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Immobilization of urease on mesoporous materials such as SBA-15 with a functional surface layer

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The template method was used to synthesize mesoporous polysiloxane material of SBA-15 type, which was subsequently modified in ethanol solution with primary and secondary amino groups, and thiol groups. Then, urease was immobilized by adsorption on the surface of the obtained carriers. It was determined that the nature of the functional groups on the surfaces of the carriers affects the kinetics of adsorption, the degree of binding, and residual enzyme activity.

Key words: SBA-15, functional surface layer, urease, adsorption, activity

INTRODUCTION

Enzymes due to their unique catalytic properties, namely, chemical, spatial, and chiral selectivity, are promising for applications in catalysis, separation, biotechnology, etc. [1, 2]. At the same time, the use of enzymes is limited due to their sensitivity to the changes of temperature and instability of activity after regeneration [3–8].

Immobilization of enzymes onto inorganic materials provides their high residual activity during reuse, minimizes production costs, and improves economic efficiency. Immobilization also provides operational flexibility and improves thermal and chemical stability of enzymes [9–11]. Among various methods for enzymes immobilization, the physical adsorption on the surface of the carriers is most widely used because of its simplicity and minimal disruption of the structure of immobilized enzymes.

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Mesoporous materials (MPM) have opened new perspectives in many fields of chemistry and materials science. Recently, it has been shown that MPM are more promising materials in bioimmobilization compared to conventional materials [12–15]. Mesoporous silica (MPS) is noteworthy among such MPM due to a hexagonal spatial structure, high specific surface area, significant volume, and pore diameter (ranging from 2 to 30 nm) [16–18]. The pore diameter of MPS fits well with the size of enzyme molecules. Thus, from this point of view such materials may be good substrates for the immobilization of the enzymes. It was determined that the size of mesopores, or the size ratio of mesopores and protein molecules, affects the parameters of immobilization significantly [19, 20]. Moreover, the nature of the surface groups determines the type of the enzyme adsorption isotherms and adsorption kinetics.

Hydrolytic enzyme urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia. Consequently, it is quite an interesting object for immobilization. This enzyme

has been studied for more than 80 years in enzymology, medicine, biology, chemistry and environmental studies [21]. From a biological point of view, urease was involved in the treatment of peptic ulcer and increasing alkalinity of the soil [22]. From a chemical point of view, urease was used due to its ability to hydrolyze rapidly a stable urea molecule [23, 24].

The main aim of this study was to investigate the effect of a spatial structure of MPS (SBA-15 was chosen) and its pore surface modification with the functional groups such as 3-mercaptopropyl, 3-aminopropyl and bis[3-propyl]amine on the activity of urease during immobilization.

EXPERIMENTAL

The following alkoxysilanes were used for the synthesis of polysiloxane matrices: tetraethoxysilane, $Si(OC_2H_5)_4$ (TEOS, Aldrich, 98%), 3-mercaptopropyltrimethoxysilane, $(CH_3O)_3Si(CH_2)_3SH$ (MPTMS, Aldrich, 99%), 3-aminopropyltriethoxysilane, $(C_2H_5O)_3Si(CH_2)_3NH_2$ (APTES, Aldrich, 99%), bis[(3-methoxysilyl)propyl]amine, $[(CH_3O)_3Si(CH_2)_3]_2NH$ (BTMSA, Aldrich, 99%). The surfactant Pluronic 123, hydrochloric acid (2 M) as a catalyst, absolute ethanol, enzyme urease (EC 3.5.1.5, from jack bean, 35 U/mg, Fluka, Switzerland), phosphate buffer (pH 7), 0.05 M solution of sodium EDTA (trilon B, reagent grade), 2 M urea solution (chemically pure) were used in our experiments.

Synthesis of the original matrix SBA-15

A batch of P123 (8 g) was dissolved in 60 cm^3 of water and 232 cm^3 of HCl (2 M) under constant stirring in a water bath at 40 °C. After 1 h, 18.2 cm^3 of TEOS was added under stirring. The precipitates formed within 20 min. The resulting heterogeneous system was mixed for 6 h in the water bath at 40 °C. The sediment was subjected to hydrothermal treatment (HTT) in a matrix solution for 24 h at 100 °C. Then the white precipitate was filtered and left to dry in air. The template was removed by calcination of a powdery sample with gradually increasing temperature from 100 to 500 °C for 3 h and 6 h at 500 °C. Yield 4.6 g.

Modification of SBA-15 matrix: sample SBA-15A

For the modification with 3-aminopropyl groups, a mesoporous sample (0.5 g) was poured with ethanol (50 cm^3) and APTES (0.23 cm^3, 0.001 mol) was added. The mixture was refluxed for 6 h. The precipitate was filtered and dried under vacuum for 6 h.

Sample SBA-15M. MPTMS (0.18 cm^3, 0.001 mol) was used instead of APTES.

Sample SBA-15B. BTMSA (0.32 cm^3, 0.001 mol) was used instead of APTES.

Immobilization of the enzyme on the modified SBA-15 samples was performed by static adsorption from buffer solutions. The maximum adsorption and adsorption isotherm of urease were measured after 4 h of interaction of the enzyme buffer solution with modified SBA-15 (urease immobilization at room temperature and periodic stirring for 12 h). The batch of modified mesoporous SBA-15 was 10 mg, and the concentration of urease in buffer solutions ranged from 0.25 to 3.0 g/l. A batch of matrix was poured with a phosphate buffer solution of urease (5.0 mg) in 2 cm^3 of a mixture of phosphate buffer and EDTA (9/1). Then the sediment was centrifuged from the solution and washed 5 times with 5 cm^3 of the phosphate buffer.

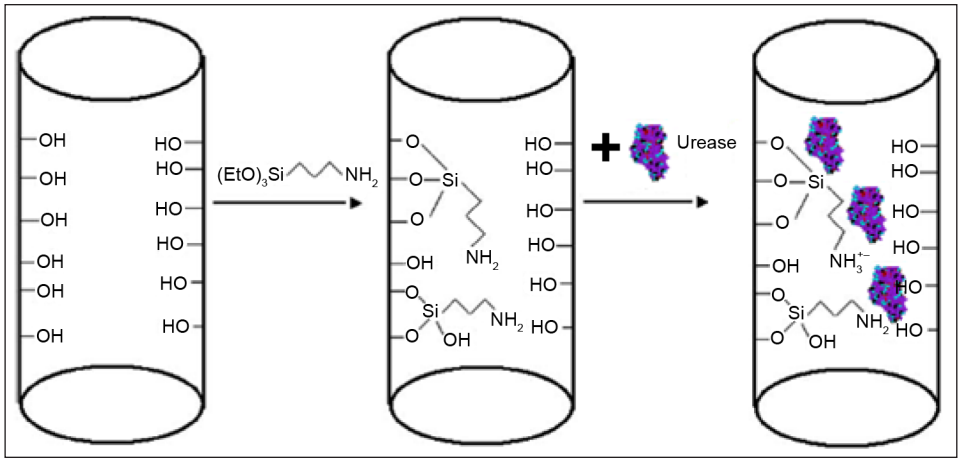
To measure the kinetics of urease adsorption, the batches of the modified mesoporous SBA-15 sample (50 mg) were shaken in test tubes with 2 cm^3 of the buffer solution with urease concentration of 1.0 g/l. The adsorption solution was analysed for enzyme content every 5, 10, 20, 30, and 60 min. In all cases, the amount of the bound enzyme was estimated by the difference between urease taken for immobilization and the amount that was found in the adsorption solution. The content of urease in the adsorption and washing solutions was determined from its activity estimation, based on the value of specific activity of native enzyme.

Urease enzyme activity was determined by the rate of ammonia formation in the urea hydrolysis reaction at 25 °C [25]. In all cases, the average of three parallel experiments (the biggest difference between them <10%) was used for activity determination. The error of the measurement using the Student coefficient at rugged probability of 0.95 is less than 10%.

Table. **Parameters of a porous structure of the obtained samples and the data on immobilization of urease**

Sample	$C_{f.gr.}$, mmol/g	S_{BET}, m^2/g^{-1}	V_s, cm^3/g	d_{eff}, nm	Urea ads., mg/g	Urease activity, units	Urease residual activity, %
SBA-15	–	580	0.53	2.0; 5.0	550	903	81.5
SBA-15A	0.72 $-NH_2$	281	0.37	5.0	648	916	68.3
SBA-15M	0.5 -SH	420	0.57	2.8; 6.6	773	815	65.2
SBA-15B	0.7 =NH	225	0.34	5.9	515	789	60.2

$C_{f.gr.}$, mmol/g – concentration of functional groups on the surface of the samples; S_{BET}, m^2/g – specific surface area of the samples; V_s, cm^3/g – specific pore volume samples; d_{eff}, nm – an effective pore diameter of the samples; Urea ads., mg/g – adsorption of urease; Urease activity, units – the retention of activity of immobilized urease (in standard units); Urease residual activity, % – the retention of activity of immobilized urease relative to native (in percentage)



Scheme. **Immobilization of urease on the functionalized carrier SBA-15**

Images of the original and modified forms of SBA-15 were obtained with a scanning electron microscope JSM 6060LA (Jeol, Tokyo, Japan) using secondary electrons at the accelerating voltage of 30 kV. Samples were placed on the surface of the object table with a deposited adhesive coating. To prevent the accumulation of the surface charge and to receive a contrast image, the surfaces of the samples were covered with thin continuous layers of gold or platinum by cathode sputtering in vacuum.

The content of amino groups in modified SBA-15 was determined by reverse acid-base titration. For this, a 0.1 g batch of the sample was poured with 20 cm^3 (0.1 M) of hydrochloric acid. After 24 h, the sample was centrifuged, and the filtrate was titrated with a 0.1 M sodium hydroxide solution in the presence of an indicator (methyl orange). The concentration of amino groups was calculated from the differences in the introduced and absorbed protons.

The content of mercapto groups in modified SBA-15 was assessed from the amount of adsorbed silver(I) ions. For this, a 0.1 g batch of the sample was poured with 20 cm^3 of (0.1 M) silver nitrate. After 24 h, the sample was centrifuged, and the filtrate was analyzed for the content of silver(I) ions. The concentration of mercapto groups was calculated from the differences in the concentration of introduced and remaining ions.

The FTIR reflection spectra were recorded in the range of 4000–400 cm^{-1} on a Thermo Nicolet Nexus FT-IR spectrometer, working in the Nexus Smart Collector mode and using a resolution of 8 cm^{-1}. The samples were pre-mixed with KBr at a ratio of 1:30. To record the IR spectra at higher temperatures (200 °C), a special thermal vacuum setup Collector II was used.

X-ray diffraction (XRD) powder patterns were collected on a DRON 4-07 diffractometer using Cu-K_α radiation, 2 h scanning was carried out in the region of 0.5–10 2θ/° at a speed of 0.5° min^{-1}.

Low-temperature nitrogen adsorption isotherms were recorded on a Kelvin-1042 (Costech Microanalytical). Samples were preliminarily degassed at 383 K in helium atmosphere. The specific surface area values were calculated using BET [26] in the relative pressure range of 0.05–0.35. The total pore volume (V_c) was determined by the amount of N_2 adsorbed at the relative pressure of 0.99 [27]. An effective pore diameter was estimated by the BJH method [27].

RESULTS AND DISCUSSION

The syntheses of silica networks were performed via hydrolytic polycondensation reaction of TEOS in the presence of surfactant P123 according to [28]. In order to obtain material with a well-developed spatial structure, the HTT of mesophase in the matrix solution at 100 °C for 24 h was carried out. The mesoporous structure of SBA-15 obtained under these conditions is formed by uniform hexagonal mesopores [28].

After the pre-activation of the surface of obtained silica by boiling in water (25 cm^3 of water per 1 g of SBA-15) and drying under vacuum (150 °C, 5 h), it was modified with 3-mercaptopropyl, 3-aminopropyl, and secondary amine groups (Scheme), followed by immobilization of urease via adsorption.

Mesoporous silica SBA-15 features a white powdery substance with available functional groups on the surface layer. The content of functional groups determined by acid-base titration in the sample SBA-15A was 0.72 mmol/g, and in the sample SBA-15B it was 0.7 mmol/g. The content of mercapto functional groups in the SBA-15M sample assessed from the amount of absorbed silver ions was 0.5 mmol/g.

Figure 1 represents SEM images of mesoporous silica specimens. As seen, the synthesized materials are composed of aggregates of cylindrical (barrel-shaped) particles of about 0.3 × 1.0 microns in size. After the deposition of the functional layer, the particles retain both their shape and size.

The X-ray diffraction patterns of an original SBA-15 sample and modified samples are presented in Fig. 2. One intense (at 0.85–0.9 2Θ/°) and two low intensity narrow reflections (at 1.2–2.0 2Θ/°) are observed at low angles. The (100), (110), and (200) reflexes are characteristic of the hexagonal phase (p6mm)

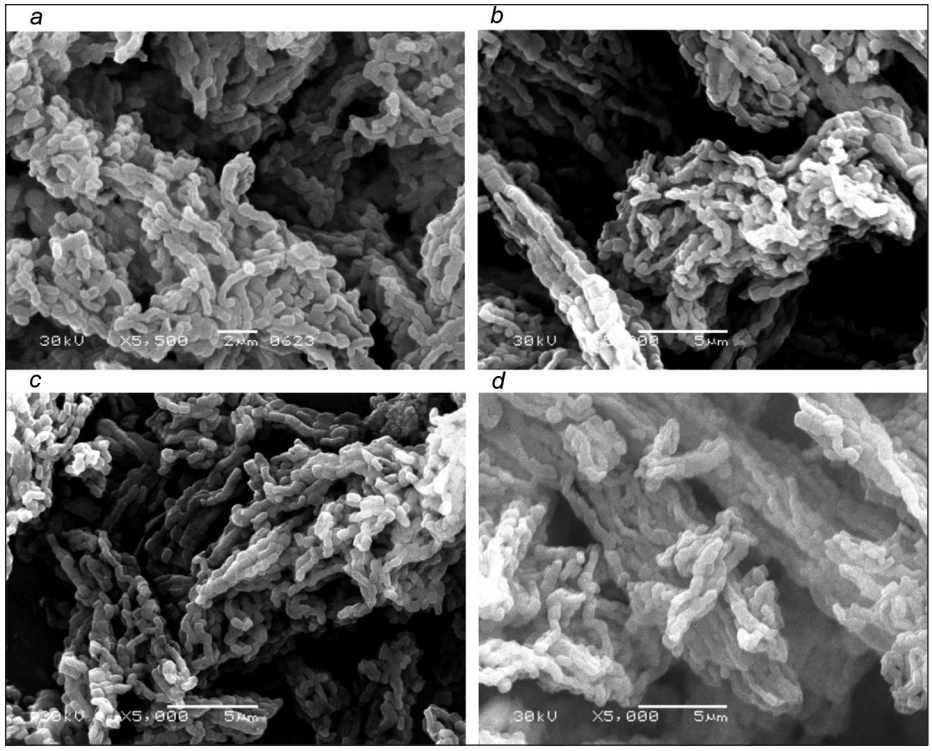


Fig. 1. SEM images of mesoporous silica: *a* – initial SBA-15; *b* – SBA-15A; *c* – SBA-15M; *d* – SBA-15B

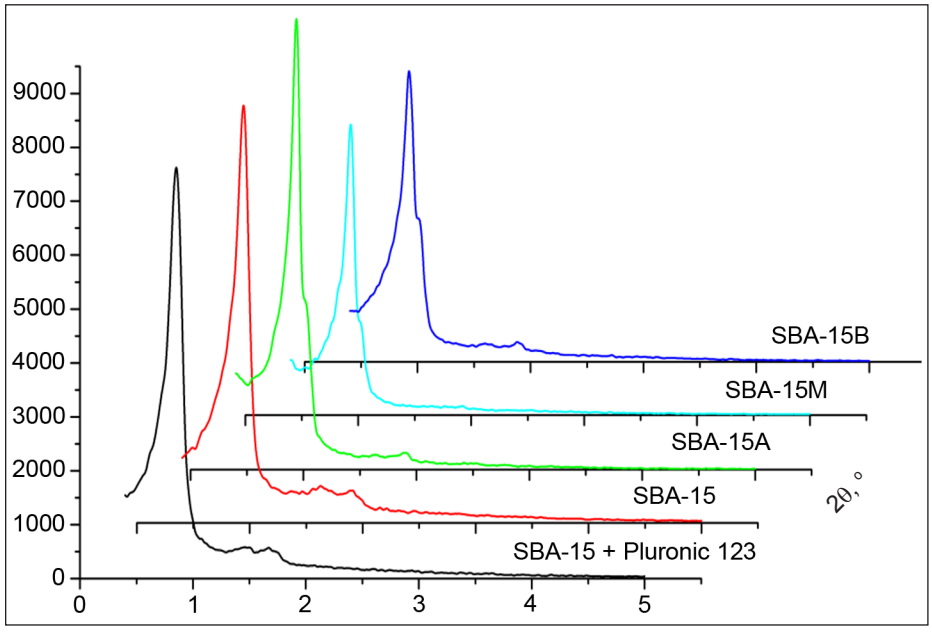


Fig. 2. X-ray diffraction patterns of the original SBA-15 sample and the functionalized samples

structure. Also, it is seen that after removing the template the samples preserve the hexagonal (p6mm) structure.

IR spectroscopy was used for the identification of the surface layers of the modified forms of SBA-15. The IR spectra of the obtained samples are typical for silica (Fig. 3). In the IR spectra of all samples the most intensive absorption band at 1 000–1 200 cm^{-1} with a shoulder in the high-frequency region related to ν_{as}(SiOSi) vibrations was observed. This indicates the presence of polysiloxane network [23]. A medium intensity band at 1 545–1 555 cm^{-1} in the IR spectrum of the sample SBA-15A refers to the deformation vibrations of the amino groups $\delta(NH_2)$ [30].

For modified mesoporous materials, three absorption bands are usually observed in the region of C-H (2 850–2 950 cm^{-1}) valence vibrations. They can be referred to $\upsilon_{s,as}$ (CH) vibrations of methylene groups, which confirm the presence of propyl chain. In addition, the IR spectrum of the sample modified with 3-mercaptopropyl groups registered an absorption band at 2 565 cm^{-1}, which can be attributed to υ(SH). For the sample modified with 3-aminopropyl groups two absorption bands of low intensity at ~3 300 and ~3 375 cm^{-1} were registered in the IR spectrum. These absorptions could be attributed to $\upsilon_{s,as}$ (NH) of amino groups. For secondary amines, only one absorption band is identified

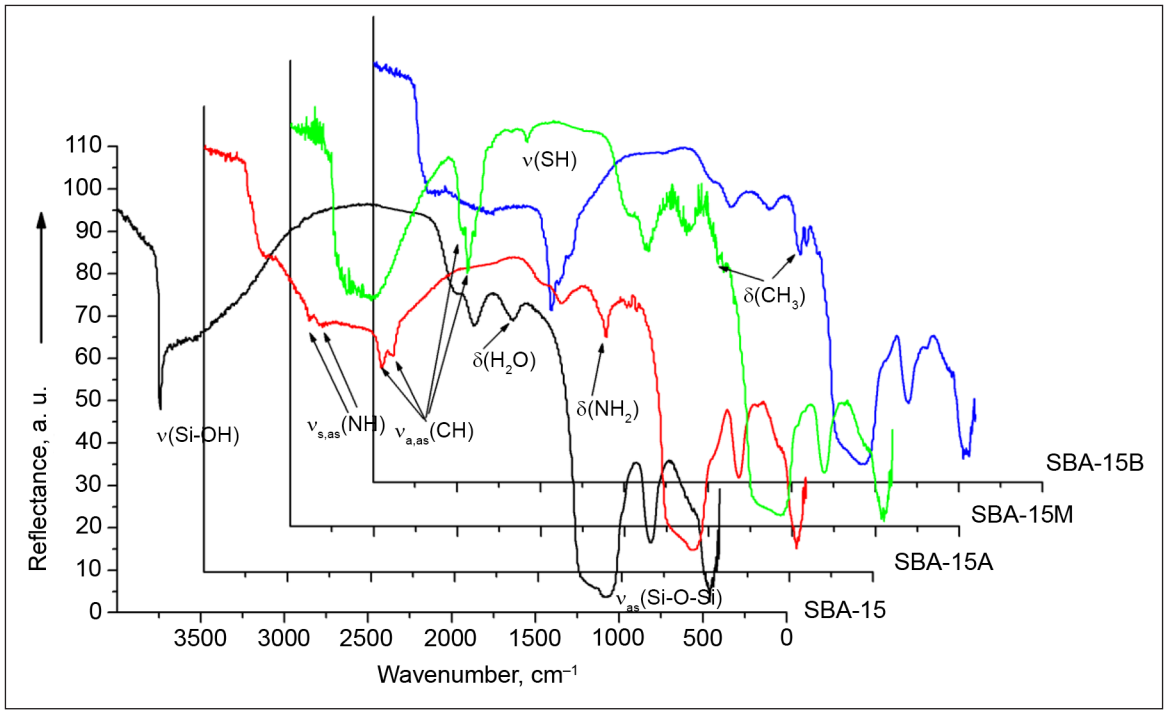


Fig. 3. IR spectra of the original SBA-15 sample and its functionalized forms

in this area. All the synthesized samples also contained water. Absorption band at ~1 630 cm^{-1} is characteristic of the deformation vibrations of H_2O molecules and a broad intense absorption band above 3 000 cm^{-1} is due to the vibrations in the OH groups of water molecules. Thus, according to the IR spectra, the surface layers of synthesized samples contain functional groups introduced during modification, uncondensed water molecules and silanol groups.

Figure 4 shows nitrogen low-temperature adsorption-desorption isotherms for all synthesized samples. The isotherms have an S-shaped character with a distinct narrow loop of a capillary condensation hysteresis at the relative pressure P/Ps > 0.5 and belong to type IV (IUPAC classification) [24]. This type of isotherms is typical of a highly mesoporous material with a hexagonal structure. A relatively narrow area of mesopores filling for these samples indicates the uniformity of their pore size. It should be mentioned that the isotherms of the sample SBA-15 and its modified forms are characterized by a steep rise in the initial area of isotherms, indicating the presence of micropores in interchannel walls. To confirm the presence of micropores in the original and modified SBA-15 the approach proposed in [25, 26] was used. The calculated pore sizes distribution curves are presented in Fig. 4. Accordingly, the obtained d_{ef} values characterize the presence of mesopores in the obtained samples. SBA-15 and its modified forms have narrow pore size distribution curves. In addition, these curves often have a low-intensity maximum in the region of 2–3 nm. According to the structural and

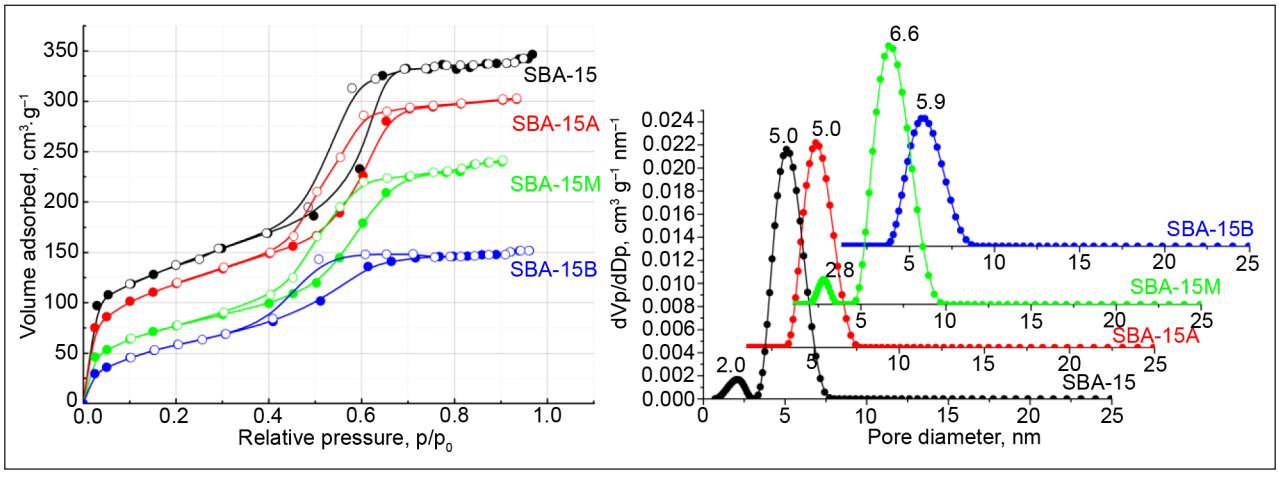


Fig. 4. N_2 adsorption-desorption isotherms and pore sizes distribution for the original and functionalized SBA-15 calculated by the method [32]

adsorption characteristics of the synthesized silica, obtained from low-temperature nitrogen adsorption isotherms (see also the Table), all the samples are characterized by well-developed porous structures. However, the modification of SBA-15 with organic groups tends to decrease their specific surface.

The results of urease immobilization via adsorption on the synthesized SBA-15 samples are presented in the Table and Fig. 5. 550 mg of enzyme per 1 g of the SBA-15 sample was immobilized, which corresponds to 81% of fixation. The residual activity of immobilized urease is 81.5%. In the transition to the modified forms of mesoporous silica, there is a slight increase in the adsorption of urease up to 90–95%. This is most likely due to the possibility of formation of more connections between the enzyme and the carrier via the interaction with the functional groups of mesoporous silica [34]. However, in this case urease residual activity is somewhat reduced (approximately 20%, see the Table) compared with the original SBA-15. As we have previously shown [20], the increased binding of urease leads to reduction in its activity. It was assumed that a large number of connections with a carrier influences the conformation of the protein molecule and, consequently, causes a decrease in its activity.

The excess urease adsorption isotherms from the buffer solution for the modified samples are presented in Fig. 6. The obtained urease adsorption isotherms belong to the Langmuir type [35]. This indicates high affinity of urease molecules to the surface functional groups of the carriers. The isotherms are all similar in shape and have a sharp rise at low concentrations of urease in the initial solution, which may indicate strong adsorbent-adsorbate interactions. It is believed that the isotherms of this type are observed when there is no strong competition between the solvent molecules or when the adsorbing molecules have strong intermolecular interactions [36]. Perhaps in this case, both of these factors are important. Urease is an enzyme with high molecular weight and due to numerous connections between the functional groups forms large associated particles in aqueous solutions.

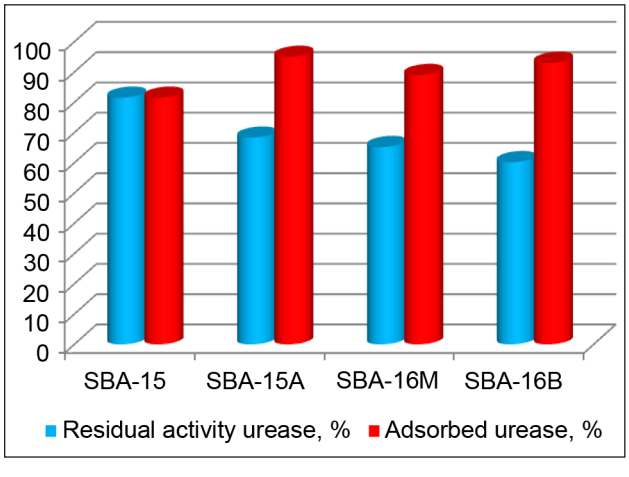


Fig. 5. Comparison of the residual activities and binding capacities of urease towards the original and functionalized forms of mesoporous SBA-15 type silica

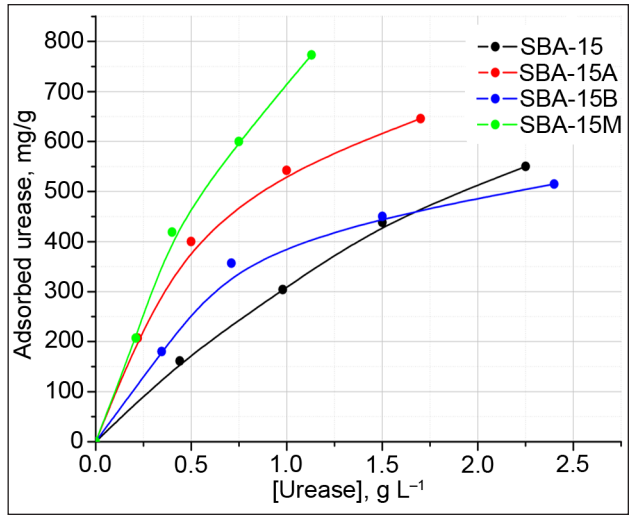


Fig. 6. Urease adsorption isotherms for samples of the original and functionalized SBA-15 forms

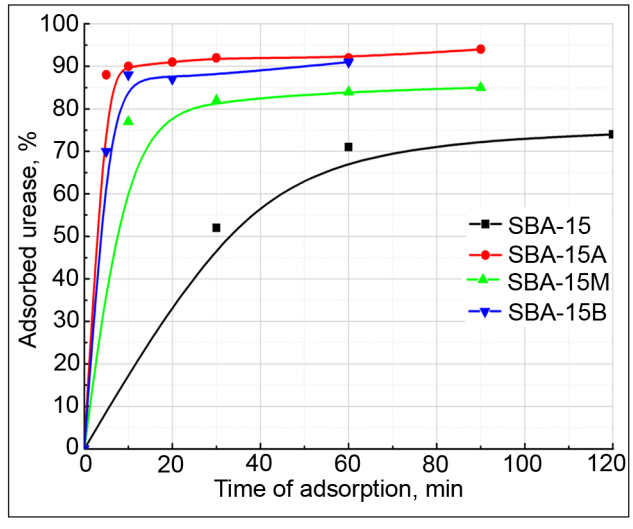


Fig. 7. Kinetics of urease adsorption on the samples of original and functionalized forms of SBA-15

Therefore, we should expect multipoint adsorption, in which the competition of water molecules is negligible.

To assess the protein binding properties of the obtained mesoporous samples urease adsorption kinetics was studied (Fig. 7). The adsorption equilibrium in the case of the original (not modified) sample SBA-15 is reached within 2 h, for the sample SBA-15M within 30 min, and in the case of samples SBA-15A and SBA-15B within 10 min. With increasing interaction time, there is no further increase in the adsorption capacity. Presumably, longer time of establishing equilibrium in the adsorption of the enzyme on the sample SBA-15M is determined by the difference in the character of the urease adsorption on protonated ammonium groups and weakly dissociated thiol groups. Moreover, the establishing of adsorption equilibrium is still longer for the original SBA-15 sample. This fact can be explained by the formation of hydrogen bonds with silanol groups and Van der Waals interaction.

CONCLUSIONS

It was shown that the mesoporous SBA-15 material synthesized using a template method and its modified forms (with amine and thiol groups) can be used as carriers for urease immobilization via adsorption. It was determined that the nature of the functional groups on the surface of the porous carrier affects the adsorption kinetics, the degree of binding and the residual activity of enzyme.

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References

1. D. Yu, Z. Wang, L. Zhao, Y. Cheng, S. Cao, *J. Mol. Catal. B: Enzym.,* **48**, 64 (2007).
2. Y. Han, S. S. Lee, J. Y. Ying, *Chem. Mater.*, **18**, 643 (2006).
3 Y. Wang, Y. L. J. Hsieh, *Membr. Sci.*, **309**, 73 (2008).
4. Y. X. Bai, Y. F. Li, Y. Yang, L. X. Yi, *Process Biochem.*, **41**, 770 (2006).
5. A. Salis, D. Meloni, S. Ligas, et al., *Langmuir,* **21**, 5511 (2005).
6. H. Ma, J. He, D. G. Evans, X. Duan, *J. Mol. Catal. B: Enzym.,* **30**, 209 (2004).
7. A. Vinu, V. Murugesan, O. Tangermann, M. Hartmann, *Chem. Mater.,* **16**, 3056 (2004).
8. A. Galarneau, M. Mureseanu, S. Atger, G. Renard, F. Fajula, *New J. Chem.*, **30**, 562 (2006).
9. R. P. Pogorilyi, V. P. Honcharyk, L. I. Kojara, et al., *Questions of Chemistry and Chemical Technology*, **6**, 103 (2005) (in Russian).
10. L. Y. Zheng, S. Q. Zhang, L. F. Zhao, et al., *J. Mol. Catal. B: Enzym.*, **38**, 119 (2006).
11. D. Bezbradica, D. Mijin, S. Siler-Marinkovic, Z. Knezevic, *J. Mol. Catal. B: Enzym.*, **38**, 11 (2006).
12. J. Lei, J. Fan, C. Z. Yu, L. Y. Zhang, et al., *Microporous Mesoporous Mater.*, **73**, 121 (2004).
13. R. P. Pogorilyi, V. P. Honcharyk, L. I. Kojara, Yu. L. Zub, A. A. Chuiko, *Ukr. Biochem. Journal.*, **78**, 1 (2006) (in Ukrainian).
14. L. N. Wang, T. Qi, Y. Zhang, J. L. Chu, *Microporous Mesoporous Mater*., **91**, 156 (2006).
15. G. Zhou, Y. Chen, S. Yang, *Microporous Mesoporous Mater.*, **119**, 223 (2009).
16. A. Sayari, B. H. Han, Y. Yang, *J. Am. Chem. Soc*., **126**, 14348 (2004).
17. P. Innocenzi, Yu. Zub, V. Kessler (eds.), *Sol-gel Methods for Materials Processing,* Springer, Boston (2008).
18. K. Kosuge, T. Sato, N. Kikukawa, M. Takemori*, Chem. Mater.,* **16**, 899 (2004).
19. Y. Kang, J. He, X. Guo, X. Guo, Z. Song, *Ind. Eng. Chem. Res.*, **46**, 4474 (2007).
20. R. P. Pogorilyi, V. P. Honcharyk, Yu. L. Zub, *Surface*, **3(18)**, 132 (2011) (in Ukrainian).
21. P. A. Karplus, M. A. Pearson, R. P. Hausinger, *Acc. Chem. Res.*, **30**, 330 (1997).
22. S. Benini, R. R. Wojciech, K. S. Wilson, S. Miletti, S. Ciurli, S. Mangani, *Struct. Fold. Des.*, **7**, 205 (1999).
23. B. P. Callahan, Y. Yuan, R. Wolfenden, *J. Am. Chem. Soc.*, **127**, 10828 (2005).
24. R. L. Blakeley, J. A. Hinds, H. E. Kunze, E. C. Webb, B. Zerner, *Biochemistry*, **8**, 1991 (1969).
25. Y. Y. Lurie, *Chemistry*, Moscow (1984) (in Russian).
26. J. S. Brunauer, P. H. Emmet, E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).
27. E. P. Barret, L. G. Joyner, P. P. Halenda, *J. Am. Chem. Soc.*, **73**, 373 (1951).
28. Ya. Li, G. Zhou, Ch. Li, D. Qin, W. Qiao, Bo. Chu, *Colloids Surf., A*, **341**, 79 (2009).
29. L. P. Finn, I. B. Slinaikova, *Colloid J.*, **37**, 4 (1975) (in Russian).
30. D. Lin-Vien, N. B. Colthup, W. G. Fateley, J. G. Grasselli, *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, Academic Press Inc., London (1991).
31. K. S. W. Sing, D. H. Everett, R. A. W. Haul, L. Moscou, R. A. Pierotti, J. Rouquerol, T. Siemieniewska, *Pure Appl. Chem.*, **57(4)**, 603 (1985).
32. V. M. Gun'ko, D. J. Sheeran, S. M. Augustine, J. P. Blitz, *J. Colloid Interface Sci.*, **249**, 123 (2002).
33. V. M. Gun'ko, V. V. Turov, J. Skubiszewska-Zieba, B. Charmas, R. Leboda, *Adsorption*, **10**, 5 (2004).
34. R. P. Pogorilyi, V. P. Honcharyk, L. I. Kojara, Yu. L. Zub, *Applied Biochem. Microbiol.*, **44(6)**, 621 (2008) (in Russian).
35. C. H. Giles, T. H. MacEwan, S. N. Nakhwa, et al., *J. Chem. Soc.*, **10**, 3973 (1960).
36. Th. S. Tsapikouni, Y. F. Missirlis, *Mater. Sci. Eng., B,* **152**, 2 (2008).

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UREAZĖS IMOBILIZACIJA Į MEZOPORINĘ MEDŽIAGĄ SVA-15 SU FUNKCINIU PAVIRŠIAUS SLUOKSNIU

S a n t r a u k a

Šablono metodas buvo naudotas mezoporinės polisiliksano medžiagos SVA-15 sintezei, kuri vėliau modifikuota etanolyje veikiant pirmines, antrines amino ar tiolo funkcines grupes turinčiais alkilalkoksisilanų junginiais. Fermentas urezė ant susintetintų nešėjų paviršiaus buvo imobilizuotas iš tirpalų adsorbcijos būdu. Tyrimo metu nustatyta, kad funkcinių grupių prigimtis ant nešėjų paviršiaus turi įtakos adsorbcijos kinetikai ir imobilizacijos laipsniui bei lemia fermentų aktyvumą.