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EFFECT OF ELECTROLYTE AND SAMPLE CONCENTRATION ON THE RELATIONSHIP BETWEEN SENSITIVITY AND RESOLUTION IN CAPILLARY ZONE ELECTROPHORESIS USING CONDUCTIVITY DETECTION

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SUMMARY

By maintaining the same ratio of ion analyte concentration to background electrolyte concentration while diluting the latter, it is shown to be possible to increase substantially the detection sensitivity without altering the resolution in capillary zone electrophoretic separations with on-line conductivity detection. Using a mixture of carboxylic acids, the limits of detection are extended to 10^{-6} *M*.

INTRODUCTION

The use of capillary zone electrophoresis (CZE) as a rapid, sensitive analytical tool has grown enormously in the last few years[1–4]. A major challenge has been in the area of detectors. Many have been developed to be used specifically for CZE and among those is the universal conductivity detector[5,6]. Some of the useful applications of the on-column conductivity detector include metal ions[6,7] and low-molecular-mass carboxylic acids[8].

A conductivity detector used with CZE can sense all ionic species having mobilities different from the background electrolyte. Moreover, it has been reported that there is a unique relationship between the retention time of a peak and its area[1,8]. The problem that is usually encountered with conductivity detection is that, if good separation is desired, the concentration of the background electrolyte must be high relative to the concentration of the sample. However, this condition (high electrolyte concentration) results in a loss in sensitivity due to the elevated background conductivity.

This study is an attempt to find the conditions that sacrifice neither resolution nor sensitivity. It is the reduction of the background electrolyte concentration, while preserving the ratio of analyte to background concentration, that permits an increase in sensitivity while maintaining resolution.

EXPERIMENTAL

Instrumentation

The CZE system with an on-column conductivity detector has been fully described elsewhere[6]. We use a polyimide-clad fused-silica capillary, 35 cm × 75 μm

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I.D. (Polymicro Technology, Phoenix, AZ, U.S.A.). A high-voltage power supply (0–30 kV with a reversible polarity output) is used (Hipotronics, Brewster, NY, U.S.A.). The current used ranges from 3 to 11 μA. Sample injection is by gravity. The capillary inlet is lifted 7 cm higher than the capillary outlet for 10 s. We prefer hydrostatic injection to electrokinetic injection for quantitative studies in order to avoid bias problems associated with the latter injection method[9].

Reagents

Reagent-grade carboxylic acids (Aldrich, Milwaukee, WI, U.S.A.) were used as received. The following carboxylic acids were used: formic, acetic, propionic, butanoic, pentanoic, hexanoic and heptanoic. All solutions were prepared from distilled, deionized water (Model LD-2A coupled with a Mega-Pure automatic distiller; Corning Glassworks, Corning, NY, U.S.A.). Stock solutions of carboxylic acids were prepared at concentrations of 1 m*M*, 0.75 m*M*, 0.50 m*M* and 0.25 m*M*. Electrolyte solutions containing 2-N-(morpholino)ethanesulfonic acid (MES) and histidine (His) were purchased from Sigma (St. Louis, MO, U.S.A.) and used without further purification. These solutions were made up at concentrations of 20 m*M*, 15 m*M*, 10 m*M* and 5 m*M* with respect to both MES and His. The electrolyte solutions were filtered through a 0.2-μm membrane (Acrodisc; Gelman Sciences, Ann Arbor, MI, U.S.A.) and in all cases 0.25 m*M* of the surfactant, tetradecyltrimethylammoniumbromide (TTAB, Sigma) and 0.05% (v/v) hydroxyethane cellulose (HEC) (Fluka, Ronkonkoma, NY, U.S.A.) were added. The pH of the electrolyte is approximately 6. Under these conditions, the relative difference in the retention times of butanoate and pentanoate at all electrolyte concentration levels was always less than 8%.

RESULTS AND DISCUSSION

Fig. 1 is a sample electropherogram demonstrating the separation of all seven of the carboxylic acids used. Baseline resolution is easily achieved in this less than 4 min operation. The early time negative peak in this figure is reproducible but has not been identified.

Starting with a 20 m*M* concentration of background buffer (MES/His), and a concentration of 1 m*M* of sample, a run was made which provided a measure of both resolution and sensitivity. Two of the carboxylic acids, butanoic and pentanoic, were used for this purpose. The resolution, R_s, was calculated using the following equation for peaks with similar widths[10]:

$$R_s = \frac{t_2 - t_1}{0.5(w_2 + w_1)}$$

where t_1 and t_2 are the retention times of the two peaks and w_1 and w_2 are their respective peak widths. At these concentrations, the peak height of butanoic acid was normalized to equal one so that the peak height at other concentrations could be made relative.

When the background electrolyte concentration was reduced to 15 m*M*, the sample concentration was also reduced to 0.75 m*M*, maintaining the same concen-

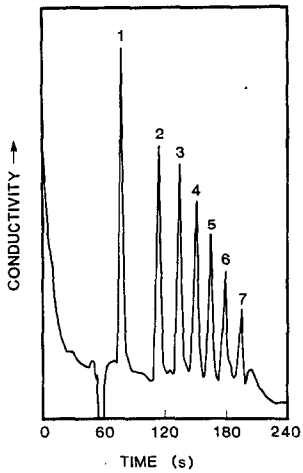

Fig. 1. Sample electropherogram of low-molecular-mass carboxylic acids. Peaks: 1 = formic; 2 = acetic; 3 = propionic; 4 = butanoic; 5 = pentanoic; 6 = hexanoic; 7 = heptanoic acid. Capillary 35 cm × 75 μm I.D.; gravity injection from 7 cm for 10 s; applied voltage 25 kV. The negative going peak at 60 s is an unidentified system peak that is reproducible.

tration ratio of sample ion to carrier ion. Under these conditions, *the resolution remained unchanged, while the sensitivity increased.* Subsequent runs reducing the background electrolyte concentration to 10 m*M* and then 5 m*M* together with concomitant reductions in sample concentration to 0.5 m*M* and 0.25 m*M*, respectively, continued to provide the same resolution and, at the same time, significantly enhanced the sensitivity.

Fig. 2 graphically displays the relationship between resolution and sensitivity at different concentrations of background electrolyte and sample while maintaining the

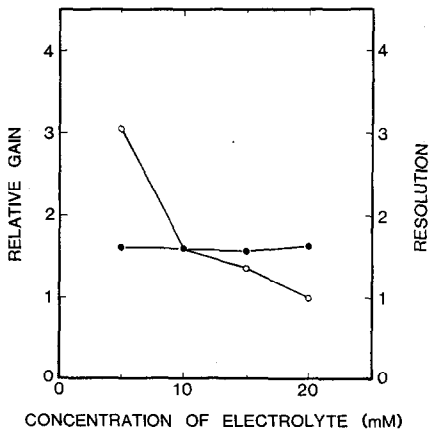

Fig. 2. Plot showing the effect of concentration of background electrolyte and sample on the relationship of resolution and sensitivity. Filled data points are resolution; open data points are relative gain. The ratio of sample concentration to background electrolyte concentration is 0.05.

same concentration ratio. Each data point in the plot represents the mean of four electrophoretic runs.

Since the ratio of sample ion to carrier ion is kept constant, the increase in sensitivity is caused primarily by a decrease in the background electrolyte conductivity. This suggests that we can simply decrease the concentration of background electrolyte to increase the sensitivity and simultaneously decrease the concentration of the sample. A fourfold decrease in the electrolyte concentration (20 mM to 5 mM), while keeping the sample concentration constant, caused an increase in absolute sensitivity of more than 12 times. It is possible to find the concentrations of sample and background electrolyte that optimize both resolution and sensitivity. If the ratio of sample concentration to electrolyte concentration is increased, the sensitivity increases while the resolution decreases.

In another series of experiments, the starting concentration of sample is decreased by a factor of 10 to 0.1 mM while the starting concentration of background electrolyte is 25 mM. In this case, the concentration ratio is decreased to 0.0004. The resolution increased, but as the concentration levels are decreased in both electrolyte and sample, the resolution remained unchanged as long as the concentration ratio is kept constant. The change in relative gain is similar to that described for the first series of experiments.

This sensitivity-enhancement procedure can permit significant improvements in quantitation but cannot be extended without limit. A practical limit arises from instrumentation and contamination.

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