PROCEEDINGS OF THE INTERNATIONAL CONFERENCE
NANOMATERIALS: APPLICATIONS AND PROPERTIES
Vol. **2** No 4, 04NABM24(2pp) (2013)

Conductometric Biosensor Based on Urease, Adsorbed on Silicalite for Determination of Urea in Serum Samples

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(Received 13 June 2013; published online 01 September 2013)

The method of enzyme adsorption on nano- and microsized zeolites, developed by us, is described. It is notable by such advantages as simple and fast performance, the absence of toxic compounds, high reproducibility and repeatability. The biosensor based on the method developed was applied for urea measurement in samples of blood serum. It was shown that the biosensor could surely distinguish healthy people from people with renal dysfunction. Good results reproducibility was proved at urea determination in real samples of blood serum (RSD = 10%). For these reasons, the biosensors based on enzyme adsorption are more suitable for standardization and production than those based on conventional methods of immobilization.

Keywords: Zeolite, Enzyme adsorption, Urease, Conductometric transducer, Biosensor

PACS numbers: 87.85.fk, 82.33.Jx

1. INTRODUCTION

Zeolites are micro- and nanosized materials that contain a crystal lattice formed by aluminum and silicon atoms. A type of zeolite that contains only silicon in a lattice is called silicalite. Zeolites are characterized by such important properties as chemical and physical stability and high surface area. Each crystal contains large amount of nanopores. This is why zeolites are considered to be good adsorbents.

Most properties of zeolites can be controlled during synthesis. It is possible to produce zeolites with different surface groups, Si/Al ratio, and size of crystal. Acidic and alcaline properties also can be changed. For these reasons, it is possible to synthesize zeolites with characteristics that are well-suited for certain application.

The oldest and the simplest method of enzyme immobilization is physical adsorption on a certain carrier. Usually enzyme adsorption implies neither additional chemical reagents nor activation; therefore, this is the least denaturing method of immobilization, which provides good retention of the enzyme activity. Besides, adsorption is commercially attractive due to lower cost of its performing as compared with other immobilization methods. An actual disadvantage of adsorption is a need in large amount of enzyme and gradual washing-out of the enzyme into working solution [2, 3,4].

A perspective approach in the improvement of adsorption is synthesis of artificial adsorbents, microparticles in particular, which are promising enzyme carriers. One of these particles, silicalite, a porous all silica crystal having two interconnected channel systems, was chosen for the present work.

2. EXPERIMENTAL

Silicalite was synthesized from a gel with composition TPAOH : 5 TEOS : 500 H2O. Tetraethylorthosilicate (TEOS, 95 %) was used as the silica source. Tetrapropylammoniumhydroxide (TPAOH, 25 %) was used as a template. Mixture was continuously stirred for 6 h at room temperature, and then resulting gel was placed in oven for 18 h at 125 °C. Resulting solid particles were centrifuged at 13,000 rpm, washed with deionized water and dried at 80 °C. The SEM images of synthesized silicalite showed that the prepared silicalite particles had size about 0.5-0.6 μm

A silicalite layer was formed on the surface of conductometric transducer by dip-coating. In our procedure 10% (w/w) silicalite solution in 5 mM phosphate buffer at pH 6.5 was used. Constant amount (0.165 μl) of silicalite solution was deposited in the active region of transducer, and then the transducer was heated for 6 min to 200 °C.

To prepare bioselective element by urease adsorption on silicalite, constant amount (0.15 μl) of 5% urease solution in 20 mM phosphate buffer, pH 6.5, was deposited on the active region of transducer (previously coated with silicalite); then the transducer underwent complete air-drying (17 min at room temperature). Next, the transducers were submerged into the working buffer for 10-15 min to wash off the unbounded enzyme.

3. RESULTS AND DISCUSSION

At the first stage of research, we studied influence of solution properties such as pH and ionic strength on the biosensor work. For example, it is known that optimal pH of the enzyme changes depending on the immobilization procedure. That is why we studied dependence of responses of the biosensor, based on adsorbed urease, on pH of working solution and compared the results with the biosensor based on urease immobilized in glutaraldehyde vapor. Working pH-optimum of the biosensor based on adsorbed urease was 6.0, while for the biosensor based on covalently bound urease it was 5.5. However, the response-pH dependences are alike for both methods tested.

An influence of ionic strength (KCl concentration) on the value of responses of the biosensor based on adsorbed urease was also studied; the responses of the biosensor decreased significantly while increasing ionic strength, i.e. at 50 mM concentration of KCl, biosensor responses (to a saturating concentration of substrate) decreased to 20% in comparison with the same responses without KCl. Thus, it is necessary to control ionic strength in order to decrease the measurement error while working with real samples.

Reproducibility and operational stability are important working characteristics of biosensors. To determine signal reproducibility, the biosensors responses to the saturation concentration of urea (2 mM) were measured during one working day with 10-15-min intervals, the sensors being kept in the continuously stirred buffer all the time between measurements. An error (relative standard deviation) of urea measurement was about 4%, what is enough for measuring real samples. After 10 days of storage, the biosensor responses decreased to 90% of initial value.

Important issues of our work were measurements in real samples of blood serum. The experiment goal was to prove that conductometric biosensors based on a new method of urease immobilization are suitable for measurement in samples of blood serum. We used the samples with excessive urea concentration taken from patients with renal failure, and two samples were taken from healthy people. Sensitivity and measurement accuracy of the biosensors used were quite sufficient for precise differentiation of the sera taken from patients and healthy people. We revealed good correlation between the values of urea concentration in samples of blood serum measured by the biosensor based on silicalite-adsorbed urease and those obtained by the traditional method of diacetyl monoxime reaction. The correlation coefficient was 0.995. It was also important to show repeatability of the biosensor signal at measurement in real assays. For this purpose, an assay with considerably higher than normal urease concentration was sequentially tested by the same biosensor ten times during one working day. The relative standard deviation between measurements was about 9 %. Certainly, in this case repeatability is significantly worse than when testing model samples, however, this is a common effect for impure real assays, in particular, blood plasma.

4. CONCLUSIONS

In the work, a possibility of efficient urease adsorption on silicalite for the purpose of biosensor creation was investigated. Procedure of urease adsorption on silicalite is notable by such advantages as simple and fast performance, non-use of toxic or auxiliary compounds. Optimal conditions for modifying transducers surface with silicalite and subsequent urease adsorption on these surfaces were selected; the working parameters of the created biosensor were optimized. The developed biosensor with adsorbed urease was characterized by good reproducibility and operational stability. The biosensor was applied for urea measurement in samples of blood serum. It was shown that the biosensor could surely distinguish healthy people from people with renal dysfunction. Good reproducibility of measurements was proved during urea determination in real samples of blood serum (RSD = 10%). For these reasons, the biosensor based on enzyme adsorption is perspective for standardization and production.

AKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of this study by Project European IRSES-NANODEVICE.

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