Chemistry for Sustainable Development **14** (2006) 295–299

Adsorption of Protein by Modified Carbon Fibres

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(Received September 6, 2005; revised February 15, 2006)

Abstract

Adsorption of bovine serum albumin by carbon fibres modified with titanium hydroxide by means of chemical and electrochemical deposition is investigated. The effect of fibre modification on changes in adsorption capacity with respect to the protein from the solutions of different composition is demonstrated. Adsorption of albumin was determined with the open-circuit potential and on the polarized surface of carbon fibres. Adsorption constants are calculated. It is established that titanium-containing fibre obtained as a result of the electrochemical deposition of titanium hydroxide is characterized by the maximal sorption capacity with respect to bovine serum albumin.

INTRODUCTION

Activated carbon fibres (ACF) are increasingly widely used in sorption processes to extract, separate and concentrate various components from technological solutions, drinking water, biological liquids [1–3]. Fibrous carbon materials possess highly developed surface, developed porosity, which is controllable within a broad range, good electrical conductance, thermal, chemical and radiation stability [1, 3]. Due to the indicated properties, the fibres are used to make various sorbents.

The sorption capacity of carbon materials depends on their porosity and surface polarity. The formation of a developed system of pores in ACF and their surface groups is carried out by means of modification, for example, by gas-vapour activation, reagent or electrochemical oxidation [4]. Modification of the surface of carbon materials, for example, with titanium hydroxide which forms strong biologically inert coatings and is stable within a broad pH range [5], allows one to change the surface properties and the porous structure of a sorbent, which results in an increase in sorption capacity with respect to proteins.

Investigation of the sorption properties of modified carbon materials becomes urgent in light of the development of new biocatalysts, in solving the problem of protein purification for medicine, pharmacology and analytical chemistry [6, 7].

Adsorption of protein molecules on a solid surface depends on temperature, composition, concentration and pH of solution, on the presence of surface groups, on the nature of ACF and on its charge [6, 8]. Change in the charge of carbon surface provides the possibility of purposeful action on the equilibrium in the system adsorbent – adsorbed substance, that is, allows one to govern the state of the adsorption system. Electrochemical polarization of ACF with the corresponding choice of the potential allows to control its adsorption capacity, to desorb the organic matter and to recover the sorption capacity of the fibrous material.

The goal of the present work was to investigate the processes involved in the adsorption of bovine serum albumin (BSA) from various solutions at the open-circuit potential and on the polarized surface of carbon fibres modified with titanium hydroxide.

EXPERIMENTAL

The sorbents were braided ACF “Aktilen” of B grade with the following characteristics: specific surface determined on the basis of nitrogen adsorption by means of BET procedure was 700 m^2/g, micropore volume 0.4 cm^3/g, mean pore radius 4 Å. We also used modified carbon fibres obtained by chemical [5] and electrochemical [9] deposition of titanium hydroxide – ACF-Ti(chem.) and ACF-Ti(el.-chem.), respectively. The BSA preparation manufactured at NIIEV (Minsk, Belarus) was used in the investigation. The BSA content of solution was determined using the biuret procedure [10]. The solutions were physiological (0.9 % NaCl) and an all-purpose buffer (sodium phosphate–acetate–borate) with pH 4.7 and 6.0, respectively.

Adsorption and electrical sorption of BSA were studied with a bipolar MB-2 membrane under static conditions in a standard three-electrode cell with the separated cathode and anode chambers. The fibrous material under investigation was placed in a gold net, which served as a current supply. The counter-electrode was a porous graphite rod. Measurements were carried out with respect to the silver chloride reference electrode. The potential at which the sorption ability of the protein was studied was chosen within the region where no electrochemical transformations of BSA occur. In order to do this, we carried out preliminary voltage-current measurements in the system: protein solution – carbon material. The amount of the protein adsorbed on the surface (Γ) was determined using equation $\Gamma = V(C_{\text{in}} - C_{\text{equil}})/m$, where V is solution volume, ml; C_{in}, C_{equil} are the initial and equilibrium protein content of solution, mg/ml; m is a weighed portion of the sorbent, g.

RESULTS AND DISCUSSION

It is known [6] that the adsorption of a protein depends on the solution pH causing the appearance or a change in the charge of protein molecules and sorbent surface. Variation of pH of the medium causes changes in the degree of dissociation of functional groups of the solid surface and of protein molecules [6], which results in the occurrence of additional electrostatic interactions affecting adsorption value.

The isotherms of adsorption of BSA on non-polarized surface of fibres at different pH of buffer solutions are shown in Fig. 1. One can see that the largest sorption of BSA is achieved at pH 4.7, which corresponds to the isoelectric point of the protein (pH = pI). To explain such a regularity, a hypothesis was proposed [11] according to which an increase in adsorption in the region of pH = pI is due to the rigid structure of the protein molecule. For this pH value, the area occupied by a BSA molecule on the surface is minimal because this conformation is to a lesser extent subjected to “flattering” under the action of surface forces. With a shift of pH from the isoelectric point to pH 6, the degree of adsorption of BSA decreases. This may be connected with changes in the conformation of the protein molecule [6], which results in an increase in the area occupied with it on the sorbent surface and a decrease in the amount of sorbed substance.

It follows from the analysis of dependences shown in Fig. 1 that, independently of solution pH, the degree of BSA adsorption on Ti-containing fibre is higher in comparison with non-modified ACF. This is likely to be due to the presence of a large number of adsorption centres on surface regions coated with titanium hydroxide [12]. The surface of fibres themselves and titanium hydroxide film coating the fibres has many binding centres of different configurations and composition for proteins that

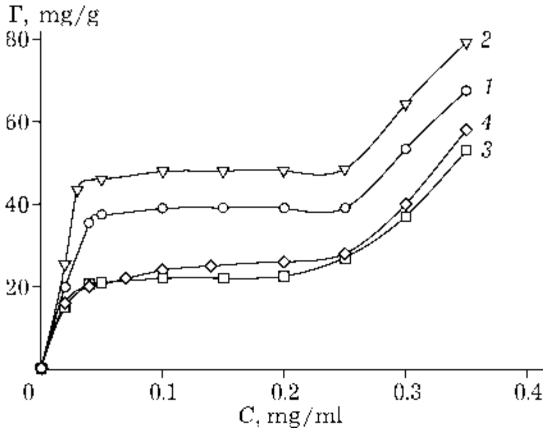

Fig. 1. Isotherms of adsorption of BSA preparation for different pH values of the buffer solution: *1, 2* – initial ACF and ACF-Ti(chem.), respectively, pH 4.7; *3, 4* – the same for pH 6.

have polyfunctional quaternary structure and therefore are able to get bound to the sorbent at several sites on its surface [10].

One can see in dependences obtained in the buffer solution that the isotherms have an inflection, which is explained in literature by reorientation of molecules and their tighter packing on the solid surface. In addition, multilayer adsorption is possible, when protein molecules interact with the layer of molecules that are already adsorbed on the solid surface [13]. However, these regularities are no longer observed when the buffer solution is replaced with the physiological one.

The isotherms of adsorption of BSA on the surface of the initial and modified carbon fibres are shown in Fig. 2, both under the open-circuit conditions and under the polarization of ACF. For BSA adsorption from the physiological solution (pH ~ 6), the isotherms have no inflections within the entire range of concentrations involved. A possible reason may be smaller competitive adsorption of electrolyte ions, which do not cause such a displacing effect as the ions of the buffer solution. As a result, adsorption isotherms have the appearance characteristic of the case when multilayer deposition of the adsorbed substance does not occur on the solid surface [13].

It should also be noted that the degree of adsorption of BSA from the physiological solution, both on the initial and on the titanium-containing fibres, is much higher than that from the buffer solution. This is likely to be a consequence of the competitive adsorption of the protein and buffer ions on the charged sites of adsorbent surface, with higher concentration of the buffer than the concentration of sodium chloride in the physiological solution [10]. So, the corresponding choice of the solution composition allows one to increase the sorption capacity of fibrous sorbents.

It follows from the analysis of curves *2* and *3* shown in Fig. 2 that the sorption capacity of titanium-containing fibre obtained electrochemically is much higher than that of the fibrous material modified by chemical deposition of titanium hydroxide. This is due to some difference in the surface properties of the sorbents obtained by means of chemical and electrochemical modification. Unlike for chemical modification, electrochemical deposition of titanium hydroxide results not only in a more uniform deposition of the latter over the whole surface of the fibrous material but, most likely, also in the formation of a larger number of adsorption centres, because electrochemical polarization process itself has an additional effect on changes in the surface properties of fibres [9, 14]. After electrochemical modification of the fibrous material, its surface area determined with the electrochemical procedure [16] increases to 770 m^2/g (for chemically modified ACF it is 720 m^2/g). An increase in the specific surface of the composite sorbent causes an increase in its sorption capacity with respect to BSA. One cannot exclude that the electrochemical modification method causes an increase in the hydrophilic properties of the surface; therefore, the hydrophilic interaction between the sorbent surface and protein molecules increases. So, the choice of the corresponding method of ACF modification allows one to increase the sorption capacity of the material used to extract the protein from solution.

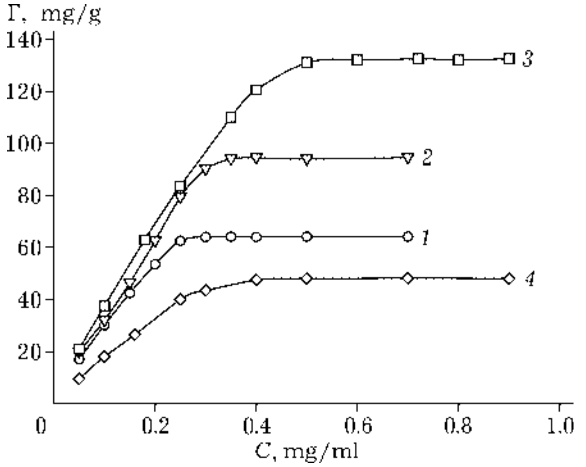

Fig. 2. Adsorption isotherms of BSA for the open-circuit potential (*1*–*3*) and polarized surface of carbon fibres (*4*) in the physiological solution: *1* – initial ACF, *2* – ACF-Ti(chem.), *3* – ACF-Ti(el.-chem.), *4* – the same for $E = -0.3$ V.

A comparison between curves *3* and *4* shown in Fig. 2 suggests that a change of the charge of sorbent surface has an essential effect on its sorption capacity with respect to the protein. A shift of the potential to 0.3 V into the cathode region decreases the capacity of titanium-containing fibre by a factor of three. Such a phenomenon is due to Faraday process occurring on the surface of the carbon material, which results in a change of the solution pH as a result of the generation of OH^- ions during cathode polarization [11]. In turn, this causes ionization of the protein molecule; its ionic form is less sorbed on the surface of the same charge. It follows from this fact that the choice of the corresponding potential of ACF polarization allows one to select the conditions for protein removal from the sorbent surface.

The composition of solution also affects the appearance of adsorption isotherms at their initial region, which provides evidence of the different interaction of BSA with the solid surface. The isotherms obtained in the buffer solution can be related to L type, while those obtained in the physiological solution are rates to C type [13]. All the adsorption isotherms obtained, except the regions with inflections on the curves, were approximated with Langmuir and Freundlich equations [13].

The results of calculation shown in Table 1 suggest that the obtained adsorption dependences are approximated with the smallest error using Langmuir equation. Henry constants [15] are shown in Table 1, too. Comparison between these values allows us to assume that the mechanism of filling the surface with protein molecules is strongly affected by the composition of solution. BSA molecules are likely to arrange themselves near the solid surface in different manners in the solutions with different salt background. Proteins are polyelectrolytes which are able to undergo conformational changes not only in solution but also when adsorbed on a surface [6, 13]. Protein molecules orientate themselves in the force field of the interface and either conserve the state close to the native one or undergo some structural changes [6] getting bound to the surface in different manners. On the basis of the values of Henry constant, one may assume that the modification method affects only the amount of sorbed BSA and does not change the mechanism of protein deposition on nonpolarized sorbent. To the contrary, changes in ACF charge during its polarization affect both the amount of sorbed BSA and the mechanism of the interaction of protein molecules with the surface.

As we have already mentioned, for cathode polarization of the fibre as a result of Faraday process, the solution of electrolyte becomes more alkaline. A change in pH in the near-electrode layer causes ionization of BSA molecules. The conformation state of these molecules will differ from BSA structure in the physiological solution with pH 6. As a result, different interaction with the surface occurs. With the replacement of the physiological solution for buffer one, judging from the values of Henry constants, the mechanism of protein interaction with the surface is affected both by the composition of the solution and by the nature of sorbent surface. This is likely to be due to the

TABLE 1

Results of approximation with Langmuir and Freundlich isotherms, and Henry constant

Sample	Langmuir			Freundlich			Henry
	s	K	Γ_{max}	s	K	n	K
			Physiological solution				
ACF-B (initial)	5.4	7.2	85.6	8.5	86.1	2.8	613
ACF-B (Ti-chem.)	8.8	4.1	144.0	12.6	134	2.1	585
ACF-B (Ti-el.-chem.)	9.0	3.3	192.0	14.5	158	2.1	624
ACF-B (Ti-el.-chem.), $E = -0.3$ V	4.1	5.3	63.9	6.8	58.2	2.6	336
			Buffer solution, pH 4.7				
ACF-B (in.)	3.6	68.7	43.4	5.1	52.2	6.3	2982
ACF-B (Ti-chem.)	4.7	82.3	52.6	6.1	62.6	7.0	4329
			Buffer solution, pH 6.0				
ACF-B (initial)	1.0	102.0	24.0	1.7	28.8	7.7	2453
ACF-B (Ti-chem.)	0.8	117.2	23.9	0.9	36.9	7.4	3339

difference in the structure of protein molecules, which get oriented near the solid surface interacting with it in different manners in the solutions with different salt backgrounds.

CONCLUSIONS

Investigation of BSA adsorption from different salt solutions on fibrous non-modified and titanium-containing materials was carried out. The data obtained allow us to estimate the promising character of the application of fibres modified with titanium hydroxide, and to extend the possibilities of the methods of protein extraction on the basis of sorption phenomena.

It was established that changes in the composition and pH of solutions affect the sorption capacity of the fibres and the mechanism of filling the solid surface with protein molecules. So, control of the composition of solution is one of the factors ensuring the most complete extraction of a protein with a solid surface.

It is shown that the titanium-containing fibre obtained by electrochemical deposition of titanium hydroxide sorbs BSA from solution most efficiently. The choice of the corresponding ACF modification method allows one to obtain a sorbent promising for protein extraction.

The effect of the negative charge of the surface on the protein adsorption was discovered. Due to this fact, it is possible to choose the conditions of BSA desorption from the surface of titanium-containing fibre and to recover its sorption capacity.

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