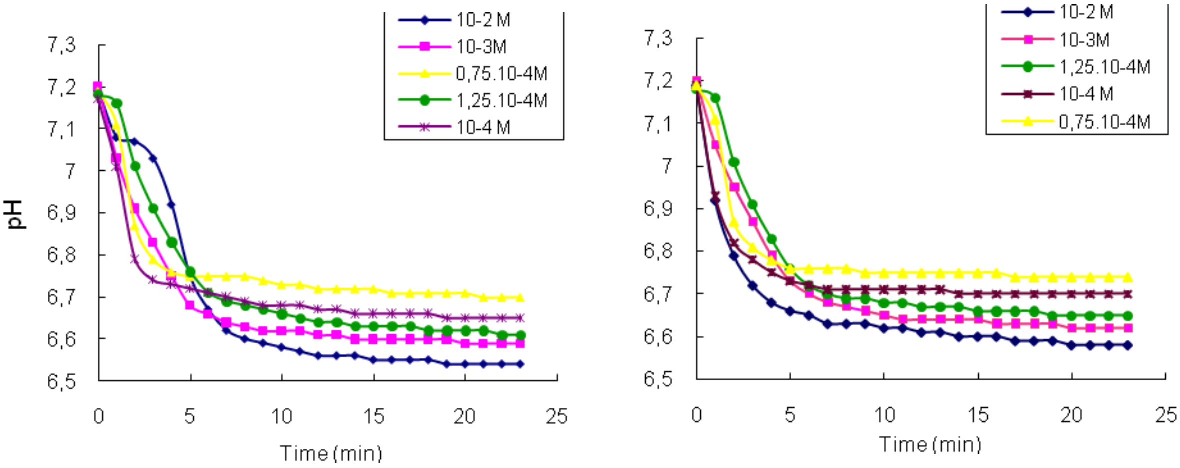

Figure 3. pH = f(t) for the *biosensor b* (*PVA*), in the first and third day of assays

As it can be seen in *Figure 2* and *Figure 3* the sensitivity of the biosensor with PVA membranous support (*biosensor b*) is increased in the range of low glucose concentrations, $0.75 \cdot 10^{-4}$ M, 10^{-4} M and $1.25 \cdot 10^{-4}$ M. A steeper decrease of the pH can be observed, meaning a higher rate of reaction for the same reaction time (more glucose is converted into gluconic acid, in the same time range). On the other hand, the diffusion through the PVA membranous support is enhanced to a larger extent and the enzymatic reaction is starting immediately. This will lead to a significant decrease of the response time of the biosensor with PVA membrane. In the case of the biosensor with cellophane membranous support, for the same low concentration range, the pH variation in time is less pronounced; hence the rate of the enzymatic reaction is lower. But the biggest inconvenience for the biosensor with cellophane membrane is the fact that, for the low glucose concentration, the reaction is starting with a time lag of at least 10 minutes, which diminish a lot the response time of the biosensor. Considering the reaction rate, the biosensor with cellophane membranous support was behaving better, in the concentration range of 10^{-3} M - 10^{-2} M, but only in the first day of assays. After three days, it registered a substantial decrease of the reaction rate. In the case of the biosensor with PVA membranous support, though in the first day the reaction rate is lower, it remained unchanged during the three days of assays, indicating a better reproducibility of data.

The calibration curves of the two biosensors, *a* and *b*, in the first and third day of assays, were plotted separately for both concentration ranges of glucose solutions ($0.75 \cdot 10^{-4}$ M – $1.25 \cdot 10^{-4}$ M and $1.25 \cdot 10^{-4}$ M – 10^{-2} M) [9]. We considered that the appropriate moment for plotting the calibration curve is t = 15 min, when the decrease of the pH in time tend to flatten for both biosensors. At this moment, the biosensors attain their utmost sensitivity [9].

Calibration lines equations for biosensors a and b, as measure of their sensibility, in the first and third day of assays, are shown in Table 1.

Table 1. Calibration lines equations for the *biosensor a* and *biosensor b*, in the first and third day of assays

Day of assay	Glucose concentration range	Calibration lines equations	
		Biosensor a (cellophane)	*Biosensor b (PVA)*
Firs day	$0.75 \cdot 10^{-4}$ M – $1.25 \cdot 10^{-4}$ M	y = 0.226x + 6.007	y = 0.311x + 5.415
	$1.25 \cdot 10^{-4}$ M – 10^{-2} M	y = 0.427x + 5.250	y = 0.036x + 6.487
Third day	$0.75 \cdot 10^{-4}$ M – $1.25 \cdot 10^{-4}$ M	y = 0.176x + 6.339	y = 0.303x + 5.478
	$1.25 \cdot 10^{-4}$ M – 10^{-2} M	y = 0.031x + 6.894	y = 0.036x + 6.527

From the calibration line slopes (Table 1) it can be seen that, in the first day of assays, on the high glucose concentration range, the sensitivity of the biosensor based on cellophane is enhanced compared to the one based on PVA ($\Delta pH/\Delta pC_M = 0.427$ and $\Delta pH/\Delta pC_M = 0.036$ respectively). But, in the low glucose concentration range, the biosensor based on PVA is more sensitive than that based on cellophane ($\Delta pH/\Delta pC_M = 0.311$ and $\Delta pH/\Delta pC_M = 0.226$ respectively). Furthermore, for diluted glucose solutions, the decrease of sensivity after three days of assays is much higher for the biosensor based on cellophane than for the one based on PVA (a decrease from 0.226 to 0.176 for *biosensor a* compared with a decrease from 0.311 to 0.303 for the *biosensor b).*

Conclusions

The results obtained in the case of the biosensor with membrane based on PVA justify us to conclude that for dilute glucose solutions assays (10^{-4} M) the replacement of the cellophane membranous support with one of PVA lead to the improvement of the glucose biosensor performances. Thus, the sensibility, the response time and data reproducibility are enhanced. These findings will allow the passage to an improved technique of enzyme immobilization, namely its entrapment in the membranous material.

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