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ENHANCING ISOTACHOPHORESIS SENSITIVITY BY LOW-CONCENTRATION ELECTROLYTE CASCADING

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SUMMARY

A method is presented which enhances isotachophoresis sensitivity through concentration cascading on commercial instrumentation. A simple modification of an LKB 2127 Tachophor allows the use of a 10 m*M*/1 m*M* leading electrolyte cascade. By employing pre-migration before the cascade is formed, little increase in analysis time occurs. Demonstration of this system with pH 6 electrolytes shows that nearly a five-fold increase in effective sensitivity for citrate can be obtained.

INTRODUCTION

In our laboratories, isotachophoresis (ITP) complements ion, gas and liquid chromatography (LC) as techniques for quantitative analysis. However, ITP often suffers from high detection limits compared with chromatographic methods. To make ITP more competitive with LC methods, we have explored lowering electrolyte concentrations as a way of enhancing sensitivity of ITP when run on commercial instrumentation.

The most attractive method of increasing ITP sensitivity is the use of narrow-bore (0.2 mm) detectors[1]. Such detectors are not commercially available and are difficult to fabricate, although simplified procedures for their construction have been published[2]. A second method for increasing sensitivity involves lowering the leading electrolyte concentration, which allows detection at reduced currents while maintaining high field strengths needed for sharp zone boundaries. Arlinger[3] demonstrated improved UV detection limits for ATP using 0.5 m*M* leading electrolytes. Quantitative investigations of both UV and conductivity detection limits by Everaerts *et al.*[4] showed that decreasing electrolyte concentration did not yield proportional decreases in detection limits, primarily due to electroosmosis and solvent ion migration. However, the ability to decrease the detection limit of the technique to near 1-ppm levels for many ions remains useful.

A major disadvantage to using a single low concentration leading electrolyte in ITP analysis is decreased separation capacity. Boček *et al.*[5] proposed forming a concentration cascade in the leading electrolyte for increasing separation capacity without decreasing sensitivity. Similarly, Everaerts *et al.*[6] discussed the use of a coupled capillary system with different leading electrolyte concentrations to improve

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sensitivity and capacity. In this paper we present a simple system, based on an LKB 2127 Tachophor, for improving ITP sensitivity by concentration cascading. Proper formation of the cascade allows runs to be made using 1 m*M* leading electrolytes with little decrease in separation capacity or increase in analysis time versus normal 10 m*M* electrolyte analyses.

EXPERIMENTAL

Isotachophoresis was performed using an LKB 2127 Tachophor modified for concentration cascading as shown in Fig. 1. A three-way valve (No. 13CCC3, Hamilton, Reno, NV, U.S.A.) was placed between the LKB 2127-140 detector and the terminating reservoir. Connection between the valve and detector was made with 40 mm × 1.6 mm O.D. × 0.5 mm I.D. Teflon® tubing while connection between the valve and the terminating reservoir was made with 170 mm × 1.6 mm O.D. × 0.8 mm I.D. tubing. Tubing and fittings were obtained from Omnifit (New York, NY, U.S.A.).

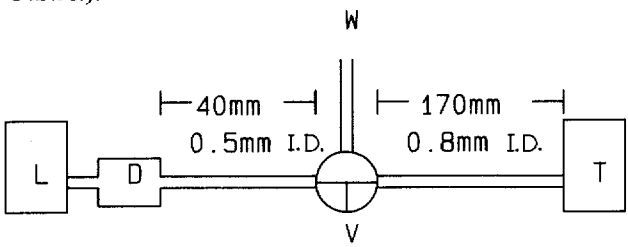

Fig. 1. Schematic of cascade system for LKB 2127 Tachophor. L = Leading reservoir; D = 2127-140 detector; V = three-way Hamilton valve; T = terminating reservoir; W = waste.

Leading electrolytes used were 10 m*M* or 1 m*M* hydrochloric acid adjusted to pH 6 with histidine and containing 0.2% hydroxypropyl methyl cellulose (HPMC). Terminating electrolyte was 10 m*M* 2-(N-morpholino)ethanesulfonic acid adjusted to pH 6 with tris(hydroxymethyl)aminomethane. All chemicals were obtained from Sigma (St. Louis, MO, U.S.A.) except for hydrochloric acid (Ultrex® grade, J. T. Baker, Phillipsburg, NJ, U.S.A.) and HPMC (Aldrich, Milwaukee, WI, U.S.A.) and were used without further purification.

RESULTS AND DISCUSSION

To test the ability of the cascade system to improve sensitivity, ITP was performed on a 1-μl injection of a 100 mg/ml citrate solution using conductivity detection. When tested under normal conditions, the apparatus was filled with the 10 m*M* electrolyte and a 255 μA current was applied for 13 min and then reduced to 55 μA for detection. For runs made with a 10 m*M*/1 m*M* cascade system, the following four-step protocol was adopted. (1) The capillary is completely filled with 10 m*M* electrolyte, the sample is injected and a 255-μA current is applied for 9 min. At this point, the leader/sample boundary is near the valve. (2) The valve is rotated 90° to allow flushing of the detector and valve with 1 m*M* electrolyte. (3) A current of 55 μA is applied for 6 min or until the sample zone nears the detector. (4) The current

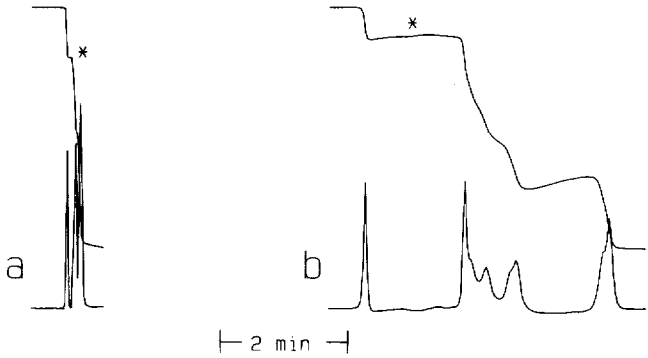

Fig. 2. Isotachopherograms of 100 ng of citrate run in (a) 10 mM leading electrolyte at 55 μA and (b) 1 mM leading electrolyte at 5 μA. * = Citrate zone; upper trace = conductivity; lower trace = differential conductivity.

is reduced to 5 μA for detection. If the cascade is formed prior to injection, the maximum current that can be applied is 75 μA (owing to the resistive 1 mM region) and analysis times become exorbitant.

Fig. 2 shows the results for 100 ng of citrate run in (a) single 10 mM electrolyte at 55 μA and in b) 10 mM/1 mM cascade at 5 μA. Observed zone lengths are 4.6 sec at 10 mM and 46.9 sec at 1 mM leading electrolyte concentration. While the zone length increase is nearly eleven-fold, it is apparent from Fig. 2b that the sensitivity to electrolyte impurities also increases and that zone definition degrades slightly. Since the "effective" sensitivity or detection limit is a combination of zone length and boundary sharpness, we define here a zone definition parameter using the differential conductivity trace

$$D = 2\,\Delta t/(w_1 + w_2) \tag{1}$$

where D is zone definition, Δt is the zone length as measured from differential con-

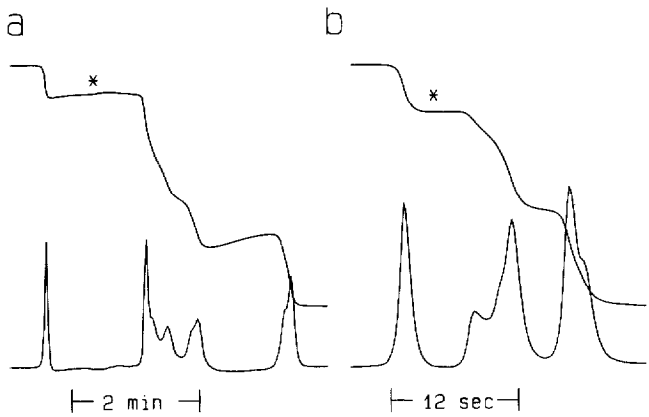

Fig. 3. Isotachopherograms of 100 ng of citrate run in (a) 1 mM leading electrolyte at 5 μA and a chart speed of 0.5 mm/sec and (b) 10 mM leading electrolyte at 55 μA and a chart speed of 5.0 mm/sec. * = Citrate zone; upper trace = conductivity; lower trace = differential conductivity; zone definition of citrate = 12.7 (a) and 2.8 (b).

ductivity peaks, and w_1 and w_2 are widths at half-height of the differential peaks corresponding to the transitions at the beginning and end of the citrate zone.

Fig. 3 shows the 1 mM and 10 mM runs recorded at different chart speeds to normalize the two zone lengths. The differential traces show that the zone definition of the citrate zone is dramatically improved upon going to the cascade system. Measurement of zone definition gives $D = 2.8$ at 10 mM and $D = 12.7$ at 1 mM. Thus, a 4.5 times increase in effective sensitivity is seen when employing the cascade system.

Inability to realize fully the increase in sensitivity offered by lower currents and concentrations arises from background solvent ion electrophoresis and electroosmosis[7]. While use of non-aqueous systems might be indicated to reduce these disturbances, concomitant decreases in sample ionization with low dielectric solvents may minimize real sensitivity gains. Addition of surfactant ions[7] to reduce electroosmosis is more promising.

A second problem with low-concentration electrolytes is increased sensitivity to electrolyte impurities. Attempts at further lowering ITP detection limits will require careful electrolyte purification procedures. Finally, as can be seen from Fig. 3, lowering of the leading electrolyte concentration alters the relative mobilities of sample, leading, terminating and impurity ions. While this fact may be exploited for achieving better ITP separations, it must be realized that the low-concentration compartment in the above cascade system offers very little capacity.

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