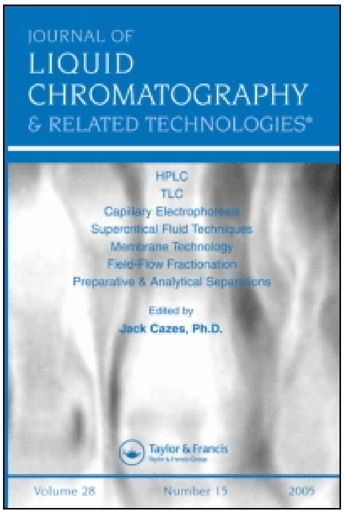

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FACTORS THAT INFLUENCE MOBILITY, RESOLUTION, AND SELECTIVITY IN CAPILLARY ZONE ELECTROPHORESIS. III. THE ROLE OF THE BUFFERS' ANION

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ABSTRACT

A study of the anion of the buffer and its effect on electroosmotic flow, mobility times, resolution and selectivity was undertaken. The anions selected acetate, borate, phosphate, citrate, carbonate, nitrate and nitrite, gave widely different currents at the same applied voltage (20 kV). Carbonate produced the lowest current, while citrate produced the highest current. Also, it was found that the anion used influences not only electroosmotic flow and mobility times but the resolution and selectivity of eight dansylated amino acids. It is hereby recommended that more attention be paid to the selection of a buffer.

INTRODUCTION

Mobility times (t_M) of charged solutes in capillary zone electrophoresis (CZE) are a function of the column chemistry, mobile phase composition, pH, ionic strength, temperature, additives.....etc. (1-3). Little attention has been paid to the effects of buffer composition. Green and Jorgenson (4) studied a series of four potassium salts (KCl, KNO_3, KBr and K_2SO_4) to see

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what effect the anion would have on the capacity factor (k′) of proteins. They concluded that when these salts were used as buffer additives at high concentrations, they yielded equivalent k′ values, suggesting that under their experimental conditions the anion does not have a measurable effect on k′. Van Orman, et al (5) determined "that buffer composition is of a little importance, while ionic strength is a primary factor in the electroosmotic flow." In two recent investigations of the role of the buffer and its effect on t_M, μ_{eo}, resolution and selectivity, it was found that the nature of the buffer and its concentration influences both μ_{eo} and t_M (6,7). For example, the t_M obtained for a test mixture of dansylated amino acids differed greatly depending on whether a monohydrogen or dihydrogen phosphate buffer having the same molarity and pH was used (6). Also, it was found that the buffer's cation had an effect on μ_{eo} and t_M. For example, μ_{eo} of the markers benzene, guanosine and mesityloxide in a lithium acetate solution differed by about 37% from that in cesium acetate solution having the same concentration and pH (7).

The objective of the present research was to investigate the influence of the buffer anion on μ_{eo}, t_M, resolution and selectivity employing the most commonly used buffers in electrophoresis. Since the higher the applied voltage the greater the resolution in CZE, one of our objectives was to identify the buffer which would give the lowest current at the same applied voltage under constant experimental conditions.

EXPERIMENTAL

Materials:

The dansyl amino acids used in this study were purchased from Sigma, and used without further purification. The sodium salts and acids were purchased from Johnson Matthey Alpha Products, Danvers, MA. Mesityl oxide was purchased from Aldrich. The salt solutions (0.05 M) were prepared by precisely weighing on an analytical balance the appropriate salt and dissolving it in distilled deionized water in a volumetric flask. The required pH was achieved by titrating with the corresponding acid.

Apparatus and Methods:

The analytical balance (Model XA200DS) and the pH meter (Model Accumet 750) were purchased from Fisher. A Beckman CZE system (Model P/ACE) equipped with a UV detector, an automatic injector, a column cartridge (50 cm x 75 μm i.d., surrounded by coolant), an autosampler and a printer was used. All experiments were carried out at 25°C using the constant voltage mode (20 kV). Injections were carried out by the pressure mode for 3 seconds. Solutes were monitored at 254 nm.

RESULTS AND DISCUSSION

The objectives of this study were (a) to see if the buffers' anion has any effect on μ_{eo}, selectivity, t_M and resolution; and (b) to find out which salt (buffer) would give the lowest current at an applied voltage. The latter point is very important for two reasons: (1) the higher the applied voltage, the higher the resolution; and (2) the heat generated inside the fused silica capillary is directly proportional to the square of the current:

$$W = IV = I^2R$$

where W is the power, V is the voltage, I is the current and R is the resistance.

The seven sodium salts selected for this study along with their pH (0.05 M solution) and resulting current, at an applied voltage of 20 kV, are listed in table 1. The unadjusted pH (column 2, table 1) of the salt solutions is mostly in the basic range and can be adjusted to pH 8 with the corresponding acid. Both sodium nitrate and nitrite were not adjusted to pH 8 because a base would have to be used which will alter the concentration of the sodium ion in solution and would lead to erroneous results (7). Table 1 shows that at 20 kV applied voltage the resulting current varies widely depending on the anion used. Sodium citrate produced a current which is 3.6 times larger than that produced by sodium hydrogen carbonate having the same concentration. This means that the power produced by using sodium citrate is approximately

Table 1: pH and current measurements at 20 kV of the sodium salts solutions (0.05 M) used in this study

Salt Compound	Measured pH	Measured Current (μAmps)	Measured Current for the solutions adjusted to pH=8	Measured Current for the solutions adjusted to pH=6
Sodium Acetate	8.0	74.5	74.0	72.5
Sodium Tetraborate	9.3	137.4	118.0	102.6
Sodium Citrate	8.9	246.5	243.3	244.0
Sodium Phosphate Dibasic	8.8	162.0	160.7	139.2
Sodium Hydrogen Carbonate	8.7	69.0	77.3	-
Sodium Nitrate	6.4	121.0	-	-
Sodium Nitrite	6.9	107.0	-	-

Table 2. Measured μ_{eo} in the five sodium buffers (0.05 M, pH 8)

Buffer	Phosphate	Acetate	Citrate	Bicarbonate	Tetraborate
$\mu_{eo}10^5 cm^2/Vs$	49.7	49.0	47.7	51.8	41.2

3.6 times larger than that produced by sodium hydrogen carbonate or sodium acetate. This also means that the heat generated by the citrate buffer is much larger than that generated by the other two. Sodium phosphate buffer, one of the most popular buffers for the separation of biological compounds, produces a current which is twice as high as that of sodium acetate. When the pH of these sodium salt solutions was adjusted to 8, there was little or no change in the current produced (compare columns 3 and 4, table 1) except in the case of sodium tetraborate because the pH was adjusted from pH 9.25 to 8. This tells us that slight adjustments in pH (less than one pH unit) may not affect drastically the current produced. When the pH was adjusted to 6, changes in current were observed for sodium tetraborate and sodium phosphate

Table 3. Mobility times of eight dansylated amino acids
sodium phosphate buffers having the same conce
pH (8.0), at an applied voltage of 20 kV

Amino Acid	Phosphate	Acetate	Citrate	Bic
Dans-Arginine	4.25	4.13	4.48	
Dans-Lysine	4.32	Coeluted	5.36	
Dans-Tyrosine	5.60	5.39	5.93	
Dans-Methionine	6.19	5.88	6.68	
Dans-Alanine	6.42	6.05	6.94	
Dans-Cystine	7.66	7.10	8.69	
Dans-Glutamic Acid	10.11	8.82	11.81	
Dans-Cysteic Acid	10.80	9.40	13.31	

Table 4. Separation factor (α) values for eight
amino acids in five sodium buffers (0.0
at an applied voltage of 20 kV

	Phosphate	Acetate	Citrate	Bicar
Lys-Arg.	1.02	-	1.20	1.
Tyr-Lys	1.30	1.31	1.11	1.
Met-Tyr	1.11	1.09	1.13	1.
Ala-Met	1.04	1.03	1.04	1.
Cys-Ala	1.19	1.17	1.25	1.
Glu-Cys	1.32	1.24	1.36	1.
Cyst-Glu	1.07	1.08	1.13	1.

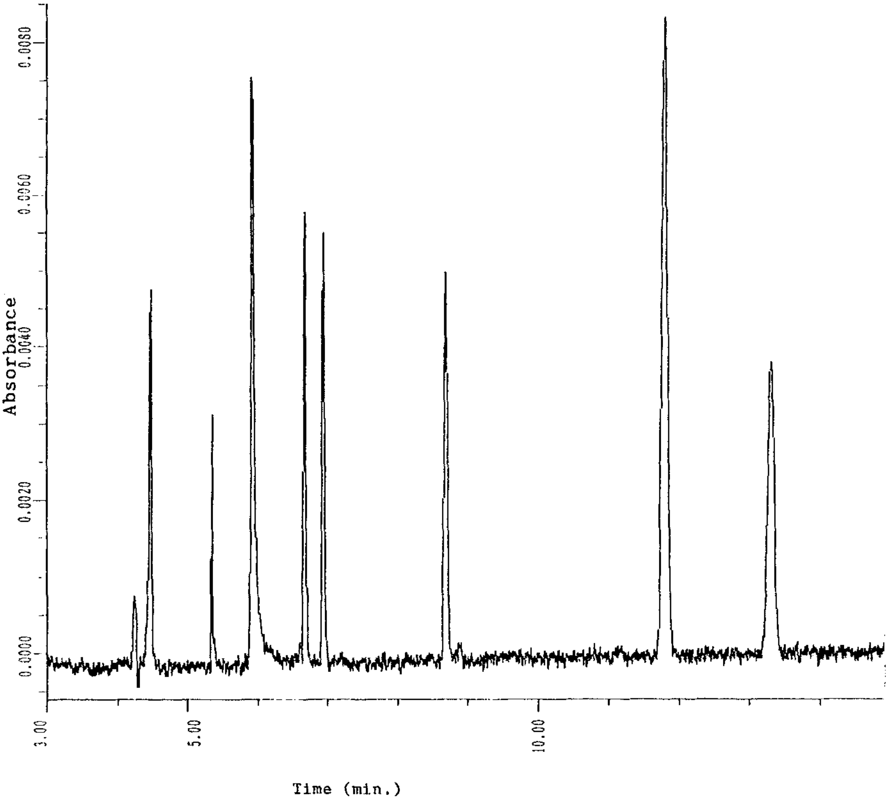

Figure 1: Electropherogram of eight dansyl amino acids. Capillary: 50 cm long, 75 μm i.d.; 0.05 M sodium citrate, pH = 8; applied voltage 20 kV; temperature constant at 25°C, detection at 254 nm. Peak assignment from left to right: Dans-Arginine, Dans-Lysine, Dans-Tyrosine, Dans-Methionine, Dans-Alanine, Dans-Cystine, Dans-Glutamic Acid and Dans-Cysteic Acid.

but not for sodium acetate or sodium citrate. The above results show that the current produced is affected by the anion used.

__Effect of buffer anion on electroosmotic flow__:

Mesityloxide was used to measure μ_{eo} in the five selected sodium buffers. The results are given in table 2.

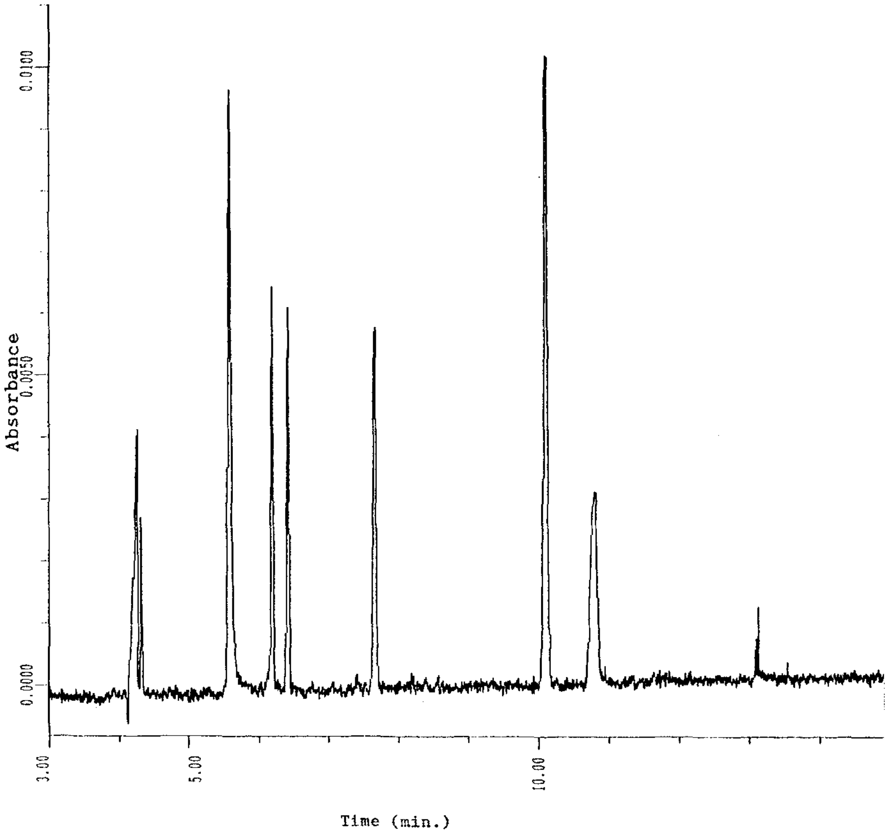

Figure 2: Same as in Figure 1 except that 0.05 M sodium phosphate solution at pH = 8 was used. The elution order of the amino acids is the same as in Figure 1.

The results show that less than a 10% change in μ_{eo} was observed for the phosphate, acetate, citrate and bicarbonate but μ_{eo} in tetraborate differed by over 20% from the other four buffers having the same concentration and pH.

<u>Effect of buffer anion on mobility, resolution and selectivity</u>:

Eight dansylated amino acids were selected to study the effect of buffer anion on t_M, R_s and selectivity. The eight amino acids and t_M's obtained in

0.05 M, pH 8.0, sodium phosphate, acetate, citrate, bicarbonate and tetraborate are listed in table 3. Examination of this table shows that differences in t_M are observed from buffer to buffer. For example, dans-cysteic acid, which eluted last in each buffer, has a difference of over 50% in t_M.

The lowest t_M was obtained in sodium bicarbonate (largest μ_{eo}), while the longest was in sodium tetraborate (lowest μ_{eo}). Also, dansylated arginine and lysine coeluted in sodium acetate but were well resolved in sodium citrate. Table 4 lists the separation factors (α) for the eight amino acids in the five sodium buffers. The table shows that the optimum separation for each pair was not realized in one specific buffer but in more than one buffer. Overall sodium citrate gave the best α values, while sodium bicarbonate gave the worst α values. Also, some pairs were resolved better in one buffer (arginine-lysine, alanine-cystine), and others were resolved equally well in all of the buffers (methionine-alanine). This indicates that differences in selectivities exist which means that the buffer anion affects selectivity under the present experimental conditions. Figures 1 and 2 are electropherograms of the dansylated amino acids in citrate and phosphate buffers.

Comparison of mobility and selectivity at pH 8 and 6:

Analysis of the eight dansylated amino acid test mixture in four of the buffers (acetate, biocarbonate, citrate and phosphate) at pH 6 revealed that the order of elution did not change from that at pH 8 (no peak crossover). However, there was a change in t_M due to the acidic buffer. For example, in the sodium citrate buffer (0.05 M; pH 6) six of the amino acids eluted at longer times than at pH 8, while dansylated glutamic and cysteic acids did not elute after 99.9 min.

CONCLUSION

The results of this study clearly show that the buffers' anion influences not only electroosmotic flow and mobility times but also resolution, selectivity, and the power produced at an applied voltage.

Therefore, attention should be paid to the selection of the buffer, in order to produce optimum results and generate minimum heat.

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