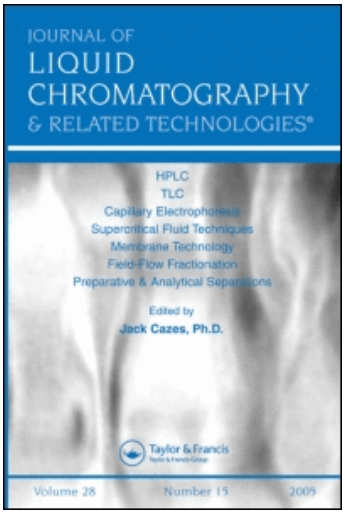

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Capillary Zone Electrophoresis of Organic Acids and Anions

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To cite this Article Kelly, L. and Nelson, R. J.(1993) 'Capillary Zone Electrophoresis of Organic Acids and Anions', Journal of Liquid Chromatography & Related Technologies, 16: 9, 2103 — 2112
To link to this Article: DOI: 10.1080/10826079308019918
URL: http://dx.doi.org/10.1080/10826079308019918

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(9&10), 2103-2112 (1993)

CAPILLARY ZONE ELECTROPHORESIS OF ORGANIC ACIDS AND ANIONS

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ABSTRACT

Two buffer systems are described for the separation of anions by capillary electrophoresis. In each system, the ions are monitored by indirect detection. Organic acids are separated with potassium hydrogen phthalate, 2-(N-morpholino)-ethanesulfonic acid and tetradecyltrimethyl ammonium bromide. Other anions are separated in 1,2,4,5 benzene tetracarboxylic acid (pyromellitic acid) and diethylenetriamine.

INTRODUCTION

Capillary Zone Electrophoresis is a powerful method to separate small analytes (1-4). Most inorganic ions do not absorb UV light and pass the detector area without a change in signal. The use of light-

absorbing ions as buffer components allows indirect detection of ions that do not themselves contain a chromophore (5,6). This report describes indirect anion detection with two buffer systems.

For indirect detection, it is the absorbance of a buffer electrolyte that is monitored by the detector, not the absorptivity that the sample might display. Because the solution in the capillary is constrained to remain electrically neutral, sample ions locally displace electrolyte on a charge-for-charge basis as the sample band migrates through the capillary. As the buffer electrolyte is diluted by the sample ions, more photons pass through the detector region. The increase in light throughput is recorded as a decrease in absorbance. The magnitude of the negative peak is dependent upon the concentration of the displacing ion, the ratio of the negative charges on the electrolyte to the sample ion, and finally, the concentration and extinction coefficient of the electrolyte.

A number of applications for separation of low molecular weight anions with various chromophore-containing electrolytes have been summarized (7). To lower detection limits below 1 ppm and to resolve some co-migrating inorganic ions, method parameters are still being improved. Electrokinetic injection can increase sample loading and sensitivity compared to hydrodynamic introduction although a bias does exist (8). Because mis-matched ionic mobilities of the carrier electrolytes and the sample ions produce peak fronting or tailing, choice of buffer components must be carefully considered (5,9). It has been previously shown that in

similar systems, pH as well as the choice and concentration of electroosmotic flow modifiers had significant influence upon selectivity (10). The influence of yet another factor, the choice of running temperature upon the selectivity of separation, is further investigated.

The use of phthalate for the separation and detection of organic acids, and of 1,2,4,5 benzene tetracarboxylic acid for anions is shown. The critical role of temperature in selectivity of the anion separation is also discussed.

MATERIALS

Capillary electrophoresis was conducted with the SpectraPHORESIS™ 1000 (Spectra-Physics Analytical, Fremont, CA). Capillaries were untreated fused silica, 70 cm x 75 μm or 44 cm x 50 μm.

All chemicals were obtained at the highest purity level available from the manufacturer, and were used without additional purification. n-Butyric acid (sodium salt), caprylic acid, D,L-malic acid, 2-(N-morpholino)-ethanesulfonic acid (MES), oxalic acid, potassium hydrogen phthalate, propionic acid (sodium salt), succinic acid, L-tartaric acid, and tetradecyltrimethyl ammonium bromide (TTAB) were obtained from Sigma Chemical Co., St. Louis MO. 1,2,4,5 benzenetetracarboxylic acid (pyromellitic acid or PMA), and

diethylenetriamine (DETA), were obtained from Aldrich Chemical Co., Milwaukee, WI. HPLC grade water from Baker or 18 MΩ Milli-Q water was used.

PREPARATION OF BUFFERS

For organic acid separations, a 10x buffer concentrate was made from 50 mM potassium acid phthalate, 5 mM TTAB, and 500 mM MES adjusted to pH 5.2 with NaOH. When diluted 1:10, the running buffer was 5 mM potassium acid phthalate, 0.5 mM TTAB, and 50 mM MES. Some samples were run without the addition of the MES. Sample injection was electrokinetic for 1 sec at -10 kV. Capillary electrophoresis was at 20° C and -30 kV, and the current was less than 20 μA using a 70 cm x 75 μm capillary. Detection was at 205 nm.

For anion separations, the running buffer was 3 mM pyromellitic acid, 0.02% DETA, 1% methanol at pH 9.6. To avoid solubility problems, the PMA was dissolved in 1 mL of methanol, added to water containing 7mM NaOH, and combined with DETA. The pH was then adjusted further with NaOH as required. Sample injection was electrokinetic for 5 sec at -10 kV. Capillary electrophoresis was at 60° C and -25 kV, and the current was less than 40 μA using a 44 cm x 50 μm capillary. Separate capillaries were maintained for each of the buffer formulations.

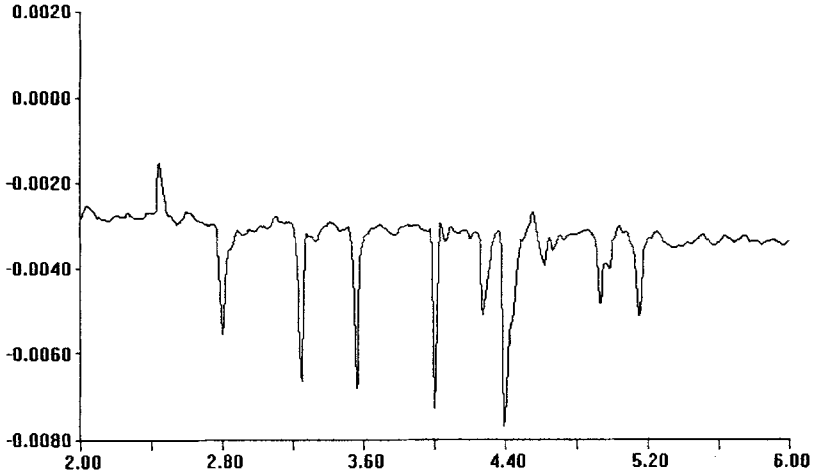

Figure 1 10 ppm Each of the organic ions oxalate, tartrate, malate, succinate, lactate, followed by the water peak at 4.6 min, then propionate. Capillary electrophoresis was at 20° C and -30 kV in phthalate-TTAB buffer.

RESULTS AND DISCUSSION

Separation of a series of organic acids is shown in Figure 1. TTAB has been added to the phthalate buffer to reverse the electroosmotic flow, so the polarity has been reversed for this series of separations. The inclusion of the Good's buffer MES into the phthalate mixture provided stabilization against pH changes as well as an improved baseline and better peak shape compared to the same mixture without MES (data not shown). A field-amplified concentration (11) of the sample bands shown in Figure 1 results from the electrokinetic injection of sample diluted in water into the

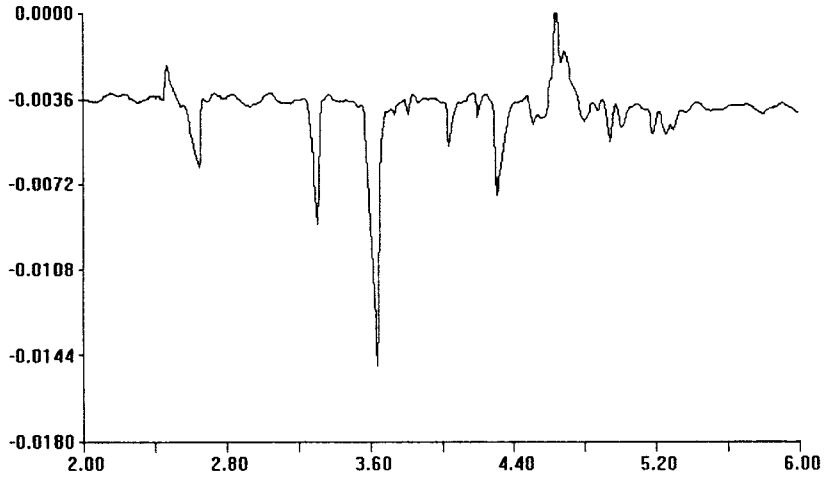

Figure 2 Tartrate and malate are the major organic acids in white wine. Conditions for the phthalate-TTAB buffer are given in **Figure 1**.

capillary containing relatively concentrated electrolyte. The presence of organic acids can be detected in beverages such as white wine and brewed coffee, as shown in Figures 2 and 3.

Smaller anions show better peak shapes when the mobility of absorbing buffer ion more closely matches that of the displacing sample ions. PMA, with two more carboxylate groups than phthalate, has a higher mobility due to its higher charge density. Mobility of the PMA is enhanced at pH 9.6, where the acidic carboxylates are predominately ionized. In the PMA system, DETA is used to slow rather than reverse the electroosmotic flow. Of the anions shown in Figure 4, peak shape is broader for the larger anions, e.g., bromate.

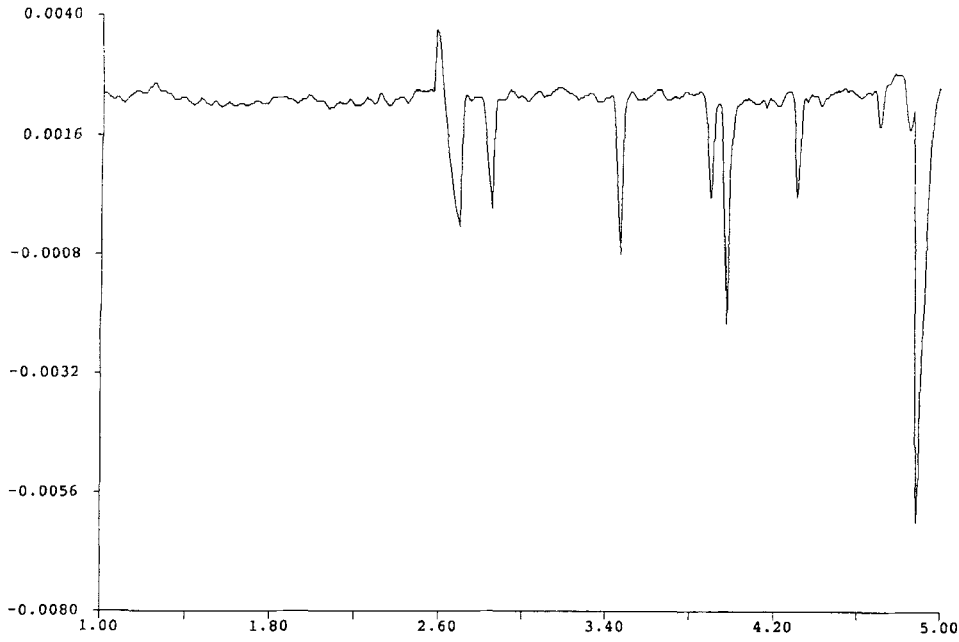

Figure 3 Organic acids in coffee, diluted 1:10 in water. Conditions for the phthalate-TTAB-MES buffer are given in **Figure 1**.

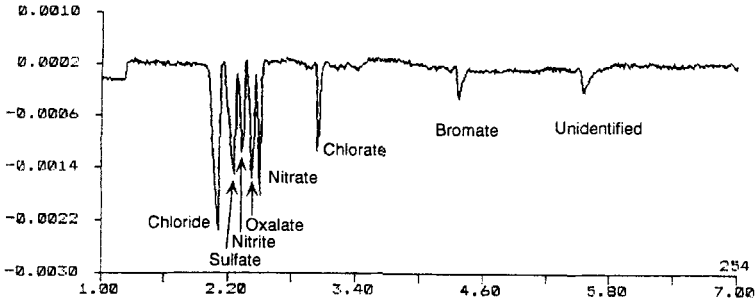

Figure 4 5 ppm Chloride, sulfate, nitrite, oxalate, nitrate, chlorate, and bromate have been separated using the PMA buffer. The broad peak at 5.6 min is unidentified. Following electrokinetic injection for 5 sec at -10 kV, capillary electrophoresis was at 60° C and -25 kV..

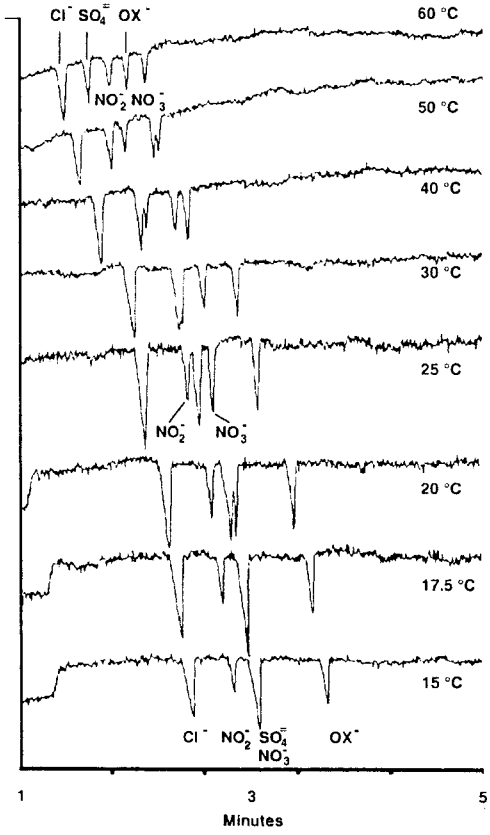

Figure 5 The effects of temperature variation upon migration times and selectivity of anions in the PMA buffer system are shown in the 15° - 60°C range. Chloride, sulfate and oxalate migrate relatively faster than the nitrite-nitrate pair as the temperature is increased. Baseline resolution of the 5 ions is only observed at 60°C. Conditions are as for **Figure 4** at the indicated temperature, except that a 1 ppm solution is electrokinetically injected for 1 sec.

Figure 5 shows the effects of temperature upon the migration times of chloride, nitrite, sulfate, nitrate and oxalate. As expected, increasing the temperature causes solution viscosity to decrease, so migration times are also decreased. However, the selectivity of the system is also observed to change with temperature. Nitrite and nitrate migrate with the same relationship to each other, while chloride, sulfate and oxalate also maintain their same relative distances. Because the nitrogen-containing ions migrate relatively slower than the other three as the temperature is increased, the elution order is a marked function of temperature. The optimal temperature for this separation is 60°C. The buffer mixture was adjusted to pH 9.6 at 23°C, and was used unadjusted for the temperature studies since temperature does not appreciably affect the ionization of carboxylates.

CONCLUSIONS

Small anions are readily separated by CE. Indirect detection is used to detect these ions, most of which do not absorb ultraviolet light. Choice of the indirect chromophore is dictated by the mobility of the ions to be analyzed, since the best resolution occurs when the mobility of the anionic buffer is close to that of the sample ions. The speed of the analysis and in some cases the selectivity are controlled by the temperature.

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