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# Theory of chiral separation in capillary electrophoresis

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ABSTRACT

The resolution of a pair of enantiomers in chiral capillary electrophoresis (CE) is described by the use of a simple model. The model shows the importance of maximising the electrophoretic mobility difference between the two enantiomers, and also the influence of electroosmotic mobility. The theory is supported by results obtained with ephedrine, atenolol and practolol.

## INTRODUCTION

The resolution attainable in any separation system is a function of both efficiency and selectivity. HPLC is commonly used in chiral separations but often suffers from the problems of poor selectivity and efficiency as well as high cost. There is therefore a great deal of interest in investigating the potential of CE which is known to be capable of generating high efficiencies and which, because of the small volumes of buffer required, should have much lower operating costs. Two recent examples of chiral CE work use (1) methyl β-cyclodextrin to determine epinephrine enantiomer ratios [1] and (2) a chiral crown ether to separate dopamine enantiomers [2].

Upon examination of the literature on chiral CE it was noted that there were trends in the change in resolution and selectivity as the concentration of chiral selector or organic solvent was varied. In two earlier papers [3,4] these trends were explained by the use of a model and the equations derived from it. In this work the model is applied to consider resolution rather than simple separation.

## BACKGROUND

The description of the theoretical relationships which govern resolution (R,) is an area which has recieved attention right from the early days of CE. Eqn. 1 for example is due to Terabe et *al.* [5]:

$$R_s = \left(\frac{V}{32\,D}\right)^{\frac{1}{2}} \cdot \left(\frac{l}{L}\right)^{\frac{1}{2}} \cdot \frac{\Delta\mu_{ep}}{(\bar{\mu}_{ep} + \mu_{eo})^{\frac{1}{2}}} \qquad (1)$$

where $V$ is the voltage, $D$ is the diffusion coefficient, $L$ is the total capillary length, I is the effective capillary length, $\Delta\mu_{ep}$ is the electrophoretic mobility difference, $\bar{\mu}_{ep}$ is the mean electrophoretic mobility, and $\mu_{eo}$ is the electroosmotic mobility.

This is an equation which assumes ideal behaviour. Other treatments cover refinements such as contributions to band broadening caused by the length of the injector plug and the length of the detector region [6]. Even more complex treatments would have to cover the influence of tailing or fronting peaks which arise from overloading the sample [7]. This work however limits itself to a consideration of the third term in eqn. 1, $R_3$, *i.e.*, that which covers the influence of the difference in electrophoretic mobility, the mean electrophoretic mobility, and the electroosmotic mobility.

In the introduction a model of chiral CE was mentioned. The model assumes that the two enantiomers and the chiral selector are in rapid equilibrium with an enantiomer-chiral selector complex which has a different electrophoretic mobility to that of the free enantiomers. The apparent electrophoretic mobility of the free enantiomers is therefore a reflection of the proportion of time that they are free and the proportion that they are complexed to the chiral selector. The apparent electrophoretic mobil-

ity of the first enantiomer (a) is described by eqn. 2:

$$\bar{\mu}_a = \frac{\mu_1 + \mu_2 K_1 [C]}{1 + K_1 [C]} \quad (2)$$

where $\mu_1$ is the electrophoretic mobility of the free enantiomers, $\mu_2$ is the electrophoretic mobility of the enantiomer-chiral selector complex (assumed to be the same for both enantiomers as a first approximation), [C] is the concentration of chiral selector, and $K_1$ is the equilibrium constant. Fig. 1 shows the results obtained from eqn. 1 by using the values $\mu_1 = 2 \cdot 10^{-4}$ cm$^2$/V s (a typical value for a small drug molecule), $\mu_2 = 1 \cdot 10^{-4}$ cm$^2$/V s (a value chosen to reflect the lower electrophoretic mobility of a complex formed between a drug and neutral chiral selector), the chiral selector concentration range 0–0.1 *M* and equilibrium constants of $K_1 = 20$ and $K_1 = 100$. Fig. 1 shows that the graphs have the same general shape of tending to the limiting value of $\mu_2$ but that the greater the value of $K_1$ the steeper the gradient. The apparent electrophoretic mobility of the second enantiomer (b) can be described by an equation to similar to eqn. 2 but which has a different equilibrium constant, i.e.,

$$\bar{\mu}_b = \frac{\mu_1 + \mu_2 K_2 [C]}{1 + K_2 [C]} \quad (3)$$

Thus $\Delta\mu_{ep}$ is the difference between eqns. 3 and 2 and $\bar{\mu}_{ep}$ is the average of them. It was shown in earlier work [3] that the apparent electrophoretic mobility difference, $\Delta\mu_{ep}$, is a function of the chiral selector concentration and is maximised when the chiral selector concentration is inversely proportional to the square root of the product of $K_1$ and $K_2$. The third term in eqn. 1 can therefore be modelled by the use of eqns. 2 and 3 and values for the electroosmotic mobility. Fig. 2 shows the curves obtained by the use of the values $K_1 = 100$, $K_2 = 110$, $\mu_1 = 2 \cdot 10^{-4}$ cm$^2$/V s, $\mu_2 = 1 \cdot 10^{-4}$ cm$^2$/V s, and $\mu_{eo} = 0$, 1 and $5 \cdot 10^{-4}$ cm$^2$/V s. The curves show that while initially resolution is expected to increase with increasing chiral selector concentration, a plateau will be reached and further concentration increases will lead to a decrease in resolution. From Fig. 2 it is also clear that for cationic analytes (where $\mu_1$ and $\mu_2$ have the same sign as $\mu_{eo}$) electroosmotic mobility will always have a detrimental effect on resolution and so should be minimised or eliminated if possible (e.g., by operating at a low pH). There are two other interesting conclusions that can be drawn from Fig. 2: (a) by comparison with ref. 3 the chiral selector concentration which gives maximum resolution is greater than that which gives the maximum electrophoretic mobility difference, and (b) as the electroosmotic mobility increases the maximum resolution occurs at slightly lower chiral selector concentrations. Both of these observations may be attributed to the influence of the $\bar{\mu}_{ep}$ term in the denominator: as the chiral selector concentration increases $\bar{\mu}_{ep}$ becomes smaller, but when $\mu_{eo}$ is large the change in $\bar{\mu}_{ep}$ is less important.

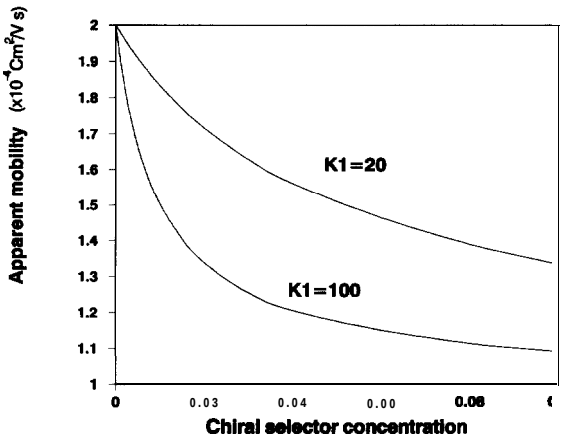


Fig. 1. Theoretical apparent electrophoretic mobility ($10^{-4}$ cm$^2$/V s) curves generated using the values $K_1 = 20$, and $K_1 = 100$, using the values $\mu_1 = 2\ 10^{-4}$ cm$^2$/V s and $\mu_2 = 1\ 10^{-4}$ cm$^2$/V s.

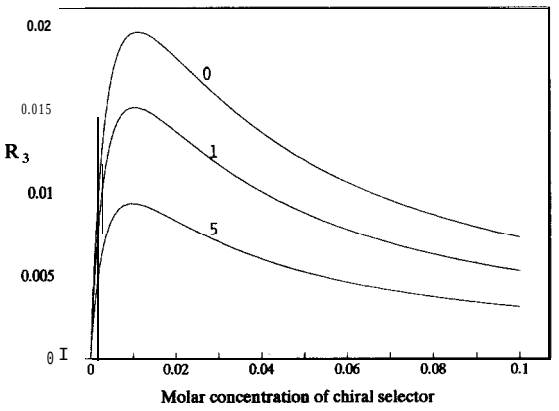


Fig. 2. Values of $R_3$ generated using the values $K_1 = 100$, $K_2 = 110$, $\mu_1 = 2 \cdot 10^{-4}$ cm$^2$/V s and $\mu_2 = 1\ 10^{-4}$ cm$^2$/V s with $\mu_{eo} = 0$ (curve 0), 1 (curve 1) and 5 $10^{-4}$ (curve 5) cm$^2$/V s.

In order to test these ideas it was decided to try and separate the enantiomers of ephedrine and those of the /?-blockers atenolol and practolol using methyl $\beta$-cyclodextrin (MeBCD) as the chiral selector. This derivatised cyclodextrin was used in preference to the parent $\beta$-cyclodextrin because of its greater solubility and favourable results of previous work. Atenolol and practolol are closely related structural isomers which differ in their hydrophobicity, atenolol has a log ***P*** value of 0.23 whereas practolol has one of 0.79 [8]. It is believed that cyclodextrins discriminate between enantiomers via inclusion into their hydrophobic cavity. On this basis therefore practolol would be expected to have a greater affinity for MeBCD than atenolol and therefore to have larger equilibrium constants. This means that the optimum MeBCD concentration should be lower for practolol than for atenolol. The work was carried out at a pH of 2.5 to reduce the level of electroosmotic mobility.

## EXPERIMENTAL

The results were obtained using a PACE 2100 system (Beckman, High Wycombe, UK) using a Beckman fused-silica capillary which had the dimensions: 50 $\mu$m internal diameter, 20 cm effective length and 27 cm total length. Atenolol and practolol were manufactured at Zeneca Pharmaceuticals and ephedrine was obtained from Sigma (Poole, UK). MeBCD was obtained from Wacker Chemicals (Halifax, UK) and had the 2, 3 and 6 hydroxy groups replaced by methoxy ones with an average degree of substitution of 1.8. The samples were dissolved in water at about 0.01 mg/ml and were loaded by a 3-s pressure injection. Separation was carried out at 15 kV and at 25°C with data collected at 200 nm and at 5 Hz. Eleven buffer systems were prepared all containing 50 m*M* lithium phosphate adjusted to pH 2.5 and a range of MeBCD concentrations from 0 to 100 m*M*. The buffers were degassed ultrasonically and filtered through a 0.2-$\mu$m filter.

## RESULTS AND DISCUSSION

The resolutions of ephedrine, practolol and atenolol enantiomers achieved at various concentrations of MeBCD are shown in Figs. 3, 4 and 5. The results are those expected from Fig. 2 with resolution initially increasing rapidly with MeBCD concentration but then reaching a maximum before declining at higher MeBCD concentrations. A comparison of the results for atenolol and practolol shows that, as expected from the log ***P*** data, the optimum MeBCD

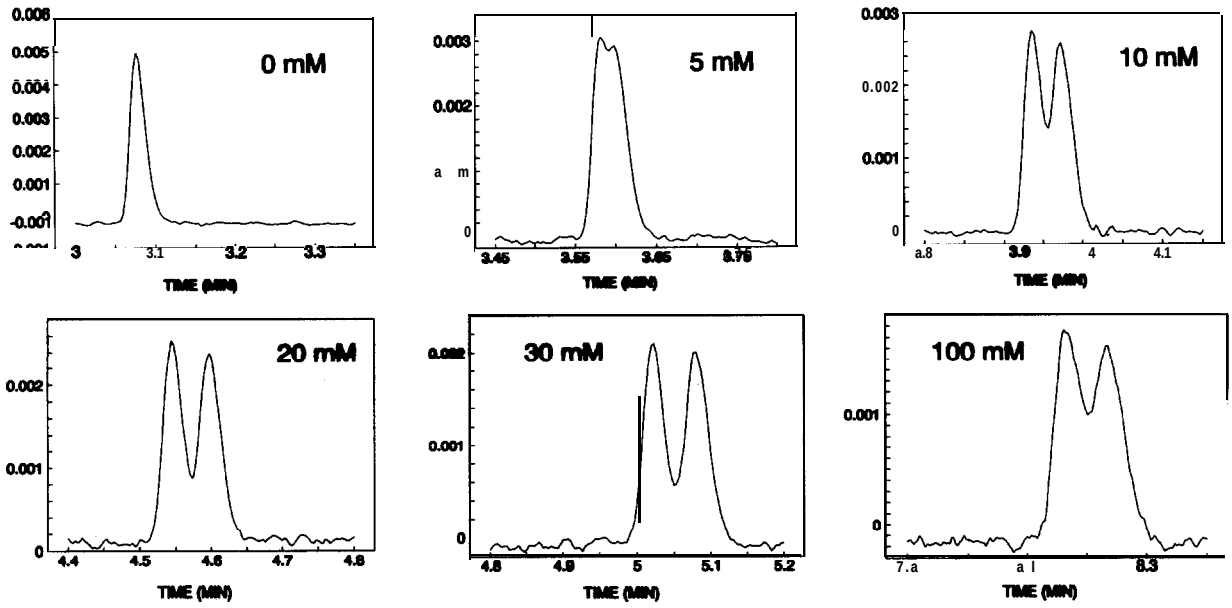


Fig. 3. Resolution of practolol enantiomers at MeBCD concentrations 0, 5, 10, 20, 30 and 100 m*M*.

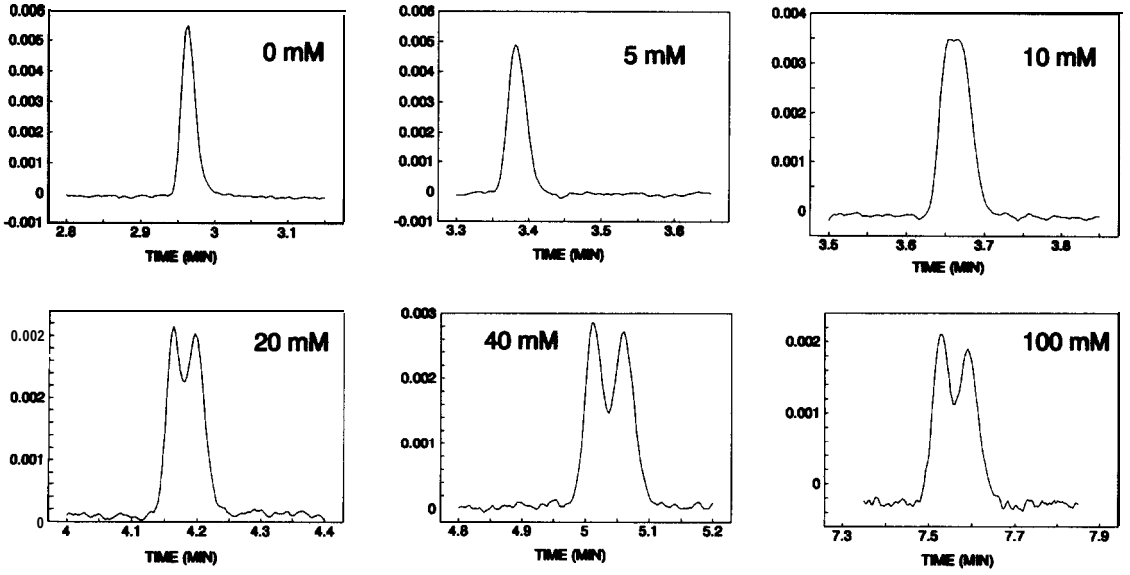


Fig. 4. Resolution of atenolol enantiomers at MeBCD concentrations 0, 5, 10, 20, 40 and 100 m*M*.

concentration for practolol is lower, *i.e.*, about 30 m*M* instead of 40 m*M*. The higher affinity of practolol for MeBCD is also shown by the results obtained at 5 m*M* MeBCD: the practolol enantiomers are just beginning to separate into two peaks whereas the atenolol peak is only slightly broadened.

Another interesting feature is that the maximum resolution for practolol is greater than that for atenolol. This implies that the percentage difference between $K_1$ and $K_2$ is greater for practolol than atenolol but it is not known why this should be. The maximum resolution for ephedrine is even higher.

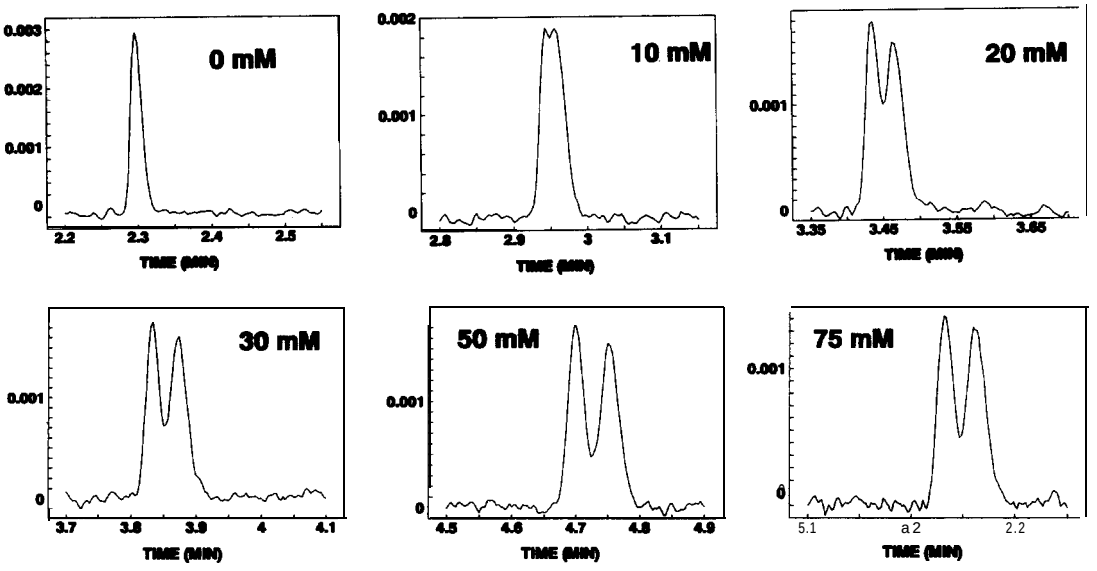


Fig. 5. Resolution of ephedrine enantiomers at MeBCD concentrations 0, 10, 20, 30, 50 and 75 m*M*.

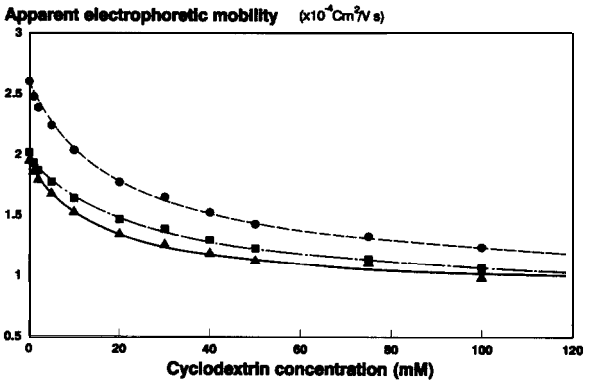


Fig. 6. Apparent electrophoretic mobility as a function of MeBCD concentration. ■ = Atenolol; A = practolol; ● = ephedrine.

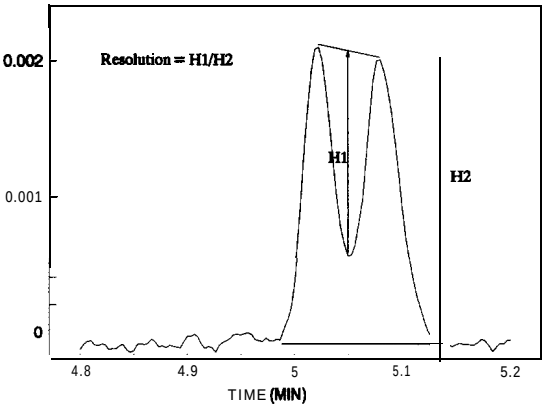


Fig. 8. Measurement of resolution by the height ratio method.

In Fig. 6 the apparent electrophoretic mobility of the fastest migrating enantiomer is shown for each of the compounds. The data are obtained from the migration times and are adjusted to compensate for increasing buffer viscosity at high MeBCD concentrations [3]. The general shape of the curves is the same as that seen in Fig. 1. The main difference between the curves is the steepness of the gradient with the curve for practolol falling away more quickly than that for atenolol.

Fig. 7 shows the apparent mobility difference between the two enantiomers for atenolol, practolol and ephedrine. The apparent mobility difference initially increases rapidly with MeBCD concentration but then levels off at a maximum before declining at higher MeBCD values. The difference between the curves is in their sharpness (a reflection of the absolute size of $K_1$ and $K_2$) and the size of the apparent mobility difference at the optimum MeBCD concentration (a reflection of the percentage difference between $K_1$ and $K_2$). The data have also been examined to measure the resolution between the two enantiomers as a function of the MeBCD concentration. Fig. 8 shows the ratio used for the measurement of resolution. This approach has been adopted in preference to that of measuring peak widths at half height because the latter is only applicable to well resolved peaks. The limitation on this height ratio method is that the maximum ratio is that of unity. The values obtained by this approach are shown in Fig. 9. The general shape of the curves is that seen in Fig. 2 although that for ephedrine

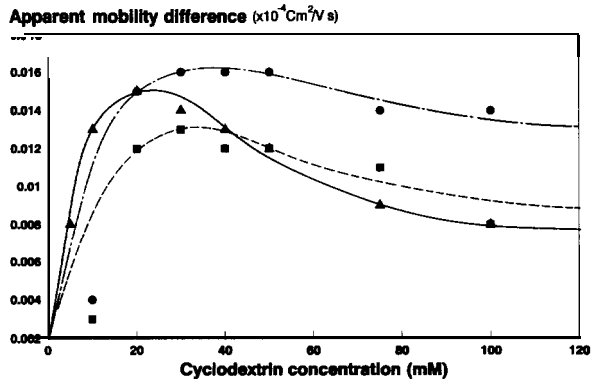


Fig. 7. The change in electrophoretic mobility difference ($10^{-4}$ $cm^2/V\,s$) with MeBCD concentration. ■ = Atenolol; A = practolol; ● = ephedrine.

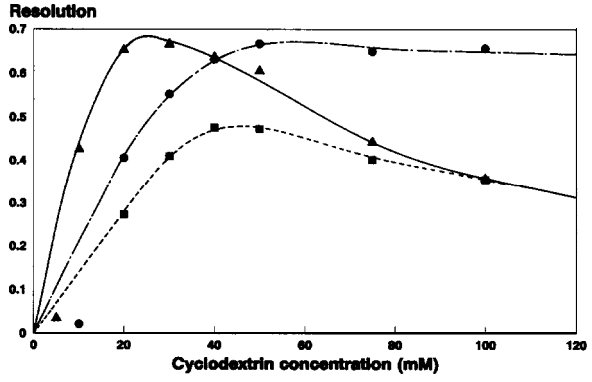


Fig. 9. Resolution as a function of MeBCD concentration. ■ = Atenolol; A = practolol; ● = ephedrine.

seems somewhat different and may warrant further investigation. The MeBCD concentration which gives maximum resolution is higher than that which gives the maximum apparent mobility difference. The maximum resolution of atenolol enantiomers for example occurs at about 50 m*M* MeBCD whereas electrophoretic mobility difference is maximised at about 30 m*M*. This result is in contrast to earlier work with propranolol [3] in which the optimum MeBCD concentration for resolution was below that for maximum electrophoretic mobility difference. In the propranolol case however the peaks tailed and this tailing was worse at longer migration times, and hence lower MeBCD concentrations were more favoured than they would otherwise have been.

## CONCLUSIONS

A model has been presented which describes resolution in chiral CE. The model is in agreement with new data obtained from the resolution of ephedrine, atenolol and practolol.

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