SILANIZATION OF SURFACES AND ITS APPLICATION TO THE ESTERASE ENZYME IMMOBILIZATION ON THE SURFACE OF NANOFIBERS

Jana Koňariková, Petr Exnar, Irena Šlamborová

Department of Chemistry, Faculty of Science, Humanities and Education, Technical University of Liberec, Czech Republic

E-mail: jana.konarikova@tul.cz, petr.exnar@tul.cz, irena.slamborova@tul.cz

Silanization of substrates with Si-OH groups on the surface is one of the current processes in material engineering. Immobilization of enzymes on the different surfaces is one of the possibility that process. On two types of nanofibers with Si-OH groups were successfully immobilization of esterase enzyme through the silanization reactions. Immobilization of enzymes was confirmed by specific colour reaction. The silanization reactions were performed in different solvents. The best results were obtained from silanization in water with rinsed of acetic acid.

Keywords: silanization, immobilization, esterase, enzyme, nanofiber.

INTRODUCTION

Role of silanization reactions were known as an important part of material sciences especially for special modifications of surfaces. The first step of the silanization is a physisorption of alkylalkoxysilanes onto the surface, followed by rapid hydrolysis of the alkoxy group (methoxy or ethoxy groups), yielding the formation of hydroxyl groups that can covalently interact with the silanol on surface [1]. Thicker layers were formed as a result of partial hydrolysis instead of monolayers if there is silanization in an aqueous solution. There might be also the reactions of colloids. Active groups Si-OH or Al-OH we needed for the proper process of silanization. Non-reactive organic groups (methyl, phenyl, hexadecyl etc.) on the surface of substrates caused only surface changes and wetting properties (hydrophobic and hydrophilic surface). When amino group were connected on silanes, the biological agents could react with this bond. This is one of the possible uses of silanization reaction.

There are a number of organosilane coupling reagents commercially available that have been utilized for the immobilization for example DNA, other biological molecules or enzymes. For example, (3-aminopropyl)triethoxysilane (APTES), (3-aminopropyl)trimethoxysilane (APTMS), (3-glycidyloxypropyl)trimethoxysilane (GPS) and haloacetamidosilanes have been extensively used [2].

Enzymes are used in pharmaceutical and chemical industries because of their high degree of specificity. Application of enzymes is limited by their instability and non-reusability. Enzyme immobilization is an effective way to overcome these limitations to some extent. The results of immobilization, including the performance of immobilized enzymes, strongly depend on the properties of supports, which are usually referred to as material types, compositions and structures. So far, different nanostructured materials have been used as supports, such as mesoporous silica and ceramics, nanofibers, and nanoparticles [3]. Nanofibers have a great potential and may be promising supports for enzyme immobilization. The enzyme-immobilized nanofibers can be used for membranes, which have functions of biocatalysis and separation [4].
Experiments proceeded with two types of nanofibers prepared on the Technical University of Liberec by the electrospinning method. First type of the nanofibers was prepared from pure SiO$_2$ and the second type was prepared from polyvinyl butyral (PVB) with addition of 1.5 vol. % tetraethyl orthosilicate (TEOS). The TEOS additive is contributing the SiO$_2$ into organic PVB nanofibers. Glass microscopy slides were used for the comparison samples.

Silanization experiments proceeded with 2% solution of 3-aminopropyl trimethoxysilane (APTMS) in various solvents for 1 hour reaction time at the ambient laboratory temperature. Reaction is schematically described in the Fig. 1. Tested solvents included water, isopropanol, and cyclohexane. The effect of the increased temperature was investigated during the experiments with cyclohexane (81 °C, with reflux condenser). After the silanization process the samples were repeatedly rinsed with pure solvent. In the experiment using the water as a solvent the sample was subsequently rinsed also with diluted acetic acid (AcOH, 1 ml of 1 dm$^3$) in order to remove the abundant reactant agent.

For the enzyme immobilization it was necessary to attach the glutaraldehyde (GA) on the amino group of reacted silane firstly. 2% glutaraldehyde solution in the phosphate buffer with pH 7.2 was used. Process proceeded for the 10 minutes at room temperature, followed by rinsing with buffer. 0.1 mg of esterase enzyme was weighed to 1 ml of phosphate buffer for the immobilization. The sample was incubated in this solution for 5 minutes and then rinsed with buffer. The enzyme attached to the free aldehydic group of glutaraldehyde. Immobilization of the enzyme on the substrate with active Si-OH functional groups is depicted on the Fig. 1.

Verification of the enzyme attachment was performed using the reaction with α-naftylacetate and o-dianisidine [2]. 1.8 mg of α-naftylacetate was weighed and subsequently dissolved in 6 ml of phosphate buffer. In the next step 30 mg o-dianiside in the 30 ml of phosphate buffer was added. The reaction of the enzyme proceeded after inserting the sample into this solution. This reaction is characterized by the change of colour of the sample, from white or colourless to pink colour at the places, where the enzyme was bound.

Fig.1 Scheme of immobilization reaction of enzyme (APTMS - 3-aminopropyl trimethoxy-silane, GA – glutaraldehyde).
RESULTS AND DISCUSSION

Changes of morphology of nanofibers in process of silanization were observed by scanning electron microscopy (SEM). The typical appearance of selected nanofibers PVB+TEOS before and after silanization are illustrated in Fig. 2. Silanization from water created continuous coating of nanofibers (2B, 2C), contrast silanization from cyclohexane created isolated particles (2D). Simple results were obtained also on pure SiO$_2$ nanofibers.

Fig. 2 The typical appearance of selected nanofibers PVB+TEOS before (A) and after silanization (B – water without rinse of acetic acid, C - water with rinse of acetic acid, D – cyclohexane in laboratory temperature).
SILANIZATION OF SURFACES AND ITS APPLICATION TO THE ESTERASE ENZYME IMMOBILIZATION ON THE SURFACE OF NANOFIBERS

J. Koňariková, P. Exnar, I. Šlamborová

Main criterion of enzyme immobilization was regularity and intensity of colour reactions with o-dianisidine. Immobilization of enzymes was confirmed on the all prepared samples. The best results were obtained after silanization in water with rinsed diluted acetic acid, and both on PVB+TEOS nanofibers and pure SiO$_2$ nanofibers. Good immobilization of enzymes was observed after silanization from cyclohexane and isopropanol (only pure SiO$_2$ nanofibers). The worst results were obtained from silanization in water without rinse of acetic acid. It can be expected that part of the enzymes were connected to abundant reactant agent and while rinsing it removed. Fundamental different between immobilization of enzymes on both type of nanofibers were not confirmed. From this results implied that enough Si-OH groups for silanization were on PVB+TEOS nanofibers.

CONCLUSION

The surfaces of both nanofibers were successfully silanization different processes. The best results were obtained after silanization from water with the rinse of acetic acid. Final immobilization of enzymes was confirmed specific colour reaction.

Acknowledgments: This work was supported by the project SGS (Technical University of Liberec) number 5843.

References