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Ion Selective and Semi-permeable Membranes for Biosensors in Biomedical Applications

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In this paper, a comprehensive review based on research, conducted by authors, related to development of biosensors in respect to different membranes applications is presented. The ion-selective membranes were described in terms of factors determining their stability, which depends on lipophilicity of the membrane components. In the case of enzymatic membranes, relation between stability of the biosensor and immobilisation of enzyme molecules in/on the membranes/surfaces is discussed. Finally, the semi-permeable membranes application in lab-on-a-chip type devices for development of a sampling probe and flow-through biosensors were described.

K e y w o r d s: biosensors, enzymatic membranes, ion selective membranes, semi-permeable membranes, lab-on-a-chip

1. Introduction

A widely used definition describes a biosensor as a device, which consists of two parts, sensing biomaterial layer that is deposited onto a transducer, which converts a (bio)chemical quantity into an electrical signal. In our research, the term biosensor means any sensors and/or analytical devices used in order to determine concentration of substances of biological interest even if they do not utilise a biological system or bioreceptors, directly. The main advantages of the biosensors are small dimensions, portability, and reduction of costs as well as simplification of assay procedure that make these devices adequate for non-professional users/operators in out-of-laboratory examination.

In general, according to a type of the transducer, the biosensors are classified as follows: (1) electrochemical (potentiometric and amperometric), (2) optical

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– spectrophotometric and colourimetric, (3) mass-sensitive – based on piezoelectric effect, and (4) thermal – based on measurement of heat stream. In biosensor research the following biological systems and/or bioreceptors: whole cell metabolism, ligand binding, antibody-antigen reaction, enzymes and DNA probes, are utilised.

To get an accurate, precise, reproducible and linear response over the useful analytical range, the (bio)receptor must be highly specific to an analyte and properly attached to the transducer. Moreover, depending on an application, the biosensor must be stable under storage conditions and/or over a large number of assays or through useful on-line monitoring period of time.

Since usually the (bio)chemical reaction depends on conditions, such as: stirring, pH, temperature, and light-exposure, the analysis procedure should include calibration performed in conditions corresponding to the conditions of sample assay.

In this paper, different kinds of membranes and their applications mainly for development of electrochemical biosensor are emphasised. In particular, it concerns membranes incorporating enzymes – as a biocatalytic material, ionophores – ligands responsive to biologically relevant ions and semi-permeable membrane. The illustrative examples of each type of the membrane, namely ion-selective, enzymatic and separating are drawn from the research conducted in our laboratory.

2. Ion-selective Membranes

Within the potentiometric class of sensors the ion-selective electrodes (ISE) and ion-selective field effect transistors (ISFETs) are dominant. Although, the principle of operation of the ion-selective electrodes and ISFETs differs, the theories for both, ISE [1, 2] as well as ISFETs [3–7] are recognised. In some areas, e.g. in clinical chemistry, the ISEs are applied successfully [8, 9]. For both, the key part is an ion-selective membrane, which is responsible for selectivity to target ions in the presence of various interfering ions from the sample. The main phenomenon responsible for generation of the response, i.e. the potential drop across the membrane, is an ion exchange between two phases: membrane/solution, which depends on the activity of the target ion (analyte) in these phases. This potential is described by the Nernst's and Nikolsky's equations.

The ion-selective membranes are usually composed of three or four components: polymeric matrix, plasticizer, lipophilic salt and ionophore, all matched in adequate proportions. Complex formation constants for the different ions and the ionophore determine selectivity of the membrane. The plasticizer polarity influences extraction properties of the membrane, while a concentration of anionic sites in the membrane depends on the lipophilic salt content. The polymers such as: poly(vinylchloride), polyurethane, polysiloxane, polyacryloamide, cellulose used for the membrane fabrication play a role of a scaffold maintaining the liquid membrane.

Since valinomycin is highly selective for potassium over sodium ions, it is the most frequently used natural ionophore. In nature, valinomycin is employed as a potassium ion specific transporter through the cellular membrane, by binding and carrying the ions, and in result reducing an electrochemical potential gradient across the membrane [10, 11]. It is a macrocyclic molecule, obtained from the cells of streptomyces strains. Compounds that enhance transport of the ions across the lipid cellular membranes are lipophilic in nature. The great majority of ionophores are synthetic products. The design of synthetic carriers takes advantage of the different elements of molecular recognition. Cavities and clefts in the ionophores make them complementary to the size and charge of a particular ion.

As mentioned above, the ion-selective membrane has a great impact on the parameters of potentiometric sensors: selectivity, sensitivity, and lifetime. In this paper, we consider a problem of enhancement of the lifetime of the potentiometric sensors, which is limited by a gradual leaching out of the membrane components: plasticizer and ionophore, mainly. For the purpose of our research, two approaches were undertaken. In the first one, a sodium ionophore (bis(phenylbenzo)-13-azocrown-5) of increased lipophylicity was synthesised at the Department of Chemical Technology of Gdańsk University of Technology, while in the second one, a plasticizer free ion-selective membrane was used.

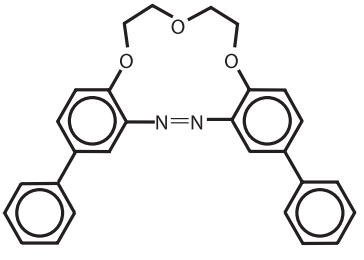

Fig. 1. Structure of a sodium ionophore – bis(phenylbenzo)-13-azocrown-5, synthesised at the Department of Chemical Technology of Gdańsk University of Technology

Poly(vinylchloride) (PVC) membranes containing a novel sodium ionophore (Fig. 1) were applied on chemically modified ISFETs (ChemFETs) [12]. The ChemFETs based on this ionophore fulfill requirements for clinical application. Moreover, they are characterised by relatively high selectivity coefficient for sodium over lithium ions. The elevated potentiometric selectivity coefficient $K(Na^+/Li^+)^{pot}$ was obtained for the ChemFETs with the PVC membrane containing *o*-nitrophenyl octyl ether (*o*NPOE) as a plasticizer.

The plasticizer-free polysiloxane type Siloprene membrane was used for fabrication of the sensitive to ammonium ions ChemFETs [13]. The ISFETs gate was covered with an intermediate hydrogel layer of poly(2-hydroxyethyl metha-

crylate), polyHEMA as first, and then the Siloprene membrane was deposited. The NH_4^+-selective membrane incorporated nonactine as an ionophore and (tetrakis-(4-chlorphenyl)borate) anions (KTpClPB) as anionic sites. The ChemFETs based on the Siloprene showed NH_4^+ responses with almost the Nernstian slope in the range of NH_4^+ ions activity from 10^{-4} to 10^{-1}in the presence of 0.1 M Na^+ and potentiometric selectivity coefficient log $K(NH_4/Na)^{pot}$ equal to –2.8. It is worth to underline that NH_4^+-ChemFETs based on the Siloprene membrane show a good reproducibility of the output signal and good long-term stability over a period of 22 months.

3. Enzymatic Membranes

The response of the biosensor is determined by properties of the membrane incorporating biocatalytic molecules (enzyme) deposited on the transducer part of the biosensors. The enzyme converts an analyte into a product that is detected at the sensor surface (Fig. 2). To obtain the biosensor response, the analyte must penetrate through the membrane to the catalytically active sites of the enzyme and then the products partially diffuse to the sensor surface and away into the bulk solution. The mass transport trough the membrane is mainly driven by the concentration gradients of species then, diffusive process is dominant. The diffusion process is considered to be two-step: (1) an external diffusion where the transport of substrates reaches the membrane followed by (2) an internal diffusion where the transport of the substrates and products, within the pores of immobilised enzyme particles, simultaneously proceeds with the reaction of enzymatic catalysis.

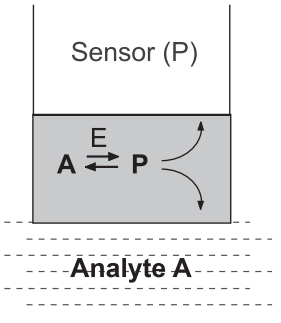

Fig. 2. Principle of operation of an enzymatic biosensor

Immobilisation of the enzymes makes them particularly applicable for biosensors. However, a process of incorporation of bioactive molecules on the top or within membrane must be reproducible and prevent their catalytic activity and accessibility of the analyte to their active sites. In some cases the enzymes are intrinsically stabilised by the immobilisation process.

The overall enzymatic process, taking place at the biosensors, is controlled by the kinetics of the electrode reaction and is classified in three control ranges [14]:

(1) Kinetic control that takes place at low enzyme concentration and enzyme activity. In this case mass-transfer parameters do not influence the sensor response and the output signal is proportional to the enzyme concentration and enzyme activity. Since the activity of the enzyme decreases as a function of time, the response of the sensor will also decrease.

(2) Diffusion control that occurs at high enzyme concentration and with low membrane permeability. This system is independent of the enzyme concentration and a linear response is obtained over a broad concentration range of analyte.

(3) Stoichiometric control that takes place if the process is limited by mediator concentration (e.g. oxygen). Under deficiency of the mediator, the sensor becomes insensitive to analyte and responds only to changes in the concentration of mediator.

Long-term use of biosensors is usually limited by loss of the enzyme molecules immobilised within the membrane or the enzyme activity along with time. The loss of the enzyme activity is related to environment: pH, ionic strength, temperature, and concentration of inhibitors while the loss of the enzyme molecules from the membrane depends strictly on method of the enzyme immobilisation. Therefore, in our laboratory several methods of the enzyme (lipase, urease, acetylocholin esterase and glucose oxidase) immobilisation for development of biosensors as well as for microreactors were investigated.

Since our aim was to obtain long-term stable biosensors and microreactors we focused on the diffusion control processes where the time dependent decrease of the enzyme activity is compensated with the excess of the enzyme participating in the reaction.

For the purpose of triglycerides determination with a biosensor, four methods of lipase immobilisation: (1) chemical coupling to the surface of silica gel beads through aminopropyltriethoxysilane and glutaraldehyde, (2) the chemical bond to the surface of glass beads coated with keratin, (3) entrapment within alginate gel beads and (4) adsorption onto nitrocellulose sheets were compared [15]. All methods of the lipase immobilisation, except the third one, were found to be effective. Immobilisation in the alginate gel beads failed due to the insufficient accessibility of the substrate to the lipase active centres. The systems consisting of the microreactors packed with surface immobilised lipase were calibrated for different substrates. The highest sensitivity was obtained for tributyrin (0.478 pH/mM for concentration < 4 mM), while the widest linear range was obtained for triacetin (up to 30 mM).

Some other methods of surface modification and supports such as: glass and polymeric beads and structured silicon surface for enzyme immobilisation were investigated as well. The surface of silicon modified with aminopropyltriethoxysilane (APTS) and 3-glycidoxypropylmethoxysilane (GOPS) with the immobilised enzyme was characterised by atomic force microscopy (AFM), time-of-flight secondary ion mass spectroscopy (ToF-SIMS) and infrared spectroscopy (FTIR) [16]. An effective

developed method of enzyme immobilisation was based on Schiff's base formation between amino groups on the enzyme surface and aldehyde groups on the chemically modified surface of the supports. The supports with immobilised urease were also tested in combination with the microreactors fabricated in silicon and Perspex, operating in a flow-through system. For the microreactors loaded with urease immobilised on glass beads (Sigma) and on polymeric ones made of polyacrylonitrile (PAN), a very high and stable signal (pH change) was obtained. It was stated that the developed method of urease immobilisation is a very effective one. The method of silicon surface modification based on the alkilation reaction between amino groups of the biomolecule and epoxy terminal on the internal wall surface of the silicon microreactors modified with GOPS was considered as a mild for biomolecules [17]. Despite of this, the performance of the GOPS method was less effective than the APTS based one. It was found that there was no significant difference between the performances of the microreactors with urease immobilised from the buffer solution of pH 7.0 and pH 8.0 [17].

4. Separation Membranes

If the biosensor is to be used for an on-line invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects, and it should also be sterilisable. In the later case, the biosensor should not be prone to proteolysis, which is very difficult in practice.

In reaction to the above-mentioned problems and biocompatibility of the biosensors, which are designed for *in vivo* monitoring, the microdialysis based sampling technique was introduced [18]. This technique provides samples that are protein- and large molecular components-free. The microdialysis probe is constructed based on a hollow fibre made of semi-permeable membrane. To withdraw a sample, the probe is inserted into an examined object e.g. tissue and then flashed with a perfusion liquid. The bidirectional mass transport through the membrane allows collecting the sample formed in the probe (dialysate). Afterwards the sample is transported to an analyser. The analytical part – sensors are actually located off-stream and measurements are performed in the pre-treated sample i.e. the dialysate.

The project realised at the University of Twente (The Netherlands) was focused on an integration of the microdialysis based lab-on-a-chip type device consisting of microdialysis probe, flow-through electrochemical sensor array and calibration facility. The microdialysis probe, so called dual-lumen or needle type probe, was made in a form of coaxial tube, where the external tube was made of a semi-permeable membrane and the internal one was a glass capillary. The internal tube was used to provide perfusion liquid (Fig. 3).

In order to realise a liquid handling system with a minimal dead volume, the microdialysis was directly connected to a glass-silicon chip. It was concluded that

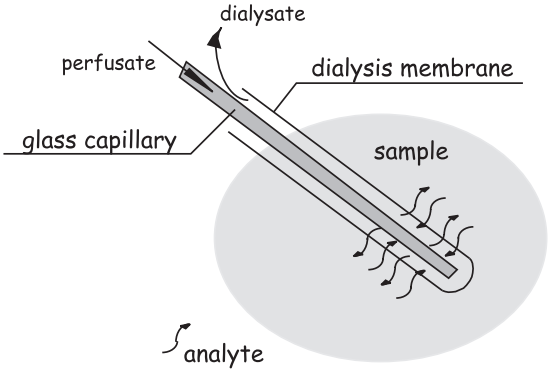

Fig. 3. Concept of a needle type microdialysis probe

in the case of the integrated micromachined microdialysis probe and sensors, the lag time was significantly reduced in comparison to a combination of sensor and conventional probe.

A specific disadvantage of conventional microsensors is their planar construction, which cause some problems with their implementation in microfluidics systems. For the purpose of integration of the microdialysis based system, generic flow-through electrochemical sensors were developed [19, 20].

As proposed, the microchannel itself is an integral part of the sensor geometry and it is formed by a tubular semi-permeable membrane. A micro reaction cell filled with enzyme solution or ionophore cocktail and internal electrolyte was formed around this membrane. This semi-permeable tubing, similar to that used in the microdialysis

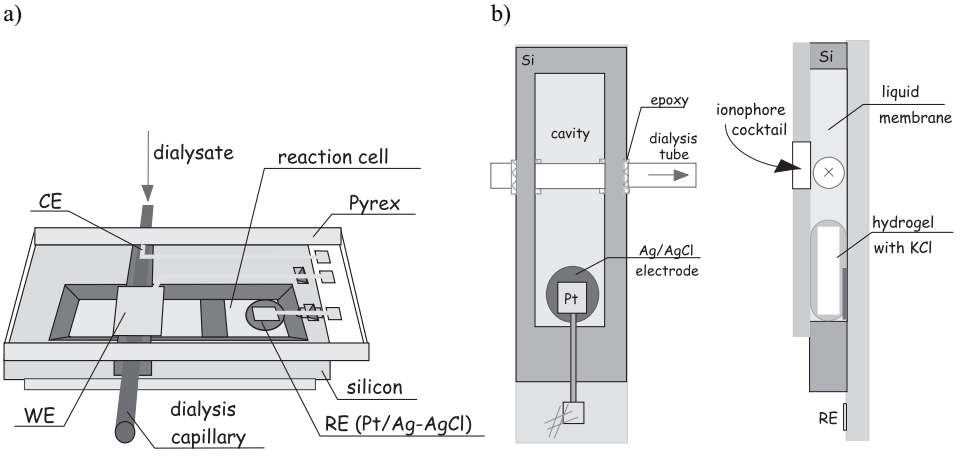

Fig. 4. Layouts of flow-through amperometric (a) and potentiometric (b) sensors based on a semi-permeable tubing made of regenerated cellulose, OD = 300 μm, implemented in silicon

probe, formed a tubular frit for the separation of dialysate to be analysed and a cavity filled with appropriate solution. It results in a stable interface between two phases, allowing only the passage of the small molecules of interests. Then, flow-through amperometric micro enzyme sensors and potentiometric sensors based on semi-permeable dialysis tubing carrying the sample were developed. As the dialysis tubing is impermeable for large molecular species such as enzymes, this approach does not require any immobilisation chemistry, and as a consequence the enzyme is present in its native free form. By adopting this approach, sensors for lactate, glutamate and glucose were successfully implemented [19]. The sensitivity of the potentiometric potassium sensors varied from 50 to 55 mV/dec with a good selectivity over sodium ions [20].

5. Summary

A comprehensive review of application of the membranes in biosensors, including the ion-selective, enzymatic and semi-permeable membranes was presented.

The ion-selective membranes were described in terms of factors determining their stability, which depends on lipophilicity of the membrane components. To reduce leakage of the membrane components, the plasticizer-free membranes and ionophore of increased lipophilicity were used.

In the case of the enzymatic membranes, stability of the biosensor depends on proper anchor of enzyme molecules to their surface or in membrane bulk. Various methods of the enzyme immobilisation in and/or within the supports with different functional groups were analysed. To obtain functional groups on the support surface, various methods of chemical modifications were applied.

Finally, to construct lab-on-a-chip type devices, the semi-permeable membranes were used as protective membranes preventing protein fouling on the biosensors' surface and to build a sampling probe. An example of use of the semi-permeable membrane made of regenerated cellulose for development of integrated microanalysis device consisting of microdialysis sampling probe and the flow-through biosensor was presented.

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