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Short Communication

Isotachophoretic analyser with options for operational electrolyte selection and repeated analysis

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ABSTRACT

A prototype of an isotachophoretic analyser with options for electrolyte selection and repeated analysis was constructed, which might be useful for the optimization of separation and the measurement of qualitative indices at different pH values for mobility and pK_a evaluation. After a sample mixture had been injected into the system it was separated and pushed back to the original position, and this cycle was repeated varying the leading electrolyte. The sample was pushed back by the use of the counter flow technique. The process of repeated analysis was monitored by the use of a 32-channel UV photometric zone detection system. It was found that the flow-rate of push-back was decisively important for good reproducibility in repeated analysis. The efficiency of the apparatus was demonstrated with a model mixture of anions.

INTRODUCTION

In electrophoresis it frequently happens that some of the sample constituents cannot be separated owing to their similar effective mobilities with the operational electrolyte system used. If the constituents are weak electrolytes, the separability might be improved by applying another appropriate electrolyte system of different pH [1,2]. On the other hand, in order to evaluate mobility and pK_a isotachophoretically, the quantitative indices must be measured under various pH conditions.

In such instances the sample must be injected and separated again after changing the operational electrolyte. Usually this process is carried out manually and it is time consuming because the electrode compartment has to be rinsed thoroughly, even if the electrolyte was ready. Recently Pospichal *et al.* [3] reported a convenient method for changing the operational pH conditions by using a three-pole column (a separation column with three electrodes), where the required operational leading electrolyte can be generated. The migration current was fed via two electrolyte

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compartments filled with solutions of different pH and the fraction of the current controlled the pH of the new leading electrolyte. A similar technique was also applied in capillary zone electrophoresis [4]. The advantage is that numerous leading electrolytes with different pH values can be generated from two basic electrolytes.

This paper describes another method for changing the electrolyte conditions, selecting from several kinds of the leading solutions prepared beforehand. The analysis of the same sample as the initially injected one can be repeated: after a sample mixture has been injected into the system, the mixture is separated and subsequently pushed back to the injection position, and the procedure is repeated varying the leading electrolyte. A well established counter-flow technique [1] is utilized for this purpose. In this paper, the design and functioning of a prototype of an isotachophoretic analyser with such a repeating option is described.

EXPERIMENTAL

Apparatus

A schematic diagram of the apparatus is shown in Fig. 1. The construction of the apparatus was not very different from the ordinary one, except for a pump to supply leading electrolyte and three T-branches (J1, J2 and J3) being added to the separation tube. The other feature of this apparatus is the electrolyte supply system: in this apparatus the electrode compartment (E in Fig. 1), which is usually the leading electrolyte compartment, no longer contains the leading solution but an arbitrary high-conductivity solution. A 10 m*M* sodium chloride solution was used in this experiment. The actual leading electrolyte was the solution filled between J1 and J2 in the PTFE separation tube (30 cm × 1 mm I.D.). The leading electrolyte selected from reservoirs 1–6 can be flowed continuously from J1 to J2 during the analysis by the use of a micropump [Model PUD-008 double-plunger type; Gasukuro Kogyo (Tokyo, Japan)]. The flow-rate was 6.4 μl/min and the linear velocity of the flow (8.2 cm/min)

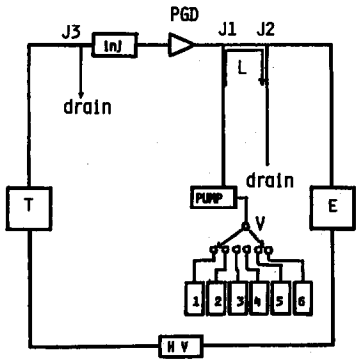


Fig. 1. Schematic diagram of isotachophoretic analyser equipped with operational electrolyte exchange system. E = electrolyte solution compartment (the actual leading electrolyte was filled between J1 and J2); T = terminating electrolyte compartment; inj = injection port; PGD = potential gradient detector; J1 = leading electrolyte inlet; J2 = leading electrolyte outlet; J3 = drain; V = valves to change operational leading electrolyte; 1–6 = leading electrolyte reservoirs; PUMP = double-plunger pump; HV = high-voltage power supply.

was 2.5 times greater than the migration velocity of sodium ions. The overflow was discarded from J2. As sodium ions migrating in the opposite direction to the samples are swept away by the flow, the isotachophoretic equilibrium was not disturbed by the use of sodium chloride solution. Consequently, the leading electrolyte in the tube connecting J1 and J2 (20 cm × 1 mm I.D.) regulated the isotachophoretic separation of the samples.

The valves and the electrolyte compartments used were those of a Shimadzu IP-1B isotachophoretic analyser. A potential gradient detector instrument (PGD) and the power supply was those for a Shimadzu IP-2A.

Method of leading electrolyte exchange

As the inner volume of the tube (J1–J2) was very small, the exchange of the electrolyte was very rapid and smooth compared with the case when the leading electrolyte was filled in the electrode compartment of E in Fig. 1. It took 5 min at the above flow-rate.

Fig. 2 illustrates the principle of the repeated separation. At time $t1$, sample (S) was separated using the leading electrolyte solution L1 and the first detection was just finished. The solution L1 was fed continuously from J1 and was drained from J2, while J3 was closed except when the counter flow technique [1] was utilized to improve the separation. The leading electrolyte L1 was replaced with the different electrolyte L2 as follows: first J3 was closed and L1 in the separation tube (J1–J2) was replaced with L2. Then at time $t2$, J3 was opened and the sample zone was pushed back on applying the migration current. At time $t3$, the sample zone was pushed back to the end of the separation tube. After J3 had been closed, the separation was repeated under the electrolyte condition of L2. The second detection was finished at time $t4$. Thus the same sample can be analysed repeatedly using different leading electrolytes. Under a typical operation, the time necessary for the electrolyte exchange and the push-back of the initial sample zone was 5–10 min.

Apparently from Fig. 1, at time $t1$ the present system has a restriction on the amount of sample, which depends on the volume of the separation tube between PGD and J1. If the leading side of the sample zone reached one of side channels J1,

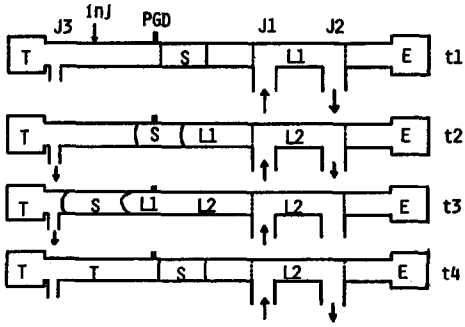


Fig. 2. Principle of the leading electrolyte exchange and repeated separation. E = NaCl solution; L1 = leading electrolyte 1; L2 = leading electrolyte 2; S = sample zone; T = terminating electrolyte. J1, J2 and J3 as in Fig. 1.

the sample would be swept away from the separation tube. Similarly at time $t3$, if the sample was pushed back too far, part of it would be drained away from J3. In the present experiment, this situation was avoided by using coloured samples. For general samples, however, an appropriate "tell-tale" detector should be placed at the leading side of J3.

The mixing of L1 and L2 in the separation tube was inevitable. However, it caused no serious problem if the pH of the leading electrolyte (pH_L) was changed appropriately, as discussed later.

Samples and electrolyte system

Two anionic model mixtures were used. One contained 4,5-dihydroxy-3-(*p*-sulphophenylazo)-2,7-naphthalenedisulphonic acid (SPADNS), monochloroacetic acid (MCA) and picric acid (PIC). The separation behaviour in repeated analysis of the mixture was monitored by the use of a Shimadzu potential gradient detector and the 32-channel UV photometric zone detection system [5]. In the latter detection system, the array of 32 photometric cells was set along the separation tube. The cell interval was *ca.* 5 mm (16.6 cm for 32 channels).

The other model mixture obtained ten constituents, oxalic acid, bromic acid, hypophosphorous acid, bromoacetic acid, succinic acid, 2-hydroxyisobutyric acid, benzoic acid, acetic acid, glutamic acid and butyric acid. The separation efficiency of the present apparatus was examined for this rather complex mixture.

The leading electrolytes were 10 m*M* hydrochloric acid buffered by adding β-alanine (pH 3.6 and 4) and ε-aminocaproic acid (pH 4.4). Hydroxypropylcellulose (HPC) was added to the leading electrolyte (0.02%). The terminator was 10 m*M* caproic acid.

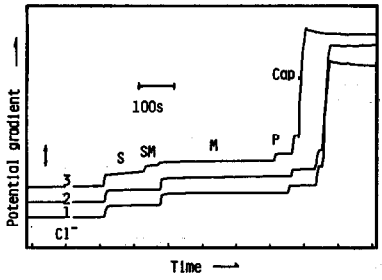
Chemicals were obtained from Tokyo Kasei Kogyo (Tokyo, Japan) in the purest form available. pH measurements were carried using a Horiba Model F7ss expanded pH meter. Data processing was carried out with an NEC PC9801VX microcomputer.

RESULTS AND DISCUSSION

Reproducibility of zone length

First the reproducibility of the time-based zone length was examined for the repeated analysis of the same kind of sample and the leading electrolyte (pH 3.6). Fig. 3 shows the PGD trace of the repeated separation of SPADNS, MCA and PIC. When the counter flow-rate for push-back was 12.2 μl/min, the total zone length agreed with that of the initial separation. On the other hand, when the counter flow-rate was 43.8 μl/min, the total zone length decreased to 80% of the initial length. This suggested that when the flow-rate for push-back was high, the separated samples mixed with the leading and/or terminating electrolytes and the initial sample amount could not be fully recovered until the next detection.

In order to establish what happened in the push-back process with a high flow-rate, the repeated analysis of SPADNS, MCA and PIC was monitored by use of the 32-channel UV photometric detector. Fig. 4 shows the isotachopherogram obtained. The base lines of the UV signals show the position of the detectors. The migration order was SPADNS (S), MCA (M) and PIC (P). It should be noted that



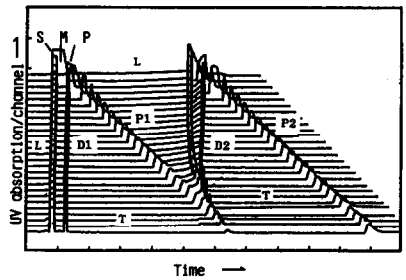


Fig. 3. Observed isotachopherograms of SPADNS (S), monochloroacetic acid (M) and picric acid (P). (1) First detection; (2) second detection (counter flow-rate for push-back = 12.2 μl/min); (3) third detection (counter flow-rate for push-back = 43.8 μl/min). The sample amounts were 7.2 nmol (S), 35.7 nmol (M) and 6.7 nmol (P). The migration current was 100 μA.

Fig. 4. Observed transient isotachopherograms of SPADNS (S), monochloroacetic acid (M) and picric acid (P) by the use of the 32-channel UV photometric zone detection system. D1 = First detection; P1 = first push-back; D2 = second detection; P2 = second push-back (counter flow-rate for push-back = 40.7 μl/min). The sample amounts were 5.8 nmol (S), 28.8 nmol (M) and 5.4 nmol (P). The migration current was 100 μA.

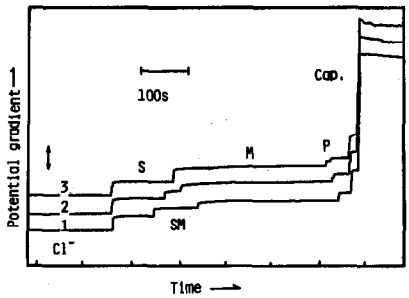
MCA (M) was UV transparent. In Fig. 4, D1 shows the first detection of the isotachophoretically separated steady zones. The boundaries between the leading and SPADNS zones were rearranged at the same abscissa position to demonstrate the exchange of the individual zone length at the transient state.

After the sample zones had reached to the final cell (No. 32), the zones were pushed back to cell No. 2 by applying a counter flow. A migration current (100 μA) was also applied during the push-back. The flow-rate was 40.7 μl/min. P1 shows the above process. Then the counter flow for push-back was stopped and the separation was restarted. D2 shows the second detection and P2 the second push-back.

Apparently from the patterns of the push-back processes (P1 and P2), the SPADNS, MCA and PIC zones, once separated, merged into a mixed zone. The gradual decrease in the UV absorption in the P1 process suggested that the concentrations of SPADNS and PIC decreased significantly, *i.e.*, the separated zones were perturbed and diluted by the counter flow. In the P1 and P2 process in Fig. 4, the left-hand side of the peaks corresponds to the boundary between the terminating zone (T) and the pushed zone. The boundary was steeper than the boundary between the leading zone (L) and the pushed zone. Moreover, the base lines of the leading zone drifted during the push-back, suggesting that the unrecovered samples existed mainly in the leading zone. This observation could explain the shortening of the zone length in Fig. 3 when the counter flow-rate was high. Considering these results, the counter flow-rate for the push-back of sample zones was in the range 10–20 μl/min, depending on the sample constituents. If a higher migration current was applied, the counter flow-rate might be increased because of the increase in the self-correction effect of the sample zone.

Counter flow technique to improve separation

When the sample amount was relatively large, the so-called counter flow technique is useful for improving the separability [1]. As the capillary length was fixed in



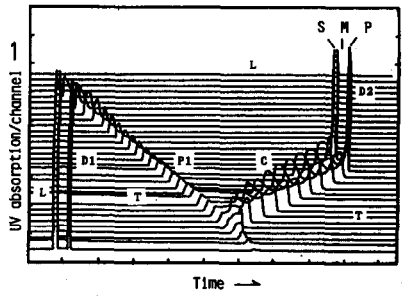


Fig. 5. Observed isotachopherograms of SPADNS (S), monochloroacetic acid (M) and picric acid (P). (1) First detection without counter flow; (2) second detection with 63% counter flow (2.2 μl/min, 8 min); (3) third detection with 17-min counter flow. The sample amounts were 28.6 nmol (S), 142.8 nmol (M) and 26.6 nmol (P). The migration current was 100 μA.

Fig. 6. Observed transient isotachopherograms of SPADNS (S), monochloroacetic acid (M) and picric acid (P) by the use of the 32-channel UV photometric zone detection system. D1 = First detection; P1 = first push-back; C = counter flow applied to improve the separability; D2 = second detection. The sample amounts were 5.8 nmol (S), 29.1 nmol (M) and 5.8 nmol (P). The migration current was 100 μA.

this apparatus, this technique was utilized. Fig. 5 shows the effect of the counter flow for a mixture of SPADNS (28.6 nmol), MCA (142.8 nmol) and PIC (26.6 nmol). As shown by trace 1 in Fig. 5, a mixed zone of SPADNS and MCA was formed (SM) when the counter flow was not applied. Then the sample zones were pushed back and separated again by applying a 63% counter flow (2.2 μl/min) for 8 min. On the trace obtained (2 in Fig. 5), however, the mixed zone still existed. Analysis was repeated by applying a counter flow for 17 min. The separation was complete, as shown by trace 3 in Fig. 5.

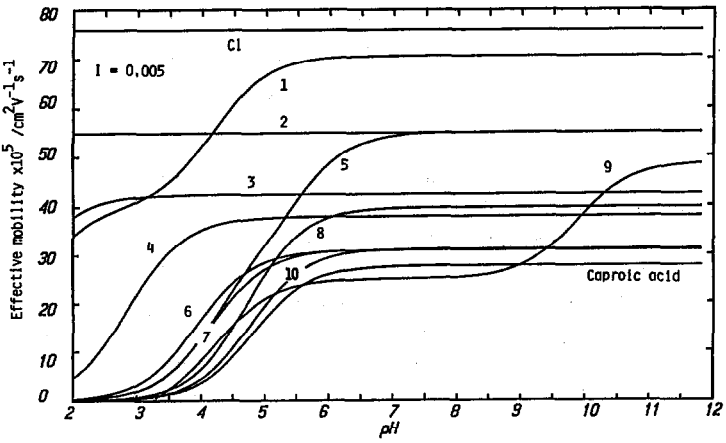


Fig. 7. Calculated effective mobility *vs.* pH curves for (1) oxalic acid, (2) bromic acid, (3) hypophosphorous acid, (4) bromoacetic acid, (5) succinic acid, (6) 2-hydroxyisobutyric acid, (7) benzoic acid, (8) acetic acid, (9) glutamic acid and (10) butyric acid. The effective mobility of the leading chloride ion and the terminating caproate ion was also plotted. Temperature = 25°C; ionic strength, 0.005.

The improvement of the separability by this method was observed by use of the 32-channel UV photometric detector for the same samples. Fig. 6 shows the observed transient isotachopherogram. D1 shows the first detection of the isotachophoretically separated zones and P1 shows the push-back process. After the sample zones had reached cell No. 2, the counter flow was reduced to 63% and the separation was restarted (C). D2 shows the second detection after the counter flow process. Apparently the separability was increased by applying the counter flow technique.

Repeating analysis using different operational systems

Finally, the isotachophoretic separation was repeated varying the leading electrolyte. Fig. 7 shows the effective mobility–pH curves for ten anionic constituents together with caproic acid as the terminator and chloride ion as the leading ion. The ionic strength was 0.005. As the curves cross each other at low pH, it seemed that complete separation with one electrolyte system was difficult. In this experiment, the pH of the leading electrolyte (pH_L) used was 3.6, 4.0 and 4.4 and was changed in the above order. As the mixing of the different leading electrolytes was inevitable in the present method, this pH_L order was very important for minimizing unfavourable effects. The buffers used were β-alanine (pK_a = 3.552, pH_L = 3.6 and 4) and ε-aminocaproic acid (pK_a = 4.373, pH_L = 4.4). Although these buffers coexisted in the separation tube when the pH_L was changed from 4.0 to 4.4, β-alanine has no serious effect on the sample zone because of its small effective mobility at pH 4.4.

Fig. 8 shows isotachopherograms observed with the use of a PGD. D1, D2 and D3 show the first, second and third detections and P1 and P2 show the PGD traces of the push-back process. The counter flow technique was utilized in order to improve the separability after changing the leading electrolyte (*ca.* 20 min). The observed isotachopherograms agreed well with those obtained individually in the usual way, confirming the smooth exchange of the electrolyte conditions in the present apparatus. Apparently from Fig. 8, the separability of the samples varied depending on the pH of the leading electrolyte. For example, the separation of samples 8 and 9 at pH 3.6, 1 and 2 at pH 4.0 and 5 and 6 at pH 4.4 could be improved at the different pH values.

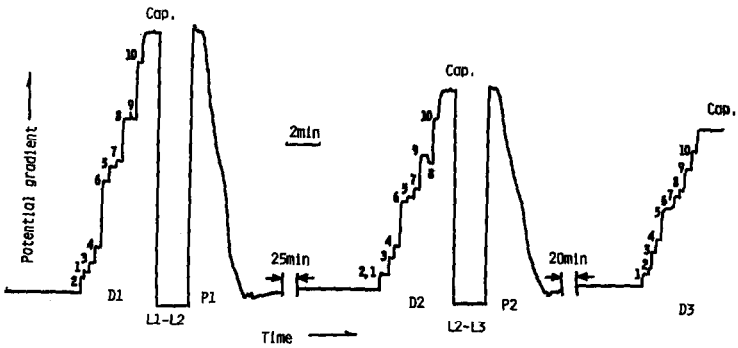


Fig. 8. Isotachopherograms observed for samples 1–10 shown in Fig. 7 obtained by the use of a PGD. The separation was repeated for the same sample, changing the leading electrolyte (pH 3.6, 4.0 and 4.4). D1, D2 and D3 = first, second and third detection, respectively; P1 and P2 = push-back process. Cap. = caproic acid (terminator). For the details, see text.

In high-performance liquid chromatography, the use of various types of eluents in a run is a common technique. Undoubtedly this technique contributes to the high separability. Similarly in electrophoresis, a new option for repeated analysis by varying the leading electrolyte might improve the separability. A fully automated repeating analyser might be possible on the basis of the proposed method in combination with a leading electrolyte generator [3].

REFERENCES

1 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, 1976.
2 P. Bocek, M. Deml, P. Gebauer and V. Dolnik, *Analytical Isotachophoresis*, VCH, Weinheim, 1988.
3 J. Pospichal, M. Deml, P. Gebauer and P. Bocek, *J. Chromatogr.*, 470 (1989) 43.
4 P. Bocek, M. Deml, J. Pospichal and J. Sudor, *J. Chromatogr.*, 470 (1989) 309.
5 T. Hirokawa, K. Nakahara and Y. Kiso, *J. Chromatogr.*, 463 (1989) 39.