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Porous glasses as a substrate for sensor elements

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The properties of porous glasses are determined by optical spectroscopy and high-resolution microscopy at different stages of immunoglobulin immobilization and after immune reaction. The influence of duration and temperature of drying between surface activation and silanization is studied. The quantity of protein immobilized on the porous glass surface is estimated by the Coomassie method. Various ways of surface silanization with the use of toluene and acetone are compared. The possibility of fabricating a microsensor element based on the porous glass for microchip is presented.

Keywords: porous glass, sensor element, laser scanning confocal microscopy, scanning near field optical microscopy, optical spectrometry.

1. Introduction

Designing microfluidic chip (MFC) devices is one of the perspective directions in the development of microanalytical systems. The chips make it possible to manipulate with picoliter volumes of samples and reagents, including dosing, mixing, carrying out chemical reactions, *etc*., [1, 2]. Microanalytical systems with the new properties and high technical characteristics can be developed by means of integrating the new elements into an MFC. These may be fabricated on the basis of porous glasses (PG) (for example, sensors elements, micropumps, columns, reactors, *etc*.)

Modern technologies made it possible to produce PG with pores of nanometer sizes (from 2 to 500 nm) and known structural characteristics [3, 4]. PG's optical properties allow using high-sensitivity methods for detection and registration of sample components.

In order to design an element of a sensor on the basis of PG it is necessary to choose optimal methods of PG surface activation, modification and sensitive substance immobilization. This requires studying optical and structural characteristics of glasses at different stages of preparation of the sensor element. So, characterization of glasses at different stages by methods of high resolution microscopy and optical spectroscopy is a topical issue.

2. Experiment

2.1. Motivation

In order to study the features of biological substance immobilization on a PG surface, the PG made of biphasic glass 8 V was used. It has a developed surface and high optical transmittance in visual and infrared spectral ranges (80–93% at a wavelength of 340–850 nm for a 0.2 mm sample thickness). The average pore radius of the samples is 85.5 nm, the specific pore surface is 88.8 m^2/g (Institute of Silicate Chemistry RAS). These parameters are suitable for designing sensor elements with good permeability for liquid probes. The PG size is 8×8×0.2 mm.

Fabrication of the sensor element required: activation of the hydroxyl groups on the PG surface, with the purpose to silanize the surface, to treat it with bifunctional reagent (glutaric dialdehyde) and to immobilize the sensitive substance (for example, biological substances like proteins, antibodies and antigenes).

In this technology, the covalent binding of the silan on the glass substrate is chosen because this immobilization type allows more firm protein fixation. But this method has a disadvantage: it makes probe diffusion difficult, so the sensor response time gets longer. Nevertheless, the PG allows using transportation of the probe to sensitive substance due to electroosmotic flow produced by the external electric field. It gives an opportunity to reduce the response time of the sensor.

2.2. Surface modification and sensor working principle

A reaction scheme for protein immobilization is outlined in Fig. 1. It was realized in static regime in four stages: surface activation (I), silanization (II), treatment with glutaric dialdehyde (III) and protein immobilization (IV) [5, 6].

For surface activation, samples were placed into 0.5 M NaOH for 0.5 hour. Silanization was carried out in two ways: *i*) immersion in a 10% solution of aminopropyltriethoxysilane (APS) in toluene at 90 °C for 2 hours; or *ii*) 4% solution of APS in acetone at 24 °C for 2 hours. Then, sample surfaces were treated with glutaraldehyde for 2 hours. Protein immobilization is based on Schiff's base formation between the amino groups on the protein surface and the aldehyde groups on a chemically modified surface of PG.

The principle of the sensor element action based on the immune reaction: IgG + (Insulin-FITC) ↔ IgG – (Insulin-FITC). Anti-insulin immunoglobulin IgG is immobilized on PG surface. Afterwards the immune reaction with insulin-FITC followed.

2.3. Measurement and instrumentation

During preparation of glasses the influence of drying conditions (time and temperature) between glass surface activation and silanization on protein immobilization was studied. At first, toluene was used for silanization, later – acetone.

At every stage of the protein immobilization and after immune reaction the characteristics of the samples were detected by transmittance spectroscopy,

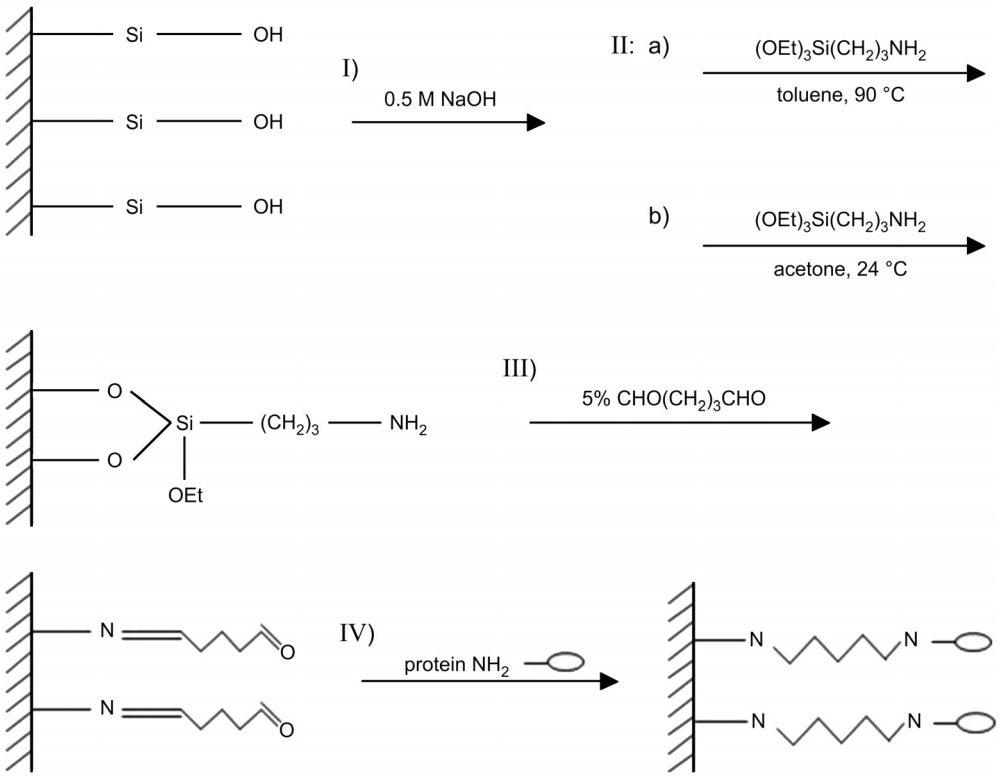

Fig. 1. Procedures for protein immobilization.

fluorescence spectroscopy, laser scanning confocal microscopy (LSCM) and scanning near-field optical microscopy (SNOM).

The optical transmittance spectra were measured by a Hitachi U-3410 spectrophotometer (Japan) at a bandpass of 3 nm, in the spectral range 350–850 nm. The fluorescence spectra were measured by a Hitachi F-4010 spectrofluorimeter (Japan) at a bandpass of 5 nm, scan speed 120 nm/min, excitation wavelength of 488 nm. Images of the surfaces of the samples were made by the laser scanning confocal microscopy TCS SL (Leica, Germany) in the mode of registration of reflection and fluorescence at the excitation wavelength of 488 nm and the scanning near-field optical microscopy NTEGRA Solaris (NT-MDT, Russia) in the modes of shear force and reflections at a wavelength of 488 nm.

3. Results and discussion

The influence of drying conditions (duration and temperature) between the surface activation (stage I) and silanization (stage II) on protein immobilization was studied. For that purpose, samples were prepared by drying for 2 hours at 50 °C, and for 1, 2 and 3 hours at 100 °C.

The images of the sample surfaces were made by laser scanning confocal microscopy. For the samples dried at 100 °C it visualized relatively large particles (with a diameter of nearly 10 mkm). After silanization (stage II) and glutaric dialdehyde surface treatment (stage III) the transmittance of the samples alters in a wide spectral range. A small "burnt out" site of the surface with lower fluorescence compared to the background on all the surfaces is observed in the images. The changes of the sample surface properties are due to laser radiation (for LSCM) at 488 nm (energy density ~500 W/cm^2).

When the sample is dried at 50 °C there are no big particles observed and "burnt site" is more degraded. Spectrophotometric measurements show that the sample dried at 60 °C has lower transmittance (~10%) in the spectral range 550–850 nm than for other samples; the sample dried for 5 hours has higher transmittance in the range 350–600 nm than for other samples. The above observations proved that drying regime exerts a significant influence on protein immobilization at PG (*i.e.*, on the volume quantity of the immobilized protein).

Protein immobilization was performed by immersing the pretreated glass samples in protein solution. The quantity of the immobilized protein was estimated by spectrophotometric detection of protein in solution before and after immobilization at PG (Coomassie method) [7]. The method was based on the dye colour change (from red-brown to blue) corresponding to absorbance peak shift from 465 to 595 nm after reaction with protein. These measurements show that only 0.202 mg (~16 mkg/mm^3) of the protein was immobilized on the sample, when toluene was used at the silanization stage (stage II, version *a*), while 0.302 mg (~24 mkg/mm^3) was immobilized when acetone was used (stage II, version *b*).

The absorbance peak at ~525 nm was observed at the spectrum of PG treated with glutaric dialdehyde. There is no such absorbance peak for the spectrum of the glutaric dialdehyde solution.

From the spectral transmittance dependences for the samples after immune reaction (Fig. 2) the fluorescence absorbance peak is observed at 495 nm. It confirms that successful immune reaction has been performed.

The treatment with glutaric dialdehyde results in reduction of sample transmittance. The immunoglobulin immobilization leads to additional transmittance reduction. The transmittance of the samples considerably increases (~15% for samples with the use of toluene for silanization (Fig. 2**a**) and ~45% with the use of acetone for silanization (Fig. 2**b**)) after the immune reaction has been carried out. This effect may be used for the future detection system design.

Figure 3 presents the normalized transmittance spectra of PG after the immune reaction and treatment with Coomassie solution. The measurements demonstrate that silanization with acetone results in immobilization of more protein compared to silanization with toluene.

Note that no fluorescence was observed in the case of initial (native) porous glass upon excitation at a wavelength of 488 nm.

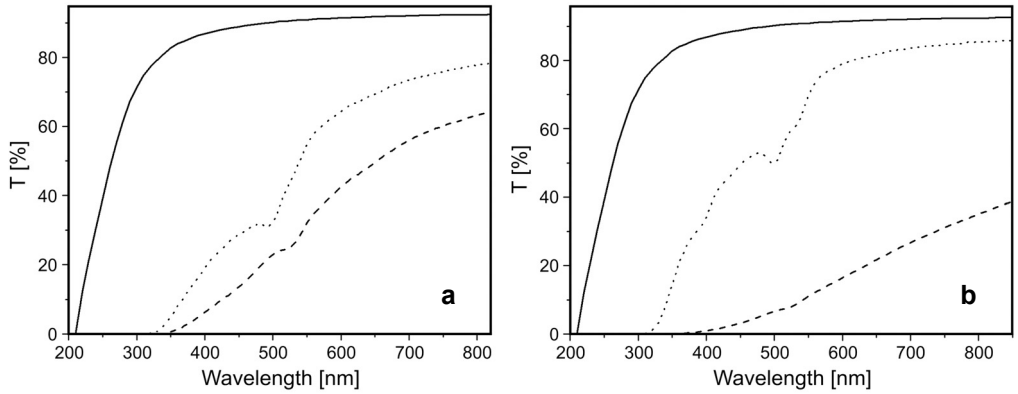

Fig. 2. Transmittance spectra of PG 8V-MAP: after silanization with toluene (**a**), after silanization with acetone (**b**); solid line – PG, dashed line – after immunoglobulin immobilization, dotted line – after immune reaction.

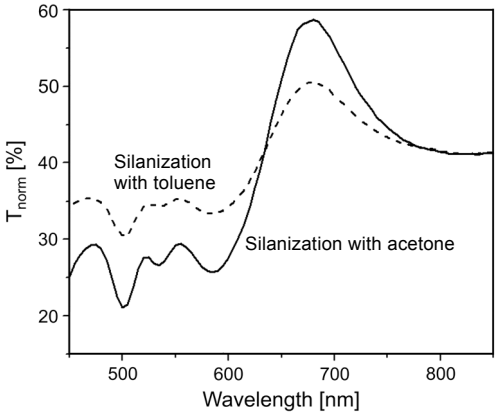

Fig. 3. Normalized transmittance spectra of PG after immune reaction and treatment with Coomassie solution.

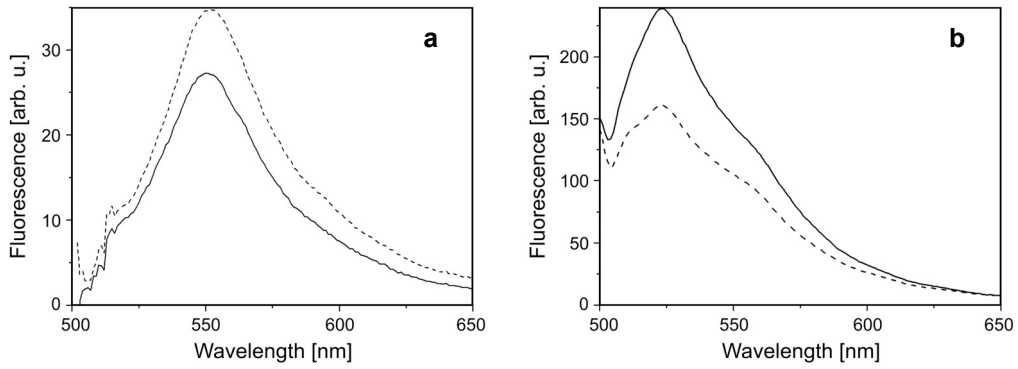

Fig. 4. Fluorescence spectra of PG: after immunoglobulin immobilization (**a**), after immune reaction (**b**); dashed line – silanization with the use of toluene, solid line – with the use of acetone.

Glutaric dialdehyde solution (which was used for protein immobilization) has a fluorescence peak at 550 nm at excitation wavelength 488 nm (Fig. 4**a**). This has made it more difficult to interpret the results obtained by laser scanning confocal microscopy because of the presence of a few fluorescence substances and not just one.

The fluorescence of insulin-FITS at 523 nm is significantly higher than the fluorescence of glutaric dialdehyde. Using acetone for silanization leads to the higher fluorescence peak of insulin-FITS for the samples after immune reaction than in the case of toluene (Fig. 4**b**). This proves that using acetone for silanization results in immobilization of more protein than in the case of using toluene.

Figure 5 presents images of the sample surfaces taken by scanning near-field optical microscopy in shear force and reflection modes. For the initial glass in both modes a near-to-smooth surface is obtained at image size 25×25 mkm. But at bigger magnification (image size 5×5 mkm) a porous structure appears. It corresponds to BET measurement (Institute of Silicate Chemistry RAS).

Groups of particles with average radius ~0.6 mkm are visualized by shear force mode after insulin immobilization at the sample surface. After immune reaction there may also be observed particles at the sample surface, but more uniform.

In the sample surface images after immune reaction (in reflection mode) one can see not only the particles formed, but the surface structure, too. Probably, this effect occurs because of the formation of a homogeneous surface film with structural elements greater than the sizes of the pores in the glass.

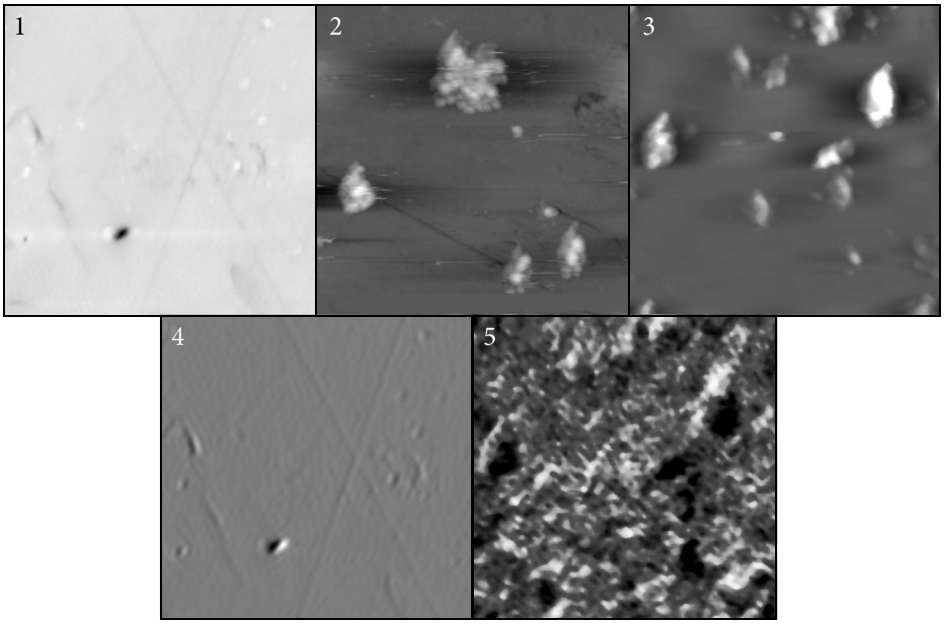

Fig. 5. Surface images of PG 8 V by SNOM. Shear force mode: 1 – initial glass, 2 – after insulin immobilization, 3 – after immune reaction; reflection mode: 4 – initial glass, 5 – after immune reaction. Images size 25×25 mkm.

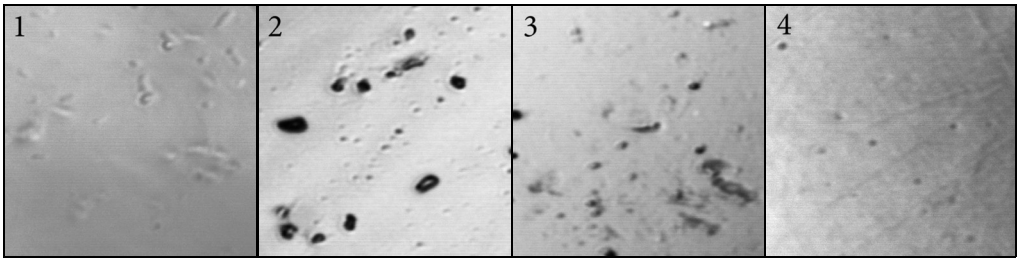

Fig. 6. Surface images of PG 8 V by LSCM: 1 – initial glass, 2 – after immunoglobulin immobilization (silanization with toluene), 3 – after immunoglobulin immobilization (silanization with acetone), 4 – after immune reaction. Image sizes 50×50 mkm.

The measurement by laser scanning confocal microscopy (Fig. 6) shows that fluorescent substances (glutaric dialdehyde/insulin-FITC) are adsorbed not only on the surface but diffused through the pores into the deep glass layers.

After immunoglobulin immobilization an PG surface some particles with significant absorption at excitation wavelength of 488 nm were visualized by laser scanning confocal microscopy. After immune reaction a homogeneous surface was observed. Obviously, this is due to the formation of a thin enough and more optically uniform layer of the immune complex on the surface. This is confirmed by measurement with the use of scanning near-field optical microscopy (Fig. 5).

4. Conclusions

Porous glass is a suitable material used in designing optical sensor elements for immune reaction detection.

Sensor element formation consists of four basic stages: surface activation, silanization, treatment by glutaric dialdehyde and protein immobilization.

Duration and temperature of drying between activation and silanization stages have a significant influence on immobilization.

According to spectrophotometric (Coomassie method) and fluorometric measurements using acetone for silanization results in immobilization of more proteins on PG in comparison with the case of using toluene.

Immune reaction leads to the formation of an optically homogeneous thin layer on the surface of glass and to an increase of transmittance, which may be used for the immune reaction detection.

Measurements by scanning near-field optical and laser scanning confocal microscopy confirmed the complex formation at the sample surfaces and the optical uniformity increase as a result of the immune reaction.

So, we can draw a conclusion about the possibility of monitoring the immune reaction with the use of sensors based on the porous glass.

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References

[1] HAEBERLE S., ZENGERLE R., *Microfluidic platforms for lab-on-a-chip applications*, Lab on a Chip **7**(9), 2007, pp. 1094–1110.

[2] HEROLD K. E., RASOOLY A., *Lab-on-a-Chip Technology (Vol. 1): Fabrication and Microfluidics*, Caister Academic Press, 2009, p. 410.

[3] KREISBERG V.A., RAKCHEEV V.P., ANTROPOVA T.V., *Influence of the acid concentration on the morphology of micropores and mesopores in porous glasses*, Glass Physics and Chemistry **32**(6), 2006, pp. 615–622.

[4] ANTROPOVA T.V., DROZDOVA I.A., *Influence of the porous glass receiving on it's structure*, Glass Physics and Chemistry **21**(2), 1995, pp. 199–209.

[5] LI XIONG, REGNIER F.E., *Channel-specific coatings on microfabricated chips*, Journal of Chromatography A **924**(1–2), 2001, pp. 165–176.

[6] PIJANOWSKA D.G., REMISZEWSKA E., PEDERZOLLI C., LUNELLI L., VENDANO M., CANTERI R., DUDZIŃSKI K., KRUK J., TORBICZ W., *Surface modification for microreactor fabrication*, Sensors **6**(4), 2006, pp. 370–379.

[7] REIGOSA R.M.J., *Handbook of Plant Ecophysiology Techniques*, Springer Netherlands, The Netherlands, 2001, pp. 283–295.

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