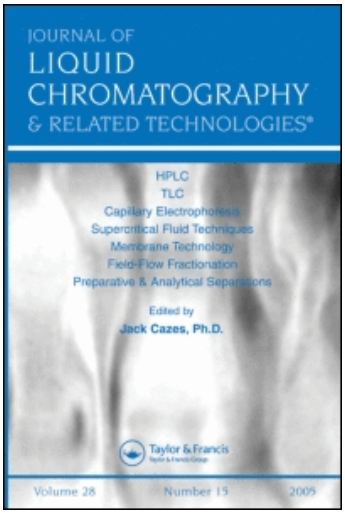

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FACTORS THAT INFLUENCE MOBILITY, RESOLUTION, AND SELECTIVITY IN CAPILLARY ZONE ELECTROPHORESIS. I. SODIUM PHOSPHATE VS. POTASSIUM PHOSPHATE

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ABSTRACT

The effect of buffer type on mobility, selectivity and resolution in capillary zone electrophoresis (CZE) was studied. The results show that the sodium phosphate buffer gave shorter mobility times (t_M) for a test dansyl amino acid mixture than the potassium phosphate buffer having the same concentration and pH. The resolution and selectivcity were also better using the sodium phosphate buffer. A comparison of resolution, t_M and selectivity using a monohydrogen and a dihydrogen sodium phosphate buffer (0.1 M, pH 7.0) showed no appreciable differences in selectivity and resolution, but the dihydrogen phosphate buffer gave t_M which are almost 45% shorter than those obtained with the monohydrogen phosphate buffer. When monohydrogen and dihydrogen potassium phosphate (0.1 M, pH 7) were used, differences in t_M, selectivity and resolution were observed.

Resolution improved with an increase in the buffer concentration (0.2 M vs. 0.1 M) but worsened and t_M got considerably shorter when the concentration of the buffer was decreased from 0.1 M to 0.05 M.

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INTRODUCTION

Andrews in the introduction to his book on electrophoresis wrote "Electrophoresis has evolved within the last thirty years from a general low resolution method of relatively limited application into a wide variety of analytical and small scale preparative techniques of unrivalled resolving power and exceptional versatility. These qualities have resulted in a virtual explosion in their use especially in the field of biochemical research. Methods are being constantly improved and modified, new variations introduced and new equipment built and yet new areas of exploitation opened up" (1). This is especially true today where one of these, Capillary Zone Electrophoresis (CZE), is emerging as a powerful analytical technique. Jorgenson and Lukacs (2) introduced CZE as a sensitive microanalytical tool. Since then many instruments have been introduced and research papers and reviews have been published (3-7). In CZE compounds are resolved according to their ability to migrate in an electric field gradient inside a fused silica capillary of 100 μm or less. In its most common mode a semiconducting liquid - generally an aqueous buffer solution - is used to establish an electric field inside the capillary and an energy exchange is established between the electric field and the liquid medium via the charged species present. Factors that affect resolution and mobility (migration) of analytes include electroosmotic flow, applied voltage, the pH, type and concentration of the buffer, ionic strength, and other buffer modifications and capillary column treatment. Altering any of these parameters may affect not only the quality of separation but also the mobility times of the analytes.

In this series of research studies we will report our findings on parameters that influence not only mobility but resolution and selectivity. Some of these parameters have been studied (2,4,8) and their effect is known, others have not.

The effect of buffer type and composition will be studied first. The study will be divided into different topics which will attempt to answer the following questions:

1. Does the buffer type play a role in determining (a) t_M, (b) analysis time, (c) resolution and (d) selectivity. What influence does the anion or the cation of the buffer have on a, b, c or d;
2. What effect the cation type and charge have on a, b, c and d;
3. What effect does the buffer type have on the current at the constant applied electric field; and finally
4. What is the relation between electric field strength, current, resolution and t_M using different buffer types.

The above questions and others need to be answered before the analyst can optimize a CZE separation. Optimization in CZE is defined as employing the mildest conditions (buffer type, pH, concentration, temperature and applied electric field) to achieve baseline separation of all the solutes in a mixture in the shortest time.

EXPERIMENTAL

Materials:

The dansyl amino acids used in this study were purchased from Sigma, and used without further purification. Sodium and potassium mono and dihydrogen phosphate buffers, phosphoric acid, potassium hydroxide and sodium hydroxide were purchased from Fisher Scientific. Buffers were prepared by exactly weighing on an analytical balance the appropriate salt and dissolving it in distilled deionized water in a volumetric flask. The pH was adjusted to pH 7 using phosphoric acid, sodium hydroxide or potassium hydroxide as required.

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 and sampler and a printer was used. All experiments were carried out at 20°C using the constant current mode 100 μA.

Injections were carried out by the pressure mode for 3 seconds. Solutes were monitored at 254 nm. All experiments were run in duplicates to insure reproducibility. Also, two different column cartridges (Beckman) were used at two different constant current settings. Constant current was used in this study because a plot of current versus $1/t_M$ gave a linear relationship, while applied voltage vs $1/t_M$ did not. This will be discussed later.

DISCUSSION

The use of phosphate buffer in CZE is very popular. However, in many published literature reports its use is casual and referred to as "phosphate buffer", without taking into consideration the effect of the cation. In preliminary studies of sodium phosphate vs. potassium phosphate, it was realized that differences were observed in resolution, mobility and selectivity of a test mixture of dansyl amino acids, under the same experimental conditions. This prompted us to study the influence of both sodium and potassium phosphate buffers.

The analyst can prepare a sodium or potassium phosphate buffer from either monohydrogen or dihydrogen phosphate by titrating with an acid or a base to achieve the required pH. Table 1 lists the pH values of the four buffers used in this study without and with the addition of acid or base to achieve pH 7.

It was decided, based on preliminary results to see what effect each of the above four buffer solutions would have on the mobility, resolution and

Table 1. Buffers used in this study

Buffer	pH	pH Adjusted to 7.0 with
NaH_2PO_4	4.23	NaOH
Na_2HPO_4	9.00	H_3PO_4
KH_2PO_4	4.17	KOH
K_2HPO_4	9.40	H_3PO_4

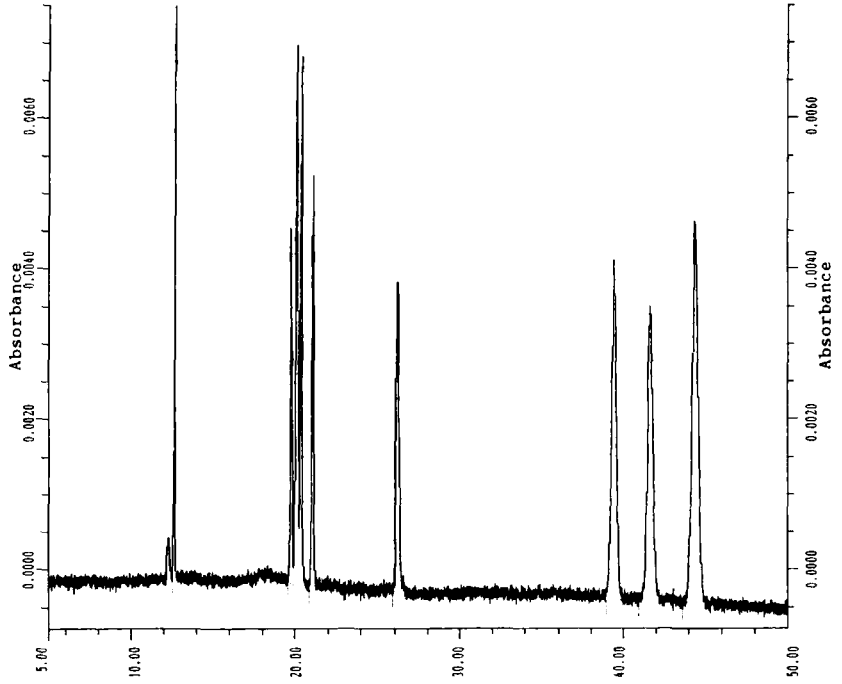

Figure 1. Electropherogram of nine dansyl amino acids. Capillary: 50 cm long, 75 μm i.d.; buffer 0.1 M NaH_2PO_4, pH 7.0; injection 0.5 psi for 3 sec; applied current 100 μA: temperature constant at 20°C detection at 254 nm. Peak assignment from left to right: arginine, leucine, proline, methionine, alanine, cystine, glutamic acid, aspartic acid and cysteic acid.

Table 2. Operating CZE parameters

Buffer	pH	Conc (M)	Current (μA)	Electric field (kV)
Na_2HPO_4	7.0	0.1	100	9.52
NaH_2PO_4	7.0	0.1	100	10.88
K_2HPO_4	7.0	0.1	100	7.40
KH_2PO_4	7.0	0.1	100	9.31

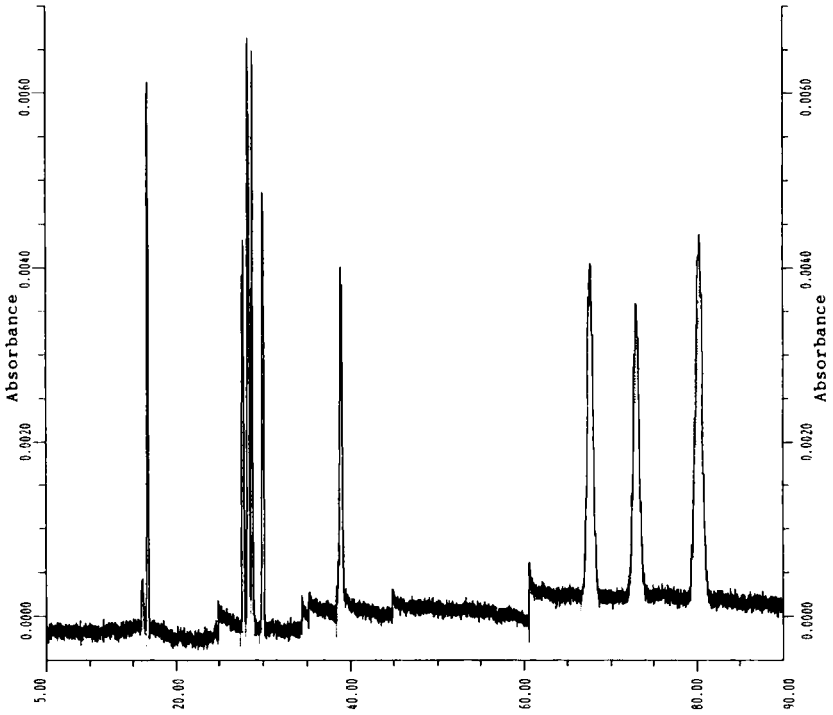

Figure 2. Same as figure 1, except 0.1 M Na_2HPO_4 buffer solution was used.

selectivity of a mixture of nine dansyl amino acids. Table 2 lists the operating parameters used in this study.

Na_2HPO_4 vs. NaH_2PO_4:

When a 0.1 M, pH 7 solution of the two buffers was used, the results indicated that although the order of elution of the nine amino acids was the same in both buffers the mobility time of the last peak was much shorter, 45 min (NaH_2PO_4) vs. 80 min (Na_2HPO_4) without any appreciable loss in resolution (Figures 1 and 2). Calculation of α (separation factor) values gave

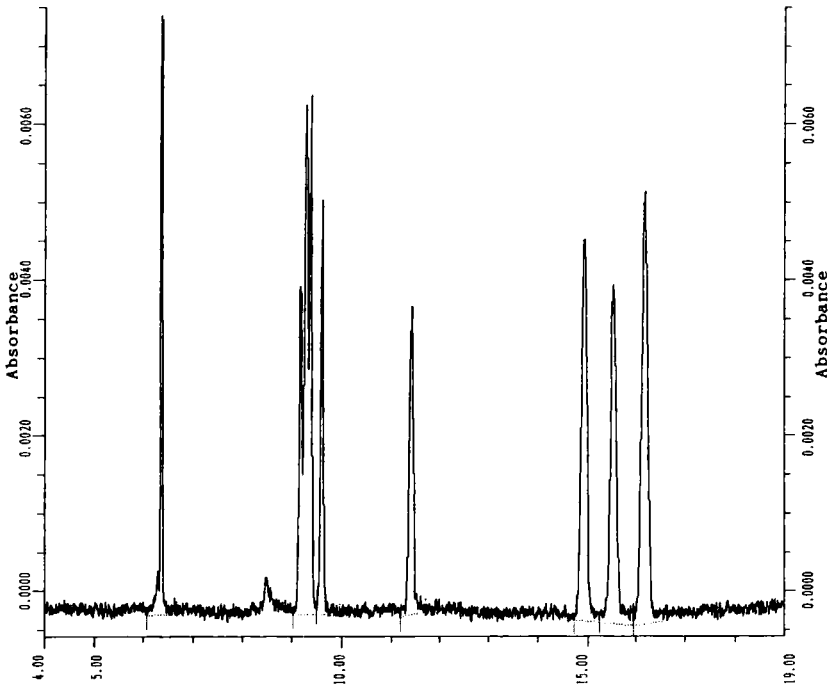

Figure 3. Same as figure 1, except 0.05 M Na_2HPO_4 buffer solution was used.

comparable results (1.06 and 1.07 vs. 1.08 and 1.10) for the last three peaks in the electropherogram. When 0.05 M Na_2HPO_4 was used in place of 0.1 M Na_2HPO_4 the retention times of the cysteic amino acid (last peak in the electropherograms) (Figures 2 and 3) the t_M was 16 min (Figure 3) vs. 80 min (Figure 2), however, there was some loss of resolution. This is evident from comparing the four peaks in the beginning of the electropherogram. The α values of the last three peaks is 1.08 and 1.10 (for 0.1 M) compared to 1.04 and 1.04 (for 0.05 M), which are baseline resolved in both cases.

0.1 M NaH_2PO_4 vs. 0.05 M Na_2HPO_4:

A comparison of t_M using 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 (Figures 1 and 2) reveals that t_M is different in each buffer solution although the pH of

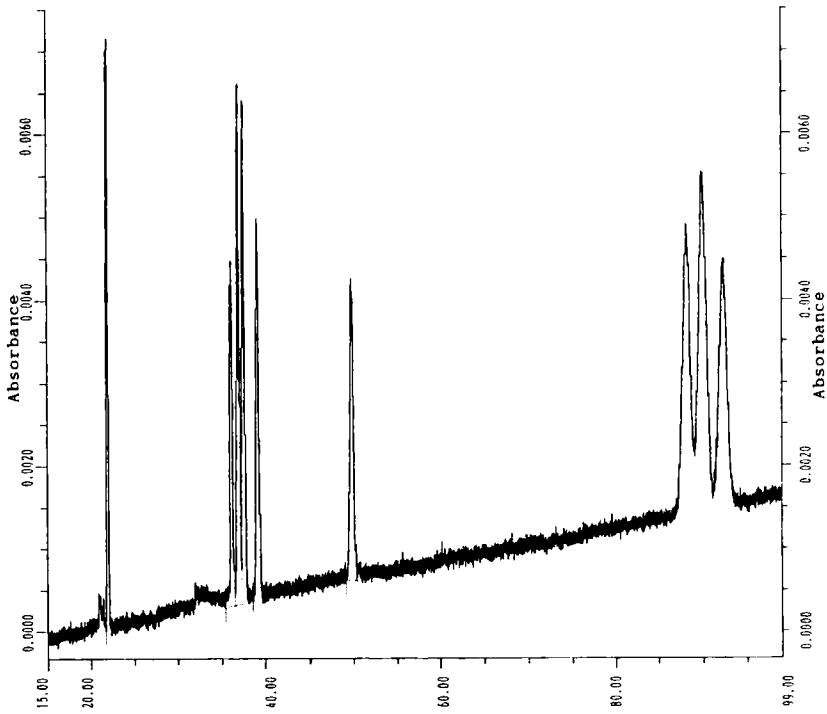

Figure 4. Same as figure 1, except 0.1 M K_2HPO_4 buffer solution was used.

both was the same. It is true that the pH was adjusted with either phosphoric acid or sodium hydroxide solution but the amounts added to adjust the pH were minimal and doubtful that they changed the ionic strength or viscosity by an appreciable amount to account for the 45% change in t_M. Therefore it was decided to reduce the number of sodium ions in Na_2HPO_4 to equal that in NaH_2PO_4. This was achieved by diluting Na_2HPO_4 solution with water (1:1 v/v). This will change the viscosity and ionic strength. The results in figure 3 show a three-fold change in t_M, from those using 0.1 M NaH_2PO_4 (Figure 1). We realize that the viscosity and ionic strength are different for 0.05 M Na_2HPO_4 than for 0.1 M NaH_2PO_4, both having a pH of 7 but can they account for such changes in t_M. Further study of this phenomenon is underway.

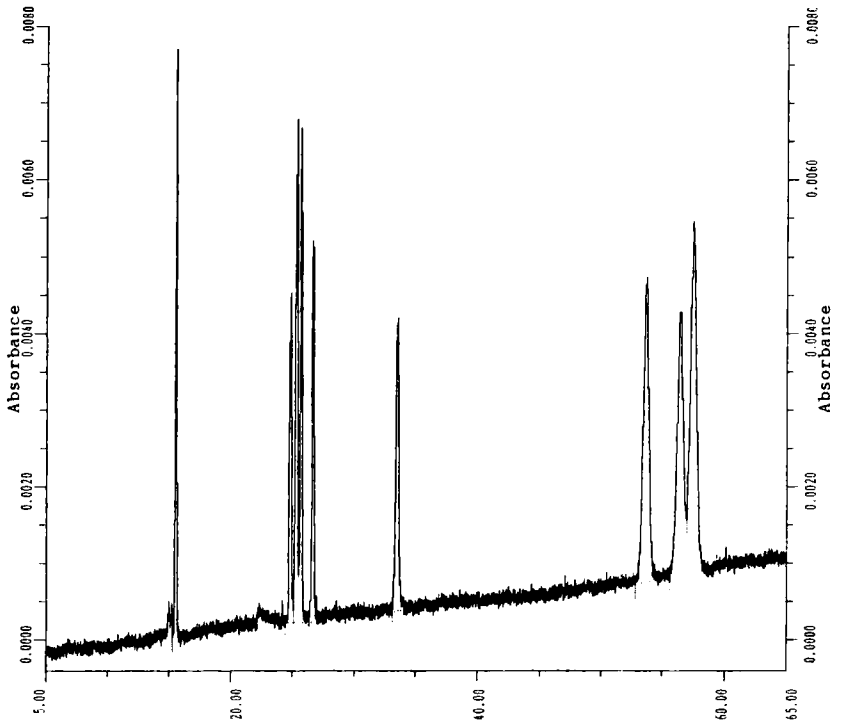

Figure 5. Same as figure 1, except 0.1 M KH_2PO_4 buffer solution was used.

K_2HPO_4 vs. KH_2PO_4:

Figures 4 and 5 are the electropherograms of the nine dansyl amino acids using 0.1 M K_2HPO_4 and 0.1 M KH_2PO_4 respectively, each at pH 7. The results indicate that t_M are shorter using KH_2PO_4. Also, the selectivity and resolution are different in each buffer judging from the last three peaks in each electropherogram. Again, the concentration of the potassium ion in K_2HPO_4 was reduced by half (0.05 M K_2HPO_4) so that the number of potassium ions is equal to that in 0.1 M KH_2PO_4. The results were different, loss of resolution and mobility (Figure 6).

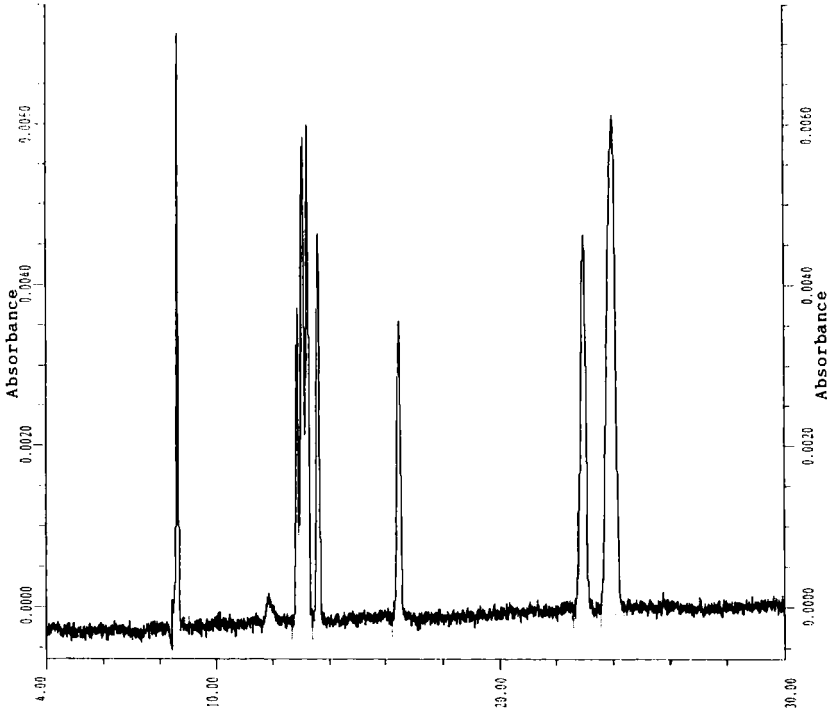

Figure 6. Same as figure 1, except 0.05 M K_2HPO_4 buffer solution was used.

Effect of buffer concentration:

It was clear from figures 2 and 3, and 5 and 6, that a decrease in buffer concentration from 0.1 M to 0.05 M at the same pH resulted in shorter t_M and loss of resolution. However, when the concentration of the buffer was increased to 0.2 M a better resolution of all the components of the mixture was realized.

Sodium or Potassium:

The question that arises from this study is which phosphate buffer to use, the sodium or the potassium? A review of the electropherograms, figures

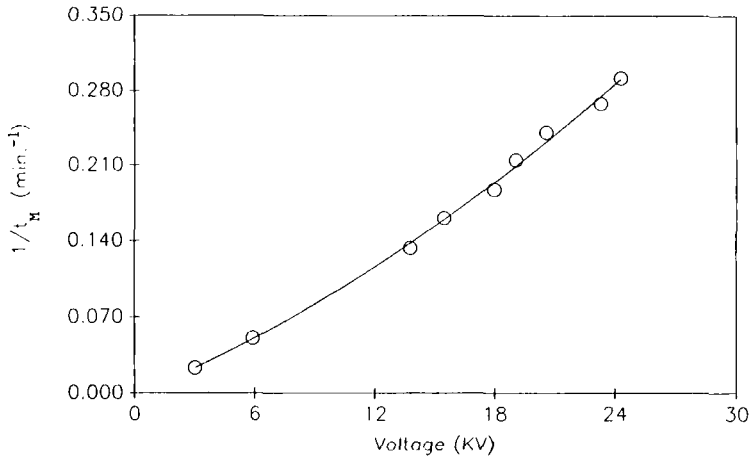

Figure 7. A plot of $1/t_M$ vs. current for the marker guanosine in 0.05 M K_2HPO_4, pH 7.15 at 20°C.

1 and 5, and 2 and 4, reveals that differences in t_M and resolution are apparent. The sodium phosphate buffer gave much better resolution of the dansyl amino acids (see last three peaks) and shorter t_M under the same experimental conditions (molarity, pH and applied current). Further, NaH_2PO_4 gave equivalent resolution but shorter t_M than when Na_2HPO_4 was used. Therefore, 0.1 M NaH_2PO_4, pH 7 is the buffer of choice for the separation of the nine dansyl amino acids in the test mixture, under the present experimental conditions. Since this study dealt with the effect of the cation of the phosphate buffer, no effort was made to optimize the separation of the nine dansyl amino acids.

Constant current vs. constant electric field:

As mentioned earlier all experiments were performed at constant current and not constant electric field. Our experimental results in both sodium and potassium phosphate buffers, using guanosine as a marker, showed that a linear relationship was obtained when $1/t_M$ was plotted against current, figure 7,

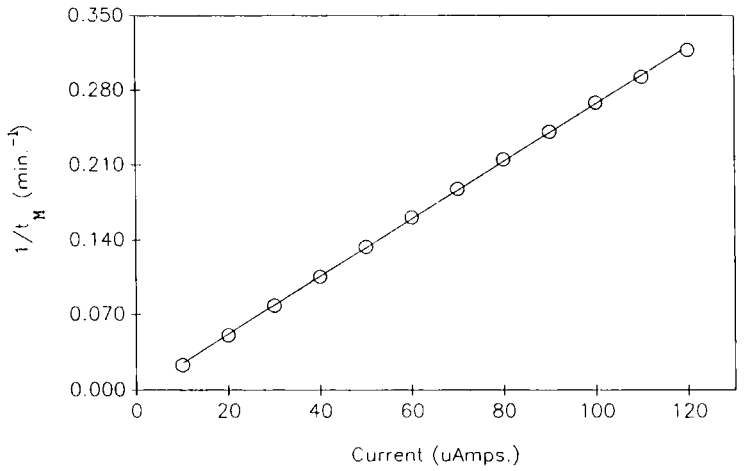

Figure 8. A plot of $1/t_M$ vs. applied electric field for guanosine, in 0.05 M K_2HPO_4, pH 7.15 at 20°C.

while a non linear relationship was obtained when $1/t_M$ was plotted against the applied electric field, figure 8. These results agree with those published earlier by Terabe et al (9) for methanol and Sudan III. Jorgenson and Lukacs (2) studied the effect of applied voltage on t_M, and reported that "as expected a linear relationship was found when $1/t_M$ vs. kV was plotted." Their published figure (figure 6 in ref. 2) indicates a non linear relationship, which agrees with our results, figure 8 and those in figure 2, ref. 9. The study of the relation between t_M, applied voltage, current and buffer type is under study and will be published separately.

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REFERENCES

1. A.T. Andrews, Electrophoresis, Theory Techniques and Biochemical and Clinical Applications, Second edition, Clarendon Press, Oxford, 1988.

2. J.W. Jorgenson and K.D. Lukacs, Anal. Chem. 53, 1298 (1981).

3. B.L. Karger, A.S. Cohen and A. Guttman, J. Chromatogr. 492, 586 (1989).

4. R.M. McCormick, Anal. Chem. 60, 2322 (1988).

5. J.C. Giddings, J. Chromatogr. 480, 21 (1989).

6. N.A. Guzman, L. Hernandez and B.G. Hoebel, BioPharm 22-37, January 1989

7. M.V. Novotny, J. Pharm. Biomed. Anal. 7, 239 (1989).

8. G.J.M. Bruin, J.P. Chang, R.H. Kuhlman, K. Zegers, J.C. Kraak and H. Poppe, J. Chromatogr. 471, 429 (1989).

9. S. Terabe, K. Otsuka, and T. Ando, Anal. Chem. 57, 834 (1985).