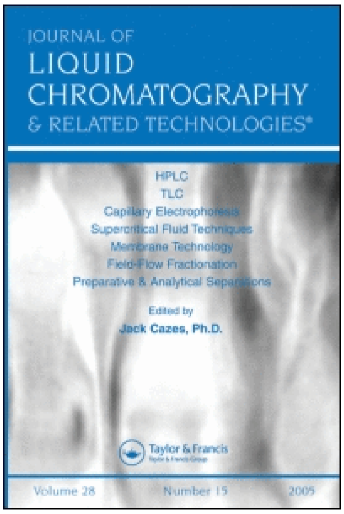

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FACTORS THAT INFLUENCE MOBILITY, RESOLUTION AND SELECTIVITY IN CAPILLARY ZONE ELECTROPHORESIS. II. THE ROLE OF THE BUFFERS' CATION

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ABSTRACT

The effect of the buffer cation on mobility, resolution and selectivity in capillary zone electrophoresis was investigated. The results show that mobility times of analyte increased with increasing cation size $Cs^+>Rb^+>K^+>Na^+>Li^+$. The increase in mobility times was verified by measuring the electroosmotic flows of benzene, mesityl oxide and guanosine in the five alkali buffers. The results show that electroosmotic flows of the three above markers decreased as the cation size increased. The separation of a test mixture of nine dansylated amino acids was better when 0.1 M cesium acetate solution was used rather than lithium acetate. Also, there was no change in the order of elution of the solutes in the test mixture in the five acetate solutions having the same pH and concentration. It was observed that at the same applied voltage (20 kV) the resulting current increased as the atomic weight of the cation increased, which is expected based on charge/size distribution.

INTRODUCTION

Capillary Zone Electrophoresis (CZE) is quickly emerging as an up and coming microanalytical technique for the separation of molecules according to their ability to migrate in an electric field gradient (1,2). Many factors affect the mobility, resolution and selectivity of the analytes. These

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include applied voltage, pH, type and concentration of the buffer, ionic strength, buffer additives, capillary column treatment and electroosmotic flow. Understanding, predicting and controlling the electroosmotic flow in CZE is of primary importance in calculating solute mobilities, and optimizing of separations. Efficiency is a function of the electroosmotic velocity (3), therefore controlling the flow will be beneficial in maximizing efficiency and resolution, which could lead to superior separations.

In a previous study (4), we reported our findings on the use of sodium vs. potassium phosphates buffers. The results indicated that there were differences in mobility times (t_M), selectivity and resolution (R_s). The present study was designed to specifically study the effect of the buffers' cation on t_M, R_s and selectivity. A series of five monocharged, alkali acetate salts, namely lithium, sodium, potassium, rubidium and cesium, was selected for this study. The investigation was carried out under different and constant pH conditions using constant current (100 μA) in one case and constant applied voltage (20 kV) in the other.

EXPERIMENTAL

Materials:

The dansyl amino acids used in this study and guanosine were purchased from Sigma, and used without further purification. Lithium, sodium, potassium, rubidium, cesium acetate, and acetic acid were purchased from Johnson Matthey Alpha Products, Danvers, MA. Benzene and mesityl oxide were purchased from Aldrich. The acetate solutions (0.1 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 dilute acetic 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, column cartridge (50 cm x 75 μm i.d., surrounded by coolant), autosampler and a printer was used. All experiments were carried out at 20°C using the constant current mode (100 µA) or the constant voltage mode (20 kV). Injections were carried out by the pressure mode for 3 seconds. Solutes were monitored at 254 nm. All experiments were run in triplicates to insure reproducibility.

RESULTS AND DISCUSSION

Table 1 lists some of the physical parameters of the five cations used in this study which may help understand their contribution in this investigation, while Table 2 lists the experimental parameters. A study of Table 2 reveals a few interesting and useful points which should be taken into consideration when a salt (buffer) is selected for CZE studies: (a) the pHs of 0.1 M solutions of the five salts gave values ranging from 6.45 for CsAc to 9.8 for RbAc, with the pHs of LiAc, NaAc and KAc falling within 0.5 pH units of each other; (b) the ionization potential of the different cations decreased with the size of the atom; and (c) widely different values for voltages and currents were observed for each of the salts when the other parameter was held constant. The values of the observed voltages at 100 µA decreased with an increase in the size of the cation (18.35 for LiAc to 11.19 for CsAc), while the current increased almost two fold (111 µA for LiAc to 215 for CsAc) when the applied voltage was held constant at 20 kV. It is important to know these values in order to control the heat generated in the capillary. A plot of Ohm's Law, i.e., current against applied voltage should give a linear relationship, however if excess heat is generated, and cannot be efficiently dissipated, a deviation from linearity will result. This deviation can lead to irreproducible results and shorten the life of the column. The manufacturer of the columns used in this study, recommend that for a 75 µm capillary the power generated should not exceed 2.5 W/meter. The power is defined as the product of voltage and current. For LiAc (0.1 M) the power generated at 20 kV is 2.2 W, while for 0.1 M CsAc it is 4.3 W. Therefore, the analyst can predict that the use of CsAc may result in deviation from linearity at a lower applied potential than LiAc.

Table I. Physical properties of group IA metals

Element	Metal Radius (A°)	Crystal Radii (A°)	Approximate Hydrated Radii (A°)	Approximate Hydration Numbers	Ionic Mobilities (5) (at Infinite Dilution, 18°C)	Average Mobility (6-8) (10^5 cm^2/V.s)
Li	1.52	0.86	3.40	25.3	33.5	40.2
Na	1.86	1.12	2.76	16.6	43.5	51.9
K	2.27	1.44	2.32	10.5	64.6	76.1
Rb	2.48	1.58	2.28	-	67.5	86.5(a)
Cs	2.65	1.84	2.28	9.9	68.0	-

(a) Based on calculations from the data in references (6-8).

Table 2. Experimental parameters

Salt	Molarity	pH	Ionization Potential (Kjmol^{-1})	Applied Current (μA)	Observed Voltage (kV)	Applied Voltage (kV)	Observed Current (μA)
LiAc	0.1	8.09	520.1	100	18.35	20	111
NaAc	0.1	8.11	495.7	100	15.40	20	147
KAc	0.1	7.59	418.7	100	12.40	20	195
RbAc	0.1	9.78	402.9	100	12.15	20	204
CsAc	0.1	6.45	375.6	100	11.19	20	215

Table 3. List of amino acids used in this study and their PK and PI values

A.A.	PK_1	PK_2	PK_3	PI
Arginine	2.02	8.80	-	10.76
Leucine	2.33	9.74	-	6.04
Proline	1.95	10.64	-	6.30
Methionine	2.17	9.27	-	5.74
Alanine	2.35	9.87	-	6.14
Cystine	1.86	8.35	10.34	-
Glutamic Acid	2.13	4.32	9.95	3.08
Aspartic Acid	1.99	3.90	10.0	2.98

It is known that R_s is directly proportional to the applied voltage. Therefore, it is important to select a salt which will allow the application of high voltage and at the same time give a reasonable current in order to avoid the generation of excess heat in the capillary.

Table 3 lists the pK values and isoelectric points (PI) of the amino acids used in this study.

Electrophoretic Separation:

The five acetate salts were dissolved in distilled deionized water and used without any pH adjustment (Table 2) and after adjustment of the pH, with acetic acid, to pH 6.45 (the pH of CsAc). Dansylated amino acids (Table 3) were used as the test solution. The results show that as the cation size increased the t_M increased and better R_s values were obtained. Two peaks which coeluted using LiAc, NaAc and KAc buffers, proline and methionine, were resolved in RbAc and CsAc. Figure 1 shows the separations obtained using LiAc and RbAc. It is worth noting that although CsAc gave the best separation of the test mixture, the t_M values were five times longer than when LiAc was used

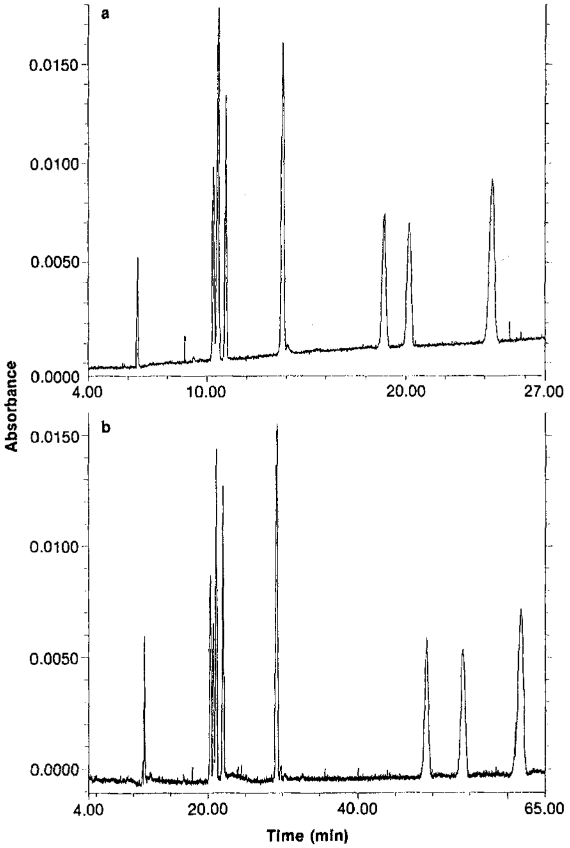

Figure 1. Effect of cation type on resolution and mobility times, for the dansylated amino acids mixture: (a) LiAc solution (0.1 M) and (b) RbAc solution (0.1 M). Constant current mode at 100 μAmps. Capillary tube: 50 cm long, 75 μm i.d.; temperature constant at 20°C; detection at 254 nm. Peak assignment from left to right: arginine, leucine, proline, methionine, alanine, cystine, glutamic acid, aspartic acid and cysteic acid.

and there was no change in the elution order of the solutes. Better resolution of the early eluting peaks was realized with an increase in the size of the cation (figure 2), which also caused the late eluting peaks to be retained for long periods of time which are unacceptable in routine analysis (see figure 1). Another observation is that the spread in elution times

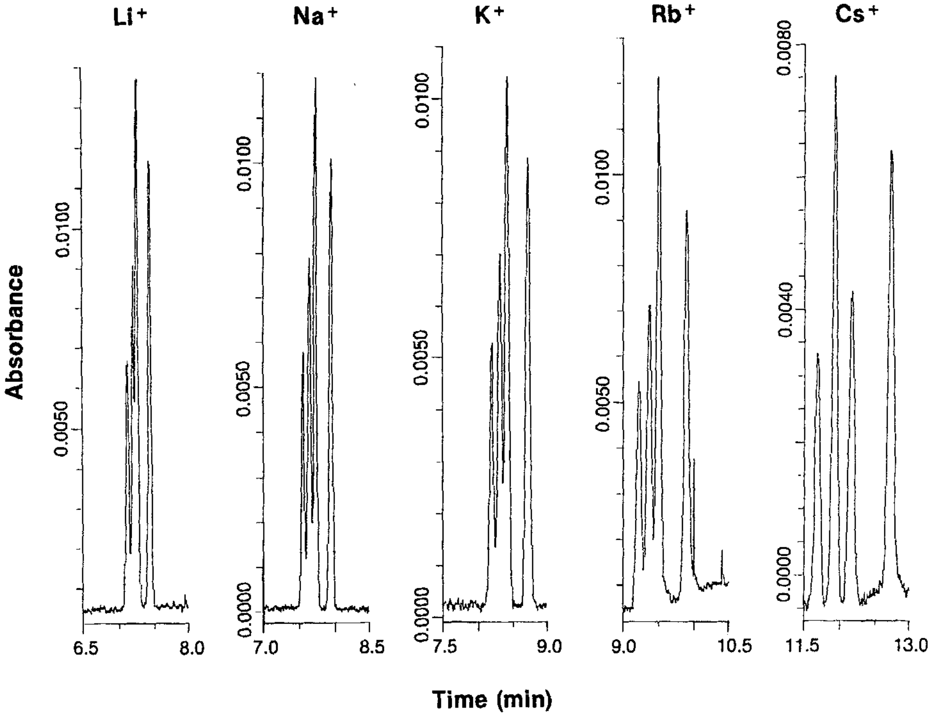

Figure 2. Effect of the cation type on the resolution and mobility times of the dansylated leucine, proline, methionine and alanine, at constant voltage of 20 kV and constant pH of 6.45. The solutions' concentrations are 0.05 M; temperature constant at 20°C, detection at 254 nm.

(selectivity) between the first and the last eluting peaks increased considerably with increase in size of the cation. These trends were observed for both pH adjusted and unadjusted solutions.

The increase in mobility times with the decrease in charge density (charge per unit volume) is supported by the Debye and Huckel theory (8,9), where the potential at certain distance from the wall of the capillary is equal to:

$$\psi_x = Ae^{-\kappa x}/x \qquad (1)$$

where x is the distance, A is a factor related to the charge of the central ion (Li^+, Na^+, K^+, Rb^+, Cs^+), and $1/\kappa$ is the thickness of the double layer.

The zeta potential at the slipping or shear plane is equal to:

$$\psi_a = (Q/Da).1/(1 + \kappa a) \qquad (2)$$

where a is distance where zeta potential is defined, Q is the charge density or valence and D is the dielectric constant of the medium. The zeta potential is linearly proportional to the charge density of the central ions (Li^+, Na^+, K^+, Rb^+, Cs^+).

The von Smoluchowski equation (8) derived for the measurement of the electrophoretic velocity of particles of any shape, is given by:

$$\upsilon = D\ \psi_a\ E/4\pi\eta \qquad (3)$$

substituting the value of ψ_a from equation 2 in equation 3 gives:

$$\upsilon = (QE/4\pi\eta a).1/(1+\kappa a) \qquad (4)$$

This important equation clearly shows the linear dependence of the electrophoretic velocity on the charge density, which in our investigation decreases as the atomic weight of the cation increases ($Cs^+<Rb^+<K^+<Na^+<Li^+$), and is also related to the ionization potential of the metals, Table 1, which decreases with size.

Since all experimental parameters were the same except for the alkali cations in the buffer solutions, μ_e, V and L are constant. Therefore, measurement of μ_{eo} in each solution should explain the difference in t_M, equation 5.

$$t_M = L^2/(\mu_{eo}+\mu_e)\ V \qquad (5)$$

where μ_{eo} is electroosmotic flow, μ_e = electrophoretic mobility, V = applied potential and L is column length.

Table 4. Measurement of the electroosmotic flow of benzene, guanosine and mesityl oxide in 0.05 M (pH 6.5) buffers at an applied voltage of 20 kV at 20°C

Solution	μ_{eo}-Benzene (10^5 cm^2/V.s)	μ_{eo}-Guanosine (10^5 cm^2/V.s)	μ_{eo}-Mesityl-oxide (10^5 cm^2/V.s)
LiAc	51.00	50.00	49.20
NaAc	47.50	47.20	46.80
KAc	46.80	44.40	45.70
RbAc	41.67	40.61	40.45
CsAc	37.00	36.40	35.70

Several methods have been published for the measurement of μ_{eo}. These methods include the use of neutral compounds such as toluene, phenol and mesityl oxide (10-12). Altria et al (13) and Everearts et al (14) measured μ_{eo} by weighing the volume of buffer transferred from the anode to the cathode (or vice versa) over a specified period of time. In this work, and in order to get an accurate value for μ_{eo}, three neutral compounds, namely mesityl oxide, guanosine and benzene, were used. These were selected to determine if the shape and size of the non-ionized molecule has any effect on μ_{eo}. Table 4 lists the μ_{eo} values of the three markers using the five test solutions at pH 6.45, and an applied voltage of 20 kV. Although different size and shape compounds were used as markers no appreciable difference in μ_{eo} was observed under the same buffer conditions. However, it is clear that μ_{eo} of each of the three markers decreases with the size of the cation in the alkali buffer solution, which accounts for the increase in t_M with increase of atomic weight of the cation.

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