

COMPARISON BETWEEN NYLON-BASED AND NITROCELLULOSE-BASED POTENTIOMETRIC BIOSENSORS IN GLUCOSE ASSESSMENT

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abstract: This paper aims at comparing the analytical performances in glucose potentiometric determination by means of nitrocellulose-based and nylon-based enzyme electrodes. Two glucose oxidase-based enzyme-electrodes were obtained by entrapping the enzyme within two different semipermeable membranes: nylon (Biodyne B) and nitrocellulose (Biotrace NT). The obtained enzyme membranes were immobilized, consecutively, on the sensitive bulb of the glass electrode. The pH variation in time was monitored for both enzyme electrodes, at different glucose concentrations; the more concentrated the solution, the greater the pH decrease, because the amount of gluconic acid generated in the enzyme-catalysed reaction is greater. Calibration graphs were obtained for both enzyme electrodes; an approximate linear range was obtained between 10^{-4} M and 10^{-3} M; the analytical signal obtained with the nylon-based enzyme electrodes was greater than the one obtained with the nitrocellulose-based enzyme electrodes, at the same enzyme loading and the same glucose concentration.

Introduction

Biosensors are selective, sensitive and rapid analytical tools, recommended, because of their advantages, for the determination of a wide range of analytes (sugars, organic acids, aminoacids, amines, alcoholic compounds) [1].

These sophisticated sensors incorporate two key components: the biocatalyst (the enzyme) and the transducer, which registers the physicochemical modifications that take place in the system, as a result of the enzymatic reaction [2].

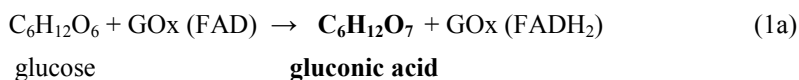
The enzymatic electrodes, the most commonly used biosensor type, can use various detection techniques: electrochemical (potentiometric, amperometric, conductometric), optical, thermal, piezoelectrical, the first mentioned being the most widely employed [3].

Different techniques were applied in order to immobilize the biocatalyst (enzyme) on the surface of the transducer: physical adsorption, gel entrapment, covalent coupling (with or without crosslinking), the use of semipermeable membranes, or electrode modification by immobilizing the enzyme in the electrode body [4].

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Potentiometry, as detection modality, makes use of different transducers: the glass electrode [5, 6], other ion selective electrodes [5], metal oxides, SnO₂ [7], field effect transistors [5, 8].

Glucose represents a key analyte in many fields, like food analysis or biomedical analysis. The reaction exploited in glucose potentiometric determination is the oxidation of this analyte, catalysed by glucose oxidase, for which FAD functions as cofactor.



The glass electrode, one of the most popular and versatile transducers, has already been used in the determination of glucose [6] and other analytes like urea, and penicillin [9].

Glucose has already been determined using semipermeable membranes like cellophane and nylon with immobilized glucose oxidase [6, 10, 11, 12]. A semipermeable membrane is known to act as a selective barrier against interfering compounds, ensuring both enzyme immobilization on the surface of the electrode and the reusability of the biocatalyst. In this paper we chose to compare two semipermeable membranes with different compositions and surface groups; we are concerned with the analytical characteristics of the obtained enzyme electrodes and with the interactions between membrane active groups and the glucose oxidase active site.

Experimental

Reagents and apparatus: glucose monohydrate analytical reagent (Reactivul Bucuresti), glucose oxidase Sigma Type X-S (21000 IU/g), monobasic potassium phosphate (Riedel de Haen), dibasic sodium phosphate (Riedel de Haen), sodium sulphate (Riedel de Haen), Biodyne B membrane (nylon 6,6 positively charged, with surface -NH₂ groups, 0,45 μm porosity), Biotrace NT membrane (nitrocellulose 0,45 μm porosity), digital pH-meter Radelkis OP-208 type (the pH-meter was calibrated using potassium biphthalate 0,01M, pH=4,01 and borax 0,01M, pH=9,18), glass electrode EGA 31 type, Germany, with calomel electrode incorporated.

Operation mode: both enzyme-pH electrodes were obtained by pouring 0,3 ml of the enzyme solution (3000IU/ml phosphate buffer, pH=7,0 [13]) in the center of the nylon/nitrocellulose membrane. The obtained enzyme membranes were fixed consecutively on the sensitive bulb of the pH electrode, after being kept for about 24 hours at 4⁰C; each time, the membrane containing the enzyme solution was held in place with a rubber ring.

The enzyme electrodes were kept for about an hour prior to use in the respective buffer solution. Measurements were carried out in buffer solutions; glucose was dissolved in phosphate buffer 0,001 M, pH=6,90, the glucose concentrations ranging from 10⁻⁴M to 10⁻³M. The buffer solutions were prepared as follows: 0,1M buffer, pH=7,0 was obtained by mixing monobasic potassium phosphate 0,1M and dibasic sodium phosphate 0,1M, in volumetric proportion, 3,90/6,10; 0,001M buffer, pH=6,9, was obtained by mixing monobasic potassium phosphate 0,001M, and dibasic sodium phosphate 0,001M, in volumetric proportion, 4,50/5,50 [13].

Prior to each determination the enzyme electrodes were equilibrated in buffer (about 10 minutes) to retain the original pH. The pH was read after reaching the steady – state.

Results and Discussions

For both enzyme electrodes we followed the pH decrease in time (Figs. 1 and 2), due to gluconic acid generation in the glucose oxidase catalysed reaction. This pH decrease monitoring was made at different glucose concentrations.

For both enzyme electrodes (see Figs. 1 and 2), the pH variation increases when glucose concentration increases, because the amount of gluconic acid generated is greater.

By analysing Figs. 1 and 2, we can notice that for the same enzyme loading and at the same glucose concentration, the analytical signal obtained with the Biodyne B-based enzyme electrode is greater than the one obtained with the Biotrace-based enzyme electrode. Nevertheless, this difference is not dramatic, and it can be considered that the use of these semipermeable membranes lead to comparable performances. To sustain this assertion, we presented the results obtained with a Biodyne A-based enzyme electrode (1200 U GOx), in a previous study (Fig. 3) [12].

Calibration graphs are also presented (Fig. 4) for the nylon-based and nitrocellulose-based enzyme electrodes.

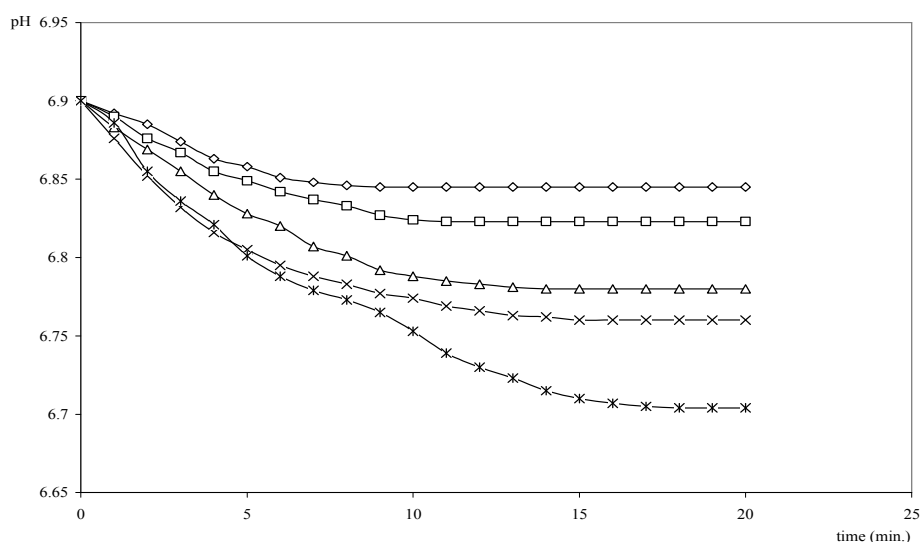


Fig. 1 pH diminution in time for the Biotrace-based enzyme electrode, at different glucose concentrations: ◇-10⁻⁴M; □-2,5x10⁻⁴M; △-5x10⁻⁴M; ×-7,5x10⁻⁴M; *-10⁻³M

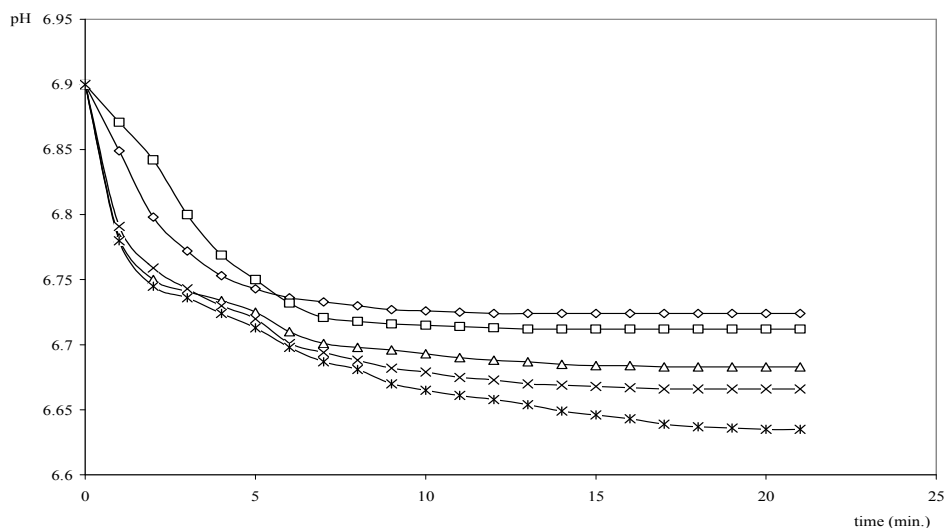


Fig. 2 pH diminution in time for the Biodyne-based enzyme electrode, at different glucose concentrations: \diamond - 10^{-4} M; \square - 2.5×10^{-4} M; \triangle - 5×10^{-4} M; \times - 7.5×10^{-4} M; $*$ - 10^{-3} M

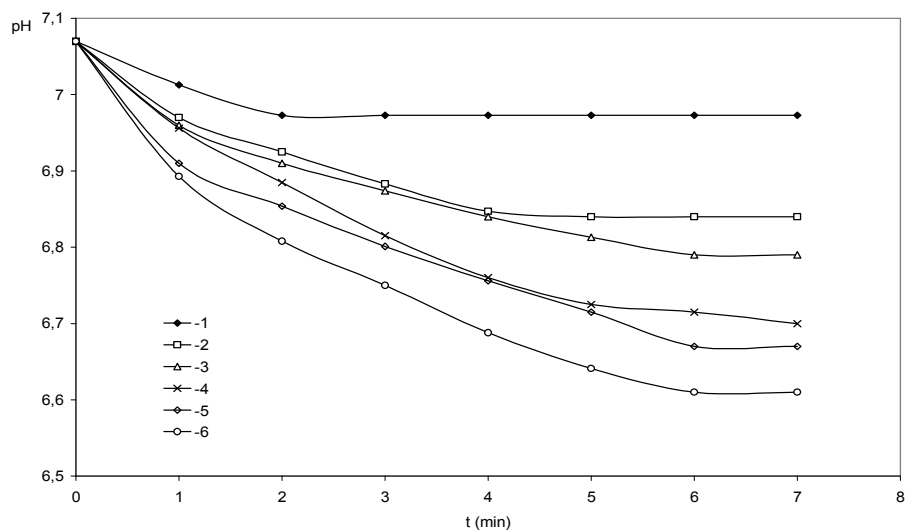


Fig. 3 pH diminution in time for the Biodyne A-based enzyme electrode (1200 U GOx) at different glucose concentrations: 1- 10^{-3} M, 2- 2.5×10^{-3} M, 3- 5×10^{-3} M, 4- 10^{-2} M, 5- 2.5×10^{-2} M, 6- 5×10^{-2} M [12]

Nevertheless, the difference between the analytical signals of the enzyme electrodes obtained in this study (nylon and nitrocellulose-based) could be explained by the fact that an eventual covalent coupling between enzyme and surface active groups ($-\text{NH}_2$) of nylon involves to a lesser extent, or does not involve at all the active site of the enzyme, which is responsible for its biocatalytical action [12].

For both obtained enzyme electrodes we notice a linear range (Nernstian dependence) between 10^{-4}M and $7.5 \times 10^{-4}\text{M}$ glucose concentration ($r^2=0.9334$ for the Biotrace-based enzyme electrodes and $r^2=0.9576$ for the Biodyne-based enzyme electrode, values calculated on the linear range).

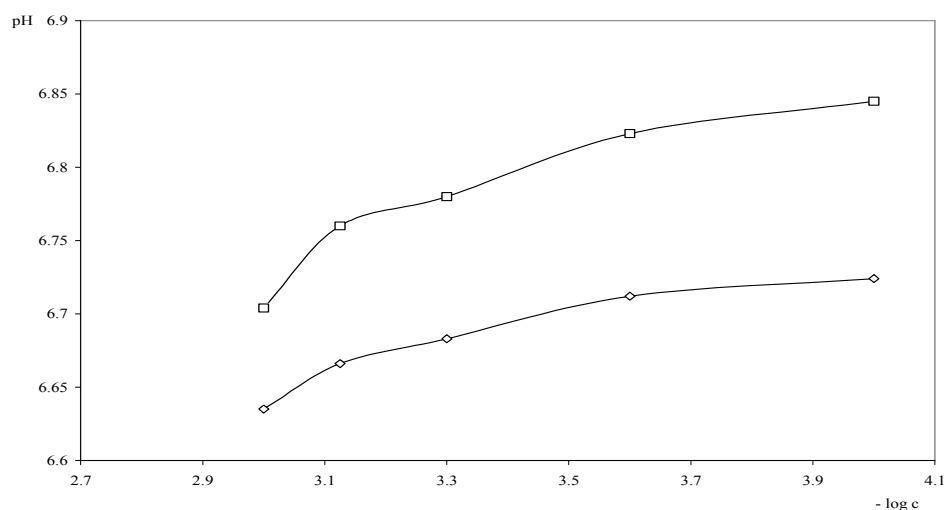


Fig. 4 Calibration graphs obtained for the Biotrace-based (□) and Biodyne-based (◇) enzyme electrodes

Conclusions

By analysing the results obtained with both enzyme electrodes, we notice that the analytical signal obtained with the nylon-based enzyme electrode is somewhat greater than the one obtained with the nitrocellulose-based enzyme electrode.

Nevertheless, the results obtained with these semipermeable membranes can be considered comparable, leading to the possibility to apply the obtained enzyme electrodes to glucose analysis in real samples.

An explanation for the slight difference obtained with these enzyme electrodes (nylon and nitrocellulose-based) could be the lack of enzyme activity diminution, because a covalent coupling between enzyme and the active groups of nylon does not involve the active site of the enzyme.

As a Biodyne-based enzymatic electrode has already been used in glucose determination in real samples [12], the object of a future study will be to apply the Biotrace-based enzymatic electrode to glucose assessment in juices and wines.

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