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Isotachophoresis in open-tubular fused-silica capillaries

Impact of electroosmosis on zone formation and displacement

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ABSTRACT

Isotachophoresis (ITP) in untreated and coated open-tubular fused-silica capillaries of 25–50 μm I.D. was examined in order to elucidate the impact of electroosmosis on zone formation and displacement. Electroosmotic flow does not hinder the formation of cationic and anionic ITP zone structures. Having untreated capillaries and a single, on-column detector towards the cathodic column end (as in different commercial instruments) permits the execution of cationic and anionic ITP analyses with the same polarity, *i.e.*, with an electroosmotic flow towards the cathode. For cationic experiments, the leader is acting as catholyte and electrophoretic and electroosmotic displacement occur in the cathodic direction. For anionic analyses, the terminator is used as catholyte and ITP develops towards the anode. The net displacement within the capillary, however, is in the cathodic direction as long as electroosmosis exceeds electrophoresis, a situation which was true for all the systems tested.

INTRODUCTION

In isotachophoresis (ITP), sample zones stack between a leading and a displacing electrolyte, display a steady-state shape and all migrate at the same velocity which is given by the properties of the leading buffer and the current density applied. Theoretical treatments predict that (i) a small amount of sample focuses non-isoelectrically as Gaussian-like peak within the migrating boundary of the discontinuous buffer system, (ii) when a system-dependent sample size is reached the sample zone forms a plateau which lengthens linearly with additional sample, (iii) the velocity of the leading boundary is independent of sample composition and (iv) in the absence of buffer flow, the position of the leading boundary depends only upon the amount of charge wich has passed[1–4]. For the past 20 years, most ITP analyses were performed in narrow-bore plastic tubes of 200–500 μm I.D. or separation channels of rectangular cross-section (about 0.3 × 1.0 mm) and with minimized electroosmosis. In

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these systems a buffer flow required for co-flow, counter-flow and continuous sampling was generated with electrolyte pumps[1,2].

Modern capillary-type electrophoretic instrumentation features coated or uncoated open-tubular fused-silica capillaries of 25–75 μm I.D. together with a single, on-column detector placed toward one end of the capillary[5,6]. With these types of apparatus the ITP configurations presented in Fig. 1 are considered. The negative surface charge of untreated fused silica causes an electroosmotic flow toward the cathode. The structure of the electroosmotic velocity field is strongly dependent on how the surface potential and the electric field vary along the column walls. In ITP, the electric field, composition of zones, pH, etc., are changing across the boundaries. Thus, electroosmosis is expected to cause a loss of separation quality. Little work has been done on the use of this new generation of capillary electrophoretic instruments for ITP[7,8]. In this paper the formation and displacement of ITP zones in open-tubular fused-silica capillaries is described. The impact of electroosmosis and its consequences for cationic and anionic ITP analyses are elucidated. The ultimate goal is the use of automated electrophoretic capillary analysers for ITP determinations of drugs in body fluids.

EXPERIMENTAL

Capillary ITP in coated fused-silica capillaries was performed with an HPE 100 apparatus (Bio-Rad Labs., Richmond, CA, U.S.A.). This instrument features capillaries of 25 μm I.D. and 20 cm length whose inside walls are coated for minimization of electroosmosis and sample adsorption. Sample injection occurs by electromigration and detection via on-line monitoring of UV absorbance close to the capillary end. No special cooling capability is provided. For ITP, the capillary and the outlet reservoir were first filled with the leader. The sample (about 50–200 μl) was then introduced into the inlet compartment prior to application of a constant voltage for 2–32 s. Subsequently, the inlet reservoir was rinsed with the terminator and the running voltage of 6–8 kV was applied. The currents were of the order of 0.2–2 μA. Isotachopherograms were monitored with a strip-chart recorder.

Capillary ITP in untreated fused-silica capillaries was performed with a Model 270A (Applied Biosystems, Foster City, CA, U.S.A.). This automated instrument features untreated fused-silica capillaries of 50 μm I.D. and about 60–100 cm length, optical absorbance zone detection toward the capillary end and a thermostated capillary compartment. Samples are placed in a autosampler from which aliquots can be vacuum-aspirated or loaded electrokinetically (at a constant 5 kV) into the capillary. The autosampler mechanism operates by alternating from wash, buffer and sample positions in a carousel at the injection end of the capillary. Prior to all experiments the capillary was washed for 3 min with 0.1 *M* sodium hydroxide solution. For cationic ITP (configuration iii in Fig. 1) leader from buffer vial B1 was aspirated into the capillary for 3 min, the sample was inserted for a specified period of time (1–30 s), and the terminating electrolyte, placed in buffer vial B2, was connected to the capillary end. For anionic ITP (configuration iv in Fig. 1) the terminator solution (vial B1) was aspirated into the separation capillary whereas the leader was in buffer vial B2. In both instances a constant voltage of 20–30 kV was applied, which produced currents of the order of a few μA. Electropherograms were monitored with a strip-chart recorder.

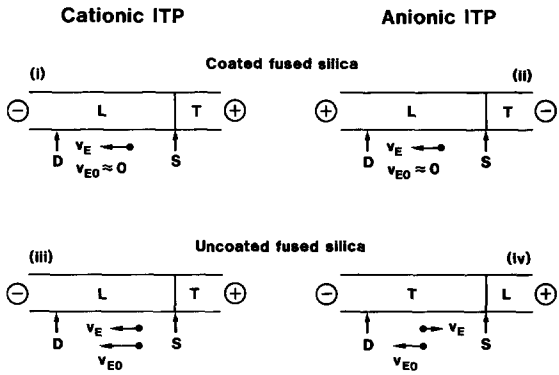

Fig. 1. Schematic representation of configurations for ITP in open-tubular fused-silica capillaries. L = Leading electrolyte; T = terminating electrolyte; S = sample inlet; D = detector position; V_{EO} = electroosmotic velocity; V_E = electrophoretic velocity.

RESULTS AND DISCUSSION

Fig. 2 depicts cationic ITP data for cycloserine (cser) between 10 m*M* sodium formate (the leader) and formic acid. This represents a model system which has been investigated previously using computer simulation and experimental validation in other instruments[9]. Fig. 2A presents data obtained with the HPE 100, the configuration with minimized or zero electroosmosis. A small amount of cser (2-s injection time) produces a peak-like zone within the sodium formate–formic acid boundary. With increasing amounts of sample, zones with a distinct plateau concentration and increasing length are established. Similar results were obtained using the ABI 270A. A typical response is shown in Fig. 2B, illustrating that electroosmosis in open tubes does not disturb the formation of this cationic ITP zone. This has also shown to be true for other cationic examples by Udseth *et al.*[8].

Under normal operation of the HPE 100 (8 kV, 2 μA for this example) and the ABI 270A (30 kV, 6 μA), distorted cser plateaus are monitored. This is best seen with long sample zones (Fig. 2C). At the high current densities applied (3000–4000 A/m^2) electrohydrodynamic forces similar to those observed in isoelectric focusing[9] and zone electrophoresis[10] could account for this behaviour. Decreasing the current density by about an order of magnitude results in an undistorted sample shape (Fig. 2D), which compares well with theoretical prediction and experimental data obtained in an instrument with a PTFE tube of 500 μm I.D.[9].

In Fig. 3 anionic ITP data for a three-component sample are shown using 10 m*M* HCl, adjusted with histidine to pH 6, as leader and 2-(N-morpholino)ethanesulphonic acid (MES) as terminator. The sample was composed of two dyes, amaranth red (A) and bromophenol bue (B), together with acetate as spacer (S). With coated capillaries (configuration ii in Fig. 1) the electropherograms shown in the upper panel are obtained. Having injection times >5 s plateau zones were detected whose step lengths increased with additional sample. Components are detected in order of migration; A migrates ahead of S and B, as is customary in conventional capillary ITP[11]. Performing the same experiment using untreated capillaries was found to be impos-

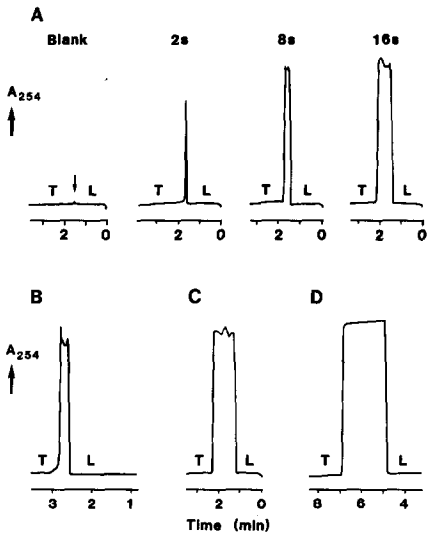

Fig. 2. Cationic capillary ITP data of cycloserine between 10 m*M* sodium formate, the catholyte (leader, L), and formic acid, the anolyte (terminator, T). A 12-m*M* sample solution was employed. The electropherograms presented were obtained in a coated capillary (HPE 100; A, C, D) and in an untreated capillary of 50 μm I.D. (ABI 270A; B). With the former instrument sample introduction occurred at 8 kV for (A) 0, 2, 8, 16 s, (C) 32 s and (D) 8 s. The running conditions were 8 kV (A and C, D during the first min only, current about 2 μA) and 0.8 kV (D, after 1 min of current flow, current about 0.2 μA). It is important to realize that the increased zone length in D originates from the small current during detection. For the experiment with electroosmosis (B), vacuum loading of the sample occurred for 30 s prior to application of a constant 30 kV (initial current, 10 μA; current during sample detection, 6 μA). The capillary length was 70 cm and the temperature was 35°C. In both instruments sample detection occurred at 254 nm.

sible because the electroosmotic flow exceeds the electrophoretic displacement. Changing the polarity and exchanging the buffers, however, allowed the anionic analysis of this sample in the presence of electroosmosis (configuration iv in Fig. 1). The corresponding electropherograms are shown in the lower panel of Fig. 3. Note the reverse order of zone detection compared with the upper panel. In this configuration electrophoretic separation occurs in the anodic direction in the presence of a strong electroosmotic flow toward the cathode which does not appear to disturb the anionic ITP process. The net displacement within the capillary is in the cathodic direction because electroosmosis exceeds electrophoretic displacement. This is similar to the zone electrophoretic analysis of anions reported by Jorgenson and Lukacs[5].

Fig. 4 displays data for an anionic ITP determination of S-carboxymethyl-L-cysteine (SCMC) in human urine using an untreated fused-silica capillary. The leader was 10 m*M* HCl, adjusted with histidine to pH 6, and the terminator was 30 m*M* MES adjusted with histidine to pH 6. This represents the same configuration as employed for the data shown in the lower panel of Fig. 3 and supplements the ITP analyses of this drug reported elsewhere[12]. Again the sequence of zone detection is reversed compared with analyses in electroosmosis-free configurations. These data provide information on the magnitude of electroosmosis via detection of the "stationary" boundary which was formed electrophoretically at the location of the initial discontinuity (marked with an asterisk in A and B).

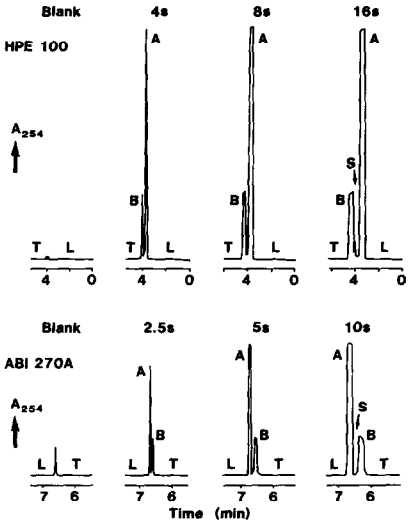

Fig. 3. Anionic capillary ITP of amaranth red (A, 1 m*M*), acetate (spacer, S, 2 m*M*) and bromphenol blue (B, 1 m*M*) between 10 m*M* HCl adjusted with histidine to pH 6 (leader) and MES–histidine at pH 6 (terminator). The electropherograms presented were obtained in a coated capillary of 20 cm length (HPE 100; upper panel; configuration ii in Fig. 1) and in an untreated capillary of 60 cm length (ABI 270A; lower panel; configuration iv in Fig. 1). In the first approach sample application occurred by electromigration at a constant 8 kV for 0 (blank), 4, 8 and 16 s. The running voltage was 8 kV and the current decreased from 2 to about 0.7 μA. In the second instrument sample insertion occurred by vacuum aspiration at the time intervals indicated. The running voltage was 30 kV, the temperature 35°C and the current decreased from 6 to 4 μA within the first 2 min of current flow and was again about 6 μA during detection of sample zones. Detection was performed at 254 nm in both instruments. Note that the sequence of zone detection is different in the two approaches.

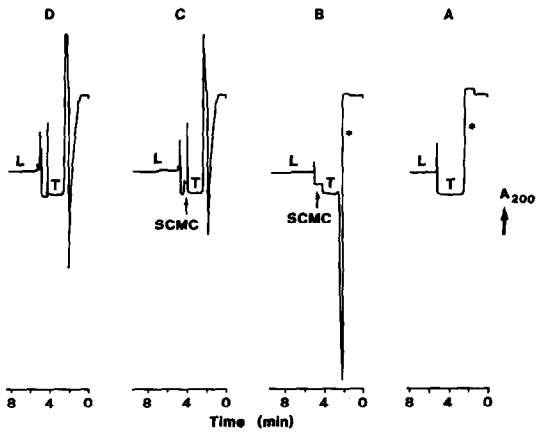

Fig. 4. Anionic ITP of S-carboxymethyl-L-cysteine (SCMC) in human urine in an untreated capillary of 60 cm length and 50 μm I.D. (ABI 270A). The buffer configuration described for Fig. 3 was used. Volume injection occurred for 15 s by vacuum aspiration. The running voltage was 30 kV, the temperature was 35°C and the detector settings were at 200 nm and 0.5 A.U. The current decreased initially from about 6 to 4 μA before increasing again to 6 μA. Electropherograms for (A) the blank, (B) a 10-m*M* SCMC sample solution, (C) urine spiked with 20 m*M* SCMC and (D) control urine are shown. The urine samples were pretreated by filtration using Sep-Pak C_{18} cartridges (Waters Assoc., Milford, MA, U.S.A.) as described elsewhere[12]. The asterisks indicate the detection of the boundary formed at the location of the initial buffer discontinuity.

As reported earlier, there are electrolyte systems which are suitable for ITP analyses in both migrational directions (dual ITP)[13]. In principle, cationic and anionic sample trains could be monitored having a single detector placed toward the cathodic end of an untreated fused-silica capillary. Fig. 5 depicts an electropherogram using the operational configuration iii in Fig. 1 with 10 m*M* sodium formate as catholyte (cationic leader) and 2.5 m*M* HCl as anolyte (anionic leader). Cser and amaranth red were employed as sample components. The cationic ITP zone of cser was monitored within a short time but the anionic zone migrated too slowly to reach the detector within 10 min (and even 60 min in other runs) of current flow. In every instance, however, the amaranth red zone (A in Fig. 5) was moved across the point of detection during the subsequent wash of the capillary. This means that the electroosmotic flow at the low pH was stronger than the electrophoretic displacement. An additional hydrodynamic flow towards the cathode could be applied and used for the detection of this anionic zone pattern under current flow.

Important for quantification by zone length measurements with a stationary, single sensor is that the current does not change appreciably during detection. Therefore, ITP data obtained at constant current are simpler to evaluate than those monitored at constant voltage. Further, data generated with electrokinetic sample application at constant voltage (as provided with both instruments used in this work) are difficult to evaluate quantitatively. In all instances the current was observed to decrease considerably during that process, and therefore doubling of the injection time did not exactly double the detected sample zone length. However, with volume injection, as is provided in the ABI 270A, a linear relationship between the time of sample application and monitored zone length was obtained.

The data presented here show that open-tubular fused-silica capillaries of 25–50 μm I.D. are well suited for rapid high-resolution ITP analyses of low-molecular-mass

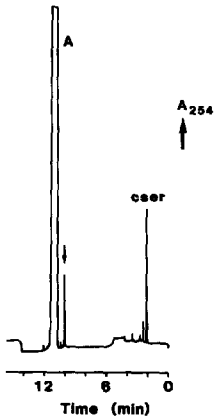

Fig. 5. "Dual" ITP with 10 m*M* sodium formate as catholyte and 2.5 m*M* HCl as anolyte. Electrophoresis took place in an untreated capillary of 70 cm length (ABI 270A). The sample was composed of cser and amaranth red, about 6 m*M* each. Sample application occurred for 6 s using vacuum aspiration, the temperature was 35°C and the monitoring was performed at 254 nm. A constant 30 kV was applied for 10 min. The current dropped from 9 to 2 μA within 7 min of power application. The cser zone (cationic ITP) passes the detector after about 2 min of current application. Amaranth red (A) passes through the detector cell during the wash cycle. The arrow marks the end of power application.

substances. Zone transitions are extremely sharp at the high current densities applied (3000–4000 A/m^2). Some distortions of zone plateaux are observed at these high current densities. Electroosmosis in open-tubular, untreated, fused-silica capillaries does not hinder the formation of ITP zones. Anionic ITP analyses can be performed with a net zone displacement toward the cathode. This also represents a configuration which is potentially suitable for the evaluation of cationic and anionic ITP sample trains in a single experiment (dual ITP).

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