

## Sorptive and Separation Properties of Ultrafiltration Membranes on the Basis of Sulfonate-Containing Polyamide with Respect to Bovine Serum Albumin

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### Abstract

Investigation of sorption of bovine serum albumin in the static mode and in ultrafiltration conditions by membranes produced from statistic copolymers of aromatic polyamides synthesized by polycondensation of the sodium salt of 4, 4'-diaminodiphenylamine-2-sulfo-acid and *m*-phenylenediamine in various ratios with chloroanhydride of isophthalic acid has been carried out. Interconnection has been established between the charge of protein macromolecules, concentration of fragments containing ionic groups in the aromatic polyamide and sorptive, separation and transport characteristics of membranes on its basis. It has been shown that dominant forces that determine membrane/protein interaction in the systems under consideration are coulomb forces, but the contribution of hydrophobic interactions is also significant. The results of mathematical processing of experimental data indicate that there is a good compliance of sorption isotherms with Langmuir's model. Depending on the concentration of fragments containing ionic groups in the polyamide and *pH* of the solution, the calculated values of maximum sorption in sorbent/sorbate systems under consideration vary in the range of 0.028 to 0.338 mg/cm<sup>2</sup>. Dynamic investigations have shown that selectivity of the membranes is 85 to 98%. To assess the sorptive activity of the membranes in the course of ultrafiltration, indicators of sorption and sorptive losses calculated on the basis of the ratio of the change of mass content of protein in the process of filtration to the initial value have been used. Depending on the material used to produce the membrane and *pH* of the solution being filtered, sorptive losses range from 5 to 33%. Their minimum value is observed when *pH* is higher than the isoelectric point of the protein, i.e. in the field where protein macromolecules and the surface of the membrane have like charges.

### Introduction

One of approaches in development of highly efficient polymer porous membrane materials involves introduction of ionic groups into their structure. Two main methods of practical solution of this objective are known: surface modification of the membrane material [1-4] and introduction of polymer electrolytes into the formulation [5-9].

Presence of ionic groups in the membranes results in a change in their electrosurface properties, which has a significant effect on the separation mechanism and transport characteristics of the systems in the course of filtration of solutions carrying a certain charge, such as protein solutions [10-12].

Presence of a fixed charge in the membrane's matrix makes it possible to produce a more loosened

active layer (as a result of electrostatic repulsion of charges on pore surfaces) and to increase the flow rate through the membrane while retaining high selectivity, due to the remote effect of electrostatic forces inside the membrane [13, 14].

Aromatic polyamides, as a special class of polymers, are actively used in production of porous polymer membranes. Their advantages include increased mechanical strength, high heat stability, good resistance to chemical and thermooxidative destruction as well as high transport and separation characteristics of membranes produced from them [15-19].

Introduction of ionic groups into macromolecular chains of aromatic polyamides makes it possible to produce polymers combining properties typical of aromatic polyamides and increased hydrophilicity and ionic selectivity typical of polyelectrolytes.

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The purpose of the present research was to explore the effect of presence and concentration of ionic groups in porous membrane materials based on aromatic polyamide on their sorptive and separation properties with respect to bovine serum albumin for the purpose of discovering the separation mechanism and revealing main factors that determine the mechanism.

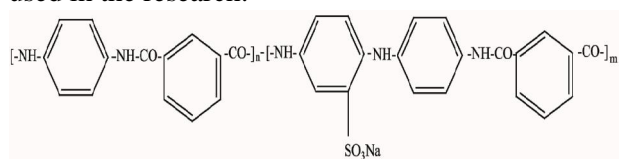
It is known that the process of protein sorption in sorbent/protein systems is rather complicated and is determined by various kinds of interactions: electrostatic, Van der Waals, hydrophobic and hydrophilic interactions between the protein and the sorbent as well as the effect of structural and steric factors [20]. The contribution of each of these components depends on both the nature of the protein and conditions of the sorption experiment (specifically, pH of the medium) as well as presence and concentration of ionic groups in the sorbent. It is the complexity of sorptive processes under consideration that results in certain inconsistency of data published in the literature. Authors of some papers [21] believe that hydrophobic interactions between the sorbent and the protein play the main role in the process of sorption, whereas in other papers [22, 23] coulomb forces are considered to be the governing factor. The inconsistency of estimates and high importance of protein sorption processes for a number of biomedical applications [24], including those involving active use of membrane technologies [25], warrant expediency of further research.

In view of the significant role of sorption in processes of membrane separation (specifically, its effect on the mechanism of separation and plugging of membranes in the process of filtration), it is considered necessary to investigate the interaction of sorptive activity, separation and transport characteristics of membranes.

It can be assumed that the use of copolymers having a varied number of fragments containing ionic groups makes it possible to deliberately control transport characteristics and selectivity of membranes on their basis.

## Experimental

Samples of ultrafiltration membranes produced by the phase-inverting wet-casting method on the basis of functionalized copolymers of aromatic polyamide (PA) having the following structure were used in the research:



$n = 99, m = 1$  mol.% (PA-1)

$n = 97, m = 3$  mol.% (PA-3)

$n = 95, m = 5$  mol.% (PA-5)

$n = 90, m = 10$  mol.% (PA-10).

Characteristics of the prepared membranes, such as permeability, bubble point and nominal molecular weight cut-off (determined by the method described in reference [26]), correspond to characteristics typical of ultrafiltration membranes. Permeability of the membranes in terms of twice-distilled water at  $p = 0.1$  MPa ranged from 0.65 to 0.80 ml/min·cm<sup>2</sup>. Bubble point was  $\sim 7.5$  atm. Nominal molecular weight cut-off ranged from 32 to 40 kDa (retention coefficient was 0.9).

Electrosurface properties of the membranes were investigated according to the method described in reference [27]. Depending on the formulation of the copolymer used for preparation of the membrane, the magnitude of its  $\zeta$ -potential ranged from -5.3 mV (PA-1) to -43.8 mV (PA-10).

Sorption of protein by membrane samples was investigated in both static and dynamic mode. Bovine serum albumin (BSA) produced by Sigma (USA) having molecular weight of 69 kDa was used in the research. BSA contains 143 free negatively charged carboxyl groups (of aspartic and glutamic acids) and 101 positively charged aminogroups including 60 primary aminogroups of lysine, 23 aminogroups of arginine and 18 aminogroups of histidine [28]. Such ratio of negatively and positively charged ionogenic groups determines the magnitude of the isoelectric point of protein  $pI$ , which is equal to 4.9 [29]. The BSA globule has dimensions of the order of  $150 \times 40\text{-}45$  Å [28].

In the case of static sorption experiment, membrane samples having the area of 25 cm<sup>2</sup> were used. The volume of the protein solution was 25 ml. In preparation of solutions the 0.04 M universal buffer was used having pH values of 8.0, 3.3 and 4.9 (above, below and conforming to  $pI$  of the protein). Sorptive properties of materials were estimated from the change in protein content in the solutions after keeping samples in the solutions until equilibrium is reached. The equilibration time in the conditions of the experiment was 3 hours. Concentration of BSA was determined using SF-2000 spectrophotometer from optical density at wavelength  $\lambda = 277$  nm. Concentration of the protein in the initial solution was 0.1 to 1.5 g/m.

Separation characteristics of membrane materials in experiments on ultrafiltration of protein solutions were investigated using a dead-end separation cell. In the experiments the operating pressure was 0.1 MPa, the protein concentration in the buffer so-

lution was 0.2 g/l. The effective area of the membrane was 11.34 cm<sup>2</sup>, the volume of the cell was 150 ml. The volume of the solution to be filtered was ~ 40 ml. The pressure in the system was maintained using compressed nitrogen. To estimate the sorptive activity of membranes, the indicators of sorption ( $S_{UF}$ ) and sorptive losses ( $\omega$ , %) were used [30, 31]. Sorptive losses were calculated from the ratio of the change in the weight content of *BSA* in the solution in the course of filtration (taking into consideration

the protein concentrations in the concentrate and the filtrate) to the initial content.

Selectivity of membranes was determined by the formula:

$$R = \frac{c_0 - c_f}{c_0} \cdot 100\%$$

where  $c_f$  and  $c_0$  are protein concentrations in the filtrate and the feed, respectively, g/l.

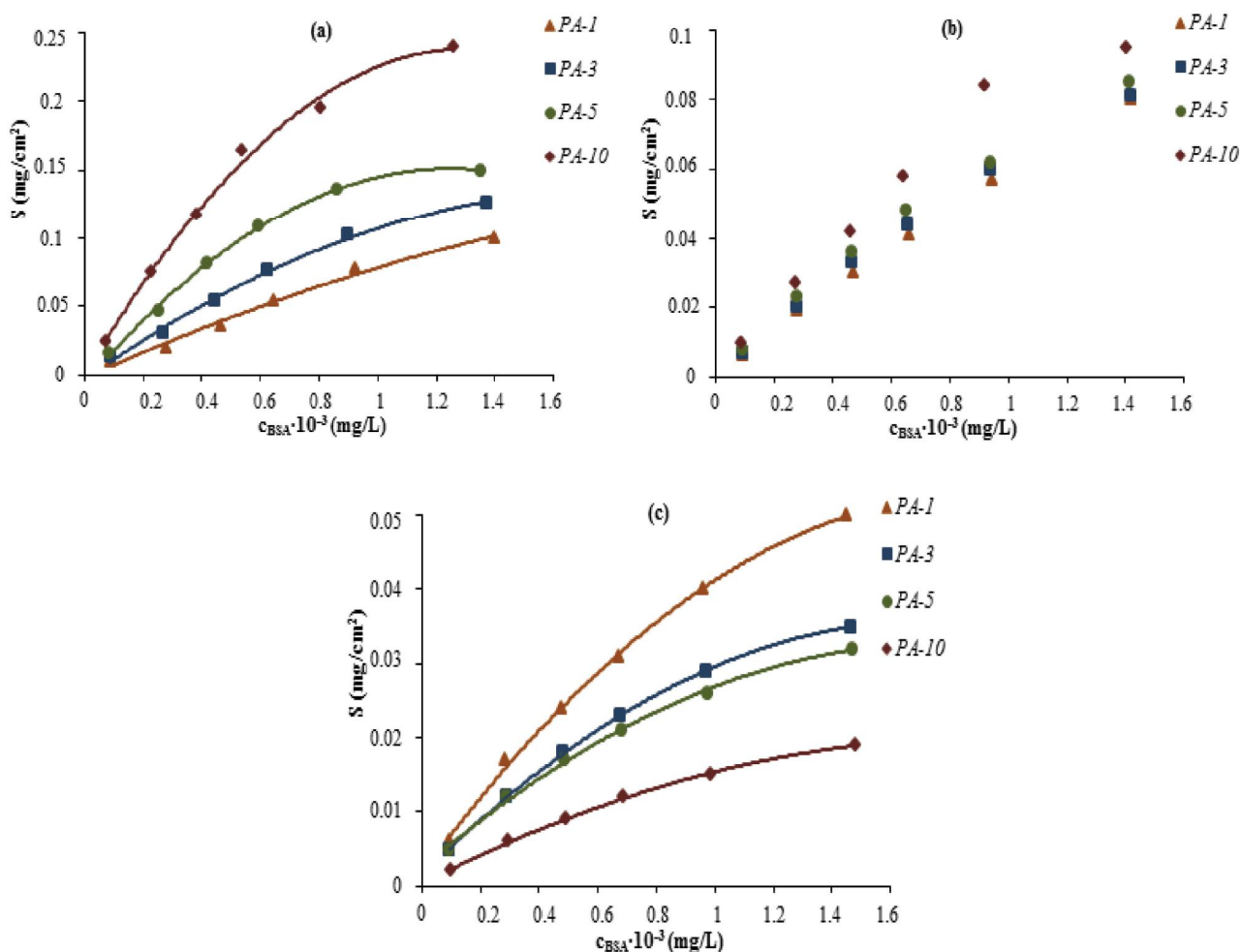


Fig. 1. Sorption isotherms of *BSA* on polyamide membranes:  $pH = 3.3$  (a), 4.9 (b), 8.0 (c).

## Results and Discussion

Figure 1 shows the isotherms of *BSA* sorption by membranes based on sulfonate-containing copolymers of aromatic polyamide of various formulations in static conditions at  $pH$  3.3 (a), 4.9 (b) and 8.0 (c).

Results of mathematical processing of experimental data indicate that the isotherms closely correspond to Langmuir's model (Fig. 2):

$$\frac{c}{S} = \frac{1}{b \cdot Q} + \frac{c}{Q}$$

where  $S$  is equilibrium sorption capacity of the membrane, mg/cm<sup>2</sup>;  $c$  protein concentration in the solution at the time of equilibrium, mg/l;  $Q$  calculated value of maximum sorption, mg/cm<sup>2</sup>;  $b$  affinity coefficient of the sorbent/sorbate system, l/mg.

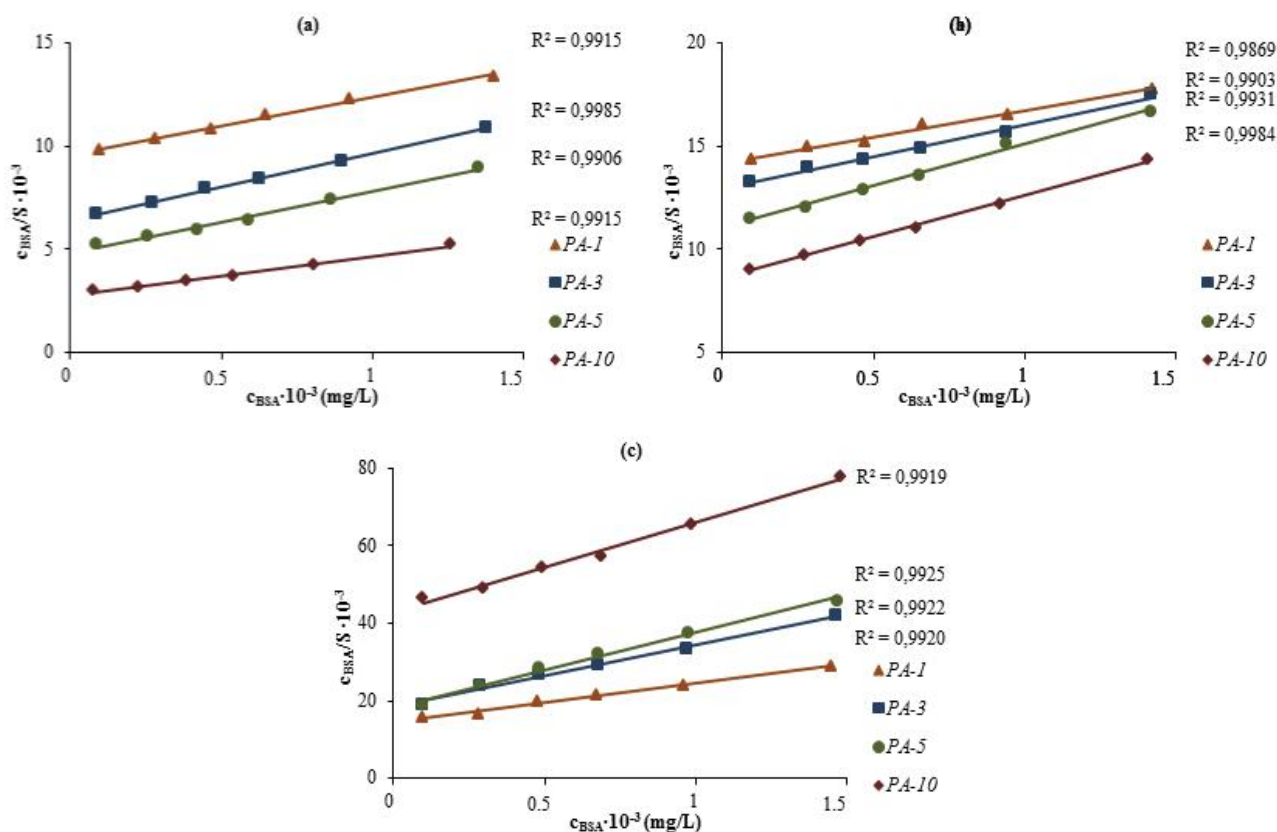


Fig. 2. Langmuir fitting of isotherms;  $pH = 3.3$  (a),  $4.9$  (b),  $8.0$  (c).

Table 1 shows isotherm coefficients of BSA sorption by membranes.

**Table 1**  
Isotherm coefficients of BSA sorption by polyamide membranes

membrane	Condition of the experiment, pH	Langmuir, s model	
		$Q, \text{ mg/cm}^2$	$b \cdot 10^3, \text{ l/mg}$
PA-1(-)	3.3	0.164	0.81
	4.9	0.121	0.75
	8.0	0.069	1.17
PA-3(-)	3.3	0.178	1.09
	4.9	0.122	0.81
	8.0	0.047	1.35
PA-5(-)	3.3	0.213	1.20
	4.9	0.124	0.84
	8.0	0.042	1.48
PA-10(-)	3.3	0.338	1.31
	4.9	0.134	0.91
	8.0	0.028	0.98

Since the membranes under consideration contain free sulfonate groups, they have a negative surface charge. When below  $pI$ , the protein is

charged positively, and the attraction forces of BSA macromolecules and negatively charged surface of the material are dominant. In these conditions, maximum sorption of protein molecules on the membrane is observed (Fig. 1 (a)). An unequivocal dependence between the formulation of the copolymer of aromatic polyamide and the sorption capacity of the material made of it in the conditions of maximum sorption (Fig. 3) is observed, which suggests that electrostatic interaction is predominant in the mechanism of this process.

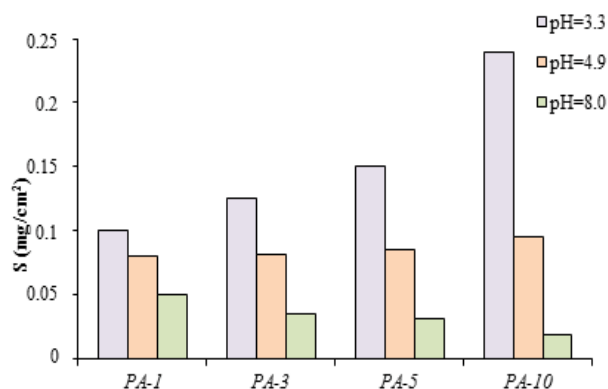


Fig. 3. Maximum experimental sorption capacity of BSA on polyamide membranes.

According to the data published in a number of publications [32, 33], at  $pH = pI_{BSA}$  the sorption is mainly determined by hydrophobic interactions, and not by electrostatic ones. For the systems under consideration the sorption capacity in this  $pH$  range is smaller than that at  $pH < pI_{BSA}$  (Fig. 1(b), 3). However, the values of  $S$  obtained in the course of experiments are rather high, where as in the case of *PA-1* the difference of sorption capacities in the  $pH$  ranges below and equal to  $pI_{BSA}$  is insignificant.

When above  $pI$  the protein is charged negatively. In these conditions the predominance of repulsive forces between likely charged membrane surface and *BSA* macromolecules results in a considerable reduction of sorption capacity of the materials under consideration (Fig. 1(c), 3). It should be noted that this trend is more pronounced in membranes produced from a copolymer having a sufficiently high concentration of fragments with ionic groups (*PA-10*).

Comparative assessment of the character of sorptive behaviour of materials produced from copolymers of various formulations, the level of values of  $S$  and the degree of intensity of the above regularities makes it possible to assume that characteristics of behaviour of the systems under

consideration in protein sorption processes are determined not only by the presence of ionic groups but also by the fact that these groups are incorporated into polymer macromolecules that are capable of intensive hydrophobic interactions. High activity of aromatic polyamides in protein sorption processes was noted, for example, by the authors of reference [34] who conducted comparative investigations of sorptive and desorptive abilities of commercial membranes produced on the basis of various polymers. The results of investigations reported in the present paper indicate that in addition to coulomb forces hydrophobic interactions should be taken into account when predicting sorptive activity of materials under consideration. It should be noted that the contribution of each of these components is determined by the nature of the protein, conditions of the sorptive experiment (such as of the medium) and the number of fragments with ionic groups in the macromolecular chain of the aromatic polyamide.

Table 2 shows the results of investigations of sorptive (in experiments on ultrafiltration) and separation properties of membranes on the basis of copolymers of aromatic polyamide having various formulations.

**Table 2**

Characteristics of membranes based on copolymers of sulfonate-containing aromatic polyamide of various formulations

Membrane	$pH = 3.3$				$pH = 4.9$				$pH = 8.0$			
	$R, \%$	$S_{UF}, \text{mg/cm}^2$	$\omega, \%$	$I, \frac{\text{ml}}{\text{min}\cdot\text{cm}^2}$	$R, \%$	$S_{UF}, \text{mg/cm}^2$	$\omega, \%$	$I, \frac{\text{ml}}{\text{min}\cdot\text{cm}^2}$	$R, \%$	$S_{UF}, \text{mg/cm}^2$	$\omega, \%$	$I, \frac{\text{ml}}{\text{min}\cdot\text{cm}^2}$
<i>PA-1(-)</i>	98	0.050	19	0.08	99	0.062	22	0.08	95	0.024	8	0.20
<i>PA-3(-)</i>	95	0.066	25	0.11	94	0.053	19	0.22	90	0.018	6	0.34
<i>PA-5(-)</i>	85	0.077	29	0.17	85	0.034	12	0.24	87	0.018	6	0.40
<i>PA-10(-)</i>	92	0.087	33	0.15	94	0.039	14	0.22	95	0.015	5	0.36

Comparative assessment of obtained data made it possible to reveal a number of regularities. First of all, it should be noted that in both series of sorption experiments (in static and dynamic conditions) a dependence is observed between the concentration of fragments with sulfonate groups in the copolymer and the sorptive activity of the membrane based on that material in the case when the protein macromolecule is charged positively. In these conditions, the highest sorptive losses and the greatest reduction of permeability of membranes are observed. Minimum reduction of permeability of materials with a relatively low level of sorptive losses and selectivity amounting to 87-95% is achieved at  $pH > pI_{BSA}$ , i.e. in the case when the protein macromolecule and the surface of the membrane are charged likely. How-

ever, the level of sorptive activity of materials in the whole range under consideration should be deemed sufficiently high which fact indicates that interactions of non-electrostatic nature in the membrane/protein system, specifically, hydrophobic interactions, should be taken into account. Nevertheless, if both participants of the sorption process (protein macromolecules and membrane surface) have a charge, coulomb forces are predominant in the mechanism of membrane plugging.

## Conclusions

An interdependence has been revealed between the formulation of copolymers of aromatic polyamide, the concentration of fragments that contain

sulfonate groups in the copolymers and sorptive properties of membranes based on the materials with respect to bovine serum albumin.

It has been shown that when pH of the solution is above (below)  $pI_{BSA}$ , the main contribution to sorption processes in the systems under consideration is made by coulomb forces.

A significant contribution of hydrophobic interaction into sorptive activity of membranes under consideration has been revealed.

It has been found that sorptive activity of membranes based on copolymers of aromatic polyamides determines to a considerable degree their separation and transport characteristics.

Minimum reduction of permeability of materials with the lowest values of sorptive losses for the systems under consideration and selectivity of 87 to 95% is observed when  $pH > pI_{BSA}$ , i.e. when the protein macromolecule and the surface of the membrane are charged likely.

The possibility of controlling processing characteristics of ultrafiltration membranes has been demonstrated by varying the formulation of copolymers used to produce them.

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