Poly(styrene/acrolein) and poly(styrene/ α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) microspheres. Similarities and differences

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Abstract: Properties of two types of polystyrene microspheres with polyacrolein and polyglycidol components in their surface layers are compared. Microspheres were prepared in batch radical emulsifier-free emulsion copolymerizations of styrene with acrolein and/or α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol macromonomer ($\bar{M}_n = 2700$). Polymerizations were initiated with potassium peroxodisulfate, and the ratio of initial concentrations of styrene and initiator was constant. Number average diameters of poly(styrene/acrolein) (P(S/A)) and of poly(styrene/polyglycidol) (P(S/PGL)) particles were in the range of 200 - 650 nm and decreased with increasing concentration of acrolein and/or polyglycidol in the polymerizing mixtures. The diameter polydispersity of synthesized particles ($\bar{D}_w / \bar{D}_n$) was usually lower than 1.02. X-ray photoelectron spectroscopy for P(S/A) and P(S/PGL) microspheres showed that surface layers of particles were significantly enriched in polyacrolein or polyglycidol segments, surface fractions of which increased with increasing concentration of the more hydrophilic comonomer in the polymerizing mixture. In the case of P(S/A) particles, the maximal fraction of polyacrolein approached 80 mol-%, whereas for P(S/PGL) particles the maximal surface fraction of PGL was 42 mol-%. Human serum albumin was used as a model protein for studies of attachment onto P(S/A) and P(S/PGL) microspheres. It has been found that for both kinds of particles, the maximal surface concentration of attached (adsorbed and/or covalently immobilized) protein decreased with increasing fraction of hydrophilic component in the particle surface layer (polyacrolein or polyglycidol units). In the case of P(S/A) particles, adsorption always accompanied covalent immobilization of proteins. To the contrary, covalent immobilization of proteins onto the P(S/PGL) microspheres proceeded without adsorption of proteins.

Introduction

The most important features of microspheres applied in life sciences, biotechnology and medicine, as the basic components of biosensors, bioseparation kits and medical diagnostics systems (e.g., latex agglutination tests) are the following ones:

- particles should be of desired size and, if possible, their diameter polydispersity $\bar{D}_w / \bar{D}_n$ (defined as the ratio of the weight average diameter to the number average diameter – for identical microspheres this ratio equals 1) should be narrow;

- chemical and colloidal stability of microspheres against pH, ionic strength and temperature should be satisfactory;
- microspheres should be equipped with chemical groups, suitable for covalent binding of proteins (antibodies) without spontaneous adsorption;
- microspheres should be able to bind antibodies (or antigens) in amounts allowing detection of chosen proteins in analyses at the required concentration range [1].

The advantage of using microspheres as protein carriers is related to their large specific surface. For example, the surface of 1 g of polystyrene particles (density of polystyrene = 1.05 g/cm³) with $\bar{D}_n = 0.5 \mu\text{m}$ (number average diameter) equals 11.4 m². There are various ways of using microspheres for medical diagnostics. Microspheres could be used simply in suspensions or as elements of more complex systems (biosensors). For the latter applications, microspheres were used for the controlled surface modification by formation of particle monolayer assemblies at interfaces [2,3].

In the last 15 years, a large number of papers has been devoted to various methods of syntheses of polymer microspheres equipped with chemical groups suitable for efficient binding of biologically active compounds. Particles with amino, hydroxyl, carbonyl, succinimide, benzotriazole, epoxide groups were usually used for this purpose [4-18]. Often, the covalent binding of proteins onto a polymer support was accompanied with the undesired (difficult to control) physical adsorption. It was established that protein adsorption was much less effective when surfaces were highly hydrophilic. Surfaces of solid supports were made hydrophilic by anchoring onto them various natural and synthetic hydrophilic oligomers and large macromolecules including terpolymers composed of methyl methacrylate, polydimethylsiloxane methacrylate and methoxypolyethyleneglycol methacrylate [19], glycosaminoglycan, hyaluronic acid and poly(glycine-valine-glycine-valine-proline) [20], alginic acid [21], phosphorylcholine derivatives [22,23], polyacrylamide and polyvinylpyrrolidone [20], and poly(vinyl alcohol) [24]. It was found that poly(ethylene oxide) (PEO) is an excellent protein adsorption reducer not only due to its hydrophilicity but also due to the very high mobility of PEO chains tethered to a surface [25]. However, modification with PEO had also some disadvantage. Namely, macromolecules of this polymer could be functionalized only at the ends. Thus, covalent immobilization of required biomolecules onto surfaces covered with PEO (via PEO end groups) was very low and often insufficient for practical applications.

In this paper we summarize results of our studies on properties of poly(styrene/acrolein) (P(S/A)) and poly(styrene/ α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) (P(S/PGL)) microspheres obtained in a one step radical emulsifier-free emulsion polymerization of styrene and one of the following water-soluble comonomers: the low molecular weight compound acrolein (MW = 56), and/or α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol (PGL), a macromonomer with number average molecular weight $\bar{M}_n = 2700$. Fig. 1 illustrates the structures of the hydrophilic comonomers mentioned above.

In our studies we were interested, in particular, in the determination of the relation between the initial ratio of comonomers in the polymerizing mixture and the chemical composition of the surface layer of microspheres. We wanted also to compare properties of P(S/A) and P(S/PGL) microspheres as protein carriers. Moreover, studies of protein binding onto both kinds of particles could provide some information on details of surface structures of microspheres, in particular on the lateral distribution of aldehyde and hydroxyl groups derived from polyacrolein and polyglycidol

monomer units, respectively. Human serum albumin (HSA) the most abundant protein in the blood serum, responsible, among other things, for the transport of hydrophobic compounds, was used as a model protein in our studies.

Fig. 1. Comonomers used for the synthesis of microspheres: (a) acrolein, (b) α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol

Experimental part

The monomers styrene and acrolein (Aldrich) were purified from stabilizers by distillation under reduced pressure at 30°C just before using them for polymerization. The macromonomer α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol (PGL) with $\bar{M}_n = 2700$ and $\bar{M}_w / \bar{M}_n = 1.03$ (determined by MALDI-TOF) was synthesized as described earlier [26, 27]. Initiator, $\text{K}_2\text{S}_2\text{O}_8$ (Fluka) was used without further purification. Human serum albumin (HSA) (Sigma, Cohn fraction V) was used as received. Diameters of microspheres were determined from scanning electron microphotographs recorded using a Jeol 35C microscope. The atomic composition of the surface layer of microspheres was determined using a Vacuum Generators MK1 spectrometer with an AEI (Marseille, France) acquisition system. On the basis of these measurements, surface fractions of polystyrene, polyacrolein and polyglycidol were evaluated according to known procedures [27,28]. The overall composition of microspheres was evaluated on the basis of the content of carbon and hydrogen determined by elemental analysis for dry samples (lyophilized and dried in a vacuum line at 10^{-4} mm Hg).

Procedures used for studies of protein attachment (adsorption in the case of P(S/PGL) microspheres and, in the case of P(S/A) microspheres with aldehyde groups that don't require activation, for adsorption and covalent immobilization) were described in our earlier papers [29,30]. Generally, they consisted of incubation (20 h at room temperature) of a protein solution with suspension of microspheres in a buffered medium (phosphate buffered saline (PBS) with pH 7.4 and ionic strength 0.2 mol/l). The amount of attached HSA was determined as the difference of the initial concentration of HSA and the concentration of HSA remaining in solution after incubation.

Covalent immobilization of HSA onto P(S/PGL) microspheres required activation of hydroxyl groups. The detailed recipe was described earlier [31]. Here we present a short description of this process. 1,3,5-Trichlorotriazine (TCT; 0.18 g) was added to 8 ml of aqueous suspension containing 0.4 g of P(S/PGL) particles. After 12 h of incubation at room temperature, microspheres were isolated by centrifugation and resus-

pended in water. Centrifugation and resuspension were repeated 4 times in order to remove unbound TCT. Then, HSA was covalently immobilized onto activated microspheres. The procedures for protein binding were common for P(S/A) and P(S/PGL) microspheres. Typically, to the suspension of microspheres (3×10^{-3} g/ml) a protein solution (in phosphate buffered saline (PBS, pH 7.4, I = 0.2 M) was added. The samples were incubated during 24 h at room temperature and then, the unbound protein was removed by centrifugation. Surface concentrations of attached proteins (here and in all further parts of this paper we use the term “attached” when no discrimination is made between adsorbed and covalently immobilized proteins) were determined according to well established procedures [29,30]. The amounts of HSA attached onto microspheres were determined either by measuring the protein contents in solution before and after incubation or directly on microspheres by a modified Lowry method [32,33]. A diode array Hewlett-Packard 8452A spectrometer was used for these measurements.

It is known that proteins (HSA and γ -globulin) adsorbed onto polystyrene microspheres could be completely washed out with 3% solution of sodium dodecyl sulfate (SDS) [29]. Thus, determination of the fractions of adsorbed and covalently immobilized HSA was possible by determination of the total (covalently immobilized and adsorbed) amount of attached protein and determination of the fraction of HSA, which could be removed from the surface of microspheres with SDS (fraction of adsorbed protein).

Results and discussion

1. Synthesis of poly(styrene/acrolein) (P(S/A)) and poly(styrene/ α -tert-butoxy- ω -vinylbenzyl-polyglycidol) (P(S/PGL)) microspheres

Syntheses of P(S/A) and P(S/PGL) were described in detail in our previous papers [27,28,34]. Here we present only a brief description. P(S/A) and P(S/PGL) microspheres were obtained in the emulsifier-free emulsion precipitation polymerization according to the recipe given in Tab. 1.

Oxygen was removed from the polymerizing mixture by bubbling nitrogen through it for 30 min. Syntheses were carried out under nitrogen, with stirring at 60 r.p.m., at 65°C, for 24 h. The traces of unreacted styrene were removed by steam stripping. Then the microspheres were purified by 4 times repeated washing with 10^{-3} mol/l HCl in water and isolation by centrifugation. Then, particles were redispersed in pure water, isolated by centrifugation and finally redispersed in water again.

Tab. 1. A recipe for the synthesis of poly(styrene/acrolein) P(S/A) and poly(styrene/ α -tert-butoxy- ω -vinylbenzyl-polyglycidol) P(S/PGL) microspheres

Ingredients	Quantity	
	P(S/A)	P(S/PGL)
Styrene	10 ml	10 ml
Acrolein	0.3-1.5 ml	--
α -tert-Butoxy- ω -vinylbenzyl-polyglycidol ($\bar{M}_n = 2700$)	--	0.9-1.36 g
K ₂ S ₂ O ₈	0.04g	0.2g
Water distilled three times, pH adjusted to 6.5 with K ₂ CO ₃	125 ml	125 ml

2. Characteristic parameters of P(S/A) and P(S/PGL) microspheres

2.1. Diameters ($\bar{D}_n$) and diameter distributions ($\bar{D}_w / \bar{D}_n$)

Number average diameters ($\bar{D}_n$) of microspheres, determined from SEM microphotographs, are listed in Tab. 2. From data collected in this table it is evident that $\bar{D}_n$ decreased with increasing content of the more hydrophilic component (acrolein and/or α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) in the polymerizing mixture. It is well known that for heterogeneous multicomponent systems in water media maximization of the interface between hydrophilic component and water is thermodynamically favorable. At very high concentrations of surface active compounds, hydrophobic monomers could be dispersed even into nanodroplets that after polymerization became converted into nanoparticles. One could expect also that microspheres with smaller diameters would be formed for increased concentrations of hydrophilic comonomer [35]. Indeed, such dependence was observed for polymerizations in many systems, e.g., for copolymerization of styrene with acrolein (hydrophilic component) [28], with 2-hydroxyethyl methacrylate [4], and with poly(ethylene oxide) macromonomers [36]. Moreover, data in Tab. 2 indicate also clearly that values of the diameter polydispersity for all populations of particles were narrow (in the range $1.002 < \bar{D}_w / \bar{D}_n < 1.022$).

2.2. Surface charge density of microspheres and stabilization of particle suspensions

Suspensions of P(S/A) and P(S/PGL) microspheres were stable as a result of the electrostatic inter-particle repulsion forces due to the anionic sulfate groups present at particle surfaces. Sulfate end groups were formed in the initiation process, during reaction of $\text{SO}_4^{\cdot -}$ radical anions (from $\text{K}_2\text{S}_2\text{O}_8$) with monomers. P(S/PGL) microspheres were stabilized also sterically, due to the presence of the hydrophilic PGL segments in their surface layers. Values of surface concentration of acidic ($-\text{OSO}_3^-$) groups for PS, P(S/A), P(A) and P(S/PGL) microspheres are given in Tab. 2. The values measured for P(S/PGL) particles indicate that the increase of surfmer (α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) concentration in the polymerizing mixture resulted in a decrease of the surface charge of synthesized microspheres. It is possible that the hydrophilic particle surface layer composed of PGL chains screens some of the anionic end groups in the P(S/PGL) microspheres. Hydrophilic PGL chains are not miscible with polystyrene and, therefore, it is reasonable to assume that due to microphase separation the microspheres with a dense hydrophobic layer, containing entrapped anionic sulfate end groups and corresponding potassium cations, covered with hydrophilic PGL layer, were formed. For P(S/A) microspheres, the surface concentration of acidic groups apparently decreases and then increases with increasing content of polyacrolein units. However, we refrain from drawing any conclusions from this dependence because aldehydes are easily oxidized and thus, adventitious formation of carboxylic groups may contribute to these observations.

2.3. Composition of surface layers of P(S/A) and P(S/PGL) microspheres

It is known that emulsion copolymerization of monomers with different hydrophilicity in water media yields microspheres with surfaces enriched in the more hydrophilic comonomer. For example, the surface layer of microspheres obtained by copolymerization of styrene and hydroxyethyl acrylate was significantly enriched in the latter monomeric units [4]. Thus, we expected that radical copolymerization of a hydrophobic vinyl monomer (styrene) and the water-soluble acrolein and/or PGL macromono-

mer would lead to microspheres with high content of the hydrophilic component in the surface layer. From XPS (X-ray photoelectron spectroscopy) measurements we found indeed that for both families of synthesized microspheres, molar surface fractions of polyacrolein units (f_A) and polyglycidol units (f_{PGL}), in P(S/A) and P(S/PGL) microspheres, respectively, increased with increasing fraction of the more hydrophilic components in the polymerization mixtures (cf. Tab. 2).

Tab. 2. Number average diameters ($\bar{D}_n$), diameter polydispersity ($\bar{D}_w / \bar{D}_n$), surface concentration of acidic groups ($-\text{OSO}_3^-$), and surface mole fraction of polyacrolein and polyglycidol units in P(S/A), P(A) and P(S/PGL) microspheres (f_{PA} , f_{PGL})

Symbol of particles	Styrene:A or styrene:PGL ^a in mol:mol	$\bar{D}_n$ ^b in μm	$\bar{D}_w / \bar{D}_n$ ^c	Surface conc. of acidic groups ^d in $\mu\text{mol}/\text{m}^2$	f_{PA}	f_{PGL}
Polystyrene microspheres (PS)						
PS	1 : 0	0.52	1.005	3.39	0	-
Poly(styrene/acrolein) microspheres P(S/A)						
P(S/A)-1	1 : 5.2×10^{-2}	0.51	1.010	2.52	0.500	-
P(S/A)-2	1 : 1.0×10^{-1}	0.49	1.002	1.38	0.630	-
P(S/A)-3	1 : 2.6×10^{-1}	0.38	1.007	2.14	0.840	-
Polyacrolein microspheres P(A)						
P(A)	0 : 1	0.30	1.003	3.24	1	-
P(S/PGL2700) microspheres						
P(S/PGL)-1	1 : 3.46×10^{-4}	0.65	1.008	1.65	-	0.216
P(S/PGL)-2	1 : 6.94×10^{-4}	0.46	1.014	1.05	-	0.255
P(S/PGL)-3	1 : 1.73×10^{-3}	0.35	1.007	0.87	-	0.334
P(S/PGL)-4	1 : 2.60×10^{-3}	0.26	1.007	0.48	-	0.423
P(S/PGL)-5	1 : 3.47×10^{-3}	0.22	1.022	0.41	-	0.426

^a Ratio of styrene to acrolein and styrene to polyglycidol in the monomer feed.

^b $\bar{D}_n = \sum n_i D_i / \sum n_i$

^c $\bar{D}_w / \bar{D}_n = (\sum n_i D_i^4 / \sum n_i) / (\sum n_i D_i^3 / \sum n_i D_i)$

^d The concentration of acidic groups on the total surface of a sample containing a known amount of microspheres was determined from a minimum on the conductometric titration curve. In the case of microspheres investigated, the position of this minimum, found by numerical differentiation and/or by intersection of linear least square fits to straight linear segments of the curve characterizing decreasing and increasing conductivity, was determined with an error of 10 - 15%. This inaccuracy constituted the major contribution to the overall error of determination of the surface concentration of acidic groups.

It is worth noting that for the investigated microspheres the ratio of styrene to acrolein and/or styrene to polyglycidol in the monomer feed allowed not only for regulation of the composition of the surface layer of microspheres, but also for controlling microsphere diameters (Tab. 2) [27,28]. The higher the content of acrolein or PGL (hydrophilic comonomer) in the monomer feed, the smaller the diameters of obtained P(S/A) or P(S/PGL) microspheres.

Fig. 2 illustrates the dependence of surface fractions of polyacrolein (PA) (Fig. 2a) and polyglycidol (Fig. 2b) on the fractions of the corresponding hydrophilic copolymers in the whole microspheres.

Fig. 2. Dependence of the molar surface fraction (f_s) of (a) polyacrolein (PA) and (b) α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol (PGL), determined by XPS, on the mole fraction of PA and PGL in the whole microspheres (f_m) (includes data from refs. [27,28])

It is worth noting that the surface fraction of polyacrolein segments in P(S/A) microspheres could be regulated in full range (from 0 to 100% of polyacrolein units), depending on the composition of the monomer feed (particles with 0% and 100% of polyacrolein content in the surface layer were obtained by homopolymerizations of styrene and acrolein, respectively). In the case of P(S/PGL) microspheres, a saturation with hydrophilic component was also achieved. However, for these particles the maximum surface fraction of PGL monomer units, determined by XPS, did not exceed 40% (Fig. 2a,b). Further studies are needed to explain why saturation occurs at such a low level. However, it is possible that, due to the increased solubility in water of poly(styrene-*co*- α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) with higher content of polyglycidol units, only chains with polyglycidol content lower than 40% can be incorporated into growing microspheres.

It is known that comonomers with surface active properties (so-called surfmers) are incorporated predominantly into surface layers of microspheres [37]. The plot in Fig. 2 indicates that in syntheses of P(S/PGL) microspheres polyglycidol acts as a surfmer.

Li et al. found that in polymerizations leading to particles stabilized with PEO the area occupied by one macromolecule of PEO with 37 monomer units of ethylene oxide is equal to 2.5 nm^2 (at saturation conditions) [38]. Assuming that the surface density of PGL molecules with $\bar{M}_n = 2700$ (corresponding to 39 monomer units) in a monolayer would be similar to that in the layer of PEO mentioned above, we estimated the thickness of a PGL monolayer to be close to 1.8 nm. XPS probes surface layers c. 5 nm thick. Thus, the measured value for the polyglycidol surface fraction at saturation (= 0.4) could be close to the one corresponding to the full coverage of polystyrene particles with a PGL monolayer (1.8 nm equals 36% of the XPS probed 5 nm layer). The considerations presented above conform to the assumption that copolymers of styrene and α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol in the surface layer of microspheres adopt a conformation with hydrophilic polyglycidol segments oriented towards the water phase and polystyrene hidden under them.

3. Adsorption and covalent immobilization of human serum albumin onto P(S/A) and P(S/PGL) microspheres

In medical diagnostics, microspheres are used mainly for protein binding. Often simple adsorption of proteins onto polymeric support does not lead to good results. Proteins are usually adsorbed oriented with hydrophobic domains towards the surface of supports, and often this orientation is not the desired one (it might happen that in this case important receptor groups may be oriented towards the particle surface and, thus, become inaccessible for any analyses). During prolonged storage, adsorbed proteins undergo slow spontaneous desorption (leaking). Thus, the covalent linking of protein onto polymeric supports is usually preferred. In the case of covalent binding, control of the amounts of attached biomolecules is easier; moreover, since covalent binding is more stable than physical adsorption, this could assure longer shelf-life of biomolecules-microsphere conjugates [39].

Proteins with amine groups could be bound to particles containing aldehyde groups (P(S/A)) directly in reaction with Schiff base formation (imino linkages). In spite of reversibility of this reaction, the binding of a protein via several imino linkages makes immobilization practically irreversible. However, the hydroxyl groups of P(S/PGL) microspheres require activation prior to immobilization of biomolecules with $-\text{NH}_2$ groups. In our studies we activated surfaces of P(S/PGL) microspheres with cyanuric chloride (1,3,5-trichlorotriazine, TCT). Reactions involved in covalent binding of proteins onto P(S/A) and P(S/PGL) microspheres are illustrated schematically in Fig. 3.

The dependence of the surface concentration of HSA adsorbed onto P(S/PGL) microspheres (without activation of hydroxyl groups) and HSA covalently immobilized onto particles activated with TCT (cf. Exptl. part) on the concentration of HSA in solution during incubation is shown in Fig. 4a,b. Surface concentrations of adsorbed (Γ_{ads}) and/or covalently immobilized (Γ_{cov}) HSA were evaluated dividing amounts of adsorbed and/or covalently immobilized protein, determined as it was described in the Exptl. part, by the total surface of microspheres in a sample.

Fig. 3. Scheme of covalent immobilization of proteins via (a) aldehyde and (b) hydroxyl groups

Fig. 4. Dependence of the surface concentration of HSA (a) adsorbed (Γ_{ads} , determined in experiments without activation of hydroxyl groups) onto P(S/PGL) microspheres, and (b) covalently immobilized (Γ_{cov}) on TCT-modified P(S/PGL) microspheres on the concentration of HSA in solution; [microspheres] = 3×10^{-3} g/ml

Experimental points plotted in Fig. 4a are characteristic of adsorption with saturation. Namely, with increasing protein concentration in solution (in the range from 0.05 mg/ml to 4.5 mg/ml) the surface concentration of protein increased until full coverage of the surface available for adsorption was achieved. Such dependencies characteristic of Langmuir type adsorption of rigid molecules were successfully applied also to proteins [30]. According to this model, the dependence of surface concentration of adsorbed HSA on HSA concentration in solution could be described by the following Eq. (1):

$$\Gamma_{\text{ads}} = \Gamma_{\text{ads}}^{\text{max}} \frac{K_A ([\text{HSA}]_0 - S \Gamma_{\text{ads}})}{1 + K_A ([\text{HSA}]_0 - S \Gamma_{\text{ads}})} \quad (1)$$

in which Γ_{ads} denotes surface concentration of adsorbed HSA, $\Gamma_{\text{ads}}^{\text{max}}$ maximal surface concentration of adsorbed HSA corresponding to the situation when the whole surface of microspheres is covered with protein and new protein molecules cannot be accommodated, K_A adsorption equilibrium constant, S total surface of microspheres, and $[\text{HSA}]_0$ initial concentration of HSA in solution during incubation. Plots in Fig. 4a illustrate fits based on Eq. (1) to experimental data. It has to be noted that for higher fractions of polyglycidol (PGL) in the surface layer (f_{PGL}) the registered maximum surface concentrations of adsorbed HSA ($\Gamma_{\text{ads}}^{\text{max}}$) were lower. They were equal to 0.76, 0.99, 0.91, 0.29, and 0.18 mg/m² for P(S/PGL)-1, P(S/PGL)-2, P(S/PGL)-3, P(S/PGL)-4, and P(S/PGL)-5 microspheres, respectively. In the same order decreased values of K_A (48.45, 14.50, 3.77, 8.32, and 7.71 ml/mg). Also for P(S/A) microspheres, the lower maximal surface concentrations of adsorbed proteins were found for particles with higher surface fraction of polyacrolein. For example, the maximal surface concentration of HSA ($\Gamma(\text{HSA})_{\text{max}}$) decreased in the order 1.60, 1.05, 1.00, 0.59, and 0.51 mg/m² for adsorption of HSA onto P(S), P(S/A)-1, P(S/A)-2, P(S/A)-3, and P(A) microspheres, respectively (i.e., according to Tab. 2, onto particles with increased surface fraction of polyacrolein). Thus, it is evident that by increasing the hydrophilicity of surfaces of microspheres it was possible to reduce the adsorption of proteins onto investigated particles.

The dependence of HAS, covalently immobilized on TCT-activated P(S/PGL) particles, on the concentration of protein in solution is presented in Fig. 4b. It is worth to note that in the case of P(S/PGL) microspheres modified with TCT we found that essentially the whole amount of attached HSA was immobilized covalently, with negligible fraction of adsorbed protein. Thus, P(S/PGL) microspheres could be used for protein immobilization in a controlled manner - “on wish”, after activation of hydroxyl groups in polyglycidol chains. This feature of polymer supports is especially valuable for medical diagnostic tests, in which any nonspecific and uncontrolled attachment of antigen (antibody) onto particles decreases the accuracy of analysis.

Dependencies of maximum surface concentration of HSA adsorbed ($\Gamma_{\text{ads}}^{\text{max}}$ -P(S/A)) and covalently immobilized ($\Gamma_{\text{cov}}^{\text{max}}$ -P(S/A)) onto P(S/A) microspheres and covalently immobilized onto the P(S/PGL) particles ($\Gamma_{\text{cov}}^{\text{max}}$ -P(S/PGL)) on the surface fraction of polyacrolein and/or polyglycidol (f_s) is shown in Fig. 5.

The values of maximal surface concentration of adsorbed and covalently immobilized protein were obtained by reading an estimated saturation value from the figure. For both investigated types of microspheres, the maximal surface concentration of immobilized HSA increases, reaches a maximum and then decreases with increasing fraction of polyacrolein or polyglycidol in the microsphere surface layer. It is well known

that HSA easily changes conformation upon adsorption and, if free space is available, tends to maximize the contact area with the surface of the substrate (side-on adsorption, partial denaturation [40]). The higher fraction of polyacrolein and/or polyglycidol in the surface layer of microspheres (for P(S/A) $f_s > 0.8$, and for P(S/PGL) $f_s > 0.4$) provides a larger number of reactive groups for protein immobilization. This leads to multivalent binding of HSA molecules that are side oriented and possibly even partially denatured. In this state, each macromolecule of HSA occupies larger space what, in effect, results in a lower surface concentration of HSA immobilized covalently onto microspheres with higher surface fraction of comonomers with reactive groups.

Fig. 5. Dependence of maximum surface concentration of HSA total ($\Gamma_{(max)}^{tot-P(S/A)}$), adsorbed ($\Gamma_{(max)}^{ads-P(S/A)}$), and covalently immobilized ($\Gamma_{(max)}^{cov-P(S/A)}$) on P(S/A) microspheres, and covalently immobilized to the P(S/PGL) particles ($\Gamma_{(max)}^{cov-P(S/PGL)}$) on the surface fraction of corresponding polyacrolein or polyglycidol (f_s)

It was reasonable to expect that, in the case of particles synthesized from a mixture of hydrophilic and hydrophobic comonomers, proteins would be adsorbed predominantly onto the hydrophobic (in our studies enriched in polystyrene) domains and covalently immobilized onto the hydrophilic domains with reactive groups (e.g., polyacrolein and/or polyglycidol). In the case of HSA attachment onto the P(S/A) microspheres we noticed that, with the exception of particles with 100% polyacrolein content in the surface layer, there was always a fraction of protein that was only physically adsorbed onto particles (fraction of HSA that could be removed with SDS). This means that on surfaces of P(S/A) microspheres there were some patches without any reactive groups capable of covalent immobilization of HSA. On the other hand, in the case of P(S/PGL) microspheres activated with TCT, all molecules of HSA were covalently immobilized. Absence of any HSA that was only adsorbed onto the P(S/PGL) microspheres (even for a surface fraction of polyglycidol units as low as 20%) indicated that the whole surface of these particles was randomly covered with polyglycidol chains, without leaving any polystyrene patches accessible to HSA adsorption.

Conclusions

Poly(styrene/acrolein) and poly(styrene/ α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol) microspheres synthesized by radical copolymerization of styrene with acrolein and/or styrene with α -*tert*-butoxy- ω -vinylbenzyl-polyglycidol macromonomer (PGL; $\overline{M}_n = 2700$) had core-shell morphology, with the core enriched in polystyrene and the shell containing hydrophilic copolymer (polyacrolein or PGL, respectively). The polyacrolein content in the surface layer of microspheres could be controlled in the range of 0 - 100%, whereas the surface fraction of PGL in P(S/PGL) microspheres could be varied only in the range of 0 - 40%. Diameter distributions of these microspheres were monomodal. Microspheres with smaller diameters were obtained for higher fractions of hydrophilic acrolein or PGL comonomers in the polymerizing mixture.

It was found that HSA could be covalently immobilized on the surfaces of microspheres directly, in reaction with aldehyde groups of P(S/A) particles and/or in reaction with TCT-activated hydroxyl groups of P(S/PGL) microspheres. The covalent immobilization of proteins onto the surface of P(S/A) microspheres was accompanied with protein adsorption, whereas the whole amount of protein attached onto TCT-activated P(S/PGL) microspheres was covalently immobilized. This conforms to the assumption that polystyrene patches (support for protein adsorption) were present on the surfaces of P(S/A) microspheres, whereas the whole surfaces of P(S/PGL) microspheres were randomly covered with polyglycidol chains.

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