Articles

Enantioseparation by Sonochromatography

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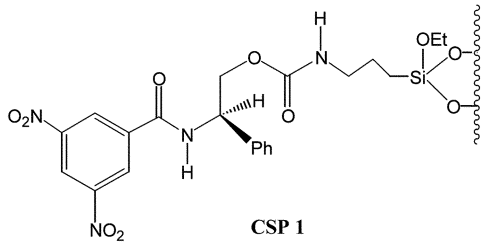
Although chiral separation has been one of the main topics of chromatographic practice for over twenty-five years, it still presents many difficulties. In this work, the ultrasonic dependence of chiral resolution was investigated at various temperatures to improve resolution and reduce analysis time. The chiral resolution was performed on recently commercialized two HPLC chiral stationary phases (**CSP 1** and **CSP 2**) with the analogues of racemic *N*-acylnaphthylethylamines (**1a-d**) and racemic amino acid derivatives (**2a-c**, **3a-c**) as analytes. The **CSP 1** was prepared from a (R)-*N*-(3,5-dinitrobenzoyl)phenylglycinol and the **CSP 2** was prepared from a (S)-*N*-3,5-(dinitrobenzoyl) leucine. From the comparison of the chromatographic results under sonic condition with those under non-sonic condition, we found that the ultrasound decreased the elution time in chiral chromatography at all temperatures and improved the enantioselectivity at high temperature (45, 50, 60 °C).

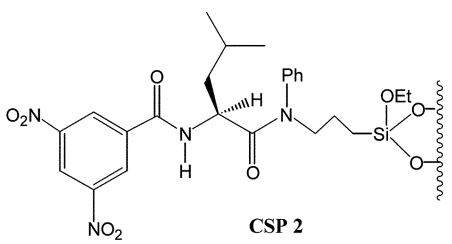
Key Words : Chiral separation, Ultrasonic dependence, Various temperature, Sonochromatography

Introduction

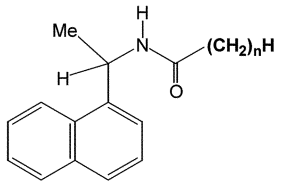
There have been numerous efforts to improve resolution and shorten analysis time in liquid chromatography. Varying pH, ionic strength and the ratio of organic solvent in the aqueous mobile phase is the most common approach to improve the selectivity in HPLC.[1-3] Examples of optimizing analysis times using controlled temperature variation have been reported in many pieces of literature,[4-6] especially, newly developed temperature-responsive stationary phases have been found useful to optimize selectivity by adjusting the temperature rather than changing the mobile phase composition.[7] Flow rate variation as a means to reduce analysis time was reported by several groups while the combination of the column temperature gradient and mobile phase flow gradient also performed with the same purpose.[8,9] Ultrasonic irradiation is widely used for various fields in chemistry.[10] It has been used to accelerate chemical reactions[11] and to extract components from particular material.[12] Ultrasound could be a potentially effective external field in



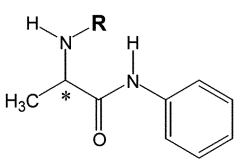
CSP 1

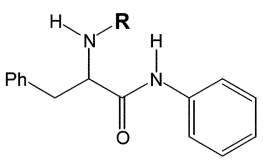


CSP 2



1a-d (n=1,3,5,11)





2a-c **3a-c**

(R= a;BOC, b; CBZ, c; FMOC)

liquid chromatography. Okada *et al.* first reported that ultrasound affects the ionic interaction between charged surfaces and analytes in ion exchange chromatography.[13] Therefore, ultrasonic vibration, which possibly affects the ionic interactions, might play an important role in changing the retention of analytes in ion exchange chromatography. However, ultrasonic vibration has not been utilized in chiral separation. In this study, we first applied ultrasonic vibration to chiral chromatography and named the ultrasound assisted chromatographic method as 'sonochromatography'.

In this work, the ultrasonic dependence of chiral resolution was investigated at various temperatures. The chiral resolutions were performed on HPLC chiral stationary phase (**CSP 1**) derived from (R)-*N*-(3,5-dinitrobenzoyl)phenylglycinol with racemic *N*-acylnaphthylethylamines (**1a-d**) as analytes[14] and **CSP 2** with the analogues of racemic amino acid derivatives (**2a-c**, **3a-c**).[15] We checked retention factors (k) and separation factors (α) of racemic **1a-d** on **CSP 1** at 8, 15, 25, 40 and 50 °C and the racemic **2a-c** and **3a-c** on **CSP 2** at 10, 20, 30, 40, 50, 55 and 60 °C with and without ultrasonic irradiation. Then, we compared the results to elucidate the influence of ultrasound on chiral chromatography.

Experimental Section

Materials. Analytical samples, **1a-d** were prepared by simply treating (R)- and (S)-naphthylethylamine with the appropriate acid chloride in the presence of triethylamine as described previously[14] and the racemic **2a-c** (alanine derivatives) and **3a-c** (phenylalanine derivatives) used in this study were available from a previous study.[15] (a; BOC = *tert*-butoxycarbonyl, b; CBZ = carbobenzyloxy, c; FMOC = 9-Fluorenylmethoxycarbinyl) All HPLC solvents used in this study were obtained from Merck.

Instrumentation. An HPLC system consisting of a Beckmann (San Ramon, CA, USA) Model 110B pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector with 20 μL sample loop, a Young In (Seoul, Korea) Model 710 absorbance detector with a 254 nm UV filter and a Young In D520B integrator was used for the HPLC analysis. Ultrasound was provided by an ordinary ultrasonic bath: BRANSONIC 5510R-DTH (Danbury, CT, USA), output power 135 watts, 42 KHz (± 6%). A column support frame was installed in the ultrasonic bath to provide the same analytical condition. The temperature in the ultrasonic bath was carefully controlled by automatic thermo-sensor. The analytical columns were densely wound with water-circulating copper tube which connected to cooling circulator (Immersion circulator, JEIO TECH CO, Seoul, Korea) for precise temperature control. The two chiral columns were purchased by K-MAC Co. (http://www.kmac.to). Column names are CHIRALRYOO-PGO100 for **CSP 1** and CHIRALHYUN-LEU100 for **CSP 2**. Column size is 25 cm length, 4.6 mm ID.

Chromatographic condition. All chromatographic data were obtained using 2-propanol/hexane (20 : 80) as a mobile phase at a flow rate of 1.5 mL/min. The column void volume was checked by injecting 1,3,5-tri-tert-butylbenzene, a presumed unretained solute,[16] obtained from Aldrich Chemical Co. All resolution data on **CSP 2** are average values of the data obtained from at least five repeated experiments.

Results and Discussion

Influence of temperature on the chiral discrimination. The chiral resolution was performed at 8, 15, 25, 40 and 50 °C to examine the influence of the HPLC analysis temperature on the optical resolution of racemic *N*-acylnaphthylethylamines (**1a-d**) with **CSP 1**. Retention factors (k) and separation factors (α) of **1a-d** at each temperature were calculated from each chromatogram obtained by the chiral HPLC. The chromatographic results are shown in Table 1.

From Table 1, it is observed that the retention time of each analyte decreased and the resolution diminished as the temperature increased. The reduced analysis time and poor resolution at high temperature (40, 50 °C) is presumed to stem from the decreased degree of interaction between the chiral selector of **CSP 1** and analytes.[17]

The retention factors and separation factors of **1a-d** were observed to decrease continuously as the alkyl chain of acyl group of **1a-d** increases in length at all analysis temperatures as shown in many similar examples.[18-20] The reduced retention factors of analytes with long acyl chains are expected to stem from the steric hindrance between analytes and the chiral selector and the favorable solute-solvent interactions between the long hydrocarbon analytes and the relatively lipophilic eluent.[17] The decreased separation factors of analytes with long acyl chains are due to the differences of the decreasing rates of retention factors as the length of acyl tails increases in each isomer. As shown in Figure 1, which illustrates the trends of the retention factor for the two isomers at 8 °C, the retention factors of more retained (S)-isomers decrease more rapidly than those of less retained (R)-isomers as the length of acyl tails increases.

The more rapid decrease in the retention factor of the (S)-isomers than that of the (R)-isomers is due to the direction of acyl tails of (S)-isomers which orient to the chiral selector tether, as explained in similar study.[17,20] So, in the resolution of the analyte **1d**, the elution order was even reversed below 15 °C as shown in Table 1.

The optical resolution of racemic **2a-c** and **3a-c** on **CSP 2** was performed at 10, 20, 30, 40, 50, 55 and 60 °C. The chromatographic results are shown in Table 2.

As shown in Table 2, it is also observed that the retention time of each analyte decreased and the resolution diminished as the temperature increased. Because all resolution data (t_R and α) on **CSP 2** are average values of the data obtained from at least five repeated experiments, the reproducibility of the chromatographic results can be checked with relative standard deviation (RSD) values. The RSD data of selectivity factor (α) on Table 2 are so small that it is concluded that the reproducibility of the α values is excellent. The RSD data of retention time (t_R) were also checked and shown a similar reproducibility with the α values but the data were

Table 1. Resolution of racemic *N*-acylnaphthylethylamines (**1a-d**) on **CSP 1** with varing temperature[a]

	8 °C		15 °C		25 °C		40 °C		50 °C	
	k	α^d	k	α^d	k	α^d	k	α^d	k	α^d
1a	9.62[b] / 17.63	1.83	8.23[b] / 14.75	1.79	6.78[b] / 11.51	1.70	4.91[b] / 7.69	1.57	3.75[b] / 5.51	1.47
1b	8.62[b] / 12.48	1.48	6.77 / 9.72	1.44	4.98 / 7.13	1.43	2.99 / 4.16	1.39	2.22 / 2.98	1.34
1c	6.49[b] / 7.83	1.21	5.35 / 6.31	1.18	5.10 / 6.15	1.21	2.33 / 2.80	1.20	1.61 / 1.90	1.19
1d	5.62[c] / 6.18	0.91	4.60[c] / 5.00	0.92	3.57 / 3.57	1.00	1.04 / 1.04	1.00	1.07 / 1.07	1.00

[a]Mobile phase was 20% IPA in hexane. Flow rate was 1.5 mL/min. Dead time (t_0) was checked by 1,3,5-tri-*tert*-butylbenzene (TTBB). [b]Absolute configuration of the first eluted enantiomer was R. [c]Absolute configuration of the first eluted enantiomer was S. [d]Separation factors were calculated from k_S/k_R, not from k_2/k_1.

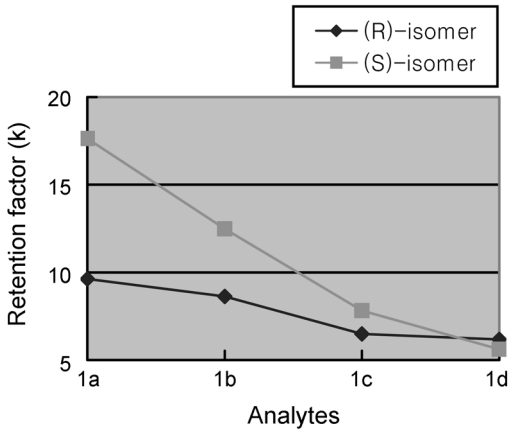


Figure 1. Trends of retention factors for resolving *N*-acylnaphthylethylamines (**1a-d**) on **CSP 1** at 8 °C.

not included in the crowded Table.

Influence of ultrasound on chiral discrimination. Chiral resolution was performed with ultrasonic radiation at 8, 15, 25, 40 and 50 °C to examine the influence of ultrasonic radiation on the optical resolution of racemic *N*-acylnaphthylethylamines (**1a-d**) on **CSP 1**. The chromatographic results are shown in Table 3.

From the comparison of the chromatographic results shown in Table 1 and Table 3, retention factors were found to decrease when the ultrasound was irradiated. The reduced retention factors at ultrasonic condition are caused by the temperature increment, which caused by ultrasonic cavitation.[10] The separation factors were slightly larger at without ultrasonic radiation than with ultrasonic radiation at 8, 15 and 25 °C, but were similar under the two conditions at 40 °C. However, at 50 °C, the separation factors were larger at sonic condition.

Chiral resolution of racemic **2a-c** and **3a-c** on **CSP 2** was performed with ultrasonic radiation at 10, 20, 30, 40, 50, 55

Table 2. Resolution of racemic amino acids derivatives (**2a-c**, **3a-c**) on **CSP 2** with varing temperature[a]

		2a	**2b**	**2c**	**3a**	**3b**	**3c**
10 °C	k_1	0.60	1.51	1.57	0.60	1.47	1.52
	k_2	2.38	12.23	14.69	2.97	12.66	14.15
	α	3.97	8.11	9.36	4.97	8.59	9.30
	RSD[b]	0.011	0.007	0.004	0.009	0.006	0.003
20 °C	k_1	0.50	1.29	1.33	0.50	1.19	1.24
	k_2	1.62	8.64	9.84	2.05	8.22	8.92
	α	3.21	6.71	7.38	4.10	6.90	7.20
	RSD[b]	0.004	0.002	0.003	0.006	0.004	0.004
30 °C	k_1	0.42	0.95	1.02	0.41	0.89	0.95
	k_2	1.16	4.82	5.64	1.40	4.74	5.16
	α	2.75	5.10	5.52	3.40	5.33	5.46
	RSD[b]	0.006	0.005	0.003	0.010	0.003	0.003
40 °C	k_1	0.38	0.85	0.91	0.36	0.77	0.84
	k_2	0.89	3.45	3.93	1.05	3.32	3.77
	α	2.35	4.04	4.33	2.92	4.32	4.50
	RSD[b]	0.011	0.003	0.001	0.001	0.002	0.002
45 °C	k_1	0.36	0.76	0.82	0.35	0.70	0.75
	k_2	0.75	2.76	3.14	0.92	2.60	2.82
	α	2.07	3.61	3.82	2.64	3.73	3.74
	RSD[b]	0.002	0.001	0.001	0.002	0.001	0.002
50 °C	k_1	0.36	0.74	0.80	0.33	0.65	0.72
	k_2	0.69	2.41	2.77	0.80	2.73	2.62
	α	1.93	3.28	3.47	2.38	3.48	3.62
	RSD[b]	0.003	0.001	0.002	0.001	0.002	0.002
60 °C	k_1	0.33	0.66	0.72	0.30	0.59	0.66
	k_2	0.58	1.85	2.08	0.63	1.75	1.98
	α	1.76	2.81	2.90	2.07	2.99	3.01
	RSD[b]	0.002	0.001	0.001	0.002	0.003	0.001

[a]Mobile phase was 20% IPA in hexane. Flow rate was 1.5 mL/min. Dead time (t_0) was checked by 1,3,5-tri-*tert*-butylbenzene (TTBB). Selectivity factors (α) were calculated from k_s/k_r, not from k_2/k_1. [b]Relative standard deviation of selectivity factor.

Table 3. Resolution of racemic *N*-acylnaphthylethylamines (**1a-d**) on **CSP 1** under ultrasonic radiation[a]

	8 °C		15 °C		25 °C		40 °C		50 °C	
	k	α^d	k	α^d	k	α^d	k	α^d	k	α^d
1a	9.31[b] / 16.90	1.82	8.31[b] / 14.84	1.79	6.05[b] / 10.21	1.69	4.40[b] / 6.86	1.56	3.56[b] / 5.05	1.51
1b	8.19[b] / 12.07	1.47	7.07 / 10.10	1.43	4.87 / 6.98	1.42	3.18 / 4.39	1.38	2.18 / 2.94	1.35
1c	6.29[b] / 7.56	1.20	5.32 / 6.24	1.17	3.96 / 4.75	1.20	2.27 / 2.73	1.20	1.57 / 1.87	1.19
1d	5.52[c] / 6.05	0.91	4.34[c] / 4.69	0.93	2.11 / 2.11	1.00	1.22 / 1.22	1.00	0.85 / 0.85	1.00

[a]Mobile phase was 20% IPA in hexane. Flow rate was 1.5 mL/min. Dead time (t_0) was checked by 1,3,5-tri-*tert*-butylbenzene (TTBB). [b]Absolute configuration of the first eluted enantiomer was R. [c]Absolute configuration of the first eluted enantiomer was S. [d]Separation factors were calculated from k_S/k_R, not from k_2/k_1.

Table 4. Resolution of racemic amino acid derivatives on **CSP 2** under ultrasonic radiation[a]

		2a	2b	2c	3a	3b	3c
10 °C	k_1	0.56	1.38	1.45	0.56	1.37	1.45
	k_2	2.15	10.55	12.39	2.58	10.80	12.35
	α	3.83	7.62	8.57	4.60	7.90	8.54
	RSD[b]	0.009	0.005	0.002	0.009	0.005	0.004
20 °C	k_1	0.50	1.28	1.32	0.50	1.17	1.21
	k_2	1.59	8.37	9.44	2.02	7.89	8.48
	α	3.18	6.55	7.18	4.05	6.72	7.01
	RSD[b]	0.003	0.002	0.001	0.005	0.004	0.007
30 °C	k_1	0.42	0.94	1.03	0.42	0.91	0.96
	k_2	1.14	4.65	5.60	1.38	4.76	5.16
	α	2.69	4.93	5.44	3.31	5.25	5.39
	RSD[b]	0.007	0.004	0.003	0.001	0.001	0.004
40 °C	k_1	0.39	0.85	0.91	0.37	0.78	0.84
	k_2	0.89	3.37	3.90	1.05	3.34	3.73
	α	2.29	3.99	4.27	2.87	4.28	4.43
	RSD[b]	0.003	0.001	0.003	0.003	0.001	0.001
45 °C	k_1	0.45	0.77	0.82	0.35	0.70	0.77
	k_2	0.76	2.80	3.16	0.93	2.61	3.01
	α	2.09	3.65	3.85	2.66	3.76	3.93
	RSD[b]	0.003	0.0002	0.001	0.002	0.001	0.001
50 °C	k_1	0.36	0.74	0.80	0.33	0.66	0.72
	k_2	0.69	2.44	2.79	0.80	2.30	2.64
	α	1.95	3.30	3.50	2.41	3.51	3.67
	RSD[b]	0.003	0.001	0.002	0.002	0.001	0.002
60 °C	k_1	0.33	0.67	0.73	0.30	0.59	0.65
	k_2	0.59	1.92	2.13	0.63	1.76	1.97
	α	1.77	2.85	2.94	2.09	3.00	3.05
	RSD[b]	0.003	0.002	0.007	0.003	0.003	0.002

[a]Mobile phase was 20% IPA in hexane. Flow rate was 1.5 mL/min. Dead time (t_0) was checked by 1,3,5-tri-*tert*-butylbenzene (TTBB). Selectivity factors (α) were calculated from k_S/k_R, not from k_2/k_1. [b]Relative standard deviation of selectivity factor.

and 60 °C to examine the influence of ultrasonic radiation more precisely. The chromatographic results are shown in Table 4.

From the comparison of the chromatographic results shown in Table 2 and Table 4, retention times were found to decrease when the ultrasound was irradiated. The chiral resolution was improved at non-sonic condition at lower temperatures but the resolution was improved under ultrasonic radiation at relatively higher temperature (45, 50, 60 °C). The differences of the separation factors on between sonic and non-sonic condition are considerably large at 10 °C. The separation factors were slightly larger at without ultrasonic radiation than with ultrasonic radiation at 20, 30 and 40 °C. However, at higher than 45 °C, the separation factors were larger at sonic condition. These are similar to the comparison chromatographic results shown in Table 1 and Table 3. It is also shown in Table 4 that the reproducibility of the α values is very excellent.

The role of ultrasound in this chiral chromatography is not clear until now. However, the improved resolution under ultrasonic radiation was observed only at relatively higher temperature. The reason for this is supposed that the effect of temperature increment induced by ultrasonic radiation is relatively small at relatively higher temperature, but it is relatively high at low temperature. As a result, when the ultrasound was irradiated to the chromatographic system, an unfavorable influence of temperature increment is expected to affect the chiral resolution more significantly at lower temperature than at higher temperature.

Conclusion

Ultrasound was first introduced to chiral chromatography. We called ultrasound assisted chromatographic technique as 'sonochromatography' because the ultrasound served as an effective external field in chromatography. From the result of this chiral sonochromatography, ultrasound appears effective at higher temperature and can be used as a tool to reduce analysis time. Thus, it can be effectively applied to large-scale separation and high temperature GPC (gel permeation chromatography) or FIA (flow injection analysis), which need a mixing process, extraction and chromatography. In such cases, it is necessary to develop a new temperature and ultrasound controlled instrument to reduce the non-column band broadening dispersion. This can be done by reducing the length of the connections before and after the column.

Even though we could not find any column bleedings during our experimental period, it might be assumed that the life of a column used at non-sonic condition would be longer than that used at sonic condition.

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