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Isotachophoretic analyser equipped with a scanning ultraviolet photometric detection system

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ABSTRACT

An istoachophoretic analyser with a scanning ultraviolet (UV) photometric detection system was constructed in order to study separation processes. A fused-silica capillary (I.D. 0.53 mm, O.D. 0.66 mm) was scanned over a length of 32 cm with an assembly of a UV lamp and a detector. The assembly was driven by a linear head with a stepping motor. One scanning cycle took 7.027 s and 5333 photometric signals (a UV position spectrum) were acquired. The practical resolution was 0.15 mm. The detection limit of picric acid was 20 pmol. Using the apparatus, the separation of a four-component mixture (SPADNS, monochloroacetic acid, picric acid and acetic acid) was studied using different electrolyte systems and the effect of the pH of leading electrolyte on the separation efficiency is briefly discussed.

INTRODUCTION

One of the important features of capillary isotachophoresis is that computer simulation of several characteristics of sample zones may afford practical guidelines for the optimization of the separation conditions. We are constructing a computer-aided separation-optimizing system utilizing these features. The system is based on the computer simulation of the steady state and the transient state. Especially the determination of dynamic features of the separation such as resolution time depends on the transient state simulation. However, the transient state has not yet been fully clarified, as is apparent from the fact that an analytical method for a three-component sample was reported only very recently [1]. Therefore, in order to improve the reliability of the simulation, the separation process of samples with various components should be observed and compared with the simulated results. In order to observe the separation process, two detection systems may be suitable, a multi-channel detection system, where many detectors are set along a separation tube, and a scanning detection system.

In a previous paper, an isotachophoretic analyser with a multi-channel UV

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detection system was reported [2]. By analysing the electropherograms observed, the velocities of the boundaries of transient mixed zones were measured accurately and were compared with theoretical results to confirm a physical model for the isotachophoretic separation of a three-component system [1,3]. However, the detection system suffered from two practical problems: the separation tube used (16.6 cm) was not long enough to observe the separation process of a complex sample system, which needed a relatively long capillary for separation, and the apparent sensitivity and signal-to-noise ratio of each cell differed significantly owing to the different UV intensities at each cell, because only two deuterium lamps were used as the UV source and the radiation from a lamp was delivered to sixteen photocells using quartz optical fibres. To avoid these problems, a scanning system was selected.

A scanning detection system for measuring the profiles of electric field distribution along the migration direction has been developed by Schumacher and co-workers [4–6]. However we avoided measuring the profiles, as the use of complicated electric circuits was inevitable for isolating potential gradient detector signals from high migration voltages. Considering this point, a scanning UV detector with a detector–amplifier pair was designed and constructed, which enabled a fused-silica capillary to be scanned over a length of 32 cm. This paper describes the design and efficiency of this system in comparison with the previous multi-channel system [2]. A typical performance was demonstrated by the separation of 4,5-dihydroxy-3-(*p*-sulphophenylazo)-2,7-naphthalenedisulphonic acid (SPADNS), monochloroacetic acid, picric acid and acetic acid using several leading electrolytes with different pH values.

EXPERIMENTAL

Apparatus

Fig. 1 shows a schematic diagram of the isotachophoretic analyser with a scanning UV photometric detection system. LE and TE in Fig. 1 are the leading and the terminating electrode compartment, respectively, where the migration current was applied; L and T are the reservoirs for the leading and terminating electrolyte, respectively. Sample solution was injected at the injection port of the sampling valve (SV in Fig. 1). The sample was separated in a fused-silica capillary separation tube (FS). The migration process over a 32-cm capillary length was monitored by a scanning UV detector (DET).

A linear head (LH) equipped with a pulse motor (PM) (Model UPH566LB3-A; Oriental Motor, Tokyo, Japan) was used for repeatitive scanning. An assembly of the detector and a UV source (UVS) was attached to the linear head. The movement of the linear head was accurately controlled by the use of a microcomputer (Model PC-9801 VX21, 80286–80287, clock 12 MHz; NEC, Tokyo, Japan) equipped with an interface board for the pulse motor [PMC-1C(98); Contec, Tokyo, Japan]. One electric pulse corresponded to a 0.72° rotation of the pulse motor, while the linear head moved by 0.02 mm. Therefore, 16 000 pulses should be sent to the pulse motor in order to scan the 32-cm capillary.

The fused-silica capillary (FS) used as a separation tube was obtained from Gasukuro Kogyo (Tokyo, Japan). The inner surface was not chemically modified. The original I.D. and O.D. were 0.53 and 0.75 mm, respectively. The polyimide coating of the fused-silica capillary was removed by burning it out in order to make

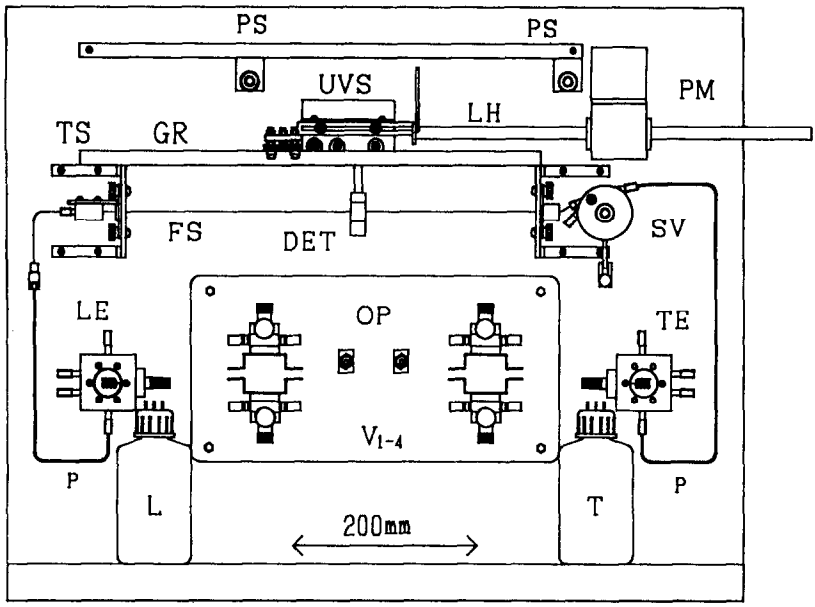

Fig. 1. Schematic diagram of isotachophoretic analyser equipped with a scanning UV photometric detection system. PM = Pulse motor; LH = linear head; UVS = UV source (deuterium lamp); DET = UV detector; FS = separation tube, fused-silica capillary (I.D. 0.53 mm, O.D. 0.66 mm); PS = position sensors; TS = tensioner for the silica capillary; SV = sample injection valve; GR = guide rail for detector assembly; LE = leading electrode compartment; TE = terminating electrode compartment; L and T = leading and terminating electrolyte reservoirs; P = PTFE tube; V_{1-4} = valves to fill or to drain the electrolytes; OP = operation board.

the capillary UV transparent. The O.D. was reduced to 0.66 mm by this treatment. The length of the capillary used was 40 cm and it was joined with PTFE tubes of O.D. 1.0 mm and I.D. 0.5 mm (P).

The silica capillary penetrated a 0.8 mm hole in the PTFE cylindrical block (O.D. 5 mm, length 15 mm) of the scanning UV detector. As the clearance between the capillary and the hole was small and the capillary without a coating was very fragile, slight vertical motion of the scanning detector caused to break it off. The important conditions for smooth scanning were as follows: a guide rail (GR) was used to support the detector assembly, ensuring straight movement of the assembly; and the silica capillary was kept tightened and the tension was adjusted at the end of the capillary by using a simple device (TS).

The UV source was a Hamamatsu deuterium lamp (Model L1626), which was driven by a power supply from a Hamamatsu Model C1518. The UV radiation from the lamp was led to the silica capillary through a short quartz optical fibre after passing through a glass filter (Toshiba Glass, Tokyo, Japan, Model D33S, λ_{max} = 330 nm). The radiation was divided into two paths, one being used for the photometric measurement and the other as the reference signal. The core and cladding diameters of the fibres used were 0.8 and 1.1 mm, respectively. The transmitted UV radiation was led to short-cut optical fibres (*ca.* 10 mm long) which was plugged into the cell.

The fibres acted like an optical slit. At the end of the fibres, a detection element was placed (Hamamatsu Model S1227-16BQ silicone photodiode). The output was connected to a differential amplifier using two LF356N operational amplifiers.

Except for the scanning device, this system had a standard construction. V_{1-4} on an operational board (OP) are valves for rinsing the separation capillary (FS) and filling the electrode compartments (LE and TE). Unless noted otherwise, the main parts used were those of a Shimadzu, (Kyoto, Japan) isotachophoretic analyser (Model IP-1B). The power supply was also that of the IP-1B.

Scanning mode

A single cycle to scan the separation tube and acquire photometric signals took 7.027 s. The speed of the linear head was set constant for the forward and the backward movement. The initial position of the head was set at the nearest position to the sampling valve (SV). An assembly of a small lamp and a photodiode was utilized as a position sensor (PS) in order to determine the initial position exactly. This sensor was also useful for preventing the overshooting of the linear head. The length of the separation tube from the injection port to the initial position of the head was 11.8 cm.

Data acquisition was successively effected during the forward movement (from the terminating side to the leading side) through an analogue-to-digital (A/D) converter. During the backward movement to the initial position, the data were stored in the 3-MB random access memory (a RAM disk system) of the microcomputer. The use of the RAM disk system was necessary in order to treat a large number of data quickly. A/D conversion of the UV signal was made every three electric pulses for the stepping motor. Therefore, the number of the data acquired in a single scan was 5333 (= 16 000/3) and one datum corresponded to 0.06 mm of the separation tube. The resolution was sufficient to obtain the exact position of the boundaries, although a minor correction should be adopted considering that there was a time delay of *ca.* 3.5 s between the first and the last data in a scan.

The scanning and data acquisition were repeated, for example, 200 times or more. After the acquisition was completed, the data in the RAM disk were transferred to a hard disk (80 MB). The data were analysed to obtain the boundary velocities as discussed below. Usually not all of the acquired data were necessary for the evaluation of the boundary velocities.

Samples

The samples were SPADNS, monochloroacetic acid (MCA), picric acid (PIC) and acetic acid (AC). SPADNS and PIC absorb visible and UV radiation. The sodium salt of SPADNS was purchased from Dojin (Kitakyusyu, Japan) in the purest form. The others (extra-pure grade) were obtained from Tokyo Kasei (Tokyo, Japan). Stock sample solutions (*ca.* 10 m*M*) were prepared by dissolution in distilled water without further purification. The sample solution was injected into the terminating electrolyte near the boundary between the leading and the terminating electrolytes.

Operational electrolyte system

The leading electrolyte was 5 m*M* hydrochloric acid. The pH of the leading electrolyte (pH_L) was adjusted to 3.6, 4.4, 4.8 and 6.0 by adding β-alanine, ε-amino-

caproic acid, creatinine and histidine, respectively. The pH measurements were carried using a Horiba (Tokyo, Japan) Model F7ss expanded pH meter.

The terminator was 10 m*M* caproic acid or N-morpholinoethanesulphonic acid (MES). The former was combined with leading electrolyte of pH_L 3.6 and the latter was used with those of pH_L 4.4, 4.8 and 6.0.

Hydroxypropylcellulose (HPC) (0.1%) from Tokyo Kasei was added to the leading and terminating electrolytes to suppress electroendosmosis, which was not negligible in a fused-silica capillary. According to the manufacturer, the viscosity of the 2% aqueous solution is 1000–4000 cP at 20°C. The apparatus was set in a thermostated room and the temperature near the detector was kept at 25 ± 1°C.

RESULTS AND DISCUSSION

Resolution and reproducibility

After inserting a copper wire (O.D. 0.3 mm) into the silica capillary, the capillary was scanned repeatedly and the position spectrum was acquired in order to check the reproducibility. The spectrum was differentiated numerically against the number of data in the scan. Then the numbers expressing the peak position in each scan were compared with each other. No drift was observed, confirming the high stability and reproducibility of the detection system.

The differential peaks had a half-width of 2.5 data (7.5 electric pulses for the stepping motor), which means the edge of the wire was detected with a resolution of 0.15 mm (= 0.02 mm × 7.5). The resolution obtained was sufficient for our purposes. The resolution was checked similarly for an actually migrating sample zone and a similar resolution was obtained.

The accuracy of the stepping distance of the linear head used was checked by measuring repeatedly the data numbers for a copper wire 150.0 mm in length. The number of data between two differential peaks was 2487 with no drift. Apparently

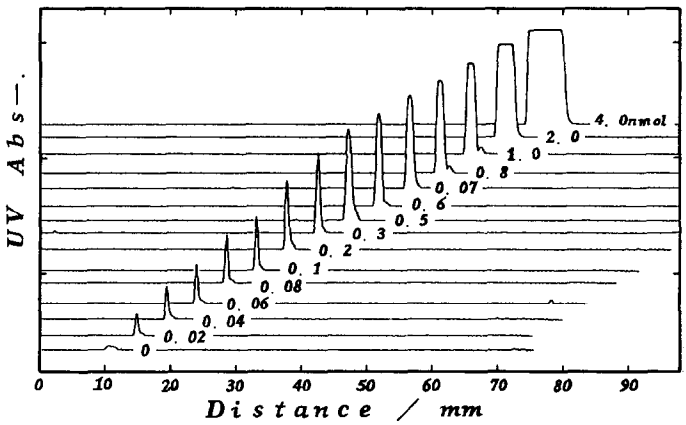

Fig. 2. Detection limit of picric acid by use of the scanning UV photometric detector (20 pmol–4.0 nmol). The leading ion was 5 mM hydrochloric acid (pH 3.6, buffer = β-alanine). The terminator was 10 m*M* caproic acid. The migration current was 49.5 μA. The I.D. of the separation tube (fused-silica capillary) was 0.53 mm.

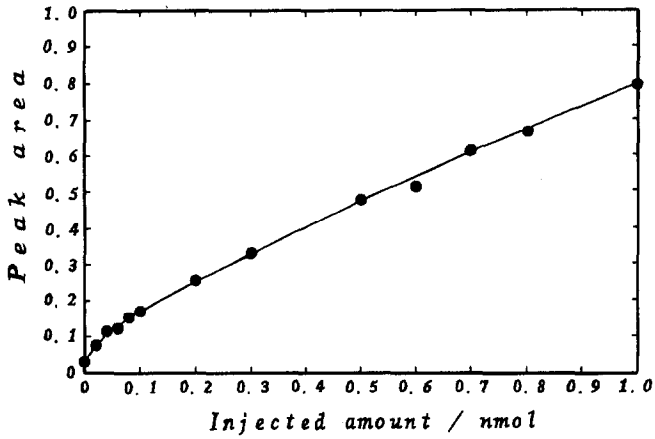

Fig. 3. UV peak area in arbitrary units *vs.* sample amount of picric acid. The sample amount was varied from 0 to 1 nmol. Operational system as in Fig. 2.

from this observation, one datum (three electric pulses) correspond to 0.0603 mm (= 150/2487), confirming the nominal value of 0.06 mm (= 0.02 mm × 3). Time retardation due to inertia effects was not apparent in our observations where the scanning speed was 100 mm/s.

Sensitivity evaluation and analytical utility

Various amounts of picric acid were separated and detected at pH_L 3.6 in order to determine the practical detection limit of the system. The sample amount was varied in the range 20 pmol–4 nmol. The injected sample concentrations were 0.01,

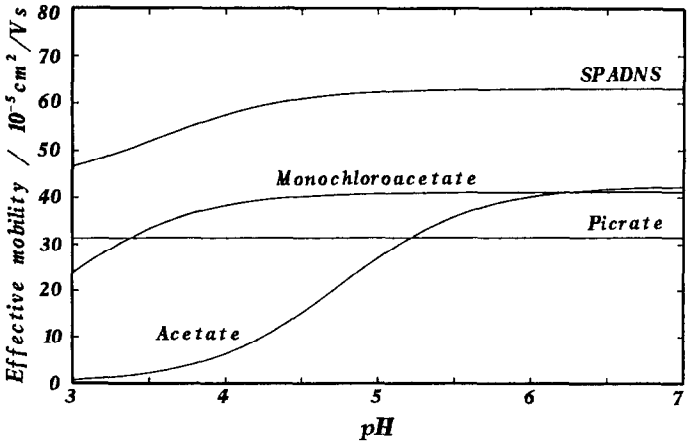

Fig. 4. pH dependence of the effective mobility of SPADNS, monochloroacetic acid, picric acid and acetic acid. Absolute mobility and thermodynamic acid dissociation constant were used. The values were as follows: SPADNS, m_0 = 21 · 10^{-5}, 42 · 10^{-5} and 63 · 10^{-5} and pK_a = −3, −2 and 3.55 (ion-pair formation was assumed for the trivalent anions); monochloroacetic acid, m_0 = 41.1 · 10^{-5} and pK_a = 2.865; picric acid, m_0 = 31.5 · 10^{-5} and pK_a = 0.708; and acetic acid, m_0 = 42.4 · 10^{-5} and pK_a = 4.756.

0.1 and 1 m*M* and the injected sample volume was not less than 2 μl. Fig. 2 shows the observed UV response for the individual sample amounts after the steady state had been achieved. The abscissa is the zone length. The minimum detectable amount was 20 pmol of picric acid, which is comparable to that with the previous system [2] and that reported by Arlinger [7]. The migration current was 49.5 μA.

In a similar manner to the previous work [2], the peak area in arbitrary units was correlated with the sample amount injected. Fig. 3 shows the relationship. Good linearity was found between the peak area and the amount injected in the range 0.1–1 nmol. The gradient of the plots below 0.1 nmol was different from that above 0.1 nmol, where the zone length of picric acid was *ca.* 0.3 mm according to our steady-state simulation.

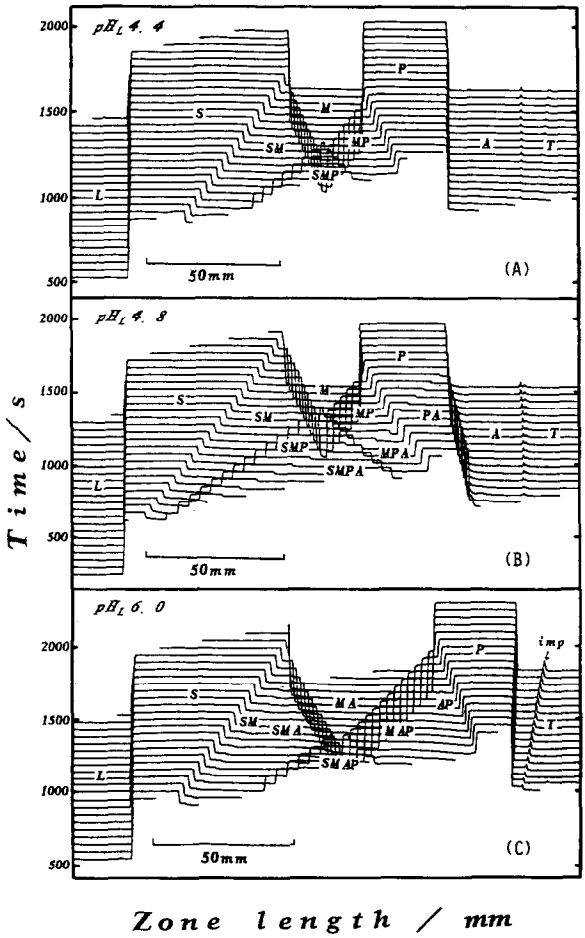

Fig. 5. Transient isotachopherograms of SPADNS, monochloroacetic acid, picric acid and acetic acid obtained by use of the scanning UV photometric detector. The total sample amount was 100 nmol. The leading electrolyte was 5 mM hydrochloric acid. The pH_L was adjusted to (A) 4.4, (B) 4.8 and (C) 6.0 by adding ε-aminocaproic acid, creatinine and histidine, respectively. The terminating electrolyte was 10 m*M* MES. The migration current was 49.5 μA. The I.D. of the separation tube (fused-silica capillary) was 0.53 mm.

Separation of the four-component mixture

Fig. 4 shows the pH dependence of the effective mobility for the components of the treated sample. Under the electrolyte conditions at pH_L 4.4, 4.8 and 6.0, different separation behaviours were expected. It was easily determined from Fig. 4 that the separation order at pH_L 4.4 (SPADNS, MCA, PIC and AC) would be the same with that at pH_L 4.8. However, the separation efficiency of PIC and AC would be higher at pH_L 4.4 than at pH_L 4.8 from the magnitude of the mobility difference. At pH_L 6.0, the migration order would change to SPADNS, MCA, AC and PIC. The separability of MCA and AC would be very low, as is apparent in Fig. 4 from the similar effective mobilities at pH 6.

Fig. 5 shows the observed transient isotacho-pherograms at each pH_L. The total sample amount was the same (100 nmol). The sample volume injected was 10 μl and the pH of the sample solution was adjusted to 3.64 by adding β-alanine. The migration current was 49.5 μA. In Fig. 5, the observed boundaries between the leading and the SPADNS zones were rearranged to the same abscissa position to show clearly the change in the individual zone length at the transient state. Considering that the UV absorption was due to SPADNS and PIC, the concentrations in individual zones were different and all of the width-decreasing zones (triangle form) were transient mixed zones, the different zones found in Fig. 5 were assigned as shown. It should be noted that the total zone length of sample was kept constant during migration and the length was *ca.* 150 mm. At pH_L = 4.4 (Fig. 5A) the SPADNS–MCA–PIC–AC four-component mixed zone (SMPA), the MCA–PIC–AC mixed zone (MPA) and the PIC–AC mixed zone (PA) were not observed, but at pH_L 4.8 (Fig. 5B) all of them were observed, suggesting a low separation efficiency at this pH. At pH_L 6.0 (Fig. 5C), the separation order was different from that in Fig. 5A and B, and no separation was achieved for the MCA–AC mixed zone (MA).

In order to determine the boundary-detected time and subsequently the boundary velocity, the observed UV signals were differentiated with respect to distance (in fact, the number of data in a scan), and the positive and negative peaks of the differentiated signals were searched. Then the distance D and the time t for the Mth datum in the Nth scan were expressed as follows:

$$D = D_{\text{unit}}\, M \tag{1}$$

$$t = t_{\text{rep}}\, N + D/V_{\text{scan}} \tag{2}$$

where D_{unit} is the unit length per datum (0.06 mm), t_{rep} is the time needed for one scanning cycle (7.027 s) and V_{scan} is the scanning velocity (100 mm/s). By the use of the time profiles of the boundaries thus obtained, the velocities of the boundaries were obtained by the least-squares method. Table I summarizes the observed resolution time evaluated by solving the boundary equations [2]. The experimental error was less than a few percent. The pH dependence of the separation efficiency is apparent from Table I.

It is concluded that the present apparatus is very useful for the observation of the isotachophoretic transient state of mixtures. This apparatus can be utilized to clarify the factors affecting the separation efficiency, *e.g.*, the number of sample components and the variation of the abundances of components. The results will be reported in due course.

TABLE I

OBSERVED RESOLUTION TIME FOR EQUIMOLAR MIXTURE OF SPADNS, MONOCHLOROACETIC ACID, PICRIC ACID AND ACETIC ACID AT pH 4.4, 4.8 AND 6.0

t_{res} = Resolution time. Zone = mixed zone. S = SPADNS; M = monochloroacetic acid; P = picric acid; A = acetic acid. The leading electrolyte used was 5 m*M* hydrochloric acid and the migration current was 49.5 μA. The sample amount was 25 nmol.

Zone	t_{res} (s)		Zone	t_{res}(s) ($pH_L = 6.0$)
	$pH_L = 4.4$	$pH_L = 4.8$		
SM	1296	1469	SM	1515
MP	1148	1220	MA	–[b]
PA	–[a]	1355	AP	1535
SMP	847	921	SMA	1391
MPA	–[a]	863	MAP	–[c]
SMPA	–[a]	719	SMAP	888

[a] Resolved before starting data acquisition.
[b] Not measured owing to a lack of resolution.
[c] Not measured owing to the unclear boundary.

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