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Preparation and characterization of monodisperse oligo(ethylene glycol) dimethacrylate polymer beads for aqueous high-performance liquid chromatography

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Abstract

Three types of oligo(ethylene glycol) dimethacrylate were polymerized by seed polymerization. The gel beads so prepared had a narrow size distribution and were macroporous. The columns packed with gel beads were suitable for aqueous size-exclusion chromatography for polysaccharides, proteins and other hydrophilic polymers. The hydrophilicity of the gel beads was almost equal to that of 2-hydroxyethyl methacrylate–ethylene dimethacrylate copolymer beads which are usually used for aqueous size-exclusion chromatography.

1. Introduction

Aqueous high-performance liquid chromatography (HPLC) is a suitable tool for the separation and analysis of natural polymers such as proteins and polysaccharides [1–7]. Polymeric packing materials used in aqueous HPLC are found to be superior stationary phases to silica-based packing materials with respect to the chemical resistance and the reproducibility [6,7]. Polymer beads used as the packing materials are currently prepared by conventional suspension polymerization. The resulting beads have broad size distributions and cannot be used for chromatography without time-consuming size fractionation. Therefore, monodisperse packing materials are needed in order to eliminate the need for the size fractionation process.

Monodisperse styrene–divinylbenzene copolymer beads have been prepared by seed polymerization with a multi-step swelling process [8–11]. It has been reported that these beads are superior as stationary phases to those prepared by conventional suspension polymerization [8,9]. Only a few other monomers have been polymerized by seed polymerization [12].

We have prepared monosized styrene–divinylbenzene gel by seed polymerization with single-step swelling and found that the gel was a good packing material for gel permeation chromatography [13]. This paper describes the preparation of monodisperse hydrophilic polymer gel beads by seed polymerization and the evaluation

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of the beads for aqueous HPLC from the viewpoint of pore size and hydrophilicity.

2. Experimental

2.1. Synthesis

Three types of oligo(ethylene glycol) dimethacrylate (degree of oligomerization 2, 3 and 3–4; abbreviated to 2G, 3G and 4G, respectively) were provided by Shin Nakamura Chemical (Wakayama, Japan) and used as received. Reagent-grade styrene was obtained from Tokyo Kasei (Tokyo, Japan) and used after distillation under reduced pressure. All other materials were used without further purification.

Monodisperse polystyrene seed particles with a diameter of 1.7 μm were prepared by the dispersion polymerization of styrene in aqueous ethanol as described in detail elsewhere [13].

In a 500-ml flask fitted with a mechanical stirrer were placed the seed dispersion (0.1 g/ml) and 50 ml of water containing 0.5% (w/w) of poly(vinyl alcohol) (PVA), and the mixture was stirred slowly. A mixture of 15 g of monomer, 15 g of butyl acetate (porogen) and 0.3 g of 2,2′-azobis(2,4-dimethylvaleronitrile) initiator was emulsified in 300 ml of 9.0% (w/w) NaCl solution containing 0.5% (w/w) of PVA with an ultrasonic disruptor (UD-200; TOMY, Tokyo, Japan) until the particle size of the oil drops became smaller than 0.5 μm. The swelling ratio was changed from 50 to 200 by using various amounts of seed particles. Then, one third portion of the emulsion was added dropwise to the dispersion of the seed particles, with stirring over 15 min. The second and third one-third portions of the emulsion were added after 1 h intervals in the same way. The mixture was stirred for 24–48 h at room temperature so that all of emulsified organic phase transferred into the polymer seed. The temperature was then increased to 80°C and the polymerization was carried out for 10 h. The resulting gel beads were washed successfully with hot water, acetone and tetrahydrofuran 2–4 times, followed by drying in vacuo.

2.2. Characterization of gel beads

Gel beads (10 g) dispersed in 50 ml of distilled water were packed into a stainless steel HPLC column (30 cm × 7.6 mm I.D.) by a slurry method. Size-exclusion chromatographic (SEC) calibrations of pullulan (Shodex, Showa Denko, Tokyo, Japan) were obtained using distilled water as an eluent (0.5 ml/min) with a JASCO (Tokyo, Japan) PU800 pump and a JASCO Model 830 refractive index detector. The standard sample concentration was 1% (w/w) and 10-μl portions of samples were injected through a Rheodyne (Cotati, CA, USA) Model 7125 loop injector. Protein samples (bovine albumin), ovalbumin, myoglobin and cytochrome *c*) were supplied by Showa Denko. The sample concentration was 0.1% (w/w) and phosphate buffer (1/15 mol/l, pH 7) eluent and a JASCO Model 880 UV detector were used. The hydrophilicity of the gel beads was evaluated from the elution times of normal alcohols (ethanol, 1-propanol and 1-butanol).

The surface morphology of the beads was analysed by scanning electron microscopy (JSM-35CF; JEOL, Tokyo, Japan).

3. Results and discussion

Table 1 presents the preparation conditions and size uniformity of the gel beads. All the gels had fairly narrow size distributions, with relative standard deviations (R.S.D.s) smaller than 17%. The size distribution of the gel tended to become broader with increase in oxyethylene units in the monomer. A possible explanation is that the solubility of the monomer in the aqueous phase increases with increasing number of oxyethylene units, which decreases the dissolution rate of the monomer in the seed particle. For the 3G gel, the R.S.D. increased with increase in the swelling ratio and decreased with increase in the swelling time.

The diameter of the gel particles (d_g) can be calculated from the diameter of the seed particle (d_s) and the swelling ratio (S):

Table 1
Preparation conditions and size uniformity of gel beads

Gel	Swelling ratio[a]	Absorption time (h)	R.S.D. (%)	Diameter (μm)	
				Observed[b]	Calculated
2G	50	24	5.8	5.5	6.3
3G-1	50	24	7.9	5.8	6.3
3G-2	100	24	15.4	8.0	7.9
3G-3	100	48	6.2	7.5	7.9
3G-4	200	48	17.2	9.7	10.0
4G	50	24	16.1	5.5	6.3

[a] Ratio of organic phase to seed.
[b] Mass-average diameter measured by Coulter multisizer.

$$d_g = d_s S^{1/3}$$

The observed and calculated gel diameters are given in Table 1. Although the observed values were slightly smaller than the calculated values, the particle size can be controlled by the initial seed diameter and the swelling ratio. The difference between the observed and calculated values can be explained by shrinkage of the beads accompanying the polymerization and drying processes.

Šmigol et al. [14] reported that the amount of the monomer phase absorbed does not exceed 65 times the volume of the polymer particles even in the best case, which are prepared using seed particles obtained by an emulsifier-free emulsion polymerization. As reported elsewhere [13], the polystyrene seed particles prepared by dispersion polymerization have a superior ability to absorb the organic phase compared with those prepared by an emulsifier-free emulsion polymerization.

Fig. 1 shows the scanning electron micrographs of 3-G-1 and demonstrate the uniformity in size and macroporous structure of the gel bands.

The prepared gel beads were packed into stainless-steel columns by a slurry method. Table 2 gives the characteristics of the columns packed with the various gel beads prepared. Each column had a pressure resistance higher than 150 kg/cm^2. The calibration graphs for the column packed with 2G, 3G or 4G beads are shown in Fig. 2. When pullulan was used as a sample and distilled water as the eluent, the samples eluted in order of decreasing molecular mass before the solvent. Each column has a molecular mass exclusion limit of over $1 \cdot 10^6$, which indicates that the columns can be used for the determination of water-soluble high-molecular-mass species. From the shape of the calibration graphs, it is found that an increase in oxyethylene units slightly decreased the pore size. For 3G gel, a slight difference in the shape of the calibration graph was observed between the gels prepared with swelling ratios of 50 and 100.

The elution behaviour of water-soluble proteins was investigated, and the chromatograms are shown in Fig. 3. Symmetrical peaks were obtained without leading or tailing. The relationships between the molecular mass and the elution volume are shown in Fig. 2. They also eluted in order of decreasing molecular mass although the elution volume is slightly higher than that for pullulan with the same molecular mass. It is clear that their elutions were governed mainly by the size-exclusion mechanism.

The hydrophilicity of the gel beads was evaluated from the elution volumes of ethanol, 1-propanol and 1-butanol using water as the eluent. The elution volume of these alcohols increased with increase in molecular mass or in the length of the alkyl chains. Therefore, the

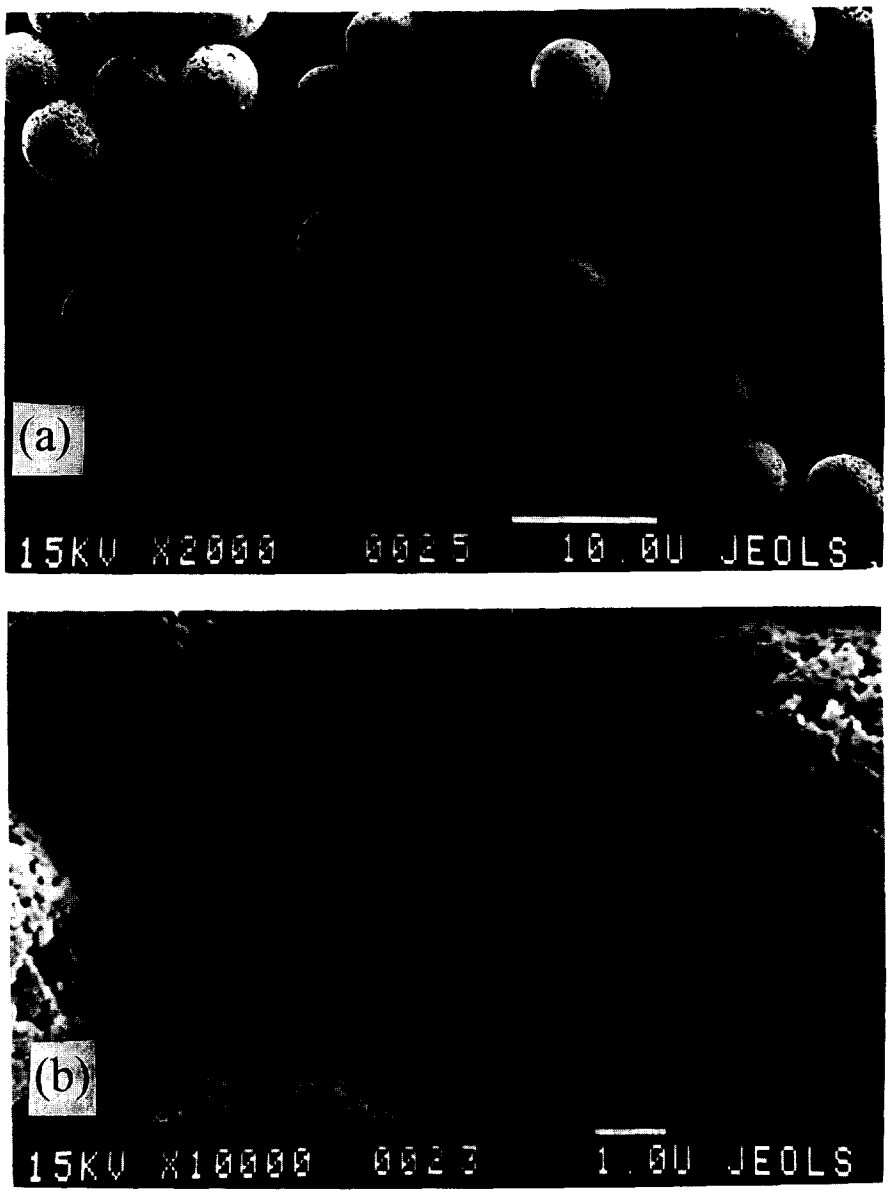

Fig. 1. Scanning electron microscope photographs of 3G-1 beads (a) ×2000; (b) ×10 000.

elution behaviour is determined not by a size-exclusion mechanism but by an adsorption or a partition mechanism. In Fig. 4, the logarithm of capacity factors is plotted against the hydrophobic parameter (log P) calculated according to the method of Leo et al. [15], which is based on the fragment factors of sample molecules. Positive linear relationships were obtained between log k' and log P for all columns examined, which indicates that the elution was governed by hydrophobic interactions between the gel beads and the alcohols. With increase in the oxyethylene units, the position of the straight line shifted downwards and the slope of the line became

Table 2
Characteristics of HPLC column

Gel	Flow-rate[a] (ml/min)	Pressure[b] (kg/cm^2)	N[c]	Exclusion limit[d] ($\times 10^4$)
2G	2.0	153	8700	>100
3G-1	1.5	160	16000	>100
3G-3	1.4	150	7500	>100
4G	1.3	155	5000	>100

[a] Final flow-rate in packing procedure.
[b] Final pressure.
[c] Number of theoretical plates determined with ethylene glycol (flow-rate 0.5 ml/min).
[d] Molecular mass of exclusion limit.

shallow. This indicates that the hydrophilicity of the gels increases in the order 2G, 3G and 4G. For comparison, the results are also plotted for the column (30 cm × 7.6 mm I.D.) packed with 2-hydroxyethyl methacrylate–ethylene dimethacrylate (HEMA–EDMA) gel, which was prepared as described elsewhere [16]. HEMA–EDMA gel is widely used as an aqueous SEC packing material. As shown in Fig. 4, the hydrophilicity of 4G beads is almost equal to that of HEMA–EDMA gel.

4. Conclusions

Hydrophilic polymer gel beads were prepared by the combination of dispersion polymerization and seed polymerization. The seed particles

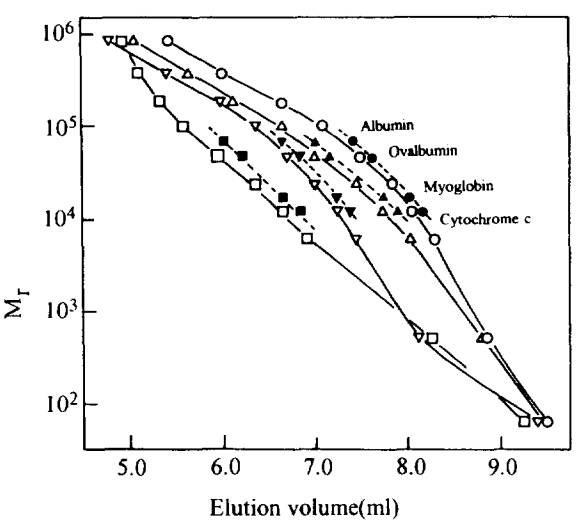

Fig. 2. Calibration graphs for columns packed with the prepared gel beads. ○ = 2G; △ = 3G-1; ▽ = 3G-3; □ = 4G. Open symbols, pullulan (water); filled symbols, proteins (phosphate buffer). Column, 30 cm × 7.6 mm I.D.; flow-rate, 0.5 ml/min.

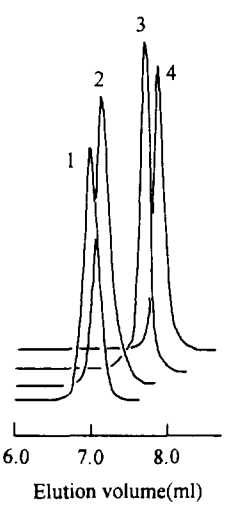

Fig. 3. Chromatograms of proteins obtained using the 3G-1 column. Peaks: 1 = (bovine albumin); 2 = ovalbumin; 3 = myoglobin; 4 = cytochrome *c*. Eluent, phosphate buffer (1/15 mol/l, pH 7); flow-rate, 0.5 ml/min.

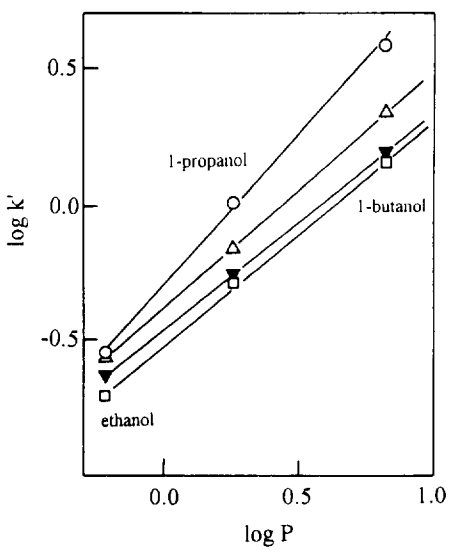

Fig. 4. Relationship between capacity factor and hydrophobic parameter ○ = 2G; △ = 3G-1; □ = 4G; ▼ = HEMA–EDMA. Column, 30 cm × 7.6 mm I.D.; eluent, distilled water (0.5 ml/min).

absorbed up to a 200-fold amount of organic phase while retaining uniformity of size. The gel beads so obtained were monodisperse with R.S.D.s less than 17%. They had pressure resistances higher than 150 kg/cm^2 and a macroporous structure. It was found that gel beads were suitable as packing materials for aqueous SEC columns. The hydrophilicity increased as the number of oxyethylene units increased. The 4G gel showed a similar hydrophilicity to a HEMA–EDMA gel.

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