NEW ENZYME IMMOBILIZATION PROTOCOL FOR DESIGN OF AMPEROMETRIC BIOSENSORS

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Immobilization of the biorecognition element is the key to the development of biosensors. Among the various enzyme immobilization protocols, an entrapment in polymer membranes is a general method for variety of transducers. Formation of the membrane from polymer solution in organic solvent on any surface is more simple and reproducible compared to chemical polymerization. We believe that the simplicity of this immobilization procedure would provide the reproducibility of the resulting biosensors strongly required for their mass production.

Polyelectrolyte Nafion has found wide use for development of the enzyme-containing membranes. Among its main advantages is that Nafion provides a biocompatible interface with mammalian tissue and hence offers the potential for use with implantable sensors. The method for membrane formation is a simple dipping of the electrode into the polyelectrolyte solution or casting a small volume of the solution onto the electrode surface and allowing the solvent to evaporate.

The use of non-conventional media for enzyme immobilization in polyelectrolyte membranes is reported. Optimal environment for glucose oxidase (GOD) in Nafion membranes is achieved using an advanced immobilization protocol based on a non-aqueous immobilization route [1]. Exposure of GOD to water-organic mixtures with a high (85÷95%) content of the organic solvent, resulted in stabilization of the enzyme by membrane-forming polyelectrolyte. Such optimal environment leads to highest enzyme specific activity in the resulting membrane, as desired for an optimal use of the expensive oxidases. Casting solution containing GOD and Nafion is completely stable over 5 days in a refrigerator providing almost absolute reproducibility of GOD-Nafion membranes (Fig.1). The activity of the GOD-Nafion membranes is almost 100% reproduced with maximum deviation in the frame of 2%, what is already a precision limit for kinetic investigations.

A glucose biosensor was prepared by casting the GOD/Nafion membranes over Prussian Blue modified glassy carbon disk electrodes. The biosensor operated in FIA mode (Fig. 2) allows the detection of glucose down to the 0.1 μM level, along with high sensitivity (0.05 A $M^{-1}cm^{-2}$), which is only 10 times lower than the sensitivity of the hydrogen peroxide transducer used. A comparison with the recently reported enzyme electrodes based on similar H_2O_2 transducers (transition metal hexacyanoferrates) shows, that the proposed approach displays a dramatic (100 fold) improvement in sensitivity of the resulting biosensor [2].

Combined with the attractive performance of a Prussian Blue based hydrogen peroxide transducer [3], the proposed immobilization protocol provides a superior performance for first generation glucose biosensors in term of sensitivity and detection limits. An introduced parameter, the ratio of sensitivity of amperometric biosensor to the lowest concentration of analyte detected was of $5 \cdot 10^5$ A $M^{-2}cm^{-2}$ for biosensor made according to the proposed approach.

Since a number of other enzymes were successfully entrapped in Nafion membranes using previous reported non-aqueous immobilization approach [1] we believe that the proposed approach is universal for immobilization of enzymes from different classes, at least for immobilization of oxidases. This investigation demonstrates how to find an optimal surrounding of the enzyme in polymer membrane, which provides its maximal efficiency.

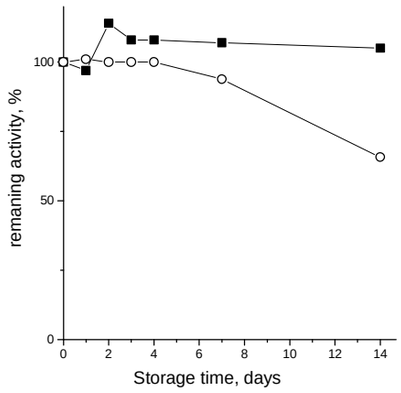

Fig.1. Shelf-life of glucose oxidase - Nafion membranes (■) and the casting solution (o) at 4 °C.

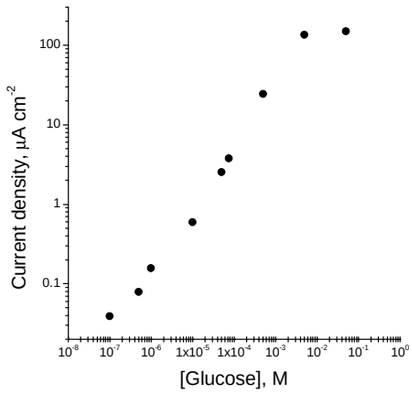

Fig.2. Calibration for glucose-sensitive biosensor: 1.5 mg ml^{-1} of glucose oxidase, 0.3% Nafion and 7.4% H_2O in casting solution; flow rate 0.5 ml min^{-1}; E = 0.0 V (Ag|AgCl|1 M KCl).

References:

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[2] A.A. Karyakin, E. A. Kotel'nikova, L.V. Lukachova, E.E. Karyakina, J. Wang. *Anal. Chem*. (2002) **74**, 1597.
[3] A.A Karyakin. *Electroanalysis* **13** (2001) 813.

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