Synthesis and Properties of Uniform Beads Based on Macroporous Copolymer Glycidyl Methacrylate–Ethylene Dimethacrylate: A Way to Improve Separation Media for HPLC

VLADIMÍR ŠMIGOL and FRANTIŠEK ŠVEC*

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia

SYNOPSIS

Monodisperse beads based on hydrolyzed macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) for use as size-exclusion HPLC packings were synthesized by the method of "activated" swelling of polystyrene seeds followed by a suspension polymerization of both methacrylates. Effects of the type and fraction of the swelling agent, inert porogenic solvent, and cross-linking monomer on the uniformity of the particles, extent of the specific surface area, pore volume, pore size, and pore-size distribution and chromatographic properties (size-exclusion limit and column efficiency) have been investigated. Trends leading to the synthesis of macroporous particles with predesigned properties for use in size-exclusion high-performance liquid chromatography in both aqueous and organic mobile phase were studied. © 1992 John Wiley & Sons, Inc.

INTRODUCTION

Organic polymeric separation media have become increasingly popular separation tools in HPLC. Their particular advantage is their stability within the whole pH range and a wide extent of surface polarity, porosity, and reactivity.[1] Synthetic polymers were introduced into chromatography in 1964[2]; since that time, most polymer commercial packings have been based on copolymers of styrene with divinylbenzene, which are available in the market in various pore and particle sizes, while polymers composed of other monomers are available only to a small extent.[3–5]

The resolution of a chromatographic column depends on the number of theoretical plates (column efficiency), on the distribution coefficients of solutes, and on V_i/V_0, i.e., on the ratio of pore volume of the separation medium V_i to the interstitial volume between the particles in the column V_0.[6] The lattermost quantity should be as high as possible; usually, for silica columns, it varies between 0.8 and 1.2. The ratio can be raised if particles with higher porosity are used (increase in V_i), or by a better arrangement of particles in the column, i.e., by improving the quality of packing (decrease in V_0). The latter depends on the uniformity of size of the particles used. The best-organized monodisperse beads occupy 74.05% of the available volume irrespective of their size.[7] The remainder (about 26%) represents the lowest possible value of V_0, which, however, can be reached only in theory.

Polymer particles obtained by suspension polymerization followed by labor-intensive size classification are never uniform. This was the reason why Ugelstad et al.[8] developed an activated two-step swelling and polymerization method that produces particles of practically uniform size. It has been mentioned in the literature that particles may be prepared from a great variety of monomers, such as styrene, vinyl acetate, methyl methacrylate, 2-hydroxyethyl methacrylate, divinylbenzene, and ethylene dimethacrylate,[9] but only the conditions of synthesis and properties of the styrene–divinylbenzene copolymer have been reported in some detail. The procedure just mentioned can also be used in

* To whom correspondence should be addressed.

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the synthesis of porous particles. Their application in size-exclusion HPLC (SEC) has been described recently.[10,11] A column packed with 5 μm styrene–divinylbenzene particles with a specific porosity of 1.04 mL/g possessed an extraordinarily high efficiency, and its V_0 was 34%.

Monodisperse polystyrene particles undoubtedly represent considerable progress in the field of separation media, but are suited only for the use in SEC in organic solvents or for the reversed-phase mode HPLC.[12] After having been modified with basic or acid groups, these materials are also employed in the ion-exchange chromatography of proteins.[13] On the other hand, however, due to their high hydrophobicity, the polystyrene polymers cannot be used in separations by the SEC mechanism in aqueous media. At the same time, this field is very attractive for the separation of natural polymers, such as proteins or polysaccharides, or of synthetic water-soluble polymers, according to the size of their molecules. This is why hydrophilic packings suitable for such separation are continuously being improved and are gradually finding their way to the market.[4]

Recently, we have contributed to the existing array of hydrophilic separation media by developing packings based on copolymers of glycidyl methacrylate with ethylene dimethacrylate[14,15] and have demonstrated their advantages in the application in an aqueous medium. These beads, however, were prepared by the classical suspension polymerization, so that even the narrow size fraction was far from monodisperse. Since all the other chromatographic properties of the methacrylate-based packings were very promising, we decided to work out a method leading to practically monodisperse beads also for the pair of monomers mentioned above. This study deals with the description and control of the chromatographic properties of copolymers of glycidyl methacrylate and ethylene dimethacrylate using cyclohexanol as the major component of the porogenic system.

EXPERIMENTAL

Synthesis

Polymerization

Monodisperse polystyrene seed particles were prepared by the emulsifier-free emulsion polymerization described in detail elsewhere.[16] To a dispersion of seed particles, an emulsion of a swelling agent is added, containing a free-radical initiator in an aqueous solution of sodium dodecyl sulfate (SDS) obtained by sonication of a mixture of the aqueous and organic phase. The mixed dispersion is stirred in a glass reactor until the drops of the swelling agent have disappeared (detection with an optical microscope), usually not longer than 16 h. The process can be accelerated by the addition of acetone, which after completion of the activation is removed under mild vacuum in a rotational evaporator. On completion of the primary swelling ("activation" of the seed particles[9]), a mixture of glycidyl methacrylate, ethylene dimethacrylate, and the porogenic solvent emulsified by sonication in a 0.25 wt % aqueous solution of SDS is added to the dispersion. The amount of the monomeric phase is calculated with respect to the required size of the final beads. At the same time, it is necessary, of course, that the "activated" beads should reliably absorb all the amount added. The mixture, while stirred, is again left at room temperature until drops of the mixture of monomers have disappeared, usually for 24 h. The final dispersion is supplemented by a 4 wt % aqueous solution of poly(vinyl alcohol) (Polyviol W 25/140, Wacker Chemie, Germany) so as to reach the required concentration needed for the stabilization of the dispersion during the polymerization. The reactor content is bubbled through with nitrogen for 20 min to remove the dissolved oxygen and the reactor is closed and heated with stirring to 70°C for 15 h. The resulting polymer is separated by repeated decantation in water and methanol, the original polystyrene seeds are extracted with toluene, and the particles are again washed with ethanol and dried.

Modification

Prior to use, the epoxy groups in the beads were hydrolyzed in 0.1 mol/L perchloric acid at room temperature for 120 h with slow stirring and washed with water until the acid reaction had disappeared.

Characterization Methods

The particle-size distribution was determined with a Coulter Counter TA II (Coulter Electronics, GB) and the specific surface area was calculated from data on the thermal desorption of nitrogen measured with a Quantasorb (Quantachrom, USA) apparatus.

Determination of Epoxy Groups

The epoxy copolymer was dispersed in solution of tetraethylammonium bromide in acetic acid and titrated with perchlorid acid in acetic acid to the blue-green end point of crystal violet indicator. The total

amount of epoxy groups was determined by IR spectroscopy.

Chromatography

Apparatus

Chromatography was performed using an LC system consisting of HPLC pump (Knauer, Germany), injection valve Rheodyne, and detector (differential refractometer RIDK, Laboratory Instruments, Prague, for water solutions of dextrane standards, or UV, Knauer, for THF solution of polystyrene standards). Particles were packed from the water suspension into a stainless-steel column 80 × 8 mm i.d. (TESSEK, Prague).

Pore-size Distribution

The pore-size distribution in the swollen state of the gel was determined by an inverse size-exclusion technique similar to that used by Halasz and coworkers[17,18] for determination of the cumulative pore-size distribution (PSD) in porous HPLC packing. The cumulative PSDs are curves of the size-exclusion coefficient $K(D)$ vs. the logarithm of the pore diameter D, where the D value associated with the $K(D)$ equal to 0.5 is the median pore diameter D_{50} of the porous packing. Values of D are calculated from the molecular weight (M_w) of polystyrene standards according to an empirically derived eq. (1)[17]:

$$D = 0.062\ M_w^{0.59} \quad (1)$$

for determinations in THF, and from the molecular weights of dextrane standards according to the eq. (2)[19]:

$$D = 0.212\ M_w^{0.511} \quad (2)$$

for determinations in water.

RESULTS AND DISCUSSION

The suspension polymerization is the most frequent technique used for production of polymeric beads. Several approaches for obtaining a dispersion of the organic phase in the aqueous continuous phase have been reported in the literature: In the traditional suspension polymerization known since 1909,[20] the dispersion is accomplished by simple stirring; spraying the organic phase into the continuous phase composed of liquid nitrogen represents the one-by-one formation of droplets,[21] and activated swelling of polymeric seeds is based on their final number while only increasing their volume.[8] The polymerization itself proceeds in the dispersed organic phase. The individual polymerizing drops may be regarded as microreactors in which polymerization, usually referred to as bulk polymerization, takes place. The polymerization process itself should be independent of the way in which the dispersion has been prepared; the product, i.e., the polymeric beads, should possess properties that depend only on the composition of the organic phase that has been subjected to polymerization. The weak effect of the continuous aqueous phase on the properties of macroporous polymers (with the exception of the particle-size distribution) has been documented in the literature.[22] All that has been said above suggests that, when passing from one procedure of preparation of the polymerizing dispersion to another, it might be advantageous to use earlier findings and to design in advance properties of particles obtained from the dispersion by emploing another method. This would indeed be true if only all conditions were identical, which unfortunately cannot be perfectly guaranteed. This is why, in optimizing the synthesis and chromatographic properties of uniform particles, it was possible to employ the known procedures, but at the same time, extensive experimental work could not be avoided, because the method chosen by us gives drops with a composition which is somewhat different from that obtained by the classical suspension polymerization, even if the monomers are the same.

The synthesis of macroporous monodisperse particles based on physicochemical principles derived by Ugelstad et al.[8] consists in the swelling of "activated" seeds with a mixture of the initiator, monomers, and porogenic solvents. Since the transport kinetics from the monomeric organic phase into the phase of polymeric seeds depends on the integrated surface area of the organic phase, it is advantageous to mix the seed dispersion with the organic phase emulsified in droplets of a size smaller than that of the seeds (below 1 μm).[23] As has been reported elsewhere,[16] the swelling of, e.g., polystyrene particles with various solvents is considerably limited, and even in the best case, the absorbed quantity does not go beyond a 65-fold volume of the polymeric particles used. This means, of course, that the particle diameter increases only about four times. On the other hand, an increase of the diameter by an order of magnitude can be achieved more easily if polymeric seeds contain a certain amount of low molecular weight liquid (or oligomers) that dissolves in water much less readily than each particular

Table I Effect of "Activating" Solvents on the Properties of Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads

Exp. #	Activator	S_g (m^2/g)	V_p (mL/g)	$N \times 10^{-3}$	V_i/V_t	$M_0 \times 10^{-3}$
G-7	Chlorobenzene	72	1.01	29.0	0.47	94
G-8	Dibutylether	72	1.08	18.5	0.48	94
G-9	Toluene	72	1.02	18.5	0.45	188
G-11	Chlorododecane	82	1.19	33.0	0.46	400
G-12	Dibutylphthalate	69	1.17	21.2	0.48	350

Reaction conditions: organic phase—0.5 vol % polystyrene seed particles 1.4 μm, activator 4.5 vol %, porogen cyclohexanol 58.1 vol %, glycidyl methacrylate 22.2 vol %, ethylene dimethacrylate 14.7 vol % of final droplets, initiator dibenzoyl peroxide 2.43 wt % in monomers; water phase— solution of dodecyl sulfate and poly(vinyl alcohol) in water; polymerization temperature 70°C; polymerization time 15 h; bead size 8.6 μm (G-7, G-8, G-9) and 10.3 (G-11, G-12). Abbreviations: S_g, specific surface area; V_p, specific pore volume; N, efficiency of packed column (theoretical plates/m); V_i, inner volume of pores; V_t, total volume of the column; M_0, upper exclusion limit of beads in SEC.

component of the emulsified monomeric phase. This liquid, the "swelling activator," can be introduced into the seeds either directly during the emulsion polymerization[24] or by swelling of the seeds on completion of the polymerization.[8,9] In the case of polymerization leading to macroporous beads, it should be borne in mind that the swelling activator becomes part of the inert porogenic system and may affect the porous structure by its properties.

The first series of experiments was therefore devoted to a description of the effect of the "activating" solvent on the properties of the macroporous product. The choice of suitable solvents is predominantly limited by the requirement of a low solutibility in water, by the ability to swell polystyrene, by miscibility with components of the mixture of monomers, and by inertness. With respect to the product, economic aspects (accessibility, price) along with toxicity, volatility, and other properties are less decisive, but of similar importance. The results of polymerizations in which various "swelling activators" were used are summarized in Table I. It shows that the effect of the chemical nature of the solvent on properties important with respect to application in chromatography is not very significant. The specific surface area varies within the limits of experimental error (75 $m^2/g \pm 9\%$). The pore volume and size (upper exclusion limit) increases somewhat if 1-chlorododecane and dibutyl phthalate are used compared with the other solvents, which can be regarded as an advantage. Since polystyrene particles swell with dibutyl phthalate seven times more than with 1-chlorododecane, the former solvent provides a wider variation range and was therefore given preference in further work. All beads thus obtained are approximately 10 μm in size, i.e., very close to the calculated dimensions, with a variation of about ±2%. When evaluating the relatively weak effect of the solvents used, one should not ignore the fact that in the experiments summarized in Table I the solvent accounts only for 4.5% of the liquid phase contained in the droplets prior to the polymerization.

Table II Effect of the "Activating" Solvent Volume on the Properties of Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads

Exp. #	DBP (Vol %)	d_p (μm)	S_g (m^2/g)	V_p (mL/g)	$N \times 10^{-3}$	V_i/V_t	$M_0 \times 10^{-3}$
G-13	0	4.13	80	1.20	14.2	0.45	122
G-14	2.3	10.3	73	1.14	22.0	0.44	93
G-15	4.5	10.7	80	1.08	20.6	0.42	252
G-16	6.8	9.9	83	1.10	19.2	0.44	162
G-17	9.0	9.9	80	1.06	20.4	0.41	122
G-18	11.3	9.9	74	1.14	36.8	0.45	124

For reaction conditions see Table I; activating solvent dibutyl phthalate. Abbreviations: DBP, dibutyl phthalate; d_p bead diameter; other abbreviations same as in Table I.

Table III Effect of Porogen Amount on the Properties of Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads

Exp. #	DBP (Vol %)	CyOH (Vol %)	S_g (m^2/g)	V_p (mL/g)	$N \times 10^{-3}$	V_i/V_t	$M_0 \times 10^{-3}$
G-33	90.9	0	> 1	0.22	7.5	0.17	0.1
G-34	12.3	22.4	≈ 1	0.26	19.3	0.18	0.1
G-35	6.6	41.8	5	0.48	20.5	0.29	2
G-112	4.5	61.2	69	1.17	21.2	0.48	350
G-36	4.2	66.1	88	1.38	25.4	0.51	800
G-37	3.9	70.9	69	1.61	24.0	0.54	7900
G-38	3.4	80.6	78	1.78	8.2	0.53	2500

For reaction conditions, see Tables I and II; bead size 10.0 μm. Abbreviations: CyOH, cyclohexanol; for other abbreviations, see Tables I and II.

However, as documented in Table II, variation of the amount of dibutyl phthalate up to 11.3 vol % is also not reflected in the extent of the specific surface area, pore volume, or uniformity of the beads (size about 10 μm, variation ±3% or less). Chromatographic properties of the matrix alone, i.e., the upper exclusion limit, pass through a maximum at a concentration 4.5 vol %. If primary latex particles swell directly with the mixture of monomers without previous activation, the droplets do not disappear completely, because seeds are not capable of receiving such a large amount of the organic phase. The product of subsequent polymerization (G-13) has properties that do not differ from properties of the other compounds in the series, except for uniformity. The size of the particles lies in the range from 4 to 13 μm. The reason can be sought in the imperfect transfer of the whole monomeric phase into the domain of primary particles. The residual part of the monomeric phase remains in the system and is polymerized by the standard suspension polymerization, which gives a polydisperse product.

Another series of experiments demonstrated that, similarly to the usual suspension polymerization of the same monomers, the properties of the product are only negligibly affected by the free-radical initiator. Various types of initiators (dibenzoyl peroxide, dilauroyl peroxide, azobisisobutyronitrile) were tested at concentrations between 0.08 and 2.42 wt % with respect to monomers. To obtain high-quality monodisperse products, it is desirable that the solubility of the initiator in water should be minimal and that no polymerization in the aqueous phase should take place. However, too low solubility requires too long a time for transferring the whole amount of initiator into the domain of "activated" primary particles. Dibenzoyl peroxide seems to be a suitable compromise with respect to the solubility in water.

The porous structure of macroporous polymers is strongly dependent on the fraction of porogenic solvent and cross-linking agent in the organic polymerizing phase. The synthesis under study allows the amount of cyclohexanol in the liquid phase, and thus also in the swollen seeds, to be varied. As expected, both the specific surface area and the pore volume and size increase with the amount of the porogen (Table III). It is known[25] that the porosity of the macroporous polymer roughly corresponds to the fraction of the inert porogenic solvent; this is also confirmed by Figure 1. Positive deviations from this rule in the domain of the low fraction of porogen are due to the presence of polystyrene dissolved in dibutyl phthalate, which to some extent acts as a porogen. Porosity therefore does not drop to zero in

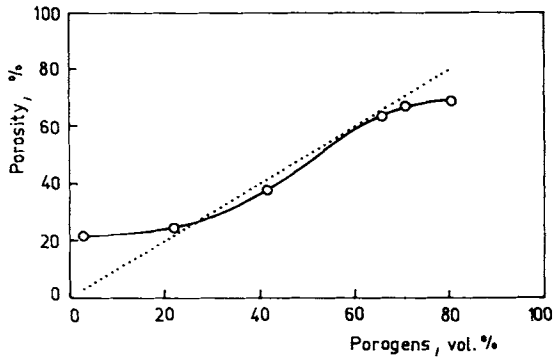

Figure 1 Effect of porogenic solvent amount in polymerization mixture on the porosity of macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate). Dotted line, theoretical dependence assuming that porosity equals volume of porogens present in the organic phase; full line, experimental data.

the absence of porogenic solvent, as might be expected. Obviously, in the domain of the high fraction of porogen in the mixture, it is not possible any more for the monomers at the given composition to form a macroporous structure with the necessary pore volume able to accommodate the whole amount of porogen. The pore volume, and thus also porosity, deviate to values below the theoretical ones. A similar trend can also be observed on curves of cumulative distribution functions obtained chromatographically (Fig. 2).

Significant changes in the upper exclusion limit given in Table III suggest an increasing pore size with increasing content of the porogenic solvent in the polymerization mixture. In addition to the chromatographic measurement, this phenomenon is also confirmed by SEM. A mere comparison between the surface morphologies of particles prepared under different conditions (Fig. 3) indicates considerable changes. Moreover, one cannot ignore the fact that the surface of polymeric macroporous particles obtained by swelling of the seeds followed by polymerization differs considerably from particles obtained by emploing the traditional suspension polymerization. Although the bead surface described in this study is distinctly porous and its morphology is defined by the polymerization mixture, the case of standard beads is quite different. The surface of the latter is coated with a layer of perfectly arranged globules that thus form a genuine envelope,[26] with pores considerably smaller than pores of beads in Figure 3. This finding is in good agreement with the theory of formation of a less porous surface shell of macroporous particles prepared by the usual suspension polymerization. The interfacial tension, which is relatively high because the aqueous phase contains only steric suspension stabilizers, compresses the bead arising by polymerization and the pressure thus evolved compresses in particular the surface layers of globules, which become more perfectly organized and form a shell.[26] In the procedure consisting of two-step swelling, the aqueous phase contains a real surfactant and the interfacial tension is much weaker. The compressive force is then missing in the polymerization, and no surface shell is formed.

The accessibility of epoxy groups to chemical reactions increases proportionally to increasing porosity and the specific surface area. Although IR spectroscopy is able to detect all epoxy groups present in the polymer, the chemical reaction affects accessible groups only. Since only the fraction of inert porogen was varied in the polymerizations, while the ratio between glycidyl methacrylate and ethylene dimethacrylate in the mixture of monomers remained unchanged, the fraction of the polymerized glycidyl methacrylate and, consequently, the concentration of epoxide groups should also remain unchanged. This finding is confirmed by the results of IR analysis, which are independent of the porogen volume (Fig. 4). Even so, however, the epoxide content determined by IR is lower by 7% than would correspond to the amount of glycidyl methacrylate used in the polymerization. This means that during the polymerization and the subsequent workup of the polymer a part of the epoxides is lost by, e.g.,

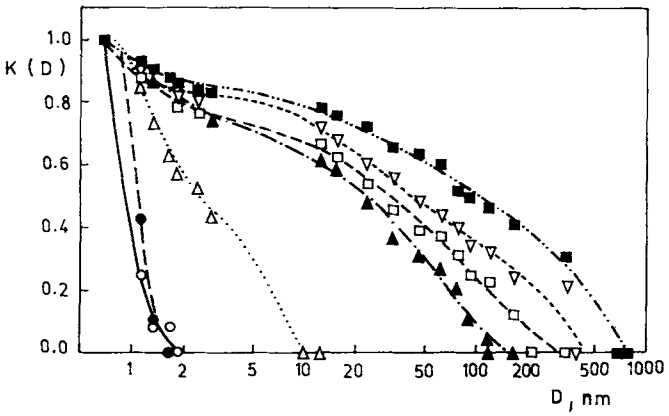

Figure 2 Cumulative distribution curves obtained from SEC of single sugars and dextrane standards in water on macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) synthesized in the presence of different fractions of cyclohexanol as porogenic solvent. Packing #: (○) G-33; (●) G-34; (△) G-35; (▲) G-12; (□) G-36; (■) G-37; (▽) G-38. For details, see Table III.

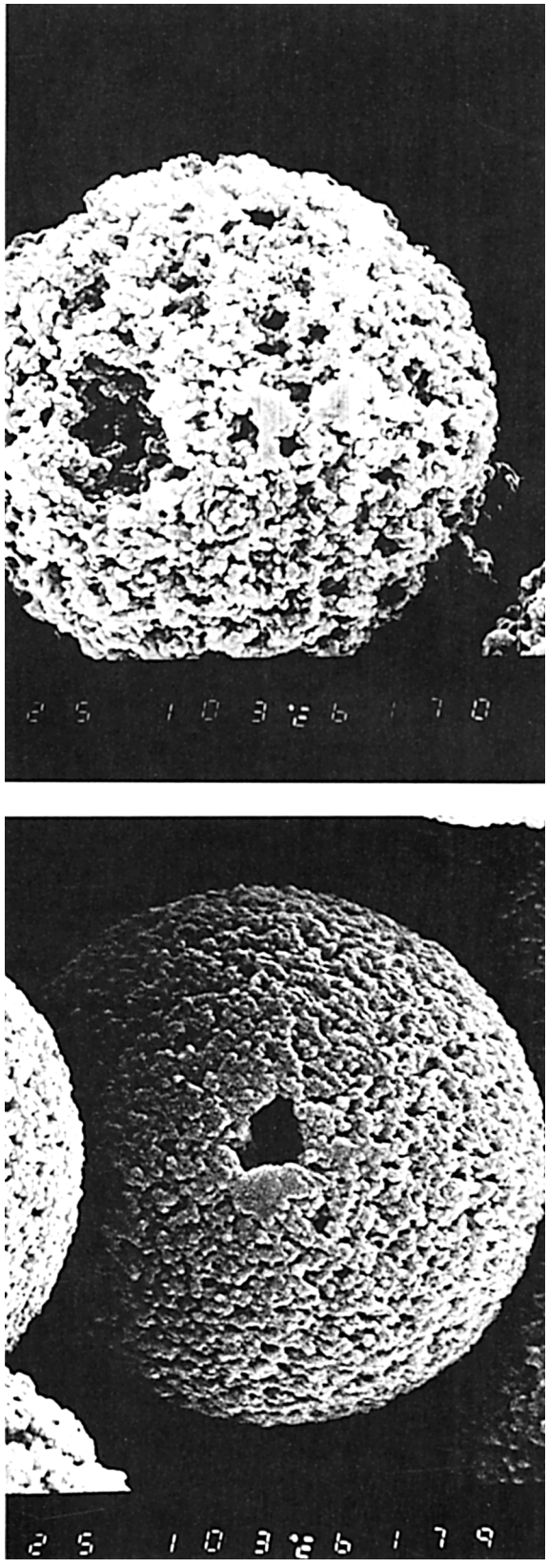

Figure 3 SEM picture of uniform macroporous particles (a) G-37 and (b) G-38.

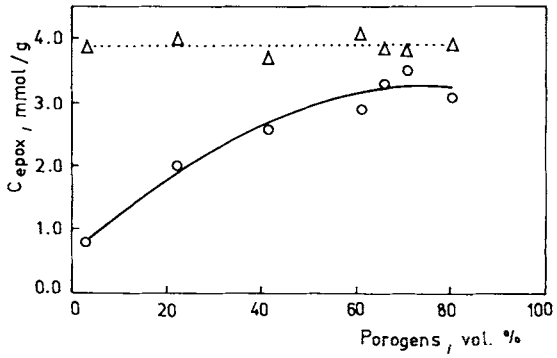

Figure 4 Content of epoxide groups in the macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) determined by (△) IR spectrometry and (○) titration as a function of porogenic solvent amount in the polymerization mixture.

hydrolysis, by the mutual reaction of groups inside the polymer, and the like. The chemical determination of the epoxides shows that in low-porosity beads most of the epoxide groups are buried inside the inaccessible polymer bulk and cannot be determined, whereas in high-porosity beads, the agreement between both types of determinations is better.

An equally important variable in the synthesis of macroporous beads is the content of the cross-linking agent in the polymerization mixture, which also significantly affects the porous properties. The largest specific surface area is found with beads synthesized from ethylene dimethacrylate alone; with decreasing content of the cross-linking agent, the specific surface area also decreases (Table IV). This is in agreement with what has been observed in the usual suspension polymerization; the reason has been reported elsewhere.[25] Chromatographic measurements made possible an estimation of the hydrophilicity of the polymer introduced into it by hydrolyzed glycidyl methacrylate units. If the latter are absent, as is the case with beads composed exclusively of ethylene dimethacrylate, the polymer should possess an explicitly hydrophobic character, whereas beads containing the predominant fraction of hydrolyzed glycidyl methacrylate should be hydrophilic. This means, in practice, that the chromatographic properties of the two should be different, depending on the mobile phase, as indeed confirmed by Figure 5(a) and (b). With respect to the use of SEC, these changes can be attributed to changes in the porous properties of packing, which, however, should be characterized in two respects, namely, the effect of the cross-linking agent on the pore size and distribution and the effect of the mobile phase used on the solvation of polymer chains of

Table IV Effect of Cross-linking Monomer Amount on the Properties of Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads

Exp. #	GMA (%)	EDMA (%)	S_g (m^2/g)	V_p (mL/g)	$N \times 10^{-3}$	V_i/V_t	$M_0 \times 10^{-3}$
G-39	0	100	483	1.23	12.3	0.47	300
G-40	20	80	260	1.23	15.8	0.45	80
G-41	40	60	132	1.16	13.3	0.43	90
G-12	60	40	69	1.17	21.2	0.48	350
G-42	80	20	30	1.09	19.4	0.49	650
G-43	90	10	28	0.48	5.0	0.30	0.5

For reaction conditions, see Tables I and II; total monomers 36.9 vol % of the final droplets, bead size 10 μm. Abbreviations: GMA, glycidyl methacrylate; EDMA, ethylene dimethacrylate; for other abbreviations, see Table I.

the beads. Although the former effect is well known (e.g., Ref. 25), the latter one has so far been neglected.

We have reported earlier that in copolymers of glycidyl methacrylate, globules that form basic morphological domains inside each bead are nonhomogeneous, and the cross-linking density decreases in the direction from the center to the shell of the globule.[27] In a simplified manner, this view can be interpreted as a hard core surrounded by "graft" linear or very weakly cross-linked chains capable of considerable solvation. In the dry state used in the mea-

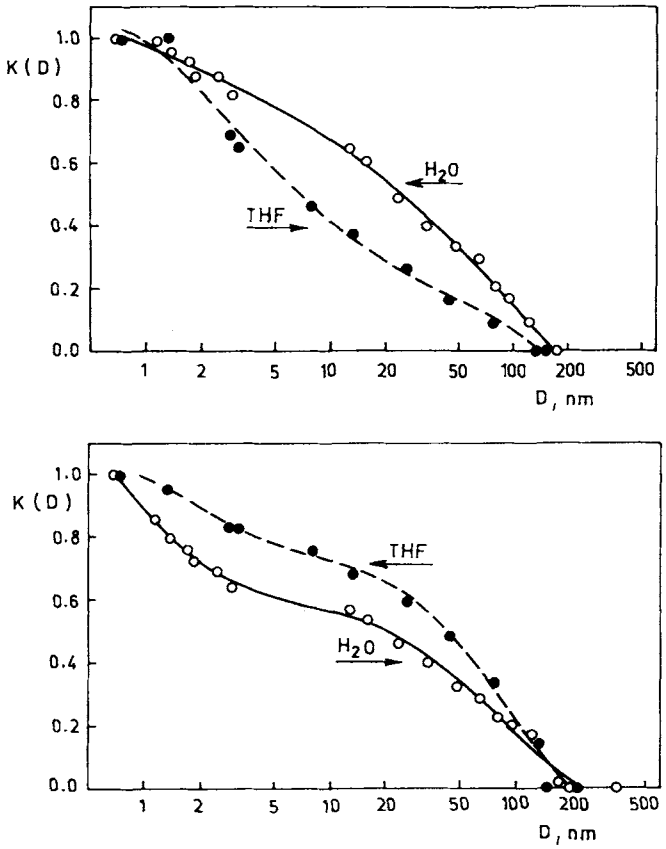

Figure 5 Cumulative distribution curves obtained from the SEC of single sugars and dextrane standards in water or polystyrene standards in THF on macroporous polymers synthesized from different monomer mixtures: (a) 100% ethylene dimethacrylate; (b) 80% glycidyl methacrylate and 20% ethylene dimethacrylate.

surement of the characteristics of the beads (dynamic nitrogen desorption, mercury porosimetry, and the like), the chains are not solvated and are seen as a part of the surface of the globules. After solvation, they swell and partly fill in the interglobular voids; both porosity and pore size consequently decrease. Solvation of free chains is a function of the solvent–polymer interaction coefficient in which, among other things, the hydrophilicity of the polymer is also reflected. Changes in the porous properties must depend on the solvent and on the composition of the polymer. This is why a copolymer containing 80% of hydrolyzed glycidyl methacrylatre has a much larger fraction of small pores in the chromatographic testing in aqueous solutions, compared with the fraction detected by measurement in THF [Fig. 5(b)]. Numerically, this means that at the specific area of 30 m^2/g the mean pore diameter in THF is 42.7 nm, while in water it is only 13.5 nm. For the explicitly hydrophobic poly(ethylene dimethacrylate) with the specific surface area 483 m^2/g, this is quite opposite [Fig. 5(a)]: In THF, the mean pore diameter is 6 nm, while in water it is 24.8 nm. This fact may also have another consequence. If the dependence of, e.g., mean pore size on the content of the cross-linking agent in THF is measured, the pore size decreases both due to the effect of the increasing content of the cross-linking agent and to the effect of increasing hydrophobicity of the surface and of its better solvation. Both effects act in the same direction, and the dependence is continuous (Fig. 6). On the other hand, though in water the increasing content of the cross-linking agent again causes a decrease in the pore size, increasing hydrophobicity restricts the solvation and the pores are not filled in with the swollen gel. In this case, the effects of cross-linking and hydrophobicity are opposite to each other, and the dependence passes through a minimum in the macroporous region. The effect of the cross-linking agent content on the pore size calculated according to the approximate formula[28]

$$D_{50} = 4000(V_p/S_g) \qquad (3)$$

where V_p is the pore volume and S_g is the specific surface area, also shown in Figure 6, is similar to the results obtained by the measurement in THF. Since the data on the specific pore volume have been calculated from chromatographic data measured in water as the mobile phase, and the extent of the specific surface area has been determined independently in the dry state, the result is partly distorted. Specifically, polymers featured by a large specific surface area contain micropores whose size, though allowing nitrogen molecules to penetrate, prevents by hydrophobic repulsive force the penetration of D_2O used as the smallest standard in the determination of the highest retention volume. At the same time, however, the smallest pores, whose contribution to the total pore volume is small, contribute most to the extent of the specific surface area. This explains why the calculated mean pore diameter is smaller for poly(ethylene dimethacrylate) than is the diameter determined in water by direct measurement. In all the other cases, however, the calculated data exceed the measured value and bring evidence that the polymer is solvated, though to a different extent, by both mobile phases used.

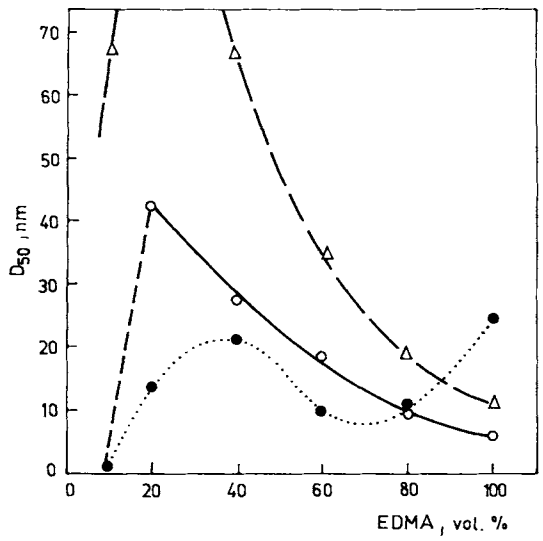

Figure 6 Mean pore size of macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) calculated from chromatographic exclusion data and from approximate formula (3) as a function of the cross-linking agent amount in the monomer mixture: (○) water, (●) THF, (△) calculation.

CONCLUSION

The results provide evidence that in spite of a certain similarity in the trends changes in the essential variables that influence the porous properties of macroporous beads of the copolymer glycidyl methacrylate–ethylene dimethacrylate have a different effect compared with the same copolymers prepared by the traditional suspension polymerization.

Chromatographic testing of the beads investigated in this study has revealed some new features of hydrophilic macroporous beads that so far have

not been detected, but are of great importance particularly for chromatographic application.

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