Theoretical aspects of chiral separation in capillary electrophoresis

I. Initial evaluation of a model

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ABSTRACT

A simple model for the separation of pairs of enantiomeric molecules in capillary electrophoresis is presented. The model shows that the degree of separation depends on the concentration of chiral selector, and that there is an optimum concentration. The size of the optimum concentration depends inversely on the affinity of the enantiomers for the chiral selector. The model is supported by experimental results using propranolol and β-cyclodextrins.

INTRODUCTION

The analysis of the different enantiomeric forms of chiral molecules is an area of increasing importance in separation science. In the more conventional chromatographic procedures such as high-performance liquid chromatography, gas chromatography and thin-layer chromatography, chiral separation is brought about by the use of chiral additives in the mobile phase or the use of a chiral stationary phase.

In the newer field of capillary electrophoresis (CE), chiral separations are being undertaken by the use of chiral additives in the running buffer. A range of additives have been employed, some of which have already been successfully used in the conventional chromatographic procedures mentioned above. Examples are bile acids [1,2], chiral surfactants [3], cyclodextrins as buffer additives [4–6], cyclodextrins incorporated into a gel matrix [7] and

cyclodextrins mixed with other chiral selectors [8]. In several of these studies it was found that the degree of resolution obtained varied with the concentration of chiral selector [5,6,8] or the concentration of organic solvent in the buffer. As the chiral selector or organic solvent concentration was increased, the resolution could either increase, decrease or increase to a maximum before decreasing. n earlier work in this laboratory it was found that there was an optimum concentration of cyclodextrin for a particular chiral separation. It was therefore decided to investigate the underlying mechanism.
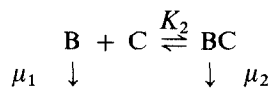
MODEL

The following simple model is proposed and used as a working hypothesis. It is intended to cover simple situations where a freely soluble analyte interacts with a single chiral selector:

$$A + C \underset{}{\overset{K_1}{\rightleftharpoons}} AC$$
$$\mu_1 \downarrow \qquad\qquad \downarrow \mu_2$$
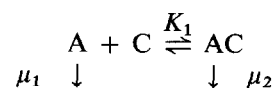
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$$\text{B} + \text{C} \overset{K_2}{\rightleftharpoons} \text{BC}$$
$$\mu_1 \downarrow \qquad\qquad \downarrow \mu_2$$

where μ_1 is the electrophoretic mobility of the analyte in free solution, μ_2 is the electrophoretic mobility of the analyte–chiral selector complex and K_1 and K_2 are equilibrium constants. A and B are a pair of enantiomers which have the same electrophoretic mobility in free solution. They interact with a chiral selector C dissolved in the buffer to form the inclusion complexes AC and BC, which are assumed to have the same electrophoretic mobility. If the two enantiomers have different affinities for the chiral selector, i.e., K_1 and K_2 are different, and the electrophoretic mobilities of the free and complexed enantiomers are different, then chiral resolution is possible. If the exchange of A between the free and bound forms is very rapid, then the apparent electrophoretic mobility of A will be a function of the proportion of the time when A is free and the proportion when it is complexed, i.e.,

$$\bar{\mu}_a = \left(\frac{[A]}{[A] + [AC]} \right) \mu_1 + \left(\frac{[AC]}{[A] + [AC]} \right) \mu_2 \qquad (1)$$

$$[AC] = K_1[A][C] \qquad (2)$$

and therefore

$$\bar{\mu}_a = \frac{\mu_1 + \mu_2 K_1[C]}{1 + K_1[C]} \qquad (3)$$

The difference in the apparent electrophoretic mobility of A and B is

$$\Delta\mu = \frac{\mu_1 + \mu_2 K_1[C]}{1 + K_1[C]} - \frac{\mu_1 + \mu_2 K_2[C]}{1 + K_2[C]} \qquad (4)$$

This rearranges to

$$\Delta\mu = \frac{[C](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1 K_2[C]^2} \qquad (5)$$

From eqn. 5, it is clear that the apparent mobility difference will be zero if $K_1 = K_2$ or $\mu_1 = \mu_2$. In addition, the apparent mobility difference will be zero if $[C] = 0$ or $[C]$ is very large. This implies that in between these two extremes some value of $[C]$ will give a maximum apparent mobility difference and hence a maximum separation of the two enantiomers.

This approach considers mobility difference rather

than resolution. This is because resolution is more complex mathamatically as it must also consider electroosmotic mobility, band broadening due to diffusion and other factors such as injector and detector length [9]. Apart from diffusion, however, these factors will generally be independent of the chiral selector concentration and the optimum resolution would be expected to occur at a value of [C] similar to that which gives optimum separation.

This model was investigated by substituting into eqn. 5 some possible parameter values. Fig. 1 was generated with $\mu_1 = 2$ and $\mu_2 = 1$ using equilibrium constants of $K_1 = 100$ and $K_2 = 105$ and $K_1 = 100$ and $K_2 = 110$. The graph shows that the apparent mobility difference reaches a maximum value as the chiral selector concentration is increased, before decreasing at higher chiral selector concentrations. The size of the difference in the apparent electrophoretic mobilities is greater the larger the difference in the equilibrium constants.

Fig. 2 shows the graphs generated by using $\mu_1 = 2$ and $\mu_2 = 1$ with three sets of equilibrium constants. The pairs in the sets differ by the same percentage value but have different absolute values. In each instance the resultant maximum apparent mobility difference is the same but the chiral selector concentration required to produce it is different. The greater the affinity of the enantiomers for the selector (the greater the equilibrium constants), the lower is the optimum selector concentration. This result is important as it indicates that the optimum concentration of chiral selector will be compound
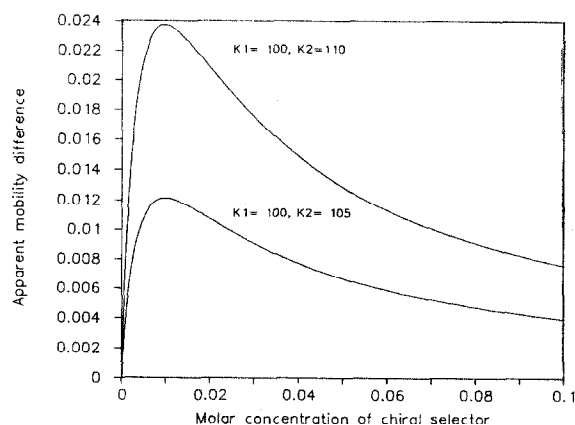

Fig. 1. Theoretical curves generated from eqn. 5 using $\mu_1 = 2$ and $\mu_2 = 1$ with the equilibrium constants $K_1 = 105$ and $K_2 = 105$ and $K_1 = 100$ and $K_2 = 110$.
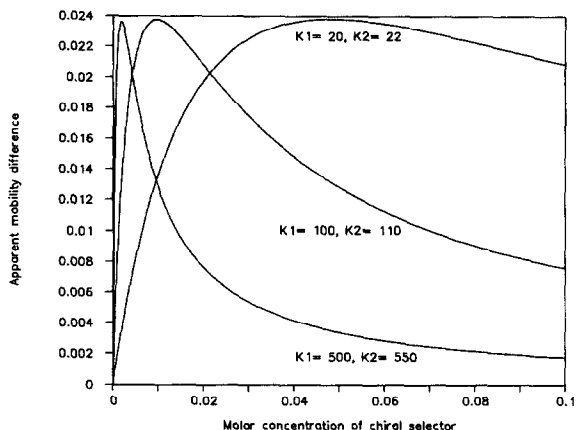
Fig. 2. Theoretical curves generated from eqn. 5 using $\mu_1 = 2$ and $\mu_2 = 1$ with three sets of equilibrium constants as shown.

dependent. For compounds that have a very high affinity for the chiral selector, the optimum concentration of chiral selector may well be much lower than the values of tens of millimoles typically mentioned in the previous references.

Fig. 3 shows the effect of keeping the analyte mobility constant and varying the apparent mobility of the analyte–chiral selector complex. It indicates that the apparent mobility difference between the two enantiomers will be greatest when the mobility of the analyte–chiral selector complex is in the opposite direction to that of the analyte itself. This
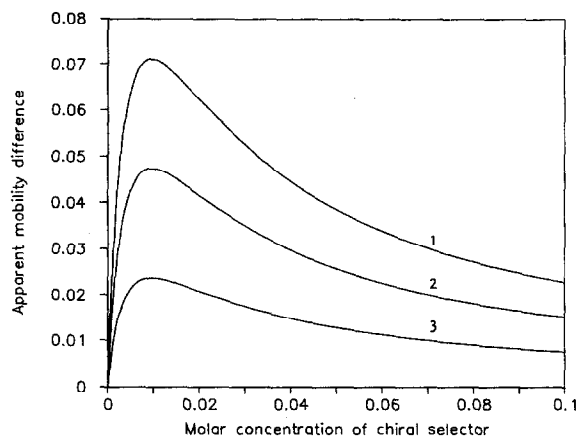

Fig. 3. Theoretical curves generated from eqn. 5 using three sets of mobility values with the equilibrium constants $K_1 = 100$ and $K_2 = 110$. Curves: (1) $\mu_1 = 2, \mu_2 = -1$; (2) $\mu_1 = 2$, $\mu_2 = 0$; (3) $\mu_1 = 2$, $\mu_2 = 1$.

suggests that chiral selectors which carry a charge opposite to that on the analyte will be useful.

The optimum concentration of chiral selector can be found from eqn. 5 by the use of differential calculus. It occurs when $d\Delta\mu/d[C] = 0$, and this condition exists when $(K_2 - K_1)(\mu_1 - \mu_2)$ $(1 - K_1K_2[C]^2) = 0$, i.e., apart from the trivial solutions when

$$[C] = \frac{1}{\sqrt{K_1 K_2}} \tag{6}$$

A knowledge of the size or likely size of the equilibrium constants will therefore be of great use in selecting the correct concentration of the chiral selector. The equilibrium constant K_2 will be some ratio of K_1, i.e., $K_2 = nK_1$. Combining eqns. 5 and 6 will give the value of the apparent mobility difference at the optimum concentration of chiral selector:

$$\Delta\mu = \frac{(n - 1)(\mu_1 - \mu_2)}{(\sqrt{n} + 1)^2} \tag{7}$$

This equation confirms the visual inferences from Figs. 1–3: the maximum apparent mobility difference between the two enantiomers will be large if the percentage difference between K_1 and K_2 is large and the mobility difference between the analyte and analyte–chiral selector complex is large.

BACKGROUND

To check the model, it was decided to examine the separation of the enantiomers of propranolol (1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol) by the use of β-cyclodextrin (BCD) and "methyl"-β-cyclodextrin (see Experimental) (MeBCD). These systems were chosen because of the good solubility of the components in water–urea or water and the results of previous work. Nuclear magnetic resonance data [10] had also shown that propranolol is included in the hydrophobic cavity of BCD and that the exchange of propranolol between the bulk solution and the cavity was rapid. Previous CE work [5,6] had shown that the resolution of the enantiomers was dependent on the concentration of BCD used and the concentration of methanol in the buffer. The work was carried out at a low pH in order to reduce the electroosmotic mobility.

EXPERIMENTAL

Experiments were carried out on PACE 2100 and PACE 2000 systems (Beckman Instruments, High Wycombe, UK). The separation capillary was fused silica with an I.D. of 75 μm, a total length of 57 cm and a length of 50 cm from the inlet to the detector. The samples were loaded by a 2-s pressure injection and separated at 25°C using a voltage of 20 kV. The data were recorded at 200 nm using a 2-Hz collection rate. Viscosity was measured using a Bohlin (Huntingdon, UK) VOR rheometer.

BCD and (R)-(+)-propranolol were obtained from Sigma (Poole, UK), lithium hydroxide from FSA Laboratory Supplies (Loughborough, UK), orthophosphoric acid from BDH (Poole, UK) and urea from Aldrich (Gillingham, UK). Racemic propranolol was made at ICI Pharmaceuticals (Macclesfield, UK) and MeBCD was a gift from Wacker Chemicals (Halifax, UK). The latter material had the 2-, 3- and 6-hydroxy groups partially substituted with methoxy groups, the average degree of substitution being 1.8. Lithium phosphate solution was prepared by adjusting the pH of a 50 mM solution of lithium hydroxide to 3.0 with orthophosphoric acid, followed by helium degassing.

The MeBCD buffers were all 40 mM in lithium phosphate (in order to reduce the current and hence power) and were prepared by mixing lithium phosphate, 370 mM MeBCD and water in the appropriate proportions to give ten buffers ranging from 0 to 74 mM in MeBCD. The BCD buffers were also 40 mM and were prepared from 50 mM lithium phosphate in 4 M urea, 80 mM BCD in 4 M urea and 4 M urea. The two most concentrated solutions were prepared directly from BCD and the other components. The buffers were measured at pH 3.12, degassed in an ultrasonic bath for 15 min, and filtered through a 0.2-μm Anotop filter (Anotec Separations, Banbury, UK). For the MeBCD work propranolol was dissolved in water at 0.05 mg ml^{-1}. The electroosmotic mobility was measured using a dilute propanone solution and was found to be very low, <0.04 · 10^{-4} cm^2 V^{-1} s^{-1}; it was therefore ignored in the calculations. For BCD propranolol was dissolved at 0.01 mg ml^{-1} in water.

Electrophoretic mobility was determined using the equation

$$\mu_{ep} + \mu_{eo} = \frac{lL}{Vt} \tag{8}$$

where μ_{ep} and μ_{eo} are the electrophoretic and electroosmotic mobilities, respectively, l is the capillary length to the detector and L is the total length, V is the operating voltage and t is the migration time. Duplicate propranolol injections were made at each buffer concentration and the average mobility value was used. The difference between the values from the two duplicates was typically 3%.

RESULTS AND DISCUSSION

Methyl-β-cyclodextrin

The separations of racemic propranolol achieved at six of the ten MeBCD concentrations are shown in Fig. 4. Clearly the degree of separation is dependent on the concentration and passes through a maximum value, as expected from the theory. By spiking a solution of (R)-(+)-propranolol into the original racemate, the (R)-(+)-enantiomer is shown to have the longer migration time and hence the greater affinity for MeBCD. The peaks tail due to overloading [11] but the efficiencies are still high with 115 000 theoretical plates being a typical value. There is no decrease in efficiency on adding MeBCD to the buffer. This supports the view that the exchange of propranolol between free solution and MeBCD complexation is rapid.

There is a significant difference in the heights of the peaks due to the two enantiomers. When peak areas are considered, however, the amounts become much closer to 50:50, and when the areas are divided by migration times, even closer. The migration times increase with MeBCD concentration. This has two causes: propranolol spends more time as the slowly moving propranolol–MeBCD complex and the buffer viscosity increases with increasing cyclodextrin concentration.

The buffer visosity will affect the mobility of all the ionic species and hence the resultant current. The adjusted electrophoretic mobility of propanolol may therefore be determined by multiplying the experimentally determined value by the ratio of the current at zero MeBCD concentration by that at the concentration of interest.

The relative viscosity values determined from measuring the current agree well with those determined by direct measurement. For the buffers without MeBCD and with 75 mM MeBCD the current values are 55.5 and 42 μA, giving a ratio of 1.32.
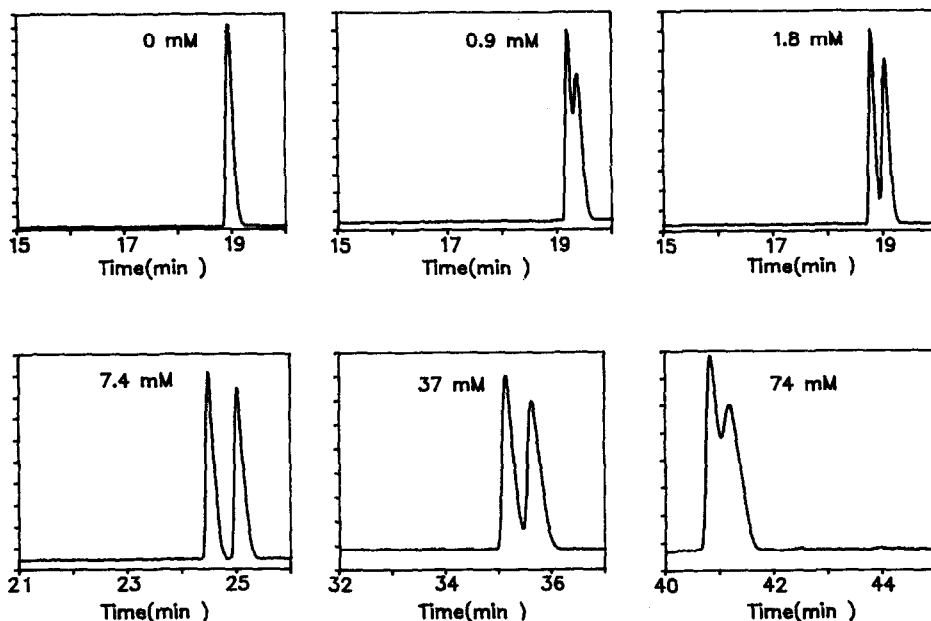
Fig. 4. Change in separation of propranolol enantiomers as the concentration of MeBCD is varied.

By direct measurement at 25°C the viscosities are 0.965 and 1.300 pascal seconds, giving a ratio of 1.35. At 35°C the relative viscosity by direct measurement is 1.34. The adjustment mentioned above was used to determine the apparent electrophoretic mobility of the (S)-(−)-enantiomer and the results are shown in Fig. 5. Initially the mobility decreases quickly before tending towards a limiting value at high MeBCD concentration. This reflects the fact that the propranolol spends an increasing amount of time complexed to MeBCD rather than in free solution.
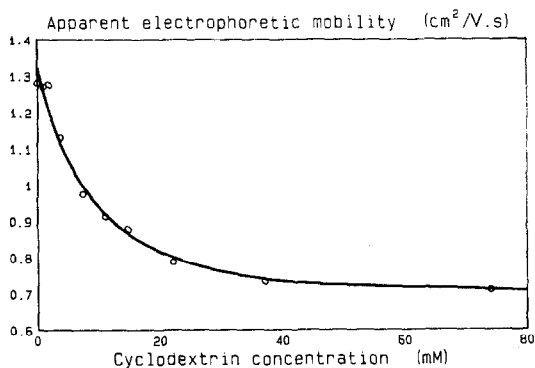
In Fig. 6 the apparent mobility difference between the two enantiomers is plotted as a function of MeBCD concentration. The graph is very similar to the theoretical plots in Figs. 1–3, which lends strong support to the model proposed in eqn. 5. The optimum MeBCD concentration is ca. 5.5 mM, which implies an average value of the two equilibri-

Fig. 5. Apparent electrophoretic mobility of (S)-(−)-propranolol as a function of MeBCD concentration.
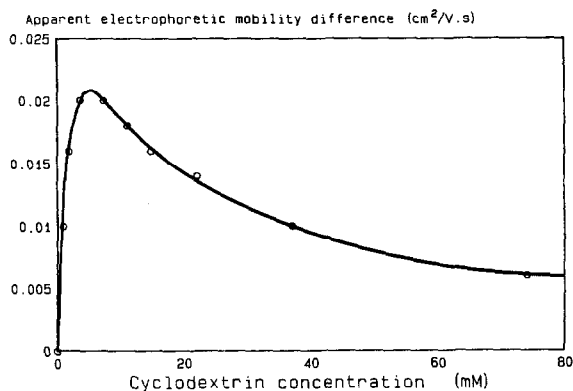

Fig. 6. Apparent electrophoretic mobility difference between the propranolol enantiomers as a function of MeBCD concentration. The apparent mobility difference was obtained via the times for the individual enantiomers after adjustment. The R form was identified by spiking (R)-(+)-propranolol into the original racemate.

um constants K_1 and K_2 of *ca.* 180 mmol^{-1}. The maximum mobility difference between the two enantiomers is *ca.* 0.02 cm^2 V^{-1} s^{-1}; the use of this value and values of μ_1 and μ_2 of 1.3 and 0.7 (from Fig. 5) implies that the two equilibrium constants differ by about 12%.

The resolution between the enantiomers is measured by the use of the equation

$$R_s = \frac{2.354\,(t_2 - t_1)}{(W_a^{\frac{1}{2}} + W_b^{\frac{1}{2}})} \tag{9}$$

where t_1 = migration time of enantiomer 1 and $W_a^{\frac{1}{2}}$ = peak width at half-height of enantiomer 1. The resultant graph is shown in Fig. 7.

As expected from Fig. 6, showing change in separation, the resolution is strongly dependent on MeBCD concentration. The optimum resolution occurs at *ca.* 4 m*M*, slightly lower than the concentration for optimum separation. The maximum value resolution is *ca.* 1.8. This was achieved in spite of overloading and compares favourably with the value of 1.4 from the use of two 25-cm β-cyclodextrin-bonded columns in series [12].

β-Cyclodextrin

The variation in the apparent electrophoretic mobility difference betweeen the two enantiomers as the BCD concentration is varied is shown in Fig. 8. The shape of the curve is again that expected on theoretical grounds. The maximum separation
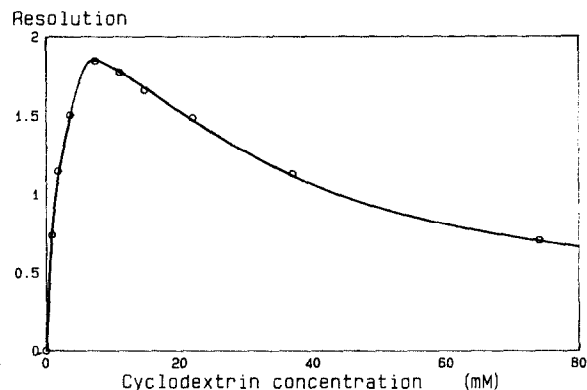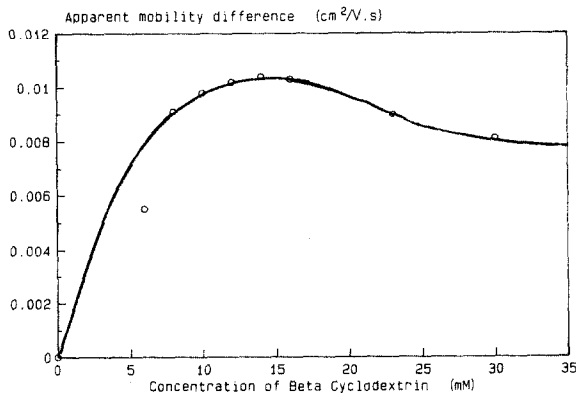

Fig. 8. Apparent electrophoretic mobility difference between the propranolol enantiomers as a function of BCD concentration.

achieved is shown in Fig. 9. A comparison with the work with MeBCD shows the optimum cyclodextrin concentration to be significantly higher and the maximum apparent mobility difference to be significantly lower. This indicates that propranolol has a lower affinity for BCD than MeBCD and that the difference between the stabilities of the two cyclodextrin–enantiomer complexes is lower. The reason for this is uncertain but it mirrors the results of Fanali's work with terbutaline [5].

Fig. 7. Resolution between the propranolol enantiomers as a function of MeBCD concentration.
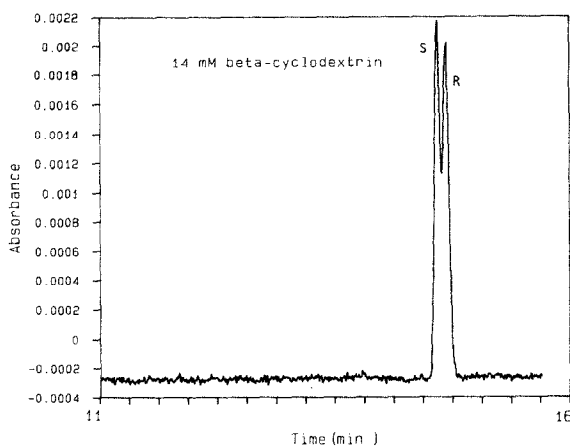

Fig. 9. Separation between propranolol enantiomers at the optimum BCD concentration.

CONCLUSIONS

A simple model for the separation of enantiomers in CE is presented. The model is of use in the choice of chiral selector concentration and is strongly supported by work on propranolol using β-cyclo-dextrin. Further work is in progress to investigate the role of the organic solvent in the buffer and to check the applicability to other chiral molecules.

REFERENCES

1 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 234.
2 R. O. Cole, M. Sepaniak and W. L. Hinze *J. High Resolut. Chromatogr.*, 13 (1990) 579.
3 K. Otsuka and S. Terabe, *J. Chromatogr.*, 515 (1990) 221.
4 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
5 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
6 G. Persson, S. Palmarsdottir, A. Walhagen and L. E. Edholm, presented at the *15th International Symposium on Column Liquid Chromatography, Basle*, June 3–7, 1991.
7 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 448 (1988) 41.
8 H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503.
9 X. Huang, W. F. Coleman and R. N. Zare, *J. Chromatogr.*, 480 (1989) 95.
10 D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 25 (1987) 208.
11 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
12 D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. E. Beesley, *Science (Washington, D.C.)*, 232 (1986) 1132.