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STUDIES OF PHYSICOCHEMICAL PROPERTIES OF THE SURFACES WITH THE CHEMICALLY BONDED PHASE OF BSA

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Abstract

The paper presents the method of preparation and determination of physicochemical properties of silica gels with a chemically bonded BSA phase as well as studies of the effect of support porosity on the synthesis. Wide-porous Z-300 and narrow-porous Z-100 silica gels were studied. The investigations showed a significant effect of pore size on the synthesis of stationary phases with BSA. Modification with protein results in changes of adsorption properties and porosity of adsorbent samples. Changes of physicochemical properties result in significant changes of geometrical and structural heterogeneity of the support (specific surface area, fractal coefficient) as well as energetic heterogeneity of the samples.

Keywords: BSA, fractal dimensions, heterogeneity, nitrogen adsorption, surface with BSA, thermogravimetry

Introduction

Protein plays a significant role in life processes – actions and structure of cells. They perform, among others, catalytic (enzymes), structural (calogene, elastin), transport (albumin) and protective (immunoglobulin) functions. They are widely applied in production on biomaterials. Rapid development of biotechnology promoted intensive studies of protein interactions with organic [1, 2] and inorganic [3–8] materials. An attempt was made to explain the mechanism of BSA adsorption on the oxide adsorbents: SiO_2 [3, 7, 8], SnO_2 and ZrO_2 [3], Al_2O_3 [9] and on the biomaterials: titanium [10] and hydroxyapatites [11–14]. The adsorption process from albumin is complicated and depends on many factors, ionic strength, medium pH, electrostatic interaction, surface charge, co-adsorption of molecularly smaller ions, isoelectric point, intermolecular forces among adsorbed molecules, solvent-solute interactions,

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functional group binding forces, sorbent surface properties and character – its energetic heterogeneity. In many cases these factors cause conformational changes of protein macromolecules as well as their denaturation.

The studies of physicochemical properties of the protein phase by infrared absorption [15–19], UV and Raman spectroscopy, NMR, reflectometry, elipsometry [20], circular dichroism CD [15, 21], atomic force microscope AFM [7, 22, 23] and sorptometry methods [24] are used very often.

Recent studies have focused on application of silica gel with immobilized protein as column packing in high performance liquid chromatography HPLC [25–27]. Separation of enantiomers is one of the most important current problems in analytical chemistry. The separation process is complicated and difficult due to large similarity in both chemical and most physical properties of enantiomers. Chiral stationary phases formed on the protein base are characterized by high enantioselectivity. Adsorbents of this type possess very interesting surface properties [28, 29] and can be applied in analysis under close to physiological conditions, separation of drugs, amino acid derivatives, etc. despite their low resistance to changes of temperature and pH. As the pionieers Alenmark *et al.* [25] used the chiral stationary phase for separation of isomers-*D* and *L*-N-acrylamino acid.

The aim of this paper is the synthesis and estimation of properties of chiral surface stationary phases through the chemical bonding of BSA molecules to various kinds of silica gels due to chemical modification. Another important task was estimation of physicochemical properties of the prepared materials by means of thermal and sorptomatic analyses. The attempt was made to explain the effect of the pore diameter of the initial silica gel on surface parameters of the modified samples and the amount of the added BSA. There were also made measurements of programmed thermodesorption of polar and apolar liquids under the quasi-isothermal conditions from the gel surface and gel after silanization to determine the effect of functional group change on the adsorption capacity for the above mentioned liquids.

Experimental

Materials

Albumin bovine (BSA, Fraction V, minimum 98%) was purchased from Sigma. It is soft protein with molecular dimension of 42x141 Å, a molecular mass of 66 267 and isoelectric point of 4.7 [30]. Silica gel Davisil 653XWP was obtained from Supelco. 2,4,6-trichloro-1,3,5-triazine and 3-aminopropyltrietoxysilane were supplied by Aldrich, ethyl glycinate hydrochloride was purchased from Merck. All other reagents were analytical grade.

Synthesis

Synthesis was carried out using the method *Zhang et al.* described in [27]. The gels (Z-300 and Z-100, 6 g) were added to HCl solution (150 mL, 20%). The mixture was

heated to 110°C with energetic stirring. After 4 h the silica was filtered off and washed with redistilled water to obtain pH=7. Activated gel was dried in vacuum at 110°C for 10 h. Then it was scattered in toluene (150 mL) under a nitrogen atmosphere, 3-aminopropyltriethoxysilane (9 mL) was added and the mixture heated to 110°C. After 12 h the toluene was filtered off and the aminopropylsilica washed several times with hot toluene as well as with chloroform and acetone before drying in a nitrogen atmosphere. Aminopropylsilica gel (4.5 g) was added to water (90 mL). The mixture was cooled to 0°C in ice bath. 2,4,6-trichloro-1,3,5-triazine (0.81 g) was added. The reaction mixture acidity was maintained at pH 4, $NaHCO_3$ if necessary, and the temperature below 5°C. After 2 h the mixture was filtered off. The modified gel was washed with water and ice several times. The synthesis product was stirred with phosphate buffer (70 mL, 0.05 M) and added into the flask with the support (c=3 mg mL^{-1}). The reaction was conducted for 2 h. The amount of the reacted BSA was determined by measuring the solution absorbance before and after the reaction. After 2 h the solution was filtered off and the precipitate stirred with 1% amino acid ethyl ester solution for the next 2 h. The adsorbent modified with BSA was washed in succession with the solution (90 mL): pH 7 phosphate buffer (0.05 M), pH 7 phosphate buffer (0.025 M) including 0.025 M NaCl, redistilled water and pH=7 phosphate buffer (50 mL).

Apparatus

The amount of the reacted modifier was determined using thermal analysis (derivatograph Q-1500 D, MOM, Hungary) and spectrophotometry UV-Vis (Specord M 42, Carl Zeis Jena). The identity of chemically bonded groups in the material was determined by means of infrared spectroscopy FTIR (Perkin Elmer, Paragon 1000). Surface coverage in individual stages of synthesis was characterized by elemental analysis (Perkin Elmer CHN 2400) and atomic force microscope (AFM Digital Instruments Nanoscope). Specific surface areas and pore volumes were determined from low-temperature nitrogen adsorption (Sorptomat ASAP 2405 V1.01). Then fractal and diffusion coefficients were calculated. From the above experimental data adsorption capacity and total surface heterogeneity of the studied samples were determined.

Liquid thermodesorption studies from the silica gel samples (Z-300) and gel after silanization (ZS-300) saturated with vapours of polar and apolar liquids including benzene, *n*-butanol, *n*-octane, redistilled water were conducted. The samples were dried in the oven at 200°C for 24 h to eliminate the hygroscopic water. Then they were placed in vacuum desiccators saturated with liquid vapours at p/p_o=1 and left for 48 h to establish the adsorption equilibrium. The prepared samples were subjected to programmed thermodesorption measurements in the temperature range 20–250 °C with an oven heating rate 6°C min^{-1} and the balance sensitivity 100 mg. The mass loss curves Q-TG were registered under quasi-isothermal conditions.

Result and discussion

Determination of adsorption properties of gel and gel after silanization

Figure 1 presents the experimental mass loss curves depending on temperature in thermodesorption of polar and apolar liquid films from the surface of pure silica gels: Z-300, Z-100 and gels after silanization: ZS-300 and ZS-100. The samples of gel with protein were not analyzed due to protein destruction at higher temperatures. Comparing the course of curves registered for different samples (gel, gel after silanization) saturated with polar and apolar liquid vapours, some differences concerning character of sorbent and kind of liquid are observed. Individual inflexions result from the presence of interactions of various forces in the sample adsorption layer: in the liquid condensation layer, in ions and under the surface layer. They are also connected with the presence of functional groups on the surface. The results of water, benzene, *n*-butanol, and *n*-octane thermodesorption from the gel surfaces (Z-300, ZS-300 after silanization) were used for calculation of: number of moles of adsorbed liquid vapour for a gram of adsorbent [mol g^{-1}], *a*, number of liquid layers on the sorbent surface *n*, and degree of surface coverage, θ. Table 1 includes the results of calculation of all the mentioned values. For the samples Z-300 and ZS-300 statistical numbers of monolayers for the systems with *n*-butanol (*n*~5) and *n*-octane (*n*~9) are comparable.

In the case of water and benzene adsorption a decrease in the amount of adsorbed liquid for the sample ZS-300 is observed whereas in the case of water the value *n* decrease six times and *Q* about five times. These changes are connected with

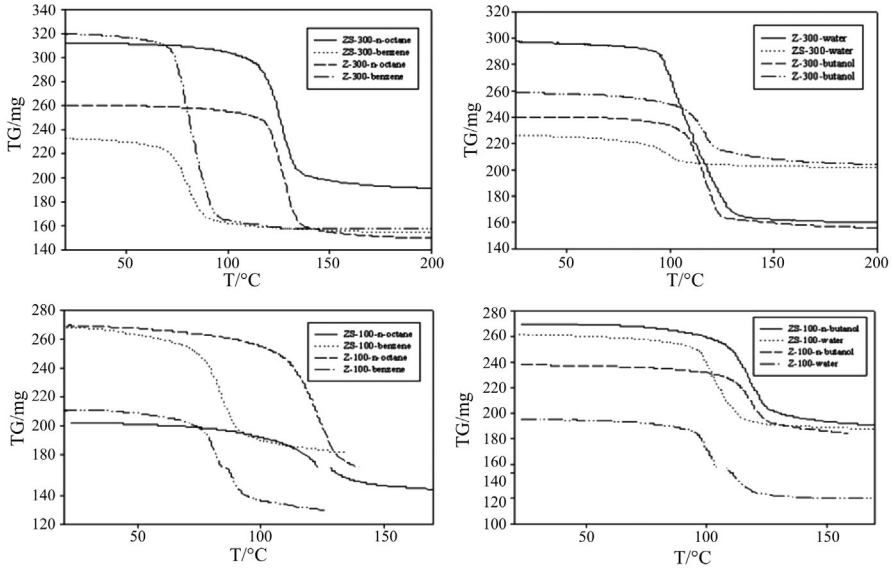

Fig. 1 The Q-TG mass loss curves of liquid thermodesorption from the sample surface

Table 1 Properties of liquid adsorption layers on the surface of pure gel and gel after silanization samples

Sample	Liquids	a/mmol g^{-1}	n	θ/mmol m^{-2}
Z-300	*n*-octane	6.80±0.21	9	0.036
ZS-300		5.51±0.30	9	0.040
Z-100		5.90±0.02	5	0.018
ZS-100		3.49±0.01	4	0.014
Z-300	benzene	12.55±0.17	14	0.066
ZS-300		7.17±0.48	10	0.052
Z-100		8.45±0.55	5	0.025
ZS-100		6.10±0.03	5	0.024
Z-300	*n*-butanol	4.62±0.43	5	0.024
ZS-300		3.60±0.21	5	0.019
Z-100		4.35±0.39	3	0.013
ZS-100		5.51±0.08	5	0.022
Z-300	water	47.15±2.08	19	0.248
ZS-300		6.91±0.24	3	0.050
Z-100		35.50±1.40	8	0.1072
ZS-100		2.19±0.08	6	0.0009

where: a – the number of moles of the adsorbed liquid vapour, n – the statistical number of liquid monolayers, θ – the degree of surface coverage

the change of functional groups on the gel surface which indicates formation of hydrogen bonds in the case of pure gel sample Z-300.

The desorption energy distribution from the Q-TG and Q-DTG curves was derived using an equation for the desorption kinetics characterised by a constant value of the desorption energy [31]:

$$-\frac{1}{1-\theta_i}\frac{d\theta_i}{dT}=\frac{\nu_i}{\beta}\exp\left(-\frac{E_i}{RT}\right) \quad (1)$$

where: $T = T_o + \beta t$, θ the degree of surface coverage, ν the entropy factor, E_i the desorption energy calculated for each temperature, T_o and T the initial and given temperatures of desorption, respectively, β the heating rate of the sample, t the time and R the universal gas constant.

The final expression for determination of desorption energy distribution $\varphi_n(E)$ can be expressed in the form [31]:

$$\varphi_n(E)=-\frac{d\theta}{dT}\frac{1}{T} \quad (2)$$

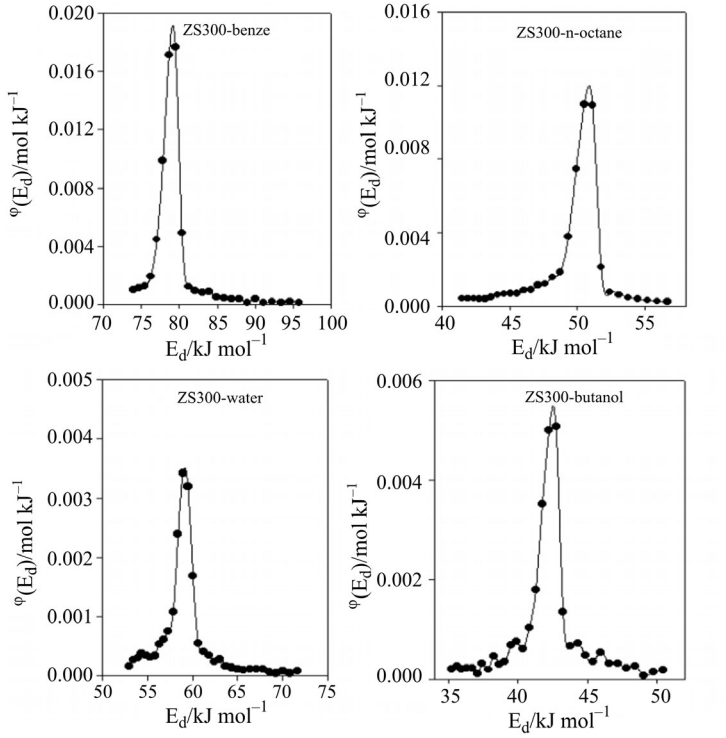

Fig. 2 Energy distribution function of liquids desorption from ZS-300 sample

The exact way of calculating the desorption energy distribution functions of *n*-octane, benzene, *n*-butanol and water from the Q-TG and Q-DTG data is presented in [31, 32]. The example desorption energy distribution functions are given in Fig. 2. Table 2 includes the values of desorption energy for individual samples.

As follows from the diagrams the dependences $\varphi_n = f(E_d)$ have a regular shape of the Gauss curve type. The values of desorption energy are differentiated and are found in the range 44-95 kJ mole^{-1} for the sample Z-300 and 37-81 kJ mole^{-1} for the sample ZS-300. The values E_d decrease in the order of the studied liquids: *n*-octane>benzene>water>*n*-butanol adsorbed for pure gel and benzene>water>*n*-octane>*n*-butanol for the samples after silanization. The value E_d for octane are 1.5 times larger for the sample Z-300 than for the sample ZS-300. The increase of adsorption energy indicates stronger interactions of *n*-octane with OH groups of pure silica. Surface hydrophobization by means of silane causes also a decrease of polar molecules (water, *n*-butanol) interaction energy. This is connected with active centers (OH^- groups) blocking by silane molecules. In the case of benzene adsorption an increase of desorption energy due to the presence of electrons π in its molecule and their interactions with hydrophobic surface is observed. In the case Z-100 and ZS-100, the value E_d decreases in the order of the liquids: water>benzene>*n*-octane>*n*-butanol adsorbed for pure gel and benzene>water≈*n*-butanol>*n*-octane for the samples after silanization.

Table 2 Changes of desorption energy of the liquids from the samples Z-300 and ZS-300

Sample	Changes of desorption energy /kJ mol^{-1}											
	Benzene			*n*-butanol			*n*-octane			water		
	E_{dmin}	$E_{d(MAX)}$	E_{dmax}	E_{dmin}	$E_{d(MAX)}$	E_{dmax}	E_{dmin}	$E_{d(MAX)}$	E_{dmax}	E_{dmin}	$E_{d(MAX)}$	E_{dmax}
Z-300	71	75	78	44	48	49	82	91	95	62	68	75
ZS-300	76	78	81	37	42	45	46	50	53	56	59	64
Z-100	44	46	49	25	27	29	35	39	42	69	74	76
ZS-100	44	49	51	38	43	46	25	26.5	29	41	43	46

Structural properties of synthesized adsorbents

From the data of nitrogen adsorption-desorption specific surface area (*S*), total pore volume (*V*) and pore diameter (*D*) were calculated. These quantities were determined from the three theories: BET, BJH and Langmuir. The samples of silica gel (Z-300, Z-100), gel after silanization (ZS-300, ZS-100) and the final product of modification with the protein BSA (ZB-300, ZB-100) deposited on the surface were sorptomatically studied. The nitrogen adsorption-desorption isotherms for all analyzed samples have a shape of type IV according to the BET classification describing the capillary condensation phenomenon. This type of isotherm is connected with multimolecular adsorption layers formation the hysteresis loop of capillary condensation with a steeply falling desorption branch was obtained in all cases. The obtained loops present type A of classification according to de Boer which indicates that capillaries have a shape of two-sided open, regular and irregular cylinders and prisms.

As follows from the data presented in Tables 3 individual modifications affect significantly structure and adsorption properties of the surface of the studied samples. In the case of Z-300 a decrease in the nitrogen adsorption capacity is observed after modification with silane (ZS samples) and BSA (ZB samples). For the other support Z-100 a decrease in the value S_{BET} after silanization and then a slight increase in the second stage of synthesis are observed. These changes are connected with blocking of pores by the large BSA macromolecules (40×140Å) and expansion of support grain surface. For better illustration of porous structure of same sorbents, a diagram of pore volume distribution in relation to the radii calculated from the desorption branch using the BJH method was made (Fig. 3). Analyzing the data in Table 3 and diagram 3 there is observed a decrease of pore radius value for gel 100 and the phases with chemically bonded protein of BSA in individual stages of synthesis. In the case of gel Z-300 an insignificant increase of pore diameter after silanization connected with the crosslinking increase is observed.

From the adsorption isotherms there were calculated fractal coefficients (Table 4) using the method presented in [33] based on the Francel–Halsey–Hill theory and Kisielev equation using the dependence:

$$D_f = 2 + \frac{\mathrm{d}[\ln \int_a (-\ln x)\mathrm{d}a]}{\mathrm{d}[\ln(-\ln x)]} \quad (3)$$

where: a is the adsorption quantity, $x=p/p_o$.

Table 3 Adsorption and structural parameters of the pure (Z), silanized (ZS) and modified by BSA (ZB) gel samples determined from the nitrogen adsorption isotherms

Sample	Z-300	ZS-300	ZB-300	Z-100	ZS-100	ZB-100
S_{BET}/m^2 g^{-1}	190.1	139.2	137	331.1	252.6	278
V_{BJHads}/cm^3 g^{-1}	1.20	0.94	0.92	1.11	0.84	0.84
$V_{BJhdess}$/cm^3 g^{-1}	1.20	0.93	0.81	1.1	0.84	0.83
D_{BET}/nm	25.1	26.6	23.6	13.4	13.3	11.9
D_{BJHdes}/nm	21.5	18.6	18.4	9.3	8.4	8.6

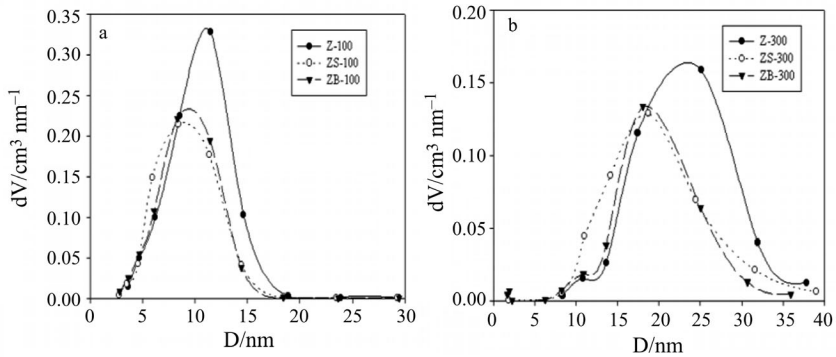

Fig. 3 Pore-size distribution functions of tested samples

Table 4 Values of fractal coefficients calculated from Eq. (3)

Sample	Z-300	ZS-300	ZB-300	Z-100	ZS-100	ZB-100
Fractal dimension	2.59	2.6	2.64	2.47	2.62	2.55

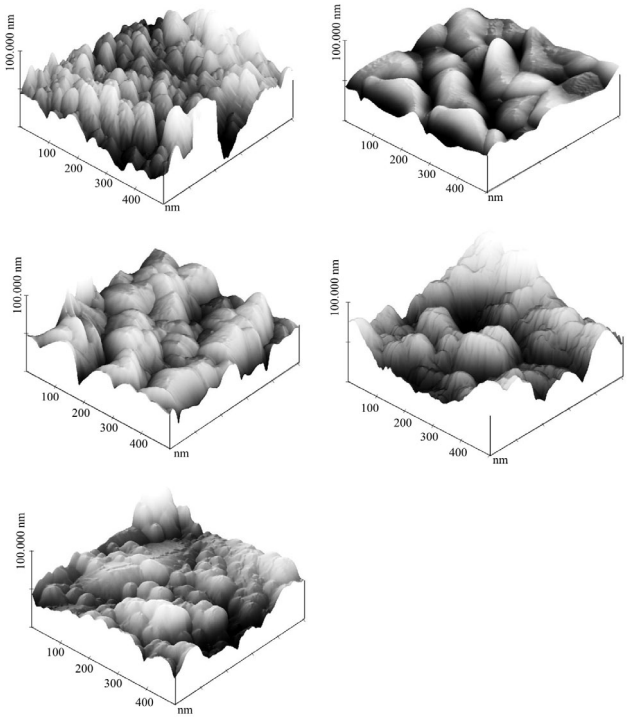

Fig. 4 AFM photographs of the sample surface: I) Z-300, II) ZS-300, III) ZB-300, IV) Z-100, V) ZB-100

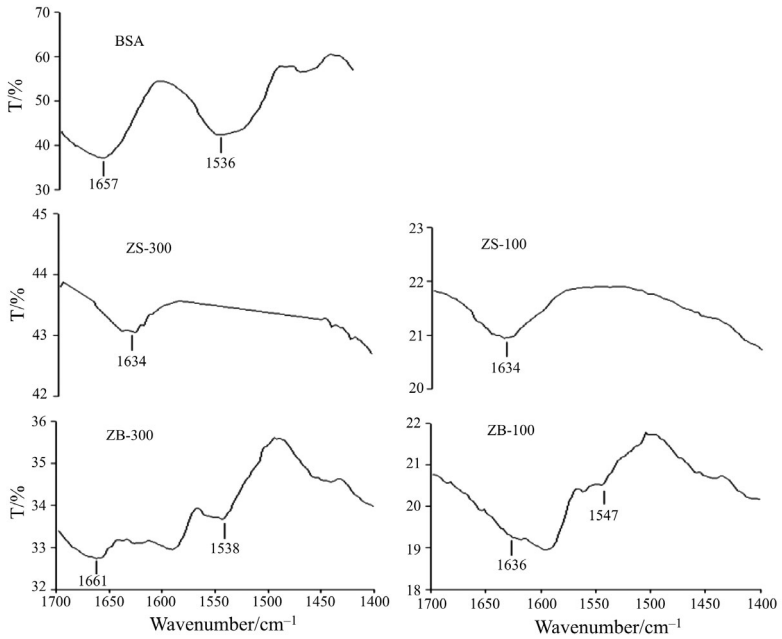

Fig. 5 Changes of FTIR spectra of pure BSA and of the samples after modification

In the case of support Z-100 the values D_f decrease in the second stage of BSA synthesis which indicates the decrease of sample porosity. It was observed that pore sizes play a significant role in synthesis. In the case of the samples on the support Z-300 the spectrophotometric studies (UV-VIS) of absorption of BSA solution before and after the reaction at the wavelength λ=280 nm showed that in the reaction 1.36 mmole of protein for 1 g of gel was bonded which is comparable with the value presented in [27]. In the case of gel Z-100 the amount of BSA diminished by half and was 0.88 mmole g^{-1}. Figure 4 shows the AFM photographs the sample surfaces on the support Z-300: pure (I), after silanization (II) and after modification with BSA (III) as well as on gel Z-100 (IV) and with deposited protein as well as with BSA (V). The photo (III) shows distinctly change of support surface porosity (coarseness) due to protein macromolecules addition. Figure 5 presents the IR spectra of the sorbent in individual stages of the synthesis. For pure BSA there are two intensive bands at 1657 cm^{-1} corresponding to the amide band I (stretching C=O) and at 1536 cm^{-1} characteristic for deformation vibration of the N–H bond (amide band II). In the spectrum of ZB-300 sample there are observed the bands 1661 and 1538 characteristic for the α-helix structure [18, 19] confirming the presence of protein on the sample surface.

Conclusions

The studies showed a great effect of pore sizes on the synthesis of stationary phases with BSA. Modification with protein results in the changes of adsorption properties and adsorbent porosity. Chemical changes cause great structural changes of the sup-

port surface (specific surface area, pore sizes, fractal coefficients). The reaction of functional groups with the gel surface results in adsorption property changes in relation to polar and apolar liquids. Surface modification with silane and BSA leads to formation of more hydrophobic rather than hydrophilic surface of the support. In the case of adsorption of benzene, *n*-butanol, and water the degree of coverage θ decreases. A statistical number of monolayers for water decreases as far as five times which indicates blocking of pure gel OH^- groups.

The shapes of Gauss curve type of desorption energy distribution function were found. The interaction energy of polar and non-polar adsorbents binding decreases in the series: *n*-octane>benzene>water>*n*-butanol for the sample Z-300, benzene> water>*n*-octane>*n*-butanol for ZS-300 and water> benzene>*n*-octane>*n*-butanol, benzene>water≈*n*-butanol>*n*-octane for the samples Z-100 and ZS-100, respectively. The data indicate a complex mechanism of liquid adsorption on the studied surfaces. In the case of adsorption systems Z-300/benzene, Z-300/*n*-butanol and ZS-300/*n*-butanol the values of pore volume are comparable with the data from sorptomate.

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