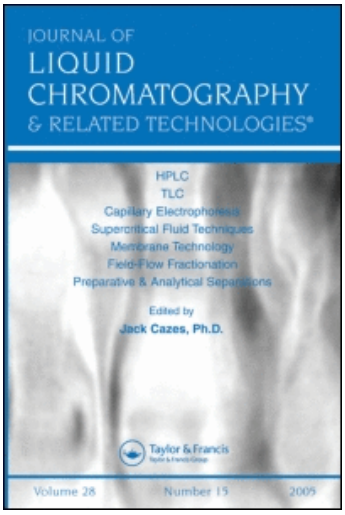

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Enantioseparation of D, L-Phenylalanine By Molecularly Imprinted Polymer Particles Filled Capillary Electrochromatography

Jin-Ming Lin[a]; Tatsuro Nakagama[a]; Katsumi Uchiyama[a]; Toshiyuki Hobo[a]
[a] Department of Industrial Chemistry, Faculty of Engineering, Tokyo Metropolitan University, Hachioji Tokyo, Japan

To cite this Article Lin, Jin-Ming , Nakagama, Tatsuro , Uchiyama, Katsumi and Hobo, Toshiyuki(1997) 'Enantioseparation of D, L-Phenylalanine By Molecularly Imprinted Polymer Particles Filled Capillary Electrochromatography', Journal of Liquid Chromatography & Related Technologies, 20: 10, 1489 — 1506
To link to this Article: DOI: 10.1080/10826079708010989
URL: http://dx.doi.org/10.1080/10826079708010989

J. LIQ. CHROM & REL. TECHNOL., 20(10), 1489–1506 (1997)

ENANTIOSEPARATION OF D,L-PHENYLALANINE BY MOLECULARLY IMPRINTED POLYMER PARTICLES FILLED CAPILLARY ELECTROCHROMATOGRAPHY

Jin-Ming Lin,* Tatsuro Nakagama,
Katsumi Uchiyama, Toshiyuki Hobo

Department of Industrial Chemistry
Faculty of Engineering
Tokyo Metropolitan University
1-1 Minami-Ohsawa, Hachioji
Tokyo 192-03, Japan

ABSTRACT

The chromatographic properties of molecularly imprinted polymer filled capillary electrochromatography (CEC) have been studied. The network polymers were prepared using L-phenylalanine anilide (L-pheAN) or L-phenylalanine as print molecules. Methacrylic acid was used as functional monomer, such that the acid function of monomer interacts ionically with the amine function and via hydrogen bonding with the carboxyl group of the print molecules. Ethylene glycol dimethacrylate was used as cross-linker and the reaction initiator was α, α′-azobis(isobutyronitrile). The obtained polymers were ground and sieved to particles<10 μm for filling into capillary for CEC and <30μm for packing into high performance liquid chromatographic columns. The separations of D,L-phenylalanine by using the molecularly imprinted polymers against L-pheAN and L-phenylalanine were compared. It was

interesting to find that the resolution of D,L-phenylalanine using L-pheAN printed polymer was higher than that using L-phenylalanine printed polymer. The selected polymers obtained were tested in CEC. Some aromatic amino acids, such as D,L-phenylalycine, D,L-tyrosine etc., could be separated to some extent. This method demonstrated that the molecularly imprinted polymer can also be used in CEC for the separation of enantiomers. The peak shape and the resolution of D,L-phenylalanine by this proposed method were better than those by the HPLC method.

INTRODUCTION

High resolution enantiomer separation by chromatography has been developed into an indispensable tool in contemporary chiral analysis. Specifically, the separation of amino acid enantiomers has been widely investigated because of their commercial significance and ease of availability. Several chromatographic techniques, such as HPLC,[1,2] GC,[3,4] and capillary electrophoresis[5,6] have been employed for the enantioseparation. Most of these techniques used chiral selectors and were largely empirical, and the mechanism of chiral recognition is not fully understood. Therefore, it is often difficult to predict the elution order of enantiomers. Molecular imprinting technique,[7-9] as a method to prepare support materials for molecular separation and concentration, has attracted much attention. Mosbach and his co-workers have made a lot of excellent contributions to this research field.[10] This technique has been demonstrated to be effective for amino acid derivatives,[11,12] metal ions,[13,14] saccharides,[15,16] organic compounds of low molecular weight,[17,18] and so on. The molecular imprinting method has been successfully used in HPLC for the separation of amino acid derivatives and some racemates.[19,20] In recent years, this technique has also been applied in CEC[21] and some interesting results have been obtained. Although, template polymerization is conceptually attractive for the preparation of highly selective adsorbents, successful demonstrations of chromatographic separation involving these materials are still relatively few. To our knowledge, although there are a lot of investigations concerned with chiral separation by using molecularly imprinted polymer, most all of them were based on HPLC method. There are almost no publications about chiral separation using molecularly imprinted polymer filled CEC. The aim of this study was to try to use molecularly imprinted polymer particles filled capillary for the enantioseparation by electrochromatography. The polymers printed with L-pheAN and L-phenylalanine were compared each other for the separation of D,L-phenylalanine, and the results from CE and HPLC are also discussed.

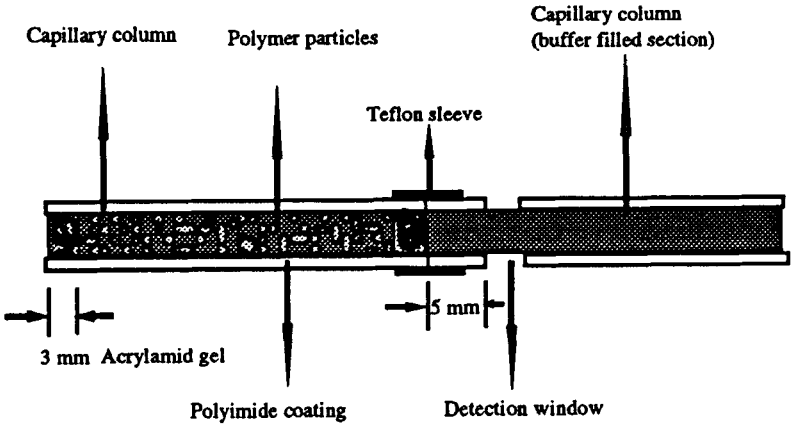

Figure 1. Schematic diagram of the connection of polymer filled capillary with an open tubular capillary.

EXPERIMENTAL

Apparatus

For capillary electrophoresis (CE) measurements, a laboratory made CE set[22] was used, which consisted of a UV-8011 detector (Tosoh, Japan), a HEL5-30P2-TTu high voltage power supply (Matsusada Precision Devices, Inc., Japan), and a C-R6A Chromatopac Recorder (Shimadzu, Japan). Separations were carried out in fused-silica capillaries (75-μm I.D. and 375-μm O.D.). The capillary was composed of two sections. One section is packed with molecularly imprinted polymer particles. The polymer particle slurry was pumped into the capillary at 200 kg cm^{-2} using a liquid chromatographic pump (Shimadzu, Japan). Another part has a 0.3 cm narrow segment detector window and is filled with the buffer. As shown in Figure 1, the total length of the capillary is 40 cm (20 + 20), and length to the detector is 20.7 cm. Before preparation, the capillary was washed with 1.0 mol L^{-1} potassium hydroxide solution, water and electrolyte buffer solution for 30 minutes, respectively.

The HPLC equipment consisted of a JASCO 88-PU pump (Japan Spectroscope Co. Ltd.), a Shimadzu SPD-6AV UV-VIS sepectrophotometric detector, and a Shimadzu C-R5R Chromatopac (Shimadzu, Japan). A Model 7125 Syringe Loading Sample Injector (Rheodyne Incorporated, California,

USA) with a 10-μL sample loop was employed. HPLC columns (150 mm x 2.1 mm I.D., GL Sciences Inc. Japan) were packed by the conventional slurry method using a Shimadzu LC-6A pump. The detection wavelength used was 254 nm or 225 nm depending on the samples. The eluent was a mixture of acetonitrile, water and acetic acid (90:5:5 v/v). The column was first purged with acetonitrile, and then with the eluent at a flow-rate of 0.2 mL min^{-1} until a stable baseline was observed.

Scanning electron micrographs were carried out in a JSM-6100 Scanning Electron Microscope (JEOL, Tokyo, Japan) operated at a voltage of 20 kV. The samples for SEM were coated with platinum in a Model 1BZ SEM coating unit (Eiko Engineering Co. Ltd, Japan). The metallic fine mesh filter series with the caves from 2-μm to 25-μm were purchased from Toyo Roshi Kaisha (Tokyo, Japan). The temperature of the capillary outside wall was measured by an IT202 Infrared Thermometer from Keyence Corporation (Japan).

Chemicals

All samples were prepared using deionized water that was double distilled. α,α'-Azobis(isobutyronitrile) (AIBN) and methacrylic acid (MAA) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Ethylene glycol dimethacrylate (EDMA) was obtained from Nacalai Tesque ,Inc. (Kyoto, Japan). Acetonitrile (MeCN), acetic acid (HAc) and chloroform are chromatographic grade and all of them were purchased from Kanto Chemicals Co. Inc. (Tokyo, Japan). D,L-Phenylalanine, L-phenyalalanine, L-pheAN and other amino acids were purchased from Sigma Chemical Company (St. Louis, Mo. USA.) and used as received. 90:5:5 (v/v) of acetonitrile, water and acetic acid mixed solution was used as the electrolyte for CEC and for the eluent of HPLC.

All solutions were passed through a 0.1μm syringe filter (Advantec, Tokyo, Japan) and then degassed.

Polymer Preparation

Polymers were synthesized by the method of Sellergren et al,[23] and the procedure is simply demonstrated in Scheme 1. Print molecule, cross-linking monomer, and the appropriate amount of functional monomer were weighed in a 20-mL borosilicate glass test tube and dissolved with 16 mL of chloroform or

Scheme 1

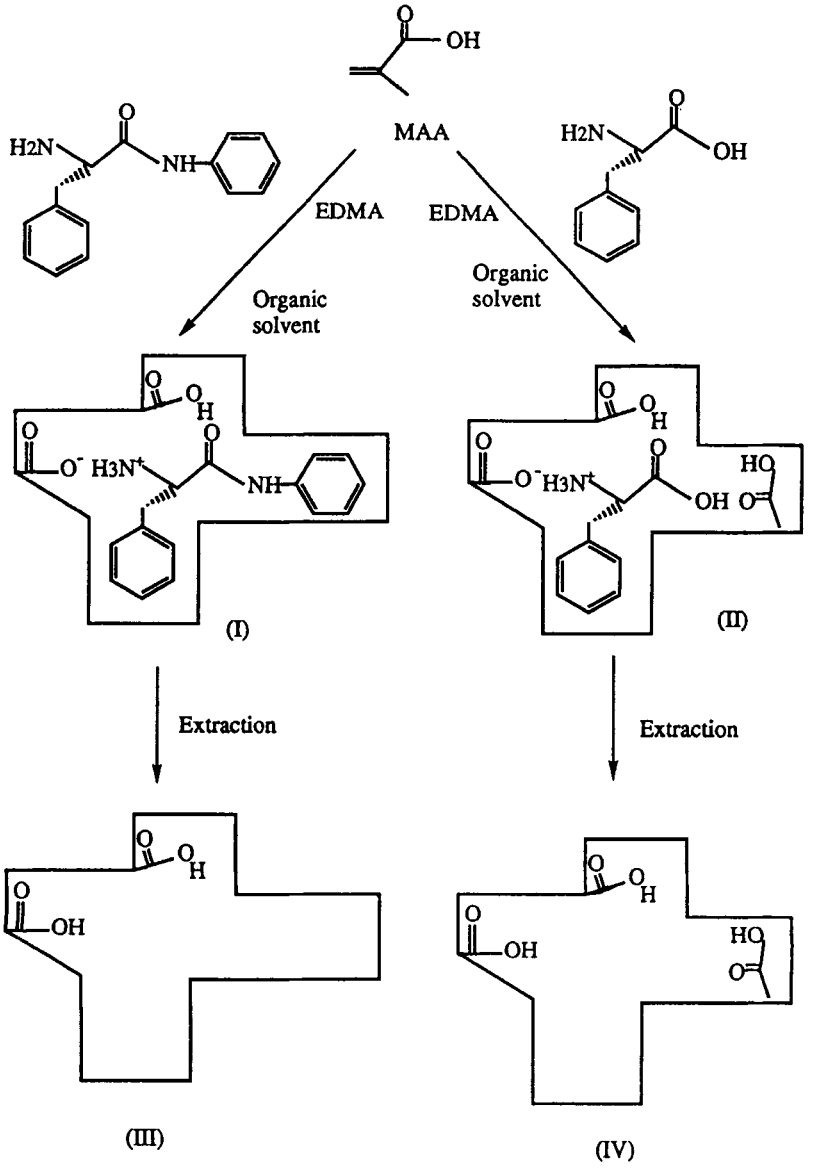

Table 1

Composition of Polymerization Mixtures

	Composition of Monomer Mixture						
Polymer	Print Molec.	Monomer	Crosslinker	Initiator	Solvent	Temp. (°C)	Time (H)
1	L-PheAN	MAA	EDMA	AIBN	Chloroform	60	24
2	L-PheAN	MAA	EDMA	AIBN	MeCN+HAc	60	24
3	L-Phe	MAA	EDMA	AIBN	MeCN+HAc	60	24
4	L-Phe	MAA	EDMA	AIBN	Chloroform	60	24

The molar ratio for EDMA:MMA:L-pheAN is 20:5:1, and for EDMA:MMA:L-phe is 20:5:0.1.

acetonitrile-acetic acid (95:5, v/v) depending on the solubility of the print molecule. The molar ratio of cross-linking monomer to functional monomer to print molecule was 20:5:0.5 (or 20:5:1). The resulting polymers were ground to particles, sieved into 2-10 μm for CEC and 20-25 μm for HPLC.

RESULTS AND DISCUSSION

Preparation of Molecularly Imprinted Polymers

The aim of this study was to try to use molecularly imprinted polymers for the separation of phenylalanine by CEC. As shown in Scheme 1, print molecule, L-pheAN or L-phenylalanine, is present at low concentrations in a mixture of MAA monomer, EDMA cross-linker, AIBN initiator and organic solvent. In this mixture, it is envisaged that print molecules would interact preferentially with carboxyl-containing vinyl monomers due to coulombic forces between positively charged amino groups of print molecules and opposite charged carboxylates of carboxyl-containing vinyl monomers. During polymerization, specific combination of molecule and vinyl monomer could be formed due to electrostatic interactions and hydrogen bond. After polymerization, loosely bound print molecules were washed from the polymer under very mild condition by extracting the polymer with an organic solvent. The obtained polymers contained imprints of the added print molecule, and the formed cavities, shaped after the print structure. These polymers were equipped with carboxyl groups that could interact specifically with the amino function of rebound phenylalanine. In this work, four kinds of polymers (Table 1) using different print molecules or organic solvents were prepared. A high concentration of cross-linking agent was present in the polymerization mixture

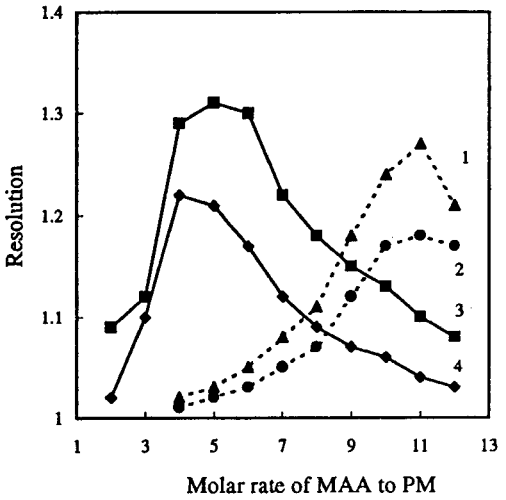

Figure 2. Effects of molar ratios of MAA to print molecule on the separation of phenylalanine. (——) presents that print molecule is L-pheAN and (-----) is for L-phenylalanine as print molecule. Curves 1 and 3 are the results from CEC under the conditions: capillary, 40cm x 75-μm I.D. x 375-μm O.D.; effective length, 20 cm; electrolyte solution, 90% MeCN-% 5 HAc-5% water; applied voltage, 350 V cm^{-1}; electroinjection, 300 V cm^{-1}, 5 s; detection, 254 nm. Curves 2 and 4 resulted from HPLC under the conditions: column, 150 mm in length and 2.1 mm I.D.; eluent, 95% MeCN-5% HAc; flow rate, 0.20 mL min^{-1}; detection, UV 254 nm.

in order to produce microporous polymers of high rigidity. The molar ratio of cross-linker (EDMA) to functional monomer was fixed at 5:1 according to the previous study.[23] The effect of molar ratio of functional monomer to print molecule on the resolution of phenylalanine was determined. As shown in Figure 2, the results indicated that the molar ratio of MAA to L-pheAN in the range of 5:1~4:1 is the most suitable for the separation of D,L-phenylalanine with both CEC and HPLC. When the polymer was imprinted with L-phenylalanine, the molar ratio of MAA to L-phenylalanine is high. The acceptable molar ratio of MAA to L-phenylalanine was 5:0.5. This may be due to phenylalanine being dissolved, a little, in acetonitrile-acetic acid or chloroform. Combined, with a previous study[23] and our results, polymer 1 and 2 were prepared from 2.510^{-3} mole of L-pheAN, 1.25×10^{-2} mole of MAA, 5.0×10^{-2} mole of EDMA and 32.0 mg AIBN in 16 mL of chloroform or 95:5 (v/v) MeCN-HAc solvent. Polymer 3 and 4 were prepared by the same method as Polymer 1 and 2, except the amount of print molecule (L-phenylalanine) was 2.5×10^{-4} mole.

The influence of solvents on the microstructure of polymer was investigated in the polymerization of methacrylic acid by Schroder.[24] The structure of the polymer prepared at 60 was found to depend on the solvent. In our experiment, we also noticed that the polymer prepared by using MeCN-HAc as solvent was harder than that using chloroform as solvent. At the same conditions, the polymer particles of Polymer 2 and 3 were more stable when MeCN-HAc was used as extraction solvent or electrolyte. This means that the usages of the same organic solvent in polymerization and eluent are beneficial to the separation. Therefore, polymer 2 and 3 were used for the following experiment.

Extraction of Print Molecule from Polymers

The extraction of print molecule from polymers is an important process, in principle, it releases the print molecules and creates the cavities capable of specific resorption of the template. It may also move other materials from the polymer, for example, residual monomers and initiator fragments. As ionic and hydrogen-bonding interactions between the amino groups of L-type amino acid and the carboxylic groups of a polymer were expected, the extraction procedure should not only facilitate the transport of print molecule from the polymer to outside, but also break the chemical interaction efficiently. This might be achieved by the use of an acid whose acidity is stronger than the template amino groups, e.g. acetic acid.

The amino group of the amino acid was more basic than that of the carboxyl group of the polymer when MAA was used as monomer. Therefore, in order to get polymer cavities with acidic properties, a mixture of acetonitrile (95 v%) and acetic acid (5 v%) was used in the extraction procedure.

Scanning Electron Microscopy

The molecularly imprinted polymer particles were examined by scanning electron microscopy (SEM) to compare the morphologies of the polymer particles. As shown in Figure 3, the polymer particles are irregular-shaped materials. The shapes of the blank polymer (without print molecule) particles and the molecularly imprinted polymer particles before and after extraction were compared. The shapes of them were almost the same. These results indicated that the polymer skeleton was stable when acetonitrile-HAc was used as extracting medium.

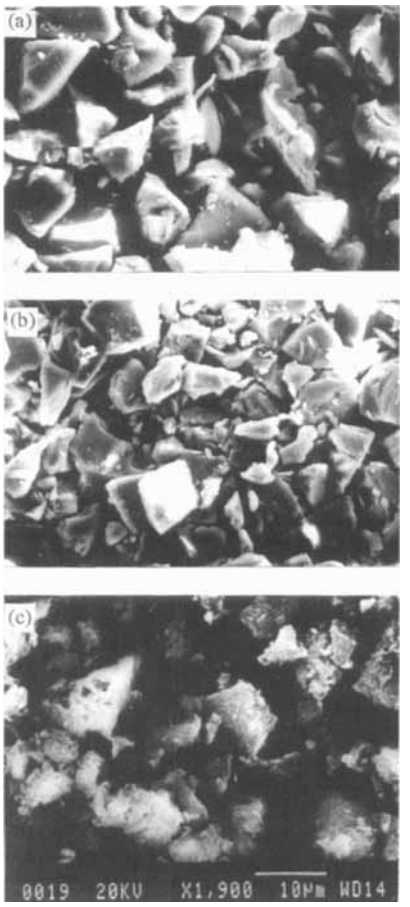

Figure 3. Scanning electron micrograph of the molecularly imprinted polymer particles. (a). the blank polymer particles which synthesized without any print molecules, (b). molecularly imprinted polymer particles without acetonitrile/HAc washing, and (c). particles washed with 95% acetonitrile-5% HAc 3 h.

The CEC resolution of D,L-phenylalanine using molecularly imprinted polymer was affected by the particle size. As shown in Figure 4, small particles are beneficial to the separation, but too small particles suffered some damage when the voltage was applied to the capillary. This influence became serious when the applied field strength was over 300 V cm^{-1}. This phenomenon can be explained by the fact that, although the polymer particles almost cannot be dissolved in acetonitrile-acetic acid solution, at high electrolyte voltage the

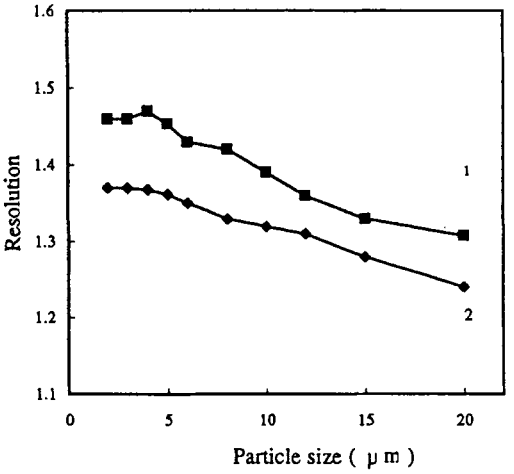

Figure 4. Effect of polymer particle size on the separation of phenylalanine by CEC. 1. polymer imprinted with L-pheAN, 2. polymer imprinted with L-phenylalanine. Separation conditions are the same as in Fig. 2.

capillary will be heated as a result of the high electric current, which will make the loss of some residual functional groups from the cavity surfaces, and may also remove some materials from the polymer. The lifetime of the capillary column will be shorter, even though a higher resolution can be achieved. On the other hand, if the polymer particle size is too large, it is difficult to fill them into the capillary and the resolution decreases. Because the effective surface area of the polymer become smaller when the particle size increases, the interaction activity between the polymer inner-surface and analytes decreased. As shown in Figure 4, the resolution gets a plateau when the polymer particle size is smaller than 5-μm. Considering the other conditions, including the fact that a longer time is needed to slurry the small polymer particle, we chose a size smaller than 10-μm for this experiment. The polymer particle sizes for HPLC were controlled in the range of 20-25 μm according to the Kempe's results,[20] and not discussed here.

A Practical Approach to Electrochromatography

The same as capillary electrophoresis, separation of phenylalanine using CEC method was also affected by several factors, such as variations of the applied voltage, buffer solution and temperature. With the exception of polymer

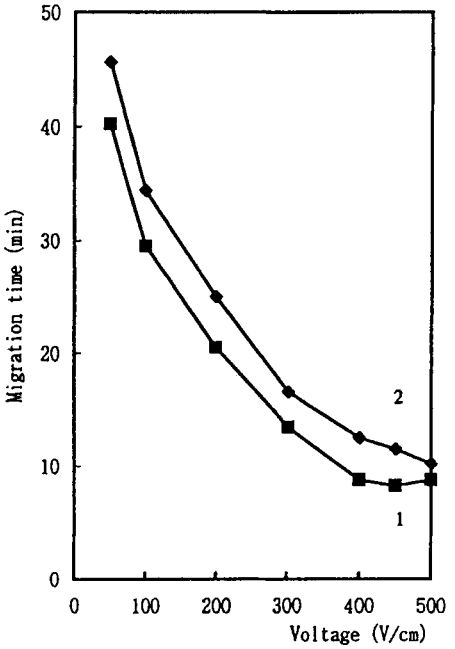

Figure 5. Migration time as a function of applied potential strength. 1. Capillary filled with L-pheAN printed polymer particles and 2. Capillary filled with L-phenylalanine printed polymer particles. Sample: D,L-phenylalanine. Other conditions are the same as in Fig.2.

properties, we investigated these factors for the determination of stability and reproducibility of the electrochromatographic performance. As illustrated in Figure 5, when the applied voltage is increased, the migration times decrease more rapidly than those predicted. Because the higher applied voltage caused the temperature increase in capillary, the interaction between carboxyl groups of molecularly imprinted polymers and re-added substrates will be affected. That is, the strength of the hydrogen bond and the ionic combination between polymer and analyte depended not only on the structure of the polymer and sample, but also on the temperature. The relationship between the separation factor with the temperature of the capillary outside wall, is shown in Figure 6. Although the temperatures between the capillary center and the outside are different, the temperature gradients from the capillary center to the outside is about 3.14 for the capillary of 75-μm I.D. and 375-μm O.D.[25]. The data show that the separation factor is lower at the high temperature which corresponds to the high applied voltage. We also determined the separation factor of the

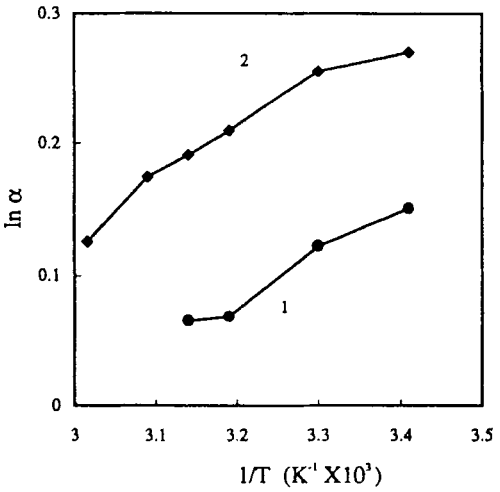

Figure 6. The relationship between column temperature and separation factor. 1, Results from CEC method and 2, data from HPLC. Polymer was imprinted by L-pheAN (Ploymer 2). Particle size for CEC and HPLC were 5-μm and 25-μm respectively. Other conditions are the same as in Fig. 2.

HPLC method when the column temperature was varied. As illustrated in Figure 6, curve 1 and 2 show some similarity. Although both of these methods were affected by temperature, the results indicated that separation factors from the HPLC method are more strongly dependent on the temperature. The reason for this, is that the recognition ability of CEC is concerned not only with the hydrogen bond between the carboxyl groups of polymer and the carboxyl groups of amino acid, but also the ionic bond between amine groups ($-NH^{+}_{3}$) of amino acid with the part of ($-COO^{-}$) groups in the polymer (see the Scheme 1 compound (I) and compound (II)), when a acidic eluent was used.

The enthalpy difference ($\Delta\Delta S^{0}$) and the entropy difference ($\Delta\Delta H^{0}$) were also calculated based on the following equation:[26]

$$\ln \alpha = - \Delta (\Delta H^{0})/RT + \Delta (\Delta S^{0})/R \qquad (1)$$

Where α is the separation factor, T is the absolute temperature, R is the gas constant. ΔH^{0} and ΔS^{0} are the different entropy and different enthalpy of the enantioselective energy, respectively.

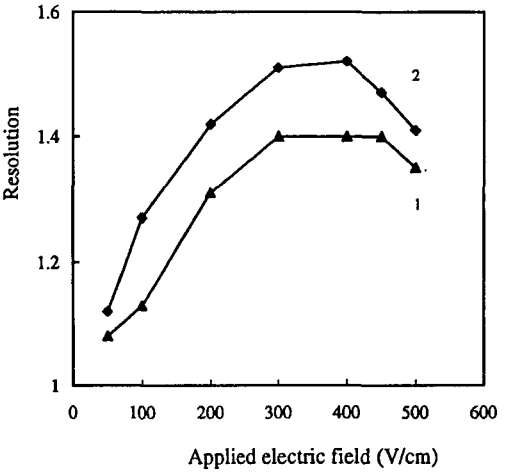

Figure 7. Effect of applied voltage on the resolution of phenylalanine by CEC. 1, using polymer 2 and 2, using polymer 3. Other conditions are the same as in Fig.2.

For CEC, the enthalpy differences and entropy differences were -8.75 J mol^{-1} K^{-1} and -2.96 KJ mol^{-1} , respectively. Similarly, ($\Delta\Delta S^0$) and ($\Delta\Delta H^0$) for the HPLC method were equal to -8.44 J mol^{-1} K^{-1} and -3.21 KJ mol^{-1}. From these results, we think that the interaction of analytes with the polymer is a diffusion-controlled process, and sufficient separation time for the recognition of chiral analytes is required. Increased thermal effect resulting from higher applied potentials can reduce the migration time, but the resolution will be decreased. In order to keep the capillary temperature constant, the environmental temperature of the CE was set at 20°C and a micro fan was also used.

Figure 7 shows the separation of D,L-phenylalanine using L-pheAN and L-phenylalanine imprinted polymers by changing the applied voltage. The applied voltage was changed from 2 to 20 kV corresponding to 50 100, 200, 300, 400, 450 and 500 V cm^{-1}, respectively. The resolution showed that the optimum is at 400 V cm^{-1}. Considering the Joule heating, peak shape, and migration time, a voltage of 350 V cm^{-1} was used in this work .

To achieve the most selective interaction between polymer and analyte, it was advantageous to use the same electrolyte solvent for CEC as that which was used for polymerization. Changes in solvent composition can not only change the strength of the interactions between substrate and interacting units in the polymer, but also, obviously, cause swelling or shrinking of the polymer.

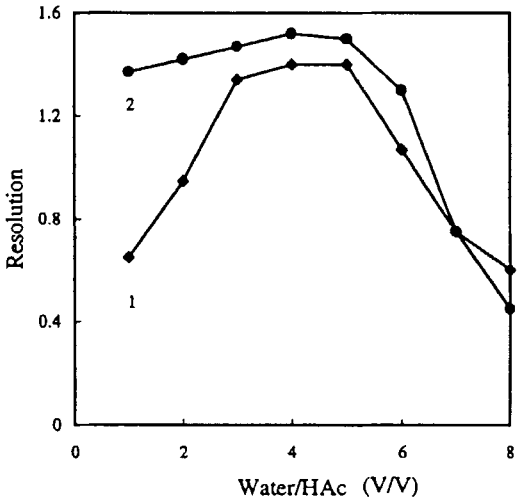

Figure 8. Effect of the eluent composition on the separation of phenylalanine by CEC and HPLC. 1, The results from HPLC and 2, results from CEC. The concentration of MeCN was 90% (v%). Total amount of water and acetic acid in the eluent was 10% (v%). Polymer 2 was used. Other conditions are the same as in Fig. 2.

We compared the solvent compositions, especially the concentration of acetic acid in the eluent and the electrolyte solution. In Figure 8, a suitable composition of solvent mixture for the electrolyte solution was chosen to be 90% acetonitrile -5% acetic acid -5% water (v%). Although this composition was slightly different from the solvent of polymerization, an appropriate amount of water in buffer solution was necessary for CEC, even if the resolution was decreased in some extent. On the other hand, increasing the concentration of acetic acid in mixed solvent is likely to result in weaker hydrophobic interaction and stronger hydrogen bond effect. The higher water content will cause the most substance strongly adsorbed on the polymer. The migration time of phenylalanine in electrochromatography was longer in the high water content electrolyte solution than, in the low one and broad peaks also appeared in the high water concentration. The concentration of water in electrolyte solution was controlled between 3%-6%. We also found that if the electrolyte contained only water and acetonitrile, separation of the analyte could not be done. Both acetic acid and water took an important role in the electrochromatography.

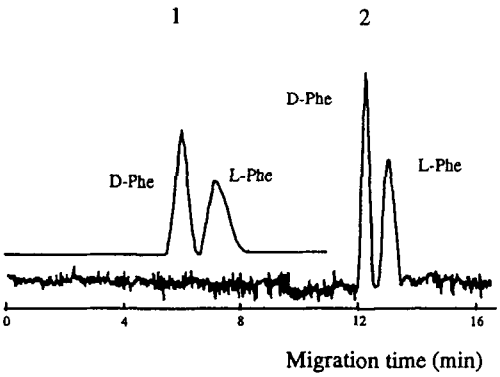

Figure 9. Typical signals of CEC and HPLC. Signal 1 got from HPLC method and Signal 2 obtained from CEC. Other conditions are the same as in Fig. 8 except the eluent (or electrolyte) is 90:5:5 MeCN/HAc/water (v/v).

Similar to most of capillary electrophoresis, degassing of the electrolyte solvent proved to be useful for the separation. Insufficient degassing led to increased baseline drift, unstable current and finally breakdown of electroosmotic flow as a result of bubble formation. These symptoms could be overcome by thoroughly degassing the eluent. The best result was obtained by a combination of purging with helium and applying vacuum under ultrasonification about 10 minute.

The typical electrochromatogram and high performance liquid chromatogram under the optimum conditions are illustrated in Figure 9. The electrochromatographic signals are much sharper than that of the liquid chromatogram. The resolution of phenylalanine by CEC was higher than that from the HPLC method. This means that the proposed method has potential for the chiral separation, and a more detailed investigation will be continued.

Resolutions of Other Amino Acids by CEC

We considered, that a polymer prepared using pheAN or phenylalanine as print molecule may also be able to separate the enantiomers of other aromatic amino acids. From the experiments, it was interesting to find that the proposed method was also a preliminary to resolving some aromatic amino acids, including D,L-phenylglycine, D,L-3-(3,4-dihydroxyphenyl)alanine, D,L-tyrosine and p-fluoro-D,L-phenylalanine. The results are summarized in Table

Table 2

Enantioseparations of Some Aromatic Amino Acids Using CEC Method

Compound	Resolution			
	Polymer 1	Polymer 2	Polymer 3	Polymeer 4
Phenylalanine	1.36	1.43	1.20	0.57
Tyrosine	0.92	0.88	0.67	0.53
p-Fluorophenylalanine	1.04	0.95	0.78	0.67
Phenylglycine	1.10	1.13	0.89	0.50
Phenulalanine anide	1.45	1.32	0	0
DOPA	0.54	0.64	0.21	0.24

2. These compounds have similar construction to phenylalanine. Although, these aromatic amino acids can be separated to some extent, the peak shapes and resolutions are not satisfactory enough for the practice application. This is a problem that needs our continued study, especially the real understanding of the separation mechanism. The molecules without the aromatic ring, for example, D,L-serine, D,L-leucine, D,L-alanine and D,L-valine, can not be recognized at all. D,L-Tryptophan and Dansyl-D,L-phenylalanine were also examined, the results proved that both of them could not be separated. The results indicated that the molecularly imprinted polymer has its special selection for the samples. The polymers printed by L-pheAN or L-phenylalanine seem to be highly specific for D,L-phenylalanine and its similar constructed samples, non-aromatic samples could not be distinguished at all. We think this advantage may be developed for other types of amino acids or chiral samples by using the CEC method.

CONCLUSION

This primary investigation successfully demonstrates that molecular imprinting method can be used for the separation of amino acid enantiomers by CEC. The polymers imprinted by L-pheAN and L-phenylalanine have different recognition characteristics. The former seems to give high resolution for D,L-phenylalanine although the structure of print molecule, L-pheAN is different from the analyte. Comparison of the results between HPLC and CEC, show peak shape and resolution by CEC were better than those of HPLC. Our present study is still in a preliminary stage and resolution improvements have not yet been addressed. Further investigation in detail will be necessary to find

a real understanding of the structure relation of molecularly imprinted polymers with analytes. We believe that the proposed technique will provide a significantly simple procedure for chiral separation. Potential applications of this technique to other template systems are also being explored.

ACKNOWLEDGMENT

The financial support of this research, by grant of the Sasakawa Science Research Foundation of Japan Science Society (Grant No. 8-149), is gratefully acknowledged.

REFERENCES

1. N. Oi, H. Kitahara, F. Aoki, N. Kisu, J. Chromatogr., **689**, 195 (1995).

2. N.Oi, et al., J. Chromatogr., **722**, 229 (1996).

3. K. Sato, K. Watabe, T. Ihara, T. Hobo, Chirality, **5**, 236 (1993).

4. I. Abe, et al., J. Chromatogr., **722**, 221 (1996).

5. H. Nishi, S. Terabe, J. Chromatogr., **694**, 245 (1995).

6. M. Novotny, H. Soini, M. Stefansson, Anal. Chem., **66**, 646A (1994).

7. K. J. Shea, D. A. Spivak, B. Sellergren, J. Am. Chem. Soc., **115**, 3368 (1993).

8. L. Fischer, R. Muller, B. Ekberg, K. Mosbach, J. Am. Chem. Soc., **113**, 9358 (1991).

9. K. J. Shea, G. J. Stoddard, D. M. Shavelle, F. Wakui, R. M. Choate, Macromolecules, **23**, 4497 (1990).

10. M. Kempe, K. Mosbach, J. Chromatogr., **694**, 3 (1995).

11. L. Andersson, D. J. O'Shannessy, K. Mosbach, J. Chromatogr., **513**, 167 (1990).

12. L. I. Andersson, K. Mosbach, J. Chromatogr., **516**, 313 (1990).

13. H. Nishide, E. Tsuchida, Makromol. Chem., **177**, 2295 (1976).

14. V. A.Kabanov, A. A. Efendiev, D. D. Orujev, J. Appl. Poly. Sci., **24**, 259 (1979).

15. G. Wulff, J.Haarer, Makromol. Chem., **192**, 1329 (1991).

16. G. Wulff, S. Schauhoff, J. Org. Chem., **56**, 395 (1991).

17. K. J. Shea, D. Y. Sasaki, J. Am. Chem. Soc., **111**, 3442 (1989).

18. K. J. Shea, D. Y. Sasaki, J. Am. Chem. Soc., **113**, 4109 (1991).

19. C. J. Welch, J. Chromatogr., **689**, 189 (1995).

20. M. Kempe, K. Mosbach, J. Chromatogr., **691**, 317 (1995).

21. K. Nillsson, J. Lindell, O. Norrlow, B. Sellergren, J. Chromatogr. A, **680**, 57 (1994).

22. J-M. Lin, T. Nakagama, H. Okasawa, X. Z. Wu, T. Hobo, Fresenius J. Anal. Chem., **354**, 451 (1996).

23. B. Sellergren, K. J. Shea, J. Chromatogr., **635**, 31 (1993).

24. V. G. Schroder, Die Makromolekulare Chemie, **97**, 232 (1966).

25. E. Grushka, R. M. McCormick, J. J. Kirkland, Anal. Chem., **61**, 241 (1989).

26. Y. Itabashi, Chemistry and Biology (Japanese), **33**, 381 (1995).

Received August 3, 1996
Accepted October 1, 1996
Manuscript 4245