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Optimizing dynamic range for the analysis of small ions by capillary zone electrophoresis

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ABSTRACT

Many applications of capillary zone electrophoresis (CZE) are for the determination of UV transparent ions. Indirect UV detection can be used for these analyses, but one problem with indirect detection is a limited dynamic range for analysis. A strategy for optimizing dynamic range for CZE analyses with indirect detection is described. Optimization involves chromaphore-electrolyte selection, concentration and wavelength optimization and tuning the electrolyte mobility through complexation. The strategy is illustrated for the analysis of aliphatic acids. We found that 2,6-naphthalenedicarboxylate is an ideal chromophore–electrolyte for aliphatic acid analysis. Through complexation with β-cyclodextrin its mobility can be tuned to match the mobility of aliphatic acids. By optimizing detection wavelength and electrolyte concentration and mobility as little as 0.025 mg/l of aliphatic acids can be detected and up to 1000 mg/l can be quantitated. Under optimized conditions CZE can be used for trace analyses.

INTRODUCTION

Capillary zone electrophoresis (CZE) has been proposed for the determination of anions such as inorganic anions, aliphatic acids, aromatic acids and anionic surfactants [1–7]. CZE can have advantages over ion chromatography for these determinations. CZE separations have very high resolution, they are typically fast, sample size is small and consumables costs (especially columns) are nearly insignificant. Neutral species are usually well separated from anions so large concentrations of neutrals, for example solvents, generally do not interfere with anion analyses by CZE. This is often not the case in ion chromatography. These advantages have been demonstrated in the cited references.

Many applications of CZE are for the determination of UV transparent ions. Indirect UV detection can be used for these analyses. A problem with indirect detection is a limited dynamic range for analysis [8,9]. The low concentration end of this range is the limit of quantitation, often defined as three times the detection limit. Photometric factors that determine detection limit for indirect detection have been investigated [9,10–15]. These authors conclude that detection limits are lowest when the indirect chromophore has a large molar absorptivity at the detection wavelength and the concentration is adjusted for optimum photometric sensitivity. For the circular cell geometry of on column detection, the background absorbance should not exceed about 0.1 AU [9]. Wang and Hartwick [15] have found, however, that fluctuations in electrolyte concentration could be the limiting factor in detection limit. They concluded there could, in theory, be situations where only the concentration of the electrolyte affected limit of detection and other situations where only the molar absorptivity of the electrolyte affected the

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limit of detection. Poppe [16] calculated that one-to-one displacement of analyte for indirect chromophore only occurs when the mobilities of these ions are equal. Therefore, mobility of the indirect chromophore also has an effect on detection limit and limit of quantitation.

The utility of CZE lies in its ability to effect high-resolution separations. For typical separations, the high end of the dynamic range for analysis is most often limited by loss of resolution due to concentration overload (electromigration dispersion) rather than photometric signal saturation [16]. This limitation can be overlooked in investigations of dynamic range that only consider a single component. The severe dispersion encountered at high analyte concentrations results from a mismatch between analyte and electrolyte mobility [16,17]. Since the driving force for separation by CZE is differences in analyte mobility, there will never be a mobility match for more than one component. Hence, the upper end of the dynamic range for a separation is generally limited by dispersion rather than photometric factors. Dispersion is negligible when there is a large difference between the concentration of electrolyte and analyte, but it broadens the analyte peaks when the analyte concentration becomes a significant fraction of the electrolyte concentration. In the limit, this dispersion destroys peak separation and establishes the effective upper limit for quantitation.

Concentration overload can be minimized, and dynamic range extended, by increasing the electrolyte concentration and optimizing the mobility of the electrolyte. Limit of quantitation can be lowered by increasing the molar absorptivity of the electrolyte, lowering its concentration to keep the background absorbance less than about 0.1 and optimizing the electrolyte mobility for the separation. The conflict of these goals is obvious.

This paper presents a strategy for optimizing dynamic range for CZE analyses with indirect detection. Optimization involves chromophore–electrolyte selection, concentration and wavelength optimization and a new concept, mobility tuning. The strategy is illustrated for the analysis of aliphatic acids. By using this strategy, as little as 0.025 mg/l of aliphatic acids can be detected and up to 1000 mg/l can be quantitated.

EXPERIMENTAL

Materials

Acetic, propionic and butyric acids and 2,6-naphthalenedicarboxylic acid were purchased from Aldrich (Milwaukee, WI, USA). β-Cyclodextrin was purchased from Advanced Separation Technologies (Whippany, NJ, USA). Water was obtained from a Millipore ultrafiltration system (Bedford, MA, USA). Adjustment of electrolytes pH was performed using sodium hydroxide purchased from Mallinckrodt (Paris, KY, USA).

Instrumentation

Analyses were performed on an ABI (Foster City, CA, USA) 270A-HT capillary electrophoresis system. The separation was performed on a 72 cm × 50 μm I.D. (injector to detector length equals 50 cm) Polymicro Technologies (Phoenix, AZ, USA) fused-silica column with an applied potential of 30 kV positive. Column chamber temperature was 30°C. Detection was by indirect UV at 240 nm or 283 nm. Sample injection was by vacuum for 5 s.

DISCUSSION

A strategy for optimizing the dynamic range of an analysis will be illustrated for the separation of acetic, propionic and butyric acids. Indirect detection is used for the detection of these analytes, but elements of the optimizing strategy can be used for direct detection as well. The strategy involves selection of the indirect chromophore–electrolyte and optimizing its concentration and detection wavelength for the analyte concentration range of interest. Dynamic concentration range for the separation is extended by matching the mobility of the electrolyte to an analyte of interest. Mobility tuning was accomplished through complexation of the electrolyte with cyclodextrin. The concentration of cyclodextrin is adjusted to where the effective mobility of the electrolyte–cyclodextrin complex mat-

ches the analyte of interest. Usually this would be an analyte of intermediate mobility.

Chromophore–electrolyte selection

An electrolyte of optimum mobility will not normally be found. While it might seem easy to find electrolytes of any desirable mobility, we have not found this to be the case. Therefore, an electrolyte is selected that has a mobility faster than the middle of the analyte range. The electrolyte should have a large molar absorptivity in the wavelength range of the detector. It should be stable in basic solutions and it must complex with β-cyclodextrin. The indirect chromophore–electrolyte selected for this separation is 2,6-naphthalenedicarboxylic acid (Fig. 1). At a pH of 9 the dianion of this acid has a mobility slightly greater than acetate and the electroosmotic flow is fast enough to achieve short analysis times.

Case 1: Analytes present at similar concentration greater than about 1 mg/l. In this case the electrolyte should be as concentrated as possible to minimize concentration overload. Column current sets an upper limit on electrolyte concentration; it should not be so high that column heating is a problem. With our system an electrolyte concentration of 7 m*M* is optimum, but this could be different for systems that have different means for column thermostating or different column dimensions and voltages. The next step is to select a wavelength so that the background absorbance is about 0.1 AU, in this case 283 nm. Under these conditions the dynamic range for the analysis is about 1 to 400 mg/l when the analytes are in equal concentrations (see Fig. 2). The sample is diluted so the analytes fall in this range. This range will depend on the mobility of the electrolyte. Ideally the mobility should fall in the middle of the range of analytes, but in this case it is somewhat greater than acetate as shown by the fronting peaks. β-cyclodextrin will complex many aromatic molecules, including 2,6-naphthalenedicarboxalate [18]. Even a small tendency to complex with the relatively large β-cyclodextrin molecule will lower the apparent mobility of the electrolyte. By changing the concentration of β-cyclodextrin the electrolyte mobility can be tuned to match any of the analytes. With no cyclodextrin, the analytes front because the electrolyte mobility is greater than the analyte mobility (Fig. 3). As the cyclodextrin concentration is increased, the peaks become symmetrical and then tail as the effective mobility of the electrolyte becomes equal to and then less than the mobility of the analytes. By tuning the mobility to match propionic acid, the upper end of the dynamic range for the separation can be increased to over 1000

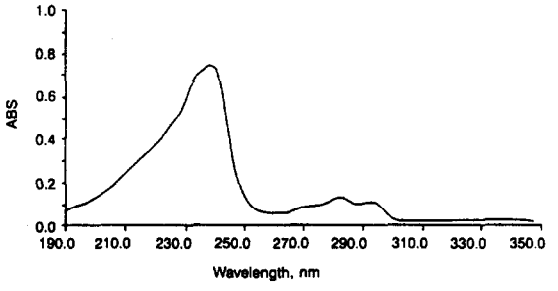

Fig. 1. Spectrum of 2,6-naphthalenedicarboxylate aqueous solution pH 9; $A_{240} = 62\,000$, $A_{283} = 11\,000$.

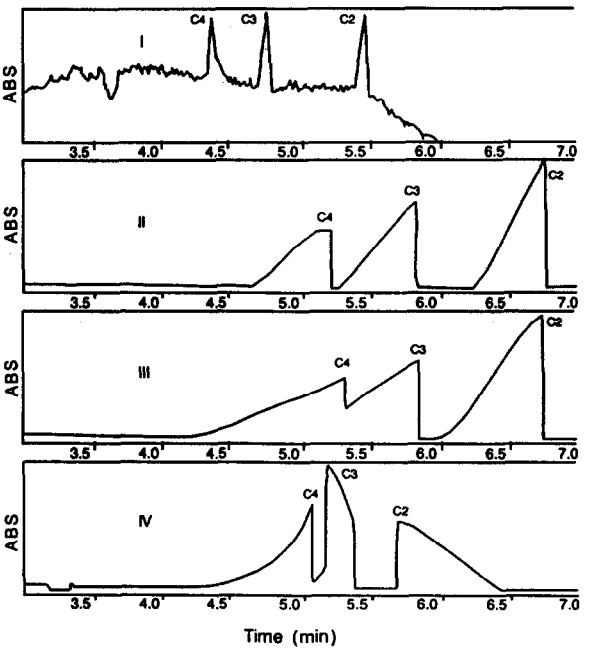

Fig. 2. Electropherograms of butyric (C4), propionic (C3) and acetic (C2) acids. Electrolyte, 7 m*M* 2,6-naphthalenedicarboxylate, pH 9; 283 nm. (I) 1 mg/l each acid; (II) 400 mg/l each acid; (III) 1000 mg/l each acid; (IV) 1000 mg/l each acid with 5 m*M* β-cyclodextrin.

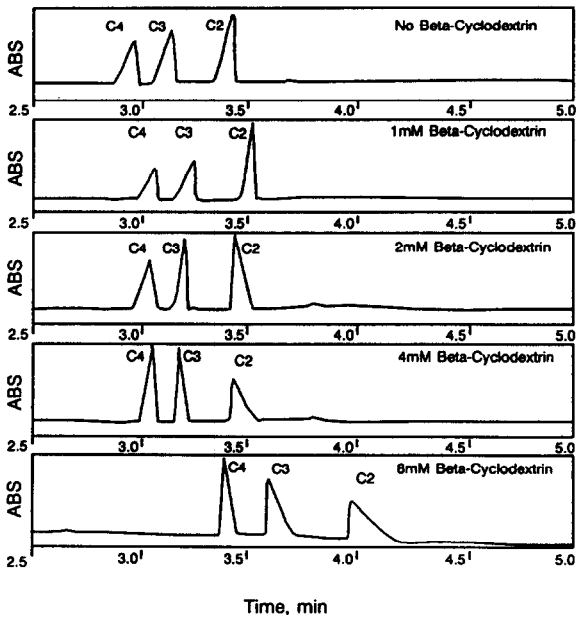

Fig. 3. Electropherograms of 10 mg/l butyric (C4), propionic (C3) and acetic (C2) acids. Electrolyte, 1 m*M* 2,6-naphthalenedicarboxylate, pH 9 with β-cyclodextrin added.

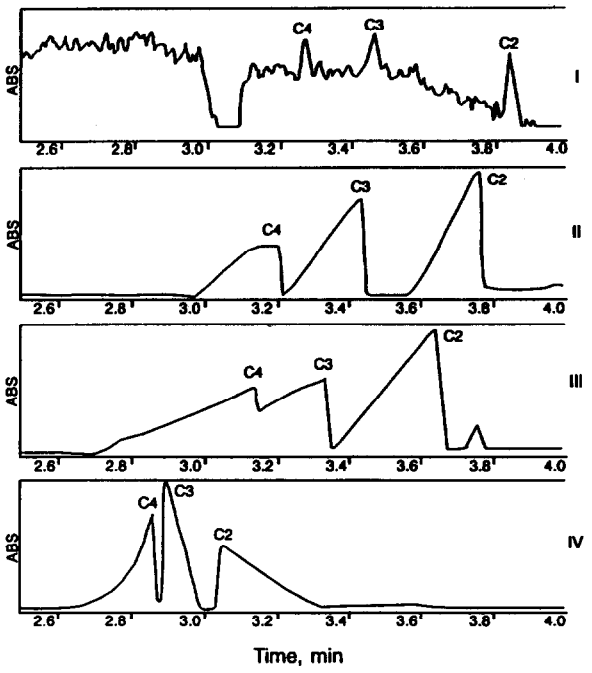

Fig. 4. Electropherograms of butyric (C4), propionic (C3) and acetic (C2) acids. Electrolyte, 1 m*M* 2,6-naphthalenedicarboxylate, pH 9; 240 nm. (I) 0.025 mg/l each acid; (II) 50 mg/l each acid; (III) 100 mg/l each acid; (IV) 100 mg/l each acid with 3 m*M* β-cyclodextrin.

mg/l (Fig. 2). The dynamic range is 1000 under these conditions, an improvement of ten-fold over previous work [8].

It should be noted that the mobility match of the electrolyte for this separation is exceptionally good without tuning with β-cyclodextrin. In cases where the inherent mobility of the electrolyte is more poorly matched the improvement is even greater.

Case 2: Analytes at similar concentration less than 1 mg/l. For this case the detection limit must be optimized at the expense of the upper concentration limit. The detector wavelength is changed to 240 nm where the molar absorptivity of the chromophore–electrolyte is largest and the concentration is lowered to 1 m*M* to keep the background absorbance to about 0.1 AU. Under these conditions the detection limit for these acids is 0.025 mg/l, but the upper limit for analysis is now only 50 mg/l (Fig. 4). Tuning the electrolyte mobility to match propionate also improves the upper separation limit in this case (Fig. 4).

These examples illustrate how the dynamic range for an analysis can be adjusted up and down to accommodate samples with different concentrations. The overall range is impressive, 0.025 to 1000 mg/l. This range is competitive with other means of determining ions and aliphatic acids.

Case 3: Analytes present at widely different concentration (trace component analysis). This is the most difficult case for analysis by CZE. For this illustration it will be assumed that the major component peak will be severely concentration overloaded to detect the trace component. By electrolyte selection, mobility tuning and flow reversal it may be possible to arrange for the trace component to be on the sharp side of the overloaded component. In this case it is best to mobility tune the electrolyte to the trace component for maximum sensitivity [16]. The improvement in sensitivity can be significant. For example, the relative molar responses, normalized for migration time, for acetate, propionate, and butyrate are 1.00:0.77:0.57 with the 2,6-naphthalenedicarboxalate electrolyte. Significant

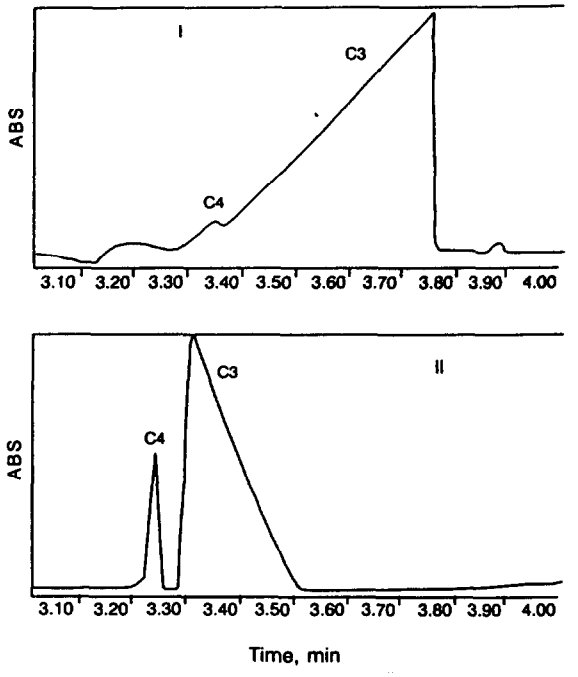

Fig. 5. Electropherograms of 10 mg/l butyric (C4) and 100 mg/l propionic (C3) acid. Electrolyte, 1 m*M* 2,6-naphthalenedicarboxylate, pH 9; 240 nm. (I) No β-cyclodextrin added; (II) with 4.5 m*M* β-cyclodextrin.

sensitivity can be gained when mobilities match. If the trace component falls in the tail or front of the major component peak, tuning the electrolyte to match the mobility of the major component may narrow this peak and expose the trace component (Fig. 5).

CONCLUSION

The optimization strategy described can be extended to other cases and mobility tuning can be applied to some analyses where direct detection is used. Electrolyte mobility tuning with cyclodextrin should be generally applicable to aromatic acid electrolytes. Other electrolytes, for example borates and phosphates, might be tuned by other complexation reactions. While the nature of the complexation reaction will vary depending on the electrolyte, the principle of continuously reducing the mobility of an electrolyte through complexation should be generally applicable. Mobility tuning will result in improved dynamic range and enable CZE to be applied to more analytical problems.

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