Polar, Monodisperse, Reactive Beads from Functionalized Methacrylate Monomers by Staged Templated Suspension Polymerization

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Porous monodisperse poly(methacrylic acid-*co*-ethylene dimethacrylate) and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads, which can be used as a platform for the production of separation media, polymeric reagents, and supports, have been prepared using the staged templated suspension polymerization process. Since the direct polymerization of methacrylic acid, which would lead to beads bearing carboxyl groups in a single step, is not compatible with the preparation technique, poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) beads were prepared and then selectively deprotected. In contrast, beads containing aliphatic hydroxyl groups can be prepared directly despite the high solubility of 2-hydroxyethyl methacrylate in water. The degree of functionalization and porous properties of the beads are largely controlled by the monomer composition in the polymerization mixture. One application for the functionalized beads is the preparation of very selective chiral separation media for HPLC of enantiomers.

Introduction

Porous polymer beads introduced in the late 1950s[1] are finding numerous applications as both commodity and specialty materials.[2] Although the former category includes ion exchangers and adsorbents, supports for solid-phase synthesis, media for heterogenization of homogeneous catalysts, and chromatographic packings fit well into the latter. Although the vast majority of current macroporous beads are based on styrene−divinylbenzene copolymers, other monomers including acrylates, methacrylates, vinylpyridines,[3] substituted vinylphenols,[4,5] vinylpyrrolidone, and vinyl acetate have also been utilized.[2] For example, porous polymers containing pendent carboxylic acid groups, such as copolymers of acrylic acid and divinylbenzene, have found a primary application as weak cation exchangers that are useful for a variety of applications including water treatment, the separation of inorganic ions, and the purification of biological molecules.[6] Hydrophilic copolymers of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) in the size range 10−100 μm have also been prepared using standard suspension polymerization techniques either in aqueous dispersion[7,8] or in fluorinated hydrocarbons.[9] The beads can be used for a variety of chromatographic techniques, including affinity and gel permeation chromatography, and as supports for enzyme immobilization.[10,11] In addition, these beads contain aliphatic hydroxyl groups that can be used for a large number of chemical tranformations.[12]

Porous beads are typically prepared by standard suspension polymerization from polymerization mixtures containing monovinyl monomer(s), a divinyl cross-linking agent, and a porogenic solvent.[13,14] Although stirring provides some control over the average size of droplets in the initial stages of a “classical” suspension polymerization,[5] the beads that are obtained have a broad distribution of sizes. As a direct consequence, most commercially available beads for high-end applications such as HPLC and solid-phase synthesis are size-fractionated, which decreases the yield of useful beads. To eliminate the considerable waste of material, several processes have been developed for the production of more uniformly sized spherical particles.

A relatively simple method that affords large beads with a relatively narrow size distribution is a suspension polymerization in which droplets are produced by pushing the monomer phase through the thin orifices of various capillaries or porous glass membranes.[15,16]

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(1) Meitzner, E. F.; Olin, J. A. US Patent 4,224,415, 1980.

(2) Arshady, R. *J. Chromatogr.* **1991**, *586*, 181, 198.

(3) Fréchet, J. M. J.; Vivas de Meftahi, M. *Br. Polym. J.* **1982**, *16*, 193.

(4) Rolls, W.; Svec, F.; Fréchet, J. M. J. *Polymer* **1990**, *31*, 165.

(5) Fréchet, J. M. J.; Eichler, E.; Ito, H.; Wilson, G. *Polymer* **1983**, *24*, 995.

(6) Helfferich, F. E. *Ion Exchange*; McGraw-Hill: New York, 1962. Dorfner K. *Ion Exchangers, Properties and Applications;* Ann Arbor Science: Ann Arbor, 1972.

(7) Coupek, J.; Krivakova, M.; Pokorny, S. *J. Polym. Sci., Polym. Symp.* **1973**, *42*, 185.

(8) Horak, D.; Lednicky, F.; Rehak, V.; Svec, F. *J. Appl. Polym. Sci.* **1993**, *49*, 2041.

(9) Zhu, D. W. *Macromolecules* **1996**, *29*, 2813.

(10) Janak, J.; Coupek, J.; Krejci, M.; Mikes, O.; Turkova, J. *Liquid Column Chromatography*; Deyl, Z., Macek, K., Janak, J., Eds.; Elsevier: Amsterdam, Oxford, New York, 1975; p 187.

(11) Kamei, S.; Okubu, M.; Matsumoto, T. *J. Appl. Polym. Sci.* **1987**, *34*, 1439.

(12) Kahovec, J.; Coupek, J. *React. Polym.* **1988**, *8*, 105.

(13) Seidl, J.; Malinsky, J.; Dusek, K.; Heitz, W. *Adv. Polym. Sci.* **1967**, *5*, 113.

(14) Guyot, A.; Bartholin, M. *Prog. Polym. Sci.* **1982**, *8*, 277.

(15) Colvin, M.; Chung, S. K.; Hyson, M. T.; Chang, M.; Rhim, W. R. *J. Polym. Sci., Polym. Chem.* **1980**, *28*, 2085.

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Table 1. Properties of Monodisperse Poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate)[a] and Poly(methacrylic acid-*co*-ethylene dimethacrylate) Beads[b]

experiment	A	B	C	D	E	F
Poly(tert-butyl methacrylate-co-ethylene dimethacrylate) Beads						
TBMA, monomer vol %	80	70	60	50	40	30
pore volume, mL/g [c]	0.57	0.64	0.64	0.88	1.01	1.03
specific surface area, m^2/g [d]	<5	<5	11	296	329	343
Poly(methacrylic acid-co-ethylene dimethacrylate) Beads						
carboxylic acid groups, mmol/g	6.3	5.0	3.7	3.0	2.1	1.1
accessible *tert*-butyl ester groups, %	86	84	78	80	75	56
pore volume, mL/g [c]	0.53	0.50	0.55	0.64	0.80	0.90
specific surface area, m^2/g [d]	<5	<5	<5	11	137	267

[a] Reaction conditions: porogen particles (shape templates, 0.10 mL 22% dispersion; dibutyl phthalate, 0.62 mL; 0.25% aqueous sodium dodecyl sulfate solution, 23 mL); TBMA and EDMA (5 mL); AIBN, 0.122 g; cyclohexanol, 4.4 mL, *tert*-butyl alcohol, 3.6 mL. [b] After hydrolysis of the corresponding poly(TBMA-*co*-EDMA) beads. [c] According to mercury porosimetry. [d] According to BET.

Another technique for the preparation of monodisperse beads pioneered by Vanderhoff and Ugelstad[17,18] utilizes an aqueous dispersion of micrometer-size uniform "seeds" that are prepared in a separate polymerization step. These small particles are swollen to the target size of the final product with a mixture of monomers and porogen, i.e., solvents, and then polymerized. The shape and size uniformity of the original seeds is preserved in the final product, and the size of the final beads is no longer determined by the stirring conditions but by the extent of swelling of the seed particles. We have recently developed a practical staged templated suspension process in which the linear polymer that constitutes the "shape template" is used as a porogen, resulting in fine control of both the monodispersity and porous structure of the beads.[19,20]

Monodisperse beads are always prepared from monomers that do not dissolve readily in water, such as styrene, its substituted derivatives,[21-24] and glycidyl methacrylate.[25] Although Ugelstad reported very small monodisperse nonporous 2.5 μm poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate-*co*-ethylene dimethacrylate) particles for radioimmunoassay applications,[26] water-soluble monomers were generally thought to be inappropriate for the staged templated suspension process.

In this report we describe the preparation of macroporous monodisperse beads with hydrophilic carboxyl and hydroxyl chemistries. The level of functionalization and porous properties of these beads can be easily controlled, leading to well-defined supports that may be used as platforms for numerous applications.

Experimental Section

Preparation of Uniformly Sized Macroporous Beads. The preparation of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) **1** and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) **2** beads using the staged templated suspension polymerization was carried out as described previously.[22] Monodisperse polystyrene shape templates (1.0 μm, 22% solid in water, 0.10 mL) were swollen by adsorption of an emulsion of 1-chlorodecane or dibutylphthalate (0.62 mL) in 0.25% aqueous sodium dodecyl sulfate (SDS) solution (30 mL). After the swelling was complete, a solution of monomers (5.0 mL), porogen (8.0 mL), and AIBN (0.12 g), emulsified in 0.25% aqueous SDS (30 mL), was added to the dispersion and the mixture was stirred slowly. After transfer of the organic droplets to the shape templates was completed, sodium nitrite (0.01 g) and a 4% solution of poly(vinyl pyrrolidone) (PVP, MW 360 000, 19.2 mL) were added and the system was purged with nitrogen for 20 min. The polymerizations were performed in sealed, 500-mL Erlenmeyer flasks placed in an orbiting shaker bath (Lab-Line) at 200 rotations/min and 70 °C for 17 h. The resulting beads were decanted repeatedly with water and methanol until the supernatant liquid remained clear. Following extraction with toluene in a Soxhlet apparatus for 36 h, the porous beads were dried under vacuum.

Poly(methacrylic acid-*co*-ethylene dimethacrylate) Beads 3. A mixture of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) beads **1** (2.8 g), *p*-toluene sulfonic acid monohydrate (0.18 g), and toluene (45 mL) was heated to 75 °C and stirred slowly for 18 h. The resulting beads **3** were filtered and washed with THF and CH_3OH and dried under vacuum. Titration of the carboxylic acid groups was accomplished by suspending the beads in THF, adding an excess of 0.1 mol/L aqueous sodium hydroxide, then back-titrating with 0.1 mol/L aqueous HCl solution to a phenolphthalein end point. The titration results are shown in Table 1.

Preparation of Carboxylated Beads 5. A mixture of poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads **2** (0.5 g), pyridine (12 mL), and succinic anhydride **4** (1.0 g) was heated to 60 °C and stirred slowly for 6 h. The beads **5** were filtered and sequentially washed with water, 0.1 mol/L HCl, water, and acetone, then dried under vacuum. The content of carboxyl groups was determined by titration as described for **3**, and the results are shown in Table 2.

Activated Beads 7. A mixture of deprotected poly(TBMA-*co*-EDMA) beads **3** (experiment D of Table 1, 3.0 mmol CO_2H/g, 3.0 g), triethylamine (4 mL), and dry THF (45 mL) was placed in a flask with a mechanical stirrer. The suspension was cooled to 0 °C, and ethyl chloroformate **6** (4.3 mL) was added slowly. After 20 min, the flask was brought to room temperature and stirred for 20 h. The beads **7** were filtered, washed with THF, and dried under vacuum. The activation is accompanied by the appearance of a broad anhydride band centered at 1805 cm^{-1} in the IR spectrum.

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(16) Omi, S.; Taguchi, T.; Nagai, M.; Ma, G. H. *J. Appl. Polym. Sci.* **1997**, *63*, 931.

(17) Cheng, C. M.; Micale, F. J.; Vanderhoff, J. W.; El-Aasser, M. S. *J. Polym. Sci., Polym. Chem.* **1992**, *30*, 235, 245.

(18) Ugelstad, J.; Kaggerud, K. H.; Hansen, F. K.; Berge, A. *Makromol. Chem.* **1979**, *180*, 737. Ugelstad, J.; Stenstad, P.; Kilaas, L.; Prestvik, W. S.; Rian, A.; Nustad, K.; Herje, R.; Berge, A. *Macromol. Symp.* **1996**, *101*, 491.

(19) Svec, F.; Fréchet, J. M. J. *Science* **1996**, *273*, 205.

(20) Wang, Q. C.; Hosoya, K.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1992**, *64*, 1232.

(21) Wang, Q. C.; Svec, F.; Fréchet, J. M. J. *Polym. Bull.* **1992**, *28*, 569.

(22) Galia, M.; Svec, F.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 2169.

(23) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *J. Liq. Chrom.* **1997**, *20*, 227.

(24) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *J. Appl. Polym. Sci.*, in press.

(25) Smigol, V.; Svec, F. *J. Appl. Polym. Sci.* **1992**, *46*, 1439.

(26) Ugelstad, J.; Mork, P. C.; Mfutakamba, H. R.; Soleimany, E.; Nor, I.; Schmid, R.; Berge, A.; Ellingsen, T.; Aune, O.; Nustad, K. *Science and Technology of Polymer Colloids*; Poehlein, G. W., Otteweill, R. H., Goodwin, J. W., Eds.; M. Nijhoff: Boston, 1983; p 51.

Table 2. Properties of Monodisperse Poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) Beads[a]

experiment	G	H	I	J	K
HEMA, monomer vol %	80	70	60	50	40
carboxyl groups, mmol/g [b]	3.9	3.4	3.2	2.8	2.2
accessible hydroxyl groups, %	63	63	69	71	70
pore volume, mL/g [c]	0.75	0.70	0.94	0.89	1.01
specific surface area, m^2/g [d]	14	32	64	91	143

[a] Reaction conditions: porogen particles (shape templates, 0.10 mL 22% dispersion; 1-chlorodecane, 0.62 mL; 0.25% aqueous sodium dodecyl sulfate solution, 23 mL); HEMA and EDMA (5 mL); AIBN, 0.122 g; cyclohexanol, 7.1 mL; 1-dodecanol, 0.8 mL. [b] After reaction with succinic anhydride. [c] According to mercury porosimetry. [d] According to BET.

Activated Beads 9. A mixture of poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads **2** (experiment J of Table 2, 2.0 g), 4-nitrophenyl chloroformate **8** (2.0 g), triethylamine (1.0 mL), and dry THF (35 mL) was heated to 65 °C and stirred slowly under nitrogen for 12 h. The modified beads **9** were filtered and washed with THF, water, and ethyl ether and dried under vacuum. Nitrogen analysis indicates that the resulting beads contain 1.45 mmol/g of 4-nitrophenyl carbonate groups ($N = 2.03\%$).

Preparation of Chiral Stationary Phases 11 and 12. L-Valine-3,5-dimethylanilide[27] **10** (2.0 g), and triethylamine (0.4 mL) were added to the activated beads (**7** or **9**, 2.0 g) suspended in dry THF (25 mL). The resulting mixture was heated to 60 °C and stirred under nitrogen for 12 h. The beads **11** or **12** were filtered and washed thoroughly with CH_3OH, water, and THF, then dried under vacuum. The selector content of the beads is 1.50 mmol/g (**11**) and 1.10 mmol/g (**12**) based on elemental analysis ($N = 4.20\%$ and 3.07%, respectively), assuming that all of the nitrogen originates from the chiral selector functionalities.

The excess carboxyl functionalities (1.5 mmol/g) on **11** were reacted with iodomethane (2 equiv) in a mixture of potassium carbonate (2 equiv) and *N,N*-dimethylformamide for 24 h at room temperature. The beads were then filtered and washed with water and acetone.

Characterization of Porous Properties. Scanning electron microscopy (JEOL JSM-35CF) was used to observe the morphology of the beads and to measure their size. The specific surface area of the beads was calculated from the BET isotherm of nitrogen, and the pore-size distribution in the dry state was determined from mercury intrusion porosimetry using an automated custom-made combined BET-sorptometer and mercury porosimeter from Porous Materials Inc., Ithaca, NY. The measurements were repeated several times, and the overall reproducibility is within 8%. Thermogravimetric measurements (Seiko Instruments Inc., TGA/DTA 220) were performed under nitrogen.

Chromatography. A Waters HPLC system consisting of two 510 HPLC pumps, a 717 plus autosampler, and a 486 UV detector, and controlled by Millennium 2010 software, was used for all of the chromatography. The chiral stationary phases were slurry-packed into 150 mm × 4.6 mm i.d. stainless steel columns.

Inverse size-exclusion chromatography was carried out in THF using benzene, toluene, amylbenzene, and polystyrene standards with molecular weights ranging from 500 to 2 950 000 in order to characterize the pore structure in a "wet" state. The peaks were monitored at 254 nm and the distribution coefficients K_D were calculated according to the equation

$$K_D = (V_R - V_0)/V_P \tag{1}$$

where V_R is the retention volume of a standard, V_0 is the void volume of the packed column (elution volume of the largest polystyrene standard), and V_P is the total pore volume available for the penetration of benzene.

Chiral separations were carried out using a hexane/dichloromethane mixture as a mobile phase. The selectivity values α were calculated using the following equation:

$$\alpha = k'_2/k'_1 \tag{2}$$

where k'_1 and k'_2 are the retention factors of the enantiomers defined as

$$k'_i = (t_R - t_0)/t_0 \tag{3}$$

where t_R and t_0 represent the retention times of the compound and 1,3,5-tri(*tert*-butyl)benzene (void volume marker), respectively.

Results and Discussion

Preparation of Functionalized Beads. Monodisperse macroporous beads containing functional groups are useful as reactive supports for a variety of applications. As indicated earlier, the staged templated polymerization procedure has previously been utilized primarily with hydrophobic monomers with low water solubility such as styrene, chloromethylstyrene, divinylbenzene, glycidyl methacrylate, and ethylene dimethacrylate.[19−25] However, only a few types of uniformly sized beads with reactive functionalities, such as epoxide or chloromethyl groups, had previously been prepared. Compounds with carboxyl and hydroxyl groups are used in a great variety of organic reactions and as the anchoring moieties of supports for solid-phase syntheses. Although monomers bearing carboxyl or aliphatic hydroxyl groups, such as methacrylic acid and 2-hydroxyethyl methacrylate, are commercially available, their use for the preparation of monodisperse porous beads had not been reported.

Poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) Beads. Although macroporous poly(acrylic acid-*co*-ethylene dimethacrylate) beads in the size range 50−150 μm have been prepared using a standard suspension polymerization in the presence of toluene as a porogen,[28] the staged templated polymerization technique does not lead to monodisperse particles because of extensive agglomeration of the polymer during the polymerization, regardless of the porogen chosen. Therefore, an alternative approach using the hydrophobic monomer *tert*-butyl methacrylate (TBMA), a protected form of methacrylic acid, was used to prepare macroporous monodisperse beads. The desired poly(methacrylic acid-*co*-ethylene dimethacrylate) beads can later be obtained following deprotection of the carboxyl groups.

Polymerization conditions were found for the preparation of uniformly sized macroporous *tert*-butyl methacrylate beads with fine control over both their porous properties and content of functional groups. Typical porogen mixtures, such as cyclohexanol and dodecanol, are not well suited for the preparation of porous poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) beads, and a porogenic mixture consisting of 45% *tert*-butyl alcohol and 55% cyclohexanol was used instead.

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(27) Liu, Y.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **1997**, *69*, 61.

(28) Tuncel, A.; Kesenci, K.; Ecevit, K.; Piskin, E. *J. Polym. Sci., Part A: Polym. Chem.* **1996**, *34*, 45.

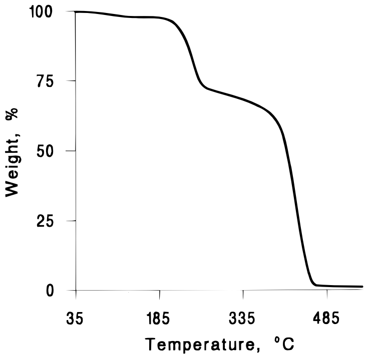
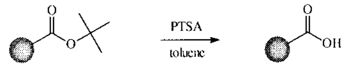


Figure 1. Gravimetric analysis of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) beads containing 50% TBMA.

Scheme 1

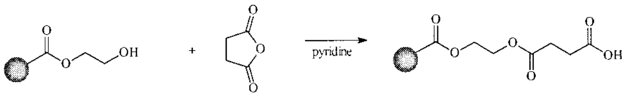


1 3

Deprotection of the Carboxyl Groups. The carboxyl groups of the macroporous *tert*-butyl methacrylate beads can be deprotected by thermolysis or acidolysis (Scheme 1). Since mass loss occurs during this deprotection, thermogravimetric analysis (TGA) can be used to determine the conditions necessary to effect deprotection. For the beads containing 50% *tert*-butyl methacrylate, Figure 1 shows a 27% weight loss occurring near 240 °C followed by a gradual loss of mass. The sharp transition corresponds to the simultaneous loss of isobutylene and water. The latter has been shown to occur for linear poly(*tert*-butyl methacrylate) polymers[29] leading to the formation of some anhydride groups at the same time as the deprotected carboxylic acid polymer. This thermolysis is later followed by an uncontrolled degradation at temperatures well above 240 °C. The formation of anhydride groups is confirmed by the appearance of a carbonyl stretch at 1810 cm^{-1} in the infrared spectrum of the thermolyzed beads after heating to 240 °C for 5 h under nitrogen.

Acidolysis of the *tert*-butyl ester groups may be accomplished at a lower temperature without the undesirable formation of anhydride groups using 4-toluenesulfonic acid, as previously demonstrated in the deprotection of linear copolymers containing *tert*-butyl methacrylate.[30,31] Under these conditions (75 °C for 18 h in toluene solution) the ester functionalities of the ethylene dimethacrylate moieties are very stable as expected from previous studies.[32] IR analysis of the beads after acidolysis confirms that the desired poly(methacrylic acid-*co*-ethylene dimethacrylate) beads are obtained. The physical characteristics of the entire series of methacrylic acid beads after their acidolysis are shown in Table 1. Direct titration of the polymers indicates that the carboxylic acid content of the beads varies over a wide range from 1.0 to 6.3 mmol/g. The percentage of *tert*-butyl ester groups removed generally decreases as the amount of EDMA in the beads increases. Therefore, the accessibility of the ester groups to the acidolytic reagent is dependent on the cross-linking density of the beads.

Scheme 2



2 4 5

Poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) Beads. Aliphatic hydroxyl functionalities provide an alternative, less polar type of reactive group to those of the carboxylic acid resins. In contrast to the direct polymerization of methacrylic acid, it is actually possible to use the staged templated polymerization method to prepare beads of hydroxyethyl methacrylate directly, since both an organic and an aqueous phase are used in this process. The solubility of the monomer in the aqueous phase is controlled by the partition coefficient, which is defined as the ratio of concentrations of the monomer in the organic and aqueous phases. If this coefficient is close to zero, the hydrophilic monomer dissolves predominantly in the organic phase, and since the concentration of the monomer in the aqueous phase is low, the staged polymerization process may proceed smoothly. The actual value of the distribution coefficient depends on a number of factors including the composition of the organic phase. Since the monomers and their percentages used for the polymerization are not a real variable, the choice of porogens in the polymerization mixture is important not only for the formation of a macroporous structure but also for the efficient transfer of the hydrophilic monomer from the emulsified monomer mixture to the swollen templates. We have found that a mixture of cyclohexanol and dodecanol is a porogenic solvent well suited for the high-yield preparation of monodisperse poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads containing up to 80% HEMA.

Content of Hydroxyl Groups. The number of hydroxyl groups originating from polymerized HEMA is an important characteristic of the porous poly(HEMA-*co*-EDMA) copolymer beads. A rough estimation of the hydroxyl content can be based on the percentage of HEMA in the polymerization mixture. However, this calculation assumes that all of the HEMA monomer is incorporated into the polymer and does not discriminate between those hydroxyl groups that are accessible and those that are buried within the highly cross-linked polymeric matrix. Since the direct determination of hydroxyl groups is difficult, we carried out an indirect analysis by treating the beads with an excess of succinic anhydride in pyridine (Scheme 2). This modification reaction involving only those hydroxyls that are accessible to the reagent leads to a reactive polymer with carboxyl functionalities susceptible to direct acid−base titration. As expected, the number of carboxyl groups found in the beads increases with increasing hydroxyethyl methacrylate content in the polymerization mixture (Table 2). These data confirm that the amount of accessible hydroxyl groups in poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads can be controlled within the broad range of 2.2−3.9 mmol/g by

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(29) Grant, D. H.; Grassie, N. *Polymer* **1960**, *1*, 125.

(30) Ito, H.; Wilson, C. G.; Fréchet, J. M. J. *Proc. SPIE—Int. Soc. Opt. Eng.* **1987**, *771*, 24.

(31) Wilson, C. G.; Ito, H.; Fréchet, J. M. J.; Tessier, T. G.; Houlihan, F. M. *J. Electrochem. Soc.* **1986**, *133*, 181.

(32) Kalalova, E.; Radova, Z.; Kalal, J.; Svec, F. *Eur. Polym. J.* **1977**, *13*, 287.

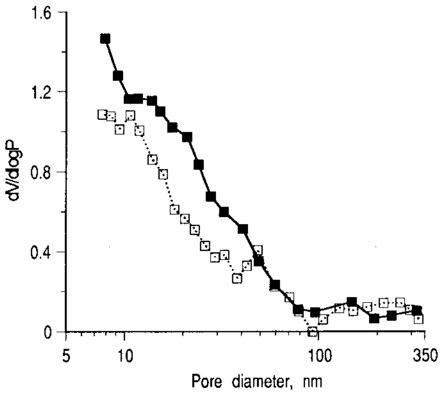

Figure 2. Differential pore-size distribution curves of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) (■) and poly(methacrylic acid-*co*-ethylene dimethacrylate) beads (□) measured by mercury porosimetry.

simply changing the composition of polymerization mixture.

It is generally assumed that reactive functionalities in highly cross-linked macroporous functional polymers are located at or near the surface of pores. If this were the case, the percentage of accessible groups should increase as the surface area of the macroporous polymer increases. Table 2 documents that this assumption does not hold for our system, since the percentage of hydroxyl groups that may be modified remains within a narrow range of 63–70% of the theoretical amount regardless of surface area.

Porous Properties. Tables 1 and 2 summarize the porous properties of two series of macroporous beads prepared from mixtures containing varying percentages of the functionalized monomers. As the amount of cross-linking monomer in the polymerization mixture is increased, both the porosity, measured by mercury-intrusion porosimetry, and the surface area, calculated using the BET equation, of the beads increase. For example, beads containing 70% TBMA and 30% ethylene dimethacrylate have a pore volume of 0.6 mL/g and a specific surface area of <5 m^2/g, while beads that contain 40% TBMA have higher values of 1.0 mL/g and 329 m^2/g, respectively. The effect of increasing the surface area of the beads by increasing the cross-linking density is in accordance with our previous work on monodisperse porous styrene–divinylbenzene polymer beads.[22] In addition, there is a large drop in the surface area of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) beads when the percentage of *tert*-butyl methacrylate exceeds 50%. This can be attributed to the formation of fewer pores less than 10 nm in size during polymerization. These pores are not reflected in the mercury-porosimetry data, since they are too small to allow mercury penetration. The pore-size distribution profiles for beads containing 50% TBMA (Figure 2) and 50% HEMA (Figure 3) do not have a sharp maximum but show a broad distribution of pores below 100 nm.

Acidolysis of the *tert*-butyl groups leads to a decrease in both the porosity and the surface area of the desired poly(methacrylic acid-*co*-ethylene dimethacrylate) beads as measured in the dry state. This is unexpected because any loss of mass within the rigid beads should be accompanied by an increase in pore volume, and therefore, a partial collapse in the porous structure must accompany deprotection and drying. The pore-size distribution profile of the beads before and after deprotection (Figure 2) remains similar, but the frequency of the individual pores is decreased, indicating again that it is only the overall volume of the pores and not their distribution that has changed.

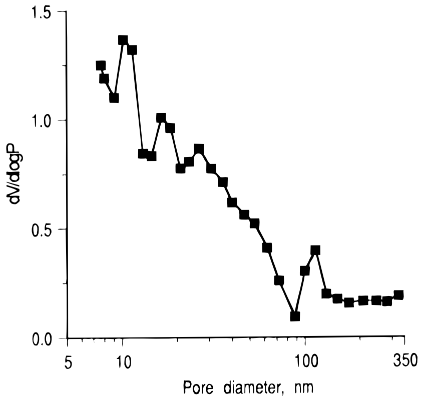

Figure 3. Differential pore-size distribution curve of poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads measured by mercury porosimetry.

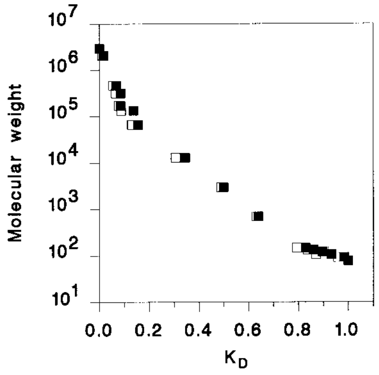

Figure 4. Size-exclusion calibration curves of poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) (■) and poly(methacrylic acid-*co*-ethylene dimethacrylate) beads (□) with polystyrene standards and alkylbenzenes in tetrahydrofuran: beads as in experiment D; columns, 150 mm × 4.6 mm i.d.; flow rate, 1 mL/min; UV detection at 254 nm.

In addition to mercury porosimetry, which characterizes the beads in the dry state, inverse size-exclusion chromatography (ISEC) can be used to determine the porous properties of the beads in a “wet” environment that is more typical of their use.[33] The ISEC calibration curves for poly(*tert*-butyl methacrylate-*co*-ethylene dimethacrylate) with 50% TBMA and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) with 50% HEMA measured in tetrahydrofuran with polystyrene standards are shown in Figures 4 and 5, respectively. These curves essentially confirm the pore-size distributions determined by mercury porosimetry. The beads have few large macropores as indicated by the lack of separation of standards with a molecular weight exceeding 10^5. In contrast, more than 80% of the total pore volume is available for the separation of polystyrene molecules with a hydrodynamic size smaller than 12 nm, a value that corresponds to a molecular weight

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(33) Tanaka, N.; Kimata, K.; Mikawa, Y.; Hosoya, K.; Araki, T.; Ohtsu, Y.; Shiojima, Y.; Tsuboi, R.; Tsuchiya, H. *J. Chromatogr.* **1990**, *535*, 13.

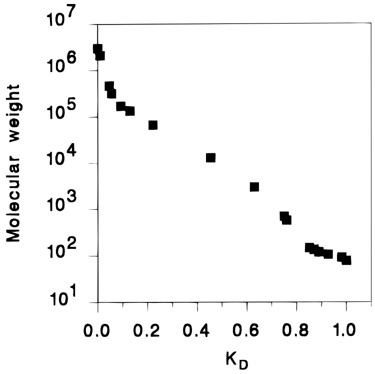


Figure 5. Size-exclusion calibration curve of poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads with polystyrene standards and alkylbenzenes in tetrahydrofuran: beads as in experiment J; column, 150 mm × 4.6 mm i.d.; flow rate, 1 mL/min; UV detection at 254 nm.

of less than 10^5. This correlates well with the observed preponderance of mesopores with a diameter range 2−50 nm and the small number of macropores determined by mercury porosimetry.

The size-exclusion calibration curves for both the original and the deprotected poly(TBMA-*co*-EDMA) beads (Figure 4) are essentially identical, which indicates that the decrease in pore volume determined by mercury porosimetry only occurs after the deprotected beads are dried. In the fully swollen state, the pore volumes and size distributions for the original and deprotected beads are the same.

Scanning electron micrographs of the poly(methacrylic acid-*co*-ethylene dimethacrylate) and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads shown in Figure 6 confirm their monodispersity and reveal a typical macroporous structure. The surface of the beads is covered with indentations that result from the incompatibility of the polystyrene-shape templates with the polymerizing methacrylate matrix.[34]

Chiral Stationary Phases. One of the numerous applications for the functional monodisperse beads is the preparation of highly selective chiral separation media for the HPLC of enantiomers. Our earlier findings with chiral separation media revealed that the nature of the support significantly affects the selectivity of chiral separations.[27] Therefore, we used both the poly(methacrylic acid-*co*-ethylene dimethacrylate) and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads for the preparation of chiral separation media.

Chiral stationary phase (CSP) **11** is easily prepared by the coupling of a valine-based chiral selector to the poly(MAA-*co*-EDMA) beads using a two-step reaction sequence shown in Scheme 3. A similar chiral selector derived from proline had earlier proven to be effective in the separation of π-acidic compounds.[35] Although a rather high quantity of the selector (1.5 mmol/g) can be loaded onto the surface of beads **11** as determined by elemental analysis, 50% of all available carboxyl groups remain unfunctionalized. To avoid nonselective interactions from these unreacted carboxyl groups, which might detract from the efficiency of the chiral stationary phase, they were converted to the more innocuous methyl ester by reaction with iodomethane. Following this capping step, titration data suggest that 95% of the remaining carboxyl groups are now esterified, but even repeated esterification reactions do not lead to complete capping.

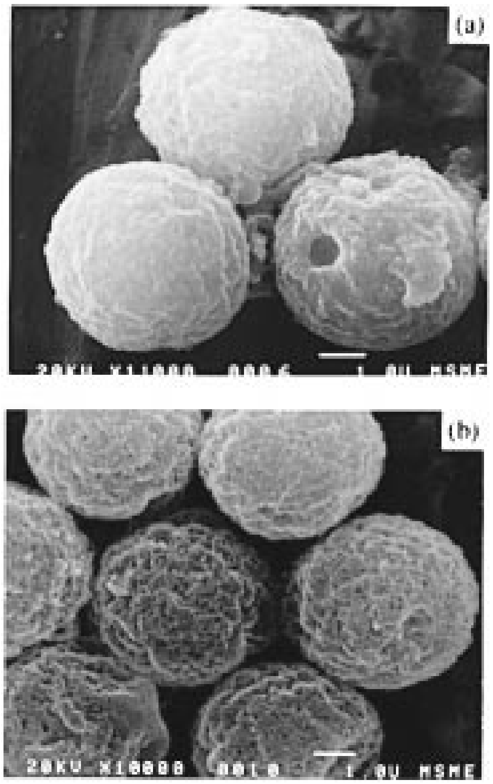
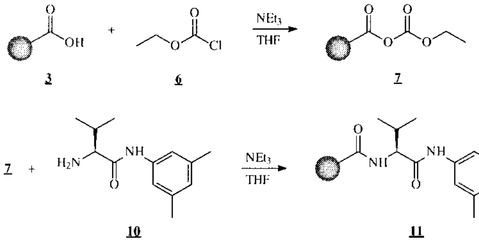


Figure 6. Scanning electron micrographs of 5.0 μm poly(methacrylic acid-*co*-ethylene dimethacrylate) (a) and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads (b).

Scheme 3



CSP **11** was used for the separations of several derivatives of racemic α-amino acids. Table 3 summarizes the results of these chromatographic measurements. This chiral stationary phase exhibits only a modest enantioselectivity characterized by selectivity factor $\alpha = 1.4-2.6$ for most of the analytes under normal-phase chromatographic conditions. Although these selectivities are in the range typical of many commercial separation media, they do not reach the level expected for polymer-based CSPs, most likely as a result of nonspecific interactions resulting from the presence of residual carboxylic acid moieties.

Chiral stationary phase **12** was prepared by coupling the valine-based chiral selector to poly(HEMA-*co*-EDMA) beads using the simple reaction sequence shown in Scheme 4. In contrast to the poly(methacrylic acid)-

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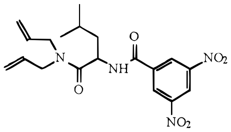
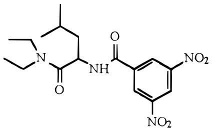
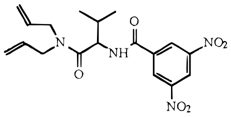
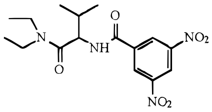
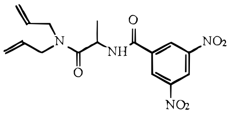
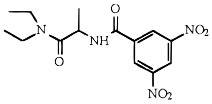
(34) Hosoya, K.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 2129.

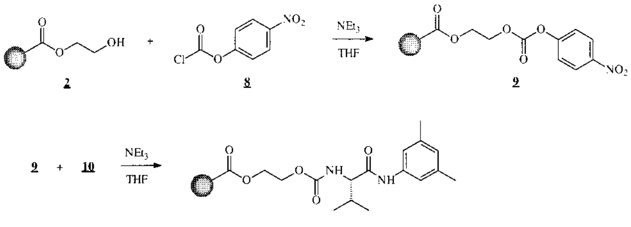
(35) Pirkle, W. H.; Murray, P. G. *J. Chromatogr.* **1993**, *641*, 11.

Table 3. Retention Factors *k′* and Separation Factors α for Enantioselective Separations of Racemic Compounds on 11 and 12[a]

	11		12	
	k'_2	α	k'_2	α
	0.78	1.39	2.75	12.11
	0.82	1.50	2.87	13.20
	0.64	1.00	1.15	3.59
	0.71	1.05	0.85	3.64
	1.38	2.27	1.93	9.05
	1.37	2.31	2.02	9.48

[a] Chromatographic conditions: column, 150 mm × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min; void marker 1,3,5-tri(*tert*-butyl)benzene; UV detection at 254 nm.

Scheme 4



based phase, CSP **12** exhibits excellent enantioselectivities for all of the separations attempted, with a separation factor α reaching values up to 13. These values are much higher than those obtained for CSPs we prepared earlier by a less controlled multistep process from glycidyl methacrylate beads.[27] The lack of nonspecific interaction sites, combined with a high selector loading results in considerably enhanced separation factors (Figure 7). It can be assumed that these values more closely approach the intrinsic capability for enantioselectivity of the chiral selector itself based on the differences in free energies (ΔΔG) of the two disastereomeric complexes formed between the selector and two enantiomers under the given conditions. A detailed description of the chromatographic separations themselves will be published elsewhere.[36]

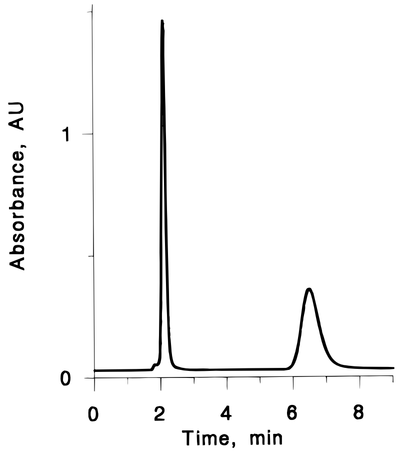


Figure 7. Separation of 3,5-dinitrobenzamidoleucine-*N*,*N*-diallylamide enantiomers on chiral stationary phase **12**: column, 150 mm × 4.6 mm i.d.; mobile phase, 20% hexane in dichloromethane; flow rate, 1 mL/min.

Conclusion

Porous monodisperse poly(methacrylic acid-*co*-ethylene dimethacrylate) and poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) beads with controlled porous properties and well-defined contents of functionalities, are easily accessed through the staged templated suspension polymerization. Although the preparation of the former beads requires the copolymerization of *tert*-butyl methacrylate followed by a deprotection reaction, the latter can be prepared readily in a single polymerization step. The desired porous properties and content of reactive functionalities can be achieved by controlling the composition of the polymerization mixture. In contrast to common perception, the number of accessible functionalities does not depend much on the magnitude of the bead surface area. It is anticipated that these beads may be useful not only for the preparation of very selective chiral separation media but also in the preparation of polymeric reagents or of supports for solid-phase chemistry.

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CM9705505

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(36) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.*, submitted.