Separation Characteristics of Molecular Imprinted Poly(methacrylic acid) for Retinoid Derivatives

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ABSTRACT: Methacrylic acid monomer was crosslinked in the presence of a template of either all-*trans*-retinoic acid or all-*trans*-retinol to prepare molecular imprinted polymers. The prepared molecular imprinted polymers showed good performance in the high-performance liquid chromatography (HPLC) separation of objective molecules from retinoid derivatives. The retention time increased with an increasing ratio of monomer to template, as more binding sites were statistically generated between host molecules and the template. The column capacity factor and selectivity were determined from the HPLC data. Not only specific interactions such as hydrogen bonding but also a tailor-made spatial cavity were the main functions of the separation of objective molecules. Thermal stability and prepared polymer particle shape were also investigated. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1081–1087, 2003

Key words: molecular imprinting; templates; separation techniques; chromatography

INTRODUCTION

Retinoids, including retinoic acid, retinol, retinal, and retinol acetate, are produced either by either biometabolism or chemical synthesis.[1] Retinoids can control cell cultivation and division, and thus they may have positive effects on the prevention of or immunity from cancer, amblyopia, and other medical disorders. Separation of retinoid derivatives is essential for effective medical treatments, as each retinoid derivative has unique medical and physical properties. All-*trans*-retinoic acid has been reported to be very effective as a remedy for leukemia and its isomer 13-*cis*-retinoic acid as effective for bladder inflammation, but other isomers, such as 13-*cis*-4-oxo-retinoic acid and all-*trans*-4-oxo-retinoic acid, have not.[2,3]

Gas chromatography–mass spectroscopy (GC-MS),[4,5] high-performance liquid chromatography (HPLC),[6,8] and high-performance liquid chromatography–ultraviolet (HPLC-UV)[9–12] spectrophotometer were applied for the separation of a number of chemical mixtures. Generally, the separation features of chromatographic methods are mostly affected by the physicochemical properties of column-packing materials. Although many successful separations of chemical mixtures have been achieved and reported from using chromatographic techniques with commercial packing columns, there are still uncertainties in the separation of many chemical systems, especially when composed of similarly structured molecules such as chiral isomers.

The molecular imprinting technique[13–24] involves the preparation and processing of molecularly recognizable materials with tailored bonding sites to the objective molecules. Molecular imprinted polymers (MIPs) are generally obtained by synthesizing the host materials, or crosslinked polymers, in the presence of a template, or objective molecule. Elimination of the template after polymer synthesis provides molecular and ionic recognition ability with host materials. With this molecular and ionic recognition ability, MIPs can be used in chromatographic column-packing materials.

In this study MIPs were synthesized from the monomer of methacrylic acid and the template of either all-*trans*-retinoic acid or all-*trans*-retinol. Their performance as chromatographic column-packing materials was analyzed for the separation of retinoid derivatives. As retinoids have been reported to be very sensitive to light and oxidation, HPLC, a relatively mild separation technology, was employed for this analysis.

EXPERIMENTAL

Preparation and characterization of MIP

In 7.5 mL of chloroform (Daihung Chemical Company, Japan) were dissolved 4 mmol of the monomer methacrylic acid (MAA, Sunjung Chemical Company, Japan), 25 mmol of the crosslinking agent ethylene

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glycol dimethacrylate (EGDMA; Aldrich Chemical Company, Milwaukee, WI), and 1 mmol of the template of all-*trans*-retinoic acid (Aldrich). The reaction mixture was ultrasonified in a vacuum to eliminate gas and then placed in a 25-mL three-necked flask. Then 0.05 g of the initiator, 2,2′-azobisisobutyronitrile (AIBN, Aldrich), was added, the radical polymerization reaction was conducted at 75°C for 48 h and then at 90°C for another 72 h under an argon gas environment in a glove box to prevent oxidation of retinoic acid. The synthesized polymers were crushed into particle form using a mortar and then separated according to their size using molecular sieves. The products were washed with chloroform four times at 4-h intervals to eliminate the unreacted monomers, and then with methanol at least six times at 60°C for 24 h to eliminate the residual template. The final polymer products were filtered at room temperature and then dried in a vacuum oven at 37°C for at least 24 h. The large polymer particles were recrushed into smaller ones.

All-*trans*-retinol was also introduced instead of retinoic acid as template material in the preparation of MIP. The experimental conditions and procedure were the same as above.

HPLC column packing

The HPLC column, 250 mm (L) × 4.6 mm (D), was packed with MIP particles using a column packer (model 1666, Altech Corporation, Flemington, NJ). MIPs were prepared in a slurry state by dispersing them in chloroform. The slurry was treated ultrasonically to prevent particle adhesion. After the column was attached to the bottom of a storage tank, it was filled with fine MIP slurry from the two column ends. The slurry was compressed by N_2 gas and then injected into the column from a slurry reservoir. The solvent permeated out through a porous metal frit, which retained the MIP particles packed in the column. Figure 1 shows a schematic of the column-packing procedure.

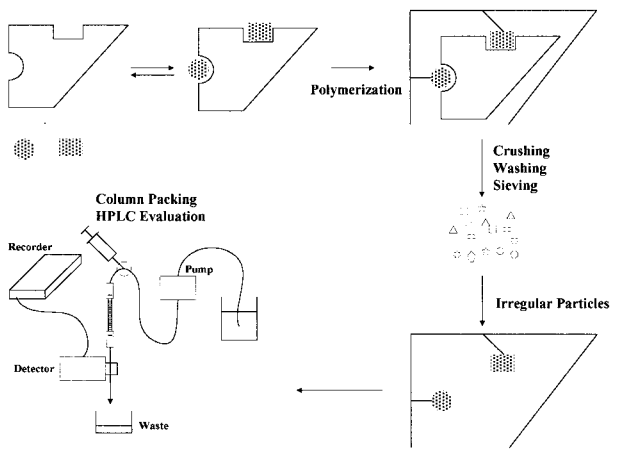


Figure 1 Schematics of column-packing apparatus and procedure.

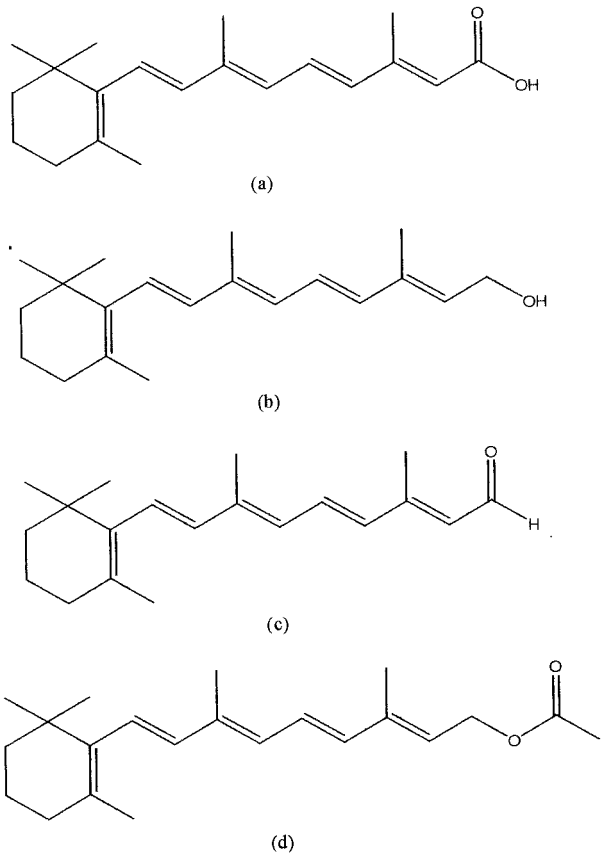


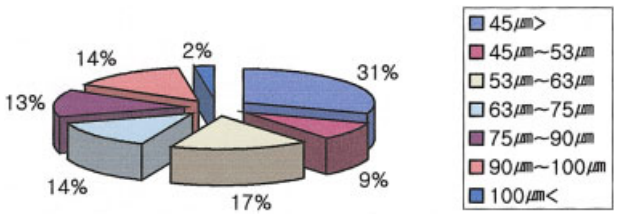
Figure 2 The molecular structures of retinoid derivatives used in the present experiment: (a) all-*trans*-retinoic acid, (b) all-*trans*-retinol, (c) all-*trans*-retinal, and (d) all-*trans*-retinol acetate.

Characterization

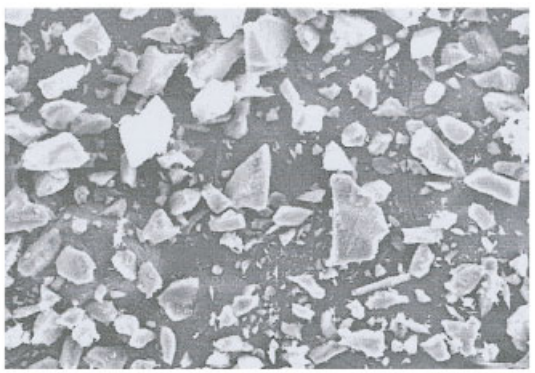
Molecular sieves (models 170, 200, 230, 270, and 325; Chunggye Sanggongsa, Korea) and an optical microscope (model CSB-HP3; Samwon Science, Korea) were used to measure the size distribution and shape of the MIP particles prepared.

A thermogravimetric analyzer (TGA7, Perkin–Elmer, Boston, MA) was used to investigate the thermal stability of both template and MIP. The measurements were conducted at temperatures ranging from room temperature to 400°C at a ramping rate of 10°C/min.

A HPLC (model 486, Waters, Milford, MA) was used to separate retinoids at room temperature. The column was packed with MIP by the method mentioned above. After the retinoid mixture, composed of all-*trans*-retinoic acid, all-*trans*-retinol, all-*trans*-retinal, and all-*trans*-retinol acetate on an equimolar basis, was diluted in chloroform to a concentration of 400



(a)



(b)

Figure 3 (a) The size distribution and (b) the shape of the MIPs prepared.

ppm, it was injected into the HPLC column. For its operation, the flow rate of the mobile phase was 0.8 mL/min, and the injection volume was 10 μL. Separation features of the injected mixtures were detected in the UV mode at 368.4 nm. Before injection of the retinoid solution mixture, each component solution was prepared and injected for the reference. Pure chloroform was injected for at least 40 min at 0.8 mL/min between individual component injections. The molecular structure of each retinoid derivative used in the present experiment is shown in Figure 2.

RESULTS AND DISCUSSION

MIP particle size and distribution

Figure 3(a,b) shows the size distribution and shape of the crosslinked poly(methacrylic acid) particles after being imprinted with the template of all-*trans*-retinoic acid. The particle diameter was less than 100 μm with a relatively narrow distribution.

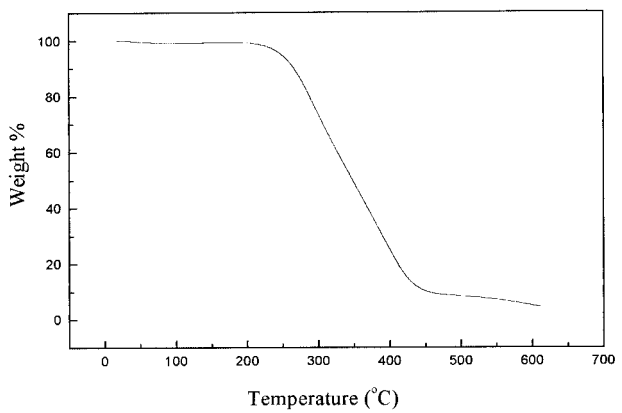
Thermal stability

TGA data of the template of all-*trans*-retinoic acid and the MIP of poly(methacrylic acid) are shown in Figure 4(a,b), respectively. Both the template and MIP were thermally stable up to 230°C; hence, no thermal degradation of the template and the MIP was assured during the preparation and manipulation of MIPs in the present experiment. The MIPs prepared using other templates showed a similar thermal behavior.

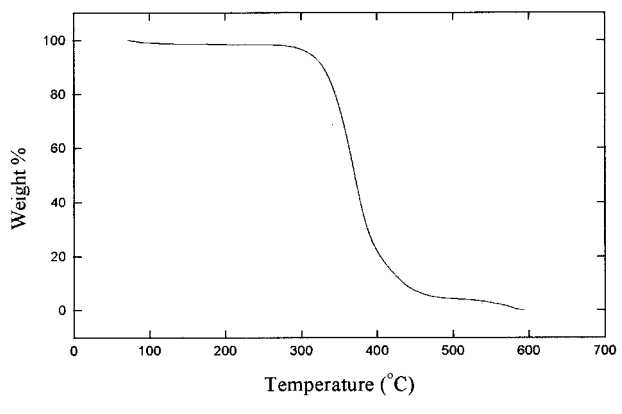
Separation characteristics of MIPs

The HPLC separation characteristics of the MIPs prepared in the presence of the template of all-*trans*-retinoic acid are shown in Figures 5 and 6. Figure 5 shows the retention characteristics of retinoid derivatives in the MIP-packed columns when each component was injected into the HPLC separately. More retention time was required for the target molecule of the all-*trans*-retinoic acid to pass through the column, as it was held longer at the binding site in the host molecules by hydrogen bonding, as shown in Figure 7. No distinguishable retention time difference was observed among the other derivatives of all-*trans*-retinol, all-*trans*-retinal, and all-*trans*-retinol acetate.

Figure 6 shows the separation peaks from HPLC when the retinoid mixture, composed of all-*trans*-retinal, all-*trans*-retinol, all-*trans*-retinoic acid, and all-



(a)



(b)

Figure 4 TGA data of (a) the template of all-*trans*-retinoic acid and (b) the MIP of poly(methacrylic acid).

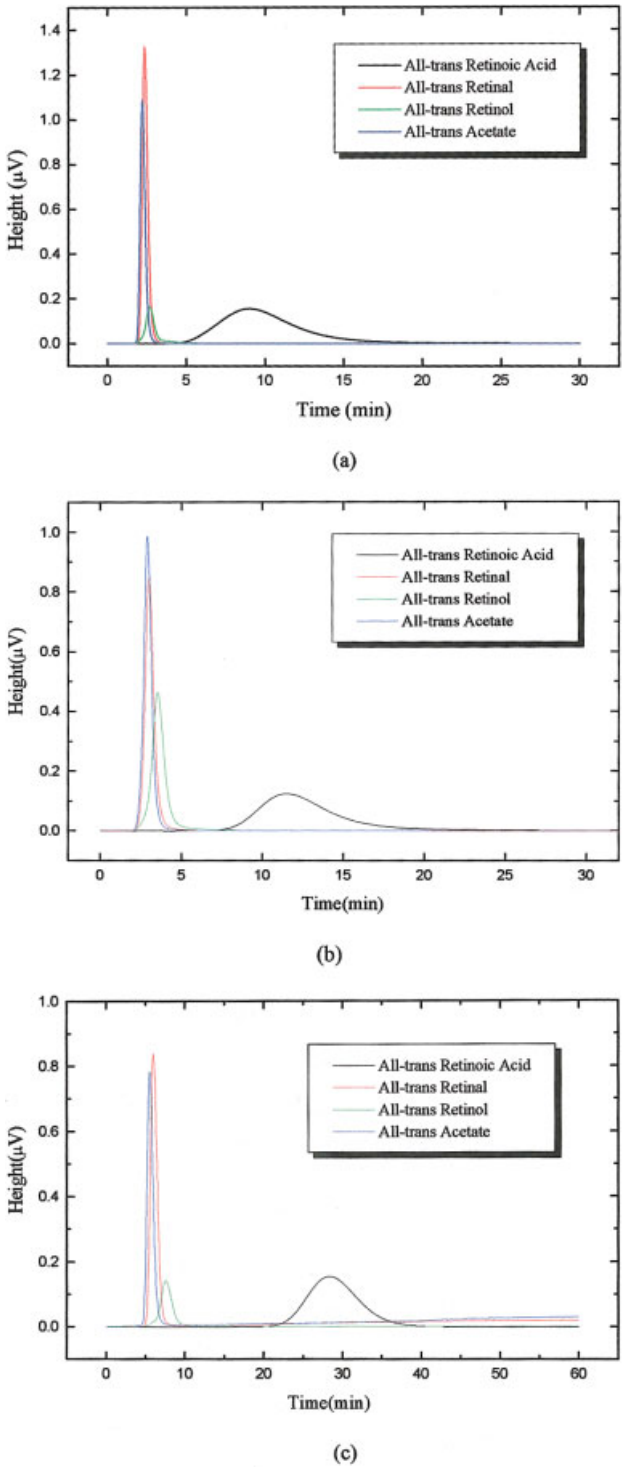


Figure 5 Separation characteristics of retinoid derivatives in the column packed with the MIP prepared using the template of all-*trans*-retinoic acid when each derivative was injected into HPLC separately, for monomer-to-template ratios of: (a) 4:1, (b) 8:1, and (c) 12:1.

trans-retinol acetate, was injected simultaneously. The peaks originating from all-*trans*-retinal, all-*trans*-retinol, and all-*trans*-retinol acetate were superimposed, as the characteristic peak of each component was observed at almost the same retention time in Figure 5. This result implied that the separation of an objective specimen from the retinoid mixture was very accurate using MIP. Also, it is expected that extension of the application of MIP to the separation of other retinoid derivatives such as all-*trans*-retinal, all-*trans*-retinol, and all-*trans*-retinol acetate would be possible.

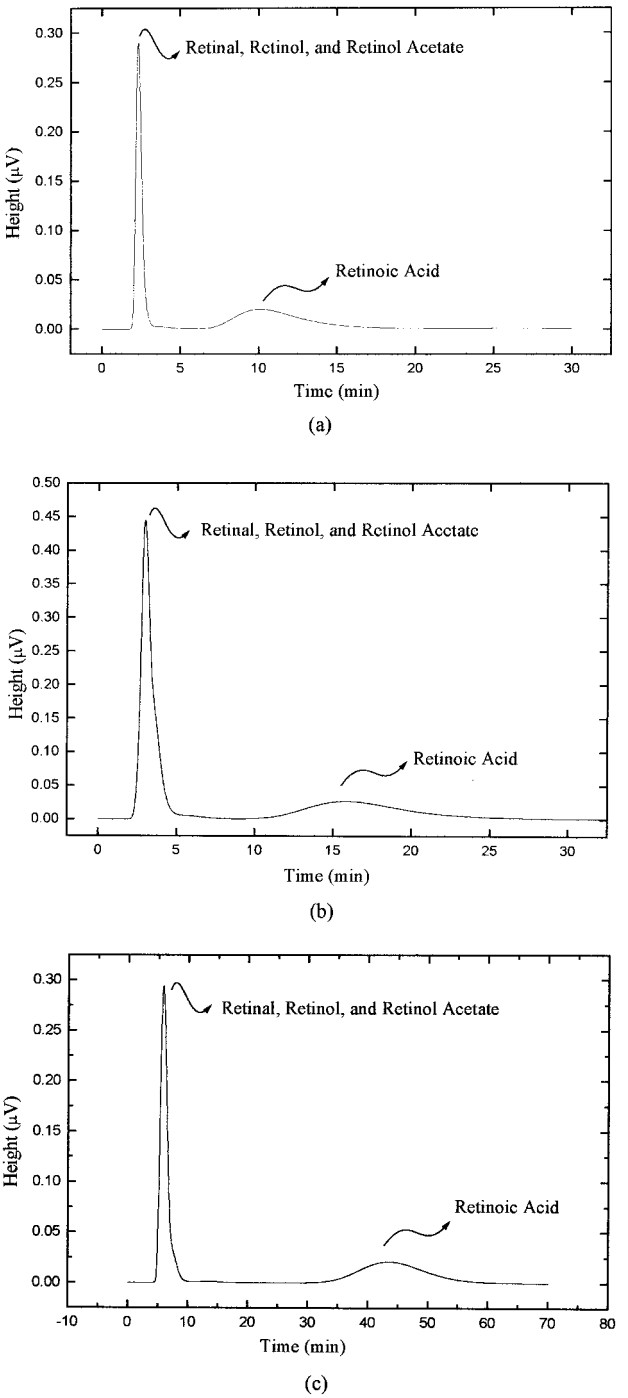


Figure 6 Separation characteristics of retinoid derivatives in the column packed with the MIP prepared using the template of all-*trans*-retinoic acid when the retinoid mixture was injected simultaneously, for monomer-to-template ratios of: (a) 4:1, (b) 8:1, and (c) 12:1.

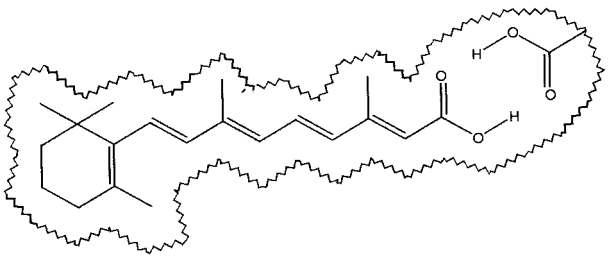


Figure 7 Hydrogen bonding between all-*trans*-retinoic acid and methacrylic acid molecules.

A comparison of Figures 5(a–c) and 6(a–c) shows that the retention time increased with increasing monomer-to-template ratio for all retinoid components. This is because more hydrogen bonding is statistically available with the presence of more methacrylic acid monomer when the template amount is fixed.

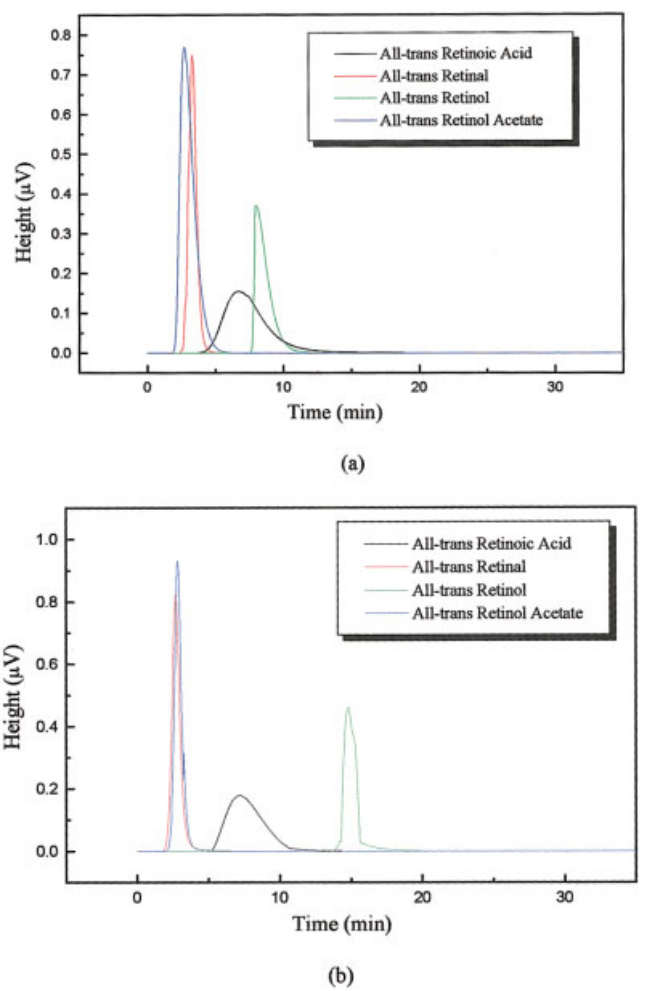


Figure 8 Separation characteristics of retinoid derivatives in the column packed with the MIP prepared using the template of all-*trans*-retinol when each derivative was injected into HPLC separately, for monomer-to-template ratios of: (a) 4:1 and (b) 8:1.

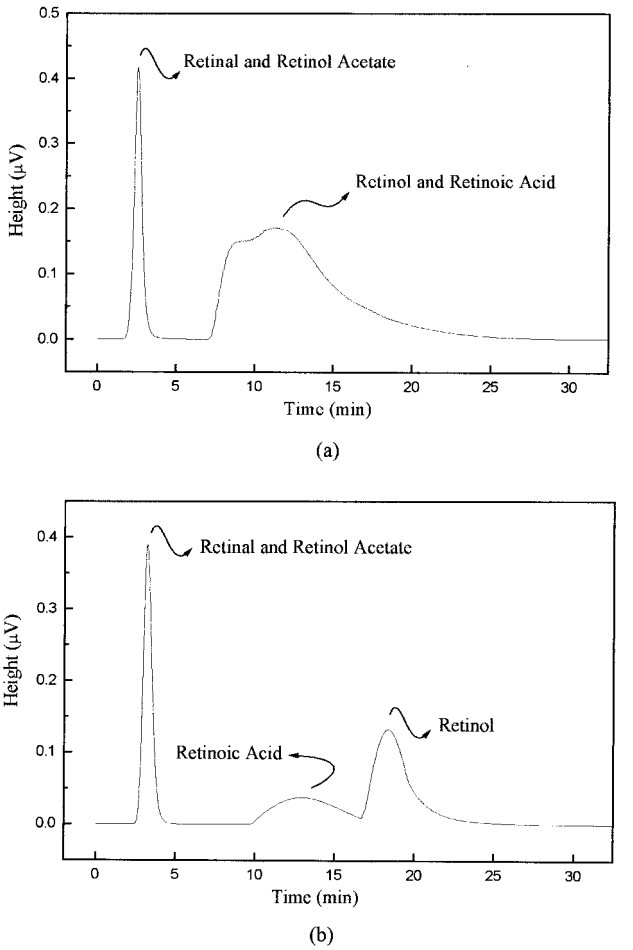


Figure 9 Separation characteristics of retinoid derivatives in the column packed with the MIP prepared using the template of all-*trans*-retinol when the retinoid mixture was injected into HPLC simultaneously, for monomer-to-template ratios of: (a) 4:1 and (b) 8:1.

Figures 8 and 9 show the HPLC separation characteristics of MIPs prepared with the template of all-*trans*-retinol. Different from the separation features of MIPs prepared with the template of all-*trans*-retinoic acid, being well separated from other components is a feature when the template is of all-*trans*-retinol. Also, even if all-*trans*-retinoic acid was not employed as the template, its separation was still observed. That is because hydrogen bonding inherently exists between template and host molecules even without its employment. Except for all-*trans*-retinoic acid there was no chemical or physical interaction between retinoid components and monomer molecules in the absence of a template being introduced. The tailor-made spatial cavity was the main cause of the separation of all-*trans*-retinol in this case. Other behavior was similar to that of the previous MIP system. A higher retention time was observed for a higher monomer concentration.

Simultaneous injection resulted in separation behavior similar to separate injection except that the

TABLE I
Capacity Factor, Separation Factor, and Resolution of HPLC Column Packed with MIPs Synthesized with the Template of All-Trans-Retinol

Ratio of retinol to monomer	Retinoid derivatives	Capacity factor(k′)	Separation factor(α)	Resolution (Rs)
1:4	Retinol	8.4297	1	—
	Retinal	2.8783	2.9287	2.9200
	Retinoic acid	6.9429	1.2141	0.3413
	Retinol acetate	2.2395	3.7640	2.5259
1:8	Retinol	18.7059	1	—
	Retinal	2.3193	8.0652	8.2452
	Retinoic acid	8.4958	2.2018	2.2111
	Retinol acetate	2.4034	7.7832	8.0749

Note: values of α and Rs were calculated based on retinol.

peaks corresponding to all-*trans*-retinal and all-*trans*-retinol acetate were superimposed because of no significant retention time difference between them. For the same reason, the peaks originating from all-*trans*-retinoic acid and all-*trans*-retinol were also superimposed for the template-to-monomer ratio of the 1:4 system, but those were clearly separated for the 1:8 system.

Capacity factor, separation factor, and resolution are the main quantitative parameters determining HPLC separation performance. Table I shows the capacity factor, separation factor, and resolution of HPLC columns packed with MIPs that were prepared using the template of all-*trans*-retinol. The capacity factor increased with an increasing monomer-to-template ratio for all-*trans*-retinol and retinoic acid components because of increased tailor-made spatial cavity and hydrogen bonding. The separation factor, α defined by eq. (1), decreased with increasing monomer concentration for the same reason.

$$\alpha = \frac{k_2'}{k_1'} \tag{1}$$

where k_2' indicates the capacity factor for the component with the higher retention time.

The resolution of column, R_S, is defined by eq. (2).

$$R_s = 2\left(\frac{t_{R_2} - t_{R_1}}{W_1 + W_2}\right) \tag{2}$$

where the subscript 2 indicates the component for the higher retention time, and t_R and W are the retention time and baseline bandwidth, respectively.

No significant difference in characteristic inclination was observed except the absolute values.

CONCLUSIONS

Molecular imprinted polymers can provide tailored binding to specific molecules. Highly crosslinked poly(methacrylic acid) imprinted with the template of either all-*trans*-retinoic acid or all-*trans*-retinol was prepared in particle form. The diameter of polymer particles was less than 100 μm. The MIPs prepared in this study showed good separation capability for the target molecules when those were used as packing materials in an HPLC column. Separation efficiency increased with increasing monomer concentration as greater molecular interaction was generated between monomer and template molecules during polymer synthesis. This separation was caused not only by the specific chemical or physical interaction between host molecules and guest molecules but also by the tailor-made spatial cavity provided in host molecules during synthesis.

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