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The Influence of Temperature, Ultrasonication and Chiral Mobile Phase Additives on Chiral Separation; Predominant Influence of β-Cyclodextrin Chiral Mobile Phase Additive Under Ultrasonic Irradiation

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This paper introduces a technique for resolving amino acids that combines the advantages of the conventional CSP (chiral stationary phase) method with the CMPA (chiral mobile phase additive) method. A commercially available chiral crown ether column, CROWNPAK CR(+), was used as the CSP and three cyclodextrins (β-CD, γ-CD, HP-β-CD) were used as the mobile phase additives. Chromatographic resolution was performed at 25 °C and 50 °C with or without sonication. A comparison of the chromatographic results under ultrasonic conditions with those under non-ultrasonic conditions showed that ultrasound decreased the elution time and enantioselectivity at all temperatures. In the case of the β-CD mobile phase additive, the elution time and enantioselectivity under ultrasonic condition were significantly higher than under non-sonic condition at all temperatures. Commercially available Chiralpak AD, Whelk-O2 and Pirkle 1-J columns were used as CSPs to examine more meticulously the effects of ultrasonication and temperature on the optical resolution. The optical resolution of some chiral samples analyzed at 25 °C and 50 °C with or without sonication was compared. As in the previous case, the enantioselectivity was lower at 25 °C but similar enantioselectivity was observed at 50 °C.

Key Words : Enantioselectivity, Ultrasound, Temperature, Mobile phase additive, Chiral stationary phase

Introduction

Chiral separation using a chiral stationary phase (CSP) is the most popular and applicable method. A wide range of chiral stationary phases, such as Pirkle Type CSPs,[1] protein CSPs, cyclodextrin CSPs,[2] cellulose CSPs, synthetic polymer CSPs, chiral ligand exchange chromatography CSPs, chiral crown ether CSP, vancomycin or teicoplanin CSP, and cyclofructan CSPs,[3] as well as more than 100 different stationary phases are commercially available.[4,5] Chiral mobile phase additives (CMPAs) are used for chiral separation by adding a chiral selector to the eluent, which has already been applied successfully to chiral chromatography.[6,7]

Okada reported that ultrasound affects the selectivity in ion exchange chromatography. Moreover, it causes an increase in temperature, and affects the retention time and resolution through an interaction with the samples because the ultrasonic energy absorbed by both the stationary and mobile phase is converted to thermal energy.[8,9] The influence of ultrasonication on chiral separation has been reported.[10]

This study examined how the separation temperature and ultrasonication affect chiral separation during the separation process, where CSP is used in combination with CMPA. The enantioseparation of racemic amino acids including methionine, ethionine and DOPA was performed on a CROWNPAK CR(+) column. CSP and CMPA methods were carried out under the same chromatographic conditions using β-CD, γ-CD and HP-β-CD as the chiral mobile phase additives. The enantioseparation on chiral columns with and without ultrasonic irradiation was examined at 25 °C and 50 °C to determine the effects of temperature and ultrasonication. Based on the primary result, the study was extended to Chiralpak AD, Whelk-O2 and Pirkle 1-J as the chiral stationary phases. Each result obtained when the enantioseparation of racemic mixtures was performed at 25 °C and 50 °C with and without ultrasonic irradiation was compared to determine the effects of temperature and ultrasonication on the enantioseparation of chiral compounds.

Experimental

The high performance liquid chromatography (HPLC) system consisted of the following: Beckmann (SanRamon, CA, USA) Model 110B pump, Rheodyne (Cotati, CA, USA) Model 7125 injector with a 20 μL sample loop, Young In (Seoul, Korea) Model 710 absorbance detector with a 254 nm UV filter and Young In D520B integrator. Ultrasound was provided by an ordinary ultrasonic bath (BRANSONIC 5510R-DTH, Danbury, CT, USA) with a frequency and nominal output power of 42 KHz (± 6%) and 135 watts, respectively. A column support frame was installed in the ultrasonic bath to provide the same analytical conditions. The temperature in the ultrasonic bath was controlled carefully using an automatic thermo-sensor. For precise temperature control, the analytical columns were wound densely with a water-circulating copper tube connected to a cooling

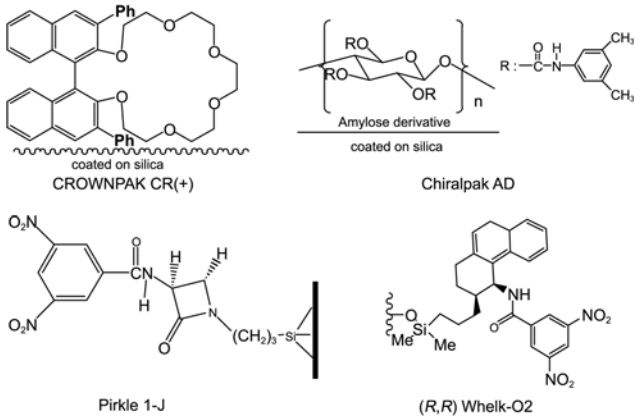

Figure 1. Structures of the chiral stationary phases used in this study.

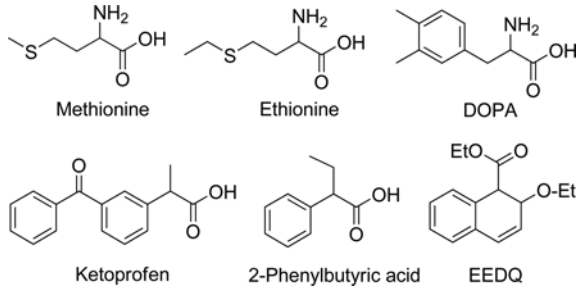

Figure 2. Structures of the chiral samples used in this study.

circulator (Immersion circulator, JEIO TECH CO, Seoul, Korea). The chiral columns used in this study were as follows: CROWNPAK CR(+): 15 cm × 4.0 mm I.D, Daicel, Japan, CHIRALPAK AD: 25 cm × 4.6 mm I.D, Daicel, Japan, (*R*, *R*) Whelk-O2: 25 cm × 4.6 mm I.D, Regis, U.S.A, (3*R*, 4*S*) Pirkle 1-J: 25 cm × 4.6 mm I.D, Regis, U.S.A. Figure 1 shows the structures of the chiral columns used in this study.

All amino acid samples and cyclodextrin, as a selector, were purchased from Aldrich Chemical Co. (Steinheim, Germany). All water (conductivity < 1 μs/cm) was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). The remaining solvents used for HPLC, *i.e.* methanol, ethanol, 2-propanol, *n*-hexane, were obtained from Merck and used as received. Perchloric acid (70%) of special reagent grade was supplied by Junsei Chemical Co. Ltd. Figure 2 shows the structures of the chiral samples.

All chromatographic data was obtained using the following mobile phases at a flow rate of 0.8 or 1.0 mL/min. A CROWNPAK CR(+) column (10 mM $HClO_4$(aq), pH 2,

Table 1. Enantioseparation of amino acids on a CROWNPAK CR(+) column with or without CMPA[a]

Sample	Temp (°C)	Chiral mobile phase additive (CMPA)	Non-ultrasonic			Ultrasonic			Selectivity increment[b]
			k_1	k_2	α(N)	k_1	k_2	α(U)	
Methionine	25	no	0.69	1.46	2.12	0.68	1.37	2.02[c]	95.3 (–4.7%)
		β-CD	0.67	1.31	1.96	0.81	2.01	2.39[c]	121.9 (+21.9%)
		γ-CD	0.62	1.37	2.19	0.73	1.52	2.10	95.9 (4.1%)
		HP-β-CD	0.51	1.21	2.54	0.50	1.05	2.12	83.5 (–16.5%)
	50	no	0.41	0.58	1.44	0.40	0.58	1.43	99.3 (–0.7%)
		β-CD	0.52	0.58	1.46	0.29	0.44	1.54	105.5 (+5.5%)
		γ-CD	0.38	0.56	1.49	0.87	0.55	1.41	94.6 (–5.4%)
		HP-β-CD	0.25	0.37	1.48	0.39	0.54	1.38	93.2 (–6.8%)
Ethionine	25	no	1.74	3.50	2.02	1.78	3.42	1.93	95.5 (–4.5%)
		β-CD	1.50	2.71	1.81	1.94	4.02	2.02	111.6 (+11.6%)
		γ-CD	1.50	3.18	2.11	1.88	3.91	2.10	99.5 (–0.5%)
		HP-β-CD	1.53	3.29	2.14	1.56	3.29	2.11	98.6 (–1.4%)
	50	no	1.04	1.46	1.40	1.01	1.41	1.39	99.3 (–0.7%)
		β-CD	0.43	0.62	1.43	0.92	1.38	1.51	105.6 (+5.6%)
		γ-CD	1.06	1.48	1.41	1.03	1.44	1.39	98.6 (–1.4%)
		HP-β-CD	0.79	1.14	1.45	0.85	1.18	1.40	96.6 (–3.4%)
DOPA	25	no	1.08	1.80	1.65	1.11	1.70	1.54	93.3 (–6.7%)
		β-CD	0.82	1.20	1.46	1.08	1.89	1.75	119.9 (+19.9%)
		γ-CD	0.81	1.33	1.65	1.14	1.74	1.53	92.7 (–7.3%)
		HP-β-CD	0.86	1.41	1.64	0.89	1.38	1.55	94.5 (–5.5%)
	50	no	0.48	0.60	1.24	0.47	0.58	1.24	100 (0)
		β-CD	0.44	0.59	1.35	0.40	0.57	1.43	107.5 (+7.5%)
		γ-CD	0.49	0.63	1.28	0.49	0.61	1.23	96.1 (–3.9%)
		HP-β-CD	0.26	0.38	1.43	0.33	0.42	1.28	89.5 (–10.5%)

[a]Flow rate: 0.8 mL/min, detection: 254 nm, mobile phase: pH 2 $HClO_4$(aq) including 1 mM of β-CD, γ-CD, HP-β-CD or not. [b]100(%) × α(U)/α(N). [c]The data shown in Figure 3.

flow rate = 0.8 mL/min); CHIRALPAK AD column (hexane/IPA/HOAc = 80/20/0.5, flow rate = 1.0 mL/min); (*R*, *R*) Whelk-O2 column (hexane/IPA/HOAC = 80/20/0.5, flow rate = 1.0 mL/min) and (3*R*, 4*S*) Pirkle 1-J column (hexane/IPA = 90/10, flow rate = 1.0 mL/min) were used. The column void volume was checked by injecting 1,3,5-tri-*tert*-butylbenzene (normal phase) or sodium nitrate (reverse phase), which is a presumed unretained solute.[11] Both compounds were purchased from Aldrich Chemical Co. All resolution data are the mean of at least five experiments.

Result and Discussion

Enantioseparation of Racemic Amino Acids Using Only the Chiral Stationary Phase Method. Initially, the chiral separation of racemic amino acids was performed at 25 °C and 50 °C using the CSP method only under ultrasonic conditions or not. This study was carried out using CROWNPACK CR(+) CSP as the chiral stationary phase and a pH 2 $HClO_4$(aq) solution was used as the mobile phase. Table 1 lists the results in the first row of each sample.

From Table 1, chiral separation was better at 25 °C and under non-ultrasonic conditions than under the other conditions. At 50 °C with ultrasonic irradiation, the elution time and enantioselectivity(α) were reduced. The decreased resolution under ultrasonic conditions was attributed to the increase in temperature by ultrasonic irradiation.[8-10]

In this chiral resolution, crown ether used as a chiral selector was bonded covalently to silica for the chiral stationary phase, and chiral recognition was achieved by a complex formed between the ammonium ion induced from the samples and crown ether. Therefore, this column is available for enantioseparation with regard to compounds with primary amino groups around a chiral center as well as amino acids.[12] As a result, the mobile phase should be acidified to form ammonium ions and separation can be performed. Previous studies suggested that a mobile phase with a lower pH leads to higher enantioselectivity. Similarly, in this study, the pH of the mobile phase was 2, making it a strong acid. The small amount of strong acid was helpful for the UV detection of methionine and ethionine at 214 or 254 nm.

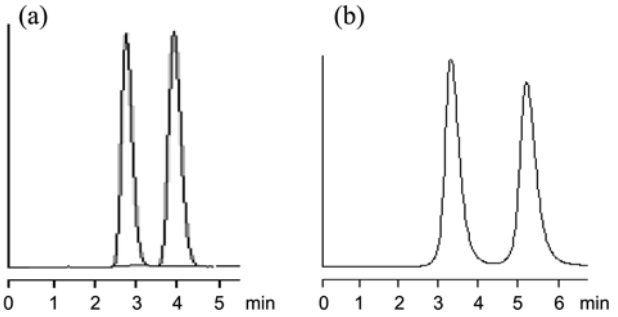

Figure 3. Resolution of methionine under ultrasonication at 25 °C by (a) CSP method with CROWNPAK CR(+) column and (b) the combined CSP (CROWNPAK CR(+) column) and CMPA (β-CD) methods. Flow rate: 0.8 mL/min, detection: 214 nm, mobile phase: pH 2 $HClO_4$ in water.

Enantioseparation of Racemic Amino Acids by Performing the CSP and CMPA Methods Simultaneously. The same CROWNPAK CR(+) column was used as the chiral stationary phase to determine if the enantioselectivity(α) was improved by the combined CSP and CMPA methods. 1 mM of β-CD or γ-CD or HP-β-CD was added to the mobile phase at 25 °C and 50 °C with or without ultrasound. Figure 3 shows the resolution of methionine under ultrasonication at 25 °C on (a) a CROWNPAK CR(+) column only and (b) the combined CSP (CROWNPAK CR(+) column) and CMPA

Table 2. Influences of ultrasound on enantioseparation of racemic ketoprofen, 2-phenylbutyric acid and EEDQ on three different chiral stationary phases[a]

Sample	Temp (°C)	CSP	Non-ultrasonic			Ultrasonic			Selectivity increment[b]
			k_1	k_2	α(N)	k_1	k_2	α(U)	
Ketoprofen	25	Chiralpak AD	1.17	1.38	1.68	1.26	1.51	1.20	71.4 (–28.6%)
		Whelk-O2	4.28	4.80	1.12	4.20	4.71	1.12	100 (0)
	50	Chiralpak AD	0.90	0.97	1.08	0.94	1.01	1.08	100 (0)
		Whelk-O2	2.40	2.61	1.09	2.48	2.70	1.09	100 (0)
2-Phenyl-butyric acid	25	Chiralpak AD	0.40	0.44	1.12	0.36	0.39	1.08	96.4 (–3.6%)
		Pirkle 1-J	0.94	1.10	1.17	1.02	1.18	1.16	99.1 (–0.9%)
		Whelk-O2	0.88	1.05	1.19	0.90	1.07	1.19	100 (0)
	50	Chiralpak AD	0.25	0.25	1.00	0.35	0.35	1.00	100 (0)
		Pirkle 1-J	0.07	0.07	1.00	0.32	0.32	1.00	100 (0)
		Whelk-O2	0.72	0.80	1.12	0.65	0.73	1.12	100 (0)
EEDQ	25	Chiralpak AD	0.41	0.46	1.12	0.36	0.40	1.10	98.2 (–1.8%)
		Pirkle 1-J	0.93	1.09	1.17	0.96	1.19	1.16	99.1 (–0.9%)
	50	Chiralpak AD	0.31	0.31	1.00	0.35	0.35	1.00	100 (0)
		Pirkle 1-J	0.40	0.40	1.00	0.49	0.49	1.00	100 (0)

[a]Flow rate: 0.8 mL/min (Chiralpak AD) or 1.0 mL/min (Pirkle 1-J and Whelk-O2), detection: 254 nm, mobile phase: hexane/IPA = 90/10 (except for Ketoprofen on chiralpak AD; hexane/IPA/HOAc = 80/20/0.5). [b]100(%) × α(U)/α(N).

(β-CD) methods.

As shown in Figure 3, the combined method was much better than the CSP method only. When β-CD was added as a mobile additive to the CROWNPAK CR(+) column without ultrasonication, the selectivity of each sample was lower at 25 °C but slightly higher at 50 °C. On the other hand, the enantioselectivity(α) was improved considerably under sonication at any temperature. This means that β-CD is helpful for the ultrasound-assisted chiral interaction between the chiral samples and stationary phase. The enantioselectivity(α) was improved slightly when γ-CD or HP-β-CD were added as a mobile additive to the CROWNPAK CR(+) column. In addition, the elution time was shorter when HP-β-CD was used as a mobile phase additive.

Ultrasound is a new powerful, technology that is not only safe and environmentally friendly but is also efficient and economical, and can be applied to existing processes in a range of industrial processes to reduce or eliminate the need for chemicals and/or heat. Ultrasound achieves its chemical or mechanical effects by generating bubbles within a liquid/slurry reaction medium; a process known as cavitation.[13,14] Despite β-CD, γ-CD and HP-β-CD having different chemical and physical properties, hydrophobic sizes, shape of hydrophilic sites, solubility, *etc*., the reason for the predominant influence of β-CD on chiral separation under ultrasonic irradiation is unclear. To determine the effect of ultrasound on chiral separation in more detail, we need much more data related to this work.

Chiral Separation of Some Racemic Samples on Amylose-derived and Two Pirkle-type Chiral Stationary Phases. Three different chiral samples and three different chiral columns were additionally used for the ultrasound assisted chiral separation experiment to determine if the enantioselectivity(α) is improved by ultrasound at different temperatures. Table 2 lists the enantioseparation results of an *anti*-inflammatory drug (ketoprofen), 2-phenylbutric acid and EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) on a Chiralpak AD column, Pirkle 1-J, and Whelk-O2 columns.

In the case of ketoprofen on a Chiralpak AD column at 25 °C, the enantioselectivity(α) was significantly lower under ultrasonic radiations than others, but no decrease in enantioselectivity(α) was observed at 50 °C. As listed in Table 1 and a previous study,[10] the enantioselectivity was decreased under sonic conditions but the enantioselectivity of 2-phenylbutyric acid on the Whelk-O2 column was not changed after sonication at any temperature. This means that the influence of ultrasound on chiral separation can be changed with the type of analyte and chiral selector.

Conclusion

This study examined the effect of temperature and ultrasonication, and compared the resolution when the CSP method was combined with the CMPA method by carrying out chiral separation using a range of commercially available chiral columns. At 50 °C, chiral separation was unfavorable, which means that higher temperatures can reduce the interaction between two substances and make chiral separation difficult. Slightly higher chiral selectivity was achieved using the CSP and CMPA method simultaneously than when using the CSP method only. In particular, the enantioselectivity(α) was improved considerably using β-CD as a mobile phase additive under ultrasonic conditions. The reason for this is unclear but it is believed that the increased molecular interactions between the chiral selector and chiral samples by ultrasonication was favorable in the presence of the β-CD mobile phase additive and can help improve the enantioselectivity(α). Although ultrasound had a profound effect on the chiral separation, the reason for the different enantioselectivity is unclear. To determine the effect of ultrasound on chiral separation in more detail, it will important to change the power (frequency) of ultrasound with more chiral samples.

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