Journal of Chromatography, 346 (1985) 167–176
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 957

MULTICHANNEL SPECTROPHOTOMETRIC DETECTOR FOR FUSED-SILICA CAPILLARY TUBE ISOTACHOPHORESIS

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(Received June 7th, 1985)

SUMMARY

Isotachophoresis carried out in a 0.25 mm I.D. fused-silica capillary tube yielded high resolution. The use of an ultraviolet visible multichannel spectrophotometer with photodiode array as detector together with a cross flow cell (volume 0.01 μl) was investigated. The system enables excellent resolution of organic acids and nucleotides, and was applied to micro spectrophotometric identification of the iron(II)–*o*-phenanthroline complex.

INTRODUCTION

In capillary tube isotachopohoresis, thermal, conductivity, potential gradient and single-wavelength UV absorption detectors are usually used. However, all these detectors are generally unsatisfactory for identifying the species of each zone. Multichannel photodiode array UV–visible detectors have recently been developed and used in high-performance liquid chromatography (HPLC)[1]. They enable multi-wavelength detection and yield spectral information in a single chromatographic analysis. We have developed a multichannel spectophotometric detector with parallel and cross flow cells for micro HPLC[2,3].

In isotachophoresis, PTFE (polytetrafluoroethylene) and FEP (fluorinated ethylene–propylene) tubes with an inside diameter of *ca.* 0.5 mm are commonly used for the migration tube. A decrease in the inside diameter of the migration tube will result in a decrease in the temperature difference between the centre of the tube and the wall, and the temperature profile will thus decrease. Moreover, the absolute increase in temperature is smaller, which reduces the convective disturbances. Therefore the use of narrow-bore tubes with smaller inside diammeters will increase the resolution[4].

In the present work, a fused-silica capillary tube of 0.25 mm I.D. was used as

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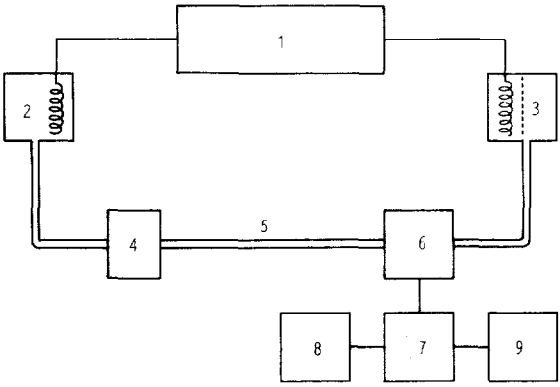

Fig. 1. Block diagram of the apparatus. 1 = Constant power supply; 2 = leading electrolyte; 3 = terminating electrolyte; 4 = sample injection port; 5 = capillary migration tube; 6 = multichannel photodiode array UV–visible detector; 7 = micro computer; 8 = monitorscope; 9 = plotter.

the migration tube in isotachophoresis, and a miniaturized multichannel photodiode array UV–visible detector was developed for capillary tube isotachophoresis. The system was applied to the analysis of real samples and to the micro spectrophotometric identification of the components.

EXPERIMENTAL

A block diagram of the apparatus is shown in Fig. 1. For isotachophoresis, an IP-2A instrument (Shimazu Seisakusho, Kyoto, Japan) was employed after replacing a FEP tube of 0.50 mm I.D. with a fused-silica tube of *ca.* 0.25 mm I.D. as the migration tube. The fused-silica tubing was obtained from SGE (Melbourne, Australia). For detection, a micro gate photodiode array detector MGPD, a micro computer MC-800, a monitorscope MC-910 and a plotter MC-920 (all from Union Gi-

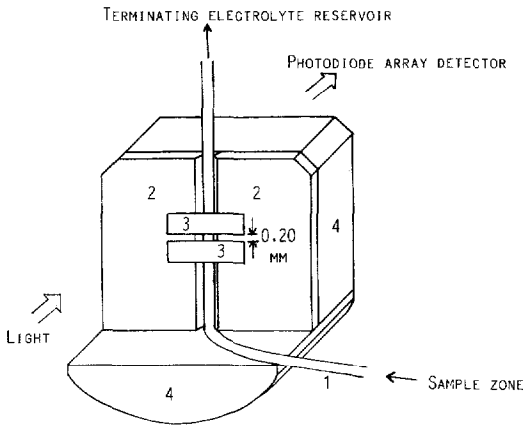

Fig. 2. Schematic diagram of the micro cross flow cell for capillary isotachophoresis. 1 = Fused-silica capillary tube (0.25 mm I.D.); 2 = stainless-steel slit block; 3 = aluminium slit tape; 4 = stainless-steel cell block.

ken, Osaka, Japan) were used. The MGPD system comprised 840 active photodiode array elements, two of which controlled each channel. Its spectral range and bandwidth were 200–800 nm and 1.4 nm, respectively.

Most UV–visible detectors used in conventional HPLC have a parallel flow cell, in which the light path is parallel to the direction of flow, and the typical cell volume of conventional flow cells is 8 μl (light pathlength 10 mm). On the other hand, a cross flow cell is chiefly employed in micro HPLC to reduce the cell volume. The structure of the micro cross flow cell employed in isotachophoresis is illustrated in Fig. 2. Two stainless-steel slit blocks were fixed on the stainless-steel cell block, having a hole in the centre for the light path and a horizontal slit width of *ca.* 0.20 mm. The material coating the fused-silica capillary migration tube (0.25 mm I.D.) was removed near the outlet parts by use of a small flame, and the capillary was mounted between the two stainless-steel slit blocks. The vertical slit width was set at *ca.* 0.20 mm by two aluminium slit tapes. Thus, the cell volume was reduced to 0.01 μl and the maximum light pathlength reduced to 0.25 mm, leading to a decrease in the observed light intensity. The latter problem was solved in this MGPD system by decreasing the number of optical parts such as lenses and mirrors, and reducing the distance between the light source and the flow cell and that between the flow cell and the grating.

For anion analysis, 0.01 *M* hydrochloric acid, β-alanine, Triton X-100 (pH 3.6) and 0.01 *M* sodium *n*-caproate (pH 7.5) were used for the leading and terminating electrolytes, respectively. Seven organic acids, maleic, fumaric, salicylic, phthalic, lactic, *p*-aminosalicylic and benzoic acid, and three nucleotides, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP), were used for the anion standard samples. Commercially available apple juice (30%) and cosmetic lotion were employed as the real samples without any pretreatment. For cation analysis, 0.005 *M* potassium acetate, acetic acid (pH 5.0) and 0.005 *M* γ-amino-*n*-butyric acid (pH 5.0) were used for the leading and terminating electrolytes, respectively. Iron(II)–*o*-phenanthroline complex (10 μM) was prepared by mixing 0.01 *N* iron(II) ammonium sulphate and 0.01 *M* *o*-phenanthroline in the ratio of 1:4, and diluting in distilled and deionized water.

Sodium *n*-caproate, *p*-aminosalicylic acid and *o*-phenanthroline were purchased from Tokyo Chemical Industry (Tokyo, Japan), Kishida Chemical Industry (Osaka, Japan) and E. Merck (F.R.G.), respectively. Other reagents were obtained from Wako Chemical Industries (Osaka, Japan).

RESULTS AND DISCUSSION

Isotachophoretic separation with fused-silica capillary tube

Verheggen *et al.*[4] demonstrated the importance of the inside diameter of the migration tube by using PTFE tubing. They tested PTFE narrow-bore tube with approximate inside diameters of 0.4, 0.2 and 0.1 mm, and found that with an I.D. of *ca.* 0.2 mm to be almost optimal, owing to the construction of the injection system and conductivity detector. On the other hand, the electroosmotic flow at low (centimolar) concentrations of electrolyte was found to be far from negligible with Pyrex glass tubes having inside diameters smaller than 0.4 mm. Since fused-silica tubing is suitable for "on line" UV detection, we tried to use a narrow-bore fused-silica tube

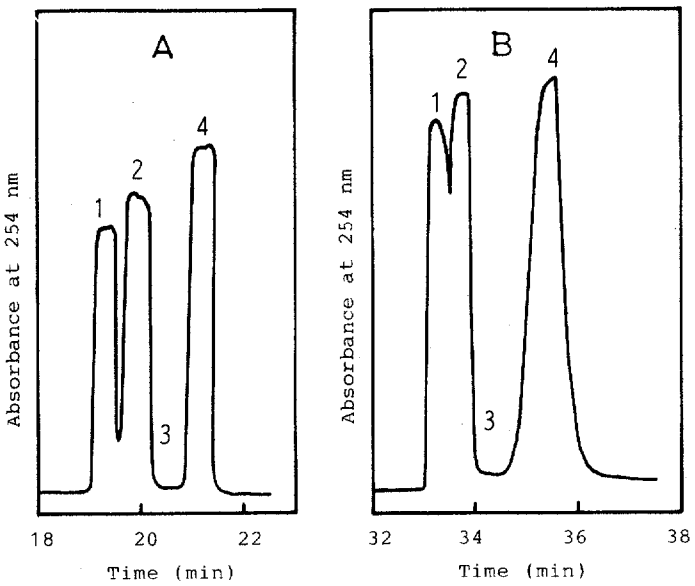

Fig. 3. Comparison of isotachophoretic separation with different migration tubes. A, Fused-silica tubing (200 × 0.25 mm I.D.); B, FEP tubing (75 × 0.50 mm I.D.). Zones: 1 = ATP; 2 = ADP; 3 = succinic acid; 4 = AMP. Sample amount injected: 2 μl of each 500 ppm mixture. Migration current: 25 μA. Slit diameter: 0.17 mm (A), 0.30 mm (B).

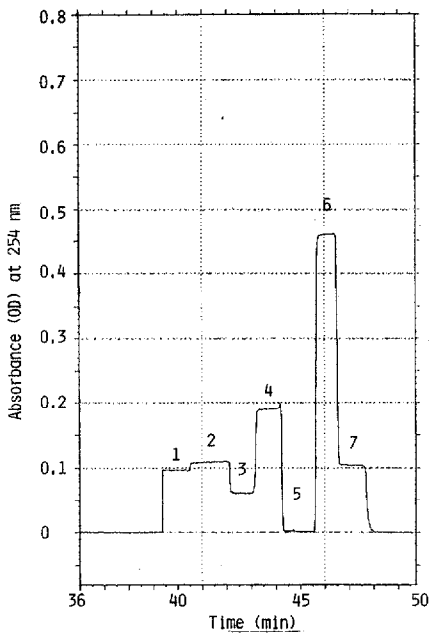

Fig. 4. Isotachophoretic separation of a standard mixture of seven organic acids with a fused-silica capillary tube. Zones: 1 = maleic acid; 2 = fumaric acid; 3 = salicylic acid; 4 = phthalic acid; 5 = lactic acid; 6 = *p*-aminosalicylic acid; 7 = benzoic acid. Sample amount injected: 2 μl of each 500 ppm mixture. Leading electrolyte: 0.01 *M* hydrochloric acid, β-alanine, Triton X-100 (pH 3.6). Terminating electrolyte: 0.01 *M* sodium *n*-caproate (pH 7.5). Migration current: 25 μA. Migration tube: fused-silica tubing (500 × 0.25 mm I.D.).

as the migration tube. Fig. 3 shows a comparison of isotachopherograms of a mixture of AMP, ADP, ATP and succinic acid with a FEP tube of *ca.* 0.50 mm I.D. and a fused-silica tube of *ca.* 0.25 mm I.D. In this case, a conventional single-wavelength UV detector UVD-10A (Shimazu Seisakusho) was used for zone detection with slit diameters of 0.30 mm for the FEP tube and 0.17 mm for the fused-silica tube. Clearly, the use of the fused-silica tube increased the resolution at a shorter analytical time, compared with that of the conventional FEP tube.

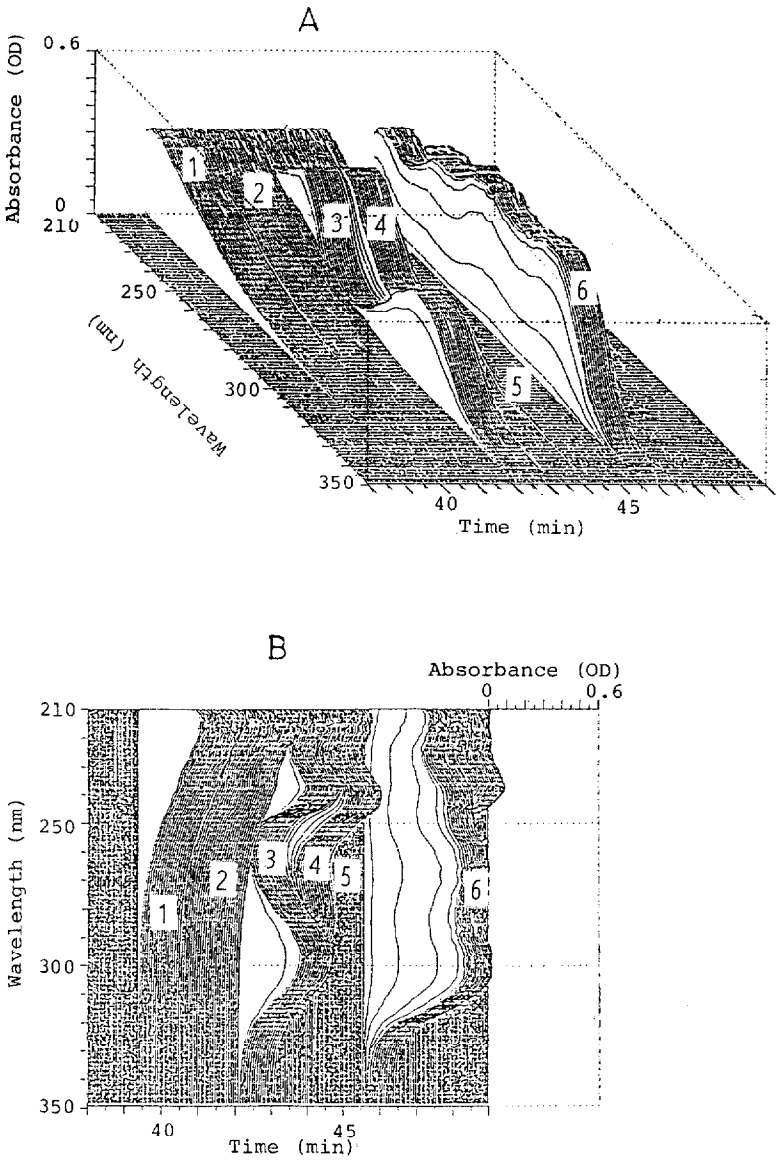

Fig. 5.

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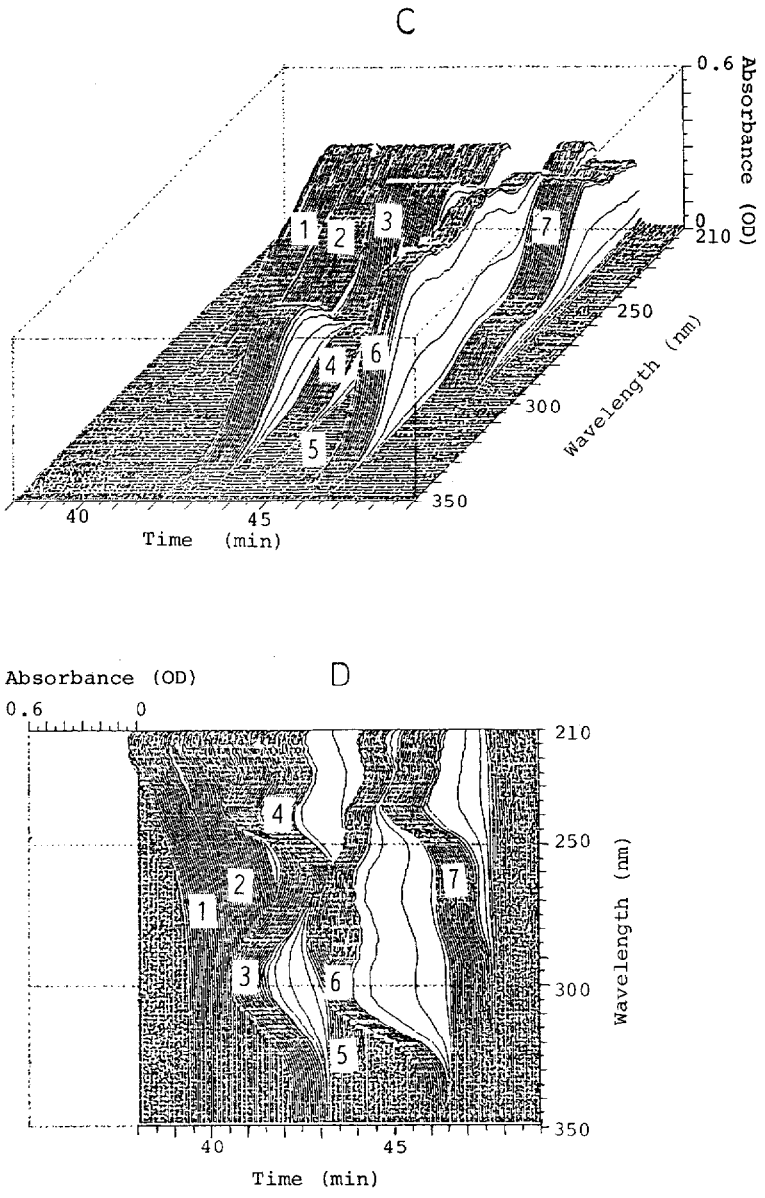

Fig. 5. Different three-dimensional isotachopherograms of a standard mixture of seven organic acids. A, Forward three-dimensional plot at 45°; B, forward plot at 90°; C, backward plot at 45°; D, backward plot at 90°. The number of zones and experimental conditions are as in Fig. 4.

Multichannel spectrophotometric detection in isotachophoresis

An isotachophoretic separation of a standard mixture of organic acids was carried out in a fused-silica tube (500 × 0.25 mm I.D.) by using the multichannel photodiode array UV–visible detector and a cross flow cell (volume 0.01 μl). Fig. 4

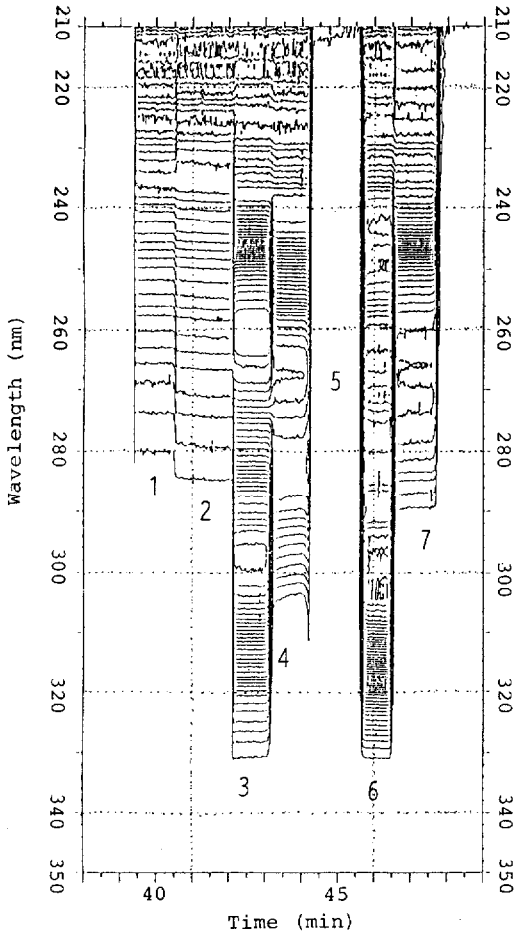

Fig. 6. Contour plot for isotachophoresis of a standard mixture of seven organic acids. The number of zones and experimental conditions are as in Fig. 4. Absorbance step: 0.01.

shows the conventional isotachopherogram monitored at a single wavelength, 254 nm. The seven organic acids were very well separated in the fused-silica capillary tube at a migration current of 25 μA. However, it is rather difficult to identify each migration zone, except for lactic acid which has no UV absorption, from the isotachopherogram at the single wavelength. Fig. 5 shows three-dimensional isotachopherograms obtained in the proposed system: A and B are the forward isotachopherograms, C and D the backward isotaschopherograms; A and C are the 45° angle plots, B and D the 90° angle plots, respectively. Thus, the addition of the wavelength axis to the absorbance and time axes in the isotachopherogram dramatically improves the identification within each migration zone. The zone of benzoic acid could not be found in the forward isotachopherograms, since the UV absorbance of *p*-aminosalicylic acid was too large, while it was clearly seen in the backward isotachopoherograms. The 45° angle plots were useful for basic quantitative and qualitative information, while the 90° angle plots gave exact qualitative information. Fig. 6 shows

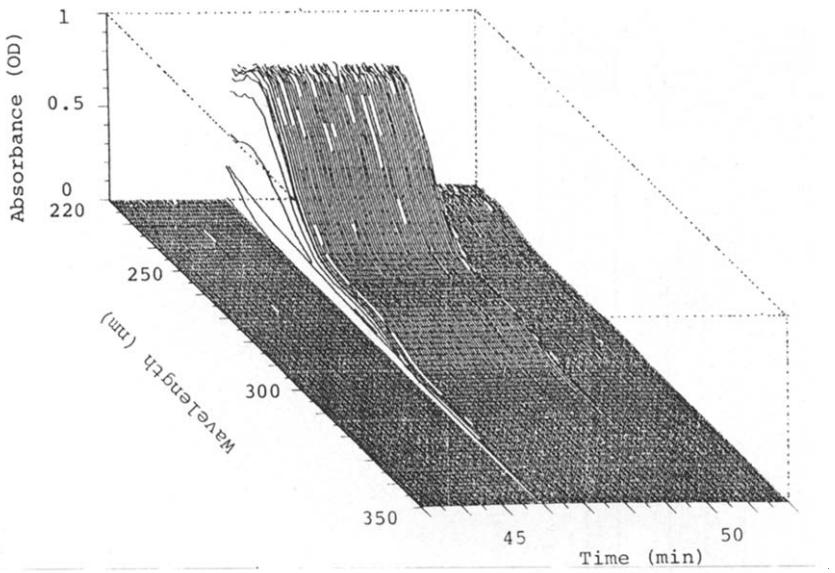

Fig. 7. Multichannel spectrophotometric detection of benzoic acid in a cosmetic lotion. Sample amount injected: 1 μl of raw cosmetic lotion. Other experimental conditions as in Fig. 4.

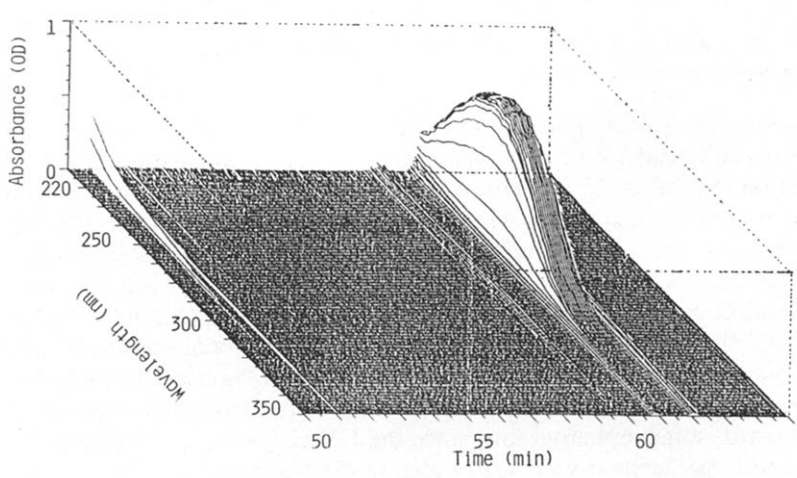

Fig. 8. Multichannel spectrophotometric detection of ascorbic acid in apple juice. Sample amount injected: 2 μl of raw apple juice (30%). Other experimental conditions as in Fig. 4.

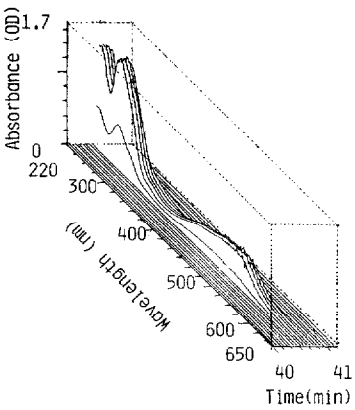

Fig. 9. Three-dimensional isotachopherogram of the iron(II)-*o*-phenanthroline complex obtained with a fused-silica capillary tube. Sample amount injected: 10 μl of 10 μM (5.6 ng as iron). Leading electrolyte: 0.005 *M* potassium acetate, acetic acid (pH 5.0). Terminating electrolyte: 0.005 *M* γ-amino-*n*-butyric acid, acetic acid (pH 5.0). Migration current: 25 μA. Migration tube: fused-silica tubing (500 × 0.25 mm I.D.).

contour plots at absorbance steps of 0.01 units for isotachophoresis of the organic acid mixture. It should be noted that a rectangular shape is characteristic in isotachophoresis, while a founded shape is observed in HPLC. This plot was useful to check the purity of each migration zone.

The proposed system was applied to the determination of organic acids in real samples. Figs. 7 and 8 show the three-dimensional isotachopherograms at 45° angle of cosmetic lotion and apple juice (30%) directly injected into the described system. Benzoic acid and ascorbic acid in each sample could clearly be identified by using the UV absorption spectra.

Application to micro spectrophotometric identification of samples

According to the Kohlrausch equation, in isotachophoresis, the sample injected can be concentrated or diluted to an extent related mainly to the concentration of leading ion used. The proposed system was applied to a very dilute sample in order to obtain the complete UV–visible spectrum by means of isotachophoretic concentration. Fig. 9 shows the three-dimensional isotachopherogram of 10 μl of 10 μM iron(II)–*o*-phenanthroline complex which could be separated from excess of *o*-phenanthroline. In this case, 10 μl of sample were concentrated to about 0.2 μl by isotachophoresis. As shown in Fig. 9, four complete absorption spectra were obtained with a sample ammount of only 5.6 ng (as iron).

CONCLUSIONS

The use of a fused-silica capillary tube with an inside diameter smaller than the *ca.* 0.5 mm commonly used improves the resolution without an appreciable electroosmotic flow. A multichannel photodiode array detector can be employed. A cross flow cell of volume only 0.01 μl is sufficient to detect migration zones, since the sample is generally concentrated by migration in isotachophoresis, while it is diluted by diffusion in HPLC or zone electrophoresis.

The system can be applied to micro spectrophotometric identification of samples; a full UV–visible absorption spectrum of iron(II)–*o*-phenanthroline can be obtained with a sample amount of *ca.* 1 ng (as iron).

ACKNOWLEDGEMENT

The authors thank Dr. H. Sasagawa of Union Giken for his technical assistance.

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