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Registration of heavy metal ions and pesticides with ATR planar waveguide enzyme sensors

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Abstract

The proposed novel type of enzyme optical sensors is based on a combination of $SiO_2/Si_3N_4/SiO_2$ planar waveguide ATR (attenuated total reflection) transducer, fabricated by standard silicon planar technology, with the composite polyelectrolyte self-assembled coating containing both organic chromophores and enzyme molecules. Such devices were deployed to monitor typical industrial and agricultural water pollutants, such as heavy metal ions and pesticides, acting as inhibitors of enzyme reactions. The sensitivity of registration of these pollutants in the range of 1 ppb was achieved. The use of different enzymes in the sensitive membrane provides a background for pattern recognition of the above pollutants.
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Keywords: Attenuated total reflection; Planar waveguide; Enzyme reactions; Inhibition of enzyme activity; Heavy metal ions; Pesticides

1. Introduction

Control of the pollution of the environment with heavy metals, pesticides and herbicides, emitted as a result of industrial and agricultural activities, is one of the most important ecological problems nowadays. The development of a wide range of very sensitive and specific to every particular polluting agent sensors is very difficult if not impossible task. An alternative approach lies in the development of universal sensing elements capable of the registration of a wide range of analytes of different chemical nature. Enzyme sensor is one of the possibilities to explore, since the catalytic activity of enzymes can be selectively suppressed (inhibited) by some environmental pollutants [1].

A number of enzyme sensors, mostly electrochemical (either amperometric or potentiometric) are known and commercially available nowadays [2–4]. A good example of enzyme sensors for environmental pollutants control is the $Si/SiO_2/Si_3N_4$/electrolyte capacitor sensor array having different enzymes immobilised in disposable cellulose membranes [5]. However the sensitivity of the above sensors is limited on the level of 10^{-6} M or 1 ppm. At the same time the European environmental legislation demands the concentration of heavy metals and pesticides in drinking water not to exceed tens of parts per billion. This can be achieved with much more sensitive optical transducers and particularly with planar waveguides. During last decade planar waveguides have been exploited in different transducing regimes, such as interferometry [6,7], fluorescence [8–11] and attenuated total reflection (ATR) [12–14], for different chemical and bio-sensing applications.

Another important part of sensor devices is the sensitive membrane. A relatively new method of

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polyelectrolyte self-assembly [15–17] provides a unique opportunity of building composite membranes, which may contain optically and bio-active components, for example, chromophores and enzymes.

Our previous study showed that the combination of a sensitive ATR planar waveguide transducer with composite polyelectrolyte film containing chromophore/enzyme pairs provides a very sensitive technique capable of registration of enzyme reactions as well as their inhibition by heavy metal ions and pesticides in ppb range of concentration. In the present work, we extended the range of analytes by using different enzymes and therefore made a step towards the development of sensor arrays.

2. Experimental

2.1. Planar waveguide transducer

The main idea of the proposed optical transducer lies in multiple reflections of the light beam in the planar waveguide, having a large difference in the refractive indices of the core and cladding. A typical waveguide of this kind, shown in Fig. 1, consists of a silicon nitride (Si_3N_4) core layer having thickness $d_2 = 190$ nm sandwiched between two thick silicon oxide (SiO_2) layers ($d_2 = 190$ μm). The refractive indices are typically $n_1 \approx 1.46$ and $n_2 \approx 2$ for SiO_2 and Si_3N_4 layers, respectively. The above parameters for core and cladding layers have been chosen to

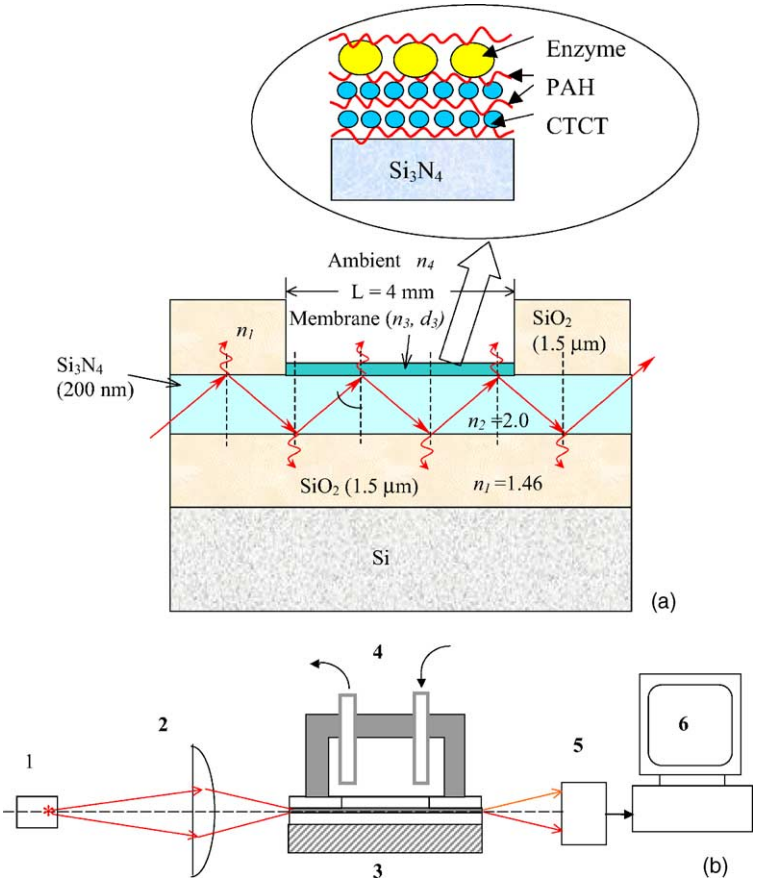

Fig. 1. (a) The structure of the planar waveguide and the light propagation scheme. Composite membrane is schematically shown in inset. (b) The experimental set-up for ATR measurements comprising a laser diode ($\lambda = 630$ nm) (1), semi-cylindrical lens (2), planar waveguide (3) with the reaction cell (4), photodetector (5), interfaced to PC (6).

provide a single mode light propagation [6]. Such waveguides were produced by standard silicon planar technology. A sensing window of 4 mm × 6 mm, etched in the top SiO_2 layer, can be coated with a sensitive membrane having refractive index $n_3 \approx 1.4$, which is typical for organic films.

The angle of light propagation through the above SiO_2/Si_3N_4 waveguide can be calculated using two following equations for the mode number m of the transverse magnetic (TM) wave [18]:

$$m = \frac{2n_2 d_2}{\lambda}\cos\theta_m - \frac{\phi_m}{\pi} \quad (1)$$

and for the phase change ϕ_m on reflection at the boundary with the SiO_2 layer [18]:

$$\phi_m = 2\tan^{-1}\left(\frac{\sqrt{\sin^2\theta_m - (n_2/n_1)^2}}{(n_2/n_1)^2\cos\theta_m}\right) \quad (2)$$

Here θ_m is the angle of incidence at the interface between the Si_3N_4 and SiO_2 layers. Using the wavelength $\lambda = 630$ nm, the self-consistent solution of Eqs. (1) and (2) gives the values of $\theta_m \approx 47^\circ$ and $\phi_m \approx 16^\circ$ for the single mode propagation. The number of reflections in the waveguide can be calculated as

$$N = \frac{L}{2d_2}\cot\theta_m \quad (3)$$

yielding $N \approx 10^4$ for the waveguide length $L = 4$ mm.

If the sensitive membrane has a non-zero absorption coefficient, the optical loss will occur each time reflection takes place at the core/membrane interface. In this case attenuation coefficient will be governed by the Beer's law:

$$I = I_0\exp(-\alpha_3 d_3 N) \quad (4)$$

where α_3 and d_3 are the absorption coefficient and thickness of the membrane, respectively. It was assumed that d_3 is much smaller than the penetration depth of the evanescent field, which is right for the 10–12 nm thick membranes used in our experiments.

Multiple reflections in a planar waveguide can provide an extremely sensitive tool for the registration of small changes in optical absorption in the membrane. As has been shown earlier [19], the above ATR transducer provides the sensitivity in about 700 times higher than conventional UV-Vis spectroscopy.

2.2. Experimental set-up, samples preparation and measurements routine

The experimental set-up, shown schematically in Fig. 1b, was described previously in detail [19]. The sensitive membranes were produced using the technique of electrostatic self-assembly, as described in detail in [19,20], and consist of 3–4 bilayers of polyallylamine hydrochloride (PAH) (Aldrich)/cyclotetra-chromotropylene (CTCT) and a layer of enzyme (either urease or acetylcholine esterase (AChE) (both from Sigma-Aldrich) deposited on top. The resulted membrane structure is shown as inset in Fig. 1a.

The enzyme reactions were studied by recording the outcoming light intensity during the injection the 100 mM aqueous solution of respective substratum into the cell. The urea and acetylcholine chloride (both from Sigma-Aldrich) were used as respective substrata for the enzymes urease and acetylcholine esterase.

The effect of heavy metal ions Cd^{2+} and Pb^{2+} as well as pesticides imidacloprid (from Wilkinson's) and 2,2-dichlorovinyl dimethyl phosphate (DVDP) (from PestAnal) was studied in the mixed aqueous solutions containing either respective salts $CdCl_2$ or $Pb(NO_3)_2$ or above pesticides with substratum. The concentrations of the above heavy metal salts and pesticides, varied in the range from 100 ppm down to 1 ppb, were obtained by multiple diluting the initial 10 mM (10 000 ppm) solutions. Alternatively, the samples were first soaked in the solutions of the above pollutants followed by the registration of the enzyme reactions in pure substrata solutions. In both cases the residual activity of enzymes $\Delta R\,(\%) = |\Delta I|/|\Delta I_0|$ was analysed, where $|\Delta I_0|$ and $|\Delta I|$ are the absolute values of changes in the light intensity in the initial pure substratum solution and after the contact with pollutants, respectively.

3. Results and discussion

Typical responses of planar waveguides coated with composite CTCT/urease films are shown in Fig. 2a. The intensity of the outcoming light is going down on course of the reaction of urea decomposition catalysed by enzyme urease (curve b). The observed attenuation of the light intensity is a consequence of

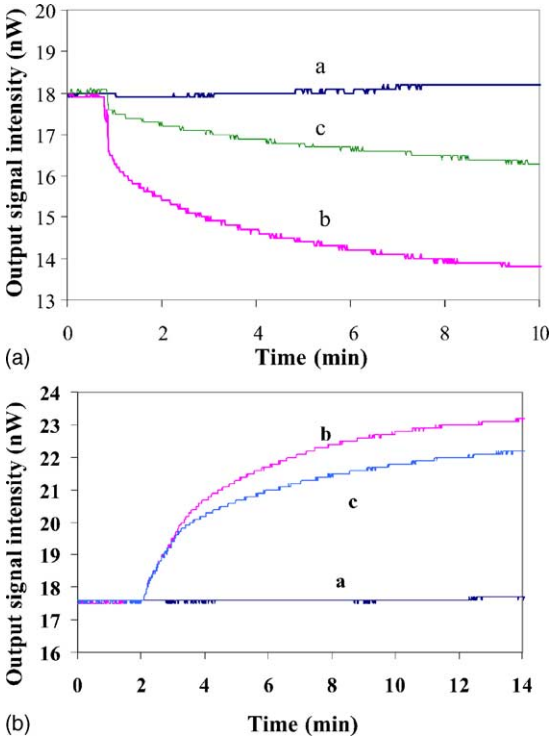

Fig. 2. Typical responses of the planar waveguide enzyme sensors on the injection the respective substratum solutions into the cell: (a) the reaction with 100 mM urea solution: (curve a) CTCT membrane without enzyme urease; (curve b) CTCT/urease membrane; (curve c) CTCT/urease membrane after exposure to 10 ppb solution of imidacloprid. (b) The reaction with 100 mM acetylcholine solution: (curve a) CTCT membrane without enzyme AChE; (curve b) CTCT/AChE membrane; (curve c) CTCT/AChE membrane after exposure to 1 ppb solution of DVDP.

the increased absorption coefficient at 630 nm due to the deprotonation of CTCT chromophore [21,22]. Injection of the buffer solution containing 10 ppb of imidacloprid also resulted in a decrease in the signal (curve c) though smaller as compared to untreated membrane (curve b). The observed behaviour can be understood in terms of the inhibition of the activity of enzyme urease by imidacloprid pesticide. The test experiment shows that the membrane without urease gave no response to injection of urea solution (curve a), which excludes the effect of pH of mixed substrata/inhibitor solutions. Two different experimental approaches to study the inhibition effect gave similar results, which also excludes the of pH changes in mixed solutions.

Similar experiments were undertaken on waveguides coated with CTCT/AChE membranes, and the typical responses are shown in Fig. 2b. This time, the injection of the substratum acetylcholine causes an increase in the outcoming light intensity (Fig. 2b, curve b). Such behaviour reflects the decreased optical absorption in the membrane at 630 nm due to the protonation of CTCT chromophore. The presence of 1 ppb DVDP pesticide inhibits the activity of AChE and therefore reduces the sensor response (Fig. 2b, curve c). The test experiment on the membrane without enzyme AChE showed no response (Fig. 2b, curve a).

The effect of both imidacloprid and DVDP pesticides on the planar waveguides coated with CTCT/urease membrane is illustrated by the bar-chart diagram in Fig. 3a. As one can see, DVDP is a stronger inhibitor for urease as compared to

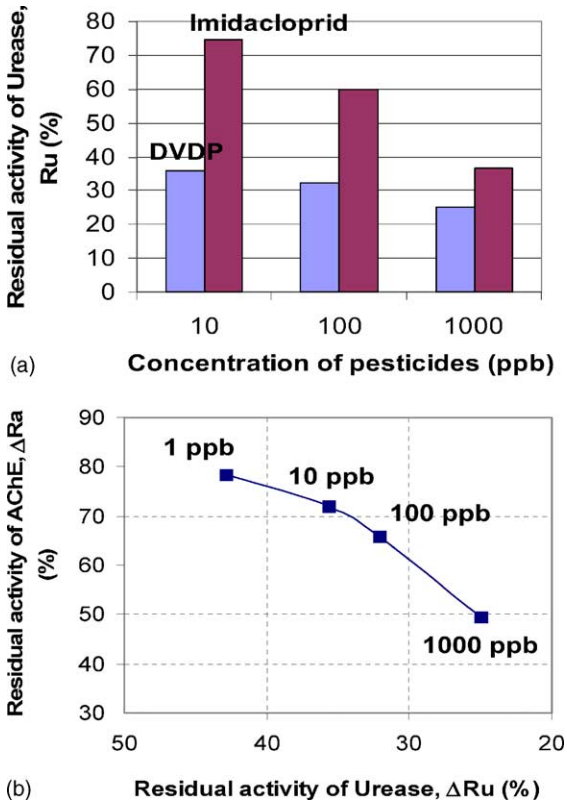

Fig. 3. (a) Bar-chart diagram of the residual activity of urease inhibited by pesticides DVDP and imidacloprid; (b) comparison of the residual activities of enzymes urease and AChE after their inhibition by DVDP.

imidacloprid. The comparison of the two sensors having CTCT/urease and CTCT/AChE membranes, presented in Fig. 3b, shows, that AChE is much less affected by DVDP than urease. The results presented in Fig. 3b contradict previous observations of the inhibition of AChE by phosphoorganic pesticides [1,5]. However the concentrations of inhibitors studied in those works were in three orders of magnitude higher than in our case.

Another important issue is the sensor recovery. Our study shows, that DVDP causes permanent inhibition of the activity of both studied enzymes, while the effect of imidacloprid on urease is not permanent. Almost full recovery of urease activity was registered after flushing the cell with pure buffer solution [19]. Because of this, the response of CTCT/urease membranes on imidacloprid was studied by injection of mixed urea/imidacloprid solutions. The above comparison proves that imidacloprid is less toxic than DVDP, and its commercial usage as a substitution of DVDP is justified.

A series of experiments on the inhibition effect of Cd^{2+} and Pb^{2+} ions were carried out on planar waveguides coated with both CTCT/urease and CTCT/AChE membranes. The dependence of the residual activity of enzyme urease on different concentrations of Cd^{2+} and Pb^{2+} ions is illustrated by the bar-chart diagram in Fig. 4a. As one can see, lead is a stronger inhibitor for the enzyme urease than cadmium. The comparison of responses of CTCT/urease and CTCT/AChE membranes, given in Fig. 4b, shows that Pb^{2+} ions cause much less effect on AChE activity than that on urease. The effect of heavy metal ions on the activity of both urease and AChE is permanent; since there was no recovery of the initial sensor response after thorough flushing the system with water and pure buffer solution. We have to accept the fact that the proposed devices operate as single-shot sensors or probes. In order to reduce the cost, further work should be focused on the development of disposable enzyme containing membranes, as it has been done in Ref. [5].

The obtained results showed an obvious possibility of pattern recognition of heavy metal ions (Cd^{2+} and Pb^{2+}) and pesticides (imidacloprid and DVDP) by using two planar waveguides coated with urease or acetylcholine esterase because of their different responses to different pollutants. Further work towards the development of the enzyme sensor array based upon ATR planar waveguide transducers is currently underway.

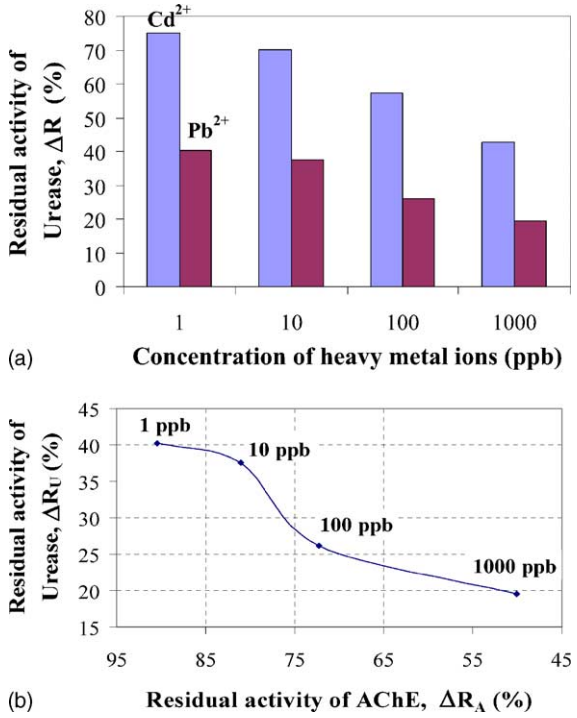

Fig. 4. (a) Bar-chart diagram of the residual activity of urease inhibited by Pb^{2+} and Cd^{2+} ions; (b) comparison of the residual activities of enzymes urease and AChE after their inhibition by Pb^{2+} ions.

4. Conclusions

The ATR transducer based upon $SiO_2/Si_3N_4/SiO_2$ planar waveguide having large difference between refractive indices of core and cladding provides a sensitive tool for the registration of small changes in light absorption in the membrane.

The composite membranes containing pairs of organic chromophore (CTCT) and enzymes (either urease or acetylcholine esterase) were deposited on the sensing window of the above transducers using the universal technique of polyelectrolyte self-assembly.

The above sensor is capable of registration of the enzyme reactions and their inhibition by heavy metal

ions (Cd^{2+} and Pb^{2+}) and pesticides (DVDP) and its recent and less toxic substitution imidacloprid). The remarkable result is that the traces of the above pollutants in concentrations down to 1 ppb can be registered with the proposed sensor. The results of the present study provide a background for further development of sensor arrays, containing different enzyme/chromophore pairs, capable of registration and recognition of different industrial and agricultural pollutants in extremely small concentrations. One of the possible applications is the control of water quality in the supply and natural resources.

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