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Short Communication

Resolution of chiral compounds by HPLC using mobile phase additives and a porous graphitic carbon stationary phase*

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Introduction

The examination of drug enantiomers is currently accepted as one of the most important steps when studying the pharmacokinetic, pharmacodynamic and xenobiotic properties of drugs with chiral centres [1]. However, in order to study these properties, the initial step involves the development of a suitable method for their separation. High-performance liquid chromatography has played a large part in this work, for which a number of chiral stationary phases (CSPs) have been developed. Although these CSPs, which are characterized by having a chirally active molecule chemically bonded to the support material, are very effective for some separations, there are a number of drawbacks, including: cost, flow rate restrictions, stability in some cases [2], limitations in choice of mobile phase and pH stability. Additionally, CSPs are often limited in applicability and sometimes are only suitable for the resolution of particular classes of compounds.

As a useful alternative, when a chiral selector is introduced into the mobile phase used with an achiral column, it offers the advantage of flexibility, a wide range of possible additives and often lower cost compared with the equivalent CSP [3]. One of the most widely used chiral mobile phase additives is the cyclodextrin group (CDs). These are based on a defined number of glucopyranose sugars linked at the α_{1-4} position to give between six and 12 glucose units. However, only the α-, β- and γ-CDs containing six, seven and eight glucose units, respectively, are commercially available. The CD-molecule is characterized by a toroidal shape with a central hydrophobic cavity. On the outer rim of the cavity there are secondary hydroxyl groups capable of hydrogen bonding with a guest molecule. Enantiomers can be resolved by differential inclusion in the form of a so-called "host" and "guest" relationship, with the guest molecule sitting more or

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less tightly in the cavity of the host. Chiral resolution occurs through differences in the steric interaction and hydrogen bonding, conforming to the classical three-point interaction model. Of the three principal CDs (namely α, β and γ), β-CD is the most readily available and has found use in a number of pharmaceutical applications. This is due, at least in part, to the fact that the size of the hydrophobic cavity matches that of a number of drugs which have the molecular size of two fused benzene rings [4].

Most of the previous work with β-CD has involved bonded ODS-silica as the achiral stationary phase [5]. However, this material is subject to pH-range restrictions which limits the flexibility in choice of mobile phase pH. Over the last 5 years a number of less pH-sensitive stationary phases have been developed for conventional HPLC; these have the potential for improved chiral resolution and decreased peak-tailing effects for certain solutes, which is due to the capability of adjustment to the extremes of the mobile phase pH range. Of these materials, PGC, developed by Knox and Gilbert [6] has been shown to be particularly relevant in this area since it is robust to extremes of mobile phase pH, as for example in the case of aromatic amino acids and benzodiazepines, where ion-suppression is required [7]. As an extension to this work, chiral drug separations have been achieved with β-CD as a chiral selector and PGC as the achiral stationary phase, where pH extremes have also been used [8].

In general though, there are problems associated with the use of CDs which are due to their poor solubility in aqueous organic solvents. In this respect, however, it has been shown by Ishii [11] that improved chiral separations can be achieved by increasing CD concentration. When considering PGC, this particular problem is accentuated due to its hydrophobic character, which requires higher organic modifier concentrations to achieve suitable retention properties. One solution to this is to add urea to the mobile phase [4] but in the authors' work this led to problems with baseline stability and high viscosity. An alternative route to improve the CD solubility is available through derivatization of the CD structure at the secondary hydroxyl groups. Methylated, hydroxyethyl, hydroxypropyl and acetylated β-CD are now available and some of these have been examined as chiral additives in the present work on a number of chiral drugs of current interest.

Experimental

Equipment and methods

A single piston Gilson pump solvent delivery system (Model No. 302, Gilson, Villiers-le-Bel, France) was connected via a Gilson manometric pressure damper (Model 302C) to an injection valve (Model 7125, Rheodyne, Berkeley, CA, USA) fitted with a 20-μl loop. The 100 × 4.6 mm stainless-steel column was packed with 7-μm PGC (Shandon-Scientific, Cheshire, UK). A Philips (Pye Unicam PU 4025) UV–variable wavelength detector (Philips Scientific, Cambridge, UK) was employed and the detector was connected to a Hewlett Packard HP 3369A, integrator-plotter (Hewlett Packard Analytical Instruments, Cheadle Heath, Cheshire, UK).

The optimized mobile phase employed for each separation was: for the analysis of racemic glycyl-DL-phenyl alanine: methanol–5 mM disodium hydrogen orthophosphate containing 5 mM β-CD at pH 3.0 (30:70, v/v) at a flow rate of 0.5 ml min^{-1}; for propranolol: acetonitrile–3 mM potassium dihydrogen orthophosphate (pH 6) containing 2.5 mM β-CD (90:10, v/v) at a flow rate of 1 ml min^{-1}; for chlorpheniramine hydrogen maleate: methanol–5 mM disodium hydrogen orthophosphate buffer (pH 4.5), containing methylated β-CD (15–25 mM) (35:65, v/v) at 0.5 ml min^{-1}.

Reagents and materials

Disodium hydrogen orthophosphate was used as received (AnalaR grade, BDH Chemicals Ltd, Poole, UK). The phosphate buffer was prepared at specified pH values using doubly glass-distilled water and filtered through a 0.45 μm filter using an all-glass apparatus. The organic modifiers used were methanol and acetonitrile (HPLC grade, Rathburn Chemicals, Walkerburn, Midlothian, UK). The mobile phases were degassed before use under vacuum in an ultrasonic bath for 12 min in each case. Propranolol and racemic glycyl-DL-phenylalanine were all obtained from Sigma (Sigma Chemical Company, St. Louis, MO, USA) and dissolved in their corresponding mobile phases. Chlorpheniramine hydrogen maleate was obtained from Glaxo (Glaxo Operations UK Ltd, Barnard Castle, UK). The derivatized β-CD was obtained from Wacker (Consortium für Elektrochemische Industrie GmbH, Munich, FRG).

Results and Discussion

Chiral separations with PGC as an achiral stationary phase in conjunction with a β-CD mobile phase additive, which operates on the basis of inclusion complexation chromatography can give significant advantages when used with drugs which have ionization constants (pK_a) at the extreme ends of the pH range. One example of this is the enantiomeric resolution obtained at pH 3 for the β-blocking drug propranolol (pK_a 9.5) (Fig. 1). In this case, the chromatographic resolution factor (CRF), as introduced by Kaiser [9] was used as a measure of resolution and a typical CRF for enantiomers is illustrated in Fig. 1. This parameter was considered important in assessing the effect of changes in mobile phase composition, on separation during mobile phase optimization.

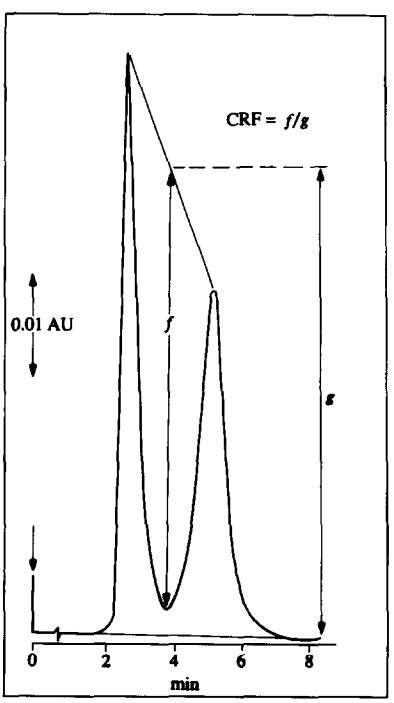

Figure 1
Separation of the enantiomers of propranolol on PGC and the measurement of chromatographic response function (CRF): where (f) is the midpoint between the line joining the apex of the peaks and the valley depth and (g) the distance between the midpoint and the chromatographic baseline. The mobile phase was acetonitrile–3.0 mM disodium hydrogen orthophosphate (pH 3) (90:10, v/v) containing 2.5 mM β-CD. The detection wavelength was 280 nm and the flow rate, 1.0 ml min^{-1}.

This particular analysis of propranolol isomers is of significance, as each of the isomers undergoes different metabolic pathways [10]; D-propranolol undergoes ring hydroxylation while L-propranolol undergoes side chain oxidation.

A further important parameter in the separation of drugs and related compounds is the ionic strength of the buffer and the mobile phase pH, which, when ionization of the analyte is present, could result in the loss of resolution. This point is exemplified in Fig.

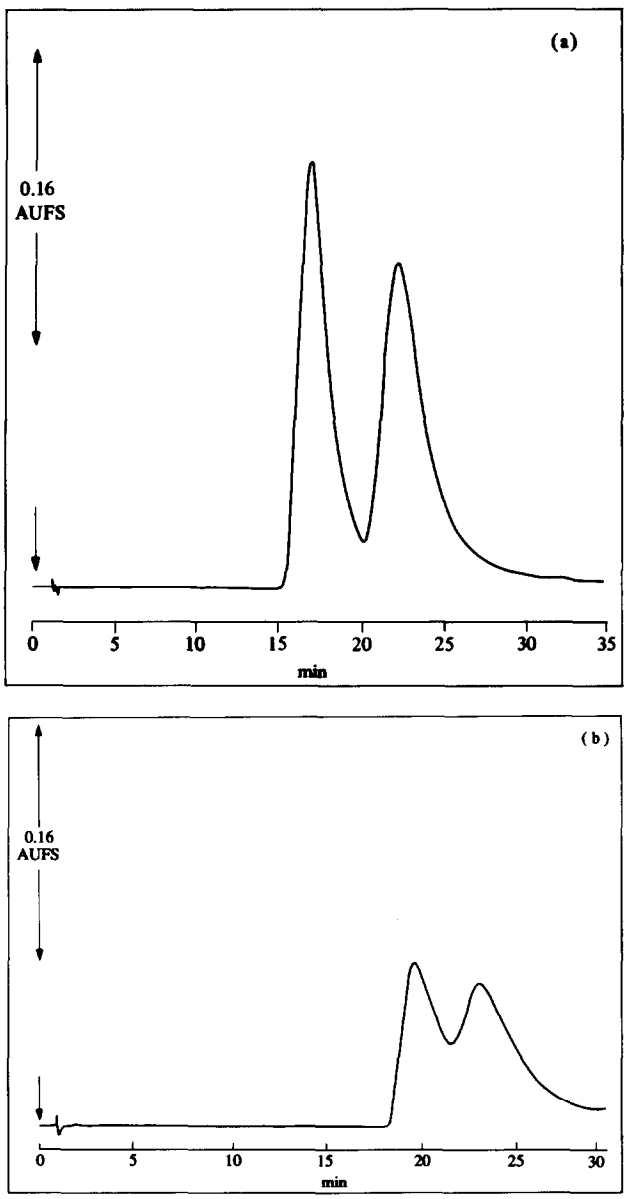

Figure 2
Separation of the enantiomers of glycyl-DL-phenyl alanine (100 $\mu g\ ml^{-1}$) on PGC. The mobile phase consisted of methanol–5 mM disodium hydrogen orthophosphate (30:70, v/v) containing 5 mM β-CD. The flow rate was 0.5 ml min^{-1} and detection wavelength, 230 nm. The mobile phase pH was: (a) pH 2.5 and (b) pH 6.0.

2(a) and (b) for the peptide glycyl-DL-phenylalanine at pH 2.5 (CRF 0.4) and pH 6 (CRF 0.9), respectively.

Previously, enantiomeric resolution has been shown to increase when applied with an achiral reversed-phase packing material and an increasing CD concentration [11]. However, as PGC is highly hydrophobic and suitable retention is generally only achieved with high organic modifier concentrations, increases above 10 mM were not possible in this system. An alternative was to use a derivatized CD where solubility is greatly improved (up to 40 mM achieved). As an example of this (Fig. 3) increased resolution was observed for the antihistamine, chlorpheniramine, when methyl β-CD was added over the concentration range 15–25 mM to the mobile phase. It is, however, important to consider, that with the derivatized CDs the apparent diameter of the toroidal cone rim can be expanded with the introduction of a more bulky group, by the selective substitution of some of the hydroxyl groups. Overall, the effects would be expected to affect the extent of interaction with the side chain of a correspondingly small or bulky analyte, respectively. This point is further exemplified by the hydroxyethyl and hydroxypropyl β-CD derivatives, which can lead to further improvements in solubility. However, as stated above this advantage is tempered with increased bulk of these groups which would be expected to lead to a reduction of interaction with the secondary hydroxyl groups on the edge of the CD cavity and result in a reduction of enantiomeric resolution (Fig. 4).

It has been illustrated above that the stability of PGC to extremes of pH can be advantageous in mobile phase additive experiments. In chiral drug separations, it has been found that improvements in stereoselective recognition can be achieved when using CDs with PGC as stationary phase, relative to the regularly used ODS-silica column packings. In addition enantiomeric resolution can be further improved when the CD-chiral selector concentration is increased, but there is the limitation due to the precipitation of the chiral additive with the higher modifier concentrations used with PGC. This has been overcome by using a more water-soluble CD and a consequent

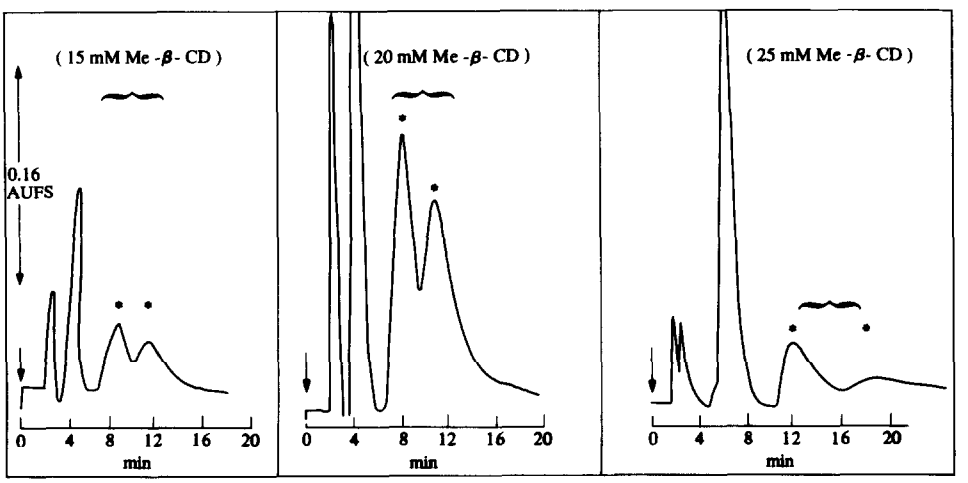

Figure 3
The use of methyl β-CD in the mobile phase for the separation of the enantiomers of chlorpheniramine hydrogen maleate on PGC. The mobile phase consisted of methanol–5 mM disodium hydrogen orthophosphate at pH 4.5 (35:65, v/v) and contained 15, 20 and 25 mM methylated β-CD, respectively.

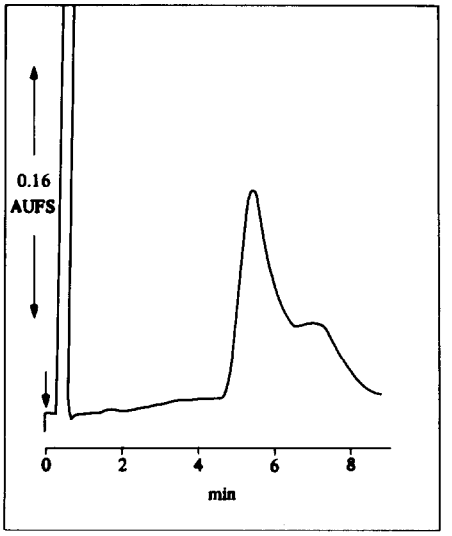

Figure 4
The effects on the separation of the enantiomers of chlorpheniramine hydrogen maleate, when hydroxyethyl β-CD was used in the mobile phase. The mobile phase consisted of methanol–5 mM disodium hydrogen orthophosphate at pH 4.5 (35:65, v/v) and contained 20 mM hydroxyethyl-β-CD and the flow rate was 0.5 ml min^{-1}.

improvement in resolution is achieved, but this advantage has to be balanced against the potential loss of resolution through the reduction in the number of the hydroxyl groups needed for chiral interaction. Further work on this interesting use of derivatized CDs is continuing in this laboratory and will be reported at a later date.

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References

[1] E. J. Ariens, *Trends Pharmacol. Sci.* May, 200–205 (1986).
[2] R. C. Williams, J. F. Vasta-Russel, J. L. Glajch and K. Golebioski, *J. Chromatogr.* **371**, 63–70 (1986).
[3] D. W. Armstrong, *J. Liq. Chromatogr.* **3**, 895–899 (1980).
[4] D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. F. Beesley, *Science* **232**, 1132–1135 (1986).
[5] M. Gazdag, G. Szepesi and L. Huszar, *J. Chromatogr.* **351**, 128–135 (1986).
[6] J. H. Knox and T. Gilbert, U.K. Patent, 7939449 (1979).
[7] J. E. Mama, B. J. Clark and A. F. Fell, *Anal. Proc.* **26**, 71–73 (1989).
[8] A. F. Fell, T. A. G. Noctor, J. E. Mama and B. J. Clark, *J. Chromatogr.* **434**, 377–384 (1988).
[9] R. E. Kaiser, *Gas Chromatographie*. Geest and Portig, Leipzig (1960).
[10] U. K. Walle, T. Walle and S. A. Bai, *Clin. Pharmacol. Therap.* **34**, 718–723 (1983).
[11] T. Takeuchi, H. Asai and D. Ishii, *J. Chromatogr.* **357**, 409–503 (1986).

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