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Theoretical aspects of chiral separation in capillary electrophoresis

III. Application to P-blockers

Stephen A.C. Wren* and Raymond C. Rowe

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

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ABSTRACT

Enantiomers of the β-blockers propranolol, atenolol, metoprolol, and oxprenolol have been separated by the addition of methyl-β-cyclodextrin (MeBCD) to the operating buffer. There is an optimum concentration of MeBCD for separation for each of the &blockers, the magnitude of which could be ranked by the use of a mathematical model and log *P* data -an indication of the hydrophobicity of the molecule.

INTRODUCTION

Capillary electrophoresis (CE) is a rapidly growing area in the field of separation science. One aspect of current interest is the use of CE to perform chiral separations by the addition of chiral selectors to the buffer. Examples in the literature are: cyclodextrins [1–6], oligosaccharides [7], cyclic ethers [8,9], bile acids [10,11], chiral surfactants [12] and copper complexes using ligand exchange [13]. An alternative approach is to use a cyclodextrin bound in a gel matrix [14] or a capillary which is coated with a cyclodextrin stationary phase [15].

A noteworthy feature of several of the publications is that the degree of separation of the enantiomers is a function of the chiral selector concentration in the buffer. Fanali [2] working with terbutaline and using β-cyclodextrin and di-o-methyl-j3cyclodextrin as chiral additives found that while initially resolution increased with increasing cyclodextrin concentration, a point was reached beyond which further increases in concentration actually led to a decrease in resolution. Similarly, Kuhn et *al.* [8] found that the resolution of D- and L-DOPA was dependant upon the concentration of [18]-crown-6 tetracarboxylic acid in the buffer. Initially resolution increased strongly with concentration but then levelled off at a maximum value.

A similar pattern of behaviour was noted by Sepaniak et *al.* [6] who separated dansylated enantiomers of phenylalanine using hydroxy propyl-β-cyclodextrin. In two earlier papers [16,17], these observations were explained by the use of a mathematical model describing the separation process in chiral CE. The model was supported by new work on the separation of the enantiomers of the p-blocker propranolol using β-cyclodextrin and "methyl"-β-cyclodextrin (MeBCD). In this work the mathematical model has been extended further by the application of MeBCD to the separation of the B-blockers

* Corresponding author.

atenolol, metoprolol and oxprenolol and the results compared with those obtained for propranolol.

MODEL

The proposed mathematical model has been fully described in an earlier paper [16]. However it is important, in the context of this study to reiterate the key features.

The model is intended to cover the more simple situations where a freely soluble analyte interacts with a single chiral selector:

$$\begin{array}{lll} A \xrightarrow{\mu_1} & & B \xrightarrow{\mu_1} \\ + & & + \\ C & & C \\ \uparrow\downarrow K_1 & & \uparrow\downarrow K_2 \\ AC \xrightarrow{\mu_2} & & BC \xrightarrow{\mu_2} \end{array}$$

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 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 A is free and the proportion it is complexed, *i.e.*,

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

Eqn. 1 and a similar expression which describes the apparent electrophoretic mobility of B can be manipulated to produce an equation which describes the difference between the apparent electrophoretic mobilities of A and B, *i.e.*,

$$\Delta\mu_{apparent} = \frac{[C](\mu_1 - \mu_2)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \qquad (2)$$

Eqn. 2 is important as it is the difference in apparent electrophoretic mobilities which governs the separation between the two enantiomers.

The optimum concentration (c) of chiral selector is the one which maximises the apparent mobility difference and it can be found from eqn. 2 by the use of differential calculus. It occurs when

$$\frac{d\Delta\mu}{dc} = 0 \qquad (3)$$

It can be shown that in addition to the non useful solutions the condition in eqn. 3 is satisfied when:

$$c = \frac{1}{\sqrt{K_1K_2}} \qquad (4)$$

This is an important result because it predicts that no single chiral selector concentration will be ideal for all separations and that the optimum chiral selector concentration will vary from case to case according to the affinity of the analyte for the chiral selector.

BACKGROUND

The model presented predicts that there will be an optimum concentration of chiral selector and that the concentration will depend inversely upon the affinity of the analyte for the chiral selector. It was decided to check this by using the same chiral selector with a range of chiral analytes. The p-blockers atenolol, oxprenolol and metoprolol (Fig. 1) were selected on the basis of the successful results obtained in earlier work with propranolol which has been included as a comparison. The chiral selector chosen was "methyl"-β-cyclodextrin (MeBCD).

Unfortunately for these systems the values of the equilibrium constants, K_1 and K_2 are not available. Cyclodextrins are believed to act as chiral selectors via inclusion of enantiomers into their hydrophobic cavity. For a series of closely related analytes such as the @blockers it seems reasonable to assume that the size of the equilibrium constants will be related to the hydrophobicities of the analytes. In this case we would expect the values of the equilibrium constants to correlate with the values of the widely used

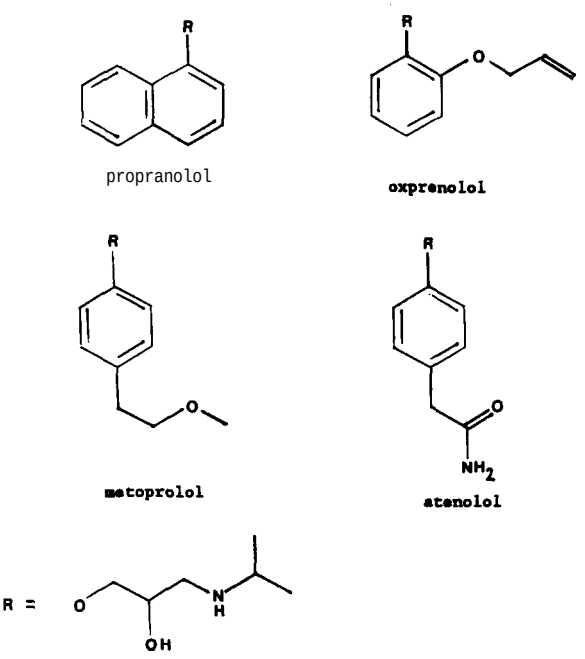

Fig. 1. The p-blockers propranolol, atenolol, metoprolol, and oxprenolol.

hydrophobicity measure, log *P* (octanol-water partition coefficient).

On the basis of the data in Table I it is to be expected that of the p-blockers, propranolol will have the greatest tendency to include into MeBCD and hence have the largest values of K_1 and K_2. Conversely atenolol should have the least tendency to include and so the smallest values of K_1 and K_2. From this analysis it follows that the optimum concentration of MeBCD will be least for propranolol and greatest for atenolol and will be intermediate for the other two molecules.

TABLE I

HYDROPHOBICITY OF DIFFERENT B-BLOCKERS [18]

P-Blocker	Log *P*
Atenolol	0.23
Metoprolol	2.15
Oxprenolol	2.18
Propranolol	3.65

EXPERIMENTAL

Work was carried out on a PACE 2100 system (Beckman Instruments, High Wycombe, UK). Using a fused-silica capillary (Beckman) with an internal diameter of 75 μm, a total length of 57 cm and a length of 50 cm from inlet to detector. Samples were loaded by a two-second pressure injection and separated at 25°C using a voltage of 20 kV. Data was recorded at 200 nm using a 2-Hz collection rate. Viscosity was measured using a Bohlin VOR rheometer (Huntingdon, UK). Propranolol and atenolol were manufactured at ICI Pharmaceuticals (Macclesfield, UK) and metoprolol and oxprenolol were obtained from Sigma (Poole, UK). Samples of the /?-blockers were dissolved in water at 0.01 mg ml^{-1} except for propranolol which was at 0.05 mg ml-'. MeBCD was a gift from Wacker Chemicals (Halifax, UK) and had the 2,3- and 6-hydroxy groups substituted by methoxy with an average degree of substitution of 1.8. Stock solution of lithium phosphate was prepared by adjusting a 50 m*M* solution of lithium hydroxide (FSA, Loughborough, UK) to pH 3.0 with orthophosphoric acid (BDH, Poole, UK).

The MeBCD solutions used were all 40 m*M* in lithium phosphate and were prepared by mixing in the appropriate proportions the following stock solutions: 50 m*M* lithium phosphate at pH 3.0; 370 m*M* MeBCD in water; and water. The ten solutions prepared ranged from 0 m*M* to 74 m*M* MeBCD and were degassed ultrasonically and filtered through a 0.2 μm filter.

Apparent electrophoretic mobilities were determined by using eqn. 5

$$\mu_{eph} + \mu_{eo} = \frac{lL}{Vt} \quad (5)$$

where l is the length to the detector, L is the total capillary length, V is the operating voltage, t is the migration time and μ_{eph} and μ_{eo} are the electrophoretic and electroosmotic mobilities. The electroosmotic mobility was found to be less than 0.04. 10^{-4} $cm^2/V \cdot s$ and was therefore ignored in the calculations. Duplicate injections of the &blockers were made at each of the MeBCD concentrations and the average mobility values used. The reproducibility between the duplicates was typically 3% or less.

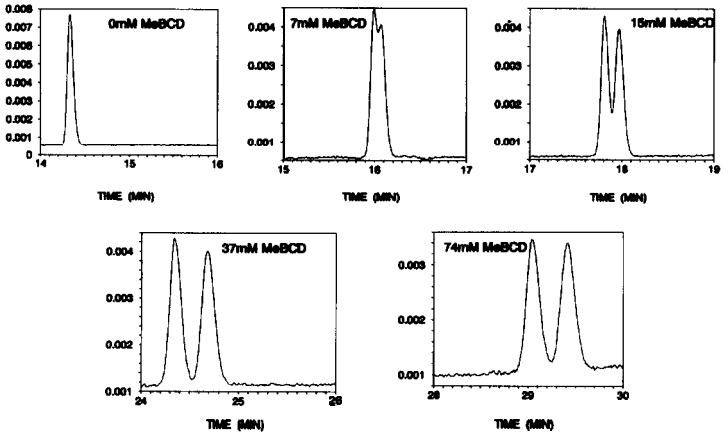

Fig. 2. Separation of atenolol enantiomers at different MeBCD concentrations.

RESULTS AND DISCUSSION

Fig. 2 shows the separation of the enantiomers of atenolol as the concentration of MeBCD is varied. The result is that expected from the theory: separation increases with increasing MeBCD until a maximum at around 37 m*M* MeBCD, with a further increase in MeBCD concentration resulting in a slight decline in separation. The efficiency is unaffected by the concentration of MeBCD with 175 000 theoretical plates being obtained both at 0 m*M* and 37 m*M* MeBCD. This supports the view that the exchange of drug between the free and bound forms is very rapid and does not lead to additional band broadening. In addition this value is between 1 and 2 orders of magnitude greater than that which might be expected from a chiral HPLC separation, indicating great promise for chiral CE.

From Fig. 2 it is clear that the apparent electrophoretic mobility of atenolol decreases with increasing MeBCD concentration. This has two causes: (i) atenolol spends more time as the more slowly moving inclusion complex, and (ii) the buffer viscosity increases with MeBCD concentration. The buffer viscosity affects the electrophoretic mobility of all species and hence the current. Because of this the viscosity affect mentioned in (ii) was compensated for by multiplying the measured apparent electrophoretic mobility, by the ratio of the current at 0 m*M* MeBCD over the current at the MeBCD concentration of interest. The adjustment factor ob-

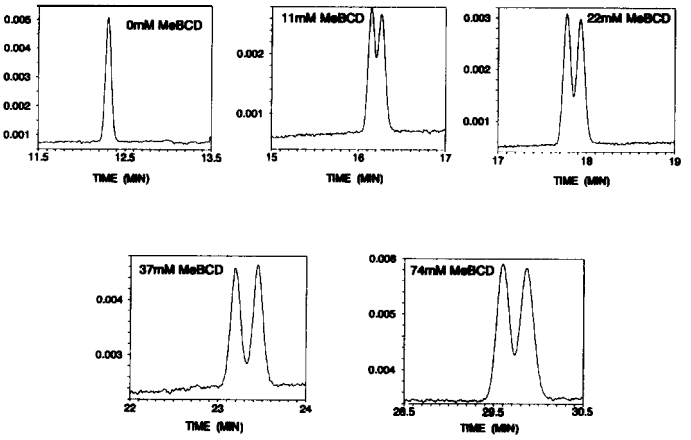

Fig. 3. Separation of oxprenolol enantiomers at different MeBCD concentrations.

tained is in close agreement with the value from the ratio of the absolute viscosities obtained using a rheometer. For example, the buffers containing 75 m*M* MeBCD and 0 m*M* MeBCD had relative viscosities of 1.32 by the current method, in comparison to a value of 1.34 obtained by rheology.

Fig. 3 shows the separations of oxprenolol enantiomers obtained at five of the MeBCD concentrations. The results follow the same pattern as that seen for atenolol and are those expected from the model proposed, with the separation increasing to a maximum at around 37 m*M* MeBCD before declining at higher MeBCD concentrations. The efficiency was again found to be independent of the MeBCD concentration.

The measured apparent electrophoretic mobility differences between the enantiomers as a function of MeBCD concentration are shown for each of the P-blockers in Fig. 4. The figure shows a number of interesting features. The general shape of the curves is the same as that expected from the theory (see ref. 16) and this therefore lends great support to the model proposed. The optimum separation occurs at different MeBCD concentrations for the different P-blockers, with the lowest concentration being required for propranolol and the highest for atenolol. The correlation between the log *P* of the P-blocker and the optimum concentration of MeBCD is that expected from the background discussion with compounds which are most hydrophobic (highest log *P*) requiring the least cyclodextrin. The exception to this general pattern is that of oxprenolol. The affinity of oxprenolol for MeBCD is similar to that of atenolol and is lower than expected. This may well be due to a shape factor with the 2-substituted oxprenolol fitting less well into the MeBCD cavity than the 4-substituted atenolol and metoprolol. Another interesting feature is that the degree of separation of the enantiomers at the optimum MeBCD concentration is different for the different /?-blockers. The order of maximum separation is: propranolol > atenolol > oxprenolol > metoprolol with the maximum separation of the propranolol enantiomers being approximately three times greater than the maximum separation of the metoprolol enantiomers. This indicates that the percentage difference between the equilibrium constants K_1 and K_2 is much smaller for metoprolol than for propranolol. This means that for metoprolol the *R* and *S* forms interact with MeBCD to very similar extents, whereas for propranolol the differences are larger. The reason for this is unknown.

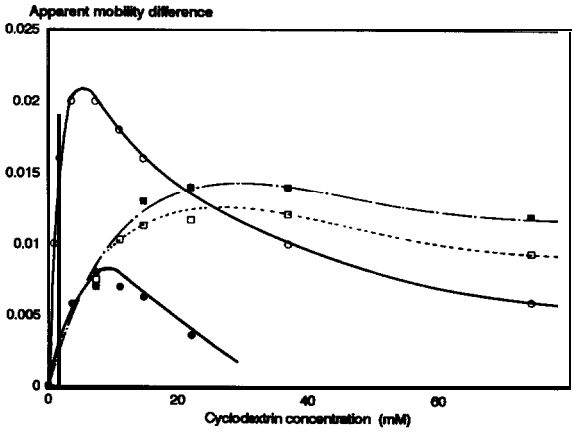

Fig. 4. Experimentally determined apparent mobility difference (10^{-4} cm^2/V·s) for the different β-blockers at different MeBCD concentrations. 0 = Propranolol; ■ = atenolol; ● = metoprolol; □ = oxprenolol.

CONCLUSIONS

Chiral separation of the enantiomers of P-blockers using MeBCD has been successfully explained by the use of a mathematical model, which correctly predicts an optimum MeBCD concentration. The size of the optimum MeBCD concentration correlates reasonably well with the log *P* value of the #I-blocker although factors such as molecular shape are also very important.

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