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Preparative isotachophoretic analyser equipped with a dropwise fractionating device

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ABSTRACT

A preparative isotachophoretic analyser with a series of four separation tubes of I.D. 0.5–5 mm was constructed and its fundamental efficiency was evaluated. The maximum injectable sample volume was 2.5 ml. The heat convection in the separation tube (I.D. = 5 mm) was suppressed by adding hydroxypropylcellulose to the leading electrolyte (1%) and sucrose to the terminating electrolyte (20%). The entire separated zones were fractionated dropwise (5.4 μl each) through a narrow-bore nozzle by a counter flow of the leading electrolyte. Variations in the course of dropping due to electrostatic forces were suppressed by a simple electrostatic device. The recoveries of several micrograms of separands were determined by photometric and PIXE analysis and were almost 100%. The separability and apparent sensitivity were very good; *e.g.*, 150 ppb (10^9) Sm^{3+} (10^{-6} *M*, 2 ml) was separated from a mixture with Dy^{3+}, Tm^{3+} and Lu^{3+}.

INTRODUCTION

Capillary isotachophoresis (IP) is an important method for preparative separations because of its high separation power. In addition to the purification of a sample by fractionation, preparative isotachophoresis is useful for various purposes such as the determination of unknown constituents in combination with different analytical methods. In such a case, preparative isotachophoresis is regarded as a pretreatment technique with high selectivity for further characterization and identification.

Preparative capillary isotachophoretic analysers can be classified into two types with respect to the method of fractionation. A type for the fractionation of the entire sample zones was reported by Arlinger[1]. The zones were swept gradually by a counter flow of the leading electrolyte on applying a migration current and the fractions were fixed on a cellulose acetate strip. For this purpose, the apparatus was equipped with a pump to provide the counter flow and a strip-winding device. The fractions on the strip are eluted and the fractions are analysed by different methods.

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On the other hand, in the type reported by Kobayashi *et al.*[2], the separated sample zone was fractionated using a microsyringe. They reported a potential gradient detector equipped with a sample removal port to fractionate the target zone immediately after the tail of the zone was detected. Although the method was not intended for the successive fractionation of the entire sample zones the operational facility is notable. Kaniansky *et al.*[3] reported a similar discontinuous fractionation technique with the use of a specially designed fractionating valve. The fractionated solution itself is convenient as a sample for further analysis.

Considering the characteristics of these methods, an isotachophoretic analyser was constructed to fractionate the entire separated zones dropwise by using a counter flow of leading electrolyte. Complete recovery of the mobile components in the injected samples was expected with the minimum risk of loss and contamination, although the mixing of adjacent sample zones could not be avoided in principle.

The other aim was to increase the amount of sample that can be separated for trace analysis. Dolnik *et al.*[4] suggested strategies for isotachophoretic trace analysis and developed an isotachophoretic analyser with rectangular tubes. In trace analysis, a large sample volume is required with application of a high electric charge[5]. There are three ways to increase the load of the leading electrolyte according to the sample amount to be separated[4]: as (1) a hydrodynamic counter flow; (2) a concentration cascade; and (3) enhancement of the separation volume. We used method (3), utilizing the column coupling technique[6].

In this paper the design, operation and basic efficiency are reported for a preparative isotachophoretic analyser using a separation tube of I.D. 5 mm. The maximum sample volume is 2.5 ml.

EXPERIMENTAL

Instrumentation

Fig. 1 shows a schematic diagram of the apparatus. The double lines represent the electrolyte tubing and single lines the electric wiring. Valves are represented by circles. Sample migration in this apparatus can be divided into three stages. In the first stage, the sample solution (2.5 ml or less) was separated roughly. We shall call this stage the "preseparation stage". The second stage was used for the complete separation of the preseparated samples or the separation of the relatively small amounts of samples of the order of 10^2 nmol or less. Part of the unnecessary constituents migrating in front of the target zones was isolated at this stage. We shall call this stage the "separation stage". In the last stage, the separated zones were fractionated dropwise by a counter flow of leading electrolyte.

Four tubes with different inner diameters (I.D.) were coupled to increase both the separable amount of sample and the volume of the sample solution; the I.D.s were 5, 2, 1 and 0.5 mm and typical lengths for effective separations were 10, 10, 15 and 30 cm, respectively. The lengths of three tubes were easily adjustable except for the tube with an I.D. of 5 mm.

The tubes with I.D. 1 and 0.5 mm were connected by using a PTFE joint for a Shimadzu IP-2A with a tapered structure. The other joints for the tubes with larger I.D. were made from a block of acrylic resin. The migration around the joint was carefully checked visually using dyes; good separation and recovery could not be

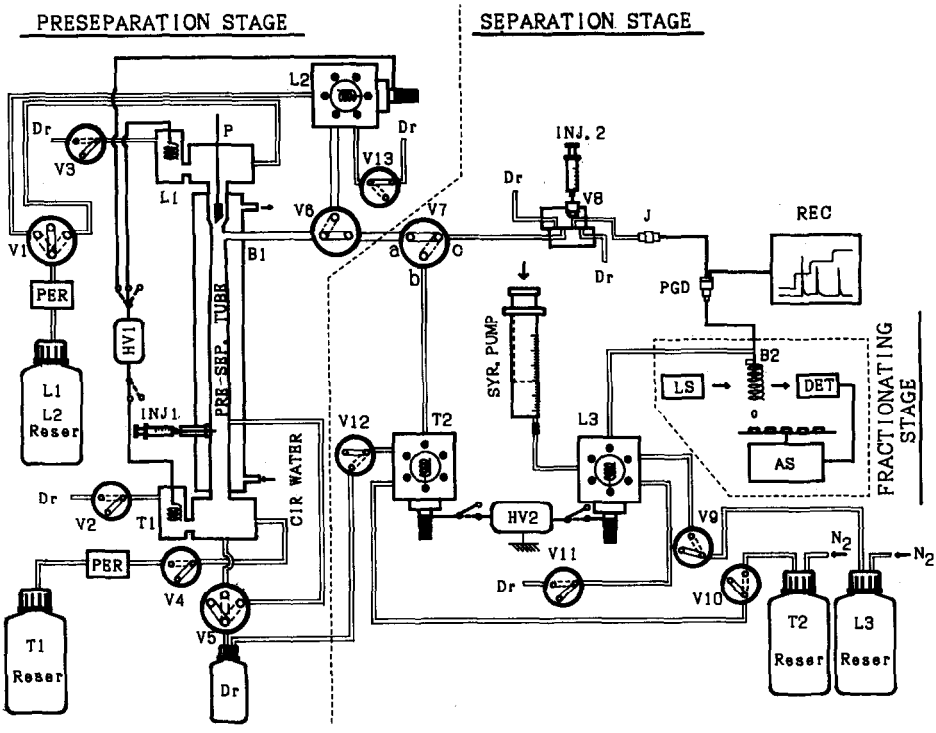

Fig. 1. Schematic diagram of preparative IP equipment. L1,L2,L3 = Leading electrolyte compartments; T1,T2 = terminating electrolyte compartments; V1–V5, V9–V13 = valves to fill and to drain the electrolyte compartments; V8 = injection valve; J = tube joint; V6,V7 = valves to change the current path; INJ1,INJ2 = injection ports; P = PTFE plug to cut off L1 compartment; PRE-SEP. TUBE = preseparation tube; B1,B2 = branch of separation tubes; Reser = electrolyte reservoir; PER = peristaltic pump; PGD = potential gradient detector; REC = amplifier and recorder; SYR.PUMP = syringe pump to give a counter flow of leading electrolyte for fractionation; AS = automatic sampler driven by stepping motor; LS = light source; DET = photodetector and amplifier for triggering the automatic sampler; Dr = electrolyte drain; HV1,HV2 = high-voltage power supplies; N_2 = nitrogen pneumatic device. Separation tubes: B1–V6–V7 and V6–L2, I.D. = 2 mm and O.D. = 3 mm; T2–V7–J and B2–L3, I.D. = 1 mm and O.D. = 2 mm; J–B2, I.D. = 0.5 mm and = O.D. = 1 mm; preseparation, I.D. = 5 mm and O.D. = 7 mm.

expected if the joint had an electrophoretically dead space. The load of the leading electrolyte in the thus connected tubes was *ca.* 3.1 ml, which is at least ten times larger than that of the ordinary apparatus. This permits the separation of micromole amounts of samples, although the amount depends on the difference in the mobilities of the separands.

Three leading electrolyte compartments (L1, L2 and L3 in Fig. 1) and two terminating compartments (T1 and T2) were used for the rapid migration of samples through the different stages. The migration current was applied selectively to the electrodes of these compartments. The apparatus was equipped with two injection ports (INJ1 and INJ2 in Fig. 1). One of them was used selectively according to the amount and/or volume of sample.

The details of each stage are considered in the following sub-sections.

Preseparation stage

The preseparation tube was made of acrylic resin. The tube was tapered as illustrated in Fig. 1 and the I.D. and O.D. were 5 and 7 mm at the maximum and 3 and 4 mm at the minimum at branch B1. At the branch the tube of the preseparation stage was connected with that of the separation stage. The preseparation tube was surrounded by a water-jacket and was cooled by circulating water (room temperature) during operation.

The leading and terminating electrolyte compartments (L1 and T1) were made of acrylic resin blocks and the volume of the solution was *ca.* 30 ml. The electrode compartments (volume *ca.* 8 ml) were set apart from the electrolyte compartments and were connected with a tube (O.D. = 12 mm, I.D. = 8 mm, length = 55 mm). The electrode used was a coiled platinum wire. The hydrogen and oxygen generated were trapped in the electrode compartments and released after the operation. It seemed that some electrode reaction took place and the reactive ions formed hindered the electrophoretic migration of samples if the electrode compartments were not separated. When histidine was present in the leading electrolyte, for example, the colour of the electrolyte solution gradually turned brown during migration.

The migration voltage applied between L1 and T1 was 1000–500 V under typical operation conditions, and the current varied from 5 to 3 mA during the migration. The distance between INJ1 and B1 was *ca.* 10 cm and it took *ca.* 20 min for the migration. Then the current path was changed from L1–T1 to L2–T1 and the preseparated zones were led to the separation stage by applying 1500 V (*ca.* 1.5 mA) using the high-voltage power supply HV1. The separation tube connecting L2 and branch B1 was made of PTFE and the I.D. and O.D. were 2 and 3 mm, respectively. When the edge of the preseparated sample zone reached valve V6, HV1 was turned off.

Appropriate dyes were used as position markers to monitor the migration process and to switch the valves appropriately. When the leading edge of the sample zone reached branch B1, HV1 was turned off. The course of the separation tube (L1–T1) was blocked by a PTFE plug (P) at B1. The role of the plug was to prevent the gradual mixing of the leading ion or the rejected sample constituents with the target zones; if the plug was not used, the separation in the subsequent stage was unfavourable owing to the elution by the leading ion from L1.

As the heat conductivity of acrylic resin was poor and the wall of the preseparation tube was relatively thick (1 mm), the electrolyte in the tube could not be cooled sufficiently. Therefore, the temperature of the electrolyte near the inner surface wall was significantly different from that in the centre of the tube. To suppress heat convection, which disturbed the formation of sharp zone boundaries, the viscosity of the electrolytes in L1 and L2 was increased by adding hydroxypropylcellulose (HPC, 1%, w/w), obtained from Tokyo Kasei (Tokyo, Japan). The viscosity of the 2% aqueous solution was 1000-4000 cP at 20°C according to the specification. A 2% stock solution was prepared and used after deionization with an ion-exchange resin.

Deionized sucrose solution was added to the terminating electrolyte in T1 (20%, w/w) to prevent the heat convection in the terminating zone. Two peristaltic pumps PER in Fig. 1; Model MP-3 (EYELA, Tokyo, Japan) were used to fill L1, L2 and T1 with the leading and terminating electrolytes. First, T1 was filled with the heavy terminating electrolyte up to the level of the drain tube near INJ1. Then L1 was filled with the viscous leading electrolyte and finally L2 was filled. Owing to the

difference between the specific gravities of the leading and terminating electrolytes, the initial boundary between these electrolytes was sufficiently stable. The boundary was clearly visible, because the refractive indices of the solutions were different from each other. The sample solution was injected into the tube containing terminating electrolyte through a rubber septum at INJ1 in Fig. 1. The initial boundary was not perturbed during sample injection, provided that the injection was slow.

The temperature of the electrolyte in the preseparation tube was at most *ca.* 50°C at the narrowest position. This was seen from the fact that the solubility of HPC decreased with increase in temperature and the transparency of the 1% solution was lost completely at *ca.* 50°C. In fact, the current applied was adjusted so that this temperature was not exceeded.

In the preseparation stage, a Toyo Seisakusyo Model 2515 high-voltage power supply (2500 V maximum HV1) was used in the constant-voltage mode, and stop valves V1, V2, V3, V4, V5, V6 and V13 were used for the liquid chromatograph (Gasukuro Kogyo, Models LPV-2, LPV-3 and LPV-4.

Separation stage

After the leading edge of the preseparated sample zone had reached at the valve V6, a migration current (300 μA) was applied between L3 and T1 by using HV2. After the final sample zone had passed V7, the current path was changed from L3-T1 to L3-T2, and the migration current was decreased from 300 to 150 μA. This change in current path was very important, as discussed later. Until fractionation, the sample zones migrated through the separation tube.

When the sample amount was small, the preseparation procedure was not necessary. In such cases the sample solution was injected using the second injection port (INJ2) at sampling valve V8. Coupled PTFE tubes (O.D. = 2, I.D. = 1 mm and O.D. = 1, I.D. = 0.5 mm) connected V8 with the leading electrolyte compartment L3. Half way along the tube of I.D. 0.5 mm a potential gradient detector (PGD, for Shimadzu IP-2A) and a fractionation compartment were fitted.

The electrolytes used in the separation stage were the same as those used in the preseparation stage except that the concentration of HPC in the former electrolytes was 0.2%. The temperature at this stage was not as high as in the preseparation stage because the applied current was low. The main role of HPC at this stage is to suppress not the heat convection but the electroendosmosis. Hence, if the presence of HPC in the fractions is undesirable, it should not be contained in the leading electrolyte solution.

In this separation stage, the sampling valve V8 and the stop valves V9, V10, V11 and V12 were used (Shimadzu IP-1B). The valve V7 was that used for Gasukuro Kogyo Models LPV-2, LPV-3 and LPV-4 liquid chromatographs. To fill L3 and T2, a pneumatic device was used.

Fractionation stage

After the migration in the separation stage had started, a counter flow of leading electrolyte was fed continuously by a syringe pump (Model JP-V, Furue Science, Tokyo, Japan). The leading electrolyte used was the same as that used to fill compartment L3. Until the leading edge of the sample zone reached the fractionation compartment, the overflow of leading electrolyte was discarded through a nozzle at the

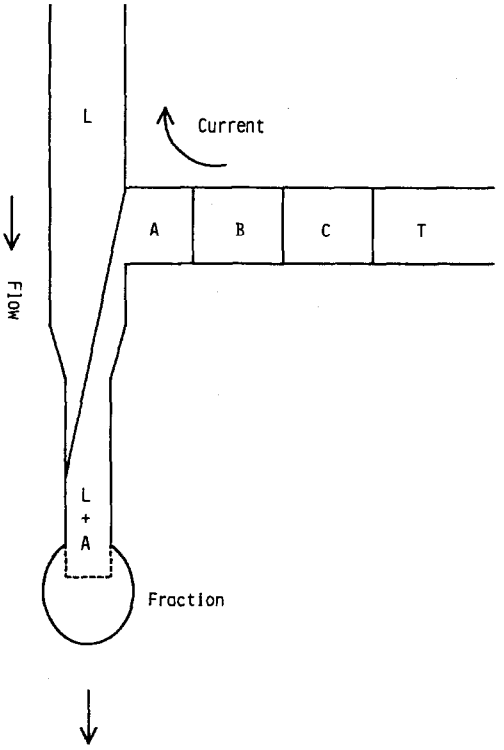

Fig. 2. T-shaped branch of the fractionation compartment and illustration of fractionation. L = leading zone; T = terminating zone; A, B and C = sample zones.

T-branch of B2 in Fig. 1. Hence it should be noted that the counter flow was not effective through the whole capillary. After the sample zones had reached B2, the dropwise fractionation was started. As shown in Fig. 2, the edge of the migrating zones was swept by the counter flow, and the samples diluted with the leading electrolyte dropped onto a sample collector through the nozzle (a platinum capillary, I.D. = 0.3 mm and O.D. = 0.5 mm). The dropping of fractions was monitored by an optical device.

The voltage applied between L3 and T2 was up to *ca.* 10 kV under typical experimental conditions. The power supply used (HV2) was a constant-current type for a Shimadzu IP-2A. As it was a bipolar type, for example, − 5 kV were applied to the leading electrode and 5 kV to the terminating electrode. If the potential at the fractionating nozzle was made equal to the ground level, there could be no electrostatic hindrance to the drop. However, it was not easy to maintain such a situation because the potential gradient varied according to the electrolyte used and during the migration process. Unless a positive counter measure was devised, the fractions scattered widely and did not form drops. To regulate such an electrostatic influence, a copper coil (six turns) was located under the nozzle and was connected to the nozzle. The O.D. of the coil was 16 mm, the length was 20 mm and the O.D. of the copper

wire was 1 mm. The size of the drops and the dropping course could be stabilized by the use of such a simple device.

An automatic sampler (AS in Fig. 1) was used for the fractional collection of the dropped samples. Twenty-five fractions were collected in small sampling tubes or on Nuclepore filters which were set on the exchangeable turntable of the sampler. The turntable was revolved by a stepping motor (Model PH264M-32, Oriental Motor, Tokyo, Japan) with a pulse generator (Model UG601) and a driver (Model UD210). The pulse generator was triggered by a delayed photometric signal caused by a fraction dropping through a light path. A small lamp was used as the visible light source (LS) and the detector (DET) used was a photodiode (Model S1226-5BQ), Hamamatsu Photonics, Hamamatsu, Japan). The distance between LS and DET was *ca.* 40 mm.

The concentration of samples in the fractionated drops or the amount of the target substance in a fraction was adjustable by changing the rate of pumping of the leading electrolyte solution. The rate was determined by considering the isotachophoretic velocity under the operating conditions and the linear velocity of the counter flow. In a typical experiment, the linear velocity of the counter flow was four times larger than the isotachophoretic velocity and the flow-rate was variable in the range 10-50 μl/min. Under the condition of 12 μl/min and a driving current of 150 μA, the time interval for fractionation was *ca.* 30 s. The averaged volume of a drop was estimated to be 5.4 μl by weighing out 200 drops of sample. The consistency of the volume was *ca.* $\pm 10\%$ when the above-mentioned difficulties were removed. The volume of one drop was affected by the diameter and seemingly by the material of the nozzle. A platinum nozzle was preferable to PTFE for making the volume small and constant.

Samples

Using the present analyser, to mark the migration process and to monitor the timing of valve opening or closing (as mentioned above), the selection of suitable dye markers is important. In this experiment, the R_E (effective mobility of leading ion/effective mobility of sample) values of eighteen kinds of cationic dyes were obtained.

Two cationic dyes, toluidine blue (TB) and astrazon pink (AP), were used in order to evaluate the basic efficiency of fractionation of the present apparatus. A mixture of Ce^{3+}, Sm^{3+}, Dy^{3+} and Tm^{3+} was also separated and fractionated for the same purpose. To exemplify trace analysis, a mixture of Sm^{3+}, Dy^{3+}, Tm^{3+} and Lu^{3+} was used. The concentration of Sm^{3+} was varied from $1.25 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ *M* whereas the concentration of Dy^{3+}, Tm^{3+} and Lu^{3+} was kept constant as $1.25 \cdot 10^{-5}$ *M*.

The dyes and other chemicals were obtained from Tokyo Kasei in the purest form available. The standard lanthanoids were purchased as the chlorides containing six molecules of water of crystallization and the purity was above 99.9%. They were dissolved in deionized water (5 m*M*).

Electrolyte system

The electrolyte system used in the fractionation and trace analysis is shown as system 1 in Table I. The concentration of HPC in the leading electrolyte for the preseparation stage was 1% and that for the separation and fractionation stages was

TABLE I

ELECTROLYTE SYSTEM USED IN ISOTACHOPHORETIC SEPARATION

HIB = α-Hydroxyisobutyric acid; CARH = carnitine hydrochloride; HPC = hydroxypropylcellulose.

Parameter	*System 1*	*System 2*
Leading electrolyte	20 m*M* KOH or 1 m*M* KOH + 19 m*M* NH_4OH[a]	10 m*M* KOH
Complexing agent	10 m*M* HIB	None
pH buffer	Acetic acid	Acetic acid
pH of leading electrolyte	4.8	4.57, 4.8, 5.0
Terminating electrolyte	20 m*M* CARH	10 m*M* CARH
Additive	0.2–1% HPC (leading) 20% sucrose (terminating)	0.2% HPC (leading)

[a] When the fractions were analysed by the PIXE method.

0.2%. The terminator (carnitine hydrochloride) was recrystallized from methanol, unless stated otherwise. The pH measurements were carried using a Horiba (Tokyo, Japan) Model F7ss expanded pH meter.

Analytical methods

The fractions of dyes were analysed with a Hitachi Model 356 double-beam spectrophotometer. One fraction (average volume = 5.4 μl) was dissolved in 5 ml of distilled water and the spectrum of the solution was obtained in the range 300–700 nm. By determining the maximum absorption at 532 and 623 nm of astrazon pink and toluidine blue, respectively, the concentration of the fractions was obtained.

PIXE (particle-induced X-ray emission) was used to analyse the lanthanoid fractions. The fractions were dropped directly on a Nuclepore filter mounted on a aluminium frame. After drying in a desiccator, they were used as the targets. The targets were irradiated by a 2.0-MeV proton beam generated by a Van de Graaff accelerator (Nisshin High Voltage, Tokyo, Japan). The PIXE spectra were obtained by a multi-channel analyser equipped with an hp (high pure)-Ge detector. A typical single run took *ca.* 1000 s (beam current = 20 nA). The details of the PIXE analysis of lanthanoids and their isotachophoretic analysis have been reported elsewhere[7,8].

In the fractionation of lanthanoids, mixed leading ions were used (1 m*M* KOH and 19 m*M* NH_4OH, see Table I) because the sensitivity of potassium is very high in PIXE analysis and it disturbed the rapid collection of the signals of the target elements. The mobilities of K^+ and NH_4^+ are of the same magnitude and the mixing has no adverse effect on isotachophoresis.

RESULTS AND DISCUSSION

Migration markers

In the measurement of the R_E values of eighteen kinds of cationic dye markers, electrolyte system 2 in Table I was used. Table II gives the observed R_E values, with carnitine as terminator and tris(hydroxymethyl)aminomethane (Tris) as an internal standard to correct the asymmetric potential of the potential gradient detector[9]. Most

TABLE II

THE OBSERVED R_E VALUES AND EFFECTIVE MOBILITIES OF CATIONIC DYES AT pH 4.57, 4.8 AND 5

R_E = effective mobility of leading ion/effective mobility of sample. $\bar{m}$ = effective mobility × 10^5 cm^2 V^{-1} s^{-1}. For the electrolyte system, see system 2 in Table I.

Dye	*pH of leading electrolyte*					
	4.57		*4.80*		*5.00*	
	R_E	$\bar{m}$	R_E	$\bar{m}$	R_E	$\bar{m}$
Bismark brown	1.47	(48.6)	1.46	(48.9)	1.48	(48.2)
Acridine orange	1.76	(40.6)	1.79	(39.9)	1.75	(40.8)
Toluidine blue (TB)	1.80	(39.7)	1.79	(39.9)	1.83	(39.0)
Neutral red	1.88	(38.0)	1.84	(38.8)	1.93	(37.0)
Pyronine Y	1.90	(37.6)	1.96	(36.4)	1.93	(37.0)
Methylene blue	1.94	(36.8)	1.97	(36.2)	1.96	(36.4)
Astrazon green	1.96	(36.4)	1.94	(36.8)	1.93	(37.0)
Astrazon blue	2.06	(34.7)	2.11	(33.8)	2.09	(34.2)
Astrazon Bordeaux	2.21	(32.3)	2.25	(31.7)	2.24	(31.9)
Safranine O	2.24	(31.9)	2.23	(32.0)	2.28	(31.3)
Crystal violet	2.25	(31.7)	2.24	(31.9)	2.25	(31.7)
Astrazon red	2.44	(29.3)	2.49	(28.7)	2.55	(28.0)
Methyl violet	2.66	(26.8)	2.70	(26.4)	2.76	(25.9)
Basic fuchsin	2.83	(25.2)	3.01	(23.7)	2.99	(23.9)
Pyronine G	2.90	(24.6)	3.04	(23.5)	3.06	(23.3)
Brilliant green	2.96	(24.1)	3.23	(22.1)	3.65	(19.6)
Astrazon pink (AP)	3.41	(20.9)	3.53	(20.2)	3.40	(21.0)
Astrazon yellow	3.69	(19.3)	3.69	(19.3)	3.69	(19.3)
Carnitine (Terminating)	5.43	(13.1)	6.18	(11.6)	6.53	(10.9)
Tris (standard)	2.66	(26.8)	2.67	(26.7)	2.67	(26.7)

of the dyes treated contained impurities. The listed R_E values were those for the coloured main components. Apparently from Table II, the R_E values of the treated dyes were not affected by changes in the pH of the leading electrolyte, except for brilliant green. It should be emphasized that most of these cationic dyes may form complexes with metal ions. When the complex-forming interaction occurs, the R_E values may be different from those listed in Table I.

Evaluation of fractionation

To examine the separability and consistency of the fractionation, 30 μl of a mixture of AP and TB (5 m*M*, 150 nmol each) was separated and fractionated. As the amount of sample was small, the preseparation stage was not used. A separation tube with an I.D. of 0.5 mm and a length of 30 cm was used, and the driving current was 150 μA. It took *ca.* 30 min to start fractionation. The observed isotachopherogram is shown in Fig. 3. The impurity zone with a large step height was due to the terminating electrolyte used, as in this particular instance the terminating carnitine hydrochloride was not purified. The impurity zone with a small step height was due to TB.

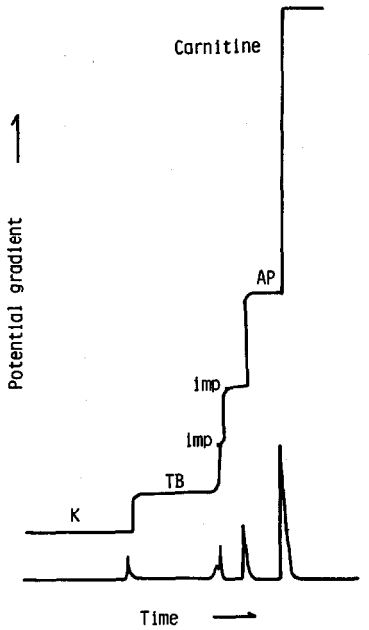

Fig. 3. Observed isotachopherogram of astrazon pink (AP) and toluidine blue (TB) mixture. Leading electrolyte, 1 m*M* KOH + 19 m*M* NH_4OH + 10 m*M* α-hydroxyisobutyric acid + 0.2% hydroxypropylcellulose, pH 4.8 (adjusted with acetic acid); terminating electrolyte, 10 m*M* carnitine hydrochloride. The migration current was 150 μA.

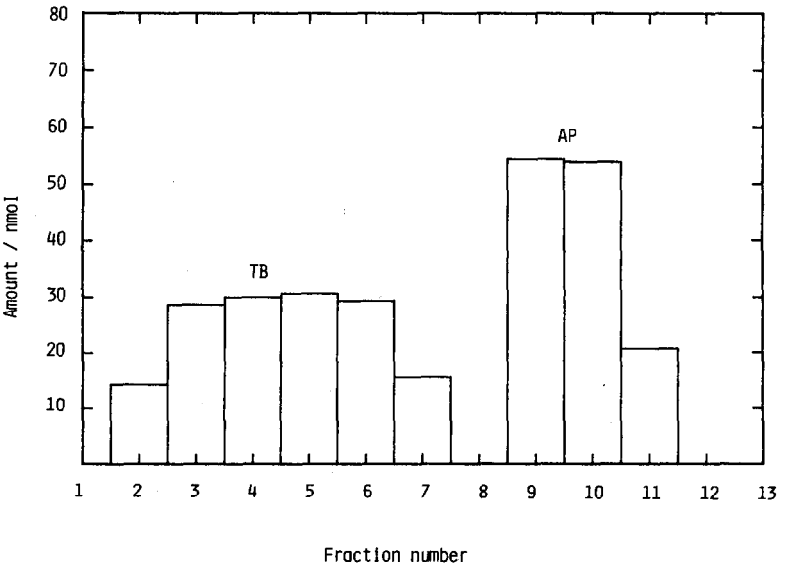

Fig. 4. Constituents and concentrations in the fractions of astrazon pink (AP) and toluidine blue (TB) mixture. Electrolyte system as in Fig. 3.

Ten fractions were obtained at a flow-rate of 24 μl/min. The visible spectra of the fractions were then obtained and the amounts of the components were determined (Fig. 4). Fractions 1–8 were TB and 9–11 were AP. The differences between the observed concentrations of TB and AP are due mainly to the differences in the effective charges of the ions. The fractionated amounts of AP and TB were 148.2 and 131 nmol, respectively, and the recoveries were 98.8 and 87.3%, respectively. The recovery of TB was relatively low because some UV-transparent impurity was present in the sample. The lack of mixing of TB and AP in the fractions was due to the existence of UV-transparent zones between the TB and AP zones.

For the same purpose, a mixture of Ce^{3+}, Sm^{3+}, Dy^{3+} and Tm^{3+} was separated and fractionated. The volume injected was 20 μl, which nominally contained 25 nmol of lanthanoids (3.50, 3.76, 4.06 and 4.22 μg, respectively). Twenty-five fractions were obtained in 6 min. Fig. 5 shows the observed isotachopherogram for Ce^{3+}, Sm^{3+}, Dy^{3+} and Tm^{3+} and Fig. 6 shows the constituents and amounts found in the fractions; these are the results of the PIXE analysis. Apparently the fractionation was very good. At the plateaux in Fig. 6, the deviation of the fractionated amounts was less than 10%. The total amounts of the lanthanoids recovered were 3.77, 4.00, 3.98 and 4.41 μg and the recoveries were 108, 106, 98 and 104% for Ce, Sm, Dy and Tm, respectively. These values are satisfactory taking into account the experimental and analytical errors of the PIXE method.

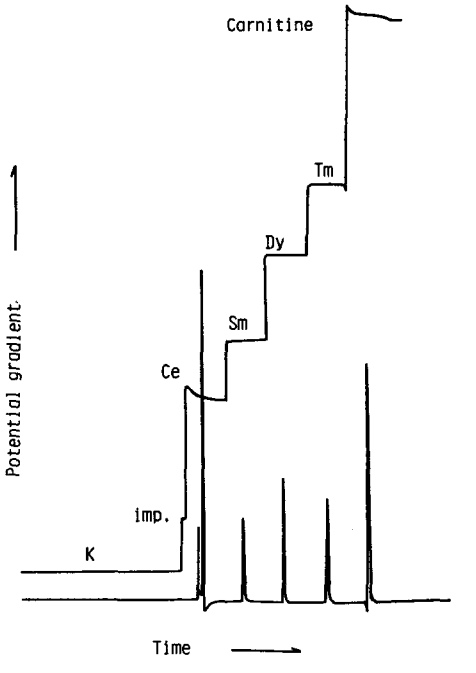

Fig. 5. Observed isotachopherogram of Ce^{3+}, Sm^{3+}, Dy^{3+} and Tm^{3+} mixture (25 nmol of each). Electrolyte system as in Fig. 3.

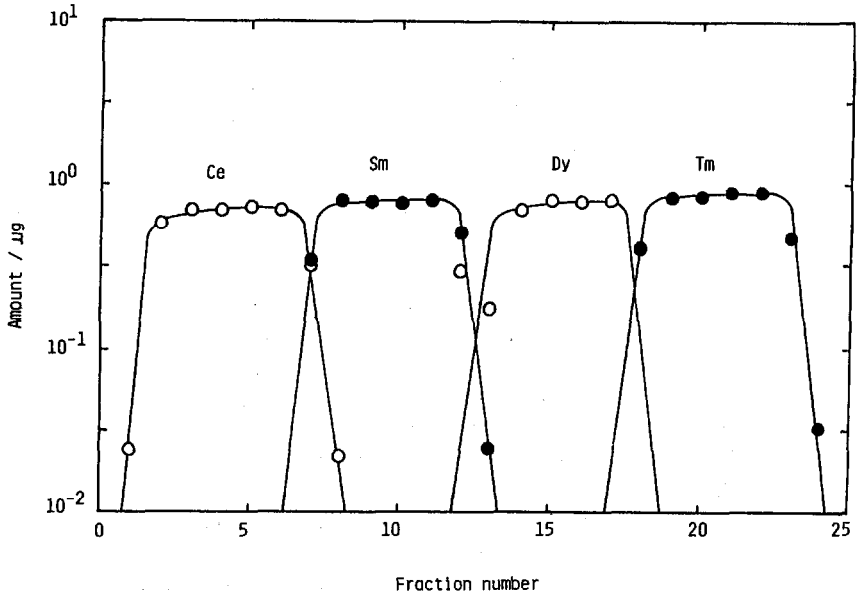

Fig. 6. Constituents of the 25 fractions of Ce, Sm, Dy and Tm mixture and the amounts evaluated by the PIXE method.

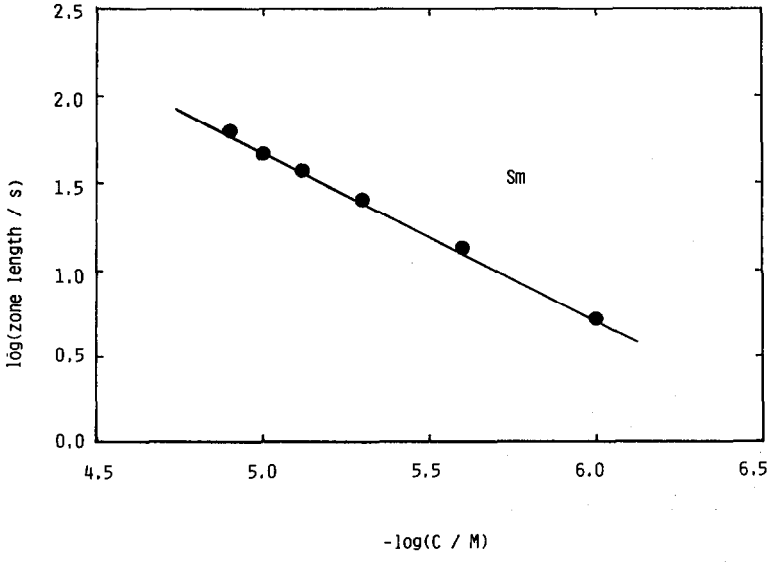

Fig. 7. Zone length of Sm^{3+} with time *vs.* the concentration of the solution injected. The solution was a mixture of Sm^3, Dy^{3+}, Tm^{3+} and Lu^{3+}. The concentrations of Dy^{3+}, Tm^{3+} and Lu^{3+} were $1.25 \cdot 10^{-5}$ *M* each. The volume injected was 2 ml. Leading electrolyte as in System 1 in Table I.

Trace analysis

As the expected performance of the developed system with respect to fractionation was confirmed, the limits were studied using a mixture of Sm^{3+}, Dy^{3+}, Tm^{3+} and Lu^{3+} as the sample. The concentration of Sm^{3+} was varied from $1.25 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$ *M* while the concentrations of the other lanthonoids were kept constant at $1.25 \cdot 10^{-5}$ *M*. The sample (volume 2 ml) was injected from the injection port in the preseparation stage (INJ1 in Fig. 1). A potential gradient detector was used and the current applied during the sample detection was 150 μA. It took *ca.* 120 min for analysis and the time-based zone lengths were in the range 63.0–5.25 s.

Fig. 7 shows the observed zone length of Sm^{3+} and the concentration of solution. Good linearity was obtained, confirming the high sensitivity and reproducibility. The minimum concentration of 10^{-6} *M* corresponds to 150 ppb (10^9). A higher sensitivity may be expected when a narrower separation tube is used.

If the migration was carried by applying a current between L3 and T1 instead of L3 and T2, the detected zone boundaries were obscure. Moreover, a linear relationship between the amounts of sample injected and the detected zone lengths could not be obtained. This can be explained as follows: during the migration in the preseparation stage, the temperature of the electrolyte between L1 and T1 increased considerably owing to the high current (3-5 mA) and the geometry of the separation tube (acrylic resin) changed slightly. After the current had been lowered to 150 μA, the electrolyte and the tube were cooled gradually and hydrostatic flow occurred owing to the contraction. Consequently, the isotachophoretic migration of the separated zone was disturbed by the flow along the direction of the capillary.

In conclusion, the present apparatus was very useful both for the fractionation of sample constituents and for the analysis of trace components. The zone of the markers formed in the preseparation stage (tube of I.D. 5 mm) was very sharp, suggesting that the use of a larger I.D. may be allowed. As the amount of electrolyte constituents which concern the electrophoretic process was relatively large, in particular in the preseparation stage, the chemicals used for the preparation of the leading and terminating electrolytes should be extremely pure. In the present experiments, a considerable amount of Na^+ was detected in KOH. Unless the HPC was deionized and the terminating carnitine was recrystallized from methanol, the disturbance due to the impurities was serious. This is really an adverse effect due to the high load of the electrolytes.

The coupled IP–PIXE method proposed here has been applied to the determination of trace amounts of lanthanoids in crude rare earth metal chlorides and misch metal. The results will be published in due course.

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