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Biosensor for urea detection based on sol-gel technology

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The sol-gel technology allows for the preparation of porous glass-like materials, which can be promising hosts for various organic molecules. In this paper the entrapment of urease in a sol-gel matrix is described. Since urease is an enzyme catalyzing the reaction of urea, it can be used for urea detection. The sol-gel bulk with immobilized urease serves as an optode of the urea fiber optics sensing system. It has been proved that urease encapsulated in the sol-gel matrix does not change its chemical activity The decomposition of urea causes an increase in pH. The changes in pH are measured by monitoring the changes in absorption of a pH indicator. In this study, the bromothymol blue was used. The results of measurements with the enzymatic sol-gel optode are presented.

1. Introduction

As the one of the main end products of protein metabolism in living organisms, urea is a primary source of organic nitrogen in soil (from animal urine, fertilizers, *etc.*). The proper level of urea is also essential for health of mammals. Therefore, monitoring the level of urea is important for medicine, as well as for environmental protection. Urease is an enzyme that breaks the carbon-nitrogen bond of amides to form carbon dioxide, ammonia, and water. This enzyme is widely used for the determination of urea in biological specimens. As it was mentioned, it catalyses the hydrolysis of urea to CO_2 and NH_3. The hydrolysis of urea in the presence of urease as a catalyst leads to an increase of the pH, proportional to urea concentration. This reaction is described below

$$\underset{\text{urea}}{(NH_2)_2CO} + 2H_2O \xrightarrow{\text{catalyst}} (NH_4)_2CO_3 \rightarrow CO_2 + 2NH_3 + H_2O.$$

Thus, by measuring the pH value, urea concentration can be found.

Enzymatic optical sensors are the subject of interest of many research groups [1]. Construction of the optode for urea biosensor requires the immobilization of protein (and pH indicator) in the host matrix. There are several methods enabling protein entrapment. One can use gels, polymers, saccharose, various meshes and membranes [2]. Recently, a new idea of enzyme entrapment has been described [3],

[4], proposing the application of sol-gel matrices. A broad range of possible applications of sol-gel derived materials has marked this technology as one of the most promising fields in contemporary material sciences [5].

The sol-gel process offers an ideal route for the production of variously shaped monolithic materials, thin films, fibers, powders, *etc.* The sol-gel derived materials provide excellent matrices for a variety of organic and inorganic compounds.

2. Preparation of sol-gel matrices

The sol-gel production process comprises several steps that are in detail described in the literature [5], [6]. First, a silicate precursor is mixed with water and a catalyst and stirred for a few hours. This part of the process leads to the hydrolysis of the Si−O−R bonds. The acids or bases catalyze the hydrolysis reaction. The longer the hydrolysis continues, the larger amount of the Si−OR groups undergoes the hydrolysis to the Si−OH form. The final product is a rigid silica network.

Taking into consideration various specific applications, it is necessary to produce material with required physico-chemical features. The sol-gel process is quite sensitive to several factors that may influence the hydrolysis, condensation or aging, thus resulting in various characteristics of the final product. One of the important factors is a molar ratio R, defined as the ratio of the solvent moles number to the precursor moles number. The molar ratio is crucial for optical quality, deciding about transmittance of light in a visible range, refractive index, and scattering [7].

Our previous studies have demonstrated that good transparency in a visible range and mechanically stable silica sol-gel bulks can be obtained from such solutions in which molar ratio R equals 15 [8]. The silica sol-gel bulks used for this study were prepared to have a cylindrical shape, 5 mm heigh and 10 mm in diameter

3. Construction of the optode for a urea biosensor

The sol-gel bulks were produced from the following components: tetraethyl-ortosilicate TEOS (98% Tetraethoxysilan from Aldrich) as a silicate precursor, double distilled water as a solvent, and hydrochloride acid as a catalyst (36% HCl, Polish Chemicals). The hydrolizate was stirred for 4 hours.

Urease (produced at the Institute of Biochemistry, University of Wrocław) was dissolved in a phosphate-oxalate buffer. The buffer was obtained out of 0.03 g of oxalic acid (Polish Chemicals) dissolved in 50 ml of water and of the suitable volume of 0.2136 g of disodium hydrogen phosphate dodecahydrate $Na_2HPO_4 \times 12H_2O$ (Polish Chemicals) dissolved in additional 50 ml of water. The phosphate-oxalate buffer was prepared with pH 6.

The solution of 0.05 g bromothymol blue (Polish Chemicals) in 100 ml of water was used as a pH indicator. This compound was chosen due to its good solubility in water and clear spectral properties depending on pH. An acid form of bromothymol blue is yellow at pH 6 and its base form (at pH 7.6) is blue.

The optode was prepared from a 10-ml mixture which contained 9 ml of cold hydrolyzate (4 °C), 1 drop of bromothymol blue solution, and 1 ml of the buffer with 0.04 g urease. Such a mixture was stirred by means of a magnetic stirrer for 15 s. Since the bromothymol blue is best acting in a pH range 6 – 7.6, the pH of the sol-gel bulks obtained in the experiment was chosen to be pH 6. Therefore, after the precursor hydrolysis, the pH of the obtained homogenous hydrolyzate was gradually brought from 2 up to 6 using a diluted ammonia solution (2.5 ml in 50 ml of water). This resulted in quick (several minutes) gelation and formation of the "wet" gel. The optodes were then aged at 4 °C for 1 week. The final material was a dry xerogel bulk, mechanically stable.

The urease activity in the sol-gel matrix strongly depends on a production process (*e.g.*, temperature and pH). The pH of the mixture is temporary as low as 2. In acid environment the activity of this enzyme may be disturbed. Therefore, mixing of the substrates was performed in a container with ice, to keep the constant temperature of 4 °C.

In our previous experiments we have stated that the addition of an organic dopant to the sol-gel increases the absorption of a matrix [9]. These changes were time-dependent and the freshly prepared bulks showed smaller absorption than those stored longer at room temperature. Higher transparency can be preserved for longer time, if optodes are stored in lower temperatures.

4. Experimental tests

The above described enzymatic optode can measure the urea concentration in liquids. The tests were performed spectrophotometrically, in visible light by means of a computer-aided Ocean Optics spectrophotometer. The experimental set-up is depicted in Fig. 1. A tungsten halogen lamp served as a light source. The light was guided via a fiber-optic cable to the optode and placed in a container with the urea solution. After passing the optode, the light was coupled to the output fiberoptic cable, which was connected at the distal end to a detector. An analogue-digital converter transmits the output signal to the computer.

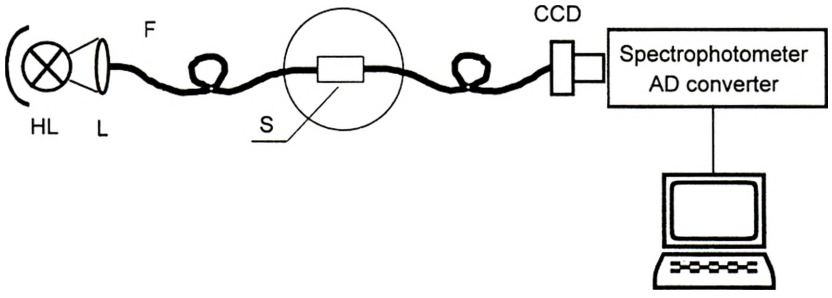

Fig. 1. Experimental set-up. HL – halogen light source, L – coupling lens, F – optical fiber, S – container with urea solution and an optode, CCD – detector connected with the computer.

Before measurements, the optodes taken from the refrigerator were incubated at 36.6 °C for 5 minutes. This temperature ensures the physiological conditions necessary for urease action. In human body the physiological level of urea may vary from 21 mg/dl to 53 mg/dl (3.5 – 9 mmol/l). For that reason, the calibration measurements were performed for a priori defined urea solutions between 21 mg/dl and 53 mg/dl. The absorbances were continuously measured and at the moment when the system became stable they were recorded.

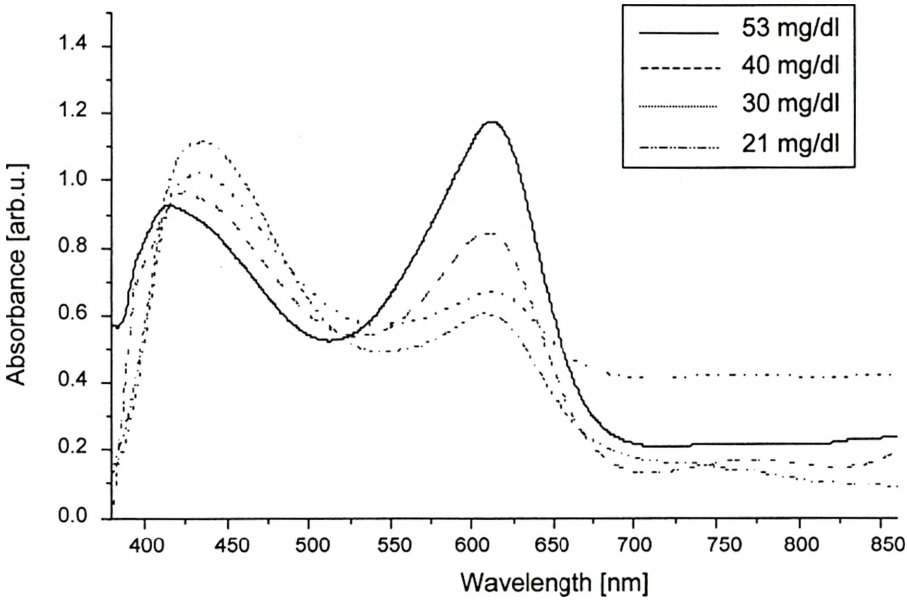

Fig. 2. Absorbance of an optode (urease and bromothymol blue as an indicator entrapped in a sol-gel matrix) in the presence of urea in the environment.

Figure 2 presents the results of absorbance measurements of the optode placed in the urea environment. The exemplary results for urea concentrations amounting to 21 mg/dl, 30 mg/dl, 40 mg/dl and 53 mg/dl, respectively, are depicted.

As one can see, the changes of the absorbance maxima depend on concentrations of urea. For higher concentrations of urea the absorption peak in the blue region decreased and in the red region increased. Thus, it is possible to determine the urea concentration either from measurements of the peak amplitude in the blue region (decreasing as the concentration grows) or from measurements of the peak amplitude in the red region (increasing as the concentration grows).

Cheap biosensors technology is essential in many applications. Undoubtedly, the light source is an importance part of each optical biosensing system and it can strongly influence the commercial aspect. Therefore, it is important to implement the cheapest light sources, if it is only possible in a given kind of

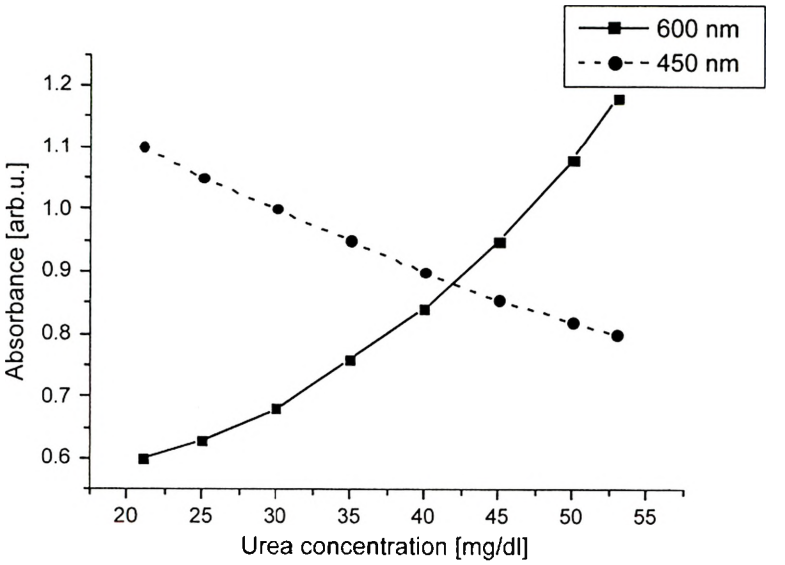

Fig. 3. Calibration curves. The absorbance *vs.* urea concentration recorder for 450 and 600 nm.

measurement. As one can see from the diagram in Fig. 2, the suitable wavelengths are 450 nm or 600 nm. The absorbance measurements for 450 nm and 600 nm are shown in Fig. 3. Such diagrams can serve as calibration curves of the optode for urea measurements.

4. Conclusions

It has been demonstrated in this work that sol-gel material can be exploited as a host matrix for the construction of an enzymatic optode. First, urease decomposes urea most rapidly in the environment of from pH 6 to 7.6. This is also the optimal range for such an indication dye as bromothymol blue. Therefore, the gelation point was chosen to be at the moment, when the pH level of mixture was equal to 6. The gelation point, *i.e.*, the moment of forming the spanning cluster initiates the creation of an interconnected silica network with pores. Moreover, pH equal to 6 results in quick gelation of an optode. Second, at pH 6 one can observe clear absorption changes (caused by the decomposition of urea), which enable accurate measurements. The porosity ensures not only the entrappment of necessary molecules (urease and pH indicator), but it results in reversible reactions with measurand (urea), as well. Immobilized active substances can react with the environment only if they are trapped in the so-called open pores. Open pores are the pores which have a direct contact with surroundings. Some active substances, however, are immobilized in closed pores, so they cannot react with the detected molecules. The proposed optode is optimally designed for the described here application. However, due to the production process each new optode should be calibrated separately.

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