High-Performance Liquid Chromatography Separation Characteristics of Molecular-Imprinted Poly(methacrylic acid) Microparticles Prepared by Suspension Polymerization

Kayon Kim, Dukjoon Kim

Department of Chemical Engineering, Polymer Technology Institute, Sungkyunkwan University, Suwon, Kyungki 440-746, Korea

Received 19 May 2004; accepted 21 September 2004
DOI 10.1002/app.21419
Published online 27 January 2005 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Molecular-imprinted poly(methacrylic acid) was synthesized with a template of retinoic acid to separate retinoid derivatives. The suspension polymerization technique was used to prepare round microparticles for high-performance liquid chromatography (HPLC) packing column materials. The effects of the types and amounts of the dispersing agents and surfactants on the structure and size of the prepared molecular-imprinted-polymer particles were investigated. The separation of retinoic acid from its derivatives was more efficient when the perfluorocarbon dispersing agent was used instead of water, as the latter reduced the binding force between the objective molecules and monomers. HPLC separation features were also affected by the size and distribution of particles loaded in the column. A higher retention volume was obtained for smaller particles with a broader size distribution. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 200–212, 2005

Key words: high-performance liquid chromatography (HPLC); molecular imprinting

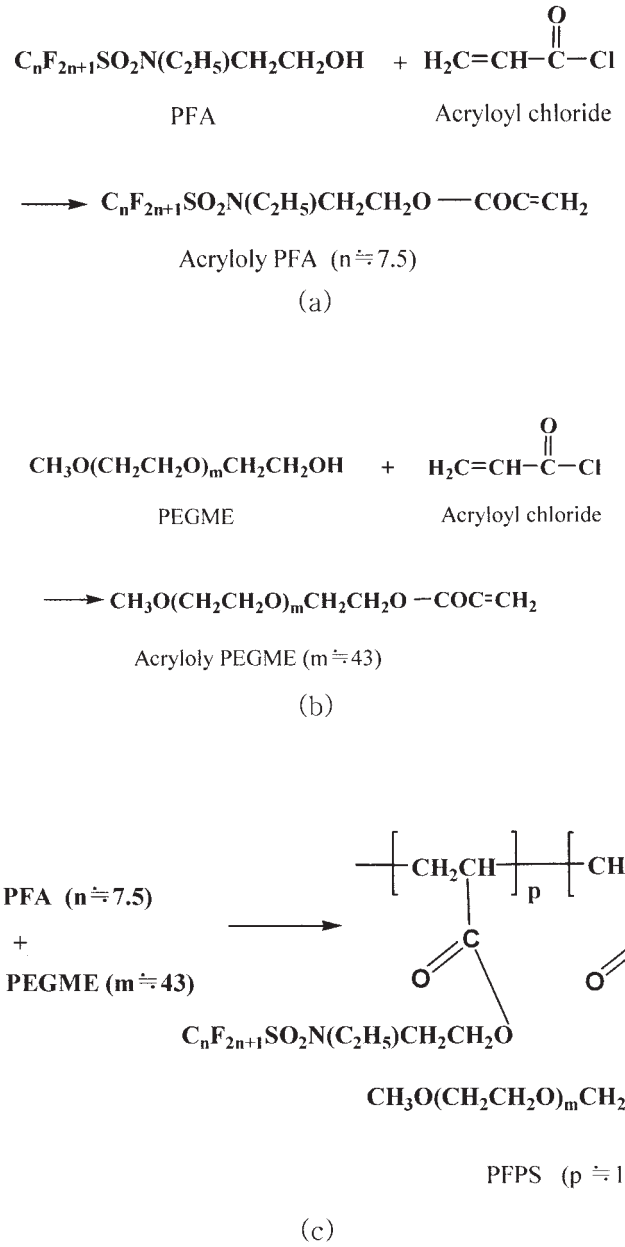
INTRODUCTION

Molecular-imprinted polymers (MIPs) are polymeric materials capable of recognizing specific molecules or ions. Molecular or ionic recognition ability is obtained during the synthesis of host polymers, usually by the crosslinking of monomers in the presence of a template and its subsequent elimination for the possession of highly specific binding sites or cavities behind it.[1–4]

MIPs can be applied in many industrial fields,[5] such as biomimetics, chromatographic and membrane separation, biosensing, and catalysis. In chromatographic applications, MIPs have often been used as high-performance liquid chromatography (HPLC) column-packing materials.[6,7] As column-packing particles are typically around 50 μm, MIPs synthesized by bulk polymerization are usually crashed and sieved before packing. Because of these crashing and sieving processes, the shapes of MIP particles prepared by bulk polymerization are irregular. Many particles, more than 50% of the total synthesized, are discarded before packing, and the column separation efficiency is very low and not reproducible.[8] As its synthetic process consumes lots of labor and time, its commercialization is hardly established.

Polymeric microparticles can be directly obtained by emulsion, suspension, or dispersion polymerization. In classical emulsion or suspension polymerization, nonpolar monomers are frequently dispersed and polymerized in polar solvents such as water and alcohols. In the preparation of MIPs, however, the use of water or a polar solvent as a dispersing agent reduces the binding site number and strength between the monomer and template by providing highly specific interactions such as hydrogen or ionic interactions between water and the monomer or template. One well-established method for overcoming this binding limitation is the aqueous two-step swelling method.[9] Although this method improves the shape regularity of particles and enhances HPLC efficiency in many ways, it still has the drawback of using water as a dispersing agent. The use of a much less polar solvent than water or alcohols is another approach to preventing a binding interaction between the monomer and solvent or between the template and solvent. As liquid perfluorocarbons are incompatible with almost all organic compounds, they are usable as dispersing agents (solvents) in suspension polymerization. A dispersed phase consisting of a monomer,

Correspondence to: D. Kim (djkim@skku.edu).
Contract grant sponsor: Samsung Research Fund (Sungkyunkwan University).



Scheme 1 Synthetic routes of (a) acryloyl PFA, (b) acryloyl PEGME, and (c) PFPS.

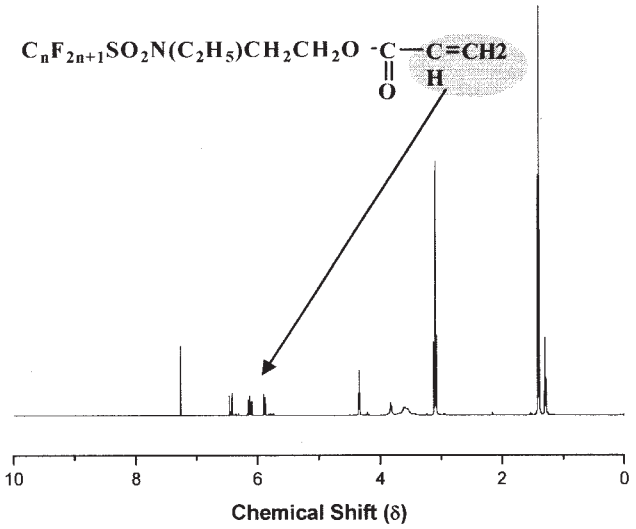
crosslinking agent, template, and porogenic solvent can be well emulsified in this kind of perfluorocarbon dispersing agent when an appropriate fluoro surfactant is used.[10] As there is no physical interaction between the dispersed and solvent phases in this system, the number and strength of binding sites are no longer reduced.

In this study, MIP microparticles were prepared by suspension polymerization with two different dispersing agents, water and perfluoro-1,3-dimethylcyclohexane. HPLC separation characteristics for retinoid derivatives were analyzed and compared for the two types of prepared MIPs. The effects of the particle size and shape on the separation efficiencies were also investigated.

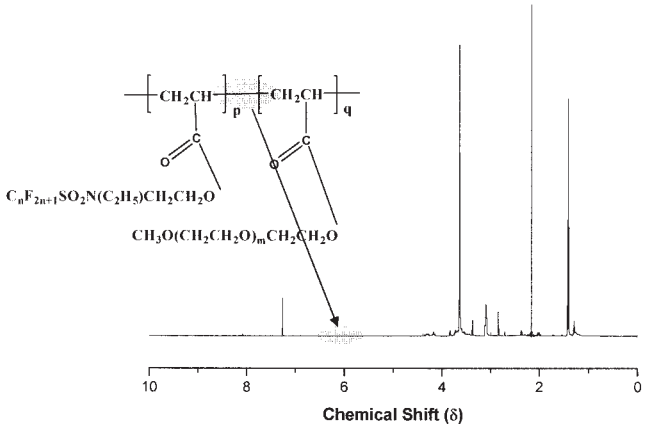
EXPERIMENTAL

Materials

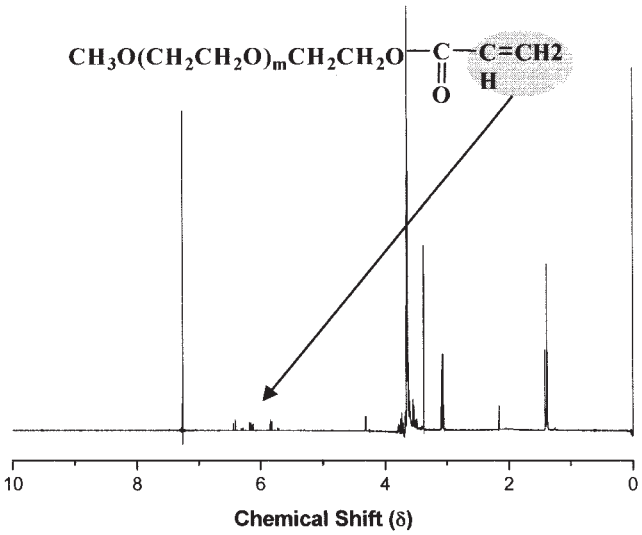
All-trans retinoic acid, the template, and its derivatives,[11] all-trans retinol, all-trans retinal, and all-trans retinol acetate, were purchased from Aldrich Chemical Co. (Milwaukee, WI). Methacrylic acid (MAA; Aldrich) was used as the monomer, ethylene glycol



(a)



(c)



(b)

Figure 1 ^{1}H-NMR spectra of surfactants: (a) acryloyl PFA, (b) acryloyl PEGME, and (c) PFPS.

dimethacrylate (EGDMA; Aldrich) was used as the crosslinking agent, and 2,2′-azobisisobutyronitrile (AIBN; Daejung, Shiheung, Korea), was used as the initiator. Poly(fluoro alcohol) (PFA; Fluorochem, Azusa, CA) and poly(ethylene glycol) methyl ether (PEGME; Aldrich) with a molecular weight of 2000 g/mol were used to synthesize the precursor of the perfluoro polymer surfactant (PFPS) by a reaction with acryloyl chloride (Aldrich) in the presence of triethylamine (Aldrich), the catalyst. Chloroform was used as the porogenic solvent, and perfluoro-1,3-dimethylcyclohexane (Aldrich) and water were used as the dispersing agents. Poly(vinyl alcohol) (PVA; Daejung) was the surfactant when water was used as the dispersing solvent.

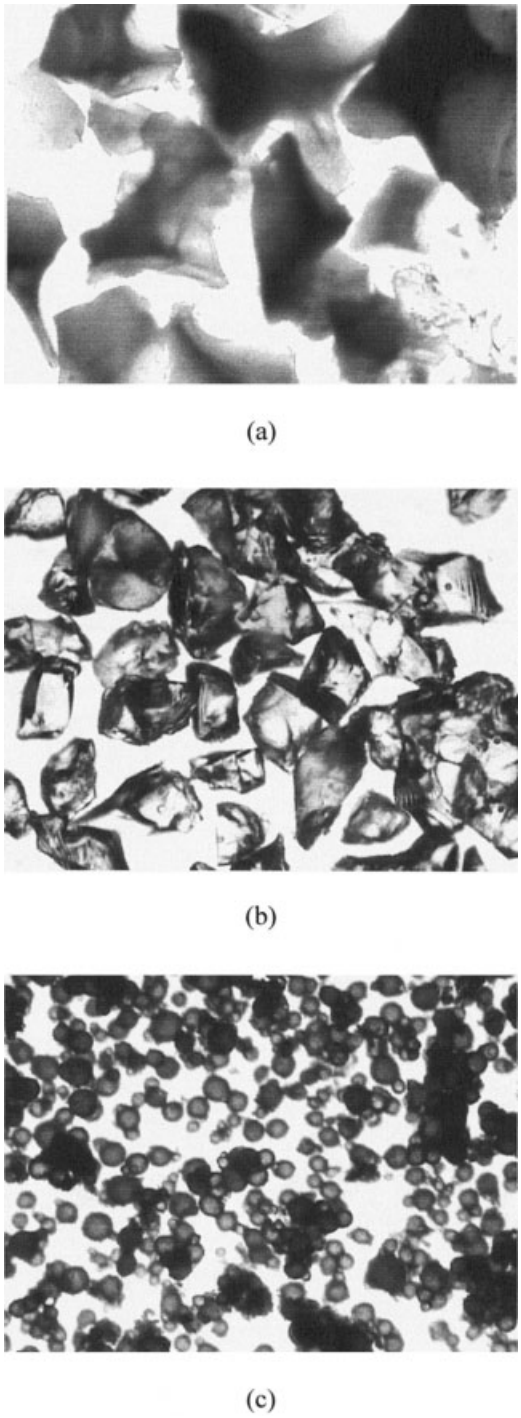


Figure 2 Shapes of MIP particles prepared with different surfactants: (a) Fluorad FC430, (b) PFA, and (c) PFPS.

Synthesis of the polymeric surfactant

PFA (39.5 mmol) was dissolved in 40 mL of chloroform with stirring from 0 to 5°C. Acryloyl chloride (43.4 mmol) and triethylamine (43.4 mmol) were dissolved in chloroform, and the mixture was slowly poured into a 500-mL, three-necked flask reactor for 5 min. The reactant mixture was stirred for 20 min at the same temperature and then again for 24 h at 20°C. Chloroform was removed with a rotary evaporator, and then triethylamine was removed by filtration. An orange-brown, viscous liquid, acryloyl PFA, was obtained when the solvents were completely removed.

The other segment of the surfactant, acryloyl PEGME, was synthesized as follows. PEGME (10 mmol) was dissolved in 20 mL of chloroform from 0 to 5°C with stirring. After acrylol chloride (11 mmol) and triethylamine (11 mmol) were separately dissolved in 5 mL of chloroform, the two solutions were slowly mixed in a reactor for 5 min. The other steps were similar to the synthesis of acryloyl PFA.

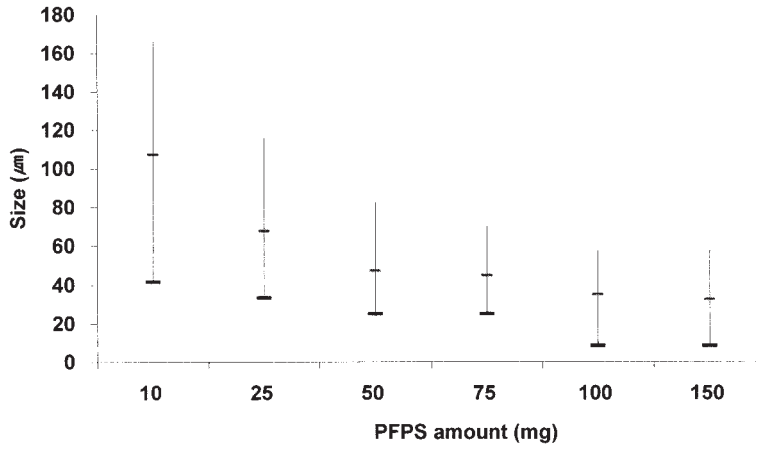
After two homopolymer products, acryloyl PFA (4 g, 7.2 mmol) and acryloyl PEGME (0.76 g, 0.36 mmol), were dissolved in 10 mL of chloroform in a vial, the initiator AIBN was added. Nitrogen gas was purged for 5 min to remove oxygen in the reactor. The copolymerization reaction was conducted at 60°C for 48 h in the completely sealed reactor, which was placed in a shaking water bath.[10,12] The products were dried at 30°C in an oven.

Fourier transform infrared (FTIR) spectroscopy (FT-IR660 Plus, Jasco, Tokyo, Japan) and Fourier transform NMR spectroscopy (Unity Inova 500, Varian, CA) were used to identify the chemical structures of acryloyl PFA, acryloyl PEGME, and PFPS.

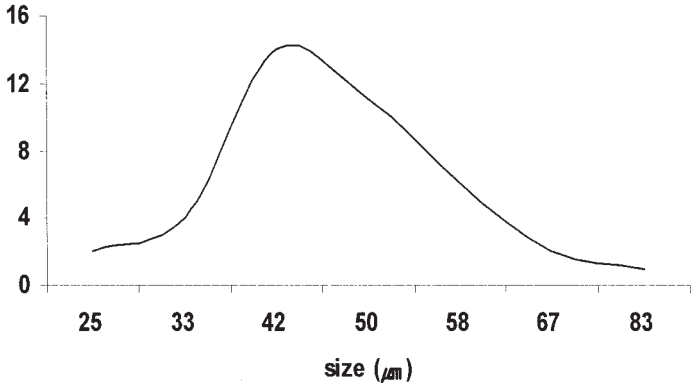
Synthesis and characterization of MIPs

Chloroform (4.2 g), a porogenic solvent, was placed in a 50-mL borosilicate glass tube, and then the prepared PFPS (40 mg) was added. Perfluoromethylcyclohexane (20 mL) was poured and stirred until the solution became opaque. MAA (0.16 g) and EGDMA (1.84 g) were added and dissolved in the solution by at least 5 min of stirring. After a purge of nitrogen gas for 5 min, the polymerization was conducted for 3 h with a lamp radiating at a wavelength of 366 nm. The UV lamp was located 5 cm from the reactor, and the radiation intensity was maximized by the wrapping of the reactor system. The polymer particles that were produced were washed with acetone, and the agglomerates were pulverized with a sonifier (Branson, Danbury, CT). The products were dried and then stored in a oven. All-trans retinoic acid (0.07 g) was introduced as a template material in this MIP preparation.

Optical microscopy (CSB-HP3, Samwon Science, Korea) and scanning electron microscopy (SEM; XL30 ESEM-FEG, FEI Co., Hillsboro, OR) were used to observe the structure and size distribution of the synthesized MIP particles. Thermogravimetric analysis (TGA; TGA7, PerkinElmer, Boston, MA) was



(a)



(b)

Figure 3 MIP particle size and particle size distribution when PFPS was used as the surfactant: (a) the surfactant concentration dependence of the average particle diameters and (b) the particle size distribution when 50 mg of PFPS was used.

used to measure the thermal stability of the polymers. The measurement was conducted from room temperature to 600°C at a ramping rate of 10°C/min. UV spectroscopy (U-3210, Hitachi Ltd., Tokyo, Japan) and energy-dispersive analysis (EDS; EDAX, Mahwah, NJ) were used to detect the presence of the template and surfactants in the final MIP products.

HPLC packing and separation experiments

A column packer (model 1666, Altech Corp., Flemington, NJ) was used to pack MIP particles in an HPLC column 250 mm long and 4.6 mm in diameter. The MIP slurries in chloroform were ultrasonified to prevent the adhesion of particles. The column was filled with fine MIP particles with compressed N_2 gas, and the solvent permeated out through a porous metal frit.

A high-performance liquid chromatograph (Agilent 1100 series, Agilent Technology, United States) was used to separate retinoid derivatives—all-trans retinoic acid, all-trans retinol, all-trans retinal, and all-trans retinol acetate—at room temperature. Each retinoid isomer and a mixture of all the isomers were diluted in chloroform to a concentration of 400 ppm. Each component solution or mixture was injected into an HPLC column at a flow rate of 0.8 mL/min. Separation features were detected in the UV mode at 368.4 nm. To obtain the separation standards, each component solution was injected before the injection of the mixture.

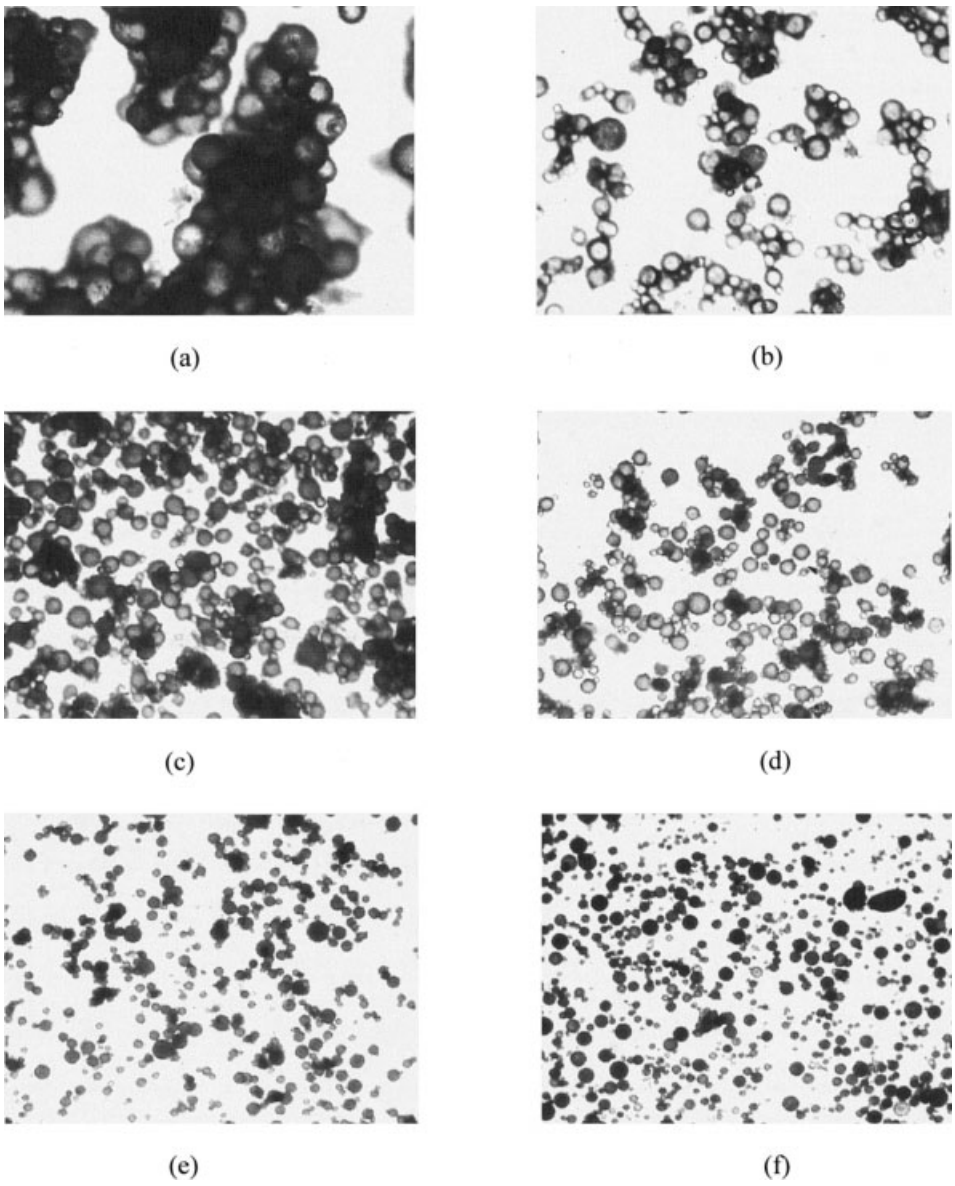


Figure 4 Sizes and shapes of the MIP particles prepared with different amounts of the PFPS surfactant: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100, and (f) 125 mg.

RESULTS AND DISCUSSION

Synthesis and characterization of polymeric surfactants

PFPS was synthesized before the preparation of MIPs. Hydrophobic and hydrophilic polymer segments of PFPS, acryloyl PFA, and acryloyl PEGME were first synthesized, according to the routes shown in Scheme 1(a,b). Acryloyl PFA was produced from the reaction of PFA and acryloyl chloride, and acryloyl PEGME was produced from the reaction of PEGME and acryloyl chloride. Hydrogen chloride was the byproduct during each condensation reaction. PFPS was synthesized by the copolymerization of acryloyl PFA and acryloyl PEGME, as shown in Scheme 1(c).

Figure 1(a–c) shows ^{1}H-NMR spectra of acryloyl PFA, acryloyl PEGME, and PFPS, respectively. The C═C double bonds, positioned at the ends of two acryloyl molecules, are shown in Figure 1(a,b) as triplet peaks at the chemical shift around 6 ppm. The disappearance of these peaks after the synthesis of PFPS in Figure 1(c) indicates that the copolymerization and its purification were well conducted.

Shape and size of the MIP particles

As the density of perfluoro-1,3-dimethylcyclohexane, the dispersing agent, was relatively high, the monomer reactants were not easily dispersed in this medium. Three types of fluoro surfactants, PFA, Fluorad FC430, and PFPS—the first two commercial and the last synthesized in this study—were used for the suspension polymerization of MIPs. Figure 2(a–c) shows the surfactant effects on the dispersion and shapes of the MIP microparticles produced. As shown in the microphotographs of MIP particles in Figure 2(a,b), the commercial surfactants could not produce well-rounded particles, as many particles adhered to the inner surface of the reactor. Their sizes were relatively large with broad distributions, and their surfaces were very rough. In comparison with the previous results, the particles prepared with PFPS were smaller but had much narrower distributions [Fig. 2(c)]. The production of well-rounded particles of uniform size was confirmed from these SEM photographs.

Figure 3(a) shows the effect of the amount of PFPS on the particle size and its distribution. The particle diameters decreased, but their distribution became narrower with an increasing amount of the surfactant. The average diameter decreased from 110 to 30 μm when the amount of the surfactant increased from 10 to 150 mg. Figure 3(b) shows the size distribution of particles prepared when 50 mg of PFPS was used. About 40% of the particles had diameters below 40 μm. Figure 4(a–f) shows SEM microphotographs of MIP particles prepared with different amounts of PFPS (10–150 mg).

Figure 5(a,b) shows the shapes of MIP particles synthesized with PFPS in different dispersing agents, water and perfluoro-1,3-dimethylcyclohexane. The structure and size were very similar, as the interaction between the monomer and dispersing agent did not affect the shape significantly. For comparison, a microphotograph of MIP particles prepared by bulk polymerization is shown Figure 5(c). In this case, the particles were irregularly shaped with sharp edges.

The TGA results for retinoic acid and MIPs are shown in Figure 6(a,b), respectively. As the retinoic acid and MIP samples were thermally stable up to 230 and 300°C, respectively, no chemical deterioration was expected during their preparation processes.

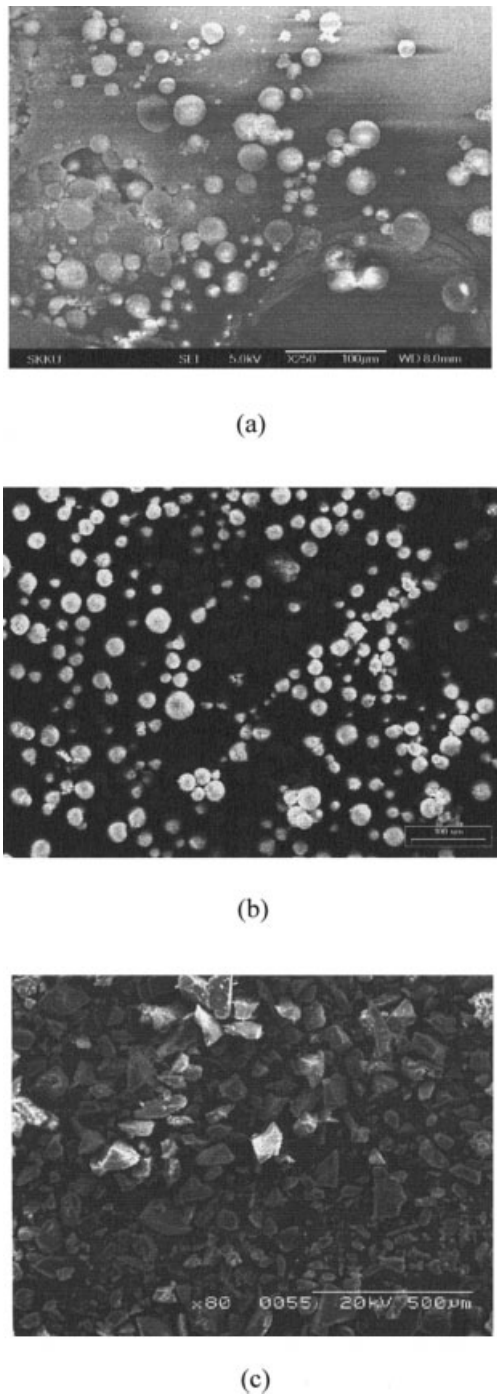
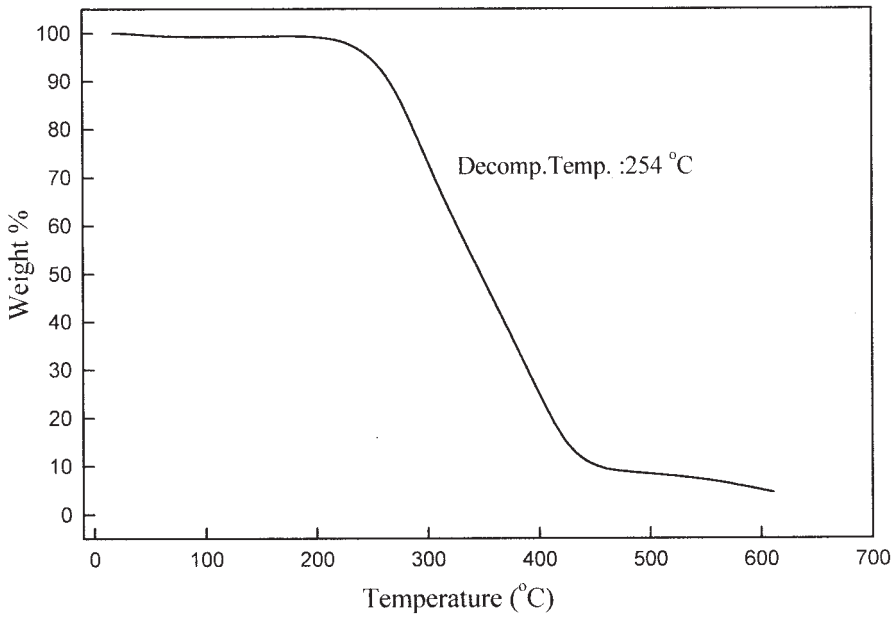


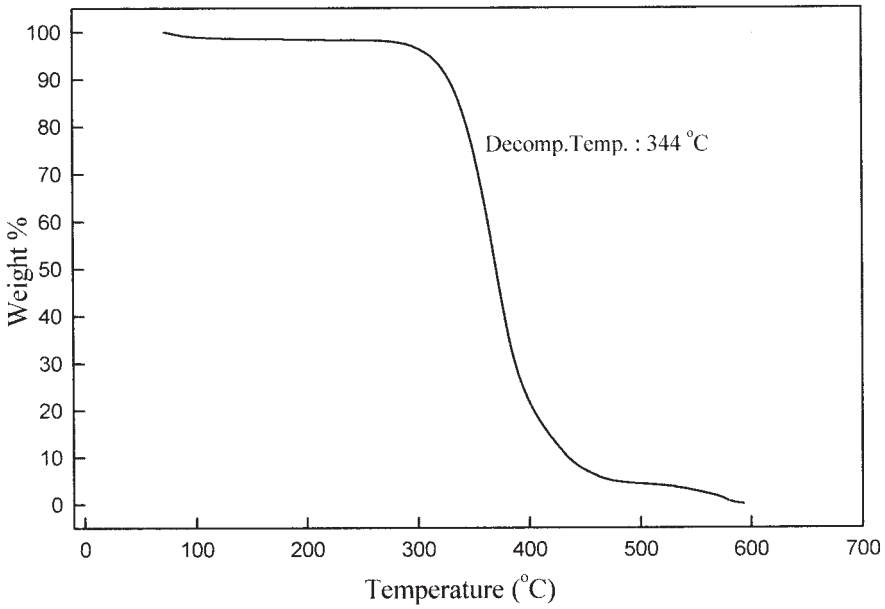
Figure 5 SEM microphotographs of MIP particles prepared by suspension polymerization with (a) water or (b) perfluoro-1,3-dimethylcyclohexane as the dispersing agent and (c) by bulk polymerization.

HPLC separation characteristics of the MIP particles

When the template molecule and surfactants remained inside the prepared MIP particles, this could reduce the chromatographic separation efficiency because of a deficiency of binding sites. UV spectrophotometry and EDS were used to detect the presence of the template and surfactant inside the MIP particles. The complete removal of the template, retinoic acid, was investigated by a comparison of the UV spectra measured before and after the washing processes. As



(a)



(b)

Figure 6 TGA results for (a) retinoic acid and (b) prepared MIPs.

shown in Figure 7, the UV peaks observed before washing disappeared after four washing processes. The elimination of the surfactant was also ensured by a comparison of the EDS spectra shown in Figure 8. The characteristic peak for the F atom in the PFPS molecule was not observed in this spectrum.

The type of dispersing agent had a significant effect on the HPLC separation characteristics. Figure 9 shows that the retention volume for all-trans retinoic acid was quite different from that for other derivatives. As retinoic acid molecules were bound to MIP sites by hydrogen bonding, its retention volume was

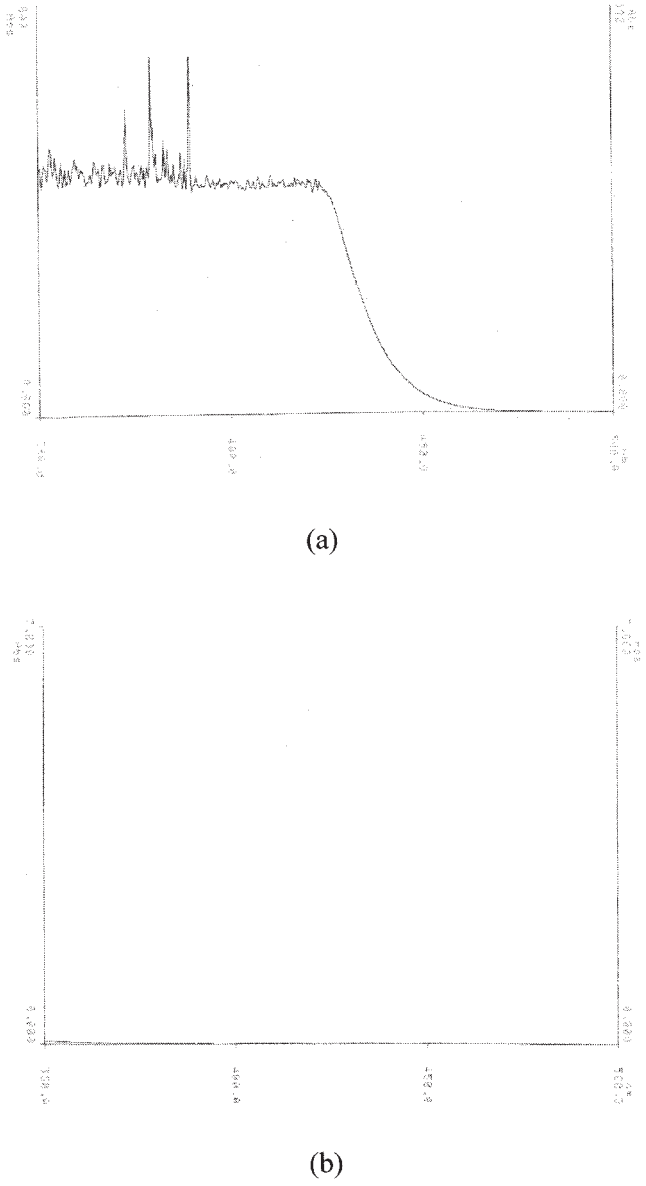


Figure 7 UV spectra of (a) retinoic acid and (b) MIPs after washing.

much greater than that of others. The separation of retinoic acid from other derivatives was more efficient when the perfluorocarbon dispersing agent was used [Fig. 9(b)] instead of water [Fig. 9(a)]. The hydrogen bonding that took place between the dispersing agent and template molecule or between the dispersing agent and monomer strongly reduced the specific interaction site numbers and forces between the objective molecule and monomer when water was used as the dispersing agent. Figure 9 was obtained when each retinoic acid derivative was injected separately, and Figure 10 was obtained when a mixture composed of all the derivatives was injected at once. As shown in Figure 10(a,b), a distinction between two peaks corresponding to retinoic acid and the other derivative mixture could be clearly observed when perfluoro-1,3-dimehylhexane was used as the dispersing agent.

The HPLC separation efficiency was also affected by the size and its distribution of particles loaded in the column. Figure 11(a–c) shows the particle size and distribution effects on the separation characteristics when the mean particle mean diameters were 105 (10 mg of PFPS), 50 (50 mg of PFPS), and 30 μm (150 mg of PFPS), respectively. A comparison of Figure 11(b,c) shows that the retention volumes for the smaller particles were higher than those for the larger particles. It was simply because longer times were required to flow through narrower channels formed by smaller particles. A comparison of Figure 11(a,b) shows, however, different results from the previous ones: the smaller particles resulted in shorter retention volumes. This result was caused by the difference in the size distribution rather than the mean diameter. When the particle sizes were too broadly distributed [Fig. 3(a)], smaller particles were easily incorporated inside

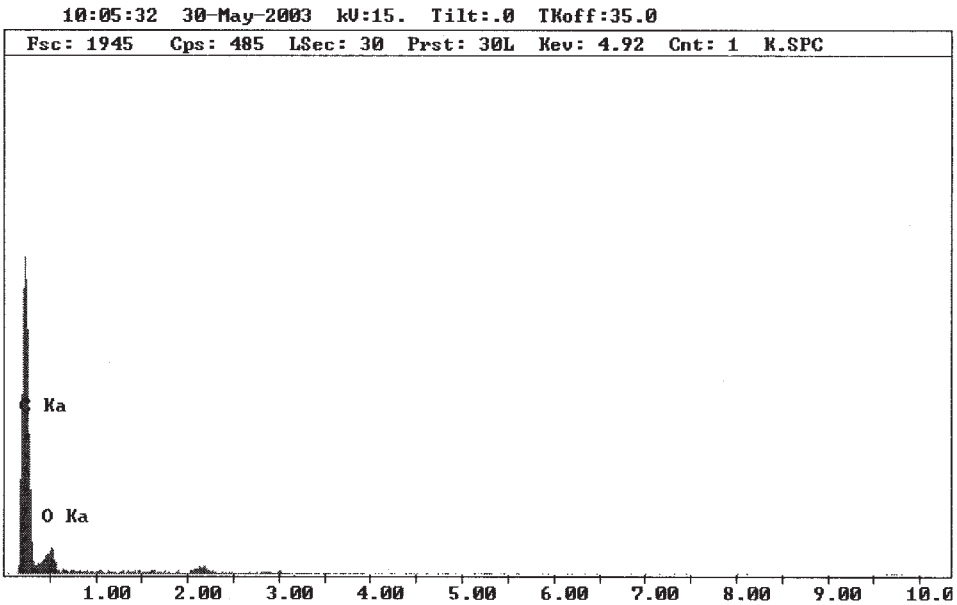
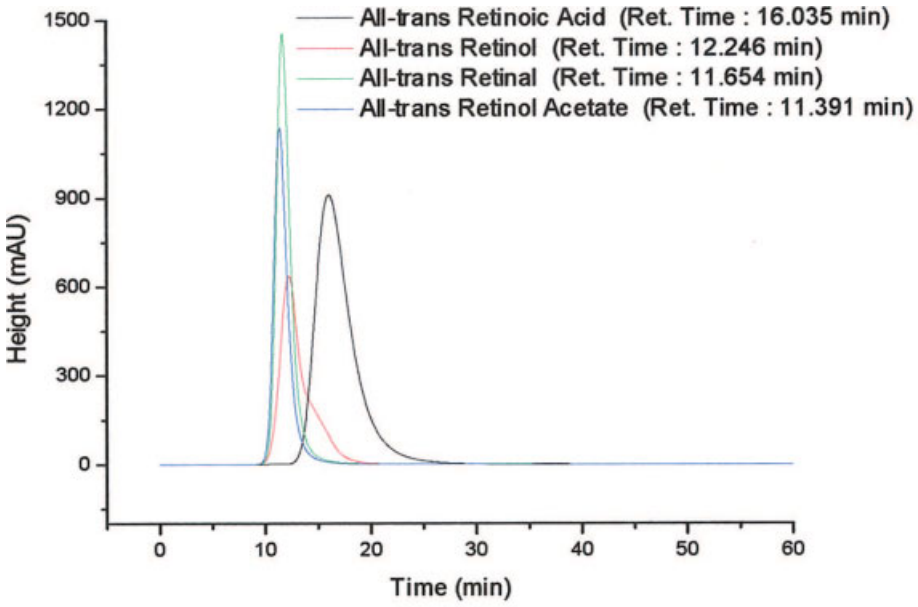
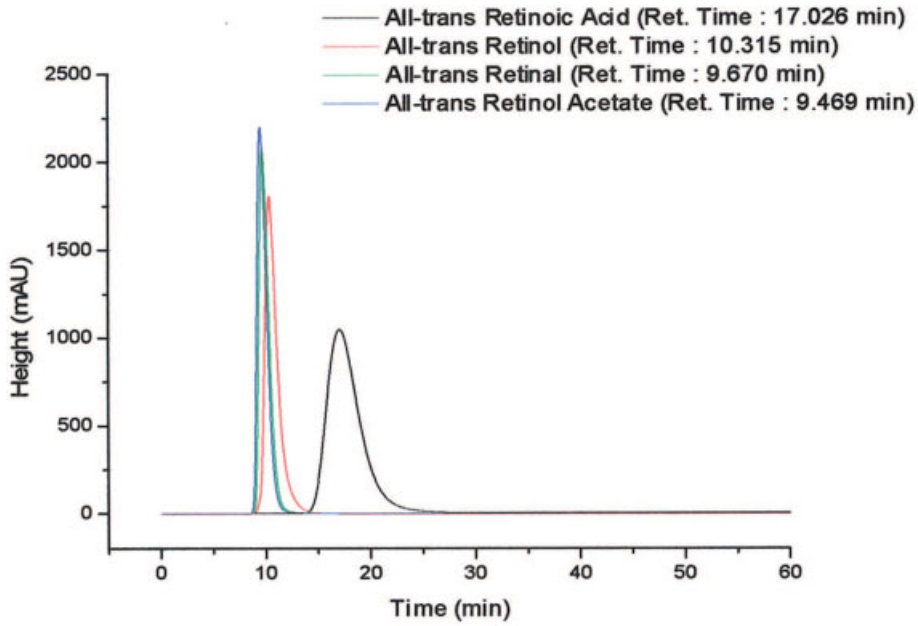


Figure 8 EDS spectra of MIPs after washing.

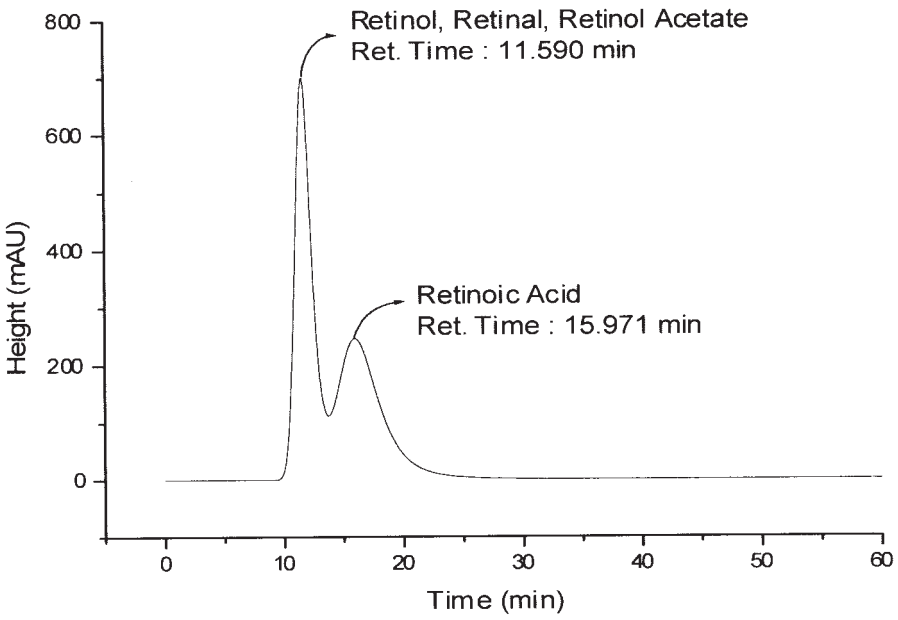


(a)

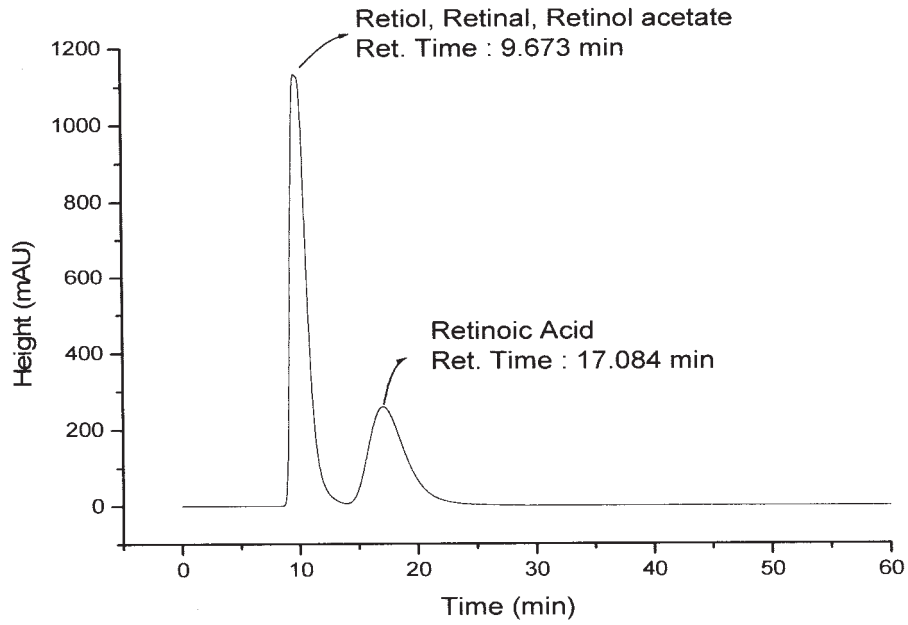


(b)

Figure 9 Separation characteristics of retinoid derivatives in columns packed with MIP particles when each derivative was separately injected into the HPLC instrument. MIPs were prepared by suspension polymerization with (a) water or (b) perfluoro-1,3-dimethylcyclohexane as the dispersing phase.



(a)



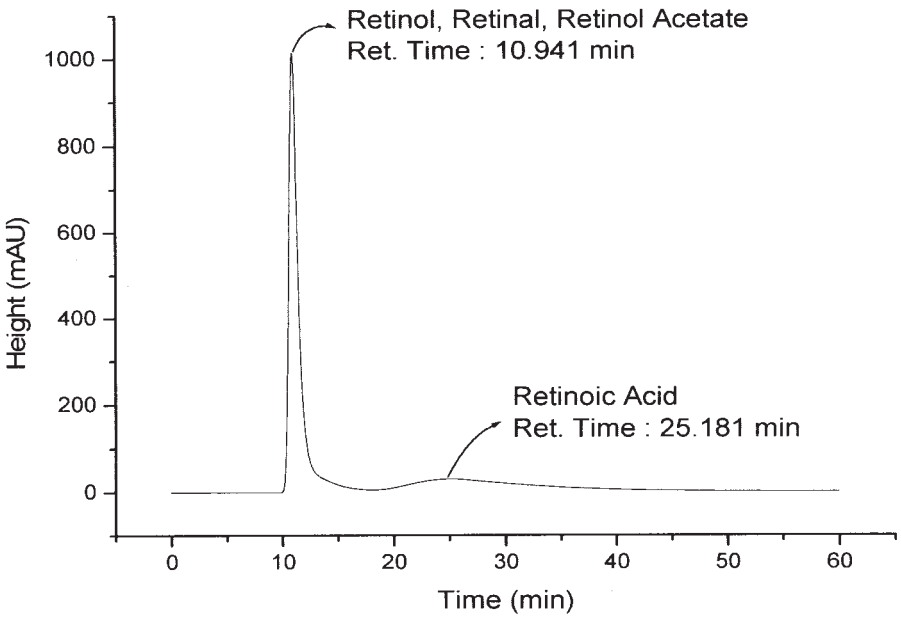
(b)

Figure 10 Separation characteristics of retinoid derivatives in columns packed with MIP particles when the retinoid mixture was injected into the HPLC instrument. MIPs were prepared by suspension polymerization with (a) water or (b) perfluoro-1,3-dimethylcyclohexane as the dispersing phase.

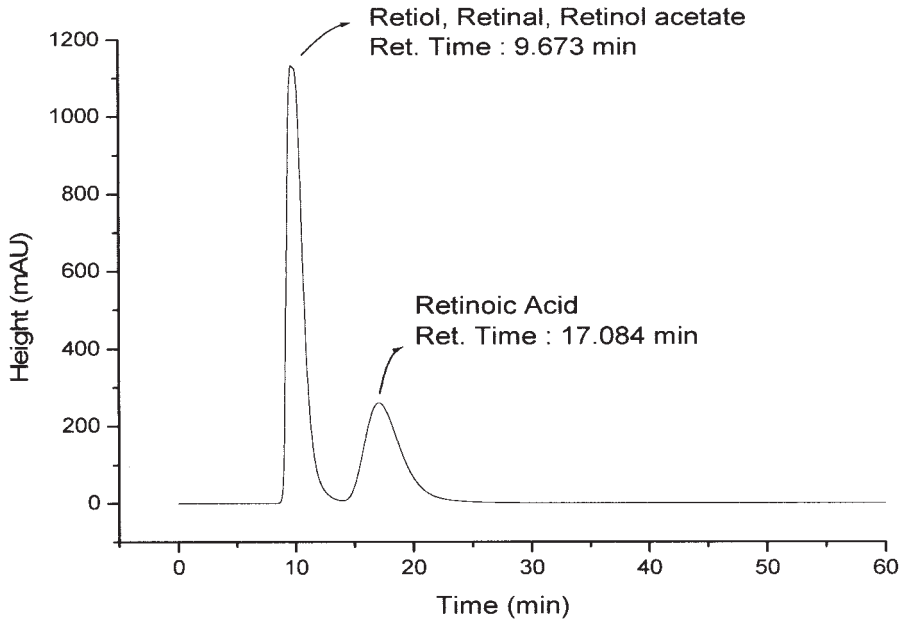
channels or cavities formed by relatively larger particles. In this case, the channels were so tightly packed that it might take much longer for mobile liquids to penetrate them.

CONCLUSIONS

MIP microparticles were prepared by suspension polymerization with two different dispersing agents, wa-



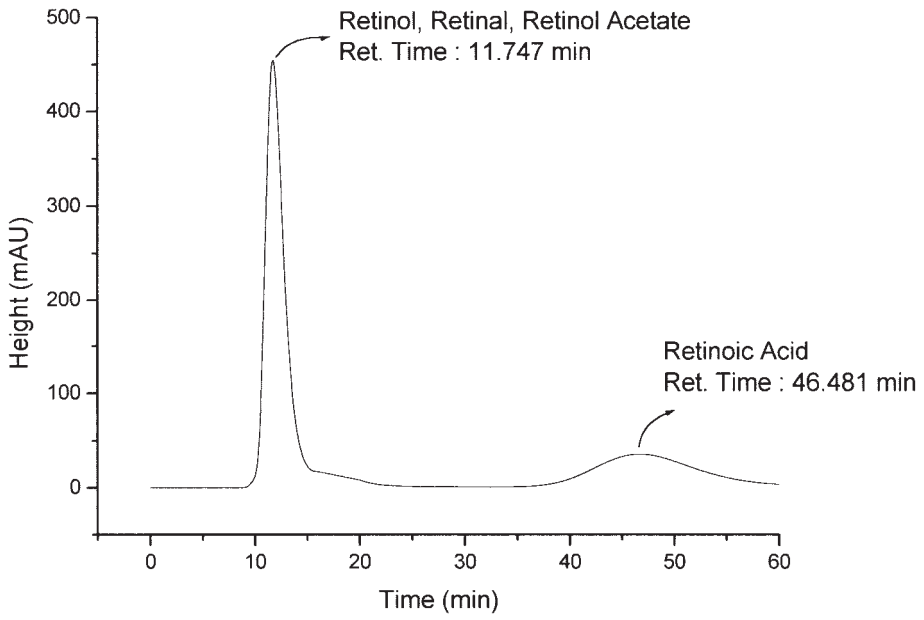
(a)



(b)

Figure 11 Separation characteristics of retinoid derivatives in columns packed with MIP particles when the retinoid derivative mixture was injected into the HPLC instrument. HPLC columns were packed with MIP samples with different mean diameters: (a) 105, (b) 50, and (c) 30 μm. The corresponding amounts of PFPS were (a) 10, (b) 50, and (c) 100 mg.

ter and perfluoro-1,3-dimethylcyclohexane. The HPLC separation features for retinoid derivatives were analyzed and compared for the two types of MIP packing materials prepared. PFPS was synthesized, and its chemical structure was identified with several analytical techniques: FTIR, NMR, EDS, and UV spectropho-



(c)

Figure 11 (*Continued from the previous page*)

tometry. The prepared particles were uniform in shape and round. The particle size was reduced and its distribution became narrower with increasing surfactant concentration. The average particle diameters decreased from 105 to 30 μm when the surfactant contents increased from 10 to 50 mg. The separation of retinoic acid from other derivatives was more efficient when the perfluorocarbon dispersing agent replaced water because it generated much stronger imprinting sites. The particle size and its distribution also affected the HPLC separation characteristics. The retention volume for smaller particles was higher than that for larger ones, simply because longer times were required to flow through narrower channels formed by smaller ones. When the particle size was too broadly distributed, smaller particles were so tightly incorporated inside the cavities formed by larger particles that much higher retention volumes might result.

References

1. Spivak, D. A.; Shea, K. J. Macromolecules 1998, 31, 2160.
2. Mayes, A. G.; Mosbach, K. Trends Anal Chem 1997, 16, 321.
3. Cheong, S. H.; McNiven, S.; Rachkov, A.; Levi, R.; Yano, K.; Karube, I. Macromolecules 1997, 30, 1317.
4. Whitcombe, M. J.; Rodriguez, M. E.; Villar, P.; Vulfson, E. N. J Am Chem Soc 1995, 117, 7105.
5. Cormack, P. A. G.; Mosbach, K. React Funct Polym 1999, 41, 115.
6. Baggiani, C.; Giraudi, G.; Vanni, A. Bioseparation 2001, 10, 389.
7. Meng, Z.; Zhou, L.; Wang, J.; Wang, Q.; Zhu, D. Biomed Chromatogr 1999, 13, 389.
8. Wang, Q. C.; Hosoya, K.; Svec, F.; Frechet, J. M. Anal Chem 1992, 64, 820.
9. Mayes, A. G. Tech Instrum Anal Chem 2001, 23, 305.
10. Ansell, R. J.; Mosbach, K. J Chromatogr 1997, 68, 3769.
11. The Retinoids: Biology, Chemistry and Medicine, 2nd ed.; Sporn, M. B.; Roberts, A. B.; Goodman, D. S., Eds.; Raven: New York, 1994.
12. Mosbach, K.; Mayes, A. G. U.S. Pat. 5,821,311 (1998).