Preparation of uniformly sized polymeric separation media potentially suitable for small-scale high-performance liquid chromatography and/or capillary electrochromatography

Ken Hosoya*, Hiroaki Ohta, Kimihiro Yoshizako, Kazuhiro Kimata, Tohru Ikegami, Nobuo Tanaka

Department of Polymer Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Abstract

Uniformly sized polymer particles were prepared by either a two-step swelling and polymerization method or a Shirasu porous glass (SPG) emulsification technique to compare their suitability as a uniformly sized packing material for small-scale high-performance liquid chromatography (HPLC) or capillary electrochromatography (CEC). The SPG emulsification technique afforded slightly worse size uniformity compared to the two-step swelling and polymerization technique. However, fairly nice spherical shapes with reproducible outward appearance and internal pore size distribution were obtained for both of 3- or 6-μm sized particles. On the other hand, the two-step swelling and polymerization method afforded broken particles with quite different outward appearances due to the polymeric porogen effects of seed polymer utilized as the shape template. In HPLC, the column packed with the 3-μm particles prepared with the SPG emulsification technique proved to have a fairly high column efficiency with good column stability, as assessed under repeated use in gradient elution. On the other hand, the column packed with the packing material obtained through the two-step swelling and polymerization method showed much poorer column stability, while initial column efficiency was compatible to that obtained using the SPG emulsification method. In addition, the packing material prepared with the SPG emulsification technique could be modified with ion-exchangeable monomers and showed fairly good column efficiency in the CEC mode. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Electrochromatography; Stationary phases, Electrochromatography; Polymer stationary phases; Polystyrene; Alkylbenzenes

1. Introduction

Capillary electrochromatography (CEC) is a combination of electrophoresis and chromatography, which can afford excellent chromatographic efficiency [1–5]. Usually, silica-based packing materials that can be utilized directly in high-performance liquid chromatography (HPLC) are used as packing materials for CEC [6,7]. Although continuous rod-type separation media derived from polymer or silica can be used directly as the separation bed, due to their easy handling in CEC column technology [8–10], packing materials having small diameters, such as 3 μm or smaller, are still applicable for CEC because of an ease in a variety of surface modifications.

We have been working on the preparation of uniformly sized polymeric separation media for HPLC [11] through a two-step swelling and poly-

*Corresponding author. Fax: +81-75-724-7710.
E-mail address: kenpc@ipc.kit.ac.jp (K. Hosoya)

merization method [12]. This method can afford uniformly sized polymer particles, however, due to the inevitability of seed polymer particle as shape template, final polymer particles must be affected in terms of physical and/or chromatographic characteristics through so-called polymeric porogen effects [13,14].

Once the seed polymer, which is usually polystyrene, can be dissolved in monomer and porogen utilized for the swelling, it acts as a polymeric porogen, which gives rise to relatively large pores in the final polymer particles. On the other hand, a hole-like crater should be formed through severe phase separation if the seed polymer cannot be dissolved in the swelling system [13]. In either case, seed polymer must affect the characteristics of the final polymer particles. Therefore, it can be easily imagined that the effects of seed polymer become more serious when smaller particles are required.

On the other hand, a special emulsification technique using a glass membrane with a relatively uniform pore has been reported for the preparation of uniformly sized polymer particles [15–18]. The so-called SPG (Shirasu porous glass) emulsification technique is one of those techniques, and was firstly reported by Nakashima et al. [19]. In this relatively new technique, no seed polymer is required, therefore, the effect of seed polymer (mentioned above) will be ignored.

We used this technique to prepare uniformly sized polymer particles having 6- and 3-μm diameters and have compared the physical and chromatographic characteristics of the particles prepared with those of the two-step swelling and polymerization method. In addition, uniformly sized polymer particles having ion-exchange groups were also prepared using the SPG emulsification technique, for use as a stationary phase for capillary electrochromatography (CEC).

2. Experimental

2.1. Materials

Styrene and divinylbenzene (tech., 80%) were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma–Aldrich Japan (Tokyo, Japan), respectively, and both were used after distillation to remove polymerization inhibitors. Methacrylic acid (MA), which was as an ion-exchange group for CEC, was purchased from Wako (Osaka, Japan) and used as received, while vinylbenzoic acid was prepared using a standard method.

Toluene, of the highest grade available, which was used as a porogenic solvent, was also purchased from Nacalai Tesque and was used as received. A radical initiator, *tert.*-butyl peroxy 2-ethylhexanoate was a gift from Japan Oils and Fats. Polyvinyl alcohol (degree of polymerization, DP=2000, saponification value=80%) was purchased from Sigma–Aldrich Japan. Sodium dodecylsulfate (SDS) was purchased from Nacalai Tesque and used as received.

2.2. Preparation of uniformly sized polymer particles

2.2.1. Two-step swelling and polymerization method

Details of the two-step swelling and polymerization method were reported previously, with detailed experimental conditions for 6-μm particles being given [13]. Particles with a 3-μm diameter were prepared as follows:

Aqueous dispersion of polystyrene seed polymer (0.06 ml in 1 ml of water), 6.4 ml was directly treated with an aqueous suspension prepared using 2.5 ml of styrene, 2.5 ml of divinylbenzene, 5 ml of toluene and 0.05 ml of *tert.*-butyl peroxy 2-ethylhexanoate in water containing 0.75 g of polyvinyl alcohol as well as 60 mg of SDS by sonication. In this case, activating solvent, which is usually used in the first swelling step, could not be used due to the size limitation of the final particles. Therefore, this preparation method is a one-step swelling and polymerization method.

The polymerization process was carried out at 70°C for 24 h with slow stirring (ca. 80 rpm) for both 6- and 3-μm particles.

2.3. SPG emulsification technique

The preparation methods for 6- and 3-μm particles using the SPG emulsification technique were as follows: A mixture containing 5.0 ml of styrene, 5.0 ml of divinylbenzene, 10 ml of toluene and 0.1 ml of

tert.-butyl peroxy 2-ethyl hexanoate was emulsified through SPG membranes having a mean pore size of 0.90 μm (for 6-μm size particle) or 0.56 μm (for 3-μm size particle). SPG membranes as well as SPG emulsification equipment (MPG micro kit) were purchased from Ise Chemical (Chiba, Japan).

The aqueous suspension of monomers obtained was then used in the polymerization process. The polymerization process was also carried out at 70°C for 24 h with slow stirring (ca. 80 rpm). The polymer particles obtained by the swelling as well as the SPG methods were repeatedly washed with methanol, tetrahydrofuran (THF) and acetone, to remove unbound impurities. The particles were packed into a stainless steel column (150×4.6 mm I.D.) using a slurry method, with a mixture of water, acetonitrile and 2-propanol as the slurry medium.

When preparing beads for CEC, methacrylic acid or vinylbenzoic acid (10%, w/w, to the amount of monomers of particles) were just added to the dispersion of 3-μm particles, 4 h after the start of polymerization of the particles. In this case, an additional 20 h of polymerization took place.

2.4. HPLC

The HPLC system was equipped with a Jasco model 880-PU pump with a Rheodyne model 7125 injector. Detection was with a Tosoh model UV 8000 detector at 254 nm and a Shimadzu model C-R3A integrator was employed.

2.5. CEC

The capillary column (300×100 μm I.D.) was packed using the slurry method, as mentioned previously, using a chromatographic high-pressure pump. The column frits were prepared using water glass by the heating technique. CEC measurement was performed with a Hewlett-Packard model G1600A. Detection was carried out at 200 nm.

3. Results and discussion

Particle size distributions before and after the polymerization are depicted in Fig. 1 and Fig. 2. As
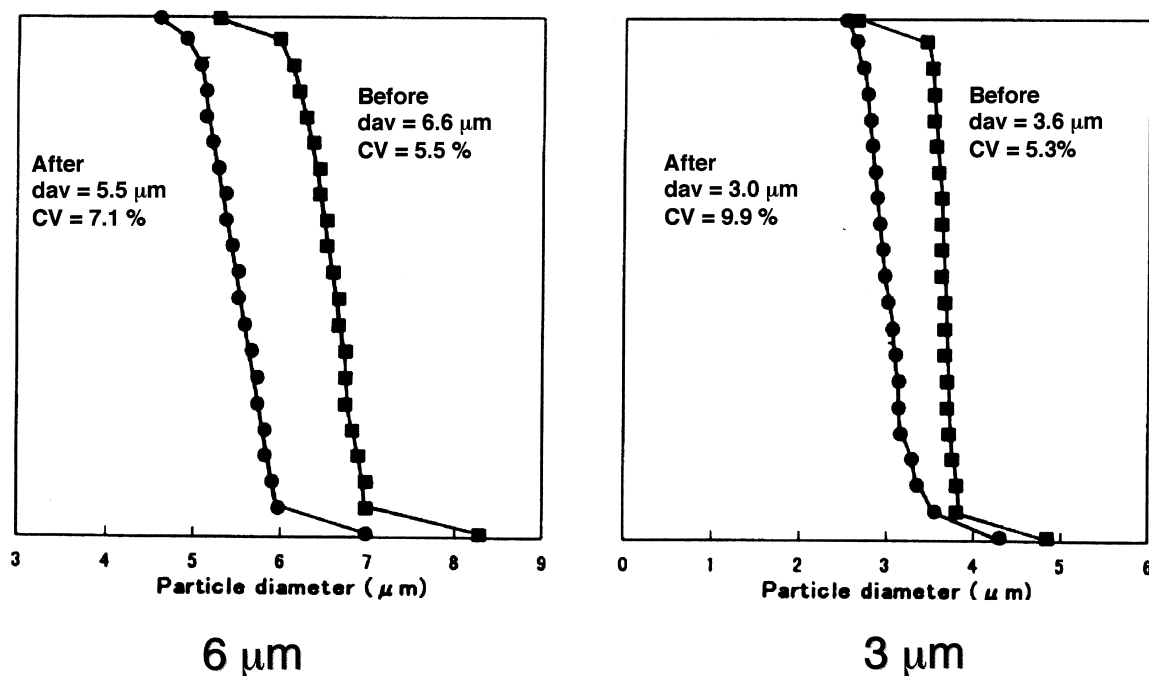

Fig. 1. Particle size distribution of the particles prepared by the two-step swelling and polymerization method. CV=RSD. dav=Average diameter of particles.
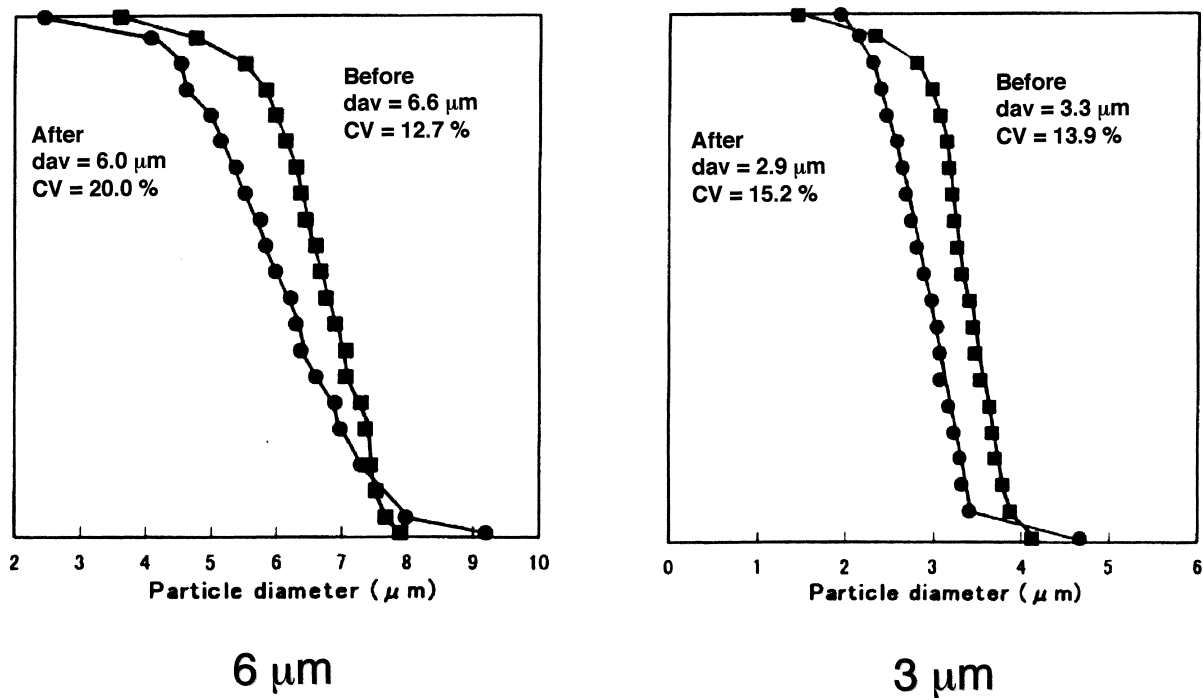
Fig. 2. Particle size distribution of the particles prepared by the SPG emulsification method.

expected, the two-step swelling and polymerization technique gave better RSD (coefficient of variation) values before and after the polymerization than those obtained using the SPG emulsification technique. This is probably because SPG membranes do not have really uniform pore sizes. In fact, the particle size distribution obtained in this paper is even compatible with those reported previously by Omi [17]. Although the RSD values observed with the SPG emulsification technique were even worse than those obtained with the two-step swelling and polymerization method, the particles obtained still had good size uniformity compared with those obtained by the traditional suspension polymerization method [13].

The relationship between the size of emulsion droplets and the mean pore size of the SPG membrane was reported previously as 6.62 [15] or 3.25 [19], while we obtained values of 7.3 for 6-μm particles and 5.8 for 3-μm particles. These values were slightly different from the reported values. This difference was probably due to our technical inexperience, but the chemical composition of the par-

ticles must be identical to each other regardless of the mean particle size obtained.

Scanning electron micrographs of the four kinds of particles are demonstrated in Figs. 3 and 4. Obviously, the outward appearance of the particles of two different sizes prepared by the two-step swelling and polymerization method looked quite different, while the two different sized particles prepared by the SPG emulsification method looked similar in appearance. In this case, the monomer and porogen used were styrene and divinylbenzene, and toluene, respectively, therefore, the seed polymer (polystyrene) must be dissolved in the swollen system in the two-step swelling method.

During the following polymerization process, the dissolved polymer acts as a polymeric porogen, resulting in different rather rough surface between the 3- and 6-μm particles [13]. If we think about the magnitude of swelling in the 3- or 6-μm particles, the concentration of the seed polymer in the swollen system is quite different. In the case of 6-μm particles, it makes up less than 1% of the concentration, whereas it makes up 4% of the con-
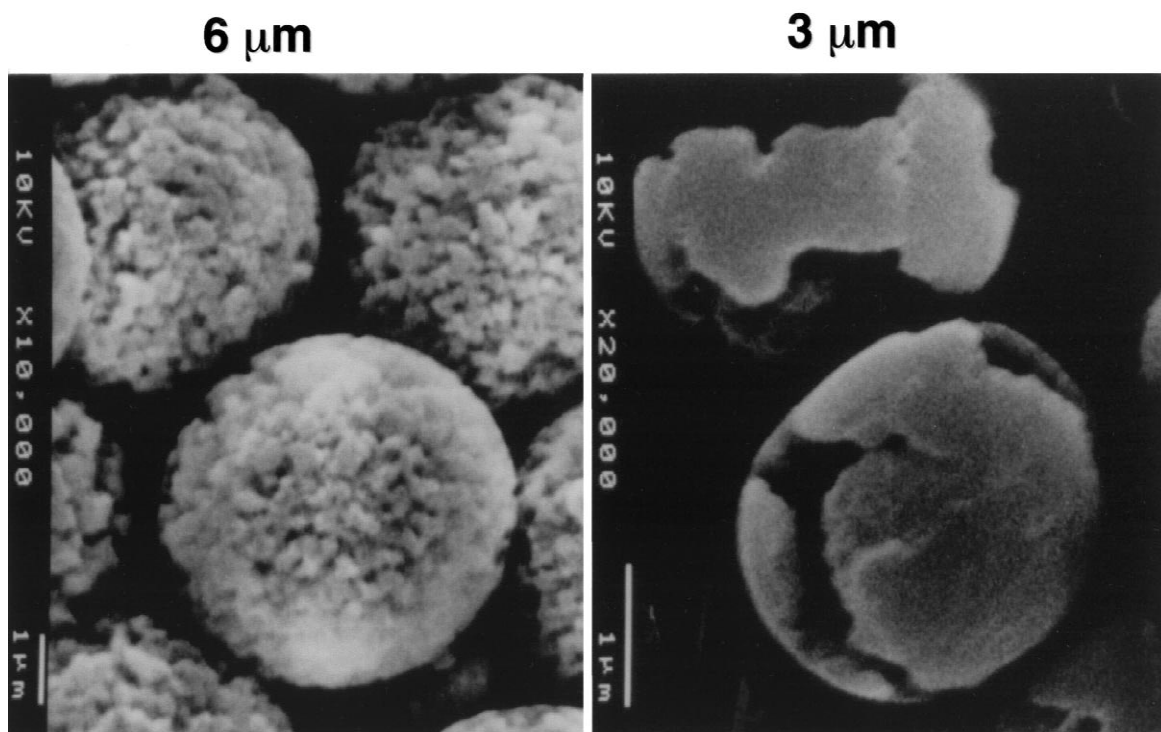
Fig. 3. SEM pictures of the particles prepared by the two-step swelling and polymerization method.

centration in the 3-μm particles. Therefore, the effect of the seed polymer was not the same, resulting in the rather different outward appearances of the particles [20]. This has clearly been a problem in the two-step swelling and polymerization method and is more serious when 3-μm and even smaller particles are prepared.

Pore size and distribution were studied using a size-exclusion method (in THF) with standard polystyrene samples as well as a series of alkylbenzenes. In the case of 3-μm particles prepared using the two-step swelling method and the SPG method, the observed calibration curves in the size-exclusion mode were rather different when larger volumes of macropores were involved for particles prepared by the two-step swelling method (Fig. 5). This can be clearly explained by the effect of polymeric porogen, which tends to create larger pores compared with small molecule porogen [20]. On the other hand, the calibration curves observed for both 3- and 6-μm particles prepared by the SPG method were similar to each other, as shown in Fig. 6. This finding proves

that the SPG method does not affect the structural characteristics inside the particles or the outward appearance, as demonstrated before.

Using an optimized packing technique, both types of 3-μm particles were packed into stainless steel columns (150 mm×4.6 mm I.D.) to compare their chromatographic efficiencies. As shown in Fig 7, even the use of the broken particles, i.e., the packing material prepared by the two-step swelling and polymerization technique, showed excellent chromatographic efficiency under reversed-phase conditions. Interestingly, the column efficiency obtained for the particles prepared by the two-step swelling and polymerization technique was almost equal to the column efficiency obtained for the particles prepared with the SPG emulsification technique. However, this is not surprising as even non-spherical particles can have equal column efficiency to spherical particles if their mean particle size is similar [21]. In addition, the column pressure drop found on both columns was also identical under reversed-phase conditions, being around 7 MPa.
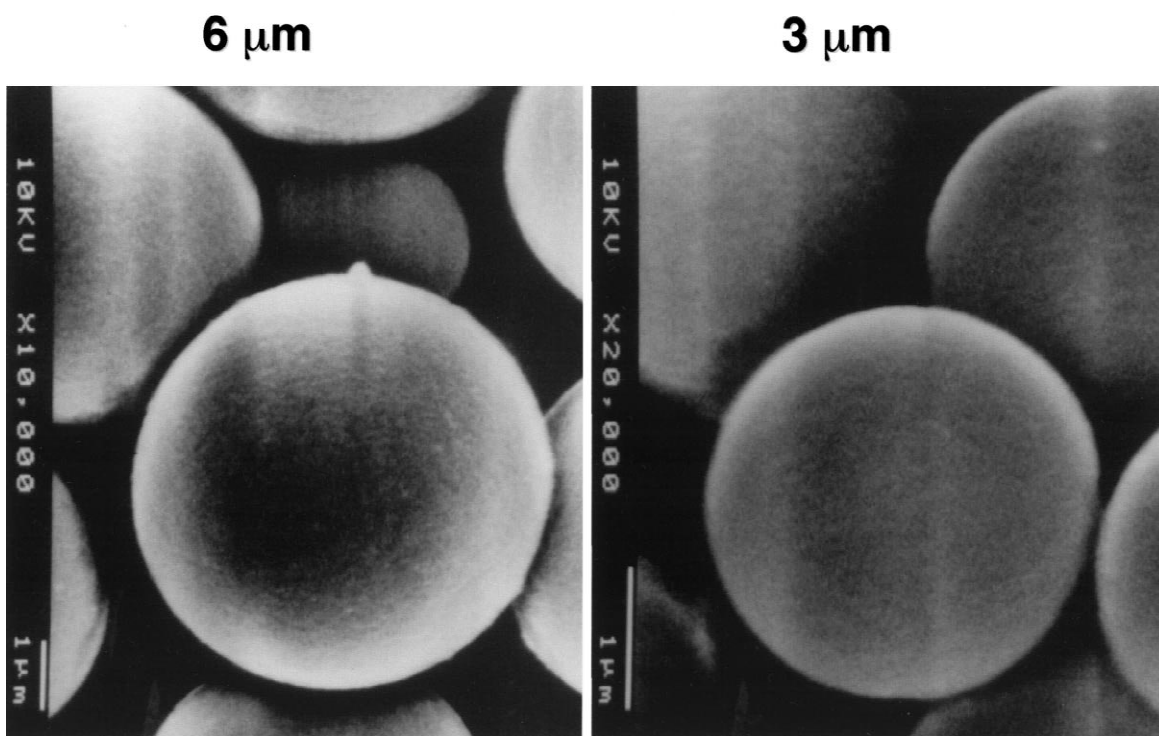
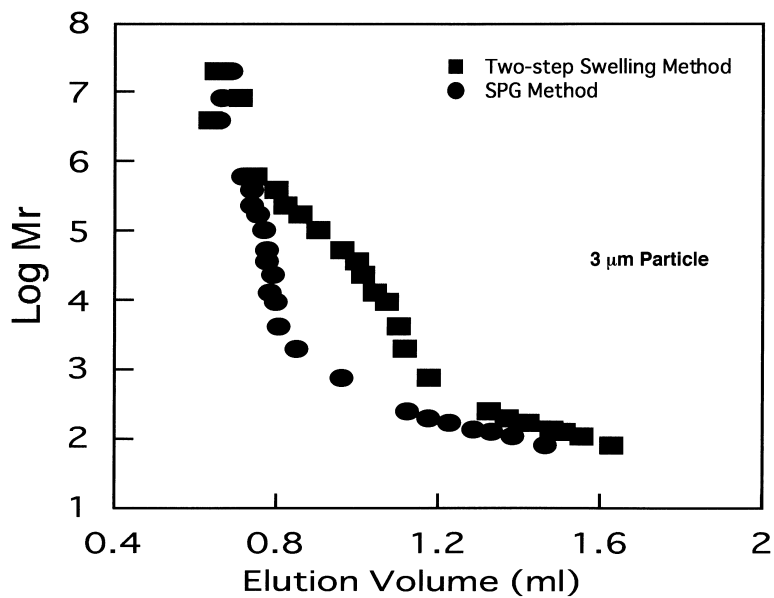Fig. 4. SEM pictures of the particles prepared by the SPG emulsification technique.

Fig. 5. SEC calibration curves for 3-μm particles Chromatographic conditions: mobile phase, tetrahydrofuran; flow-rate, 0.5 ml/min.; samples, polystyrene standards and alkylbenzenes; detection, UV at 254 nm.
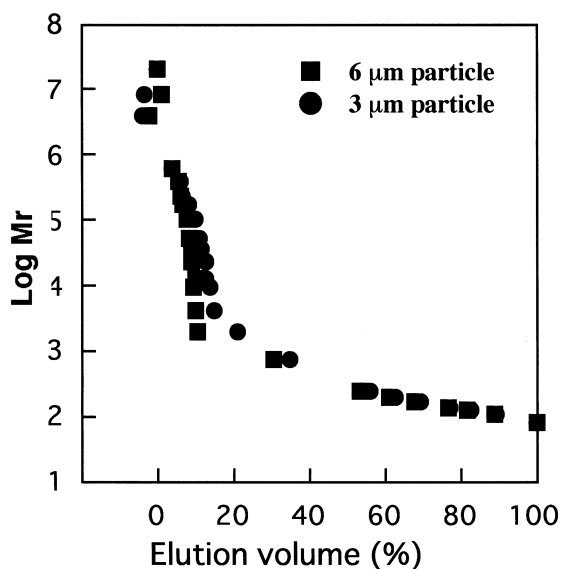
Fig. 6. SEC calibration curves for 3- and 6-μm particles prepared by the SPG emulsification method.

One of the main problems of non-spherical packing materials reported is instability of the packed column bed. In fact, if the packed two columns were tested through gradient reversed-phase conditions where column pressure drop should be changed due to the mobile phase composition. The column efficiency of the particles prepared by the two-step swelling and polymerization technique was dramatically decreased (larger h values), while quite stable phenomena were observed on the column packed with the particles prepared using the SPG emulsification method, as depicted in Fig. 8. These kinds of problems have not been encountered with 5 μm particles or larger, because the particle shape was found to be spherical, as shown in Fig. 3 in this study.

In situ surface modification [22] can also be achieved with the particles prepared using the SPG emulsification technique and, in this study, we applied vinylbenzoic acid or methacrylic acid as
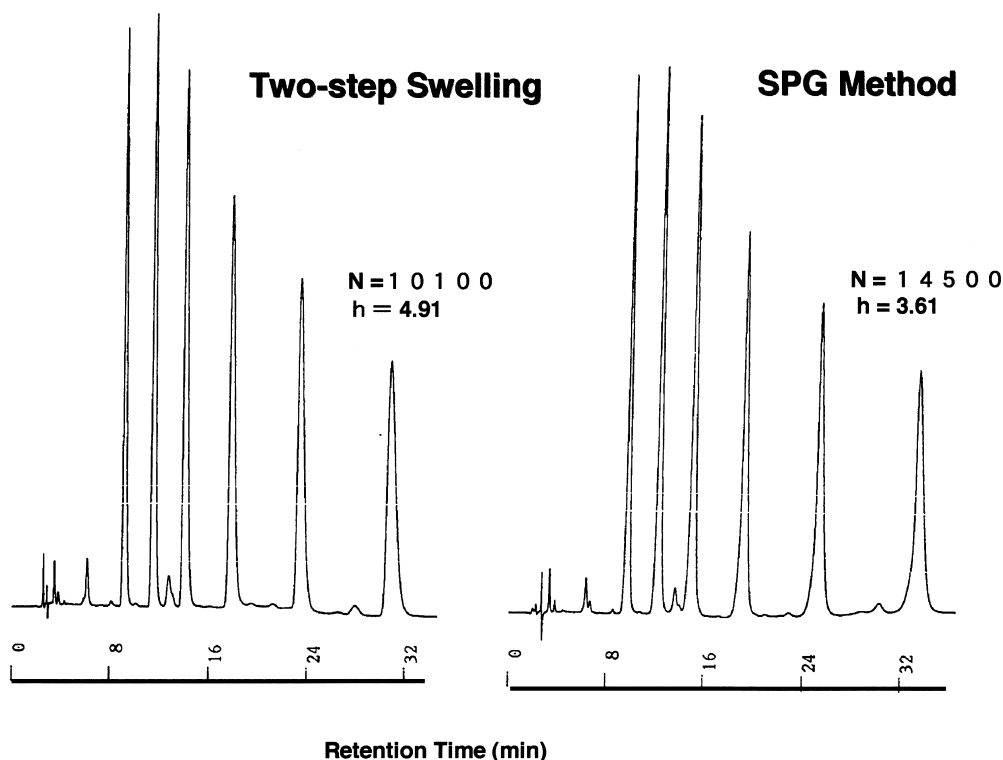

Fig. 7. Chromatograms of the separation of alkylbenzenes. Chromatographic conditions: mobile phase, 80% aq. acetonitrile; flow-rate, 1.0 ml/min.; detection, UV at 254 nm; samples, alkylbenzenes, benzene to pentylbenzene at 30°C.
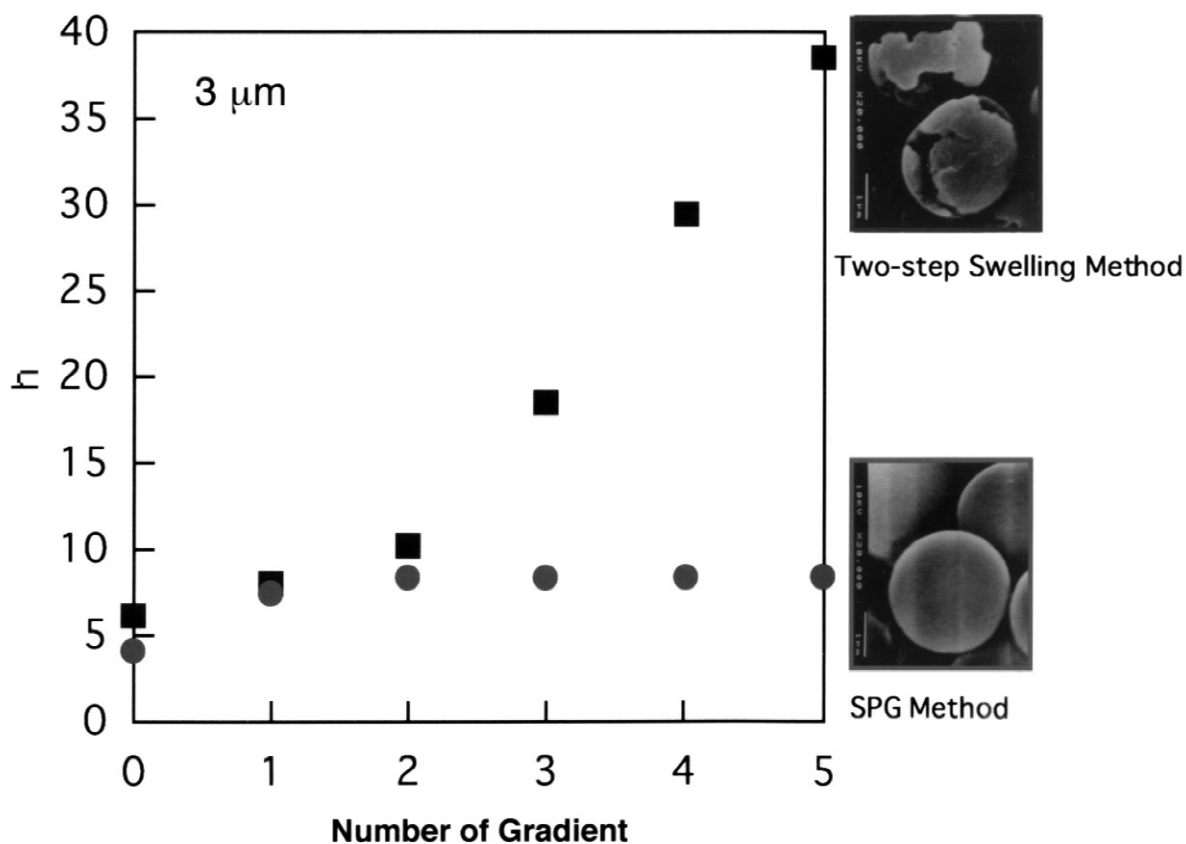
Fig. 8. Tests to determine column stability by repeated use under gradient elution conditions. Gradient: from 80 to 50% aq. acetonitrile over 10 min at 0.5 ml/min. Sample, propylbenzene.

acidic monomers in CEC mode. In this modification method, functional monomer for a desired modification can be added directly during the polymerization process of the uniformly sized monomer dispersion. In this case, the modification yield was nearly quantitative, as determined using a reported titration method.

The prepared surface-modified polymer particles were directly packed into capillaries (100-μm I.D.) using the slurry technique to evaluate chromatographic efficiency under CEC conditions. The separation of alkylbenzenes in 80% acetonitrile with buffer solutions is depicted in Fig. 9. In preliminary work, the theoretical plate heights obtained were over 100 000 plates/m, which are reasonable numbers for the preliminary work in this case.

4. Conclusion

The SPG emulsification technique afforded uniformly sized polymer particles with slightly worse size uniformity compared with the two-step swelling and polymerization method. On the other hand, the chromatographic efficiency obtained was excellent, with better column stability with 3-μm particles compared with that obtained using the two-step swelling and polymerization method. The surface-modified polymer can be also utilized as a packing material in CEC mode, resulting in reasonably good column efficiency.

This work is still at a preliminary stage and the obtained packing material for CEC was not optimized in terms of the preparation method and the
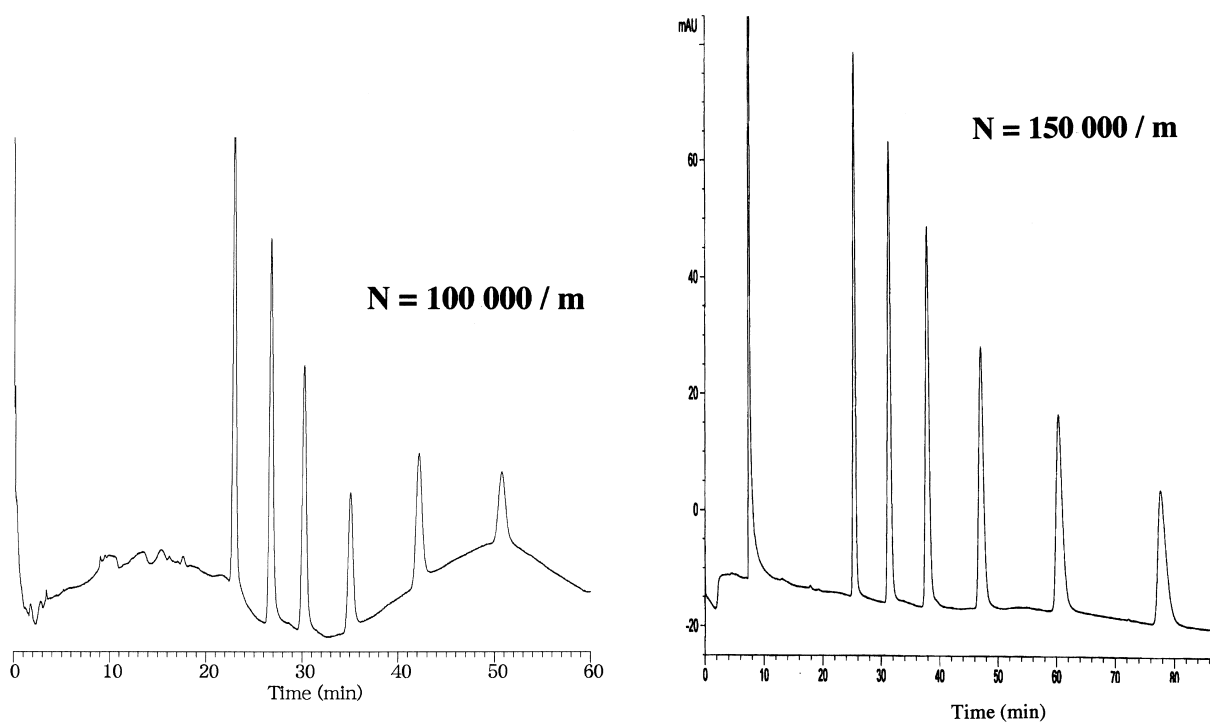
Fig. 9. Separation of alkylbenzenes in CEC mode. Chromatographic conditions: acetonitrile–50 mM Tris–HCl, pH 8 (90:10, v/v), 30 kV with 8 bar (both sides) at 20°C; detection, UV at 200 nm.

surface-modification method, including functional monomer. Further work is now in progress to elucidate the role of uniformly sized polymers with even smaller particle sizes ($<$3 μm) for CEC separation.

Acknowledgements

References

[1] V. Pretorius, B.J. Hopkins, J. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukcs, J. Chromatogr. 218 (1981) 209.
[3] T. Tsuda, K. Nomura, G. Nakagawa, J. Chromatogr. 248 (1982) 241.
[4] T.S. Stevens, H.J. Cortes, Anal. Chem. 55 (1983) 1365.
[5] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[6] M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC·GC 13 (1995) 800.
[7] M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, A. Lab. 3 (1996) 34.
[8] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 70 (1998) 2288.
[9] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 70 (1998) 2296.
[10] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3646.
[11] K. Hosoya, J.M.J. Fréchet, J. Liq. Chromatogr. 16 (1993) 353.
[12] J. Ugelstad, K.H. Kaggerud, F.K. Hansen, A. Berge, Makromol. Chem. 180 (1979) 737.
[13] K. Hosoya, J.M.J. Fréchet, J. Polym. Sci., Part A: Polym. Chem. 31 (1993) 2129.
[14] Q. Wang, K. Hosoya, F. Svec, J.M.J. Fréchet, Anal. Chem. 64 (1992) 1232.
[15] S. Omi, K. Katami, A. Yamamoto, M. Iso, J. Appl. Polym. Sci. 51 (1994) 1.
[16] S. Omi, K. Katami, T. Taguchi, K. Kaneko, M. Iso, J. Appl. Polym. Sci. 57 (1995) 1013.
[17] S. Omi, Colloid Surfaces 109 (1996) 97.

[18] Y. Hatate, H. Ohta, Y. Umemura, K. Ijichi, H. Yoshizawa, J. Appl. Polym. Sci. 64 (1997) 1107.

[19] T. Nakashima, M. Shimizu, M. Kukizaki, Emulsification operation manual, Ads. Ind. Res. Inst. Of Miyazaki (1991) 11.

[20] J.C. Moore, J. Polym. Sci., Part A 2 (1964) 835.

[21] L.R. Snyder, J.J. Kirkland, in: Introduction to Modern Liquid Chromatography, 2nd ed, Wiley, New York, 1979, Section 5.

[22] K. Hosoya, E. Sawada, K. Kimata, T. Araki, N. Tanaka, J.M.J. Fréchet, Macromolecules 27 (1994) 3973.