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Influence of organic dopant – urease on sol-gel matrix optical properties in visible range

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The preparation of sol-gel derived materials in the form of bulks and thin films as well as bulks with entrapped urease as organic dopant is described. The absorbance spectra of urease solutions and sol-gel matrices were recorded in transmission mode. The absorbance of sol-gel matrices with embedded enzyme was measured in reflection mode.

1. Introduction

Urea is 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 monitoring of 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 determination of urea in biological specimen. As was mentioned, it catalyses the hydrolysis of urea to CO_2 and NH_3. Hydrolysis of urea in the presence of urease as catalyst gives an increase in pH proportional to urea concentration. Thus, by measuring pH, urea concentration can be calculated.

Construction of the optode for urea biosensor requires immobilisation 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 [1]. Recently, a new idea of enzyme entrapment has been described [2], [3], 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 [4].

The sol-gel process offers an ideal route for production of variously shaped monolithic materials, thin films, fibres, powders, *etc.* The sol-gel-derived materials provide excellent matrices for a variety of organic and inorganic compounds. In the paper, we report on the optical properties of two types of sol-gel matrices with entrapped enzyme molecules. Since the projected sensor measures urea based on the visible changes in pH, absorbance is studied in visible spectrum.

2. Preparation of sol-gel matrices

The chemistry of the sol-gel process comprises several steps that are thoroughly described in the literature [4]. First, silicate precursor (*e.g.*, tetraethylortosilicate

TEOS) is mixed with water and catalyst and stirred for a few hours. This process leads to hydrolysis of the Si–O–R bonds. Acids or bases catalyze the hydrolysis reaction. The longer the hydrolysis, the larger amount of the Si–OR groups undergoes hydrolysis to the Si–OH form.

After hydrolysis the pH of the homogeneous hydrolysate gradually brought up to ca. 6 by means of diluted ammonia solution. This results in quick (several minutes) gelation and formation of the "wet" gel. Subsequently, the gel obtained can be aged for a few days in water or ammonia solution. This process reduces the mechanical stress during the drying of the gel and prevents, to some extent, the risk of the sample cracking.

For the purpose of our study, the enzyme urease was immobilised in sol-gel derived matrix. Two different types of the sol-gel matrices, bulk and thin film, were produced. The bulks were prepared from precursors in the following proportions: 24 ml H_2O:19 ml TEOS:1 drop of 36% HCl (TEOS – tetraethoxysilan 98% from Aldrich). The thin films were obtained from 85 ml of ethyl alcohol, 10 ml of TEOS, 3.2 ml of triton X-100 (Aldrich), 2 drops of 36% HCl. The enzyme solution was added to the matrices just after the hydrolysis process. All samples were dried for two weaks.

3. Absorbance measurements

The measurements of absorbance were performed using computer aided Ocean Optics spectrophotometer in the set-up depicted in Fig. 1. Tungsten halogen lamp served as the light source. The light was guided via fibre optics cable to the sample. After passing through the sample the light was coupled into the output fibre optics cable, which was connected at a distant end to the detector. Analogue-digital converter transmitted the output signal to the computer.

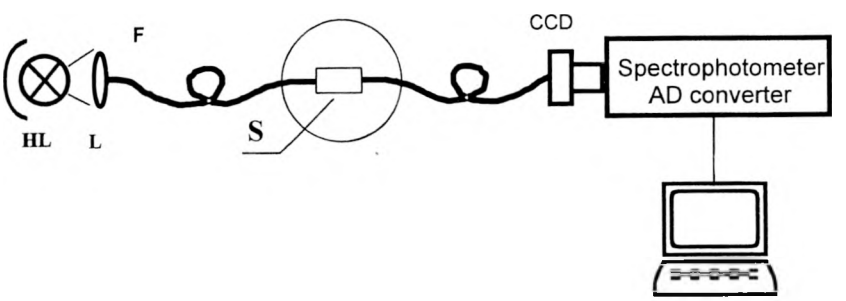

Fig. 1. Schematic set-up for absorbance measurement in transition mode. HL – halogen light source, L – coupling lens, F – optical fibers, S – sample holder for transparent solids and liquids, CCD – detector connected with the computer.

First, the absorbance of the urease solution in distilled water was measured. For reference, the spectrum of distilled water was taken. Two samples of urease solutions were studied: one of 0.2% and the other of 0.08% concentration. When examined with the naked eye, the transparent samples did not show any differences in visual

region. The measurement revealed the fact that the absorbance is slightly bigger in blue region and depends on concentration according to the formula

$$A = -\log(S/R) = \varepsilon cd$$

where: A denotes absorbance, S is the light intensity after passing through the sample, R – the reference intensity, ε – the extinction coefficient, c – the concentration of the absorbing sample, d – the path-length of the absorption. The spectra of urease solutions are presented in Fig. 2.

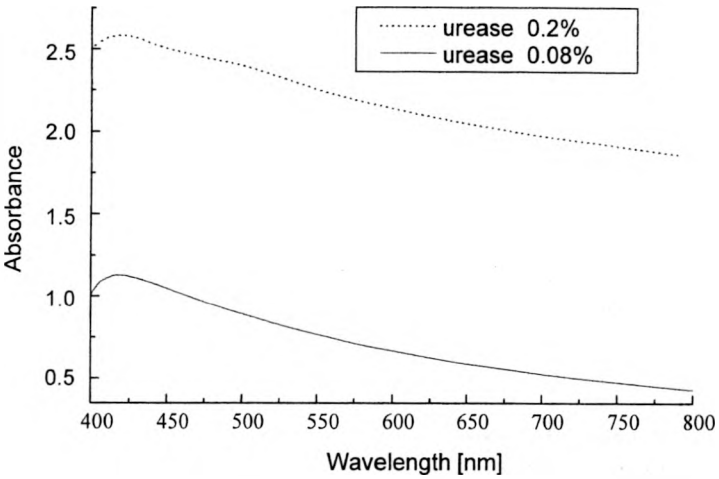

Fig. 2. Absorbance of urease solutions in the distilled water. The absorption length was 1 cm.

In the same set-up as in Figure 1, the absorbance spectra of the sol-gel matrices were recorded. The material was prepared according to the description given above. We produced sol-gel bulks and films in the form of cylinders of 1.5 cm in diameter and 5 mm in height. Additionally, a thinner (3 mm) sol-gel bulk was also prepared and examined. All the samples were placed in the holder so that the optical axis was perpendicular to the cylinder base. Although, when checked visually all the samples were highly transparent, they showed slightly different absorption. The lowest absorbance was observed for a thin sol-gel bulk, the highest for 5 mm thick bulk (see Fig. 3).

The samples with urease were produced by adding 1 ml of 0.08% urease solution to the mixture, just after hydrolysis. After gelation, the material turned to an opaque one, with milky colour. The samples were cylindrical with the same diameter as the matrices and the height equal to 3 mm. The spectra were recorded in the set-up shown in Fig. 4. The integrating sphere with built-in tungsten halogen lamp and coupled fiberoptic cable was used for absorbance measurement in the reflection mode.

The diagrams in Figure 5 illustrate the absorbance of matrix – 3 mm thick sol-gel bulk (solid line) and 3 mm thick bulks with entrapped urease. The dotted line

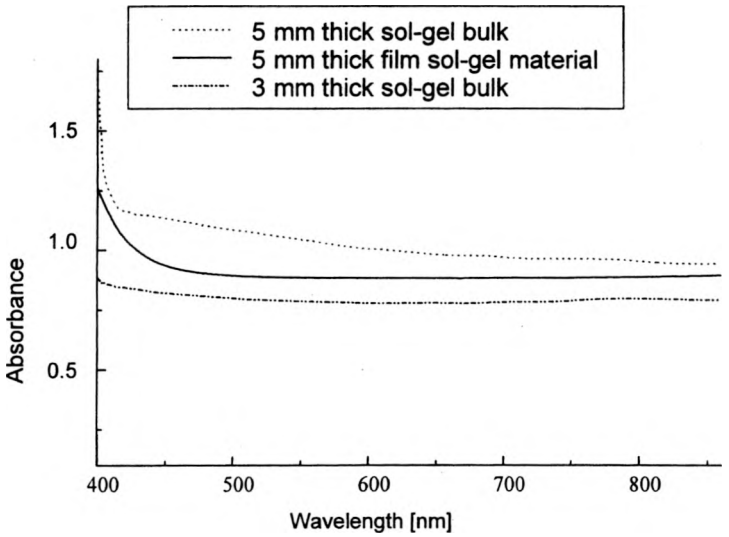

Fig. 3. Absorbance of the sol-gel materials. The dashed line represents the 3 mm sol-gel bulk. The absorbance in visible region remains almost constant.

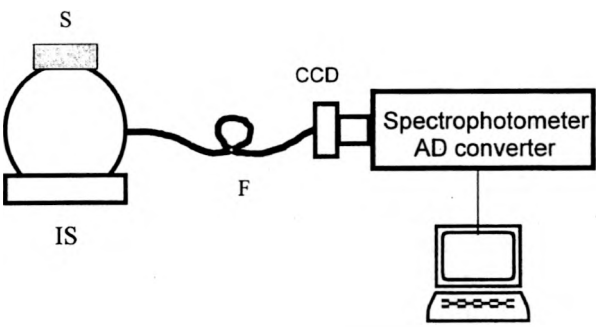

Fig. 4. Schematic set-up for absorbance measurement in reflection mode. IS — integrating sphere with built-in halogen light source, F — optical fibers, S — opaque sample, CCD — detector connected with the computer.

stands for the new material (two weeks old) and the dashed line for the three-month -old bulk. One can notice that the old material shows absorbance almost 4 times greater compared to the new material. Such behaviour was not observed in the case of pure sol-gel matrices. The aging of pure material had less significant influence on the absorption properties.

4. Final remarks

In the paper, we reported on absorbance measurement in visible spectrum of sol-gel derived materials. It was observed that the addition of organic dopant to the sol-gel matrix enhanced the absorbance. These changes were related to the aging time. This can be explained by changes of the pore sizes in the matrix, which tend to decrease

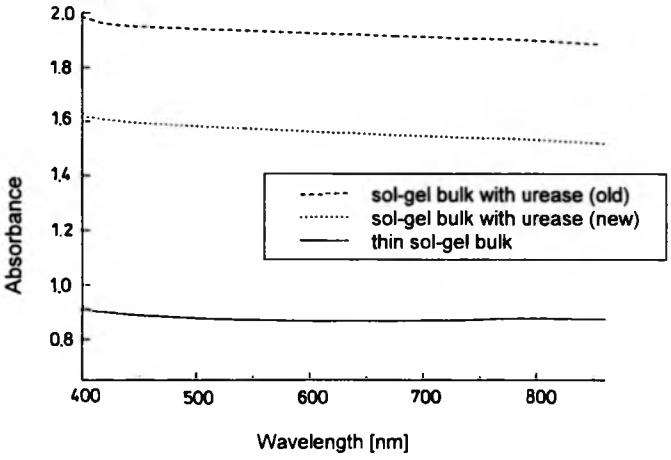

Fig. 5. Absorbance of urease entrapped in 3 mm thick sol-gel bulks compared to the pure matrix.

with prolonged drying. Most probably, this influences also the enzyme structure. For the future investigation some protecting molecules should be aided, *e.g.*, albumin or glycerol solutions. The characteristic properties of some proteins are visible in UV light, and UV spectrophotometric examination should be performed, as well.

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