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Isotachophoresis in open-tubular fused-silica capillaries with on-column multi-wavelength detection

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ABSTRACT

The use of a fast-scanning multi-wavelength detector for isotachophoresis in open-tubular fused-silica capillaries is described. It is demonstrated that it allows sophisticated characterization of isotachophoretic sample zones, identification of compounds in isotachophoretic zones, investigation of zone purity and confirmation of separation in isotachophoresis.

INTRODUCTION

Photometric detection has been employed for many years in isotachophoresis (ITP) with capillary tubes of 200–500 μm I.D. The combination of this selective detection technique with conductance or electric field measurements has been demonstrated to be extremely helpful for the evaluation of complex isotachopherograms [1]. Two sensors placed in series at the end of the separation column were employed and implemented in commercial capillary-type instruments. Although the application of dual-wavelength detection [2] and photodiode-array sensing [3] has been discussed, fixed-wavelength detectors have been primarily used. This is a major limitation, as only one or two wavelength resolution elements are registered at a time, which is insufficient for the identification of the components of an ITP zone. The gathering of complete absorption spectra in times of the order of milliseconds, *i.e.*, the simultaneous detection of a large number of spectral resolution elements, adds an additional dimension of information. The data in digital form can be readily stored, manipulated and presented in any desired format, making it possible to obtain three-dimensional pherograms within the time needed for a single run.

In a recent paper [4], it was shown that anionic and cationic ITP analyses can be performed in untreated and coated open-tubular fused-silica capillaries with very small inside diameters. In this paper the use of a fast-scanning detector for solute monitoring and confirmation of the ITP steady state via comparison of absorption

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spectra is discussed in conjunction with ITP analyses in an untreated, open-tubular fused-silica capillary, *i.e.*, in the presence of an electroosmotic flow.

EXPERIMENTAL

Chemicals

All chemicals were of analytical-reagent or research grade.

Electrolyte system

For all the experiments presented here, a cationic model system was employed, consisting of 0.01 *M* potassium acetate and acetic acid (pH_L = 4.75) as the leading electrolyte (catholyte) and 0.01 *M* acetic acid as the terminating electrolyte (anolyte). No additives were used.

Instrumentation and running conditions

The instrument used featured a 75 μm I.D. fused-silica capillary of about 90 cm length (Product TSP/075/375; Polymicro Technologies, Phoenix, AZ, U.S.A.) together with a Model UVIS 206 PHD fast-scanning multi-wavelength detector [5] with an on-column capillary detector cell No. 9550-0155 (both from Linear Instruments, Reno, NV, U.S.A.) towards the capillary end. The effective separation distance was 70 cm. Two 50-ml plastic bottles served as electrode vessels and a Model VacTorr 150 vacuum pump (CGA/Precision Scientific, Chicago, IL, U.S.A.) was used to rinse the capillary with cleaning solution (0.1 *M* sodium hydroxide) and leading electrolyte. Current was applied at a constant voltage (20 kV) with a Model HCN 14-20000 power supply (FUG Elektronik, Rosenheim, Germany). The cathode was on the detector side. Samples were applied manually via gravity by lifting the anodic capillary end, dipped into the sample vial, by *ca.* 34 cm for a specified time interval. Multi-wavelength data were read, evaluated and stored employing a Mandax AT 286 computer system and running the Model 206 detector software package version 2.0 (Linear Instruments) with windows 286 version 2.1 (Microsoft, Redmont, WA, U.S.A.). Conditioning for each experiment was effected by rinsing the capillary with 0.1 *M* sodium hydroxide solution for 3 min and with leading electrolyte for 5 min. Throughout this work the Model 206 detector was employed in the high-speed polychrome mode by scanning from 195 to 320 nm at 5-nm intervals (26 wavelengths). With these settings the sampling rate for each wavelength was 3.69 data points/s.

For comparison, experiments were also performed on a Tachophor 2127 analyser (LKB, Bromma, Sweden). This instrument was equipped with a 28-cm × 0.5 mm I.D. PTFE capillary and a conductivity and a UV detector (277-nm filter) at the column end. The measurements were performed at a constant current of 150 μA. Samples were injected with a 10-μl syringe (Hamilton, Bonaduz, Switzerland). The data were registered with a Model PM8252A two-channel strip chart recorder (Philips, Eindhoven, The Netherlands).

RESULTS AND DISCUSSION

As a first example, the cationic ITP analysis of ephedrine, procaine and cycloserine was studied. Data obtained on the Tachophor analyser (Fig. 1) illustrate that the

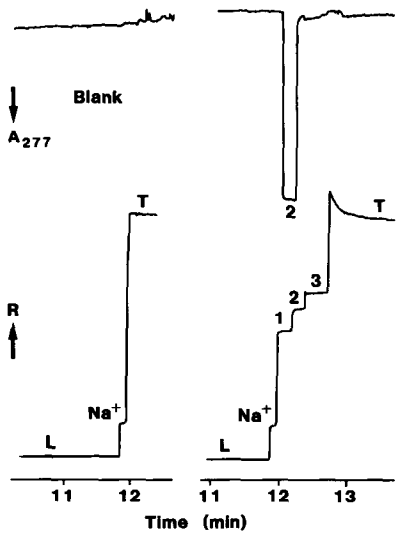

Fig. 1. Blank and cationic ITP analysis of (1) ephedrine, (2) procaine and (3) cycloserine using the Tachophor analyser. A 1-μl volume of an aqueous sample solution containing 10 mM of each compound was injected. Tracings of the conductivity expressed as increase in resistance R (lower graph) and the absorbance measurement at 277 nm (upper graph) are shown. L and T refer to leading and terminating electrolyte, respectively.

three compounds are well separated under the given conditions. With a PTFE column ephedrine (1) migrated ahead of procaine (2), as reported previously by Fanali *et al.* [6] using a similar electrolyte system, and cycloserine (3) was the slowest. Together with a sodium impurity, the three compounds were readily detected via the stepwise response of the conductivity probe. With UV absorbance detection at 277 nm, however, only procaine could be monitored. With these modes of detection and performing a single experiment only, identification of zones and confirmation of complete separation are not achieved [7]. Time-consuming procedures of running single substances and/or various sample loads of the mixture may help in the identification problem and in the recognition of complete separation [8]. The former problem can, however, be solved only for a known sample composition and if all sample components are available as standard substances.

The use of a multi-wavelength detector permits the gathering of three-dimensional isotachopherograms and identification of the sample zones. Fig. 2 depicts such data for the analysis in Fig. 1 performed with a 75 μm I.D. fused-silica capillary in the presence of electroosmosis. The three sample components migrated in the same order as in the Tachophor analyses. All three panels were generated from the data of a single run, illustrating the versatility of data presentation. The projection plot of absorbance *vs.* time relationships between 195 and 320 nm permits the selection of a suitable wavelength for the detection of all three compounds (bottom panel in Fig. 2), the extraction of a complete absorption spectrum for each zone as a so-called time slice (Fig. 3), and thus unambiguous characterization of all three zones. With such data, a computer library of reference spectra can be established, thus allowing qual-

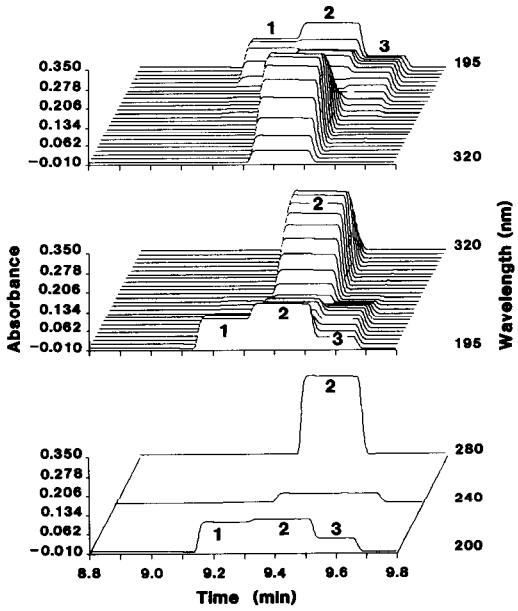

Fig. 2. Three-dimensional isotachopherogram of the cationic analysis of (1) ephedrine, (2) procaine and (3) cycloserine using the fused-silica capillary instrument. The sample solution was the same as in Fig. 1 and the sample application time was 15 s. The initial and final (at detection time) currents were 11 and 3 μA, respectively.

itative studies, such as the identification of substances in steady-state zones, the investigation of the purity of ITP zones and the attainment of the ITP steady state, *i.e.* whether or not the ITP separation process is terminated at the time of detection.

An analysis of the same three compounds with a 3.7-fold higher sample load is shown in Fig. 4. A four-zone structure is monitored, which is clearly seen in the three-dimensional data plot in panel A. The normalized absorption spectra of zones 1, 2 and 3 from Fig. 3 are in complete agreement with those obtained from the data in Fig. 4 (panel B). This proves the identity of the composition of these zones in the two experiments. The normalized time slice of the zone denoted by M was found to be

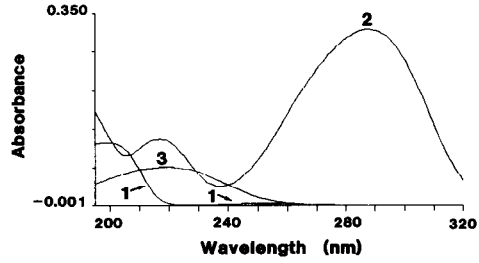

Fig. 3. Absorption spectra of (1) ephedrine, (2) procaine and (3) cycloserine obtained as time slices from the data in Fig. 2.

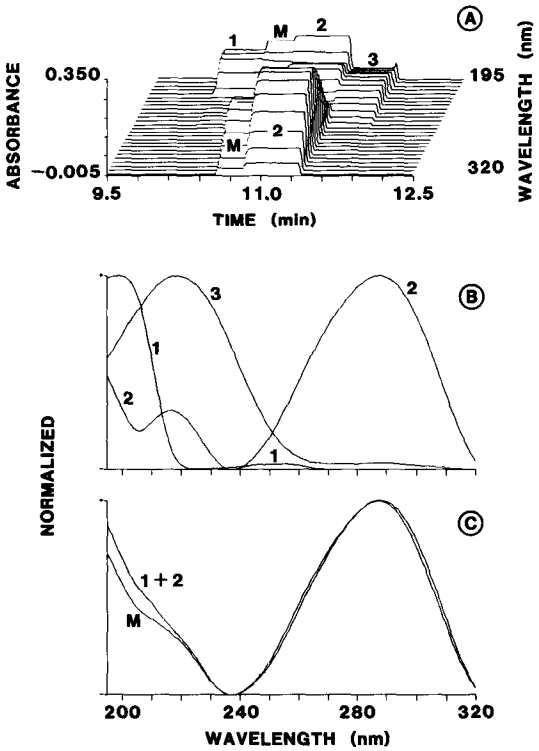

Fig. 4. Three-dimensional isotachopherogram of the same analysis as shown in Fig. 2 but with a sample application time of 55 s. For further details, see text. Note that the normalized spectra in panel B represent an overlay of the data of two different experiments (Figs. 2 and 4).

almost identical with the sum of those of zones 1 and 2 (panel C). In this way we can easily and unambiguously identify zone M as a mixed zone composed of ephedrine and procaine which was not completely resolved before reaching the location of detection. Experiments with 50- and 60-s injection times showed similar patterns having a shorter and longer mixed zone, respectively, than monitored in Fig. 4, whereas with an 80-s injection a five-zone structure containing two different mixed zones was obtained (data not shown). With this sample, the load capacity of the column was reached with an injection time of about 45 s.

Another example illustrating the superiority of multi-wavelength detection over monitoring at one wavelength only is shown in Fig. 5, depicting the purity control of a synthetic peptide, L-histidyl-L-phenylalanine (L-His-L-Phe). Again, all the graphs can be generated from the data from one experiment. The single-wavelength data at 255 nm (panel A) are comparable to those obtained on the Tachophor analyser [9]. Zones 1 and 3 represent impurities and zone 2 the dipeptide. The spectra of the three zones are very different, as is shown in the three-dimensional data plot in panel B and the time slices at (1) 12.174, (2) 12.818 and (3) 13.745 min in panel C. It is interesting that with monitoring at 200 nm (inset in panel A) the impurities are not readily recognized. This is important, as purity control of peptides is typically performed

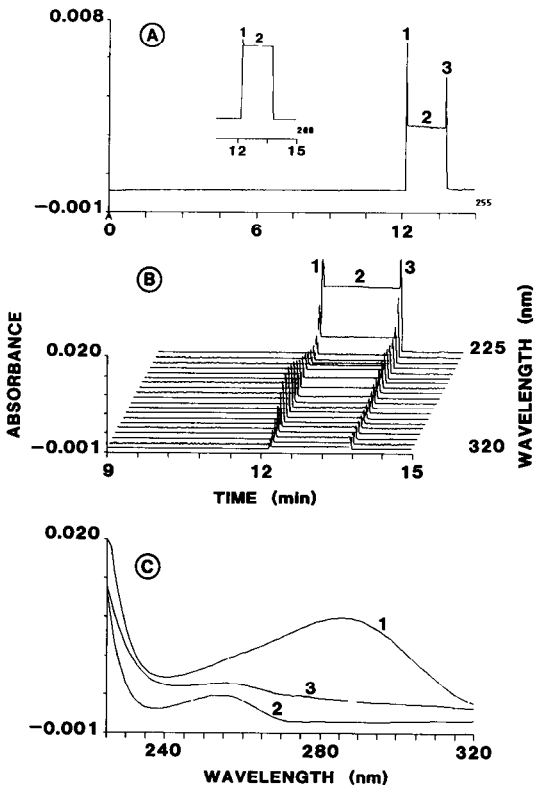

Fig. 5. Cationic analysis of L-His-L-Phe (zone 2) in the fused-silica capillary apparatus with multi-wavelength detection. A *ca.* 100 m*M* sample solution in the leading electrolyte was introduced during 5 s. The current dropped from 10 to 3 μA. Single-wavelength data at 255 and 200 nm (insct with 50-fold larger absorbance scale) are depicted in panel A, a three-dimensional data plot between 225 and 320 nm in panel B and the absorbance spectra of the three zones in panel C. Zones 1 and 3 represent impurities.

around 200 nm [9]. Data reduction on the basis of the ratio of absorption at any two measured wavelengths (as was described for dual-wavelength detection [2]) is not shown here but is easily performed with the Model 206 software.

In conclusion, the use of multi-wavelength detection over a relatively wide range of wavelengths allows a sophisticated characterization of ITP sample zones, the identification of compounds in ITP zones if complete separation is attained, the investigation of zone purity and the confirmation of separation in ITP. Having more than one such detector along the capillary permits the attainment of the ITP steady state to be followed as described previously for sensors measuring a universal sample property [7].Thus, capillary instruments featuring multi-wavelength detectors do not require a second sensor, such as a conductivity or electric field probe, for proper zone assignement in most ITP work. The requirement for optical absorption represents the only limitation to employing this technology as a general and versatile detection principle in ITP.

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REFERENCES

1 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis–Theory, Instrumentation and Applications,* Journal of Chromatography Library, Vol. 6, Elsevier, Amsterdam 1976.
2 J. C. Reijenga, Th. P. E. M. Verheggen and F. M. Everaerts, *J. Chromatogr.*, 267 (1983) 75.
3 M. Goto, K. Irino and D. Ishii, *J. Chromatogr.*, 346 (1985) 167.
4 W. Thormann, *J. Chromatogr.*, 516 (1990) 211.
5 K. Weinberger, *Am. Lab.*, December (1989), 12.
6 S. Fanali, F. Foret and P. Boček, *J. Chromatogr.*, 330 (1985) 436.
7 W. Thormann, *J. Chromatogr.*, 334 (1985) 83, and references cited therein.
8 P. Boček, M. Deml, P. Gebauer and V. Dolník, *Analytical Isotachophoresis*, VCH, Weinheim, 1988.
9 M. A. Firestone, J. Michaud, R. H. Carter and W. Thormann, *J. Chromatogr.*, 407 (1987) 363.