Colloid Polym Sci 279:916–924 (2001)

ORIGINAL CONTRIBUTION

T. Basinska
S. Slomkowski
A. Dworak
I. Panchev
M. M. Chehimi

Synthesis and characterization of poly(styrene/α-*t*-butoxy-ω-vinylbenzyl-polyglycidol) microspheres

Received: 26 September 2000
Accepted: 19 February 2001

T. Basinska · S. Slomkowski (✉)
Center of Molecular and Macromolecular Studies, Polish Academy of Sciences
Sienkiewicza 112, 90-363 Lodz, Poland
e-mail: staslomk@bilbo.cbmm.lodz.pl

A. Dworak · I. Panchev
Institute of Coal Chemistry
Polish Academy of Sciences
Sowinskiego 5, 44-102 Gliwice, Poland

M. M. Chehimi
Institut de Topologie et de Dynamique des Systemes, Universite Paris 7
Denis Diderot, CNRS, 1 rue Guy de la Brosse, 75005 Paris, France

Abstract Polystyrene microspheres with polyglycidol (polyGL) in a surface layer were synthesized in batch radical emulsifier-free emulsion copolymerizations of styrene and surfmers, α-t-butoxy-ω-vinylbenzyl-polyGL macromonomers (VB-polyGL). Macromonomers with number-average molecular weight $\bar{M}_n = 950$ (VB-polyGL950) and $\bar{M}_n = 2700$ (VB-polyGL2700) were used for these polymerizations. In all syntheses the initial concentrations of styrene and initiator ($K_2S_2O_8$) were constant. The initial macromonomer-to-styrene ratios were varied from 1.10×10^{-3} to 1.64×10^{-2} mol/mol and from 3.46×10^{-4} to 3.47×10^{-3} mol/mol for VB-polyGL950 and VB-polyGL2700, respectively. The diameters of microspheres obtained were smaller for the syntheses with higher concentrations of macromonomers. Syntheses with VB-polyGL950 yielded microspheres with number-average diameters ($\bar{D}_n$) from 216 to 900 nm and with a bimodal diameter distribution. The number-average diameters of microspheres obtained with VB-polyGL2700 varied from 220 to 650 nm, depending on the initial concentration of macromonomer. Their diameter distributions were monomodal, with a diameter polydispersity parameter (ratio of weight-average and number-average diameters) in the range $1.007 \leq \bar{D}_w/\bar{D}_n \leq 1.022$. For each type of microsphere the fraction of polyGL in a surface layer and the surface concentration of sulfate anions were determined. The fraction of polyGL in the surface layer was related to the initial monomer composition in the polymerizing mixture. Adsorption of human serum albumin onto surfaces of some poly(styrene/VB-polyGL) microspheres was up to 10 times lower than for the polystyrene microspheres obtained in a similar emulsifier-free emulsion polymerization of styrene.

Key words Microspheres · Polystyrene · α-*t*-Butoxy-ω-vinylbenzyl-polyglycidol macromonomer · Surfmer · Emulsifier-free radical polymerization

Introduction

Polymer microspheres tailored for applications in medical diagnostics as components of assays and elements of diagnostic devices should allow the covalent immobilization of bioactive compounds, without their undesired adsorption. Such microspheres are often obtained by adsorption of surfactants with hydrophilic blocks, for example, Pluronics, onto hydrophobic primary particles, for example, polystyrene microspheres, [1] or by copolymerization of surface-active comonomers (surfmers) containing hydrophilic

blocks, for example, poly(vinyl alcohol) [2], poly(*n*-isopropylacrylamide) [3], poly(ethylene oxide) and/or poly(butylene oxide) [4,–7], poly(2-oxazoline) [7] and disaccharide units [8]. Core–shell nanoparticles with poly(ethylene oxide) in the surface layer and a poly(γ-benzyl L-glutamate) core were obtained by Cho and et al. [10] from organic solutions of the poly(ethylene oxide)-*b*-poly(γ-benzyl L-glutamate) copolymers by the diafiltration method.

The biological activity of covalently immobilized proteins and the extent of their nonspecific adsorption onto microspheres depend, among others, on the hydrophilicity of particle surfaces related to the surface concentration of hydrophilic blocks in their surface layers and on the surface charge. It was found that the increase in the surface concentration of the poly(ethylene oxide) blocks results in a decrease in the protein adsorption [11]. Sofia et al. [12] studied the adsorption of cytochrome c, human serum albumin (HSA) and fibronectin onto poly(ethylene oxide)-grafted silicon surfaces [$\bar{M}_n$ of poly(ethylene oxide) of 3,400, 10,000 and 20,000]. The efficiency of protein adsorption was determined as a function of the poly(ethylene oxide) grafting density. For surfaces with grafting densities approaching 100 ng/m^2 the protein adsorption dropped to zero for all kinds of poly(ethylene oxide)-modified silicon surfaces.

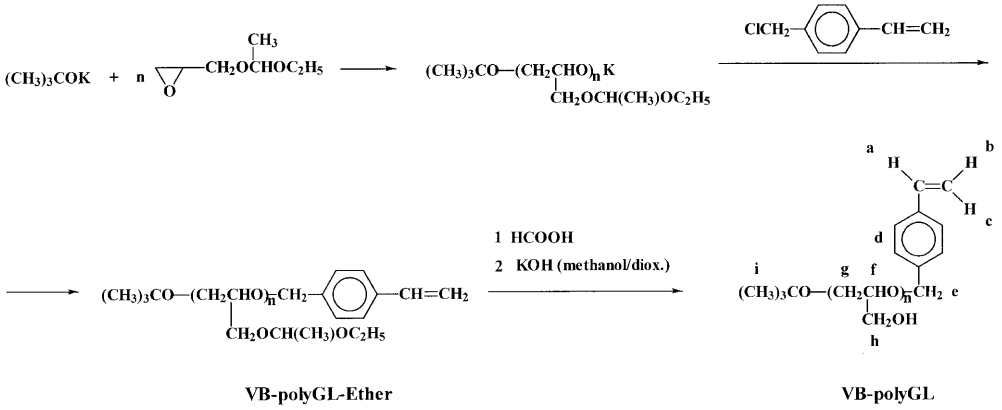
Poly(ethylene oxide) macromolecules covalently immobilized onto surfaces of microspheres can be equipped only with one functional group at the end. The goal of our study was to find a method of synthesis of particles having at the surface polyglycidol (polyGL), a polymer with main chain similar to poly(ethylene oxide) but with a high number of side hydroxyl groups. Macromolecules of polyGL when present in a surface layer of microspheres should protect them from the nonspecific adsorption of proteins.

This article describes the synthesis and basic properties of particles obtained directly by the emulsifier-free radical polymerization of styrene and the amphiphilic macromonomer α-*t*-butoxy-ω-vinylbenzyl-polyGL (VB-polyGL). We expected that during polymerization a substantial portion of the hydrophilic polyGL would be incorporated into the surface layer of the microspheres. With the purpose to control this process we wanted to determine the relation between the composition of the surface layer of the microspheres and the fraction of the macromonomer in an initial polymerizing mixture. We were also interested in finding out whether the presence of polyGL in the surface layer of polystyrene microspheres reduces protein adsorption. For these preliminary studies we selected HSA, the most abundant protein in the blood serum, known to be efficiently adsorbed onto polystyrene microspheres [13–15].

Experimental

Styrene was purified from the stabilizer (4-*t*-butylcatechol) by distillation at 30 °C under reduced pressure. $K_2S_2O_8$ (Fluka) was used as the initiator without further purification. HSA (Sigma, fraction V) was used as received. VB-polyGL macromonomers ($\bar{M}_n = 950$ and $\bar{M}_n = 2700$) were synthesized as described earlier [16]. Briefly, VB-ethoxy ethyl polyGL ether (VB-polyGL-Ether) was synthesized via termination with *p*-chloromethylstyrene of a polymer obtained in an anionic polymerization of ethoxy ethyl glycidyl ether initiated with potassium *t*-butoxide. Subsequent hydrolysis of the product leads to VB-polyGL. The reactions involved in the synthesis of the macromonomer are illustrated in Scheme 1.

The intermediate product (VB-polyGL-Ether) was characterized by gel permeation chromatography. A set of three columns (two PLgel mixedC and one PLgel 100C) was used, along with the eluant tetrahydrofuran (low rate 1 ml/min), a refractive index detector and calibration for polystyrene standards. Two VB-polyGL-Ether macromonomers were synthesized, one with $\bar{M}_n = 2000$ and the second with $\bar{M}_n = 6500$ (apparent molecular weights related to polystyrene standards). The VB-polyGL macromonomers, obtained after hydrolysis of VB-polyGL-Ether, were characterized by ^{1}H NMR (Bruker AC 200 spectrometer at 200 MHz) and matrix-assisted laser desorption ionization time of flight (MALDI-TOF) studies (Voyager Elite mass spectrometer, PerSeptive Biosystems). The following signals were found in the ^{1}H NMR spectra of VB-polyGL (Scheme 1): a 6.71 (m), b 5.23 (d), c 5.78 (d), d 7.38 (m), e 4.60 (d), f,g,h 3.45–3.64 (m), and i 1.12 (s). For registration of the



Scheme 1

MALDI-TOF spectra the samples were dissolved in a chloroform solution of 2,5-dihydroxybenzoic acid containing NaI and then dried in air. The spectra shown in Figs. 1 and 2 were recorded in a linear mode.

The spectrum shown in Fig. 1 comprises three series of signals. The main one at 213 + n·74 corresponds to VB-polyGL with attached Na^+. The two other, much weaker, signals were assigned as follows. The first one is VB-polyGL with a deblocked terminal *t*-butyl group (173 + n·74) complexed with K^+ cations and the second one is VB-polyGL complexed with K^+ (229 + n·74) overlapping with signals of VB-polyGL with a deblocked terminal *t*-butyl group (157 + n·74) and complexed with Na^+. The values of $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ calculated from this spectrum were 950 and 1.10, respectively. Subsequently this macromonomer we denoted as VB-polyGL950. The spectrum shown in Fig. 2 contained a similar signal series, however, with a much higher proportion of VB-polyGL with deblocked terminal *t*–butyl groups and with a higher proportion of macromolecules with bound K^+ cations. For the second macromonomer, calculations based on the MALDI-TOF spectrum yielded $\bar{M}_n = 2700$ and $\bar{M}_w/\bar{M}_n = 1.03$. This macromonomer was denoted as VB-polyGL2700. Both macromonomers were copolymerized with styrene.

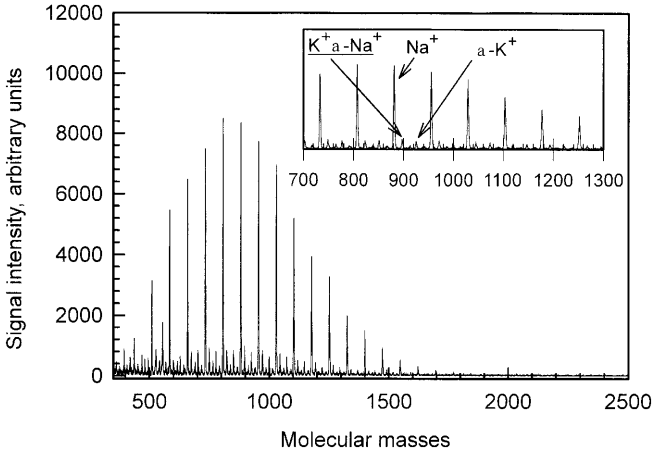


Fig. 1 Matrix-assisted laser desorption ionization time of flight (*MALDI-TOF*) spectrum of VB-polyGL950. Signals in the series a $-Na^+$ and a $-K^+$ denote macromonomers with deblocked *t*-butyl end groups

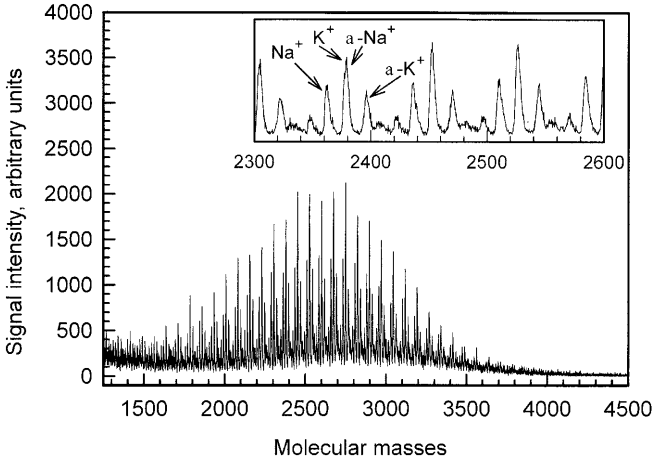


Fig. 2 MALDI-TOF spectrum of VB-polyGL2700. Signals in series the a $-Na^+$ and a $-K^+$ denote macromonomers with deblocked *t*-butyl end groups

Poly(styrene/VB-polyGL) microspheres were synthesized in the following way [17]. Known amounts of VB-polyGL (with $M_n = 950$ or 2,700) were dissolved in 70 ml water (distilled three times, with the pH was adjusted to 6.5 by addition of K_2CO_3). Styrene (9.09 g) and $K_2S_2O_8$ (0.2 g) were added to this solution. Oxygen dissolved in the water was removed with argon. Polymerizations were carried out under argon, at 65 °C, with stirring at 60 rpm, for 28 h. The unreacted styrene was removed from the suspension of the microspheres by steam stripping. Thereafter, the particles were isolated by centrifugation, washed with 10^{-3} mol/l HCl, then with new portions of water. The centrifugation/washing steps were repeated four times. The concentrations of the microspheres in the final suspensions were determined by drying a sample with a known volume at 50 °C to a constant weight.

The diameters of the poly(styrene/VB-polyGL) microspheres were determined from micro-photographics obtained using a Jeol 35C scanning electron microscope (SEM). Examples of SEM microphotographs of the microspheres are given in Fig. 3a and b.

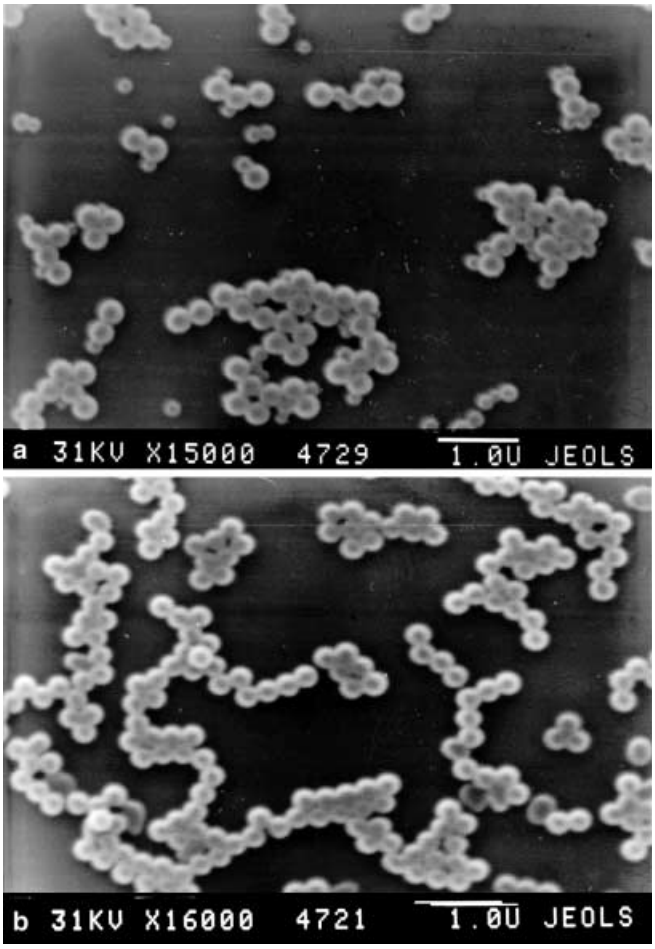


Fig. 3 Scanning electron microscope microphotograph of poly(styrene/VB-polyGL) microspheres: **a** microspheres synthesized with VB-poly950, molar ratio styrene:VB-polyGL950 in the polymerizing mixture of $1{:}1.09 \times 10^{-2}$; **b** microspheres synthesized with VB-poly2700, molar ratio styrene:VB-polyGL2700 in the polymerizing mixture of $1{:}3.47 \times 10^{-3}$

The concentration of acidic groups on the surface of the microspheres was determined by conductometric titration. Prior to the surface charge density measurements, samples of microsphere suspensions were transferred through a Dowex 50WX4 ion-exchange resin. The conductometric titrations of samples containing 0.15 g microspheres in 3 ml water were performed with 10^{-3} mol/l KOH.

UV spectra for determination of the HSA concentrations were recorded using a Hewlett-Packard 8452A diode array spectrometer. A Vacuum Generators MK1 spectrometer with an AEI (Marseille, France) digital aquisition system was used for recording the X-ray photoelectron spectra. The base pressure in the X-ray photoelectron spectrometer chamber was 10^{-8} mbar. A Mg anode (1253.6 eV) was used with an applied power of 100–200 W. The spectrometer was operated in constant energy mode (pass energy 20 eV). At these conditions poly(styrene/VB-polyGL) microspheres did not degrade during the recording of the spectra. Standard data reduction procedures were used: peak areas were obtained by numerical integration, the spectra were simulated by least-squares fitting of Gaussian components. The charging effect was corrected by assigning a 285.0 eV binding energy to the main C1*s* signal. Atomic abundances were obtained from peak areas with experimental atomic sensitivities deduced from the analysis of several polymers and organic compounds of known stoichiometry. The relative atomic sensitivities used were the following: $C1s = 1$, $O1s = 2.6$.

Adsorption of HSA onto the poly(styrene/VB-polyGL) microspheres was carried out by incubating the microspheres suspended in 5 ml aqueous HSA solutions for 20 h at 25 °C. In all experiments, the concentration of the microspheres was 5 g/l and the concentrations of the HSA solutions were varied from 5.00×10^{-2} to 4.05×10^{-1} g/l. The surface concentrations of HSA adsorbed onto the microspheres were determined by a Lowry method according to the described earlier procedure [18, 19].

Results and discussion

Diameters and diameter distributions of microspheres

The values of the number-average diameters ($\bar{D}_n$) and of the diameter polydispersity parameters ($\bar{D}_w/\bar{D}_n$, where $\bar{D}_w$ denotes the weight-average diameter) of the poly(styrene/VB-polyGL) microspheres are given in Table 1.

It was found that the diameters of the microspheres decreased for an increasing fraction of VB-polyGL in the polymerizing mixture, regardless of the molecular weight of the macromonomer. The diameter polydispersity parameter for microspheres synthesized with VB-polyGL2700 macromonomer was in the range 1.007–1.022, indicating that these particles were monodisperse. The diameter distributions of the microspheres synthesized with VB-polyGL950 were bimodal (Fig. 4) and the values of the diameter polydispersity parameter for these particles were high ($1.011 < \bar{D}_w/\bar{D}_n < 1.108$).

It is worth noting, however, that for each subpopulation of microspheres the diameter distribution was relatively narrow ($\bar{D}_w/\bar{D}_n \leq 1.08$) (cf. Fig. 4 and data in Table 2 illustrating the diameter distribution of the fraction of smaller particles).

The increase in the initial concentration of VB-polyGL950 in the monomer feed resulted in a higher fraction of particles with smaller diameters. A bimodal distribution of microspheres was observed earlier by Ottewill and Satgurunathan [20] for the copolymerization of methoxy poly(ethylene glycol methacrylate) (with $\bar{M}_n = 2100$) and styrene carried out in water and initiated with potassium persulfate. However, the authors of this study did not propose any explanation of this phenomenon.

The presence of two subpopulations of poly(styrene/VB-polyGL) microspheres, each with a relatively narrow diameter distribution, excludes the possibility of continuous particle nucleation because for continuous particle nucleation one should expect a very broad diameter distribution with tailing at the small diameter side. It is worth noting that the number-average diameter of the small-diameter fraction was almost

Table 1 Number-average diameters ($\bar{D}_n$), diameter polydispersity ($\bar{D}_w/\bar{D}_n$), and surface concentration of the acidic groups for poly(styrene/VB-polyGL) microspheres

Symbol of particles	Styrene:VB-polyGL in monomer feed (mol:mol)	$\bar{D}_n^a$	$\bar{D}_w/\bar{D}_n^b$	Surface concentration of acidic groups $[SO_4^-]$ (mol/m^2)
Microspheres with surfmer VB-polyGL950				
950-1	$1:1.09 \times 10^{-3}$	0.89	1.032	3.04×10^{-7}
950-2	$1:2.19 \times 10^{-3}$	0.77	1.011	4.82×10^{-7}
950-3	$1:5.48 \times 10^{-3}$	0.28	1.079	8.11×10^{-7}
950-4	$1:1.09 \times 10^{-2}$	0.26	1.050	8.18×10^{-7}
950-5	$1:1.64 \times 10^{-2}$	0.22	1.108	9.98×10^{-7}
Microspheres with surfmer VB-polyGL2700				
2700-1	$1:3.46 \times 10^{-4}$	0.65	1.008	1.65×10^{-6}
2700-2	$1:6.94 \times 10^{-4}$	0.46	1.014	1.05×10^{-6}
2700-3	$1:1.73 \times 10^{-3}$	0.35	1.007	8.77×10^{-7}
2700-4	$1:2.60 \times 10^{-3}$	0.26	1.007	4.85×10^{-7}
2700-5	$1:3.47 \times 10^{-3}$	0.22	1.022	4.13×10^{-7}

[a] $\bar{D}_n = \sum D_i n_i / \sum n_i$

[b] $\bar{D}_w = \sum D_i^4 n_i / \sum D_i^3 n_i$

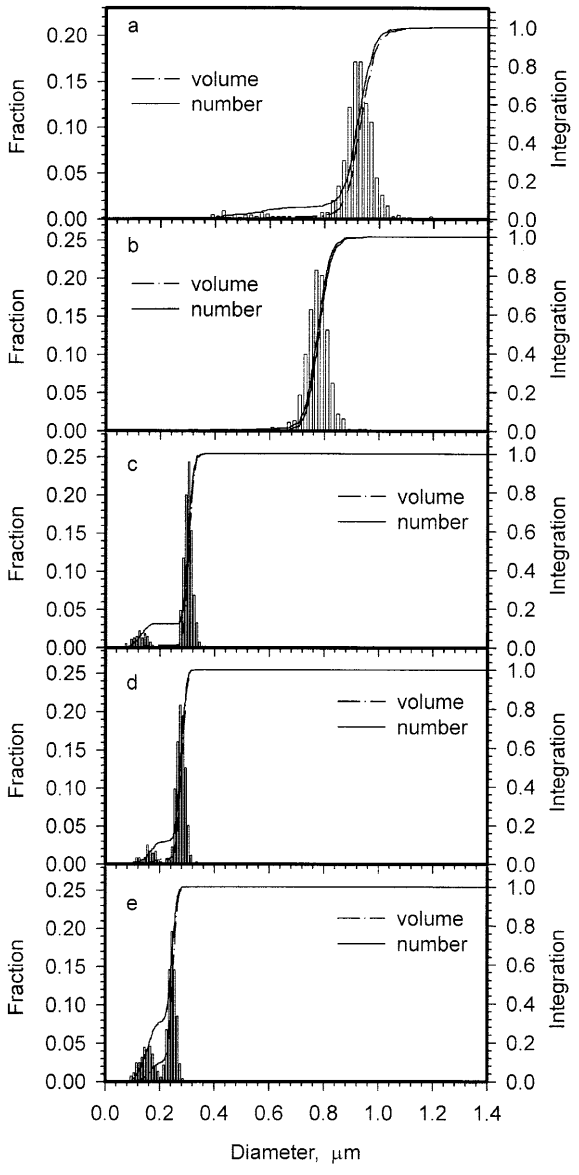


Fig. 4 Diameter distributions of poly(styrene/VB-polyGL950) microspheres with various contents of VB-polyGL950 in the monomer feed. Styrene:VB-polyGL950 molar ratios: **a** $1{:}1.09 \times 10^{-3}$; **b** $1{:}2.19 \times 10^{-3}$; **c** $1{:}5.48 \times 10^{-3}$; **d** $1{:}1.09 \times 10^{-2}$; **e** $1{:}1.64 \times 10^{-2}$

Table 2 $\bar{D}_n$ and $\bar{D}_w/\bar{D}_n$ of a second crop of poly(styrene/VB-polyGL950) particles

Symbol of particles	$\bar{D}_n$	$\bar{D}_w/\bar{D}_n$	Fraction (number) of second crop of particles in the whole suspension of the latex
950-3	0.13	1.09	0.12
950-4	0.16	1.05	0.11
950-5	0.15	1.08	0.30

independent of the initial VB-polyGL950 concentration, whereas the diameters of the large-diameter fraction significantly decreased with increasing concentration of VB-polyGL950. This observation suggested that the microspheres in these subpopulations were formed according to different mechanisms. The decrease in $\bar{D}_n$ with the increase in the hydrophilic component (VB-polyGL950) observed in our studies for subpopulation of particles with larger diameters was typical for emulsion polymerization [2, 4]. The independence of $\bar{D}_n$ on the concentration of VB-polyGL950 that was noticed for microspheres of subpopulations with smaller diameters might suggest that these particles were formed by a miniemulsion polymerization mechanism. However, light scattering studies indicated that in the concentration range from 5.26×10^{-4} to 1.15×10^{-2} mol/l the VB-polyGL950 macromonomer did not form micelles in water. With respect to this property VB-polyGL950 macromonomers were different from the poly(ethylene oxide) nonpolymerizable models with $\bar{M}_n$ in the range 2,000–3,000 for which the critical micelle concentration was below 7.1×10^{-5} mol/l [5]. It is possible, however, that a certain fraction of poly(styrene/VB-polyGL950) copolymers with suitable composition and molecular weight forms monomer-swollen macromolecular micelles suitable for contribution to particle formation by the microemulsion mechanism. We hope to make a final conclusion on the contribution of the microemulsion mechanism to the copolymerization of styrene and VB-polyGL only after more extensive experimental work exceeding the scope of studies described here.

It is worth noting that a subpopulation of microspheres with smaller particle diameters was absent for polymerizations with VB-polyGL2700 macromonomer. Apparently, macromolecules with long polyGL grafts did not form macromolecular micelles.

Surface charge density

Suspensions of poly(styrene/VB-polyGL) microspheres were stabilized against aggregation electrostatically, owing to the repulsion of anionic sulfate groups at their surfaces, and sterically, owing to the presence of hydrophilic polyGL segments in their surface layer. Sulfate end groups in the polymer chains were formed in the process of initiation with $SO_4^{\bullet-}$ radical anions derived from $K_2S_2O_8$ initiator. The values of the surface concentration of acidic groups determined for poly(styrene/VB-polyGL950) and poly(styrene/VB-polyGL2700) microspheres are given in Table 1. The data for these two sets of particles were apparently inconsistent; namely, for poly(styrene/VB-polyGL950) microspheres the surface concentration of anionic groups increased with the increasing macromonomer concen-

tration, whereas for poly(styrene/VB-polyGL2700) the opposite dependence was observed. Tentatively, we propose the following explanation for this phenomenon. The hydrophilic surface layer composed of polyGL chains screens the anionic end groups of the poly(styrene/VB-polyGL) main chains. On the other hand, the hydrophilic polyGL grafts drag polystyrene chains towards the interface, exposing their anionic end groups. Thus, these factors act in the opposite directions and the prevalence of the first or the second one for a given type of macromonomer may result in the decreasing or increasing surface concentration of anionic groups with the increasing concentration of macromonomer. Apparently, macromonomers with shorter chains yield poly(styrene/VB-polyGL950) macromolecules with segments which are more soluble and, thus, with sulfate end groups moved towards the liquid phase. On the other hand, in poly(styrene/VB-polyGL2700) with the same weight fraction of polyGL the copolymer macromolecules bear few, but long, hydrophilic chains. Such a structure could facilitate microphase separation and thus the formation of microspheres with a dense hydrophobic layer containing entrapped anionic end groups covered with hydrophilic polyGL.

Composition of the microspheres

The overall composition of the microspheres was determined on the basis of elemental analysis data for the dried samples. The dependence of the polyGL content in the microspheres on the fraction of VB-polyGL in the monomer feed is illustrated in Fig. 5.

For molar fractions of VB-polyGL2700 up to 4×10^{-3} the previously mentioned dependence for both kinds of macromonomers could be approximated with a straight line with slope 0.633.

For the macromonomer molar fraction in the monomer feed exceeding 5×10^{-3} the polyGL content in the poly(styrene/VB-polyGL950) microspheres approached a plateau (Fig. 5). It is worth noting that above this fraction of VB-polyGL950 the subpopulation of the microspheres with smaller diameters increased significantly. This observation conforms to the assumption that these microspheres were formed by the miniemulsion mechanism and, thus, contain polyGL predominantly in the surface layer, leaving the excess of macromonomer in solution.

It was important to find out whether polyGL in the microspheres was incorporated by copolymerization of VB-polyGL with styrene or simply by adsorption of this compound onto the polystyrene particles. With the purpose to discriminate between these two possibilities we attempted to dissolve the microspheres in methylene dichloride. The poly(styrene/VB-polyGL950) and poly(styrene/VB-polyGL2700) microspheres did not dissolve in methylene dichloride even after 2 days, whereas the polystyrene microspheres synthesized in the absence of VB-polyGL dissolved in a few minutes. Thus, it was clear that the microspheres were composed of poly(styrene-*co*-VB-polyGL).

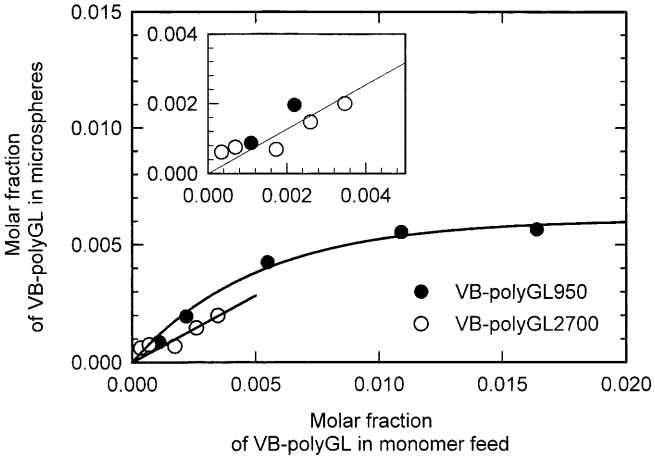


Fig. 5 Relation between molar fraction of VB-polyGL in the monomer feed and in the microspheres

Composition of the surface layer of poly(styrene/VB-polyGL) microspheres; studies by X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) is known to be a useful tool for the determination of the atomic composition of about 5-nm-thick surface layers of plates and powders [7, 21–25]. The X-ray photoelectron spectra of poly(styrene/VB-polyGL) particles contained a group of signals (maxima from 285 to 292 eV) due to an electron emission from the $1s^2$ orbitals of carbon atoms in different chemical environments and a signal at 532.8 eV due to the electron emission from the $1s^2$ orbital of oxygen atoms. A low-resolution spectrum of poly(styrene/VB-polyGL950) microspheres is shown in Fig. 6.

Computer deconvolution of the complex C1*s* signal from 282 to 288 eV indicated the presence of three components. The main signal, at 284.7 eV, (Fig. 7) was assigned to the emission from the aliphatic carbon atoms. The weak one, at 283.3 eV, resulted from the static charge shift due to the charging of the sample surface during the recording of the X-ray photoelectron spectra. The component at 287 eV was assigned to the numerous CH_2OH groups in the polyGL grafts. The assignment of these signals was made according to the literature data [7, 21].

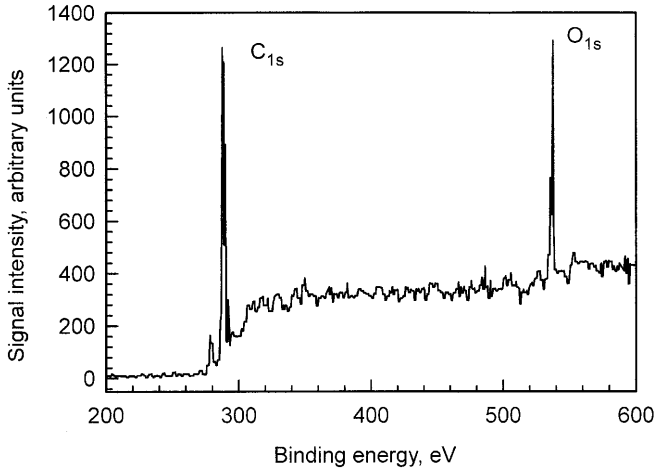


Fig. 6 Low resolution X-ray photoelectron spectroscopy (*XPS*) spectrum of poly(styrene/VB-polyGL950) microspheres. Styrene:VB-polyGL950 ratio in monomer feed of $1{:}5.48 \times 10^{-3}$

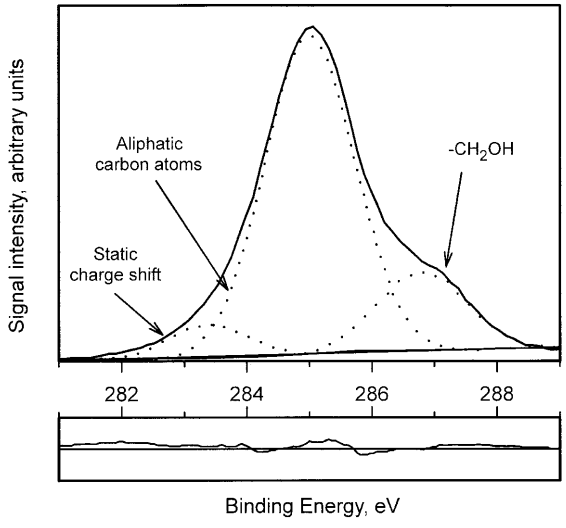


Fig. 7 C1*s* signal in the high-resolution XPS spectrum of poly(styrene/VB-polyGL950) microspheres. Styrene:VB-polyGL950 ratio in monomer feed of $1{:}5.48 \times 10^{-3}$

It is worth noting that the intensity of the signal component at 287 eV increased for the microspheres obtained with a higher proportion of VB-polyGL in the polymerization mixture, indicating an increase in the fraction of the polyGL units in the surface layer of these particles. Additionally, all spectra showed a small shake-up satellite at 291.6 eV arising from the aromatic ring of the styrene and benzyl groups of the macromonomer units. The intensity of this signal decreased with the increase in the fraction of VB-polyGL in the microspheres, indicating screening of polystyrene segments with polyGL chains. This decrease in intensity of the shake-up signal was accompanied with an increase in the overall carbon atom signal intensity.

In principle, the fractions of the polyGL units in the surface layer of the microspheres could be determined from the C1*s* signal intensities by measuring the ratio of the CH_2OH component intensity to the total intensity of the signals of the carbon atoms. Unfortunately, we found that the errors in the intensities of the components calculated by deconvolution of the original signals were too large to use this approach. Thus, the determination of the fractions of the polyGL units in the surface layers of the microspheres was based on integrals of the signals due to carbon and oxygen atoms. Before making any calculations based on the intensities of carbon and oxygen atoms we estimated whether it was necessary to take into account the contribution of oxygen atoms from the SO_4^- end groups (from $K_2S_2O_8$ initiator) to the total XPS oxygen signal. One has to remember that a significant concentration of SO_4^- end groups in the surface layer should lead to the presence of S2*p* signals in the XPS spectra. Thus, the absence of S2*p* signals indicated that the contribution of SO_4^- end groups to the composition of the surface layer of poly(styrene/VB-polyGL) microspheres was negligible.

The chemical composition of the surface layer of poly(styrene/(VB-polyGL)) particles is illustrated schematically in Scheme 2. In this scheme x denotes the fraction of the polystyrene units, $y = 1-x$ the fraction of the VB-polyGL units, and z the number of polyGL units in the macromonomer chain. For VB-polyGL950 and VB-polyGL2700 z was 10 and 34, respectively.

In the polystyrene unit there are eight carbon atoms and in the VB-polyGL unit the number of carbon atoms was $13 + 3z$. Thus, the integrated (from 283 to 293 eV) intensity of all carbon atoms, $\sum S(\mathrm{C})$, can be expressed by

$$\sum S(\mathrm{C}) = \alpha_{\mathrm{C}}[8x + (13 + 3z)(1 - x)] \,, \qquad (1)$$

in which α_{C} denotes the sensitivity factor for carbon atoms.

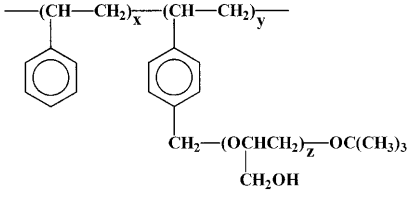
For the integrated intensity of the oxygen atoms one can write

$$\sum S(\mathrm{O}) = \alpha_{\mathrm{O}}(1 - x)(1 + 2z) \,, \qquad (2)$$

where α_{O} denotes the sensitivity factor for oxygen atoms.

From Eqs. (1) and (2) one can calculate the fraction of macromonomer units:

$$y = 1 - x = \frac{8}{\frac{\alpha_{\mathrm{O}} \sum S(\mathrm{C})}{\alpha_{\mathrm{C}} \sum S(\mathrm{O})}(1 + 2z) - 3z - 5} \,. \qquad (3)$$



Scheme 2

Table 3 Molar fraction of polyGL units in the surface layer of microspheres (determined by X-ray photoelectron spectroscopy)

Styrene:VB-polyGL950 in the microspheres (mol:mol)	Fraction of polyGL units in the surface layer of the microspheres	Styrene:VB-polyGL2700 in the microspheres (mol:mol)	Fraction of polyGL units in the surface layer of the microspheres
8.73×10^{-4}	0.245	6.16×10^{-4}	0.216
1.96×10^{-3}	0.141	6.94×10^{-4}	0.334
4.26×10^{-3}	0.383	7.59×10^{-4}	0.255
5.54×10^{-3}	0.432	1.48×10^{-3}	0.423
5.65×10^{-3}	0.444	2.00×10^{-3}	0.426

For XPS signals of carbon and oxygen atoms the relative sensitivity factor $\alpha_O/\alpha_C = 2.6$.

In an average macromolecule of poly(styrene/VB-polyGL) the number of polystyrene units is proportional to $x = 1 - y$ and the number of polyGL units is proportional to zy. Thus, in the surface layer of the microspheres the fraction of polyGL units, f(polyGL), can be calculated using the following formula:

$$f(\text{polyGL}) = \frac{yz}{y(z-1)+1} \quad . \tag{4}$$

The values of the fraction of the polyGL units in the surface layer of the microspheres are given in Table 3.

The relation between the fraction of polyGL units in the surface layer and the fraction of polyGL units in the whole poly(styrene/VB-polyGL) microsphere is illustrated in Fig. 8.

The general tendency for two sets of microspheres analyzed was very similar. Initially, the fraction of polyGL units in the surface layer of the microspheres rapidly increased with increasing fraction of polyGL in the whole particle and then reached plateau at about 44% of polyGL units. Moreover, for particles containing VB-polyGL950 a higher fraction of macromonomer was needed (5.54×10^{-3} mol/mol in relation to styrene) to saturate the surface in the hydrophilic macromonomer than in the case of microspheres with longer chains. However, for both macromonomers saturation was achieved when the molar fraction of polyGL units in whole microspheres approached 5%.

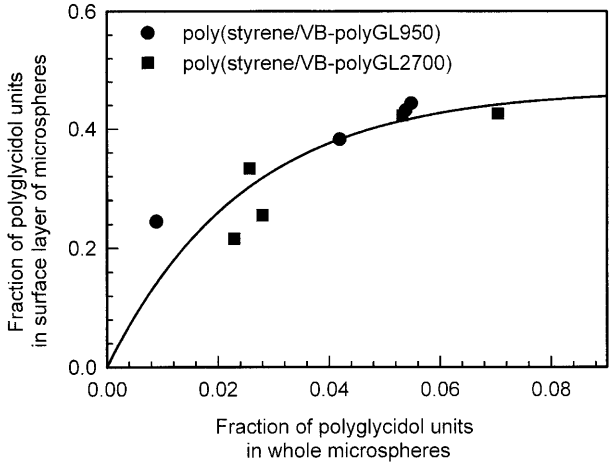


Fig. 8 Fraction of polyGL units in the surface layer of the microspheres as function of the fraction of polyGL units in the whole particles

Adsorption of HSA onto the poly(styrene/VB-polyGL) microspheres

Here we present preliminary information on the maximum uptake of HSA by poly(styrene/VB-polyGL) particles. The adsorption experiments were carried out by gently mixing of 5 ml phosphate-buffered saline containing 1.5×10^{-2} g of the corresponding microspheres and HSA (protein in the range 2.50×10^{-4}–2.25×10^{-3} g). The data in Table 4 show that with increasing fraction of polyGL in the surface layer of the microspheres the maximum surface concentration of adsorbed protein decreases to a value about 10 times lower than for the bare polystyrene particles.

This decrease was more significant in the case of particles synthesized with VB-polyGL2700 particles. It

Table 4 Maximum surface concentrations of human serum albumin (*HSA*), Γ_{HSA}(max), adsorbed onto the polystyrene and poly(styrene/VB-polyGL) microspheres

Symbol of particles	Molar fraction of polyGL units in the surface layer of the microspheres	Γ_{HSA}(max) (g/m^2)
Polystyrene microspheres		
Polystyrene	0	1.44×10^{-3}
Poly(styrene/VB-polyGL950) microspheres		
950-1	0.245	5.12×10^{-4}
950-2	0.141	8.71×10^{-4}
950-3	0.383	1.88×10^{-4}
950-4	0.432	1.73×10^{-4}
950-5	0.444	1.75×10^{-4}
Poly(styrene/VB-polyGL2700) microspheres		
2700-1	0.216	8.05×10^{-4}
2700-2	0.255	8.30×10^{-4}
2700-3	0.334	5.77×10^{-4}
2700-4	0.423	2.21×10^{-4}
2700-5	0.426	1.56×10^{-4}

was also found that for both types of microspheres with surfaces saturated with polyGL units (synthesized with VB-polyGL950 and VB-polyGL2700) the maximum surface fractions of HSA that could be adsorbed were similar and were 1.75×10^{-4} and 1.56×10^{-4} g/m^2, respectively.

The decreased protein adsorption for particles with higher content of VB-polyGL indicated that the hydrophilic, hairlike polyGL chains incorporated into the surface layer of the particles investigated protected them efficiently from the protein adsorption.

Conclusions

Emulsifier-free copolymerization of styrene and VB-polyGL macromonomers yielded microspheres with a surface layer enriched with polyGL units. The fraction of the macromonomer units in the surface layer increased with increasing concentration of VB-polyGL in whole particles. Microspheres with smaller particle diameters were obtained by increasing the VB-polyGL content in the polymerizing mixture. The diameter distributions of particles synthesized using VB-polyGL2700 as comonomer were monomodal. In the case of VB-poly950, microspheres with a bimodal diameter distribution were obtained. This phenomenon suggests the presence of two different mechanisms of particle formation during polymerization. Adsorption of HSA onto poly(styrene/VB-polyGL) microspheres with a fraction of polyGL units higher than 0.4 is about 10 times lower than for polystyrene microspheres synthesized without VB-polyGL.

Acknowledgements This work was supported by the Polish Committee for Scientific Research, grant no. 3T09A 07016.

References

1. Basinska T, Caldwell KD (1999) In: Provder T (ed) Chromatography of polymers. Hyphenated and multidimensional techniques, ACS Symposium Series 731. American Chemical Society, Washington, DC, pp 162–177
2. Charleux B, Pichot C (1993) Polymer 34:195
3. Meunier F, Elaïssari A, Pichot C (1995) Polym Adv Technol 6:489
4. Schipper ETWM, Sindt O, Hamaide T, Lacroix Desmazes P, Müller B, Guyot A, van den Enden MJWA, Vidal F, van Es JJGS, German AL, Montaya Goñi A, Sherrington DC, Schoonbrood HAS, Asua JM, Sjöberg M (1998) Colloid Polym Sci 276:402
5. Ito K, Hashimura K, Itsuno S, Yamada E (1991) Macromolecules 24:3977
6. Kawagushi S, Winnik M (1995) Macromolecules 28:1159
7. Brindley A, Davies MC, Lynn RAP, Davis SS, Hearn J, Watts JF (1992) Polymer 33:1112
8. Kobayashi S, Uyama H, Choi JH, Matsumoto Y (1993) Polym Int 30:265
9. Charreyre M-T, Boullanger P, Pichot C, Delair T, Mandrand B, Llauro M-F (1993) Makromol Chem 194:117
10. Cho C-S, Nah J-W, Jeong Y-I, Cheon J-B, Asayama S, Ise H, Akaike T (1999) Polymer 40:6769
11. Li J, Carlsson J, Huang *S*-C, Caldwell KD (1996) In: Glass JE (ed) Hydrophilic polymers; Performance with environmental acceptability. ACS Advances in Chemistry Series 248. American Chemical Society, Washington, DC, pp 61–78
12. Sofia SJ, Premnath V, Merrill W (1998) Macromolecules 31:5059
13. Basinska T, Slomkowski S (1993) In: Guilbault G, Mascini M (eds) Uses of immobilized biological compounds. Kluwer, Dordrecht, pp 453–462
14. Basinska T, Kowalczyk D, Miksa B, Slomkowski S (1995) Polym Adv Technol 6:526
15. Miksa B, Slomkowski S (1995) J Biomater Sci Polym Edn 7:77
16. Dworak A, Panchev I, Trzebicka B, Walach W (1998) Polym Bull 40:461
17. Center of Molecular and Macromolecular Studies of the Polish Academy of Sciences and Institute of Carbochemistry of the Polish Academy of Sciences (1999) Polish patent application P-333294, May 21
18. Lowry DH, Rosenbrough NJ, Farr AL, Randall RJ (1951) J Biochem 193:265
19. Basinska T, Slomkowski S (1991) J Biomater Sci Polym Ed 3:115
20. Ottewill RH, Satgurunathan R (1987) Colloid Polym Sci 265:845
21. Okubo M, Yamamoto Y, Kamei S (1989) Colloid Polym Sci 267:861
22. Briggs D (1990) In Briggs D, Seah MP (eds) Practical surface analysis, vol 1. Angle and X-ray photoelectron spectroscopy, 2nd edn. Wiley, New York, pp 437–483
23. Davies MC, Lynn RAP, Hearn J, Paul AJ, Vickerman JC, Watts JF (1995) Langmuir 11:4313
24. Maeda S, Corradi R, Armes SP (1995) Macromolecules 28:2905
25. Scholes PD, Coombes AGA, Illum L, Davis SS, Watts JF, Ustariz C, Vert M, Davies MC (1999) J Controlled Release 59:261