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Hydrogels for Treatment of Water Polluted with Macromolecules: Effect of Network Polyelectrolyte Composition on the Sorption Rate

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Abstract—Globular proteins uptake kinetics by hydrogels of oppositely charged network polyelectrolytes was studied with relation to the polyelectrolyte composition. It was shown that introduction of hydrophilic neutral units of acrylamide, inert towards proteins studied, significantly accelerated the uptake process, with no effect on the polyelectrolyte capacity towards proteins (with respect to ionic groups). The results obtained allow for optimization of the sorbent composition in order to enhance the polymeric pollutants uptake and/or tune their release rate.

Keywords: Crosslinked polyelectrolyte, interpolyelectrolyte reaction, kinetics, protein uptake.

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The crosslinked (network) polyelectrolytes are so called smart materials, their properties being strongly dependent on the external conditions: temperature, pH, ionic strength, and the solvent composition [1–4]. Due to the presence of ionic groups, crosslinked polyelectrolytes efficiently interact with the oppositely charged compounds via interpolyelectrolyte reactions [5–7].

The ability of polyelectrolyte gels to absorb the oppositely charged species from external solution is widely used for wastewater treatment in order to separate such pollutants as metal ions, dyes, polycyclic phenols, and humic acids [8]. Polyelectrolyte gels are used for soil protection towards erosion [9]. In the latter regard, the hydrophilic nature of gels is important as it allows to accommodate significant amount of water in the soil, thus partially resolving the issue of watering in arid regions; the ability to keep the charged compounds inside the material and to release them with controlled rate is used in the prolonged fertilization.

All the above-listed applications being concerned, the key issues are efficiency of hydrophilic gels interaction with the oppositely charged species and the factors influencing the process rate. To date, numerous studies have revealed that the sorbate nature strongly influences the uptake rate and the equilibrium state of polyelectrolyte gels reactions [10]. In particular, low molecular mass surfactants and metal ions are in general absorbed and released much faster than the polymeric species. Among macromolecular sorbates, the linear polymers, capable of reptation movements in the gel phase, are absorbed relatively fast, whereas globular proteins, moving as a whole, show up slower diffusion. Nevertheless, the role of the network polyelectrolyte nature has been scarcely studied so far.

As far as water treatment is concerned, the rapid absorption of the pollutants is a must, independently of their molecular mass. In this regard it is of interest to study if it is possible to accelerate the macromolecular species absorption by changing the crosslinked polyelectrolyte composition. This paper reports on this issue considering absorption of the two model globular proteins, cytochrome *c* and lysozyme.

From a number of previous studies on polyelectrolyte–protein interaction, covering the issue

of enzymes immobilization within crosslinked polyelectrolyte matrix, it is known that in general the interaction with polymer leads to decrease of enzyme activity, however increasing its stability towards denaturation [11, 12].

It was demonstrated [6, 13] that the introduction of nonionic acrylamide units into the charged crosslinked polymer did not affect the efficiency of its interactions with oppositely charged globular proteins. Extending that approach, in this work the influence of nonionic hydrophilic acrylamide units on the kinetics of interpolyelectrolyte reaction was studied.

EXPERIMENTAL

Materials

Acrylamide AAm (Fluka) and N,N'-methylenebisacrylamide BIS (BioRad) were recrystallized from chloroform, acrylic acid AA (Khimmed) was distilled under reduced pressure. Potassium persulfate, sodium metabisulfite, sodium hydroxide, 2-acrylamido-2-methylpropanesulfonic acid AMPS, lysozyme from chicken egg white and cytochrome *c* from horse heart (all Sigma) were used as received.

Crosslinked Polyanions Preparation

Hydrogels of crosslinked copolymers of sodium 2-acrylamido-2-propanesulfonate AMPSNa and AAm were prepared by radical copolymerization of the corresponding monomers in the presence of bifunctional BIS [14]. The mixtures with BIS concentration of 1.2 or 2.5 mol % with respect to the monofunctional monomers were used, the fraction of AMPSNa being of 0 to 100 mol %. After the polymerization, the gel was washed with excess of water several times and then kept in water till the swelling ratio was constant. The reactivities of momomers in the AMPSNa/AAm mixture under the preparation conditions were close to unity [14]; therefore, the copolymer composition was the same as that of the monomers mixture, and random distribution of the units was expected.

Hydrogels of crosslinked copolymers of sodium acrylate ANa and AAm were prepared by radical copolymerization and AA and AAm [6] in 10 wt % aqueous solution. The crosslinker BIS concentration was of 0.5, 1, or 2.5 mol % with respect to the monofunctional monomers. AA fraction in the monomers mixture was 5 to 80 mol %. After polymerization, the prepared gel was washed with excess of water several times, neutralized with sodium hydroxide, and incubated in water till the swelling ratio was constant. As the monomers reactivities under polymerization conditions were not equal to unity, the copolymer was somewhat enriched with AA. Data on the copolymer composition was taken from [6].

Copolymers Samples Coding

The crosslinked copolymers were coded as follows throughout this work: #P stood for crosslinked nature, then the monomers were listed in parentheses, and finally the fractions of the ionic comonomer and the crosslinker were given separated by slash. For example, **#P(ANa-AAm)-83/1** was a copolymer of sodium acrylate and acrylamide with 83 mol % of acrylate units crosslinked with 1 mol % of BIS.

Sorption Experiments

A weighed specimen of equilibrium swollen hydrogel (0.2–0.5 g) was immersed into a protein solution of a certain concentration. The solution was regularly sampled, and the concentration of protein was determined. The experiments were performed at pH of 8–9 and (except for determination of the activation energy) at room temperature. The conversion of hydrogel into the polyelectrolyte complex F was calculated as the ratio of protein amount absorbed at a given moment ν_t to the sorption capacity of the gel sample under conditions of the experiment ν_{max}. Such normalization with respect to the sorption capacity (dependent on the ionic units concentration and the sample size [6, 13]) allowed comparison of the sorption rate with different copolymer gels.

Other Methods

The equilibrium swelling of gels was characterized by the ratio of water mass in the swollen sample and the corresponding dry polymer mass:

$$H = (m_1 - m_2)/m_2,$$

with m_1 swollen specimen mass and m_2 dry specimen mass.

In order to determine the swelling ratio, the weighed specimen of swollen hydrogel was dried to constant mass at 50°C.

Proteins concentration was determined by means of spectrophotometry. The molar absorptivity of cytochrome *c* was of 25220±100 L mol^{-1} cm^{-1} at λ = 280 nm and of 108500±500 L mol^{-1} cm^{-1} at 408 nm. In the case of lysozyme, molar absorptivity at λ = 281 nm was of 33200±100 to 37600±150 L mol^{-1} cm^{-1}

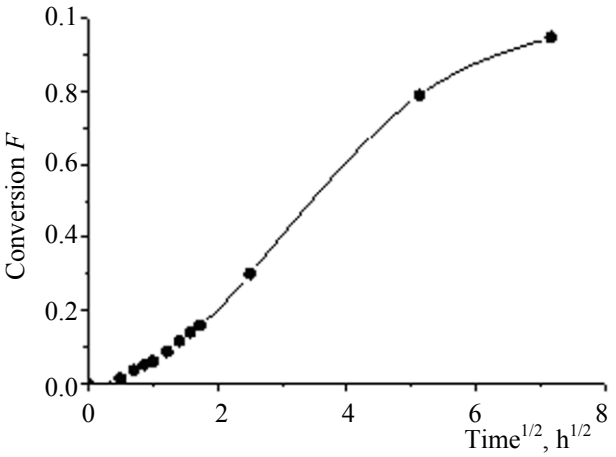

Fig. 1. Kinetics of cytochrome *c* sorption by the #P(AMPSNa-AAm)-30/2.5 hydrogel in the $F - t^{1/2}$ coordinates.

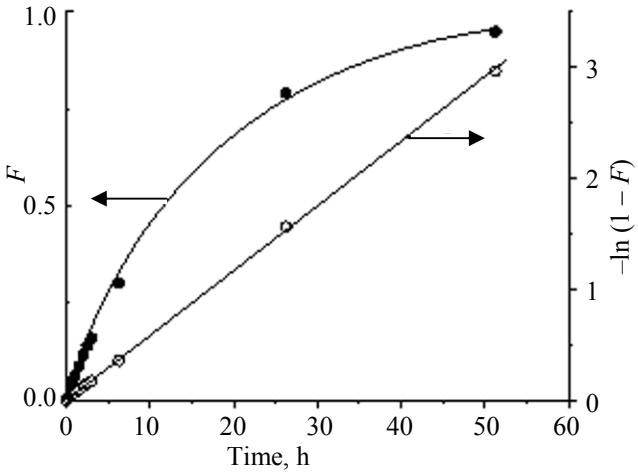

Fig. 2. Kinetics of cytochrome *c* sorption by the #P(AMPSNa-AAm)-30/2.5 hydrogel in the –ln (1– *F*) – *t* and *F* – *t* coordinates

depending on the lot number. The amount of proteins was expressed in moles of globules.

RESULTS AND DISCUSSION

Mechanism of Proteins Sorption with Crosslinked Polyelectrolyte

Sorption of an oppositely charged sorbate with a polyelectrolyte hydrogels includes in general at least three stages: diffusion of the sorbates towards the gel surface, formation of the interpolyelectrolyte complex at the interphase boundary, and sorbate diffusion inside the gel sample [15]. Depending on the sorbate concentration in the external solution, the observed sorption rate can be determined either by the passive diffusion towards the gel or by the sorbate transport within the gel phase [16]. In this work, the proteins sorption kinetics was studied at initial proteins concentration of $>4\times10^{-5}$ mol/L; under such conditions the influence of passive diffusion on the observed sorption rate was eliminated. Thus, all the observed effects were connected with the effect of crosslinked polyelectrolyte of the protein sorption activated by interpolyelectrolyte reaction.

If the sorbate diffusion inside the gel phase is passive, the sorption kinetic curves should be linear in the $F - t^{1/2}$ coordinates [17]. As seen from Fig. 1 (the representative case is shown for illustration of the common trend), the sorption of proteins with oppositely charged hydrogels could not be described by kinetic laws of passive diffusion.

It was previously suggested [7] that the kinetics of proteins interaction with oppositely charged crosslinked homopolymers could be described as frontal heterogeneous reaction at the contracting interphase boundary between the initial gel (central part of the specimen) and the formed polyelectrolyte complex (specimen shell). If that was held, the kinetic curve was linear as –ln (1 – *F*) function of *t*; the curve should pass through the axes origin, its slope being proortional to the rate constant of the interpolyelectrolyte exchange reaction occurring in the gel phase. In this work it was shown that the model described in [7] was applicable to the proteins sorption by the oppositely charged copolymers, even by those containing a fairly low fraction of the charged units, 5 mol %. This is illustrated in Fig. 2, showing the kinetic curve from Fig. 1 in the $F - t$ and $-\ln(1 - F) - t$ coordinates.

Hereafter, the proteins sorption rate by different gels was characterized by the effective rate constant k_s, calculated by fitting of the experimental data with the following equation

$$\ln(1 - F) = -\frac{3}{2} k_s t \text{ [16].}$$

Influence of Ionic Units Content in the Copolymer on the Proteins Sorption Rate

The effective rate constants of lysozyme sorption with crosslinked copolymers of ANa and AAm of varied composition (0.5 mol % of BIS) are shown in Table 1. It is to be seen that sorption was accelerated with decreasing fraction of the charged units, down to

Table 1. Effective rate constant of lysozyme sorption by the #P(ANa-AAm)-*X*/0.5 hydrogels with varied fraction of the charged units *X*

ANa molar fraction, %	k_s, s^{-1}
6	$(19.5 \pm 1.4)\times10^{-5}$
14	$(10.8 \pm 0.3)\times10^{-5}$
45	$(9.1 \pm 0.3)\times10^{-5}$
52	$(3.1 \pm 0.2)\times10^{-5}$
83	$(0.26 \pm 0.01)\times10^{-5}$

Table 2. Effective rate constant of cytochrome *c* sorption by the #P(AMPSNa-AAm)-*X*/2.5 hydrogels with varied fraction of the charged units *X*

AMPSNa molar fraction, %	k_s, s^{-1}
5	$(2.9 \pm 0.1)\times10^{-5}$
20	$(1.59 \pm 0.03)\times10^{-5}$
30	$(1.08 \pm 0.01)\times10^{-5}$
50	$(0.58 \pm 0.01)\times10^{-5}$

6 mol % of ANa in the copolymer. Similar trends were revealed in the case of cytochrome *c* sorption by the crosslinked copolymers of AMPSNa and AAm (2.5 mol % of BIS) (Table 2). Kinetics of lysozyme sorption with AMPSNa-containing crosslinked copolymers showed the same trend; for example, with decreasing of the ionic units fraction *X* in #P(AMPSNa-AAm)-*X*/2.5 from 30 to 5 mol %, the effective rate constant was increased from $(8.7\pm0.1)\times10^{-6}$ s^{-1} to $(33\pm2)\times10^{-6}$ s^{-1}.

Thus, acceleration of the globular proteins sorption with hydrogels of the oppositely charged crosslinked copolymer with decreasing fraction of the charged units was a general trend. The effect was ascribed to the increasing mobility of the crosslinked copolymer chains with decreasing their linear charge density (and thus, the chains rigidity). That proved that the network polyelectrolyte chains were not simply a medium providing for routes of the protein diffusion; on the contrary, the mobility of network chains played important part in the overall sorption process.

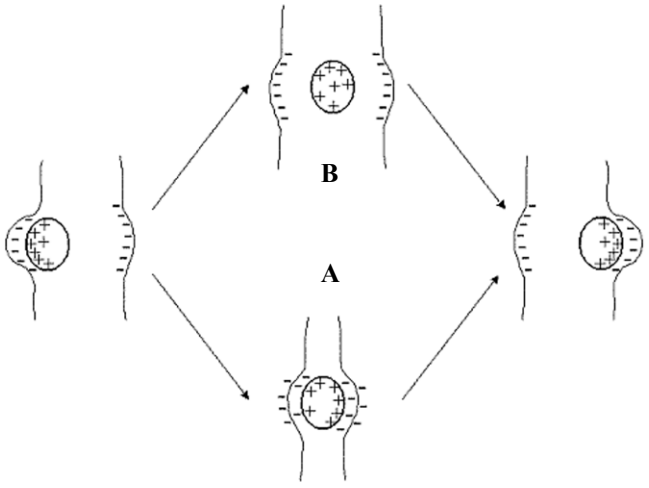

Fig. 3. Scheme of the possible mechanism of the activated diffusion of protein in the oppositely charged crosslinked polyelectrolyte phase.

In particular, in the polyelectrolyte complex with crosslinked polyelectrolyte, each protein globule is bound to 7–9 ionic groups of the copolymer [13]. The most probable mechanism of the bound globule transfer between the network chains presumes the transition state at which the globule is bound to the both network chains simultaneously (**A** in Fig. 3). The alternative mechanism, implying complete dissociation of all the ionic bonds between the globule and the network chains (**B** in Fig. 3) seems less probable. If so, indeed, the increase of the flexibility of crosslinked copolymer chains with decreasing fraction of the charged units should have led to the globule transfer facilitation.

The acceleration of proteins sorption with changing the copolymer composition was known in the case of interpolyelectrolyte reactions of pairs of oppositely charged linear polyelectrolytes. In particular, the decrease of poly(N-ethyl-4-vinylpyridine) alkylation degree from 90 to 60% increased the observed rate constant of the polyelectrolyte exchange reaction by more than two orders of magnitude [12, 18].

In the case of nonionic crosslinked polymers, the interpolyelectrolyte addition reaction between the gel and the sorbate was impossible, and the sorption should have occurred via passive diffusion rather than via diffusion activated by interpolyelectrolyte reaction. In the latter case, the sorption capacity was determined by the composition of the formed interpolyelectrolyte complex, and in the excess of the protein no additional sorption was observed [6]. On the contrary, in the course of passive diffusion, the maximal amount of the protein absorbed by hydrogel is determined by the total protein concentration in the system as well as by the ratio of the phases volume.

Table 3. Initial sorption rate of cytochrome *c* sorption by the #P(AMPSNa-AAm)-*X*/2.5 hydrogels with varied fraction of the charged units *X*. The rate is given as moles of protein globules absorbed during 1 hour, with respect to the moles of monomeric units (r_0) or to 1 m^2 of the gel specimen surface area (f_0)

AMPSNa molar fraction, %	$r_0\times10^5$, mol unit^{-1} h^{-1}	$f_0\times10^5$, mol m^{-2} h^{-1}
0	7.4×10^{-5}	0.83×10^{-5}
5	210×10^{-5}	2.9×10^{-5}
20	290×10^{-5}	3.0×10^{-5}
30	265×10^{-5}	2.5×10^{-5}
50	205×10^{-5}	1.5×10^{-5}
100	380×10^{-5}	0.79×10^{-5}

Comparison of the rate constants of cytochrome *c* sorption by the #P(AMPSNa-AAm)-*X*/2.5 hydrogels (Table 3) revealed that even at fairly high protein concentration (4.8×10^{-5} mol/L) its sorption by the nonionic crosslinked copolymer gel was characterized by noticeably lower transfer rates than that in the case of transport activated by interpolyelectrolyte reaction.

From the above-given consideration it followed that the protein sorption should have been accelerated with decreasing of the fraction of ionic units as far as the diffusion activated by the interpolyelectrolyte reaction was the prevailing mechanism. When passive diffusion would become the predominant mechanism, the sorption should have been slowed down.

Indeed, the non-monotonous dependence of the sorption rate on the copolymer composition was experimentally revealed in the case of the #P(ANa-AAm)-*X*/1 – lysozyme system (Table 4). The difference in behavior as compared to the #P(ANa-AAm)-*X*/0.5 – lysozyme system (Table 1) was explained by decreasing of the average number of ionic groups at the chain fragment between the adjacent crosslinks with increasing BIS content. Therefore, at some low fraction of the ionic groups in the denser crosslinked copolymer several network chains would form the polyelectrolyte complex with a single protein globule. The increase of the network fragment volume participating in the elementary sorption stage (Fig. 3) was the factor slowing down the sorption. Indeed, in the case of the #P(ANa-AAm)-*X*/0.5 copolymer, lysozyme sorption was accelerated with decreasing *X* down to 6 mol % ANa, whereas in the case of #P(ANa-AAm)-*X*/1 – lysozyme system the highest sorption rate was achieved at *X* = 14 mol %, and in the case of #P(ANa-AAm)-*X*/2.5 – lysozyme system the sorption rate was slowed down at *X* < 27 mol % of ANa.

Table 4. Effective rate constant of lysozyme sorption by the #P(ANa-AAm)-*X*/1 hydrogels with varied fraction of the charged units *X*

ANa molar fraction, %	k_s, s^{-1}
6	$(11.6 \pm 0.3)\times10^{-5}$
14	$(21.6 \pm 1.9)\times10^{-5}$
20	$(11.4 \pm 0.2)\times10^{-5}$
27	$(9.8 \pm 0.5)\times10^{-5}$
45	$(0.6 \pm 0.2)\times10^{-5}$

Interestingly, the maximum at the sorption rate profile as function of copolymer composition was not observed in the cases of cytochrome *c* sorption with all the studied hydrogels (Table 2) and in the cases of lysozyme sorption by the #P(AMPSNa-AAm)-*X*/2.5 hydrogels, even though at *X* = 20 mol % of AMPSNa the average number of ionic groups between the adjacent crosslinks was of 4 smaller than the number of AMPSNa units bound to a single polymer globule in the complex (6–7). On the contrary, the monotonous sorption acceleration was observed in the cases of the mentioned systems with *X* decreasing down to 5 mol %. That could be explained by the copolymer heterogeneity with respect to the crosslinks distribution. Probably, the regions with crosslinking density below average across the whole specimen existed in the gel; if so, the protein sorption could occur through such lighter crosslinked regions.

The Copolymer Composition Influence on the Sorbates Kinetic Differentiation

The proteins used in this work were quite similar with respect to their physico-chemical parameters, such as molecular mass (14300 Da in the case of lysozyme and 12400 Da in the case of cytochrome *c*), globule size (lysozyme globule is an ellipsoid with semiaxes of 35 and 45 Å, cytochrome *c* globule is a ball with diameter of 30 Å [19]), and total charge under the experiment conditions (+7 in the case of lysozyme [19] and +9 in the case of cytochrome *c* [20]).

Nevertheless, lysozyme was generally absorbed slower than cytochrome *c* (as was shown in [7] in the

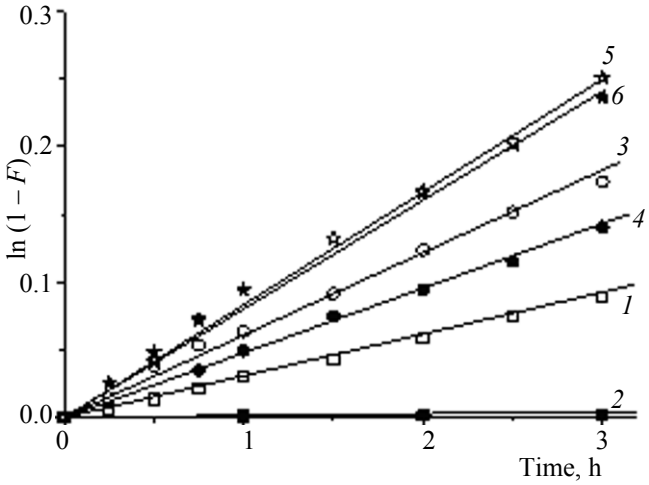

Fig. 4. Kinetics of cytochrome *c* (*1, 3, 5*) and lysozyme (*2, 4, 6*) sorption by the #P(AMPSNa-AAm)-*X*/2.5 hydrogels with the ionic units fraction *X* = 100 mol % (*1, 2*), *X* = 30 mol % (*3, 4*), and *X* = 20 mol % (*5, 6*).

case of #P(ANa)-100/1 gel). In the case of the crosslinked ANPSNa homopolymer, the difference in the sorption rate was of several orders of magnitude as well (Fig. 4). It was confirmed that the self-aggregation in the solution was not the cause of the difference. Hence, the observed difference could only be ascribed to the patterns of the charged groups localization at the proteins globule.

With decreasing the fraction of ionic groups in the copolymer, the difference in the sorption rate of lysozyme and cytochrome *c* became less significant and practically vanished in the case of the #P(AMPSNa-AAm)-20/2.5 gel (Fig. 4).

Thus, the acceleration of sorption of the globular proteins with the oppositely charged crosslinked polymer was accompanied by the leveling off the individual features of the sorbates. Indeed, with less of the ionic groups in the copolymer, its chains became more flexible and seemingly participated in the globules transfer more and more actively, to become finally the predominant factor influencing the sorption rate.

Activation Energy of the Globular Proteins Sorption

From the temperature dependence of rate constant

$$\ln k = -\frac{E_a}{RT} + \ln k_0$$

the temperature coefficient of sorption rate could be determined taking advantage of the Arrhenius equation:

The results of processing of kinetic data at 5–35°C in the cases of several selected systems are shown in Table 5.

Most of the calculated activation energies ranged at 20–30 kJ/mol in the case of globular proteins sorption. The values were lower than the activation energy of poly(N-ethyl-4-vinyl pyridinium) chloride sorption (system 8) but higher than that in the case of dodecylpyridinium chloride (system 9).

Analysis of data in Table 5 revealed that with sltering the flexibility of AMPSNa-containing

Table 5. Temperature coefficient of the sorption rate ("activation energy") E_a and the effective sorption rate constant k_s in the case of selected crosslinked polyelectrolyte systems

Run no.	Sorbate	crosslinked polyelectrolyte	$k_s \times 10^6$, s^{-1}(22°C)	E_a, kJ/mol
1	Cytochrome *c*	#P(AMPSNa)-100/2.5	5.5±0.1	22±2
2	Cytochrome *c*	#P(AMPSNa-AAm)-50/2.5	5.8±0.1	27±3
3	Cytochrome *c*	#P(AMPSNa-AAm)-30/2.5	11±1	26±2
4	Cytochrome *c*	#P(AMPSNa-AAm)-5/2.5	29±3	24±3
5	Lysozyme	#P(AMPSNa-AAm)-30/2.5	8.7±0.1	52±2
6	Lysozyme	#P(AMPSNa-AAm)-5/2.5	33±2	20±5
7	Lysozyme	#P(ANa-AAm)-30/2.5	14±1	13±6
8	Poly(N-ethyl-4-vimylpyridinium) bromide	#P(ANa)-100/1		~40 [16]
9	Dodecylpyridinium chloride	#P(ANa)-100/1		~10 [21]

copolymers, the effective rate constant was changed by 5 times (systems 1–4); at the same time, the activation energy was not changed. On the contrary, change of the sorbate (systems 3 and 5) led to significant change of the activation energy at practically the same rate constant. In the case of proteins sorption by the most flexible copolymer (systems 4 and 6), the kinetic parameters of sorption were independent of the individual sorbate features. The combined analysis of rate constants and activation energy will likely lead to discovery of more details on the mechanism of proteins interaction with the crosslinked polyelectrolytes.

CONCLUSIONS

This work demonstrated the applicability of the kinetic model of heterogeneous reaction at the contractibg interphase boundary to description of sorption of globular proteins with oppositely charged crosslinked copolymers in wide range of compositions of the latter. The extracted rate constant was perfectly described by the Arrhenius law.

With decreasing of the ionic units fraction in the copolymer (achieved by incorporation of the nonionic acrylamide units) the proteins sorption was significantly accelerated. Simultaneously, the individual kinetic features assigned to the sorbates structure leveled off. With decreasing the fraction of ionic groups in copolymers down to extremely low values, the sorption rate and (in some cases) the sorption capacity were, however, decreased. Therefore, if rapid sorption independently of the sorbate nature is desired, the flexible-chain copolymers with nonionic hydrophilic comonomer are advantageous over the homopolymer polyelectrolyte. On the contrary, selective isolation of the sorbates is likely most successive with using the homopolymer crosslinked polyelectrolytes with rigid chains.

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