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SURFACE PROPERTIES OF SILICA GEL SAMPLES MODIFIED BY SELECTED PROTEINS

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The adsorption of a polar (water) and a non-polar (*n*-octane) liquid on silica gels, modified by adsorption of proteins, has been studied by thermal analysis. Silica gels with physically adsorbed BSA and ovalbumin layers were used. Thermodesorption energies were determined from Q-TG and Q-DTG curves recorded under quasi-equilibrium conditions. Significant differences in liquid desorption were observed from the surfaces due to heterogeneous changes (energetic and geometrical) as a result of modification. These results are compared with those obtained for the samples heated at 160°C for 1 h.

Keywords: *adsorption, albumin, fractal dimension, heterogeneity, protein, surface, thermogravimetry Q-TG*

Introduction

The adsorption and desorption of proteins on solid surfaces involve complex processes, resulting in possible changes to their conformation [1–3] and denaturation [4, 5]. The penetration of protein molecules into the surface layers is an important factor in the process [6]. The change in the protein molecules from a globular to an elongated chain conformation results in changes to the Gibbs free energy of adsorption. This energy depends largely on pH, concentration, protein type and preparation techniques of materials [7].

Extensive studies of protein adsorption are being intensively investigated [8–13]. Their relevance to problems of health and to applications in producing biosensor chiral stationary phases [13–22] is important.

Albumin adsorption from solution depends on many factors [23–26]: ionic strength, pH of medium, electrostatic interaction, surface charge, co-adsorption of ions of small molecules, material, isoelectric point, intermolecular forces between adsorbed molecules, solvent–solute interactions, bonding power of functional groups, character and surface properties of a sorbent and its energetic heterogeneity. Energetic heterogeneity of sorbents is caused by differences in topology of adsorption centres, pore size distribution and other factors. In many cases these factors result in conformation changes of protein macromolecules and their denaturation. Protein denaturation can be affected by physical [27–31] or chemical [32] factors. It can be caused by increased temperature, ionic strength of the solution, pH, precipitation, ultraviolet, X-ray and radioactive radiation or ultrasound action. Chemical denaturation proceeds under the effect of compounds capable of hydrogen bond disruption of guanidine hydrochloride, action of acid or bases (pH value below 3 or above 9) and also of surfactants [32]. It leads to decreases in biological activity (changes in the native structure) while maintaining the first order structure. During denaturation, hydrogen bonds are damaged and in the presence of reducing agents disulfide bonds are disconnected. Denaturation results in changes of some physicochemical properties of the systems under investigation, among others, solubility, viscosity leading to aggregation of protein polymer molecules and their removal. Solid–albumin interactions also result in complications. Transition from a low energetic state of the biologically active form to the denatured one is acompanied by an increase of entropy.

This paper reports on differences in physicochemical properties of adsorbents with chemically and physically bonded albumin. Liquid thermodesorption measurements made under quasi-isothermal conditions were used for the study of differences in polar and non-polar liquids. Using the method based on the kinetic model described elsewhere in detail [33], thermodesorption energy and surface fractal dimensions were determined from a single Q-TG curve, which facilitated significantly the calculation and measurements.

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Experimental

Materials

Albumin bovine (BSA, Fraction V, minimum 98%) and ovalbumin were purchased from Sigma. Silica gel Davisil 653XWP was obtained from Supelco. All other reagents were of analytical grade.

Modification

Silica gel (10 g) was added to HCl solution (200 cm^3 20%). The mixture was heated to 110°C and vigorously stirred for hours. It was then filtered and washed with redistilled water to obtain a pH of 7. The activated gel was dried at 200°C for 5 h. Albumin (BSA and ovalbumin) stock solution (8 cm^3, 3 mg cm^{-3}), phosphate buffer (or KCl solution) and activated silica gel (0.25 g) were added into plastic reactors. The mixtures were precipitated at 25°C with 100 rpm for 2 or 24 h. Then the adsorbent was separated from the solution, washed with buffer solution, dried at room temperature for 3 days, heated at 45°C for 2 days and dried in the desiccator with molecular sieves. The solution was centrifuged and the adsorbed protein concentration was determined (by measuring the absorbance before and after modification at λ=279 nm). The phosphate buffer was prepared by mixing measured quantities of Na_2HPO_4 (0.05 M) and NaH_2PO_4 (0.05 M) solution to obtain a given pH value. The solutions based on KCl of a determined pH value were prepared likewise from KOH (0.01 M) and HCl solutions (0.01 M).

Methods

The thermodesorption of a polar (water) and a nonpolar (*n*-octane) liquid from the surface of the synthesized material was studied using a Derivatograph of Q-1500D type (MOM, Hungary). The samples were saturated with liquid vapours in a vacuum desiccator where p/p_0=1 for 24 h, to establish the state of liquid adsorption equilibrium. The gel samples were removed to the measuring crucible placed in the furnace of the Derivatograph and 3 measurements were made in the temperature range 20–250°C with a heating rate 6°C min^{-1}; measurements close to the average value were taken for calculations.

The amount of reacted modifier was determined by the UV-Vis spectrophotometry and the type of chemically bonded groups in the material was determined by infrared spectroscopy (FTIR, Perkin Elmer, Paragon 100). Albumin conformation changes were also determined from low-temperature nitrogen adsorption (Sorptomat ASAP 2405 V1.01); specific surface areas and pore volume were determined and then fractal dimensions were calculated. Based on the above experimental data, adsorption capabilities as well as total surface heterogeneity of the studied samples were determined and images of the surface AFM (Digital Instruments Nanoscope III) were recorded.

Results and discussion

Silica gel modification leads to changes of surface energetic heterogeneity and above all to the change of samples' geometrical heterogeneity. Table 1 presents the values of specific surface area (S_{BET}), pore volume (V) and pore diameter (D) of the samples modified with ovalbumin and BSA determined by the low-temperature nitrogen adsorption–desorption sorptometry. Albumin addition causes a decrease in specific surface area and pore volume compared with those of the initial materials.

In the case of gel modification with the BSA solution, the effect of concentration and pH are observed (Table 1). For adsorption from KCl solutions with small pH values, it was observed that there was an increase of specific surface area (S_{BET}) and decrease of total pore volume in the range 1.3–1.02 for pH 4.9 and 8.5. This results from protein conformation changes rather than from increased protein adsorption. For adsorption from phosphate buffer solutions, decrease in pore volume with decreasing pH was observed (Table 1) due to significant difference in the amount of added albumin; this is the largest at the lowest pH.

For ovalbumin adsorption, decrease of specific surface area (compared with BSA) and pore volume, as well as significant changes of pore diameter for both acidity stabilizers, were observed (Table 1). Studies of gel samples modified with albumin for 24 h showed insignificant differences in the amount of adsorbed protein and in the surface parameters, compared with the materials modified for 2 h. In the case of 24 h modifications, BSA and ovalbumin adsorption from the 0.05 M phosphate buffer solution of pH 7, micropore formation was observed. This effect results from interactions of adsorbed molecules.

Surface heterogeneity of the albumin/SiO_2 system increases due to heating of adsorbents. Heating for 1 h at 160°C causes increase in the volumetric fractal dimension: D_v=2.66 due to hydrogen bonds destabilization.

From the Q-TG curves (Figs 1–4) and Q-DTG data the statistical number of liquid monolayers, n_{aver}, and desorption energy distribution functions were determined using the dependence [33]:

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

Table 1 Adsorption and porosity parameters of silica gel samples (pure and modified by BSA)

t_{mod}/	Protein	Solution	Liquid	Amount adsorbed protein/	N (liquid)/	n_{aver}	S_{BET}/	S_{mic}/	V_{BJH}/	D/	E_{d_p}/	$E_{d,max}$/	E_{d_k}/	$\varphi_{E_{d,max}}\cdot10^6$/	D_v	D_p
h				mg g^{-1}	mol		m^2 g^{-1}		cm^3 g^{-1}	nm		kJ mol^{-1}		mol kJ^{-1}		
2	BSA	0.1 M KCl pH 4.9	*n*-octane water	86.5±4.7	0.0051 0.0494	7 20	185.3		1.30	27.93	14 20	46 49	70 69	42.5 90.2	2.61	–
2	BSA	0.1 M KCl pH 7	*n*-octane water	77.54	0.0059 0.0392	9 18	162.4		1.10	27.18	13 16	30 49	42 70	35.5 65.5	2.63	–
2	BSA	0.1 M KCl pH 8.5	*n*-octane water	58.17	0.0052 0.0308	9 16	146.3		1.01	27.75	18 20	45 43	70 60	42.8 55.5	2.62	–
2	BSA	0.05 M phosphate buffer pH 4.9	octane water	90.7±4.61	0.0040 0.0500	7 25	150.1		1.01	27.03	13 17	42 60	70 83	27.2 84.5	2.61	2.45
2	BSA	0.05 M phosphate buffer pH 7	*n*-octane water	40.07	0.0070 0.0458	12 23	147.7		1.05	28.39	17 20	38 51	60 80	19.3 89.7	2.60	2.50
2	BSA	0.05 M phosphate buffer pH 8.5	*n*-octane water	20.66	0.0030 0.0396	5 20	152.3		1.07	28.18	20 30	43 43	80 57	18.9 58.5	2.62	2.53
2	BSA	0.05 M phosphate buffer pH 7, 1 h, 160°C	*n*-octane water	40.07	0.0036 0.0427	5 18	173.7		1.23	28.65	18 17	44 65	80 92	17.8 76.3	2.66	2.58
2	ovalbumine	0.05 M phosphate buffer pH 7, 1 h, 160°C	*n*-octane water	12.67	0.0031 0.0537	4 20	201.2		1.44	28.80	17 17	53 54	84 80	17.9 86.3	2.66	2.32
2	ovalbumine	0.05 M phosphate buffer pH 4.9	*n*-octane water	84.83	0.0193	9	170.8		1.09	22.88	16	44	64	33.5	2.59	2.45
2	ovalbumine	0.05 M phosphate buffer pH 7	*n*-octane water	12.67	0.0007 0.0277	1 12	171.8		1.23	28.45	14 20	26 60	60 80	2.11 50.12	2.64	2.40
2	ovalbumine	0.1 M KCl pH 4.9	*n*-octane water	79.6±4.16	0.0004 0.0131	1 6	171.4		0.92	24.68	11 17	18 77	35 120		2.61	–
2	ovalbumine	0.1 M KCl pH 7	*n*-octane water	73.6±2.2	0.0005 0.0234	1 10	172.4		1.19	28.04	14 15	20 71	35 110		2.58	2.44
24	BSA	0.05 M phosphate buffer pH 7	*n*-octane water	51.64		–	150.1	3.8	1.05 0.0008	28.08	–	–	–	–	2.61	2.42
24	ovalbumine	0.05 M phosphate buffer pH 7	*n*-octane water	18.9		–	172.1	9.1	1.23 0.0033	28.38	–	–	–	–	2.63	2.40
–	–	pure SiO_2	*n*-octane water	–	0.0068 0.0470	9 19	190.1		1.2	25.17	26 24	54 37	90 55	106.9 74.01	2.59	2.63

t_{mod} – time modification, N – the number of moles of the adsorbed liquid vapour, n_{aver} – the statistical number of liquid monolayers, S_{BET} – specific surface area, V_{BJH} – the pore volume, D – pore diameter, D_v – pore and D_p – surface fractal dimensions, $\varphi_{E_{d,max}}$ – value energy distribution function in maximum

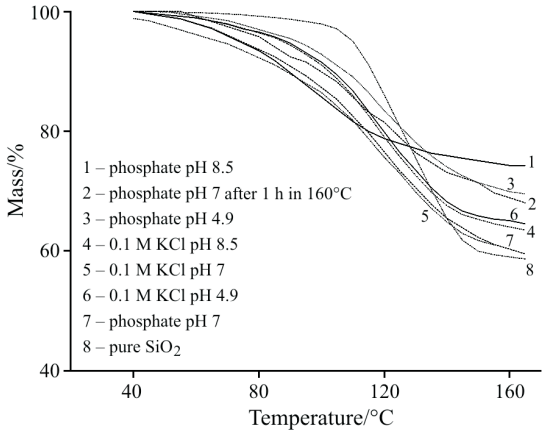

Fig. 1 Q-TG curves of *n*-octane thermodesorption from the surface of pure and modified (by BSA) samples of silica gel

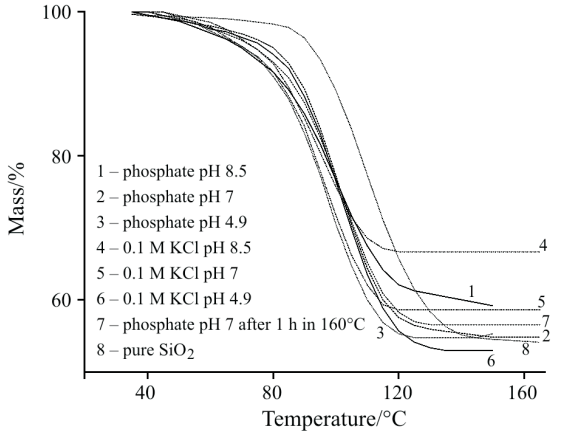

Fig. 2 Q-TG curves of water thermodesorption from the surface of pure and modified (by BSA) samples of silica gel

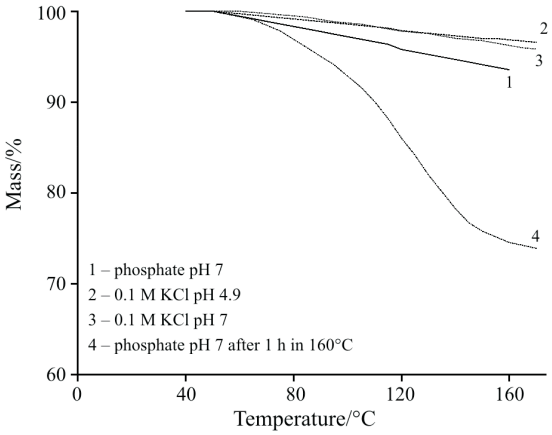

Fig. 3 Q-TG curves of *n*-octane thermodesorption from the surface of pure and modified (by ovalbumine) samples of silica gel

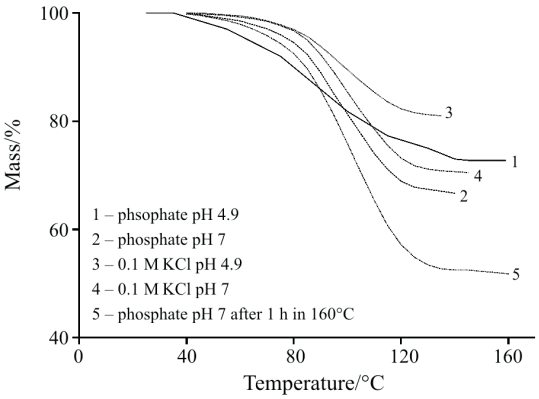

Fig. 4 Q-TG curves of water thermodesorption from the surface of pure and modified (by ovalbumine) samples of silica gel

where $T=T_0+\beta t$, θ – degree of surface coverage, ν – entropy factor, E_i – desorption energy calculated for each temperature; T_0 and T – initial and given temperatures of desorption, respectively, β – heating rate of the sample, t – time and R – universal gas constant and:

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

Table 1 gives the ranges of E_d value changes for individual systems. Material energy distribution functions based on BSA and ovalbumin differ in desorption energy values and shape of curve (Figs 5 and 6) which indicates a complex desorption mechanism and influence of adsorbed protein concentration on it. The higher the value of the distribution function, the greater is the number of active centres of a given energy on the surface. Increase in the $E_{d,max}$ value also indicates increase of adsorbate–adsorbent interactions. In the case water adsorption from the BSA immobilized samples there was observed an increase in the statistical number of monolayers with an increase in the amount of protein on the surface (Fig. 7) and also an increase in adsorbent–adsorbate interactions accompanying it (Fig. 8). One of the parameters characterizing solid geometrical heterogeneity is fractal dimension. These are calculated by the methods based on the theory by Frenkel, Halsey and Hill as well as by Kiselev from the following dependences [34]:

$$D_v = 2 + \frac{d\left[\ln\int_\alpha (-\ln x)da\right]}{d[\ln(-\ln x)]} \tag{3}$$

$$D_v = 3 - d[\ln a(x)]/d[\ln(\ln x)] \tag{4}$$

where a is the amount adsorbed, $x=p/p_0$.

Table 1 presents the mean values of fractal dimension calculated from nitrogen adsorption isotherms based on Eqs (3) and (4) (volumetric fractal dimensions D_v) and from AFM data. The value of volumetric fractal dimension, (D_v), lies within a narrow range, 2.6–2.63, for BSA adsorption and 2.58–2.64 during ovalbumine modification. BSA adsorption re-

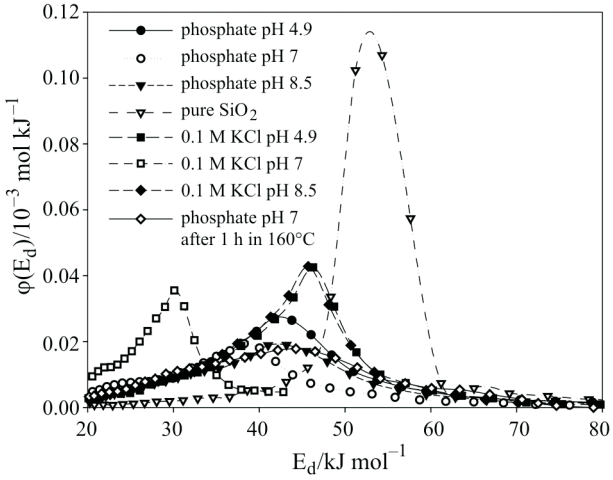

Fig. 5a Desorption energy distribution function of *n*-octane from pure and modified by BSA of samples

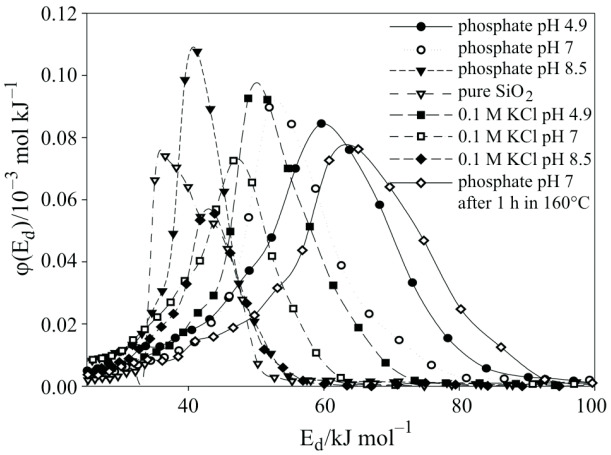

Fig. 5b Desorption energy distribution function of water from pure and after modifications by BSA samples

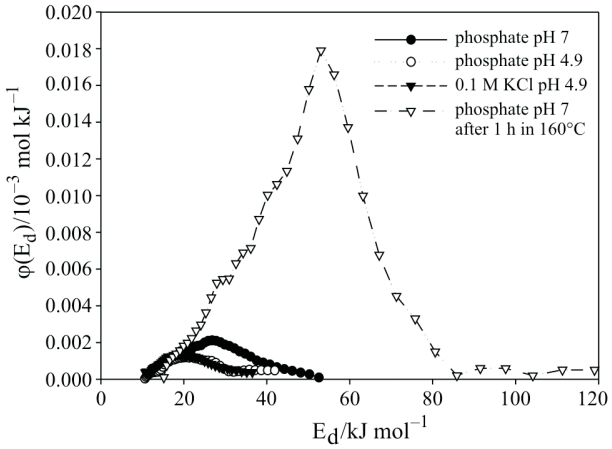

Fig. 6a Desorption energy distribution function of *n*-octane from pure gel and after modifications by ovalbumin samples

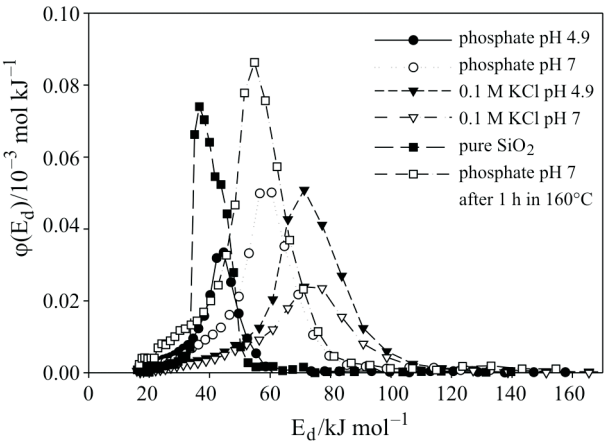

Fig. 6b Desorption energy distribution function of water from pure silica gel and after modifications by ovalbumin samples

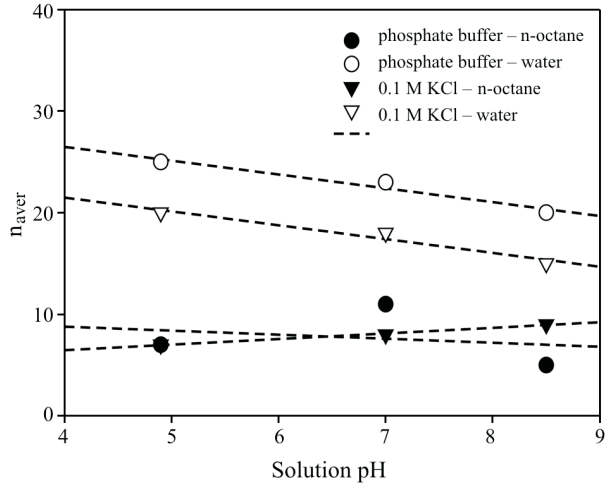

Fig. 7 Liquid monolayer statistical number variation with pH for modification samples by BSA

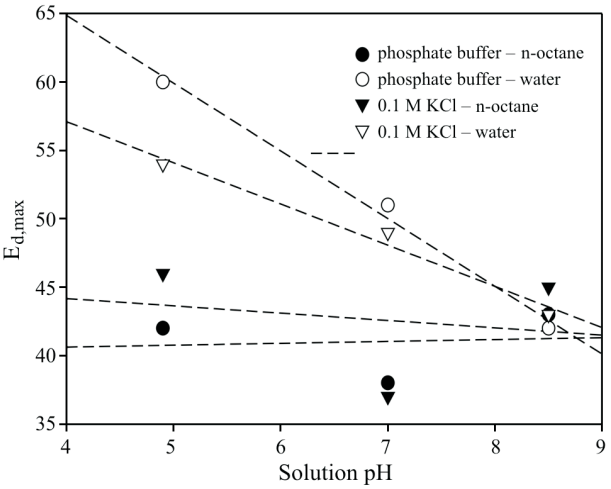

Fig. 8 Desorption energy value in the distribution function maximum – variation with pH for modification samples by BSA

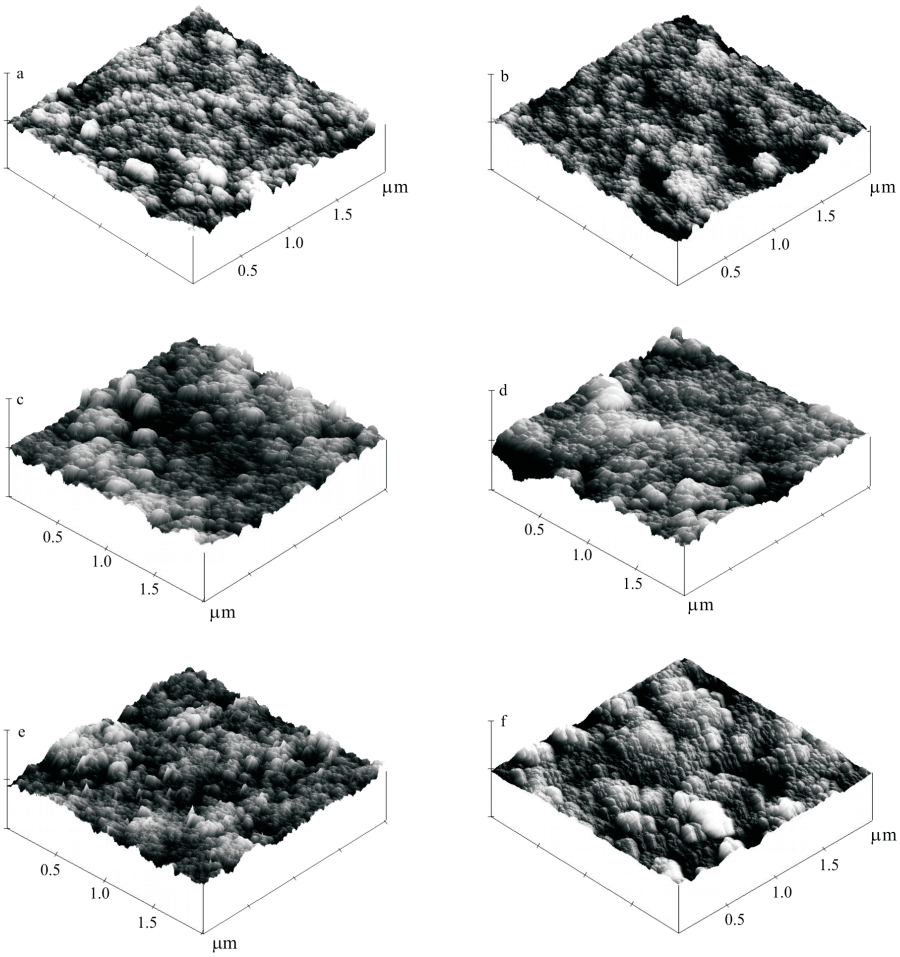

Fig. 9 AFM images of silica gel samples modified by ovalbumine. a – phosphate, buffer pH 7, 24 h; c – phosphate buffer, pH 7 after 1 h in 160°C; e – phosphate, buffer pH 7, 2 h and BSA; b – phosphate, buffer pH 7, 24 h; d – phosphate buffer, pH 7 after 1 h in 160°C; f – phosphate, buffer pH 7, 2 h

sults in greater changes to surface properties compared with ovalbumin adsorption. However, in contrast, the modification time and acidity stabilizer do not play a significant role (small D_f changes) (Table 1) in BSA adsorption, compared with ovalbumin adsorption. Albumin adsorption causes layer changes in fractal dimensions depending on pH, acidity stabilizer and immobilization time. The largest D_v changes were observed for the adsorbents modified in phosphate buffer solutions of pH=7 after 2 and 24 h (D_v=2.63). The samples modified with ovalbumin from KCl solutions are characterized by D_v increases with the increasing amount of adsorbed protein.

With increasing D_v value, the relative contribution of pore size values close to the minimum value increases compared with the pore size values close to the maximum value. The distribution becomes more heterogeneous. The increase in amount of adsorbed protein results in decrease of surface fractal dimension (D_p) and hence in surface corrugation (Fig. 9).

Conclusions

The surface parameters of silica gel are modified by adsorption of protein. The adsorption of BSA depends significantly on the adsorption conditions: pH and stabilizer. The closer pH value is to the isoelectric point, the larger is the amount of adsorbed albumin. Chemical composition of the studied albumins and the amount of adsorbed protein has a great effect on total heterogeneity of the adsorbent. BSA modified adsorbents have smaller specific surface areas compared with those of the initial material. Surface porosity of the adsorbents depends significantly on the stabilizer acidity and pH of the modifier solution. Material obtained after modification by BSA from solution in phosphate buffer medium of pH 7 after 24 h adsorption showed formation of micropores in the wide-pore material. Because chromatographic separation using silica gel as the stationary phase depends significantly on its surface properties, these results are important in liquid chromatography. The studies

show that protein modified phases possess properties, which depend largely on the conditions of modification (bulk solution pH, acidity stabilizer, etc.). They show that the mechanism of albumin interactions with sorbents is complicated. Proteins are denaturated at higher temperatures, which cause changes in the native protein structure leading to smaller or greater loss of biological activity or other characteristic feature of the proteins.

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