Preparation and Properties of Uniform Beads Based on Macroporous Glycidyl Methacrylate–Ethylene Dimethacrylate Copolymer: Use of Chain Transfer Agent for Control of the Pore-Size Distribution

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SYNOPSIS

Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads were prepared by both standard suspension polymerization and suspension polymerization in which the suspension of monodispersed droplets of the polymerization mixture was prepared by swelling of the shape-template polymer particles with porogen diluents and monomers. The effect of butanethiol, a chain-transfer agent in radical polymerization, on pore-size distribution, specific surface area, and chromatographic properties was investigated and the mechanism of its action explained. The synergic effects of the chain transfer, cross-linking monomer concentration, and content of porogenic diluent were documented. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

The choice of porous separation media for size-exclusion chromatography (SEC) made of synthetic polymers is broad.[1] Since they are based mainly on copolymers of styrene and divinylbenzene, they are compatible with a wide range of solvents at almost any pH, but they cannot be used for separation of proteins due to extensive adsorption. Their swelling porosity is negligible and their mechanical strength is sufficient for application in HPLC columns. A closer look reveals, however, that the number of packings for HPLC based on synthetic polymers having narrow fractionation limits in range under 100,000 or, even more specifically, under 20,000 is rather small. The reason has to be in the preparation technique. All the macroporous polymeric beads are produced by a suspension polymerization from a mixture composed of monomers, free-radical initiator, and porogenic diluent. In this process, the number of variables available for powerful control of the pore-size distribution, but that do not decrease simultaneously the pore volume, is limited. The major effects provide changes in the concentration of the cross-linking monomer in the monomer mixture and in the type and fraction of the porogenic diluent in the polymerization mixture.[2–4]

On the other hand, organic gels based on polysaccharides, such as dextrane or agarose and polyacrylamide, have been commercially available in many pore sizes for more than three decades.[5] They reveal excellent compatibility with proteins, and are chemically stable but do not have any permanent porosity (macroporosity) and the pores generate during swelling. Their use in column chromatography is limited by lack of rigidity to linear flow velocities less than 25 cm/h. Attempts made to decrease compressibility of the beads resulted in production of gels cross-linked with polyacrylamide chains.[1]

It is obvious from the description of the properties of common polymeric separation media for SEC that a combination of rigid matrix typical for a highly cross-linked synthetic polymer with a soft gel may result in a stationary phase strong enough for HPLC but at the same time exhibiting separation ability characteristic for gels packings. Such a trend was recently predicted as one of the most promising in

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the preparation of new separation media.[6] Some examples are already available. Wulff et al.[7] derivatized pores of wide-pore silica with styrenic double bonds and polymerized them free radically together with free styrene soaked in the pores. The product had excellent chromatographic properties and the column was very stable. There are other examples in the chromatographic literature describing modification of pores in porous separation media using polymers.[8] Typically, the polymer is grafted or polymerized in the pores of an already existing matrix. The preparation of the matrix and its modification are clearly separated.

In a previous report,[9] we described the effects of porogenic diluent and cross-linking monomer on chromatographic properties. This paper concerns attempts to show preparation of a material containing both a rigid matrix and embedded gel domains in only a single polymerization step using typical polymer chemistry.

EXPERIMENTAL

Polymer Preparation

Uniformly Sized Porous Beads

Monodispersed polystyrene shape-template particles with a diameter of approximately 1 μm were prepared by the emulsifier-free emulsion polymerization described in detail earlier.[10] The shape-template particles were swollen with a solution of free-radical initiator (AIBN) in an activating solvent (dibutyl phthalate), emulsified by sonication in an aqueous solution of sodium dodecyl sulfate (SDS), and added to a dispersion of the shape-templates. This swelling dramatically increases uptake of the polymerization mixture into the latex seed particles in the following swelling step,[11] which was started after the total disappearance of droplets of emulsified liquid observed in an optical microscope. It occurs typically in less than 16 h.

In the second swelling step, a mixture of glycidyl methacrylate, ethylene dimethacrylate, cyclohexanol, and, optionally, butanethiol emulsified in 0.25 wt % aqueous SDS solution was added to the activated seeds. The volume of added monomers and diluents was calculated to obtain particles of the requested size. The mixture was slowly stirred in a round-bottomed reactor until the small emulsified droplets disappeared and the dispersion contained only swollen seeds, usually less than 24 h. To the dispersion was added 4 wt % water solution of poly(vinyl alcohol) (Polyviol W 25/140, Wacker Chemie, Germany) in such an amount to adjust final concentration of this suspension stabilizer in the mixture to 1 wt %. The mixture was deoxygenated by purging nitrogen for 15 min and the reactor was sealed. The polymerization was carried out under continuous stirring (150 rpm) at 70°C for 15 h. After the polymerization was completed, the beads were transferred to a beaker containing water. The resulting polymer was washed several times with water and methanol until the supernatant liquid was no longer turbid. The beads were extracted by toluene in a Soxhlet apparatus for 48 h, washed again with methanol, and dried.

Suspension Polymerization

For comparison purposes, similar macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads were prepared by a standard suspension polymerization technique. The organic phase consisting typically of 60 vol % cyclohexanol, 24 vol % glycidyl methacrylate, 16 vol % ethylene dimethacrylate, and azobisisobutyronitrile (1% w/v to monomers) and, optionally, butanethiol was stirred in an 1 wt % aqueous poly(vinyl alcohol) at 70°C for 15 h. The work up of the beads was done as described above except for the extraction step.

Hydrolysis of Epoxide Groups

Since the epoxide groups may react with the solutes during the chromatographic separation process, they were quenched by hydrolysis. The chemical treatment also enhances the hydrophilicity of the separation medium. The beads were dispersed in 0.1 mol/L perchloric acid and under occasional stirring kept at ambient temperature for 120 h. The beads were filtered off and washed with water until the acidic reaction had disappeared, then washed with methanol and dried.

Characterization of Beads

The bead-size distribution was measured with a Coulter Counter TA II (Coulter Electronics, U.K.), and the specific surface area was calculated according to the B.E.T. equation from data on the sorption and desorption of nitrogen (Quantasorb, Quantachrome, U.S.A.). Chromatographic experiments were carried out using a chromatograph consisting of an HPLC pump (Knauer, Berlin, Germany), a Rheodyne 7125 valve loop injector, and an UV (Knauer) or RI detector (Laboratory Instruments, Prague, Czechoslovakia). The beads were packed in water into stainless-steel columns 80 × 8 mm i.d. (TESSEK, Prague). The pore-size distribution was

calculated from size-exclusion chromatography of defined polystyrene or dextrane standards with narrow molecular weight distribution according to Halasz and co-workers.[12]

RESULTS AND DISCUSSION

Copolymerization of mono- and divinylic monomers resulting in a macroporous product is a specific type of heterogeneous cross-linking polymerization. Initially, the polymerization mixture is a homogeneous solution of monomers, porogenic diluent, and a free-radical initiator. As the polymerization proceeds, the cross-linking agent enters the polymer chains, its second double bond reacts, and the cross-link occurs. This can be both intra- and intermolecular. The cross-linking results later in gel formation and causes separation of the polymer gel phase. The separated microgel elements are nuclei of globules that are later the basic morphological unit of the macroporous polymer. Typically, a high content of the cross-linking agent is required in the preparation of macroporous polymers to produce materials that do not swell in solvents and well resist mechanical forces. The globular morphology of macroporous polymers always shown in SEM pictures represents the polymer in the dry state. This picture may totally differ from that of a solvated polymer. It has been already confirmed experimentally for styrene, methacrylate, and other polymers that the surface shell of the globules has lower cross-linking density than that of the core.[13–15] The origin of this can be sought in the mechanism of polymerization under conditions leading to a macroporous polymer. The surface layer behaves more like a gel in which density depends on the porogen type used in the polymerization.[15] A simplified view presents the inside of a macroporous polymer in a solvent as an array of hard, highly cross-linked cores with grafted chains of highly solvated polymer. The extent of the gel phase influences accessibility of groups located in macroporous network polymers, but, again, the spontaneous process may hardly be controlled by other means except for porogen.

Polymer chemistry, however, proposes several methods for control of the cross-link density affecting the molecular weight of a polymer and also provides other means than simple changes of concentration of divinylic monomer. Chain transfer, which causes termination of growing polymer chain and starts growth of a new one, is the most powerful method. The polymer network cannot grow infinitely in the presence of a chain-transfer agent and its density is greatly reduced. In presence of a chain-transfer agent, the cross-linking polymerization gives a network that swells more than the network prepared in the absence of the transfer agent because the molecular weight of the terminated unit as well as the number of cross-links is lower. According to this concept, the chain-transfer agent should provide an effect on cross-linking density reflected in the changes of porous structure in the macroporous polymers.

Results of experiments in which butanethiol was added to the polymerization mixture agree with the starting idea of a forced decrease of cross-linking efficiency caused by chain transfer. The pore-size distribution changes as documented in related properties like exclusion limit in the size-exclusion chromatography (Table I), mean pore size determined by inverse size-exclusion chromatography (Fig. 1), specific surface area (Fig. 2), and pore volume (Fig. 3). Figures 1–3 also show the very important effect of volume of the porogenic diluent in

Table I Effect of the Chain Transfer Agent on the Exclusion Limits of Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) Beads

Butanethiol (wt %)	Exclusion Limit $\times 10^{-3}$
Monodispersed beads[a]	
0.0	> 3000
0.2	2235
0.4	560
0.6	450
0.8	95
1.0	88
1.4	43
Monodispersed beads[b]	
0.0	350
0.1	150
0.2	72
0.4	43
0.8	7
1.2	7
Polydispersed beads[c]	
0.0	80
0.2	28
0.4	13
0.8	13
1.2	11
1.6	3
2.0	3

[a–c] Polymerization mixture contains: [a]3.9 vol % dibutyl phthalate and 70.9 vol % cyclohexanol; [b]4.5 vol % dibutyl phthalate and 61.2 vol % cyclohexanol; [c]60 vol % cyclohexanol.

the polymerization mixture. This fact was already described in the previous paper.[9] Here, however, the effect is more stressed. For example, beads prepared in the presence of 61 vol % cyclohexanol and 0.8 vol % butanethiol in the polymerization mixture do not possess any reasonable specific surface area (S_g less than 2 m^2/g) and the pore volume represents only half of the value with no butanethiol present. If the volume of diluent is increased by 10% to 71 vol %, keeping constant all other conditions, the specific surface area and the pore volume do not change within the butanethiol concentration ranging from 0 to 0.8 wt % while the pore size decreases.

It should be stressed that even the most dramatic changes in the porous structure do not affect the shape and mechanical properties of the beads. They still may be packed into columns and used in the size-exclusion HPLC. The efficiencies of columns packed with 11 μm beads vary between 20,000 and 30,000 plates/m. This value compares favorably to polymer columns currently available. The back pressure in chromatographic measurements typically range between 1 and 2 MPa and does not exceed 2.5 MPa at a flow rate 1 mL/min.

The mechanism of formation of the macropores in cross-linked polymers prepared in the presence of an inert diluent has been known for a long time.[2,3] At the beginning, the polymerization of mono- and divinylic compound proceeds in solution. The chains formed at this stage of the polymerization are soluble, though they may be branched. Depending on the porogenic diluent, the polymer precipitates from the solution. In thermodynamically poor solvents, the separation of the second phase may occur even

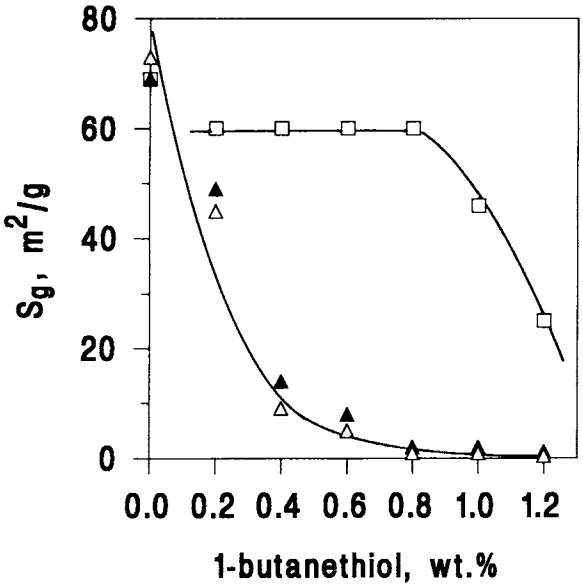

Figure 2 Specific surface area (S_g) as a function of the butanethiol content in the polymerization mixture. (△) Standard suspension polymerization, diluent 60 vol %; swelling and polymerization method in presence of (▲) 61 vol % and (□) 71% of diluent.

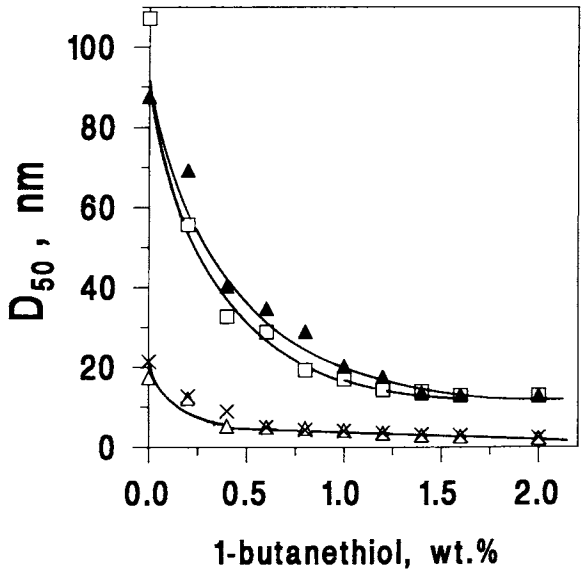

Figure 1 Effect of the butanethiol concentration in the polymerization mixture on the mean pore size D_{50} of porous beads prepared by (△) standard suspension polymerization or the swelling and polymerization method in presence of (x) 61 vol % and (□, ▲) 71 vol % of diluent. Determination by SEC in (△, x, □) water or (▲) THF.

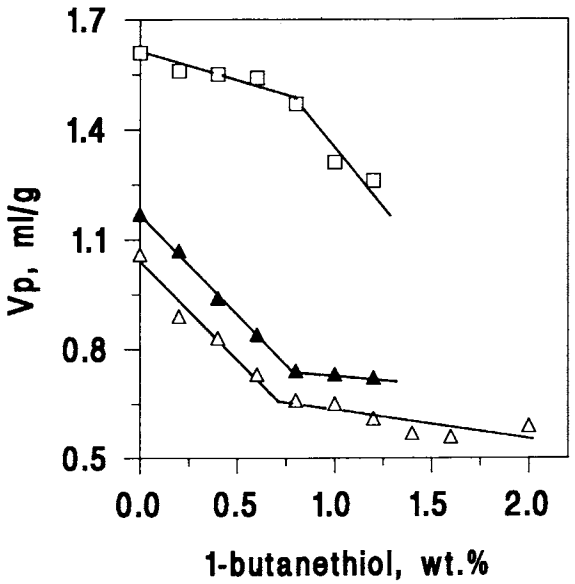

Figure 3 Pore volume (V_p) as a function of the butanethiol content in the polymerization mixture. (△) Standard suspension polymerization, diluent 60 vol %; swelling and polymerization method in presence of (▲) 61 vol % and (□) 71 vol % of diluent.

before the gel point. Once the separated microgel entities are present in the polymerizing system, they become nuclei, which, in later stages of polymerization, grow and transform into globules. In the poor solvent, the polymeric chains of the nuclei incline to solvate with monomers that are thermodynamically better solvents. The thermodynamic quality of solvent surrounding the swollen nuclei decreases. Because of higher concentration of monomers in solvated domains, the nuclei become loci of the polymerization and grow up to the size of globules (10–20 nm).

If the polymerization mixture contains a chain-transfer agent, it proceeds in a similar way. Since the chain-transfer reaction terminates the growing chains much earlier than that which occurs under standard conditions, the polymers stay dissolved in the liquid consisting of monomers and porogenic diluent. The higher the concentration of the chain-transfer agent, the shorter the chains and the longer they remain soluble. Chain transfer occurs until the agent is exhausted. In that moment, the polymerization proceeds according to the standard mechanism described in the previous paragraph. This causes not only formation of nuclei but also precipitation of dissolved oligomers and polymers produced during the initial stage of polymerization that is controlled by the chain-transfer agent. The largest molecules precipitate earlier than do the oligomers. These polymers that separate from solution aggregate with already-present nuclei or preglobules and remain a part of their surface through reaction of pendant double bonds, mutual epoxy groups, or chain-transfer reactions. When the concentration of chain-transfer agent exceeds some limit, the polymerization does not lead to any porous beads. The specific surface area approaches zero, and all pores are filled up even in the dry state. The SEM picture in Figure 4(b) shows nonporous structure in contrast to typically macroporous morphology of polymer prepared without any chain transfer [Fig. 4(a)].

In comparison to beads prepared under standard conditions, the beads prepared in the presence of a chain-transfer agent differ only in the layer of gel-like polymer attached to the surface of globules. The typical macroporous structure does not vary, which results in nearly equivalent mechanical properties.

The presence of the lesser cross-linked chain layer on the surface of globules manifests itself in different ways according to the environment of the polymer. In the dry state, in which the specific surface area is measured, the layer is almost "invisible." The surface chains adhere to the globule and do not interfere in the pores between globules. The whole

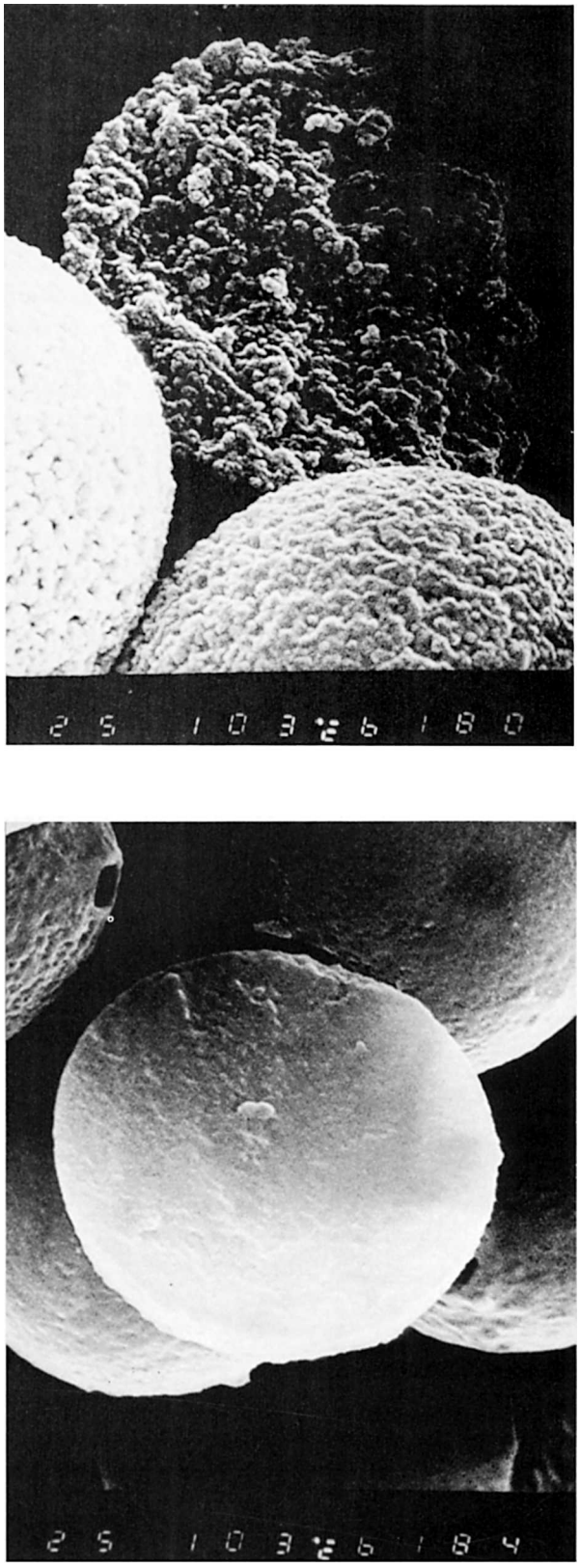

Figure 4 SEM of porous beads prepared by a swelling and polymerization method from glycidyl methacrylate and ethylene dimethacrylate (6:4) in the presence of cyclohexanol (a) without and with (b) 0.4 wt % butanethiol.

surface of the globules, which mainly contributes to the surface area, is accessible.

In contrast to the morphology in the dry state, macroporous polymers prepared in the presence of butanethiol tested under chromatographic conditions behave differently from standard beads. For example, the beads prepared in the presence of 61 vol % of cyclohexanol and 0.8 vol % butanethiol do not exhibit any porosity characterized with pores sized over 50 nm (macropores). They even contain almost no pores ranging from 50 to 2 nm (mesopores). The beads allow permeation of molecules with molecular weight up to 5000 only. In a simplified view, the beads may look like a standard macroporous matrix, pores of which are not free but filled up with a swollen polymer gel, exhibiting only swelling porosity. The permeability is proportional to the amount of chains in the pores. A high amount of chain-transfer agent produces much of the gel and the pores are crowded with the swollen polymer chains, preventing other molecules from penetration.

Stacking of the beads will be reduced when the pores are bigger and the pore volume higher. It occurs when the fraction of porogenic diluent in the polymerization mixture is increased. The beads prepared without any butanethiol with 61% cyclohexanol have a pore volume determined by size-exclusion chromatography of 1.2 mL/g and an exclusion limit of 350,000, whereas 71% of cyclohexanol provides a pore volume 1.6 mL/g, i.e., 25% more, and an extrapolated exclusion limit well over 2 million.

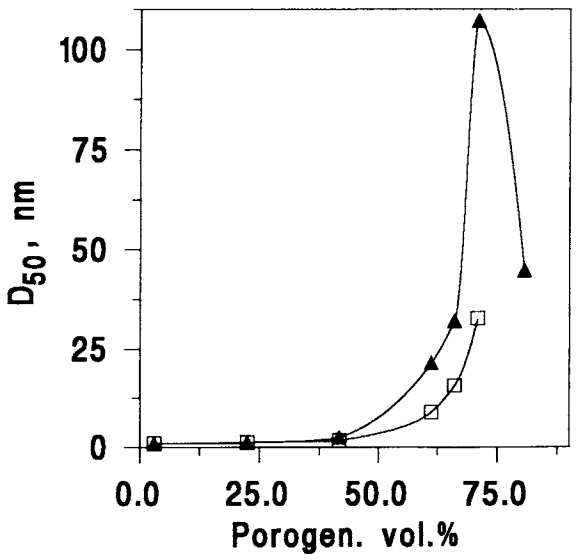

Figure 5 Effect of the porogen volume on the mean pore diameter (D_{50}) of porous beads prepared (▲) without and (□) with 0.4 wt % butanethiol as determined by SEC in water.

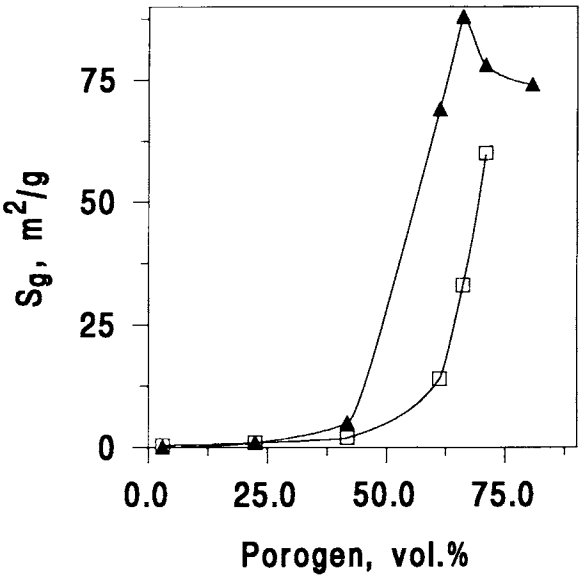

Figure 6 Effect of the porogen volume on the specific surface area (S_g) of porous beads prepared (▲) without and (□) with 0.4 wt % butanethiol.

The effect of porogen fraction and butanethiol in the polymerization mixture on the mean pore size and specific surface area is shown in Figures 5 and 6.

From the explanation of the particular mechanism of polymerization in the presence of a chain-transfer agent, it follows that the amount of the "gel" phase located on the surface of globules and, consequently, in the pores may be reduced when more

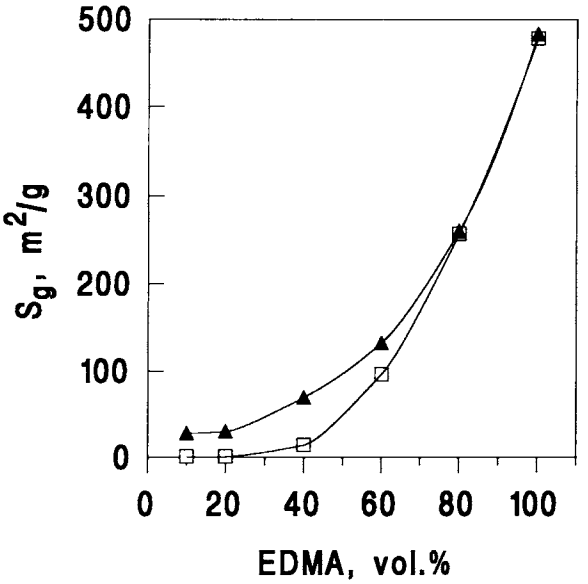

Figure 7 Effect of the cross-linking agent concentration on the specific surface area (S_g) of porous beads prepared (▲) without and (□) with 0.4 wt % butanethiol.

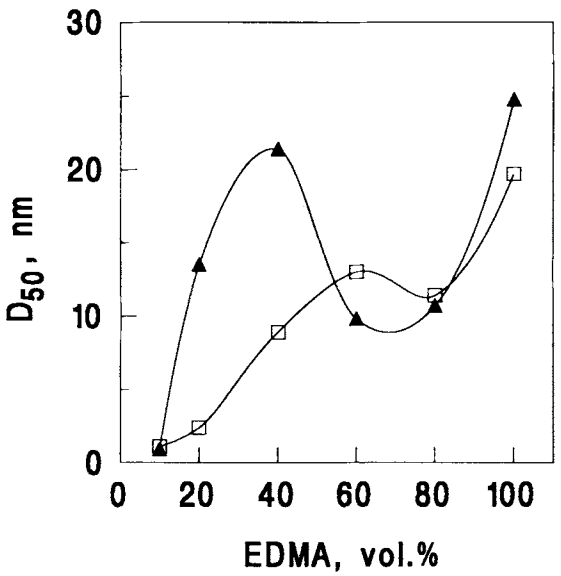

Figure 8 Effect of the cross-linking agent concentration on the mean pore diameter (D_{50}) of porous beads prepared (▲) without and (□) with 0.4 wt % butanethiol as determined by SEC in water.

cross-links develop and the phase separation occurs earlier. This can be simply achieved with a higher content of cross-linking monomer. Figure 7 documents that 0.4% butanethiol in the polymerization mixture affects the specific surface area of polymers containing less than 60% of ethylene dimethacrylate. There is, however, no difference at a content exceeding 80%. An effect would probably be achieved at much higher butanethiol concentration.

The dependence of mean pore size on ethylene dimethacrylate concentration in polymers prepared in the presence of butanethiol differs from that for standard polymers (Fig. 8). Polymers prepared with less than 50% of cross-linking agent contain higher fractions of gel-like polymer, formed mainly from hydrophilic poly(2,3-dihydroxypropyl methacrylate). The positive effect of higher cross-linking on the mean pore size is reversed by increased swelling of the loose hydrophilic chains. The main pore size seems to be smaller when butanethiol is applied. An increased content of ethylene dimethacrylate increases hydrophobicity of all the polymers, including the less cross-linked gel structures, and the mean pore size is not affected to any large extent.

CONCLUSION

Porous structures of macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) prepared by both traditional suspension and an activated two-step swelling method is strongly affected by addition of a chain-transfer agent in the polymerization mixture. The chain transfer provides a novel and powerful tool for control of pore-size distribution in polymeric separation media. A selection of contents of the cross-linking agent, porogenic diluent, and chain-transfer agent may result in beads having porous properties better engineered for particular chromatographic separation like size-exclusion chromatography of small molecules.

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