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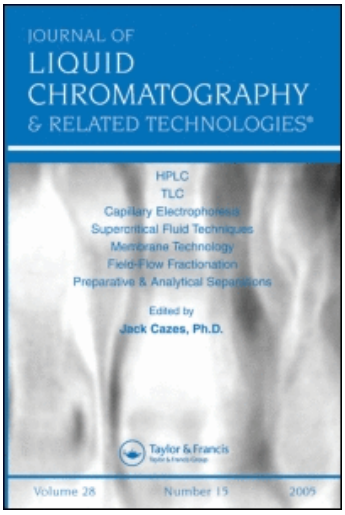

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The Effect of Column Temperature on the Migration Time of Peptides in Free-Solution Capillary Electrophoresis

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THE EFFECT OF COLUMN TEMPERATURE ON THE MIGRATION TIME OF PEPTIDES IN FREE–SOLUTION CAPILLARY ELECTROPHORESIS

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ABSTRACT

The effect of column temperature on the migration times of peptides in free — solution capillary electrophoresis (FSCE) is discussed. It has been found that there are parallel linear relationships between the logarithm of the migration time and the reciprocal of column temperature. The changes in migration time with temperature for the peptides are predominantly due to the temperature — induced viscosity changes of water. The electrophoretic selectivity remains constant for a specific pair of peptides despite a considerable variation in the migration time for each of the peptides in FSCE.

INTRODUCTION

High — performance capillary electrophoresis (HPCE) has experienced a significant growth during the past several years. Among

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its rapidly increasing number of practical applications, the separation of peptides has often been emphasized[1—3]. Optimization of the electrophoretic behaviour of solutes such as peptides in HPCE requires further understanding of the relationship between the migration time and the experimental parameters[2—3]. The most common way of decribing such relationships is by the function of the electrophoretic mobility, which is defined as the steady—state velocity of a peptide under unit field strength. Therefore, there are many studies of the effects of solution parameters as well as operation parameters on electrophoretic mobility[4].

In this paper, the effect of column temperature on the migration time of the peptides is discussed in free—solution capillary electrophoresis (FSCE). A quantitative linear relationship between the logarithm of the migration time and the reciprocal column temperature has been found. The electrophoretic selectivity factors were also studied as a function of the column temperature.

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EXPERIMENTAL

Reagents

The peptides used in this study were purchased from Serva Feinbiochemica Company (Meidelberg, Germany). All chemicals and solvents used were of analytical grade. Water was distilled twice. NaH_2PO_4 and phosphoric acid were of analytical grade. All solutes were dissolved in the buffer which was used for the experiments.

Apparatus

A SP—500 HPCE system equipped with a UV detector, an automatic injector, capillary column cartridge (with fused capillary column in 375μm(O. D.) * 75μm I. D. * 75cm), autosampler was

used in this study. A model Cole —Parmer Chemcadet 8986—50 pH meter was used for pH measurements. The fused capillary column was provided by SP company (Spectra—Physics Analytical, Fremont, California 94537, USA). The capillary was stored overnight for 16 hrs with 0. 5M pH2. 5 phosphate buffer before acquiring data in order to obtain better migration time reproducibility, before and after performing a run, the capillary was washed with the buffer. The experiments were carried out at various column temperatures and were run at least twice to ensure reproducibility. Injections were made using the hydro mode for 3 seconds. Standard peptides were monitored at 210 nm.

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RESULTS AND DISCUSSION

The theoretical concepts of capillary electrophoresis are relatively simple. Separations in FSCE are achieved via the specific migration velocities of the analytes under the influence of an electric field. A more general parameter that specifies an analyte is its electrophoretic migration velocity (ν_{ep}), which can be expressed as[5]:

$$\nu_{ep}=\frac{2\varepsilon E\xi_a}{3\eta}f(\kappa\alpha)$$

where E(=V/L) is the local electric field, V is the applied potential and L the length of the capillary. ξ_a is the ξ—potential of the analyte, ε is the dielectric constant of the medium, η is the viscosity coefficient.

κ, the reciprocal of the analyte double layer thickness, α, the "radius" of the analyte and $f(\kappa\alpha)$ are functions of the molecular shape and $\kappa\alpha$ of the analyte in the buffer.

The electroosmotic velocity can also be represented by eqn. (1):

$$\nu_{eo}=\frac{\varepsilon\xi_c}{\eta}E \qquad (2)$$

where ξ_c is the ξ—potential of the inner wall of the capillary. The net migration velocity of an analyte:

$$\nu_{ni}=\frac{\varepsilon E}{\eta}\left[\xi_c+\frac{2\xi_a}{3}f(\kappa\alpha)\right] \qquad (3)$$

It follows that the analyte migration time is:

$$t_m=\frac{\eta L}{\varepsilon E\left[\xi_c+\frac{2\xi_a}{3}f(\kappa\alpha)\right]} \qquad (4)$$

Temperature of the capillary plays a significant role in the electrophoretic behaviours of the peptides in the migration equation (4); term η is temperature—dependent; all the other terms ξ_c, ξ_a, ε and $f(\kappa\alpha)$ are all relatively temperature insensitive. Phosphate buffer has a relatively small temperature coefficient (ca. —0.004 pH/°C). The difference in pH of the buffer at column temperature range 15 to 60°C can be considered insignificant.

As the viscosity of the medium has the following simplified relationship with the temperature[6]:

$$\eta=A\cdot 10^{B/T} \qquad (5)$$

where A, B are constants characterizing the properties of the buffer, T is column temperature.

Therefore we have:

$$\log t_m=\log Q+\frac{B}{T} \qquad (6)$$

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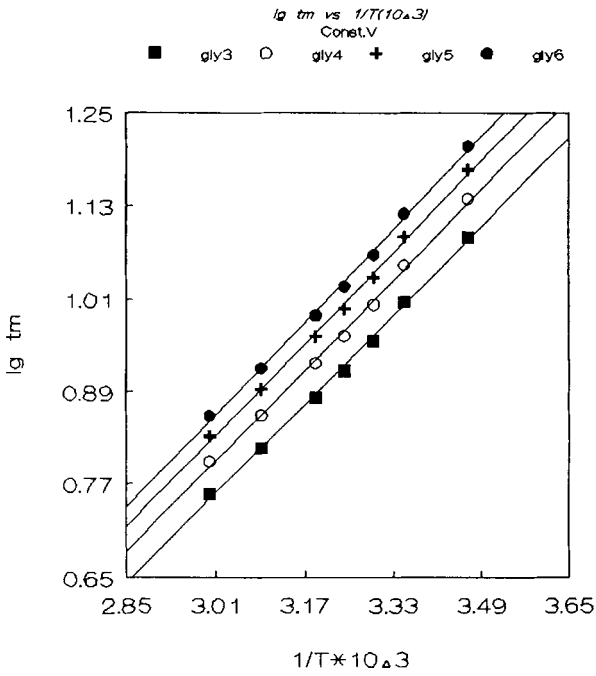

FIGURE 1.

The linear relationships between $\log t_m$ and 1/T for $(gly)_n$ peptides in FSCE, 75cm×75μm I. D., fused silica, constant voltage (25kV).

$$\text{where } Q=\frac{AL}{\varepsilon E[\xi_c+\frac{2\xi_a}{3}f(\kappa\alpha)]}$$

Therefore, under the condition that separations were carried out at constant voltage or applied voltage does not significantly change with column temperature, simplified linear relationships between log t_m and 1/T with the same slope have been derived (see Fig. 1). Under the mode of constant current, increasing the

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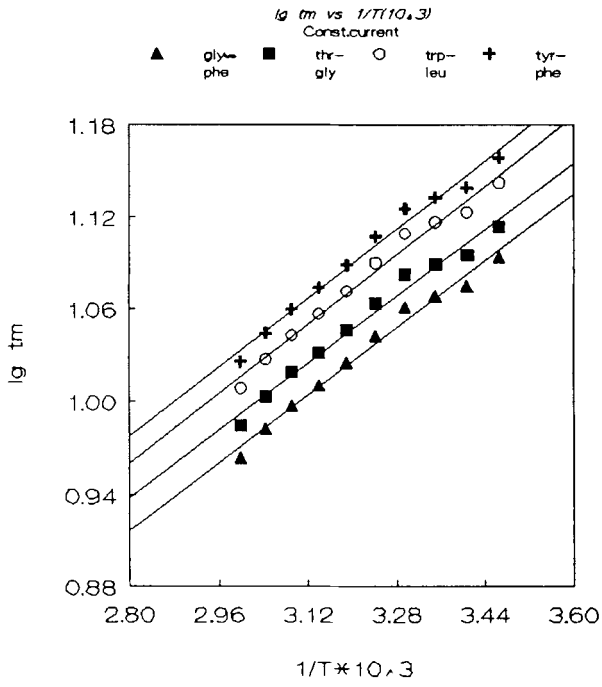

FIGURE 2.

The parallel linear relationship between $\log t_m$ and 1/T for 4 dipeptides under constant current in FSCE. For electrophoretic conditions see Table I.

column temperature results in a decrease in the applied voltage, but, the logarithm of the applied voltage under constant current is linear proportional to the reciprocal of column temperature, therefore, an approximately linear relationship between lg tm and 1/T has also been obtained (see Fig. 2).

As is seen from the fingures all the lines have the same slope, the peptides have parallel electrophoretic behaviour, which shows that the changes in electromigration time with temperature for

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TABLE I.

The variation of the migration time and relative migration time of 4 dipeptides with the column temperature *

C	Gly—phe	thr—gly		trp—leu		tyr—phe	
	t_m	t_m	γ	t_m	γ	t_m	γ
15	12.41	12.99	1.05	13.89	1.12	14.41	1.16
20	11.89	12.45	1.05	13.28	1.12	13.78	1.16
25	11.70	12.27	1.05	13.08	1.12	13.58	1.16
30	11.51	12.08	1.05	12.85	1.12	13.35	1.16
35	11.03	11.58	1.05	12.30	1.12	12.79	1.16
40	10.59	11.12	1.05	11.79	1.11	12.26	1.16
45	10.24	10.75	1.05	11.40	1.11	11.85	1.16
50	9.93	10.44	1.05	11.04	1.11	11.48	1.16
55	9.60	10.07	1.05	10.65	1.11	11.07	1.15
60	9.20	9.64	1.05	10.20	1.11	10.61	1.15

* 75cm * 75μm I. D., fused silica capillary column, 100 μA, relative migration time were calculated based on gly—phe.

dipeptides should be predominantly caused by temperature induced viscosity changes of aqueous buffer.

The decrease in viscosity of running buffer with temperature increasing results in the increase in velocity of the analyte, which thus leads to a decrease in migration time.

Table 1 shows the variation of migration time for 4 dipeptides with the column temperature.

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The date of Table 1 shows that, increasing the column temperature, which results in a decrease in the viscosity of the medium, leads to a decrease in migration time of the peptides in FSCE. The electrophoretic selectivity (γ) is defined as the ratio of the migration time of the ionic component (t_m) to that of the reference standard ($t_{m(r)}$) which can have the following expressions:

$$\gamma = \frac{t_m}{t_{m(r)}} \tag{7}$$

$$\gamma = \frac{\xi_c + \frac{2\xi_a(r)}{3} f(\kappa\alpha)(r)}{\xi_c + \frac{2\xi_a}{3} f(\kappa\alpha)} \tag{8}$$

Examination of Eq(8) reveals that no term is temperature-dependent. γ is independent of the column temperature. The results in Table 1 support this assumption. In spite of a considerable variation in the migration time for each of the dipeptides, the electrophoretic selectivity remains constant for a specific pair of dipeptides.

The electrophoretic selectivity between each pair of peptides is independent of the temperature, which is characteristic of the properties of the peptides and the capillary column. Therefore, electrophoretic selectivity can be used as a reliable index for peak identification in FSCE.

CONCLUSION

The changes in the migration times of the peptides were found to be predominantly due to temperature-induced viscosity changes of the aqueous buffer. A quantitative linear relationship between log t_m and $1/T$ was found. This quantitative relationship may be used to predict the migration times of peptides in FSCE when the

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applied column temperature changes. The electrophoretic selectivity factors were independent of the applied column temperature.

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