PECULIARITIES OF GLUCOSE OXIDASE ADSORPTION BY MESOPOROUS ALUMINOSILICATES SYNTHESIZED IN PRESENCE OF BIOTEMPLATES

Z.V. Smelaya and Yu.G. Goltsov

L.V. Pisarzhevsky Institute of Physical Chemistry, National Academy of Sciences
Pr. Nauky 31, 03039 Kyiv, UKRAINE

Abstract

A study of glucose oxidase adsorption has been undertaken from a solution of the enzyme (0.125 mmol dm^{-3}) in acetate buffer (pH 4) onto mesoporous aluminosilicates synthesized in the presence of biomolecules (lecithin, glucose oxidase). These aluminosilicates displays a high sorption capacity (up to 150 mg/g). The physical adsorption of the enzyme showed clear dependence on pore diameter of the materials. The porous structure of such inclusion compounds was detailed. The glucose oxidase cannot be removed from the aluminosilicates with repeated washing in the acetate buffer.

Introduction

Mesoporous silicates and aluminosilicates applied to biological systems open many potential applications due to their large, controllable pore sizes, high internal surface areas, chemical inertness and the ability to modify their surfaces. Some general areas where such materials could be applied are in the separation of proteins or other large biochemical molecules, and as supports for biocatalytic systems and in biosensor application [1-6]. They provide many advantages over conventional dextran polymer and sol-gel based immobilization techniques, the most significant being their highly ordered rigid structures and their resistance to microbial attack due to their inert inorganic nature. Mesoporous silicates and aluminosilicates possess tunable narrow pore size distribution in the range of 1.5-12 nm. These dimensions are of the same size as proteins and other biomolecules and thus makes such solids attractive candidates to host encapsulated/immobilized enzymes.

Balkus *et al* [4-6] has immobilized cytochrome c, papain and trypsin onto MCM-41, SBA-15 and layered niobium oxide NB-TMS4. They have shown that the physical adsorption process is dependent on pH of the adsorption buffer and the pore size of the material. For instance peroxidase adsorption onto MCM-41 was limited due to the enzyme being larger than the pore diameters [5]. In more recent study it has been established that immobilized cytochrome c is stable under what would normally be denaturing conditions and cyclic voltammetry has shown cytochrome c to be active for several months [6]. Adsorption and desorption characteristics of cytochrome c onto different ordered mesoporous silicates have been detailed in [1]. Adsorbed protein was not desorbed with repeated washing in buffer but polyethylene glycol and/or ammonium sulfate in buffer caused considerable desorption. Glucose oxidase (GO) is the most widely used enzyme in the field of biosensors. However, the efficiency of the glucose sensors is limited, mainly due to the heterogeneity of the enzyme distribution in the biosensor membrane, which makes difficult the creation of the effectively functioning compact analytical devices [7]. In order to improve the molecular architecture of

the biosensor the approach of using of ordered mesoporous materials with tunable narrow pore size distribution might be fruitful.

Recent studies indicate that a biomimetic approach based on the main constructional processes of biomineralization results in the development of new strategies in controlled synthesis of high ordered inorganic materials. Imitation of the basic processes of biomineralization - super molecular preorganization (formation of organic matrices by spatial organization of an assembly of macromolecules) and molecular recognition (templating) - permits the preparation of valuable products under relatively mild conditions, the regulation of structure formation, and also exclusion of the use of toxic intermediates [8-9]. Variation in the nature of the templates used for mineralization and also the composition of the reaction mixture may permit materials, which differ in the size of the pores, the nature of the active sites, the composition of the skeleton and other characteristics of the porous substances suitable for the inclusion of various macromolecules and their ensembles [10-11].

In this paper the results of a study of the adsorption of glucose oxidase onto mesoporous aluminosilicates synthesized in the presence of biotemplates (lecithin, GO) are described. Glucose oxidase is dimer of ca. 160 000 molecular weight, containing two identical and no interacting flavin groups [12-13]. The isoelectric point of the native GO is 4.44 [14]. According to X-ray crystallography data [14] the dimeric partially deglycosylated GO molecule is compact spheroid with approximate dimensions $6.0 \times 5.2 \times 7.7$ nm. It has been found with the use of dynamic light scattering that the hydrodynamic diameter of the native GO molecule is 7.6 nm [14]. This value corresponds to a molecular cross section area of about 45 nm^2. At cubic packing of the protein in a surface monolayer, the area occupied by one molecule is about 58 nm^2.

Experimental

Aluminosilicate MCM-41 materials were synthesized by using sodium silicate, aluminum sulfate, and cetyltrimethylammonium bromide (CTMAB). The compositions of the reaction mixtures were: $16.2SiO_2:Al_2O_3:5.4Na_2O:13.8CTMAB:1767H_2O$ (sample SA2, pH of the reaction mixture was close to neutral) and $116SiO_2:Al_2O_3:38Na_2O:49CTMAB: 9101H_2O$ (sample SA1, pH$\approx$9). The hydrothermal synthesis was carried out at 423K for 48 h. The obtained product was washed with water, dried in air and calcined at 823K for 6 h in air [10-11].

Aluminosilicate mesoporous materials in the presence of lecithin (L) were synthesized by adding lecithin (17% of L-α-phosphatidyl choline, Sigma) or its combinations with CTMAB or octadecylamine (ODA) instead of CTMAB to the reaction mixture, so that the L:CTMAB and L:ODA molar ratios in a reaction mixture were 0.47 or 1.40 and 0.34 or 1.04, respectively. The hydrothermal synthesis was carried out at 353K for 72 h. The obtained products were washed with water, dried in air and calcined at 823K for 6 h in air [10].

Aluminosilicate mesoporous materials in the presence of GO were synthesized from the reaction mixture of sample SA2. Glucose oxidase (from *Aspergillus niger*, Sigma) was added to the reaction mixture (1) without changing the amount of CTMAB in the mixture so that the mass ratio of GO:CTMAB=0.5 (sample SA2-G01) (2) without changing the overall amount of template in the mixture, i.e., GO replaced part of the CTMAB so that the mass ratio GO:CTMAB=0.25 (sample SA2-G02). The hydrothermal synthesis was carried out at 423K for 48 h. The obtained product was washed with water, dried in air and calcined at 823K for 6 h in air [11].

The Si:Al ratios in the samples were close to them in the reaction mixtures.

The sorption of glucose oxidase on obtained mesoporous aluminosilicates was carried out from acetate buffer of pH=4 (0.02 g GO per 1 ml of buffer; 0.08 g of sorbent per 1 ml of solution) at 35°C over a period of 20 h. The samples produced were washed repeatedly (5 times) by water, then by adsorption acetate buffer and by water, and dried in air. The GO1-SA2-L2 sample was additionally washed by 0.5 M NaCl solution. The amount of adsorbed protein was calculated from the difference between the protein concentrations in the supernatants before GO adsorption and after washing procedure. The protein concentration in solutions was determined by spectrophotometric monitoring of absorption at 36000 cm^{-1} (Specord UV VIS).

The characterization of samples was carried out by the powder X-ray diffraction (automatic diffractometer DRON-3M, CuK$_\alpha$ radiation) and methanol ad(de)sorption measurements at 293K on standard vacuum set-up based on the McBain-Backr quartz spring balance (prior to measurements mesoporous samples were degassed at 390K, samples containing GO were degassed at 303K). The Kelvin equation (assuming hemispherical meniscus and zero contact angle) with the multilayer thickness correction was applied to determine the pore diameter distribution [15]. Total volume V_S of the samples were estimated from the isotherms at p/p_s=0.95 assuming that the pores have been filled with condensed liquid adsorptive. The BET surface area S_{BET} was calculated in the conventional manner, the methanol molecule area was accepted to be 25 Å^2.

Results and discussion

Peculiarities of the porous structure of mesoporous aluminosilicates (MPAS) synthesized in the presence of biomolecules have been analyzed in our earlier studies [10-11]. Mesoporous SA2-GO1 and SA2-GO2 samples with complex porous structure were obtained in the presence of combination GO:CTMAB=0.5 and 0.25 (Si:Al=8). Both samples have similar isotherms (Fig. 1) with four hysteresis loops, which begin and end at similar values of p/p_s and 3 peaks corresponding 3 effective sizes of mesopores in the 3.0-10.0 nm range (Table 1) is observed on the pore size distributions. The sample SA2-GO2 obtained in the presence of GO:CTMAB=0.25 exhibits a wider pore size distribution [11].

It was shown [10-11] that in the presence of only lecithin mesoporous substances are formed, so the supramolecular structures of lecithin molecules are template in aluminosilicate framework formation. We have found that MPAS with pore diameters up to 10.0 nm and biporous materials, which have bimodal mesopore size distribution, can be prepared in the presence of the lecithin mixtures with CTAB or ODA (Fig. 2, Table 1) as template.

The adsorption of GO onto MPAS likely involves numerous interactions between the support surface and the exterior amino acid residues of the protein. These interactions can depend on the composition of the MPAS and the tertiary structure of the protein. The presence of negative charge sites in MPAS can promote adsorption through electrostatic forces through positively (below the isoelectric point) charged amino acid residues on the enzyme. Many hydrophilic side chains, as well as covalently bound polysaccharide chains of the native glycoprotein GO molecule are located on its surface [12, 14]. Therefore, hydrogen bonding to surface hydroxyl groups can also influence on enzyme adsorption, as it has been described for adsorption of the enzyme onto platinum [16]. The high amount of adsorbed protein for the all MPAS used indicates that the GO adsorption strongly influenced by hydrophobic/hydrophilic interactions rather than electrostatically in agreement with [4].

The greatest GO sorption capacity possess MPAS with greatest total adsorption volume (Si:Al=58, samples SA1-L3 and SA1-L5 synthesized in presence L:CTMAB=.40 and L:ODA=1.04 respectively). As shown in [10-11], such materials have mesopores of large size (D=8.0 nm and D=11.4 nm, accordingly) and identical, most likely, of channel constitution.

The larger part of adsorption volume (approximately 70 and 80 %, accordingly) corresponds to the pores with D>8.0 nm (the dynamic diameter of GO molecule). The SA1-L5 material also has sub-group of pores of sizes ranging from 20-50 nm with very small, compared with pores D=11.4 nm, adsorption volume. Such sample (Table 2) adsorbs about 130 mg/g of GO. After GO adsorption, the total adsorption volume of the SA1-L5 material is reduced in 1.7 times, but adsorption volume of pores with D<8.0 nm does not vary.
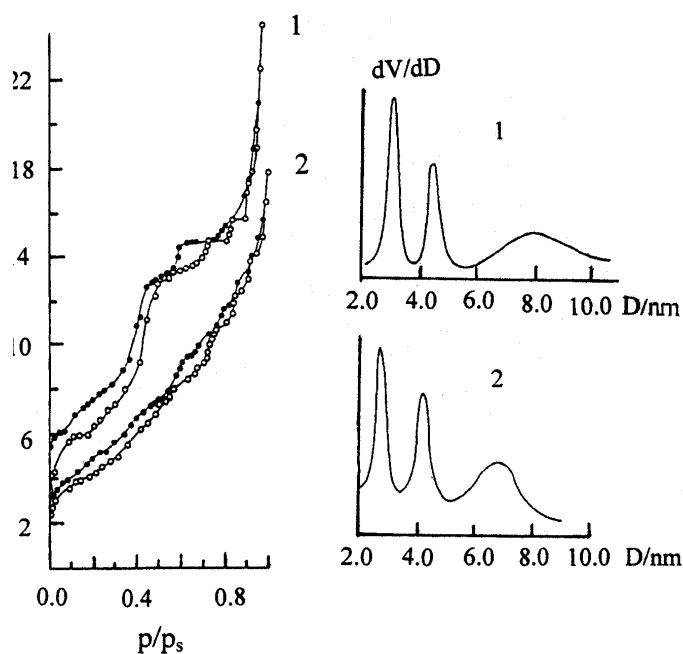

Fig. 1. Methanol ad(de)sorption isotherms and pore size distributions for samples SA1-GO1 (*1*) and SA2-GO2 (*2*).

Table 1. Peculiarities of the porous structure of mesoporous aluminosilicates synthesized in the presence of biotemplates.

Mesoporous aluminosilicate	Si:Al[1]	Template	D, nm	ΔD, nm	$V_{0.78}$[2], cm^3/g	V_S, cm^3/g	S_{BET}, m^2/g
SA1-L3	58	L:CTMAB=1.40	8.0	2.5÷15.0	0.40	1.33	342
SA1-L5	58	L:ODA=1.04	5.8	2.5÷7.5	0.22	1.23	256
			11.4	7.5÷17.5			
SA2-L2	8.1	L:CTMAB=0.47	3.6	2.0÷5.0	0.46	0.77	342
			9.8	7.5÷12.5			
SA2-GO1	8.1	GO:CTMAB=1:2	3.1	2.5÷3.8	0.58	0.84	775
			4.5	3.8÷4.6			
			8.1	6.2÷10.6			
SA2-GO2	8.1	GO:CTMAB=1:4	2.7	2.5÷3.8	0.43	0.63	544
			4.2	3.8÷5.0			
			6.8	5.5÷9.0			

[1] The Si:Al ratio in reaction mixture.
[2] Adsorption volume of pores with D<8.0 nm.

The sorption of GO onto sample SA1-L3 occurs in the same manner. Such material exhibited wide pore size distribution (ΔD=2.5-15.0 nm) with the maximum at D=8.0 nm, the considerable part of adsorption volume is in the pores with sizes ranging from 6.0-10.0 nm. After a sorption GO (151 mg/g) adsorption volume of the pores with D<8.0 nm, does not change, and the considerable part of adsorption volume is in pores with sizes ranging from 4.0-6.0 nm, the pores with D>6.5 nm practically disappear.
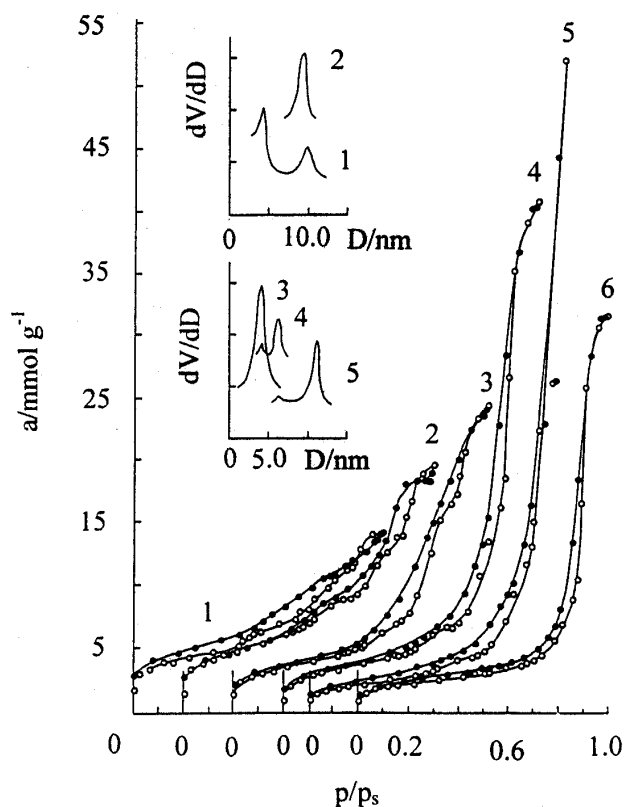

Fig. 2. Methanol ad(de)sorption isotherms for samples SA2-L2 (*2*), SA1-L5 (*6*) and synthesized in the presence of L, Si:Al=8 (*1*), L:ODA=0.34, Si:Al=8 (*3*), L:CTMAB=0.47, Si:Al=58 (*4*), L, Si:Al=8 (*5*); and pore size distributions for samples SA2-L2 (*1*), SA1-L5 (*5*) and synthesized in the presence of L:CTMAB=0.47, Si:Al=58 (*2*), L, Si:Al=8 (*3*), L:ODA=0.34, Si:Al=8 (*4*).

From such data it appears that the protein molecules penetrate into pore network, so the adsorption onto SA1-L3 and SA1-L5 occurs onto internal surface of the support. The protein loaded MPAS samples are characterized by similar type IV isotherms (Fig. 3) with wide H1 hysteresis ranging from p/p_s=0.2-0.9. However the isotherms of the GO1-SA1-L3 and GO1-SA1-L5 exhibit much shaper increase of adsorbed methanol volume, than isotherms of other protein loaded MPAS. In addition, such increase is displaced towards the high relative pressure and occurs for relative pressure p/p_s greater than 0.6. The analysis of adsorption data allows assuming, that along to cylindrical or hexagonal pores protein loaded MPAS materials contain some amount of bottle shaped pores. It is known [17] that the relationships between the relative pressure h_a, of the adsorption branch and the relative pressure h_d of desorption branch corresponding to equal adsorbed amounts, differ for different shapes of pores. Thus, for instance, for the model of cylindrical open-ended pores $h_a^2=h_d$. The relation that applies to model pore structures containing bottle shaped pores with narrow

throats the cavity radius of which is more than double the throat radius, is the inequality $h_a^2 > h_d$. For bottle shaped pores the effective cavity radius of which is less than double the effective throat radius, and for tubular pores with the same ratio of the two principal dimensions, the inequality changes to $h_a^2 < h_d$. Graphical representation of the dependence of h_a^2 on h_d. is a convenient means for studying the dependence of the shape of pores on their dimensions. As we can see from Fig. 4, all samples obtained after GO sorption contain bottle shaped pores with cavity radius larder than the throat diameter. The pores of such type can be formed owing to the GO sorption in the case if the difference between the pore size of the support and GO molecular diameter is greater than kinetic diameter of methanol molecule (0.33 nm [15]). Therefore methanol molecules can penetrate the pores of protein loaded MPAS.

Table 2. Peculiarities of the porous structure of mesoporous aluminosilicates synthesized in the presence of biotemplates after GO sorption.

Mesoporous aluminosilicate after GO sorption	Amount of GO adsorbed, mg/g	D, Nm	ΔD, nm	$V_{0.78}$[1], cm^3/g	V_S, cm^3/g	S_{BET}, m^2/g
GO1-SA1-L5	130	5.0	3.0÷7.0	0.22	0.71	210
GO1-SA1-L3	151	4.9	3.5÷6.5	0.39	0.89	336
GO1-SA2-L2	66 (60)[2]	4.1 (4.1)[2]	2.0÷8.0 (2.0÷8.0)[2]	0.35 (0.43)[2]	0.69 (0.70)[2]	502 (570)[2]
GO1-SA2-GO1	42	-	Up to 8.0 nm	0.34	0.71	498
GO1-SA2-GO2	69	4.5	2.5÷7.0	0.36	0.59	462

[1] Adsorption volume of pores with D<8.0 nm.
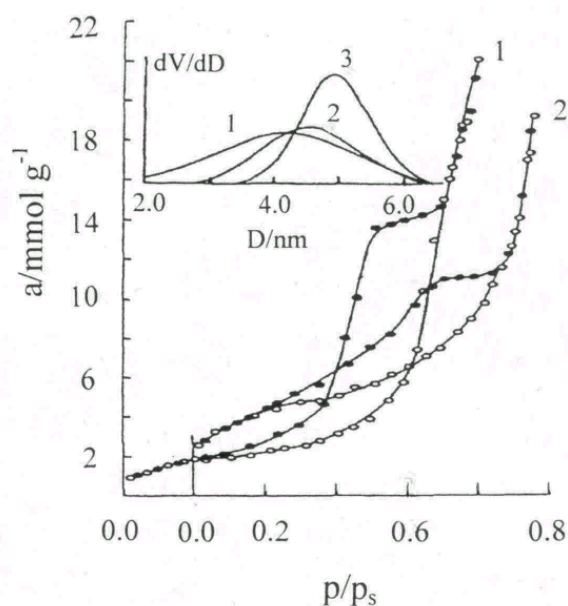[2] The characteristics of sample after washing by 0.5 M NaCl solution.

Fig. 3. Methanol ad(de)sorption isotherms for samples GO1-SA1-L5 (*1*), GO1-SA2-L2 (*2*) and pore size distributions for samples GO1-SA2-L2 (*1*), GO1-SA2-GO2 (*2*), GO1-SA1-L3 (*3*).
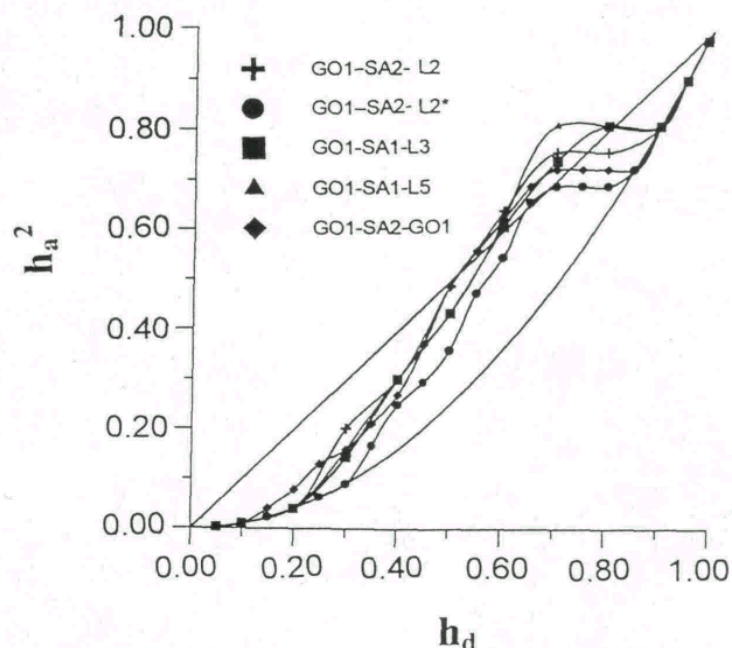
Fig. 4. The dependence of h_a^2 on h_d for samples GO1-SA2-L2, GO1-SA1-L3, GO1-SA1-L5, GO1-SA2-GO1 and for sample GO1-SA2-L2 after washing by 0.5 M NaCl solution.

The adsorbed GO was found to be stable on the MPAS materials after repeated washings in adsorption buffer and water. The protein was washed repeatedly (5 times) with UV-scans detecting no protein in the supernatant solution. The difficulty of desorption may be explained by low probability of such process due to "multipoint" bonding of protein globule with support interface while the desorption requires simultaneous rupture of all bonds (this corresponds to a considerable difference between the activation energies for the adsorption and desorption processes).

The washing up 0.5 M NaCl solution also almost does not influence on porous structure of a sample GO1-SA10-L2 (Table 2). However, the analysis of dependence $(h_a)^2$ on h_d demonstrates that the washing by 0.5 M NaCl solution results in disappearing of bottle shaped pores with narrow throats (Fig. 4).

The calculation according to Kelvin equation from desorption branch of the isotherm demonstrates that the GO1-SA1-L3, GO1-SA1-L5 samples are characterized by relative narrow throat size distributions with the peak at diameter D=5.0 nm, regardless of porous structure of the source MPAS materials (Table 2). Total adsorption volume of the samples is rather large (0.72, 0.88 см^3/g, accordingly), but considerably smaller than total adsorption volumes of source mesoporous materials, and the adsorption volume of mesopores of diameter ranging from 3.0 nm up to 7.0 nm is about 60% of total adsorption volume. The pores with D>8.0 nm are practically absent. It seems to indicate that GO molecules fill almost all pores, which being large enough to accommodate them. The size of filled pores is diminished on the magnitude of GO molecular diameter, so the source MPAS with homogeneous large pores change to bottle shaped pores material with narrow neck size distributions.

SA2-L2, SA2-GO1 and SA2-GO2 (see Table 1) possess complex porous structure and the pores with D>8.4 nm have considerably smaller adsorption volume in comparison with SA1-L3 and SA1-L5. Thereof SA2-L2, SA2-GO1 and SA2-GO2 have smaller GO sorption

capacity (66, 42 and 69 mg/g, respectively). After GO adsorption such MPAS exhibit wide pore size distributions ranging from 2.0-8.0 nm (Fig. 3, Table 2).

A decrease in surface area was measure for the protein loaded MPAS. Significantly lower surface area measured for the protein loaded GO1-SA2-GO1 and GO1-SA2-GO2, while that determined for the GO1-SA1-L3 and GO1-SA1-L5 was slightly smaller. Such results may be explained if it is supposed that the different sub-groups of the MPAS are interconnected. This is also supported by the considerable decrease of methanol adsorption volume of pores with D<8 nm for such MPAS after GO adsorption.

The protein loading and MPAS surface area before protein adsorption provide an opportunity to estimate the enzyme coverage. A monolayer of GO would consist of approximately $4.4 \cdot 10^{18}$ molecules for SA1-L5 (in the case of cubic packing). This value is very close to that calculated from the GO loading of $4.9 \cdot 10^{17}$ for this MPAS. Such surface concentration of the protein corresponds to molecular cross section area 523 nm^2, whereas maximum surface concentration of the GO at silicas is characterized by molecular cross section areas ranging from 800 nm^2 [14]. These results may encourage the application of such protein adsorbed MPAS materials as active elements of biosensors or sorbents for biomolecular sorption.

Conclusions

Studies of glucose oxidase sorption onto mesoporous aluminosilicates synthesized in the presence of biotemplates (lecithin, glucose oxidase) indicate that these sorbents possess high sorption capacity. The amount of adsorbed glucose oxidase is related to the characteristics of the mesopore diameter with most adsorption associated with materials having pore diameter in excess of the size of glucose oxidase molecule. The inclusion compounds of adsorbent-glucose oxidase type based on mesoporous aluminosilicates with large pores and great total adsorption volume (up to 1.3 cm^3/g) have the biggest content of glucose oxidase (up to 150 mg/g). The sorption of glucose oxidase occurs within the pores of effective diameter exceeding that of protein (8 nm) and results in essential reduction of methanol adsorption volume. Porous structure of such inclusion compounds is characterized by a narrow distribution of pore sizes with effective diameter at 5 nm. The inclusion compounds based on mesoporous aluminosilicates with small total adsorption volume (up to 0.8 cm^3/g), the larger part of the latter corresponding to the pores with a diameter ranging down to 8 nm, exhibit broad distribution of pore sizes and smaller content of glucose oxidase (up to 70 mg/g).

The results obtained allow us to consider aluminosilicate mesoporous materials synthesized in the presence of biotemplates as a prospect for creation of high capable and selective sorbents for biomolecular sorption or active elements of chemical and biosensors.

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