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Use of cyclodextrins in the isotachophoretic determination of various inorganic anions

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ABSTRACT

The effects of α-, β- and γ-cyclodextrins on the effective mobilities of various inorganic anions in capillary isotachophoresis were studied. The effective mobilities of several anions decreased when the concentration of cyclodextrins in an ordinary leading electrolyte was increased up to 50 m*M*, whereas the effective mobilities of a few anions remained almost constant. Hence by use of α-cyclodextrin, nitrite and nitrate ions, cyanate, thiocyanate and selenocyanate ions and chlorate and perchlorate ions were completely separated and could be determined. The effective mobilities of iodide, periodate and tetrathionate ions also decreased with increasing concentration of α-, β- or γ-cyclodextrin.

INTRODUCTION

The most important task in isotachophoresis is to control or adjust the effective mobilities of analyte ions. The mutual separation of analyte ions having approximately equal mobilities is essential in order to expand its analytical applications [1].

Recently, cyclodextrins (CDs) have been used for this purpose. The effective mobilities of analyte ions are altered by the formation of complexes of the ions with CD added to a leading electrolyte. So far most of these analyte ions have been organic [1–12]. Tazaki *et al.* [13] used α-CD for the separation of iodide ion in chloride–bromide mixtures and for the detemination of perchlorate ion in the presence of hexacyanoferrate(III) and -(II) ions. There has been no report concerning the isotachophoretic separation of inorganic ions with the use of CD apart from the one above.

It is difficult to separate nitrite and nitrate ions, cyanate, thiocyanate and selenocyanate ions, chlorate and perchlorate ions and chloride, bromide and iodide ions with ordinary leading electrolytes by capillary isotachophoresis, because their ionic mobilities are approximately equal in aqueous solutions [14]. Therefore, we have studied the effects of α-, β- and γ-CDs on the effective mobilities of nitrite, nitrate, cyanate, thiocyanate, selenocyanate, chlorate, perchlorate, fluoride, bromide, iodide,

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iodate, periodate and tetrathionate ions. The results of these experiments were applied to the simultaneous determination of nitrite and nitrate ions, cyanate, thiocyanate and selenocyanate ions and chlorate and perchlorate ions by capillary isotachophoresis with ordinary leading electrolytes containing α-CD.

EXPERIMENTAL

Apparatus

A Shimadzu Model IP-2A isotachophoretic analyser was used with a potential-gradient detector. The main column was a fluorinated ethylene–propylene (FEP) copolymer tube (15 cm × 0.5 mm I.D.), and the precolumn was a PTFE tube (15 or 20 cm × 1.0 mm I.D.). A Hamilton Model 1701-N microsyringe was used for the injection of samples into the isotachophoretic analyser. Distilled and demineralized water was obtained from a Yamato-Kagaku Model WG-25 automatic still and a Nihon Millipore Milli-QII system.

Reagents

All reagents were of analytical-reagent grade and used without further purification. Distilled and demineralized water was used throughout. α-, β- and γ-CDs were obtained from the Nacalai Tesque. Standard solutions of various anions were prepared by dissolving their sodium or potassium salts in water; those of periodate ion were prepared immediately before use because periodate is considerably hydrolysed.

Procedures

Volumes of 5-μl of solutions containing 1.0 m*M* of each anion are injected into the isotachophoretic analyser. The migration current is maintained at 150 μA for the first 14 min and then reduced to 50 μA. The leading electrolyte is an aqueous solution containing 5 m*M* histidine hydrochloride, 0.01 wt.-% Triton X-100 and an appropriate amount of CD, and the terminating electrolyte is 10 m*M* sodium acetate solution. The concentration of α- or γ-CD in the leading electrolyte is increased to 50 m*M* and that of β-CD to 15 m*M* owing to the poor solubility of β-CD compared with that of α- and γ-CD in water [15].

The potential unit (*PU*) value [16] (relative step height [7]) which is generally used as a parameter of identification of the analyte ions, and is defined by the equation

$$PU = (PG_{\mathrm{A}} - PG_{\mathrm{L}})/(PG_{\mathrm{T}} - PG_{\mathrm{L}}) \tag{1}$$

where *PG* is the potential gradient and the subscripts denote quantities relating to analyte (A), leading (L) and terminating (T) ions, respectively, is calculated. The relationship between the *PU* value and the effective mobility of the analyte ion ($\bar{m}_A$) [16] is given by

$$PU = (a - 1)^{-1}\, \bar{m}_{\mathrm{L}}/\bar{m}_{\mathrm{A}} - (a - 1)^{-1} \tag{2}$$

where $\bar{m}$ is the effective mobility and $a = \bar{m}_{\mathrm{L}}/\bar{m}_{\mathrm{T}}$. Eqn. 2 indicates that the *PU* value becomes larger when the effective mobility of the analyte ion ($\bar{m}_{\mathrm{A}}$) becomes smaller, and the former becomes smaller when the latter becomes larger. That is the change in the effective mobility of the analyte ion can be indirectly determined in terms of the *PU* values.

RESULTS AND DISCUSSION

Effective mobilities of analyte ions [1]

The effective mobilities of analyte ions A and B ($\bar{m}_A$ and $\bar{m}_B$, respectively) in the presence of an electrically neutral ligand N are expressed as follows:

$$\bar{m}_A = (m_A + m_{AN}K_{AN}\,[N]_A)/(1 + K_{AN}\,[N]_A) \tag{3}$$

$$\bar{m}_B = (m_B + m_{BN}K_{BN}\,[N]_B)/(1 + K_{BN}\,[N]_B) \tag{4}$$

where m_A, m_B, m_{AN} and m_{BN} are the ionic mobilities of the free analyte ions A and B and the complexed analyte ions AN and BN, respectively, K_{AN} and K_{BN} the complex-formation constants of A and B, respectively, with the ligand N and $[N]_A$ and $[N]_B$ the ligand concentrations in each zone. Eqns. 3 and 4 indicate that A and B can be separated from each other even when they have the same ionic mobilities ($m_A = m_B$) if the complex-formation constants with N are appreciably different ($K_{AN} \neq K_{BN}$): if $K_{AN} > K_{BN}$, $\bar{m}_A < \bar{m}_B$ and if $K_{AN} < K_{BN}$, $\bar{m}_A > \bar{m}_B$. In addition, $\bar{m}_A$ and $\bar{m}_B$ decrease with increasing $[N]_A$ and $[N]_B$, respectively.

In this study, N corresponds to CDs. The stability of the inclusion complex of the analyte ion with CD depends on the hydrophobicity, size and geometric arrangement of the analyte ion [1,7,17].

Determination of nitrite and nitrate ions

The limiting molar conductivity of nitrite ion is approximately equal to that of nitrate ion, as shown in Table I. Therefore, it is difficult to separate these ions from each other with ordinary leading electrolytes. These ions have been separated with an

TABLE I

LIMITING MOLAR CONDUCTIVITIES (λ_-^∞) OF VARIOUS ANIONS IN AQUEOUS SOLUTION (25°C) [14]

Anion	λ_-^∞ (S cm^2 mol^{-1})
NO_2^-	71.8
NO_3^-	71.5
OCN^-	64.6
NCS^-	66.5
$NCSe^-$	64.7
ClO_3^-	64.6
ClO_4^-	67.4
F^-	55.4
Cl^-	76.3
Br^-	78.1
I^-	76.8

aqueous leading electrolyte containing inorganic or organic co-counter ions [18,19] and with non-aqueous leading electrolytes [20,21].

Fig. 1 shows the effect of α-CD concentration on the *PU* values of various anions. The *PU* value of nitrate ion slightly increased linearly with increasing concentration of α-CD up to 50 m*M*. On the other hand, the *PU* value of nitrite ion remained almost constant. This indicated that the nitrate ion formed a weak inclusion complex with α-CD, whereas the nitrite ion did not. The separation of the nitrate ion from the leading ion (chloride ion) was insufficient when the α-CD concentration was relatively low, and the difference between the *PU* values of nitrite and nitrate ions decreased when the α-CD concentration increased. Therefore, 25 m*M* α-CD was selected as the optimum concentration in the leading electrolyte for the simultaneous determination of nitrite and nitrate ions.

Linear calibration graphs were obtained for nitrite and nitrate ions up to 3.0 m*M* by using a leading electrolyte containing 25 m*M* α-CD. The regression equations of these graphs for nitrite and nitrate ions were $y = 9.5x + 0.4$ ($0 \leqslant x \leqslant 3.0$, $0 \leqslant y \leqslant 28.8$) and $y = 12.7x + 0.1$ ($0 \leqslant x \leqslant 3.0$, $0 \leqslant y \leqslant 37.0$), respectively. The correlation coefficients were 1.000 and 0.998, respectively. In these equations x is the concentration of the ion in m*M* and y the zone length in mm when the recording speed is adjusted to 40 mm/min. The relative standard deviations were obtained by calculating the zone length per 1.0 m*M* at each point on the calibration graphs. They were 0.040 and 0.045 ($n = 6$), respectively. The limits of determination for nitrite and nitrate ions were $1.1 \cdot 10^{-2}$ and $7.9 \cdot 10^{-3}$ m*M*, respectively, corresponding to a 0.1-mm zone lengh.

When 5-μl volumes of mixed solutions containing various concentrations of nitrite and nitrate ions were injected and analysed by use of the calibration graphs, the

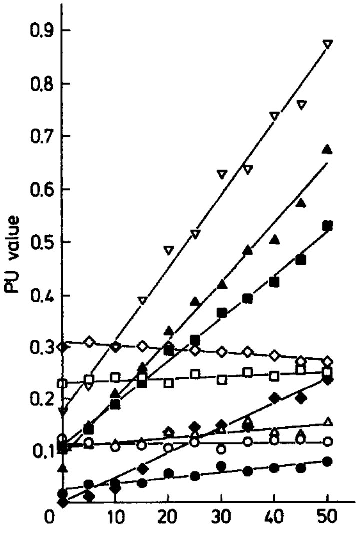

Fig. 1. Effect of α-cyclodextrin concentration on the *PU* values of various anions. ○, NO_2^-; ●, NO_3^-; □, OCN^-; ■, NCS^-; ▽, $NCSe^-$; △, ClO_3^-; ▲, ClO_4^-; ◇, F^-; ◆, I^-.

error in the determination of these ions was less than ±11%, as shown in Table II. The isotachopherogram of mixture 2 in Table II is shown in Fig. 2. Completely separated zones with sharp boundaries for the nitrite and nitrate ions were obtained with the leading electrolyte containing 25 m*M* α-CD, whereas a completely separated zone for nitrate ion was not obtained without α-CD.

Determination of cyanate, thiocyanate and selenocyanate ions

The limiting molar conductivities of cyanate, thiocyanate and selenocyanate ions are approximately equal, as shown in Table I. Therefore, their separation is difficult with ordinary leading electrolytes. There is no report concerning the simultaneous determination of these ions by capillary isotachophoresis.

The *PU* values of thiocyanate and selenocyanate ions increased linearly with increasing concentration of α-CD, whereas that of cyanate ion remained almost constant, as shown in Fig. 1. The slope of the regression line of *PU* value *vs.* α-CD concentration for the selenocyanate ion was larger than that for the thiocyanate ion. This result suggests that the complex-formation constant of selenocyanate ion with α-CD is larger than that of thiocyanate ion. It can be presumed that the α-CD concentration of 30 m*M* or above is sufficient for the mutual separation of cyanate, thiocyanate and selenocyanate ions. However, the reproducibility of separation of these ions was not sufficient in the concentration range 30–40 m*M*. Therefore, 45 m*M* was adopted as the optimum concentration of α-CD for the simultaneous determination of cyanate, thiocyanate and selenocyanate ions.

Linear calibration graphs were obtained for cyanate, thiocyanate and selenocyanate ions by using a leading electrolyte containing 45 m*M* α-CD. The regression equations for cyanate, thiocyanate and selenocyanate ions were $y = 6.7x - 0.1$ ($0 \leqslant x \leqslant 3.0, 0 \leqslant y \leqslant 20.7$) and $y = 12.3x + 0.7$ ($0 \leqslant x \leqslant 3.0, 0 \leqslant y \leqslant 36.9$) and $y = 15.8x + 0.4$ ($0 \leqslant x \leqslant 3.0, 0 \leqslant y \leqslant 47.6$), the correlation coefficients were 0.998, 0.999 and 1.000 and the relative standard deviations were 0.049, 0.053 and 0.028, respectively. The limits of determination for cyanate, thiocyanate and selenocyanate ions were $1.5 \cdot 10^{-2}$, $8.1 \cdot 10^{-3}$ and $6.3 \cdot 10^{-3}$ m*M*, respectively. The error in the simultaneous determination of these ions was less than ±14%, as shown in Table III. The isotachopherogram of mixture 2 in Table III is shown in Fig. 3. Completely

TABLE II

ANALYTICAL RESULTS FOR NITRITE AND NITRATE IONS

Mixture	Added (m*M*)		Found (m*M*)		Error (%)	
	NO_2^-	NO_3^-	NO_2^-	NO_3^-	NO_2^-	NO_3^-
1	0.50	3.0	0.47	2.9	−6.0	− 3.3
2	1.0	1.0	0.99	0.89	−1.0	−11
3	1.0	2.5	1.0	2.3	0.0	− 8.0
4	1.5	2.0	1.5	2.1	0.0	+ 5.0
5	2.0	1.5	1.9	1.4	−5.0	− 6.7
6	2.5	1.0	2.4	0.92	−4.0	− 8.0
7	3.0	0.50	2.9	0.48	−3.3	− 4.0

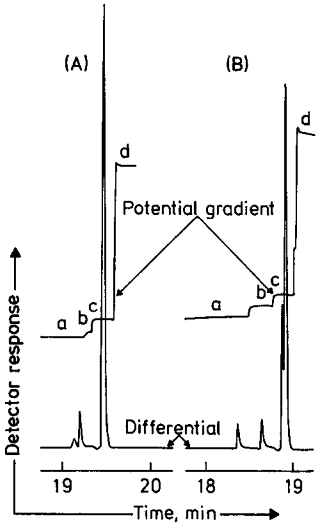

Fig. 2. Isotachopherograms for the separation of nitrite and nitrate ions. (A) Without α-cyclodextrin; (B) 25 m*M* α-cyclodextrin. (a) Cl^- (leading ion); (b) NO_3^-; (c) NO_2^-; (d) CH_3COO^- (terminating ion).

separated and stable zones with sharp boundaries for these ions were obtained by use of a leading electrolyte containing 45 m*M* α-CD, whereas thiocyanate and selenocyanate ions were not completely separated without α-CD.

Determination of chlorate and perchlorate ions

The limiting molar conductivity of chlorate ion is approximately equal to that of perchlorate ion in aqueous solution, as shown in Table I, whereas those of chlorate and perchlorate ions differ from each other in methanol (61,4 and 70.1 S cm^2 mol^{-1} at 25°C, respectively [21]). Therefore, it is difficult to separate these ions by use of ordinary leading electrolytes, whereas it is possible by use of methanolic leading electrolytes.

TABLE III

ANALYTICAL RESULTS FOR CYANATE, THIOCYANATE AND SELENOCYANATE IONS

Mixture	Added (m*M*)			Found (m*M*)			Error (%)		
	OCN^-	NCS^-	$NCSe^-$	OCN^-	NCS^-	$NCSe^-$	OCN^-	NCS^-	$NCSe^-$
1	0.50	2.5	2.0	0.43	2.4	2.0	−14	−4.0	0.0
2	1.0	1.0	1.0	0.94	1.1	0.99	−6.0	+10	−1.0
3	1.0	1.5	2.5	0.88	1.5	2.5	−12	0.0	0.0
4	1.5	0.50	3.0	1.4	0.57	2.9	−6.7	+14	−3.3
5	1.5	3.0	0.50	1.3	2.9	0.52	−13	−3.3	+4.0
6	2.0	2.0	1.0	1.8	2.0	1.0	−10	0.0	0.0
7	2.5	1.0	1.5	2.3	1.1	1.4	−8.0	+10	−6.7
8	3.0	1.0	1.0	2.8	1.0	0.98	−6.7	0.0	−2.0

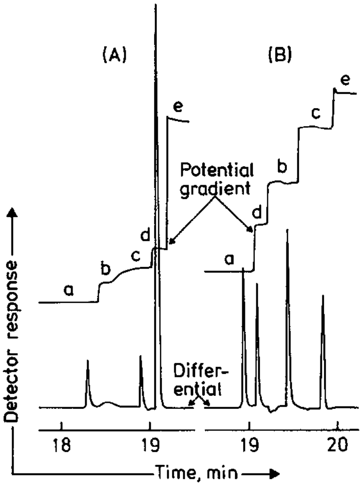

Fig. 3. Isotachopherograms for the separation of cyanate, thiocyanate and selenocyanate ions. (A) Without α-cyclodextrin; (B) 45 m*M* α-cyclodextrin. (a) Cl^-; (b) NCS^-; (c) $NCSe^-$; (d) OCN^-; (e) CH_3COO^-.

The *PU* value of perchlorate ion increased linearly with an increase in α-CD concentration, whereas that of chlorate ion remained almost constant, as shown in Fig. 1.

Linear calibration graphs were obtained for chlorate and perchlorate ions by using a leading electrolyte containing 10 m*M* α-CD. The regression equations for chlorate and perchlorate ions were $y = 10.7x + 0.4$ ($0 \leqslant x \leqslant 3.0$, $0 \leqslant y \leqslant 32.6$) and $y = 11.2x$ ($0 \leqslant x \leqslant 3.0$, $0 \leqslant y \leqslant 33.7$), respectively (correlation coefficients both 1.000). The relative standard deviations were 0.047 and 0.0074, respectively. The limits of determination for chlorate and perchlorate ions were $9.3 \cdot 10^{-3}$ and $8.9 \cdot 10^{-3}$ m*M*, respectively. The error in the simultaneous determination of these ions was less than ±14%, as shown in Table IV. The isotachopherogram of mixture 2 in Table IV is shown in Fig. 4. These ions were completely separated with a leading electrolyte containing 10 m*M* α-CD.

TABLE IV

ANALYTICAL RESULTS FOR CHLORATE AND PERCHLORATE IONS

Mixture	Added (m*M*)		Found (m*M*)		Error (%)	
	ClO_3^-	ClO_4^-	ClO_3^-	ClO_4^-	ClO_3^-	ClO_4^-
1	0.50	3.0	0.43	3.0	−14	0.0
2	1.0	1.0	0.98	1.0	− 2.0	0.0
3	1.0	2.5	0.91	2.6	− 9.0	+ 4.0
4	1.5	2.0	1.4	2.0	− 6.7	0.0
5	2.0	1.5	1.9	1.5	− 5.0	0.0
6	2.5	1.0	2.5	1.0	0.0	0.0
7	3.0	0.50	3.0	0.55	0.0	+10

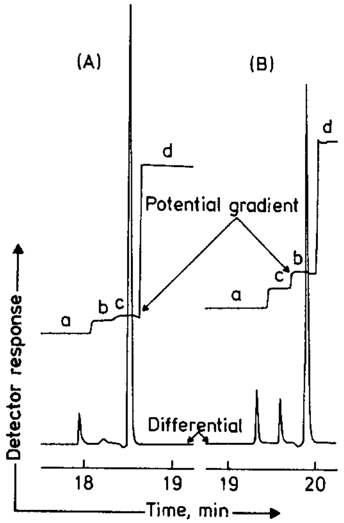

Fig. 4. Isotachopherograms for the separation of chlorate and perchlorate ions. (A) Without α-cyclodextrin; (B) 10 m*M* α-cyclodextrin. (a) Cl^-; (b) ClO_4^-; (c) ClO_3^-; (d) CH_3COO^-.

Separation of halide ions

The limiting molar conductivities of chloride, bromide and iodide ions are approximately equal, as shown in Table I. Therefore, their separation is difficult with ordinary leading electrolytes. These ions have been separated by complex formation with cadmium ion [22] or by use of a methanolic leading electrolytes [21,23,24].

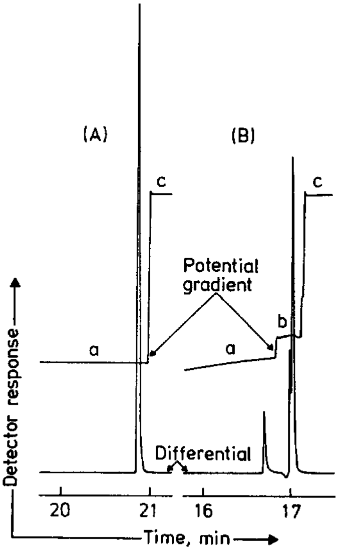

Fig. 5. Isotachopherograms for the separation of iodide ion. (A) Without α-cyclodextrin; (B) 20 m*M* α-cyclodextrin. (a) Cl^-; (b) I^-; (c) CH_3COO^-.

The *PU* value of iodide ion increased linearly with increasing concentration of α-CD, as shown in Fig. 1. Bromide ion was not separated from chloride ion (leading ion).

A linear calibration graph was obtained for iodide ion by use of a leading electrolyte containing 20 m*M* α-CD. The regression equation was $y = 11.6x + 0.1$ ($0 \leqslant x \leqslant 3.0$, $0 \leqslant y \leqslant 35.0$) and the correlation coefficient was 0.999. The relative standard deviation was 0.047. The limit of determination was $8.6 \cdot 10^{-3}$ m*M*. Iodide ion (1.0 m*M*) was completely separated from chloride ion (leading ion) with a leading electrolyte containing 20 m*M* α-CD, as shown in Fig. 5.

Further, the effect of β-CD concentration on the PU values of the above anions was examined by increasing the β-CD concentration up to 15 m*M*. This effect was smaller than that of α-CD.

Applications to other anions

The effect of α-, β- and γ-CD concentration on the *PU* values of iodate, periodate and tetrathionate ions was investigated. The results are shown in Fig. 6. The *PU* value of periodate ion increased linearly with increasing concentration of α- or β-CD. The slope of the regression line for β-CD was larger than that for α-CD. The *PU* value of tetrathionate ion increased linearly when the β- or γ-CD concentration increased. The slope of the regression line for β-CD was larger than that for γ-CD. These results indicate that the complex-formation constant of periodate ion with β-CD is larger than that with α-CD, and that of tetrathionate ion with β-CD is larger than that with γ-CD. The *PU* value of iodate ion remained almost constant when the α- or β-CD concentration increased.

It can be concluded that almost all the anions studied can be separated from each

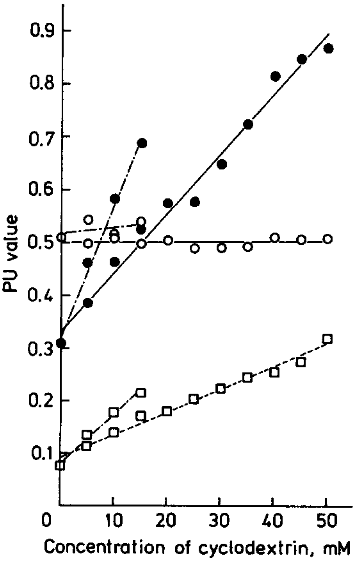

Fig. 6. Effect of cyclodextrin concentration on the *PU* values of some anions. ○, IO_3^-; ●, IO_4^-; □, $S_4O_6^{2-}$. ———, α-CD; —·—, β-CD; ......, γ-CD.

other by use of a leading electrolyte containing 45 m*M* α-CD, although the separation of nitrite and chlorate ions may be insufficient. It is concluded from the slopes of the regression lines which represent the increase in *PU* values that the complex-formation constant of anions with α-CD increases in the order nitrate, iodide, thiocyanate, perchlorate, periodate and selenocyanate ions.

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REFERENCES

1 M. Tazaki, T. Hayashita, Y. Fujino and M. Takagi, *Bull. Chem. Soc. Jpn.*, 59 (1986) 3459.
2 T. Kamimura, Y. Fujino, T. Hayashita, M. Takagi, M. Tazaki, S. Tsutsui and S. Nagahama, *Proceedings of the 3rd Isotachophoresis Symposium, Osaka, December 14, 1983,* Japan Discussion Group of Electrophoretic Analysis, Japan Society for Analytical Chemistry, Tokyo, 1983, p. 7.
3 N. Kuramoto and K. Asao, *Proceedings of the 5th Isotachophoresis Symposium, Kyoto, December 11–12, 1985,* Japan Discussion Group of Electrophoretic Analysis, Japan Society for Analytical Chemistry, Tokyo, 1985, p. 17.
4 N. Kuramoto and K. Asao, *Osaka Furitsu Kogyo Gijutsu Kenkyusho Hokoku,* 88 (1986) 18.
5 N. Kuramoto, *Proceedings of the 6th Isotachophoresis Symposium, Nagoya, December 11–12, 1986,* Japan Discussion Group of Electrophoretic Analysis, Japan Society for Analytical Chemistry, Tokyo, 1986, p. 21.
6 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 405 (1987) 379.
7 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 411 (1987) 153.
8 N. Kuramoto and K. Asao, *Osaka Furitsu Sangyo Gijutsu Sogo Kenkyusho Hokoku,* 1 (1988) 45.
9 I. Jelínek, J. Dohnal, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 435 (1988) 496.
10 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
11 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 439 (1988) 386.
12 J. Snopek, E. Smolková-Keulemansová, I. Jelínek, J. Dohnal, J. Klinot and E. Klinotová, *J. Chromatogr.*, 450 (1988) 373.
13 M. Tazaki, M. Takagi and K. Ueno, *Chem. Lett.*, (1982) 639.
14 Chemical Society of Japan, *Kagaku Binran,* Maruzen, Tokyo, 3rd ed., 1984, p. II-460.
15 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, 1978; translated by H. Hirai and M. Komiyama, *Cyclodextrin no Kagaku,* Gakkai-Shuppan-Center, Tokyo, 183, p. 8.
16 H. Miyazaki and K. Kato, *Tosoku-Denki-Eido-Ho*(*Isotachophoresis,* in Japanese), Kodansha Scientific, Tokyo, 1980, p. 30.
17 I. Tabushi, T. Nishiya, E. Kimura, K. Hattori, K. Odashima, K. Koga and F. Tota, *Host–Guest no Kagaku* (*Host–Guest Chemistry,* in Japanese), Kyoritsu-Shuppan, Tokyo, 1980, p. 15.
18 I. Matejovič and J. Polonský, *J. Chromatogr.*, 438 (1988) 454.
19 I. Matejovič and M. Bieliková, *Collect. Czech. Chem. Commun.*, 53 (1988) 3067.
20 *Shimadzu Application News,* CA 198-010, 1977.
21 *Shimadzu Application News,* CA 198-096, 1983.
22 P. Boček, I. Miedziak, M. Deml and J. Janák, *J. Chromatogr.*, 137 (1977) 83.
23 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 51 (1970) 339.
24 P. Boček and F. Foret, *J. Chromatogr.*, 313 (1984) 189.