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Screen-printed disposable urease-based biosensors for inhibitive detection of heavy metal ions

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Abstract

Low-cost and small-size, all-solid-state potentiometric pH-urease electrodes useful for determination of heavy metals ions have been developed by means of screen-printing. The biosensing thick-film is prepared using biocomposite screen-printable material composed of ruthenium dioxide, urease, graphite and organic polymer. Electric circuit and electrode insulation are also fabricated in the same thick-film technology using inexpensive commercially available pastes. All the pastes used for manufacturing of the biosensor are cured under mild conditions not dangerous for the immobilized enzyme and for the plastic flexible foil used as a substrate for screen-printing. The developed enzyme electrodes are useful as disposable potentiometric biotests for selected urease inhibitors. They enable detection of silver and copper ions at sub-ppm levels.

Keywords: Biosensor; Potentiometry; Screen-printing; Urease; Heavy metals

1. Introduction

Many enzyme-based biosensors can be used as sensors for enzyme inhibitors, although sometimes selectivity of such detection schemes is questionable [1]. Urease is a common biocatalytic receptor, recognizing selected heavy metals ions. Mechanism of the enzyme inhibition is based on interactions between heavy metal ions and thiol/methylthiol groups of cysteine/methionine presented in the active center of the enzyme. Therefore, several urease-based biosensors reported in the literature [2–11] are used for detection of these toxic ions. There are enzymatically modified optical [2,3], conductometric [4] and capacitance [5,6] devices. Potentiometric biosensors for detection of urease inhibitors are based on ammonium [7] and pH-ISFETs [8,9], as well as on conventional pH-electrodes based on iridium oxide [10] or polypyrrole [11].

There are two general strategies for analysis with enzyme inhibitor biosensors. Such biosensors can work as single-use or as reusable analytical devices. The latter way is sometimes difficult in analytical practice as a regeneration of the enzyme layer after inhibitor recognition is inconvenient and time-consuming process. Enzyme layers designed for repeatable inhibition and regeneration processes should be relatively stable, as in some cases regeneration procedures are rather aggressive. On the other hand, this need of high stability of the enzyme layer stays in opposition to the requirement of low resistivity of the immobilized enzyme on inhibitory effects.

Single-use devices seem to be an attractive alternative. In case of such, so-called “single-shot”, devices another requirements are defined. The most important are: (i) high reproducibility “from sensors to sensor” also in large-scale, (ii) low-cost and simplicity of manufacturing, (iii) possibility of mass-production and (iv) long-term storage stability. All these requirements are fulfilled by screen-printing tech-

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nology. This technology is very simple, cost-effective and naturally designed for large-scale production. Although this fabrication method is widely used for development of various amperometric sensors and biosensors [12,13], surprisingly its application in the field of potentiometric analytical devices is rather poor. In this paper we present how to manufacture by means of screen-printing very simple, cheap and reproducible urease-based potentiometric biosensors. Their utility for heavy metal ions detection is demonstrated.

2. Experimental

Graphite paste (Electrodag 421SS) and silver paste (Electrodag 725A-6S-54) were obtained from Acheson. Hydrophobic, UV-curable paste (no. 5018) was obtained from DuPont. A flexible polyester foil 125 μm thick (Type CT-5) from Autotype was used as a substrate for screen-printing.

Urease (Type IX, activity 80 U/mg) was obtained from Sigma. Fine powder of ruthenium dioxide was obtained from Polish National Mint. All other reagents were of analytical grade. Doubly distilled water was used for the preparation of all solutions.

Composition of the laboratory-made biocomposite paste, obtained by hand mixing, was: 40% of graphite paste, 59.5% of RuO_2 and 0.5% of urease. In order to obtained uniform biocomposite paste, both the powders were placed in a minute volume of toluene and ground in a mortal until dry out. The resulting homogenous RuO_2/urease powder was wetted with butyl celosolve and mixed with the graphite paste.

The enzyme electrodes were produced using a Presco semi-automatic screen-printer (model 564). All pastes were printed through 77 T mesh polyester screens with 20 μm masking. The enzyme electrodes were produced in 7 cm × 7 cm sheets. Each sheet contained set of 32 biosensors (Fig. 1).

The biosensor fabrication was a three-step process: first a thick-film of silver was deposited and dried (10 min at 120 °C). Than, the biocomposite paste was screen-printed and dried (3 h at 36 °C). Finally, a protective dielectric film was deposited and cured for 15 s by UV-irradiation.

Potentiometric measurements were performed using 16-channel instrument from Lawson Labs Inc. controlled by PC. The sets of the enzyme electrodes were connected with the instrument using edge card connectors commonly applied in computer mainboards (Fig. 1). Conventional double junction, reference electrode (Ag/AgCl/KCl(sat)/1 M KNO_3) was used in all experiments.

Common procedure for each biosensor consisted of incubation in solution containing heavy metal ions (analyte recognition step), washing with working buffer (biosensor equilibration step) and potentiometric measurement after urea addition (detection step).

Heavy metals ions were used as nitrate salts. All potentiometric measurements with the developed pH-enzyme electrodes were performed in stirred, easy to prepare, 5.0 mM phosphate buffer composed of equal amounts of KH_2PO_4 and K_2HPO_4 in 0.1 M KCl. Concentration of urea for test of biosensor response was 10.0 mM.

3. Results and discussion

Ruthenium dioxide presented in the biosensing film causes pH-dependent potentiometric sensitivity of the developed biosensor. Several mechanisms of pH-response for various metal oxide electrodes were proposed and discussed in detail elsewhere [14,15]. As the metal oxide has slightly non-stechiometric composition, most probably there is a pH-dependent redox equilibrium between lower and higher valences. Screen-printed ruthenium dioxide electrodes and their analytical properties were reported previously [16,17]. The

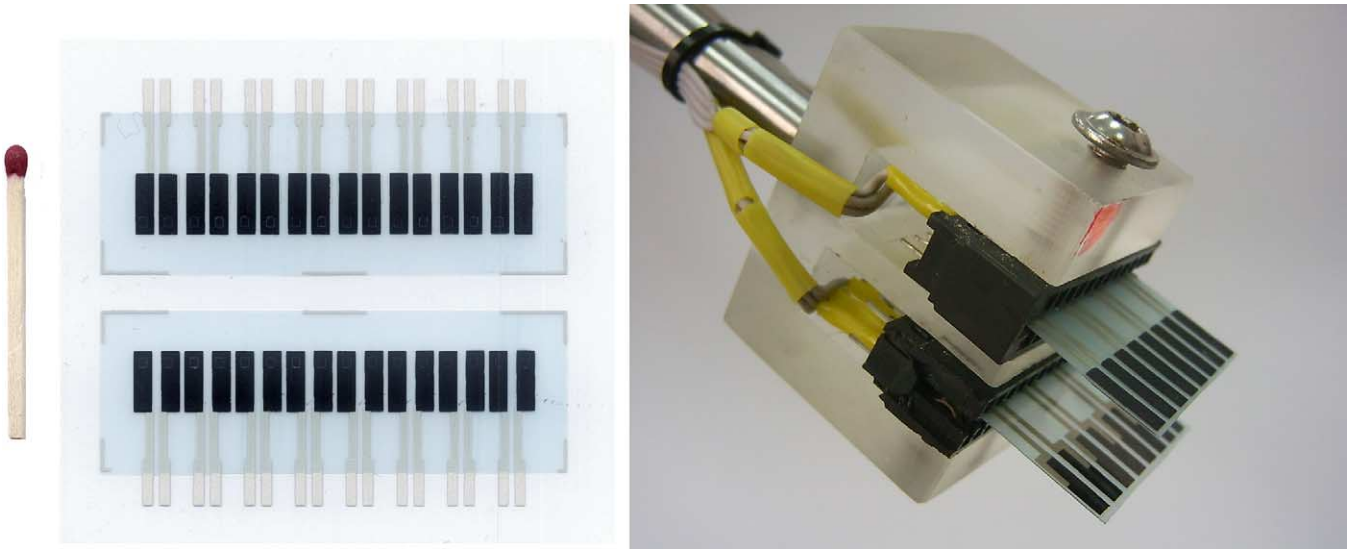

Fig. 1. Set of planar biosensors and adaptor for their connection with instrument.