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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Monodisperse Microspheres Based on Acrolein Copolymers

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Abstract—Emulsifier-free emulsion copolymerization of acrolein with styrene and methyl methacrylate in the presence of potassium persulfate was developed to prepare microspheres with surface aldehyde groups. The kinetics of monomer copolymerization was studied and the conditions for preparing monodisperse microspheres 370–1000 nm in diameter were determined.

Monodisperse polymer microspheres are widely used in immunology as supports of biologically active compounds (BACs) [1]. These BACs are either simply sorbed on the support surface or bound to it by various chemical reactions with reactive groups on the microsphere surface. For example, surface aldehyde groups react with BAC amino groups yielding Schiff bases under mild conditions. As a rule, polymer microspheres with aldehyde groups are prepared by homopolymerization of unsaturated aldehydes (e.g., acrolein, formylstyrene) [1]. The monodisperse polyacrolein particles are often prepared by the anionic or radiation-induced radical polymerization in aqueous solutions [2–5]. However, these procedures give significant amounts of oligomeric products, which, in the course of storage of the latexes and preparations on their base, migrate to the particle surface, making its surface structure unstable. At the same time, only in few works, microspheres were prepared by radical copolymerization of acrolein and styrene [6–8], though such a procedure allows control over the fraction of acrolein units in the surface layer and, probably, over the degree of polymer cross-linking via aldehyde groups. Therefore, we studied in this work the copolymerization of acrolein with more hydrophobic monomers, such as styrene and methyl methacrylate (MMA), to prepare monodisperse microspheres containing surface aldehyde groups. Data on the synthesis of poly(styrene/acrolein) (PSAC) and poly(MMA/acrolein) (PMMAAC) latexes by emulsifier-free emulsion copolymerization are of particular interest for revealing the effect of water-soluble acrolein on the course and mechanism of particle formation.

EXPERIMENTAL

Preliminarily, styrene, MMA, acrolein, dimethylformamide (DMF), methyl ethyl ketone (MEK), and methylene chloride were purified by distillation according to standard procedures. Double-distilled water served as dispersion medium. Sodium chloride NaCl, sodium tertaborate $Na_4B_2O_7$, potassium dihydrogenphosphate NH_2PO_4, analytically pure hydroxylamine hydrochloride, and HCl and NaOH standard solutions were used without additional purification.

The emulsion radical emulsifier-free copolymerization of the monomer mixtures was carried out in the presence of potassium persulfate [9]. In order to obtain monodisperse microspheres of widely varying size, the molar ratio of the initial monomers, concentration of the monomer mixture in water, *w* (wt %), temperature, and concentrations of the initiating agent and buffer salt were varied (Table 1). The residual monomers were separated from the resulting latexes by distillation with steam. The monomer conversion was determined by gas chromatography with internal reference (butanol).

The measurement error was 5%. The particle size of the resulting latexes was measured with a JEM 100 S electron microscope (JEOL, Japan). Water-soluble admixtures were removed from the latex by three cycles of centrifugation and dispersion in double-distilled water. Then the surface concentration of the carboxy and aldehyde groups was determined by conductometric titration [8]. The aldehyde groups were analyzed after preliminary treatment of the latex with

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hydroxylamine hydrochloride [9]. The content of the sol fraction in the copolymers was determined by fractional extraction with methylene chloride for 3 days. The IR spectra of the sol fraction solutions were registered in the 400–4000 cm^{-1} range. The thin-layer chromatograms of the sol fraction of the resulting polymers were studied on the silica support in comparison with the homopolymers: polystyrene (PS) and polymethyl methacrylate (PMMA). Polyacrolein is insoluble in the solvents used because of the strong intermolecular cross-linking [1] and thus cannot be used as reference. In the case of PS and PSAC, the mobile phase was toluene, whereas for PMMA and PMMAAC the mixture of toluene and MEK (5 : 7 volume ratio) was used. The chromatograms were developed using a mixture of sulfuric acid and potassium permanganate.

The procedure of the microsphere surface modification with protein is presented elsewhere [10]. As adsorbate protein we used the native bovine serum albumin (BSA); this protein is widely used in immunoassay as a carrier of small antigens and as a blocking agent, which fills the hydrophobic surface and thus hinders nonspecific interactions. The procedures for purification and analysis of serum albumin were given in [10].

The solutions of BSA before and after their interaction with the polymer particles were studied by both the traditional Lowry procedure and modern high-performance monolithic chromatography (HPMC) [11] on a CIM® Disk DEAE anion-exchange membrane (BIA Separations, Ljubljana, Slovenia). The gradient elution was performed using solution containing tris(hydroxymethyl)aminomethane hydrochloride (0.02 M) and sodium chloride (0.5 M), pH 8.0 at a rate of 3 ml min^{-1}. Albumin was detected at λ = 229 nm.

Monodisperse latexes with particle size of 370–670 nm were prepared by radical copolymerization of acrolein and styrene (Table 1). It was found that at equimolar monomer ratio the reaction proceeded most completely, whereas at a styrene : acrolein molar ratio of 2 : 1 the conversion was only 75 and 57% (Table 1, run nos. 2 and 11). These data agree with published results [8]. With the preparation temperature decreasing from 60 to 55°C, the diameter of the resulting monodisperse microspheres increases (Table 1, run nos. 1 and 3), which is due to the decrease in the decomposition rate of the initiating agent. However, upon additional decrease in the initiating agent concentration (Table 1, run no. 4), the size of the resulting microspheres did not increase further because of the significant decrease in the monomer conversion.

Table 1. Synthesis conditions, monomer conversion *K*, and diameter *D* of microspheres prepared by copolymerization of styrene and acrolein*

Run no.	Synthesis conditions			*K*, %	*D*, nm
	w, wt %	*T*, °C	$C_{salt} \times 10^{-2}$, M		
1	10	60	–	90	370
2	10	60	–	75	370
3	10	55	–	90	520
4	10	55	–	57	370
5	15	55	–	90	610
6	10	60	NaCl, 2	90	420
7	10	60	NaCl, 4	90	<700**
8	15	60	KH_2PO_4, 1	90	670
9	15	55	KH_2PO_4, 1	80	<700**
10	15	55	KH_2PO_4, 2	80	<800**
11	15	55	KH_2PO_4, 1	57	520

* Styrene : acrolein molar ratio 1 : 1, in run nos. 2 and 11 2 : 1; concentration of $K_2S_2O_8$ with respect to monomers was 1 wt %, and 0.5 wt % in run no. 4.

** The resulting latexes along with the main fraction of coarse particles contain finer particles.

In contrast, with increasing monomer concentration in the reaction mixture, their conversion reached 90%; in this case, coarser monodisperse microspheres 610 nm in diameter were formed (Table 1, run no. 5).

To make larger the resulting microspheres, we also raised the ionic strength of the reaction mixture. It was found, that with the NaCl concentration increasing to 0.02 M, the particle size grows somewhat (Table 1, run nos. 1 and 6), whereas in a more saline solution the resulting latex becomes polydisperse (run no. 7). It is well known that, during emulsion polymerization, the persulfate initiating agent reacts with water to form hydroxy radicals, and the reaction mixture becomes more acidic [12]. In our case, the pH of the resulting polymer dispersions was 2.5 after the reaction termination. The aggregation stability of the polymer–monomer particles (PMPs) in the dispersion medium with low pH and high ionic strength was insufficient owing to a decrease in the ionization of the surface sulfate and carboxy groups which can be formed during oxidation of the surface aldehyde groups in the acrolein units with persulfate.

To enhance the aggregation stability in the synthesis of coarse particles at high ionic strength of the dispersion phase, we used potassium dihydrophosphate buffer salt, which ensures a weakly acidic state of the reaction mixture. In the presence of this

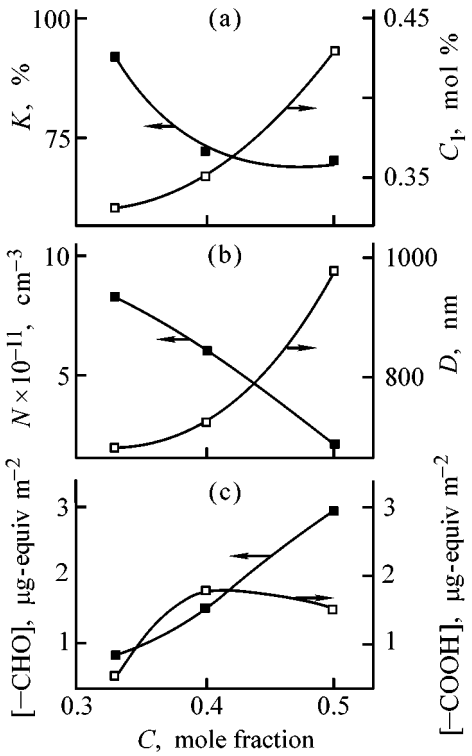

Fig. 1. (a) Acrolein conversion K and content of acrolein units in the latex C_1, (b) number of particles N and their diameter D, and (c) concentration of the surface aldehyde [–CHO] and carboxy [–COOH] groups vs. the acrolein mole fraction C in the mixture with MMA.

salt, a monodisperse latex with particle diameter of up to 670 nm was prepared (Table 1, run nos. 8 and 11). In this case, the size of the resulting microspheres also increased with decreasing temperature (Table 1, run nos. 9 and 10), but finer particles were detected along with a coarse fraction.

Table 2. Sol fraction in copolymers

Run no.	Content, mole fraction		Sol fraction in copolymer, %
	acrolein in the reaction mixture	number of acrolein units in copolymer	
Methyl methacrylate/acrolein			
12	0.33	0.33	100
13	0.40	0.35	72
14	0.50	0.43	50
Methyl methacrylate/styrene			
8	0.50	0.53	30

Copolymerization of acrolein with a less hydrophobic MMA was carried out under the conditions providing, in the case of acrolein and styrene, polymerization formation of coarse monodisperse particles and the most complete monomer conversion (Table 1, run no. 8). Since data on the emulsion copolymerization of acrolein with MMA are lacking, the effect of the monomer ratio on their conversion and the properties of the resulting latexes were studied in more detail. It was found that the acrolein conversion decreases with its increasing concentration in the reaction mixture (Fig. 1a), whereas the MMA conversion remains at a level of about 90%.

With increasing acrolein content in the initial mixture, the number of the polymer particles decreases and thus their size increases (Fig. 1b). With increasing acrolein content, the content of the surface aldehyde groups grows in parallel (Fig. 1c) with the number of acrolein units in the latex structure (Fig. 1a). However, there is no clear correlation between the concentration of the surface carboxy groups and the content of acrolein (Fig. 1c).

We found that the resulting microspheres are partially cross-linked systems and the content of the sol fraction increases with decreasing content of acrolein in the initial mixture (Table 2). These data confirm that the radical polymerization is accompanied by intermolecular cross-linking via the C=C and C=O groups of acrolein [1]. Thin-layer chromatograms (silica) of the sol fractions of the polymers prepared at equimolar MMA : acrolein or styrene : acrolein ratio show that these samples differ from, respectively, MMA and styrene homopolymers. For example, in the chromatograms with toluene eluent, PS moved in the front, whereas PSAC remained at the start. In the toluene/MEK mixture, PMMA advanced with the β-front and PMMAAC remained at the start. At the same time, in the case of MEK eluent, the PSAC and PMMAAC samples moved at the front. IR spectra of the PSAC and PMMAAC samples are shown in Fig. 2 in comparison with those of MMA and styrene homopolymers. The IR spectrum of the PSAC sample contains a strong absorption band at 1725 cm^{-1} (C=O vibrations) and a weak band at 2740 cm^{-1} (C–H asymmetric vibrations of the aldehyde group), which are absent in the IR spectrum of the PS sample. The IR spectrum of the PMMAAC sample contains a strong band at 1730 cm^{-1}, which can be attributed to vibrations of the carbonyl bond in both acrolein and MMA. A weak absorption at 2740 cm^{-1} confirms the presence of the aldehyde groups in this polymer. Hence, the PSAC and PMMAAC samples are copolymers of styrene or MMA with acrolein.

The copolymerization kinetics of acrolein with MMA and styrene at equimolar monomer mixtures is illustrated in Fig. 3. The kinetics of MMA conversion (Fig. 3a) shows that, at the start of polymerization, the contribution of this monomer is significantly smaller as compared with that of acrolein. Based on the slope of the conversion curves, the polymerization rates of MMA and acrolein during this induction period are 0.3×10^{-5} and 2.4×10^{-5} mol l^{-1} s^{-1}, respectively. The copolymerization constants for the MMA/acrolein system in dioxane are 10 and 0.2, respectively [13]. Thus, at equal concentrations of the required monomers in the reaction zone the rate of MMA polymerization must be significantly higher. The above result is probably due to the fact that the solubility of acrolein in water exceeds that of MMA: 3.7 and 0.15 M, respectively [14]. Gas chromatographic data show that, at the start of the process, nearly 70% of acrolein is in the aqueous solution. In the presence of water-soluble initiating agent, various oligomers and oligomeric radicals enriched with acrolein units are formed in solution; this provides their high solubility in water. Moreover, these compounds must possess low surface activity because of the low content of the MMA units and insignificant difference in polarity between the comonomer units. As a result, they form PMP later and their stabilization effect is smaller. Hence, we obtain coarser microspheres than in the case of acrolein copolymerization with more hydrophobic styrene (Fig. 4). Such a mechanism of PMP formation is confirmed by the fact that, at increased content of acrolein in the mixture with MMA, coarser particles are formed (Fig. 1b). In the second stage of polymerization, the rate of MMA consumption increases from 0.8×10^{-5} to 2.1×10^{-5} mol l^{-1} s^{-1}, whereas the rate of acrolein conversion remains constant. In this stage, polymerization proceeds predominantly on the PMP surface and thus the mole fraction of MMA in PMP may increase in the course of the experiment and acrolein consumption.

When the MMA conversion reaches nearly 35%, a gradual increase in the rate of MMA polymerization gives way to a pronounced gel effect: the rate of MMA consumption increases from 2.1×10^{-5} to 7.5×10^{-5} mol l^{-1} s^{-1}. Si-multaneously, the rate of acrolein consumption increases to 4.8×10^{-5} mol l^{-1} s^{-1}. This confirms the formation of a copolymer of acrolein and MMA in PMP. It should be noted that in this stage of polymerization the increase in the viscosity of the polymer-monomer mixture in the particles, which provides coexistence of several growing radicals and causes the gel effect, may be enhanced by cross-

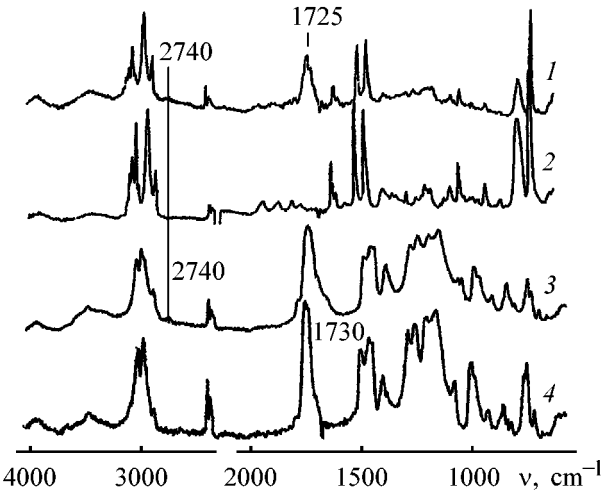

Fig. 2. IR spectra of sol fractions of (*1*) PS/AC and (*2*) PMMA/AC in comparison with IR spectra of (*3*) PS and (*4*) PMMA. (ν) Wave number.

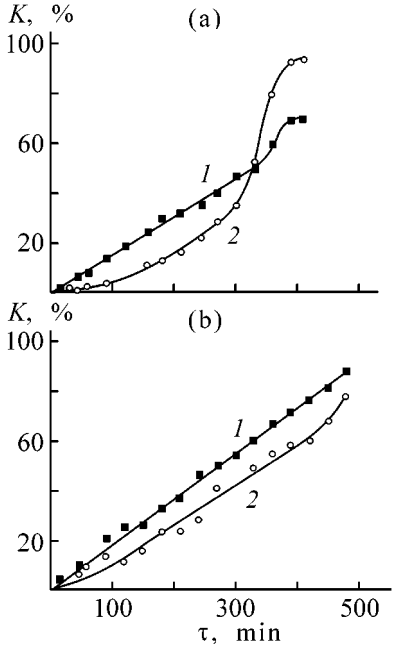

Fig. 3. Monomer conversion K vs. synthesis time τ in copolymerization of equimolar mixtures of (a) (*1*) acrolein with (*2*) MMA and (b) (*1*) acrolein with (*2*) styrene.

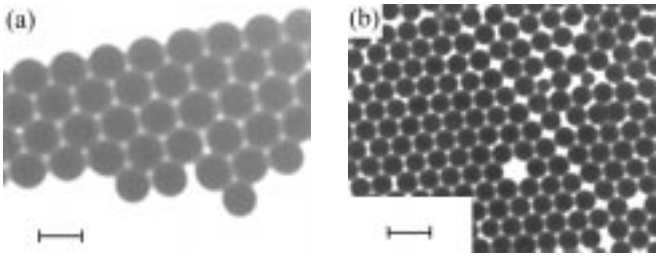

Fig. 4. Microspheres of (a) PMMA/AC and (b) PS/AC prepared by copolymerization of equimolar monomer mixtures.

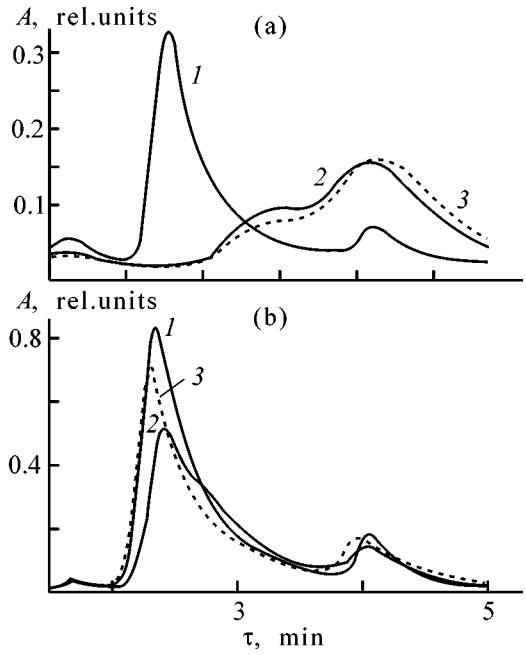

Fig. 5. HPMC curves of the albumin solutions in borate buffer (pH 9.2) (*1*) before and (*2*, *3*) after their interaction with microspheres of acrolein copolymers with (*2*) styrene and (*3*) MMA (a) without preliminary removal of oligomers and (b) after their separation. (*A*) Absorption and (τ) time. Albumin concentration, mg ml^{-1}: (a) 1 and (b) 1.5.

linking of macromolecules via acrolein units (Table 2). After the exhaustion of the monomers in PMPs, the copolymerization rate significantly decreases and plateaus are observed in the conversion curves. The acrolein conversion is incomplete, probably because its certain fraction is retained in water.

In the course of polymerization with styrene, acrolein is consumed at a constant rate of 2.7×10^{-5} mol l^{-1} s^{-1} (Fig. 3b), which is higher than the rate of its copolymerization with MMA before gelation (2.4×10^{-5} mol l^{-1} s^{-1}). This is due to the fact that copolymerization with styrene yields smaller particles, and, thus, the concentration of growing PMPs is higher. In this case, the induction period in the conversion curve of styrene is less pronounced than in the case of MMA copolymerization with acrolein. This suggests rapid formation of diphilic PMPs, which thus involve the surface-active oligomers and oligomeric radicals containing fragments of hydrophobic styrene (3.7×10^{-3} M solubility in water [14]) and hydrophilic acrolein. At moderate conversion of monomers, when copolymerization proceeds predominantly in PMPs, the rate of styrene consumption (2.6×10^{-2} mol l^{-1} s^{-1}) is close to that of acrolein. It was found [8] that, despite equal copolymerization constants of styrene and acrolein in dioxane (0.25 [13]), the number of acrolein fragments in the copolymer in the course of emulsion copolymerization grows only slightly with its increasing content in the reaction mixture and does not exceed 30% at styrene/acrolein molar ratio of 1 : 2. By contrast, we prepared in our case PSAC copolymer microspheres with acrolein content of 53 mol % even at the equimolar monomer ratio (Table 2). The similar reactivities of styrene and acrolein in this case can be accounted for by the presence of potassium dihydrogenphosphate in the reaction mixture, which decreases the acrolein solubility in water and shifts the polymerization predominantly into PMPs. As a result, the conversion of styrene in the course of copolymerization is slightly smaller than that of acrolein, which allows preparation of copolymer microspheres with high content of aldehyde groups.

To study the sorption of proteins by the surface of PSAC and PMMAAC copolymer microspheres, we performed binding of BSA with their surface aldehyde groups in alkaline medium. The residual concentration of BSA after chemisorption was determined by HPMC; the results suggested that, simultaneously with the formation of the Schiff bases on the particle surface, the polymeric chains enriched with carboxy and aldehyde groups can be washed out of the particles into solution, with their further binding with BSA in solution. As a result, broad peaks of BSA conjugates with the most soluble copolymer chains appear in the chromatograms in analysis of the residual BSA in the dispersion system after chemisorption (Fig. 5a). Their retention time is longer than that of the initial BSA, which is due to an increase in the number of anionic groups in this protein. This trend is the most pronounced under the chemisorption conditions at high concentration of the surface aldehyde groups and protein solution (pH > 9.0). This effect was eliminated when the microspheres were washed with borate buffer solution (pH 10.0) before chemisorption (Fig. 5b).

The data on the BSA chemisorption in several samples of copolymer microspheres with different copolymer compositions and surface concentrations of the functional groups are listed in Table 3. It was found that the BSA chemisorption on PSAC copolymer microspheres is somewhat stronger than that in the case of PMMAAC. This is probably due to higher surface hydrophobicity of the acrolein-styrene copolymer. The chemisorption efficiency depends on the pH of the dispersion medium. It was found that, with the pH of the albumin solution increasing in the course of chemisorption, the amount of BSA bound to the PMMAAC microspheres increases significantly,

Table 3. Conditions of albumin chemisorption

Sample no.	[–CHO]	[–COOH]	Buffer solution pH	Albumin concentration, mg ml^{-1}		Albumin chemisorption, mg m^{-2}
	μg-equiv m^{-2}			C_0	C_p	
Poly(styrene/acrolein)						
8	3.20	0.9	7.5	1.50	1.01	1.60
8	3.20	0.9	10.0	1.50	1.09	1.30
3	2.00	0.7	9.25	1.61	1.08	2.30*
3	2.00	0.7	10.2	1.64	1.10	2.08*
Poly(methacrylate/acrolein)						
14	2.96	1.7	10.0	1.50	1.23	1.27
13	1.53	1.8	7.5	1.00	0.93	0.35
13	1.53	1.8	8.2	1.00	0.90	0.65
13	1.53	1.8	9.20	1.50	1.29	0.72
13	1.53	1.8	10.0	1.00	0.79	1.10
12	0.81	0.5	8.2	0.50	0.46	0.35

* The concentrations of albumin and other polymers were determined by Lowry and HPMC procedures, respectively.

whereas in the case of PSAC microspheres the trend is reverse. It is obvious that certain fraction of BSA is physically sorbed on the surface of PSAC microspheres through the hydrophobic interactions. With increasing pH, the microsphere surface and albumin globules acquire a larger negative charge owing to ionization of their carboxy groups, and the albumin sorption must decrease because of the electrostatic repulsion and decreasing hydrophobic interaction [15]. The contribution of this mechanism to the BSA interaction with the surface of PSAC microspheres is confirmed by the fact that, with decreasing surface concentration of the carboxy groups, the albumin binding becomes more efficient (Table 3, sample nos. 3, 8); similar results were obtained in [16]. By contrast, the formation of the Schiff base becomes facilitated with increasing pH, which is observed in the course of BSA binding with the less hydrophobic surface of the PMMAAC microspheres. With increasing surface concentration of the aldehyde groups in the PMMAAC microspheres, the chemisorption efficiency also increases.

CONCLUSIONS

(1) Emulsifier-free emulsion copolymerization of acrolein with styrene and methyl methacrylate to prepare microspheres with surface aldehyde groups was developed. The copolymerization kinetics at the equimolar monomer ratios was studied. The results obtained allow control over the size of monodisperse microspheres (370–1000 nm), hydrophobicity of their surface, and content of the aldehyde groups providing the covalent binding of biologically active compounds.

(2) The albumin binding to the microsphere surface becomes stronger with increasing hydrophobicity of the polymer support, i.e., on passing from poly(methyl methacrylate/acrolein) to poly(styrene/acrolein). However, the contribution of chemisorption to the binding of the native bovine serum albumin is greater for poly(methyl metacrylate/acrolein) microspheres, because the efficiency of the albumin binding to the surface of this copolymer grows with increasing surface concentration of the aldehyde groups and the albumin solution pH.

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