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Theoretical aspects of chiral separation in capillary electrophoresis

II. The role of organic solvent

Stephen A. C. Wren and Raymond C. Rowe

Pharmaceutical Department, ICI Pharmaceuticals, Macclesfield SK10 2NA (UK)

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ABSTRACT

Previous work in chiral capillary electrophoresis has shown that the addition of methanol to the buffer can lead to either an increase or decrease in separation. This behaviour is explained by a mathematical model. The proposed model is supported by new work on the chiral separation of propranolol enantiomers using buffer systems containing methanol or acetonitrile.

INTRODUCTION

Capillary electrophoresis (CE) is a rapidly expanding separation technique which has been successfully employed in a wide range of analytical problems. One area of great promise is the field of chiral analysis. In this area separation is achieved by the use of a range of chiral selectors which are added to the buffer [1–9] or trapped in a gel matrix [10].

An interesting feature seen in several of the reports is the variation in the degree of separation of the two enantiomers as the concentration of chiral selctor is varied. In particular the separation increases with chiral selector concentration until a maximum value is achieved. Further increases in chiral selector concentration result in a decline in separation. In an earlier paper we proposed a model which explains this type of behaviour [11]. This model was supported by experimental data on the separation of propranolol using β-cyclodextrin and a methyl substituted β-cyclodextrin.

Correspondence to: Dr. S. A. C. Wren, Pharmaceutical Department, ICI Pharmaceuticals, Macclesfield SK10 2NA, UK.

In several of the reports the addition of organic solvent to the buffer was also shown to have an important effect on the separation. The reason for this has been investigated in this paper by the use of the separation model mentioned above. In addition further work on the separation of the enantiomers of propranolol involving the addition of methanol and acetonitrile to the buffer is presented.

MODEL

In a previous paper [11] a model of the chiral separation process was proposed as a working hypothesis. The model is summarised below:

$$\begin{array}{ll} A \xrightarrow{\mu_1} & B \xrightarrow{\mu_1} \\ + & + \\ C & C \\ \Updownarrow K_1 & \Updownarrow K_2 \\ AC \xrightarrow{\mu_2} & BC \xrightarrow{\mu_2}; \end{array}$$

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 enantiomer–chiral selector complexes AC and BC. If the size of the chiral selector molecule is large in comparison to that of the analyte it seems likely that AC and BC will have similar size and shape, and hence, as a first approximation, the same electrophoretic mobility. If the exchange of A between the free and bound forms is very rapid then the apparent electrophoretic mobility of A, μ_a, will be a function of the proportion of the time A is free and the time it is complexed, *i.e.*

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

Manipulation of eqn. 1 and a similar expression which describes the apparent electrophoretic mobility of B leads to an equation which describes the apparent mobility difference between the two enantiomers (see eqn. 2).

$$\mu = \frac{[C](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \quad (2)$$

From eqn. 2 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 between these two extremes some value of [C] will give a maximum apparent mobility difference and hence a maximum separation of the two enantiomers.

Eqn. 2 can be investigated by the substitution of some plausible values for the equilibrium constants, the electrophoretic mobilities $\mu_1 = 2 \cdot 10^{-4}$ cm^2/V · s and $\mu_2 = 1 \cdot 10^{-4}$ cm^2/V · s and a chiral selector concentration range covering the values typically seen in the literature.

Fig. 1 was generated by this procedure and shows the apparent mobility difference as a function of chiral selector concentration for three sets of equilibrium constants. In each of these sets K_2 is 10% larger than K_1. It is clear that the optimum chiral selector concentration depends upon the size of the equilibrium constants. The greater the affinity of the analyte for the chiral selector the lower is the optimum concentration. This means that the best chiral selector concentration for one analyte is unlikely to be the best for another.

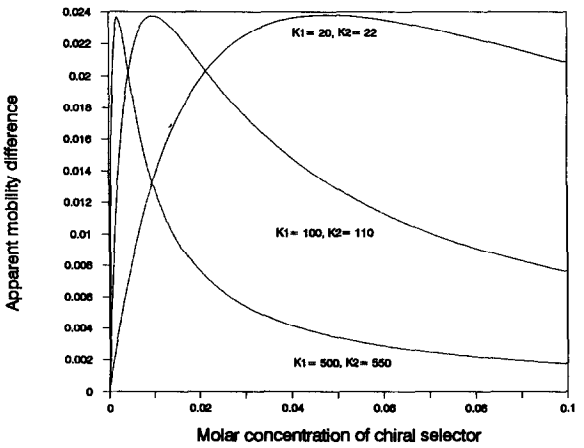

Fig. 1. Theoretical curves generated from eqn. 2 using $\mu_1 = 2 \cdot 10^{-4}$ cm^2/V · s, $\mu_2 = 1 \cdot 10^{-4}$ cm^2/V · s and three sets of equilibrium constants. From ref. 11.

BACKGROUND

Guttman *et al.* [10] used β-cyclodextrin in a gel matrix to separate the D and L forms of dansylated amino acids. They found that the selectivity changed when 10% of methanol was added to the buffer. For three of the amino acids the selectivity decreased whereas for the other nine it increased. The selectivity increase for the aromatic amino acids was noted in particular.

Fanali [5] used a buffer containing 40 m*M* of β-cyclodextrin to separate the enantiomers of the β-blocker propanolol. Methanol was added to the buffer in proportions ranging from 0 to 40%. The resolution at 0% was zero and the best value was obtained at 30%.

These two results can be examined with the aid of the proposed model.

With cyclodextrins it is assumed that the hydrophobic portion of the analyte sits inside the hydrophobic cavity. Therefore addition of methanol would be expected to reduce the affinity of the analyte for the cyclodextrin and increase it for the bulk buffer, *i.e.* to reduce the size of the equilibrium

constants K_1 and K_2. Whether this change in equilibrium constants leads to an increase or decrease in the apparent mobility difference (and hence separation) will depend upon whether the concentration of cyclodextrin is above or below the optimum value for the original system. This can be seen from Fig. 1.

Suppose for the first case we have a buffer with a chiral selector concentration of 40 mM and equilibrium constants of $K_1 = 500$ and $K_2 = 550$. If methanol were then added such that the new equilibrium constants were now $K_1 = 100$ and $K_2 = 110$ then the apparent mobility difference, and hence separation, would increase by about a factor of three.

In the second case suppose that we have a chiral selector concentration of 10 mM and the equilibrium constants for the two enantiomers were $K_1 = 100$ and $K_2 = 110$. If then another buffer was examined which contained the same chiral selector concentration but enough methanol such that the equilibrium constants were $K_1 = 20$ and $K_2 = 22$, then the apparent mobility difference would decrease to about half of the original value leading to a decrease in separation.

According to the model, therefore, in Fanali's [5] work the addition of methanol led to an increase in the separation of propranolol enantiomers as the concentration of β-cyclodextrin was above the optimum for a methanol free buffer.

The model also predicts that the addition of methanol could also lead to the opposite result, *i.e.* a *decrease* in separation of the propranolol enantiomers. This would happen if the original cyclodextrin concentration was at or below the optimum value for an organic solvent free buffer.

It was therefore decided to test this prediction by the examination of the separation of the enantiomers of propranolol (1-[(1-methylethyl)amino]-3-1-naphthalenyloxy)-2-propanol in buffers which contained different amounts of methanol or acetonitrile along with 40 mM lithium phosphate and 3.7 mM "methyl"-β-cyclodextrin (MeBCD). In a previous paper [11] it was shown that 3.7 mM MeBCD was below the concentration for the optimum separation.

EXPERIMENTAL

Experiments were carried out on PACE 2100 or PACE 2000 systems (Beckman Instruments, High Wycombe, UK). The separation capillary was fused silica with an internal diameter of 75 μm, a total length of 57 cm and a length of 50 cm from 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.

Racemic propranolol was made at ICI Pharmaceuticals, and MeBCD was a gift from Waker Chemicals (Halifax, UK). The MeBCD had the 2-, 3- and 6-hydroxy groups partially substituted with methoxy ones with the average degree of substitution being 1.8.

The buffers were all 40 mM in lithium phosphate (from lithium hydroxide and orthophosphoric acid) and were prepared by mixing stock solutions of 50 mM lithium phosphate at pH 3.0; 370 mM MeBCD in water; methanol or acetonitrile; and water in the appropriate proportions. The buffers were degassed ultrasonically and filtered through 0.2 μm filters. Propranolol was dissolved in water at 0.01 mg ml^{-1}.

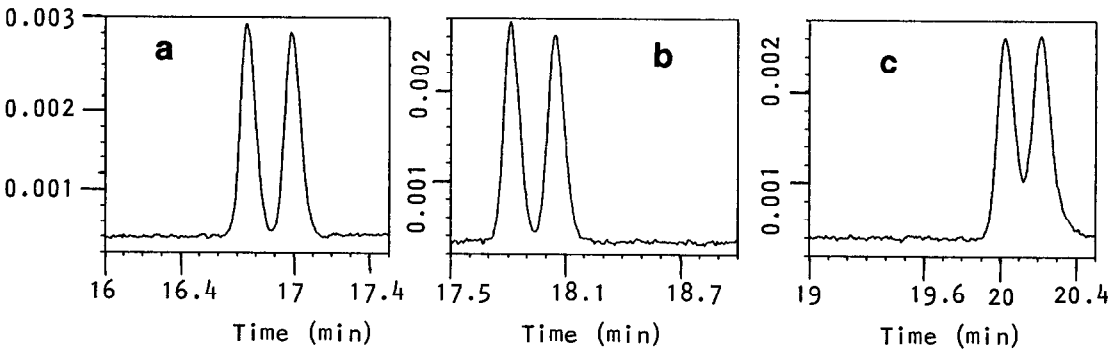

Fig. 2. The change in the separation of propranolol enantiomers with changing methanol concentration: (a) 0%, (b) 5%, (c) 19%.

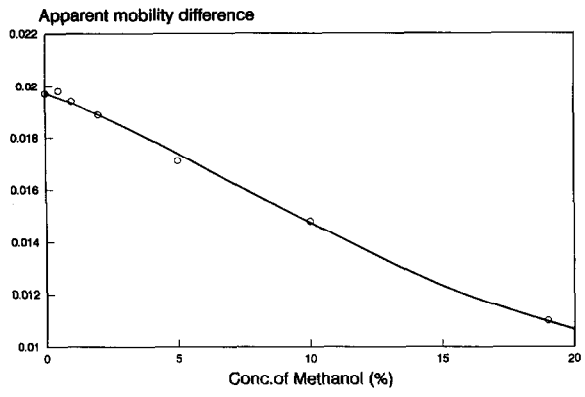

Fig. 3. The electrophoretic mobility difference (10^{-4} $cm^2/V \cdot s$) between propranolol enantiomers as a function of methanol concentration.

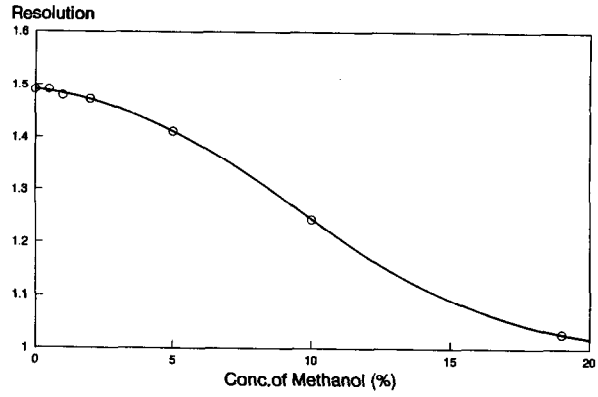

Fig. 4. The change in resolution between propranolol enantiomers as a function of methanol concentration.

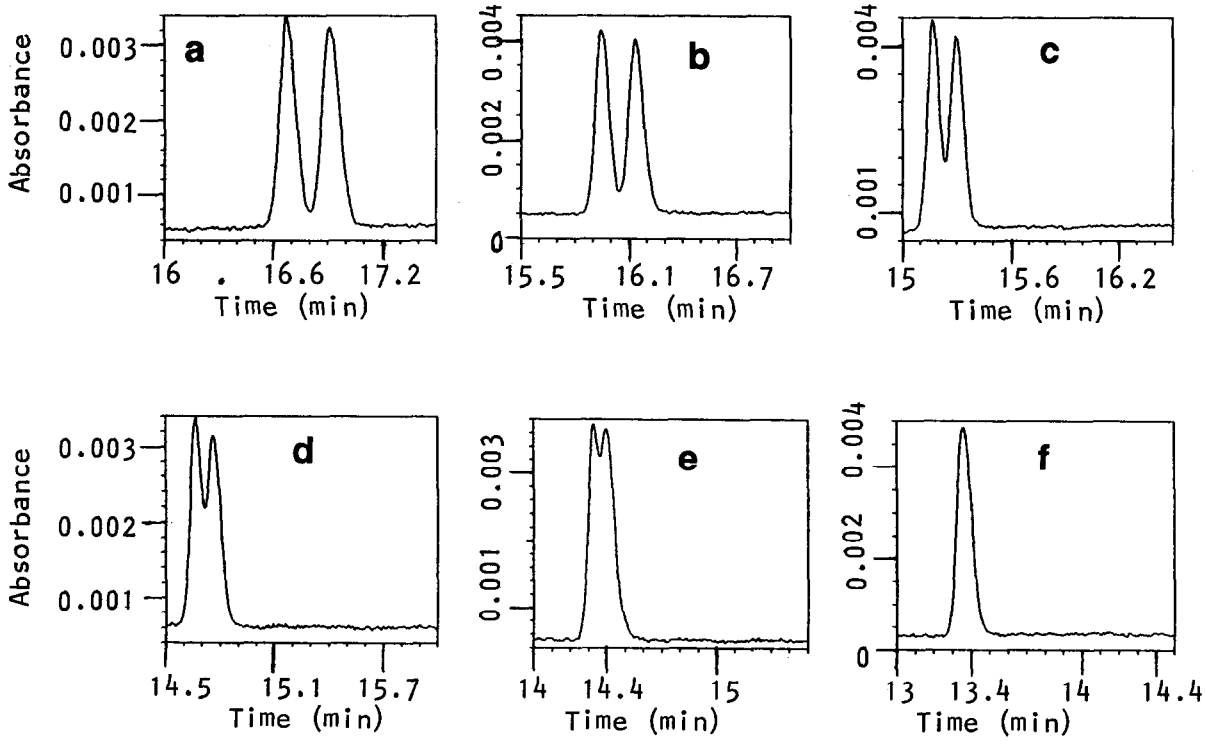

Fig. 5. The change in the separation of propranolol enantiomers with changing acetonitrile concentration: (a) 0%, (b) 2%, (c) 6%, (d) 8%, (e) 10%, (f) 15%.

Duplicate injections were made and the migration times typically varied by less than 1%.

RESULTS AND DISCUSSION

In Fig. 2 the separation at three of the seven methanol concentrations are shown. It is clear that, as expected from the proposed model, increasing the methanol content leads to a significant decline in the separation of the two propranolol enantiomers. The reason for this is the decline in apparent mobility difference. This can be seen in Fig. 3 where the measured apparent mobility difference is plotted against the methanol concentration.

The resolution (R_s) between the two propranolol enantiomers can be measured using eqn. 3.

$$R_s = 1.177 \times \frac{(t_2 - t_1)}{(W_{a\frac{1}{2}} + W_{b\frac{1}{2}})} \quad (3)$$

where t_1 = migration time of the first enantiomer and $W_{a\frac{1}{2}}$ = peak width at half height of the first enantiomer.

Fig. 4 shows how the resolution measured using eqn. 3 decreases from the baseline value of 1.5 at 0% methanol to a value of just over 1 at 19% methanol. Fig. 2 also shows that the migration times of both enantiomers increases with increasing methanol concentration.

The same experiments were also carried out using acetonitrile as the organic solvent instead of methanol. Acetonitrile is less polar than methanol and so might be expected to bring about a larger reduction in the equilibrium constants. The results obtained at six of the seven acetonitrile concentratuons are shown in Fig. 5. The trend is even more pronounced than that seen with methanol. As the acetonitrile concentration increases from 0 to 15% the resolution declines from baseline to zero. The measured apparent mobility difference is shown in Fig. 6 and from this it is clear that the loss of resolution is caused by the loss of apparent mobility difference. In contast to the results with methanol (Fig. 2) it can be seen from Fig. 5 that the addition of acetonitrile leads to a decrease in migration times. The reason for this is probably a combination of differences in viscosity [12] and the different amounts of time spent as free propranolol and the more slowly moving propranolol–cyclodextrin complex.

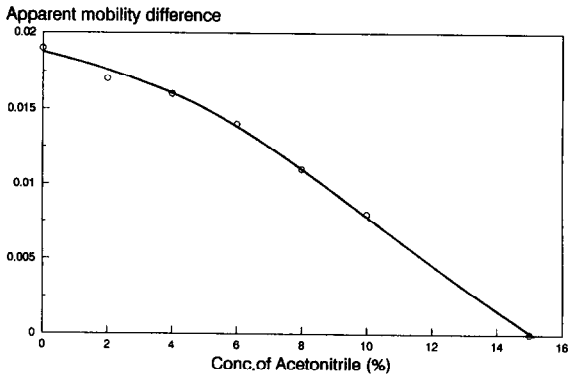

Fig. 6. The electrophoretic mobility difference (10^{-4} cm^2/V · s) between propranolol enantiomers as a function of acetonitrile concentration.

CONCLUSIONS

The influence of organic solvent on separation in chiral CE has been investigated using a simple mathematical model. The model was strongly supported by new work on the separation of propranolol in buffer systems containing methanol or acetonitrile.

Further work is under way to check the applicability of this model to other chiral molecules.

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