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Differential binding of tioconazole enantiomers to hydroxypropyl-β-cyclodextrin studied by capillary electrophoresis

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ABSTRACT

Capillary electrophoresis has been used to determine binding constants of tioconazole enantiomers with hydroxypropyl-β-cyclodexrin (HP-β-CD), after correcting for changes in mobility with increasing viscosity. The predicted and observed values of enantiomeric mobility difference were found to be maximum at a concentration of HP-β-CD equal to the reciprocal of the average binding constant.

INTRODUCTION

Capillary electrophoresis (CE) is an analytical technique that achieves separation of water-soluble analytes based on the principles of the electrically-driven flow of ions in solution, using a high-voltage potential [1,2]. The technique has been shown to be ideal for chiral separations when used in conjunction with chiral additives [3–5] which differentiate between enantiomers through dynamic equilibrium to form host–guest complexes. Previous studies using CE for chiral analysis have been limited to the achievement of satisfactory separation, though a recent paper has provided experimental and theoretical support for a simple model of optimisation of separation [6]. The present work shows how tioconazole enantiomers can be separated using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a chiral additive, and discusses the importance of an understanding of solution viscosity in quantitatively interpreting the data. Binding constants are obtained from measurements of viscosity and ionic mobilities as a function of HP-β-CD concentration.

Tioconazole (Fig. 1) is a broad spectrum imidazole antifungal agent used in the topical treatment of fungal infections [7]. A high-performance liquid chromatographic (HPLC) method has been optimised for separation of the enantiomers using a β-cyclodextrin chiral stationary phase [8]. Tioconazole has been shown by NMR to form a 1:1 complex with cyclodextrin [9].

In our CE study the use of HP-β-CD (Fig. 1) was found to be more informative than that of native β-cyclodextrin as the former has greater solubility and could be studied over the full range of complexation with weakly-binding substrates such as tioconazole. Optimum separations were obtained at pH 4.3 with 25% methanol (a 27.5% methanol–water eluent at pH 4.3 was previously found to be optimum in the HPLC separation).

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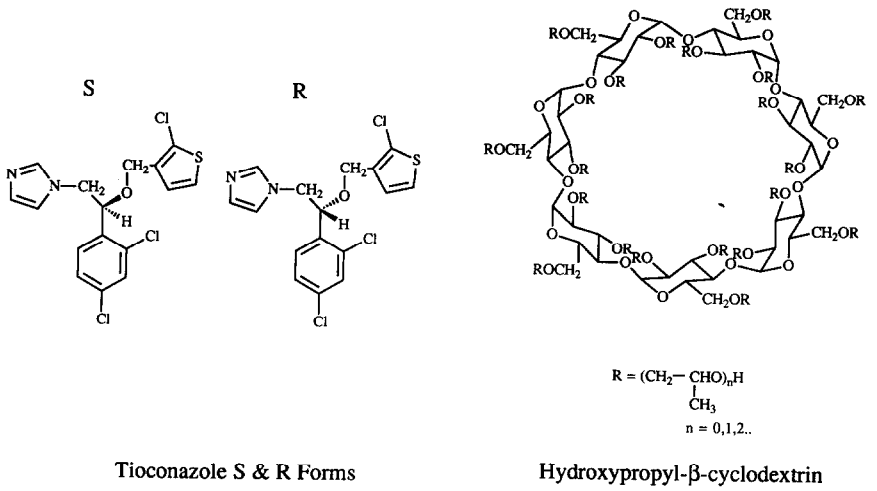

Fig. 1. Structures of tioconazole (*R*) and (*S*) forms, and structure of hydroxypropyl-*β*-cyclodextrin.

EXPERIMENTAL

Experiments were carried out on a P/ACE 2100 system (Beckman, High Wycombe, UK). The fused-silica separation capillary had an internal diameter of 50 μm, a total length of 57 cm and a length of 50 cm from inlet to detector. Detection was carried out using UV absorbance at 230 nm. The samples were loaded by a 1-s pressure injection (corresponding to an injected volume of 1 nl), from a 1-m*M* solution of tioconazole in run buffer. Each experiment was carried out in triplicate.

HP-*β*-CD (degree of substitution, $DS = 0.6$) was a gift from Wacker Chemicals (Halifax, UK). Tioconazole and its single enantiomers were provided by Pfizer Central Research. All other materials were from Aldrich (Gillingham, UK). The phosphate–citrate buffer was prepared by 5-fold dilution of a 0.1 *M* citric acid–0.2 *M* Na_2HPO_4 mixture with pH 4.3. Methanol was added in the ratio 25% methanol–buffer (25:75), giving a final ionic strength 0.03 mol kg^{-1}. HP-*β*-CD was dissolved at various concentrations in this buffer system.

Viscosity was measured using a semimicro Ubbelohde suspended-level capillary viscometer, with a bulb capacity of approximately 1.5 cm^3. This was held in a thermostatted water bath (Townson and Mercer, Croydon, UK) with temperature control to ±0.05°C. Viscosity was measured at 25.0°C, the temperature corresponding to the CE experiments. All the efflux times for the viscometer were greater than the required minimum of 100 s. An average of three or more efflux times for each concentration was recorded, and the viscosity of the solutions calculated relative to the CE run buffer without added HP-*β*-CD.

RESULTS AND DISCUSSION

Electropherograms at a series of HP-*β*-CD concentrations are given in Fig. 2. The (−) enantiomer of tioconazole was found to migrate first in the buffer system. Enantiomeric resolution of tioconazole was achieved a concentration of HP-*β*-CD of around 5 m*M*. At high concentrations of HP-*β*-CD (above 40 m*M*) the two enantiomers migrated together and there was no chiral separation, but the mobility continued to decrease with increasing HP-*β*-CD concentration suggesting that viscosity variation [10,11] was contributing to a change in migration time.

Relative viscosity, η_c/η_0, was determined using a thermostatted capillary viscometer. Viscosity increased with concentration in a power series as expected [12]. The curve fitted to the data has the form,

$$\eta_c/\eta_0 = 1 + 1.395\ c + 35.89\ c^2 \quad (1)$$

where c = HP-*β*-CD concentration. At 90 m*M* HP-*β*-D, η was found to be 40% greater than without any additive.

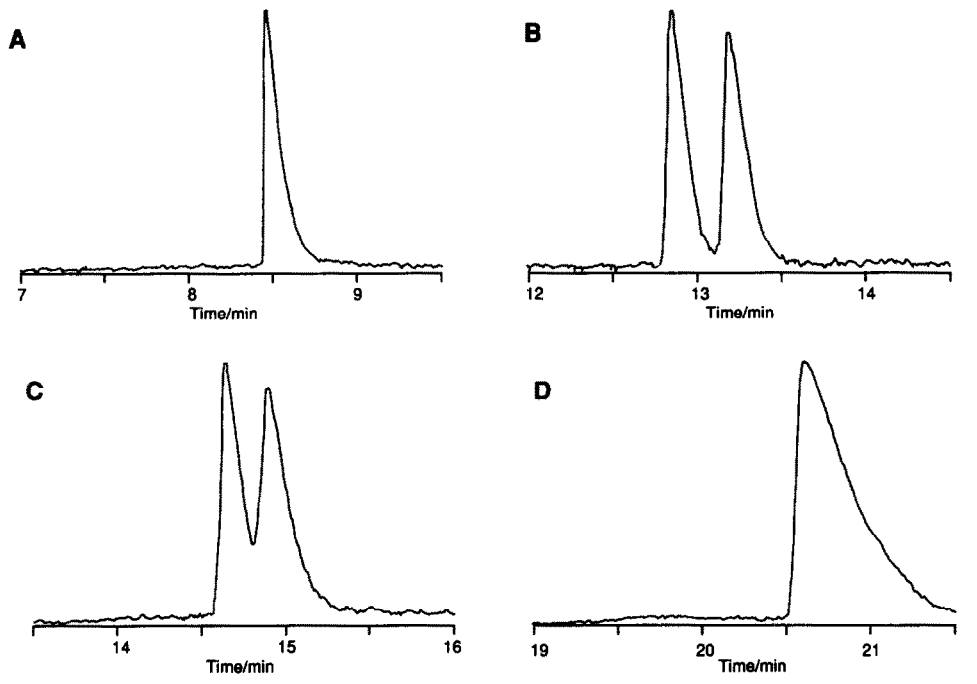

Fig. 2. Separation of tioconazole enantiomers with (A) no HP-β-CD in the buffer, (B) 10 m*M* HP-β-CD, (C) 20 m*M* HP-β-CD, (D) 60 m*M* HP-β-CD. Eluent: methanol–buffer (25:75); buffer: phosphate–citrate solution, pH 4.3; ionic strength 0.03 mol kg^{-1}. Apparatus: Beckman P/ACE 2100; capillary 50 μm I.D., 57 cm length; temperature, 25°C; detection at 230 nm.

Electrophoretic mobility, μ, was determined for tioconazole by subtracting the electroosmotic flow mobility obtained using mesityl oxide as neutral marker from the observed mobility, μ_{obs}. Electrophoretic mobilities corrected to $c = 0$ (μ') were obtained using η values from eqn. 1 (*cf.* ref. 6).

$$\mu'_c = \mu_c \eta_c / \eta_0 \quad (2)$$

Mobilities corrected in this way are given in Fig. 3. The dependence of μ'_c on HP-β-CD concentration was found to follow a binding equilibrium curve. Values of the binding constant, K, and the corrected mobility of the complex, μ'_∞, were established from a least-squares fit of the data in Fig. 3 to the function,

$$\frac{\mu'_0 - \mu'_c}{\mu'_c - \mu'_\infty} = Kc \quad (3)$$

The mobility of tioconazole ($\mu_0 = 1.42 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}) is greater than that of the tioconazole–HP-β-CD complex ($\mu'_\infty = 0.47 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}) because of its smaller size. Mobility is a function of the charge-to-size ratio, and at pH 4.3 both species bear a single positive charge since tioconazole has a pK_a of 6.5. Binding constants for the enantiomers are $K_{(-)} = 201 \pm 10\ M^{-1}$ and $K_{(+)} = 231 \pm 12$

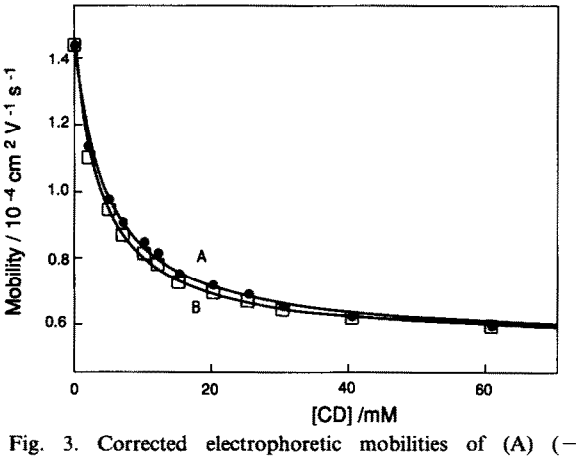

Fig. 3. Corrected electrophoretic mobilities of (A) (−) enantiomer, (B) (+) enantiomer as a function of HP-β-CD concentration. Data fitted to binding equilibrium curve, giving $K_{(-)} = 201 \pm 10\ M^{-1}$ and $K_{(+)} = 231 \pm 12\ M^{-1}$.

M^{-1}. When viscosity changes were not taken into account and the uncorrected data were fitted using μ_c and not μ'_c in eqn. 3, the binding constants were considerably lower and the variance χ^2 higher, suggesting a poorer fit of the data. This shows that it is imperative to correct for viscosity changes when determining binding constants from data for weak substrate binding to cyclodextrins. It should be noted that molecular modelling [9], which has been used to predict that the strongest binding to tioconazole will occur with β-CD in the series α-, β-, γ-cyclodextrin, would suggest a much higher binding constant than is observed.

By considering variation of μ'_c in eqn. 3 with K it may be shown that the mobility difference $\Delta\mu'$ for the enantiomers is dependent on ΔK, the difference of binding constants $K_{(-)}$ and $K_{(+)}$, via the relationship,

$$\frac{\Delta\mu'_c}{\mu'_0 - \mu'_\infty} = \frac{-\Delta K}{\bar{K}} \cdot \frac{\bar{K}c}{(1 + \bar{K}c)^2} \quad (4)$$

where $\bar{K}$ is the average binding constant. Eqn. 4 may be compared with that in ref. 6, and its use provides the most accurate method of determining the difference in binding constants between enantiomers. Fig. 4 plots both terms of eqn. 4 as a function of the variable log $\bar{K}c$, and gives $\Delta K = 34 \pm 4\ M^{-1}$ from the least-squares fit. Maximum value of the mobility difference occurs when the concentration is the reciprocal of the average binding constant, *i.e.*, $\bar{K}c = 1$, $c = 4.63$ mM. The theoretical analysis may readily be extended to show that the maximum value of the resolution [2], which is proportional to $\Delta\mu/\mu_{obs}$, occurs when

$$\bar{K}c = [(\mu_{eo} + \mu_0)/(\mu_{eo} + \mu_\infty)]^{1/2} \quad (5)$$

where μ_{eo} is the electroosmotic flow mobility. For tioconazole and its HP-β-CD complex at pH 4.3, $[(\mu_{eo} + \mu_0)/(\mu_{eo} + \mu_\infty)] = 2.06$, and for maximum resolution $c = 6.65$ mM.

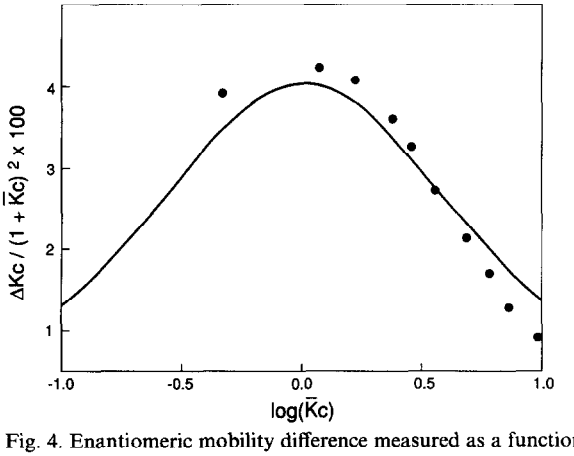

Fig. 4. Enantiomeric mobility difference measured as a function of HP-β-CD concentration (experimental points) superimposed upon a theoretical plot from which the binding constant difference ΔK is obtained. $\Delta K = 34 \pm 4\ M^{-1}$, from a least squares fit, with the average binding constant $\bar{K} = 216\ M^{-1}$.

CONCLUSIONS

The use of a CE apparatus to measure mobilities offers a very convenient way of determining binding constants of drugs to cyclodextrins. Differential binding of enantiomers is predicted and observed to be maximum when the cyclodextrin concentration is equal to the reciprocal of the average binding constant. Plans for future work include temperature dependence studies to allow enthalpy changes on binding to be calculated for individual enantiomers.

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REFERENCES

1 W. G. Kuhr, *Anal. Chem.*, 62 (1990) 403R.
2 R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr.*, 29 (1989) 1.
3 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
4 R. Kuhn, F. Stoecklin and F. Erni, *Chromatographia*, 33 (1992) 32.
5 K. D. Altria, D. M. Goodall and M. M. Rogan, *Chromatographia*, 34 (1992) 19.
6 S. A. C. Wren and R. C. Rowe, *J. Chromatogr.*, 603 (1992) 235.
7 S. Jevons, G. E. Gymer, K. W. Brammer, D. A. Cox and M. R. G. Leeming, *Antimicrob. Agents Chemother.*, 15 (1989) 597.
8 R. P. Tucker, A. F. Fell, J. C. Berridge and M. W. Coleman, *Chirality*, 4 (1992) 316.
9 M. Kinns, J. C. Berridge, M. W. Coleman, A. S. Roberts-McIntosh, M. S. Tute, A. F. Fell and R. P. Tucker, *Proc. 10th International Conference on NMR, St. Andrews, 1991.*
10 D. Y. Pharr, Z. S. Fu, T. K. Smith and W. L. Hinze, *Anal. Chem.*, 61 (1989) 275.
11 L. Paduana, R. Sartorio, V. Vitagliano and L. Costantino, *J. Solution Chem.*, 19 (1990) 31.
12 E. A. Moelwyn-Hughes, *Physical Chemistry*, Pergamon Press, Oxford, 2nd ed., 1961.