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Journal of Chromatography A, 768 (1997) 153–159

JOURNAL OF CHROMATOGRAPHY A

Mobility measurements on dansylated amino acids

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Abstract

The dansylated enantiomers of several amino acids were separated using hydroxypropyl-β-cyclodextrin (HPBCD) at both pH 2.75 and 6.0. The derivatives of the phenylalanine enantiomers showed unusual behaviour at pH 2.75 in that the mobility difference reached a plateau value which did not decrease at high concentration. This behaviour was ascribed to differences in the limiting mobilities of the two enantiomer–HPBCD complexes.

Keywords: Enantiomer separation; Electrophoretic mobility; Amino acids

1. Introduction

The field of chiral analysis is an area where capillary electrophoresis (CE) has become useful in recent years because of the advantages of high efficiency, low operating cost and simplicity. Chiral CE typically uses chiral selectors, such as cyclodextrins, added to the separation buffer. This gives the advantage of rapid method development in that a range of chiral selectors and different concentrations can be screened in a single run. One benefit of CE over techniques such as HPLC is that chiral CE can be easily modeled using the assumption of simple equilibria [1]. It is assumed that the two enantiomers interact rapidly and reversibly with the chiral selector to form complexes of differing mobility to that of the free enantiomers. It is also assumed that the mobilities of the two enantiomer–selector complexes are the same. One feature of the model is the prediction of an optimum selector concentration inversely related to affinity of the enantiomer for the chiral selector. The model also predicts that the apparent mobility difference between the two enantiomers can be described by a single continuous function. More recently, however, some workers have noted a change in the migration order of the two enantiomers with increasing chiral selector concentration. An example of this behaviour is provided by Schmitt and Engelhardt using hydroxypropyl-β-cyclodextrin (HPBCD) with dansylated phenylalanine [2]. They found that with a 20 m*M* phosphate buffer at pH 6.0, the D enantiomer had the highest electrophoretic mobility with 0.3% (w/v) HPBCD whereas the L enantiomer had the highest electrophoretic mobility with 15% (w/v). At 6.0% (w/v) HPBCD the two enantiomers had equal mobility. The authors ascribe the changeover to different separation mechanisms: at low concentration the separation is due to a differential interaction with HPBCD and at high concentrations due to the mobilities of the two HPBCD–analyte complexes.

This unusual behaviour was considered in a more general equilibrium model [3] which covered the case where the limiting mobilities of the two enantiomer–chiral selector complexes are different. The two possibilities are illustrated by the examples given in Fig. 1 Fig. 2. In Fig. 1, the enantiomer with the highest affinity for the chiral selector gives rise to the complex with the lower electrophoretic mobility. In this case the difference in apparent mobility (and

PII S0021-9673(96)00959-4

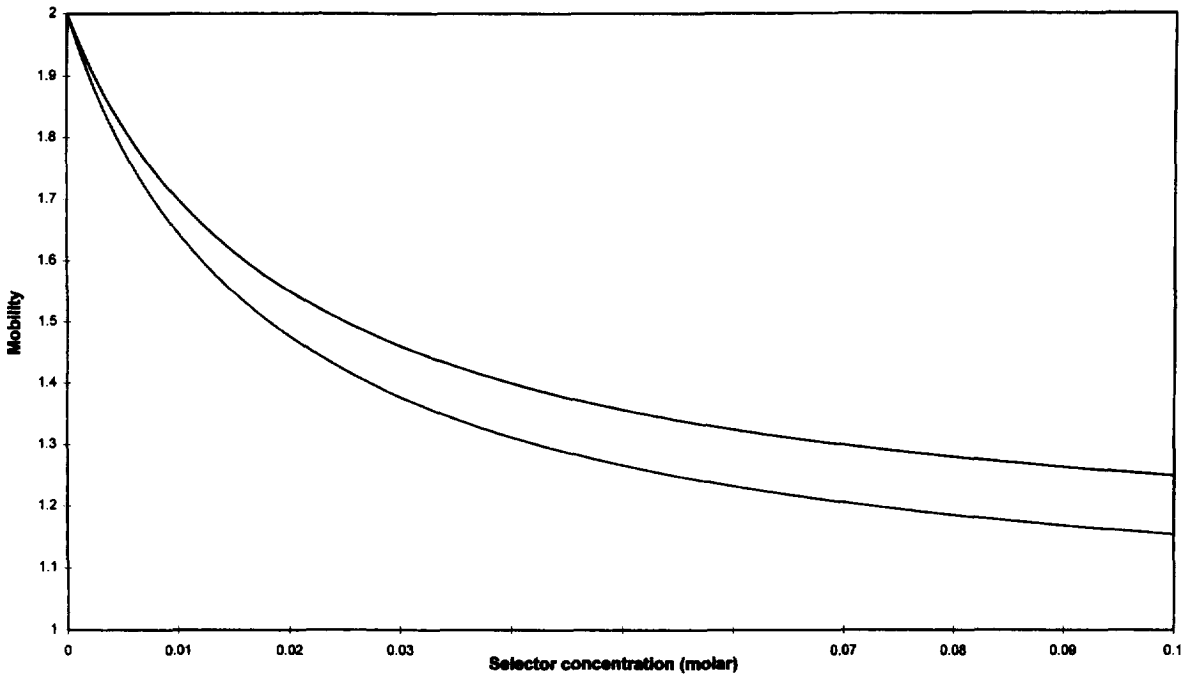

Fig. 1. Calculated apparent mobility using the equilibrium constants $K_1 = 50$ and $K_2 = 55$ (M^{-1}) with the mobilities $\mu_0 = 2$, $\mu_1 = 1.1$, $\mu_2 = 1.0$ ($\times 10^{-4}$ cm^2/V/s).

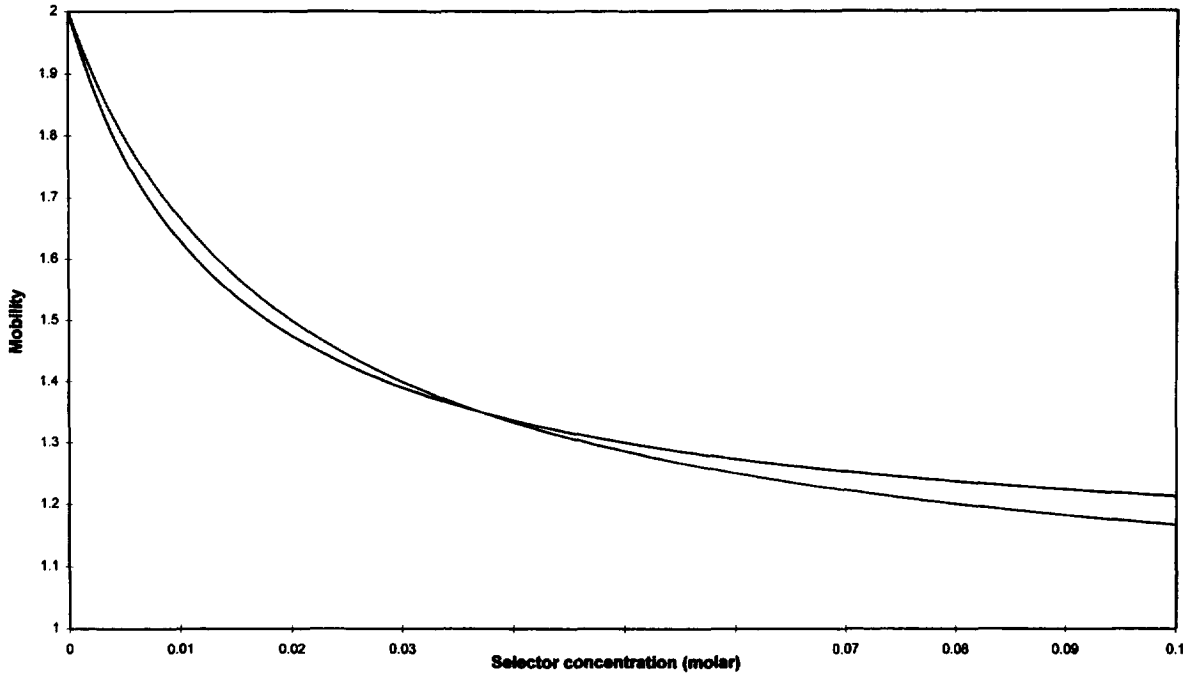

Fig. 2. Calculated apparent mobility using the equilibrium constants $K_1 = 70$ and $K_2 = 50$ (M^{-1}) with the mobilities $\mu_0 = 2$, $\mu_1 = 1.1$, $\mu_2 = 1.0$ ($\times 10^{-4}$ cm^2/V/s).

hence separation) tends to a maximum value which is determined by the electrophoretic mobility differences between the two complexes. In Fig. 2, the enantiomer with the highest affinity for the chiral selector gives rise to the complex with the higher electrophoretic mobility. This means that the migration order will change and there will be two mobility difference maxima.

2. Experimental

The experiments were carried out using a Beckman PACE 5000 instrument (High Wycombe, UK). The capillary was of fused silica with an internal diameter of 50 μm, a length of 20.3 cm to the detector and 27.0 cm overall. The samples were dissolved in buffer and loaded by a 2-s pressure injection. The separation was carried out at 25°C using 10 kV. The data were collected at 200 nm using a rate of 5 Hz. The dansylated amino acids were from Sigma (Poole, UK), racemic mixtures were used in the initial experiments and the migration order determined subsequently by spiking in one of the enantiomers. The HPBCD had an average degree of substitution of 0.8 and an average molecular mass of 1500 and was from Aldrich (Gillingham, UK) as was the benzyl alcohol. The buffers were prepared from lithium hydroxide which was taken to pH 2.75 or 6.0 using phosphoric acid. Stock buffer and HPBCD solutions were used to prepare a range of separation buffers which were all 50 m*M* in buffer but contained the following concentrations of HPBCD: 0, 0.33, 0.66, 1.32, 2.64, 5.28, 7.92 13.2, 26.4 and 66.0 m*M*. At each concentration two sample injections were made and the average mobility value used. The electroosmotic mobility was determined by including benzyl alcohol in with the dansylated amino acids. The electrophoretic mobilities were corrected for viscosity by multiplying by the ratio of the current at 0 m*M* HPBCD over the current at the HPBCD concentration of interest [1]. The curve fitting was carried out using the computer program GENPLOT (Computer Graphic Service, New York, NY, USA).

3. Results and discussion

The dansylated amino acids were examined at both pH 2.75 and pH 6.0. The purpose of examining two different pH values was to see if the degree of interaction was altered by changing from a positively charged to a negatively charged analyte. Both buffers were prepared using 100 m*M* lithium hydroxide to ensure that the lithium ion concentration was the same in all experiments.

3.1. Experiments at pH 2.75

A pH of 2.75 was chosen as a compromise between the desire to fully protonate the dansylated amino acids and to operate at a pH which gave a readily measurable electroosmotic mobility. Dansylated glycine, L-alanine, D,L-valine, D,L-leucine and D,L-phenylalanine were analysed at ten different HPBCD concentrations ranging from 0 m*M* to 66 m*M* (see Section 2 for full details). Benzyl alcohol was injected along with the analytes so that the electroosmotic mobility could be measured. With the racemic mixtures, spiking experiments were carried out at a range of concentrations so that the migration orders could be determined. There were no changes in migration order with the change in HPBCD concentration.

For dansylated D,L-valine, leucine and phenylalanine, the mobility difference between the pairs of enantiomers as a function of the HPBCD concentration is shown in Fig. 3.

The mobility difference between the dansylated enantiomers of valine and between those of leucine follow a familiar pattern. The mobility difference initially increases with increasing concentration before reaching a maximum value and then dropping off at higher values. For the dansylated enantiomers of phenylalanine the pattern is different with the mobility difference appearing to reach a plateau. It is also interesting to note that a much larger mobility difference is seen between the enantiomers of dansylated phenylalanine than between the enantiomers of the other two amino acids.

The measured apparent electrophoretic mobilities of the dansylated phenylalanine enantiomers as a function of the HPBCD concentration are shown in

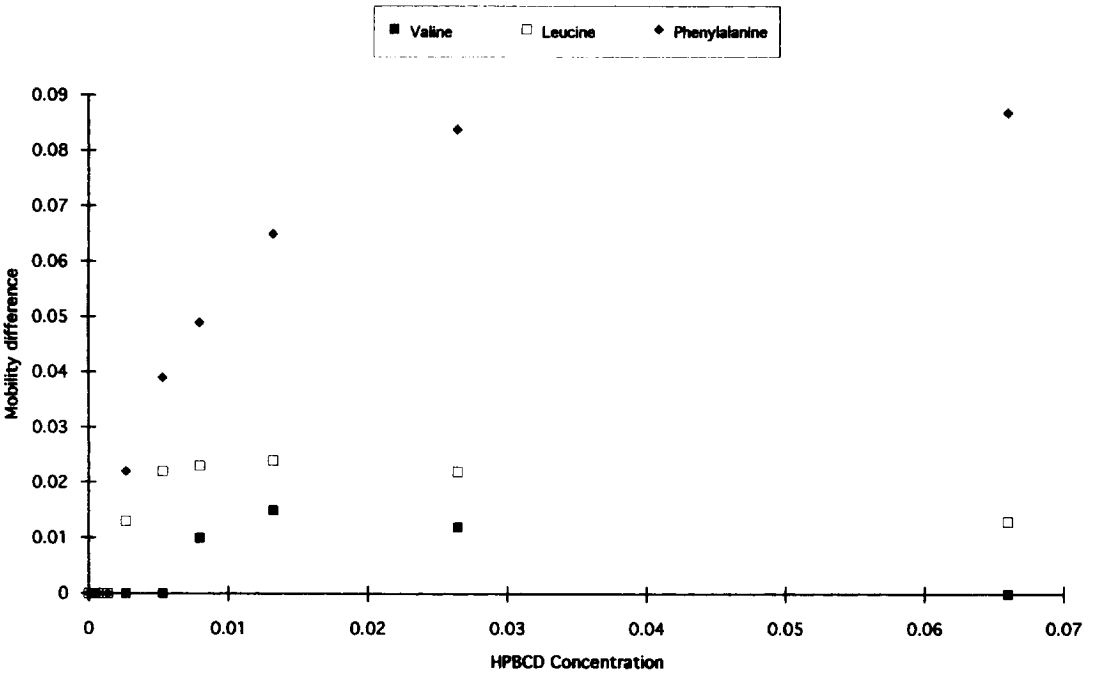

Fig. 3. Measured mobility differences ($\times 10^{-4}$ $cm^2/V/s$) as a function of HPBCD concentration for dansylated amino acid enantiomers at pH 2.75.

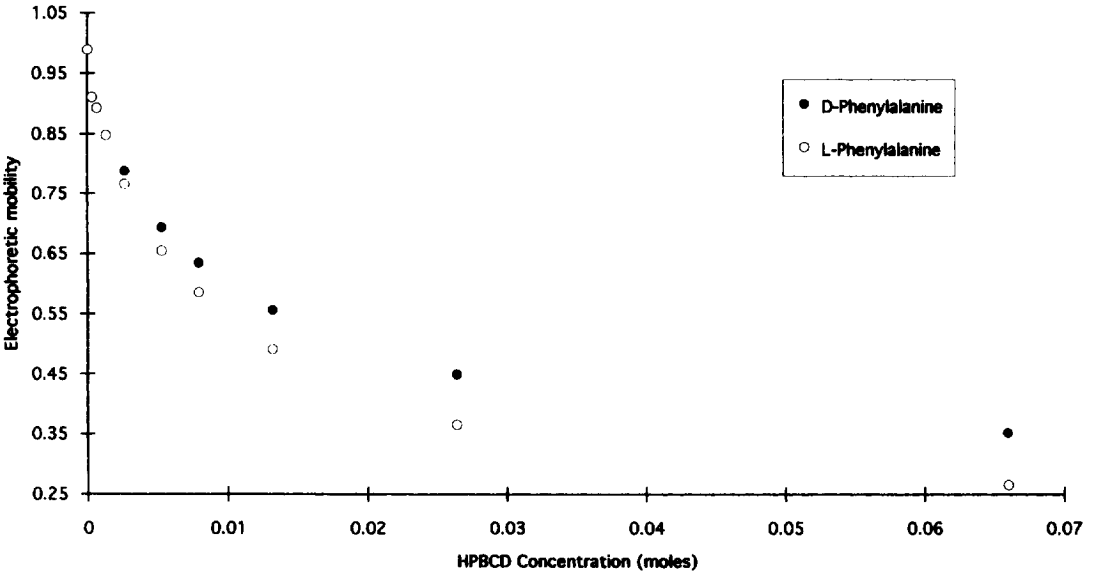

Fig. 4. Measured electrophoretic mobilities ($\times 10^{-4}$ $cm^2/V/s$) as a function of HPBCD concentration for dansylated phenylalanine enantiomers at pH 2.75.

Fig. 4. It can be seen that although the apparent mobilities seem to be tending towards their limiting values there is no sign of convergence. This is strong evidence for a difference in the electrophoretic mobilities of the two enantiomer–HPBCD complexes.

3.2. Determination of equilibrium constants and limiting mobilities

According to the theory mentioned in the introduction [3] the apparent mobility of the analyte will be a function of the selector concentration as outlined in Eq. (1).

$$\mu_a = \frac{\mu_0 + \mu_1 K_1[C]}{1 + K_1[C]} \quad (1)$$

where μ_0 is the mobility of the free analyte, μ_1 is the electrophoretic mobility of the analyte–chiral selector complex and K_1 is the equilibrium constant for the formation of the complex.

From measurement of the apparent mobility at a range of HPBCD concentrations, the values for μ_1 and K_1 can be estimated by a curve fitting approach. Equations of the form of Eq. (1) have been used widely in many branches of chemistry and some examples and a review of fitting techniques have been given recently by Rundlett and Armstrong [4]. A consideration of the curves shown in Fig. 4 shows good agreement with the form expected from Eq. (1). The values of the equilibrium constants and limiting mobilities are the mean values derived by the curve fitting program GENPLOT and are shown in Table 1 along with the associated standard errors σ_k and σ_μ.

Table 1 shows a number of interesting results. The size of the K_1 values vary by a factor of greater than three. As a rough rule, the size of K_1 increases with the size of the amino acid although the correlation is not very strong. From Fig. 3, we can see that the enantiomers of dansyl leucine give rise to a greater mobility difference than do those of dansyl valine. For the individual pairs of enantiomers, a bigger difference in the K_1 values gives rise to a larger difference in electrophoretic mobilities. This is consistent with the theory and the data in Table 1. The results for dansyl phenylalanine do not fit the previous pattern. The difference in the K_1 values for the dansylated enantiomers of phenylalanine is the same as that for those of leucine but the mobility difference is much larger. In addition, the mobility difference between the dansylated phenylalanine enantiomers appears to plateau at a high value. An explanation for this seems to lie in the values of the limiting mobilities of the HPBCD–analyte complexes (μ_1). Whilst the values for the D and L enantiomers of leucine are the same, as are those for the D and L enantiomers of valine, the D and L enantiomers of dansyl phenylalanine give limiting mobilities which are significantly different ($p<0.005$). Thus the reason for the very large difference in electrophoretic mobility of the enantiomers of dansyl phenylalanine lies in the difference in the limiting mobilities rather than the equilibrium constants.

This difference in limiting mobilities is unusual, most pairs of enantiomers give complexes with the same value (see e.g. Ref. [3]).

Table 1
Equilibrium constants and limiting mobilities with HPBCD at pH 2.75

Amino acid	μ_0 ($\times 10^{-5}$)	K_1	σ_k	μ_1 ($\times 10^{-5}$)	σ_μ ($\times 10^{-5}$)
Glycine	10.47	60	1	0.45	0.10
L-Alanine	10.42	47	3	0.59	0.27
D-Valine	10.51	59	6	1.3	0.39
L-Valine	10.51	62	6	1.4	0.35
D-Leucine	10.07	145	13	2.1	0.23
L-Leucine	10.07	155	11	2.0	0.18
D-Phenylalanine	9.89	154	20	3.1	0.28
L-Phenylalanine	9.89	144	13	2.0	0.24

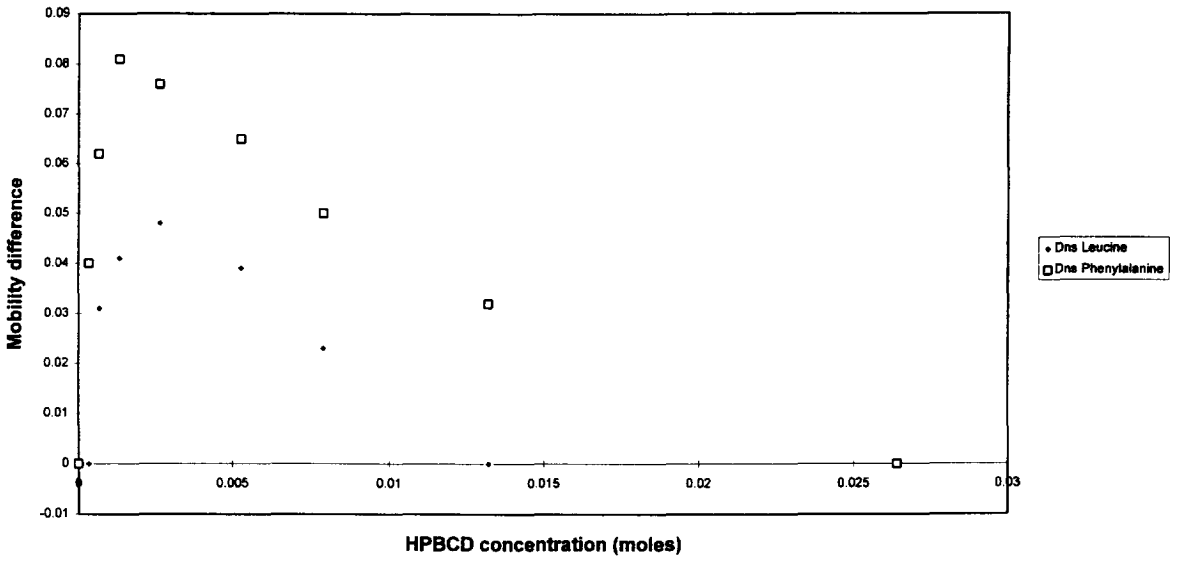

Fig. 5. Measured mobility differences ($\times 10^{-4}$ cm^2/V/s) as a function of HPBCD concentration for dansylated amino acid enantiomers at pH 6.0.

3.3. Experiments at pH 6.0

A separate set of experiments was carried out at pH 6.0 in order to replicate the conditions of Schmitt and Engelhardt [2]. In this case however, an ordinary fused silica capillary was chosen instead of a neutral one so that the electroosmotic mobility could be measured. The same HPBCD concentration as those used at pH 2.75 were employed. In addition only the dansylated enantiomers of leucine and phenylalanine were examined. The two major differences on changing the pH were that the analytes were all negatively rather than positively charged and appeared to have higher affinities for HPBCD. The higher affinities can be seen in Fig. 5, a graph of mobility difference vs. HPBCD concentration. It can be seen that the greatest mobility difference for the phenylalanine enantiomers occurs at about 1.3 m*M* and for the leucine enantiomers at 2.6 m*M*. These optimum concentrations are very small and indicate large equilibrium constants. For both pairs of enantiomers, the mobility difference decreases to zero at higher HPBCD concentrations. For the enantiomers of dansyl phenylalanine, the mobility difference does not plateau at a constant value as was seen at pH 2.75. The equilibrium constants and limiting mobilities were again obtained by curve fitting.

Table 2 shows the equilibrium constants for the enantiomers of dansyl phenylalanine to differ by a greater percentage than those of dansyl leucine (50% vs. 26%). This is consistent with the greater measured electrophoretic mobility difference shown in Fig. 5. Table 2 also shows larger equilibrium constants for phenylalanine than leucine which is again consistent with a lower optimum HPBCD concentration. A comparison with the data from Table 1

Table 2
Equilibrium constants and limiting mobilities with HPBCD at pH 6.0

Amino acid	μ_0 ($\times 10^{-5}$)	K_1	σ_k	μ_1 ($\times 10^{-5}$)	σ_μ ($\times 10^{-5}$)
L-Leucine	−15.68	414	32	−6.4	0.18
D-Leucine	−15.68	329	21	−6.2	0.16
L-Phenylalanine	−15.89	601	43	−6.5	0.16
D-Phenylalanine	−15.89	400	28	−6.3	0.17

show that the equilibrium constants for the enantiomers of both dansyl phenylalanine and dansyl leucine are smaller at pH 2.75 than at pH 6.0. This may merely be a reflection of the difference in the ionic strength of the buffers or may indicate interaction at different parts of the molecules. It is also interesting to note that the maximum mobility difference between the enantiomers of dansyl phenylalanine is similar at both pH values (at around $0.09 \cdot 10^{-4}$ $cm^2/V/s$). At pH 2.75, the difference is mainly as a result of the difference in the limiting mobility whereas at pH 6.0 it arises from the difference in the equilibrium constants.

The experiments with HPBCD did not indicate any change in the migration order of dansyl phenylalanine up to a concentration of 66 m*M*, and the measured limiting mobilities are not significantly different. Subsequent experiments at higher concentrations of HPBCD did suggest that a separation had reappeared but it was found to be difficult to obtain consistent results. This is possible due to the high viscosities of buffers containing concentrations of HPBCD greater than 100 m*M*.

Thus, although the theory is consistent with the work of Schmitt and Engelhardt, it has not been possible to demonstrate an unequivocal link.

References

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