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Application of modified cyclodextrins in capillary electrophoresis for enantiomeric resolution of propranolol and analogues

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Abstract

The enantio-resolution of propranolol and four derivatives was examined by free solution capillary electrophoresis (FSCE) using various cyclodextrin molecules. Of the three modified cyclodextrins investigated, hydroxyethyl-β-cyclodextrin provided the largest chiral resolution values for all five analytes. This may be linked to its extended hydrogen bonding chains on the cyclodextrin rim. Methyl-β-cyclodextrin and *heptakis*(2,3-di-O-acetyl) β-cyclodextrin gave lower maximum analyte resolutions, probably due to differences in their macrocyclic structure and hydrogen bonding ability. The presence of a bulky, non-polar alkyl group on the analytes was found to enhance chiral recognition. Methanol was found to have a varied effect on chiral resolutions, dependent on the type of cyclodextrin and structure of the analyte.

1. Introduction

Propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propranol] is a widely prescribed optically active β-blocker used in the treatment of various cardiovascular disorders. There is considerable interest in developing methods to separate its enantiomers [1–3], which show different pharmacological effects in vivo [4]. The effects of various modified cyclodextrins on the enantiomeric resolution of propranolol and four analogues by free solution capillary electrophoresis (FSCE) are reported here.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of between 6 and 12 α-(1,4) linked glucopyranose units, with α- (6units), β- (7 units) and γ-CD (8 units) being the only commercially available forms of the parent macrocycles. The molecules have nonpolar inner cavities and are hydrophilic in nature on their external rims due to the presence of either primary or secondary hydroxyl groups [5]. With these characteristics, CD "hosts" are able to form diastereomeric inclusion complexes with a wide range of optically active compounds, allowing the "guest" molecules to be resolved into their enantiomers. CDs have thus been used for chiral discrimination in a variety of analytical techniques [6].

FSCE involves the application of a high voltage across a narrow capillary filled with buffer in order to separate components based on their relative electrophoretic mobilities, often in the presence of electroendosmotic flow (EOF) [7].

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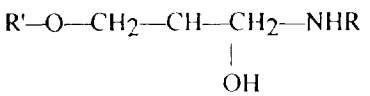

1 = R' = naphthyl, R = *i*-pro
2 = R' = naphthyl, R = *t*-butyl
3 = R' = naphthyl, R = ethyl
4 = R' = phenyl, R = *i*-pro
5 = R' = phenyl, R = *t*-butyl

Fig. 1. Structures of propranolol (1) and four analogues used in this study.

When CDs are added to a buffer containing cationic analytes, compounds which have higher association constants with the CD are expected to display increased migration times in FSCE (anode destination electrode). The CD is electrically neutral and thus possesses no electrophoretic mobility. Consequently, enantiomers which are charged may be chirally resolved if their CD stability constants, which are strongly dependent upon the structures of both CD and analyte, are sufficiently different to provide for separate migration times.

In this work propranolol and four closely related analogues (1–5 in Fig. 1) were used to study how changes in molecular structure can affect the complexation process with available cyclodextrins, selected on the basis of their different hydrophobicities and hydrogen-bonding abilities: methyl-β-cyclodextrin (Me-β-CD), hydroxyethyl-β-cyclodextrin (HE-β-CD) and *heptakis*(2,3-di-O-acetyl)β-cyclodextrin (Ac-β-CD). Possible host–guest interactions are proposed by relating the analyte and CD structures to the resulting migration times and enantio-resolutions.

A method previously applied to propranolol by Fanali [8] was also employed to rapidly assess the ability of urea solubilized β-CD to resolve the structurally related analogues. In addition, the effect of methanol on enantio-resolutions and migration times was briefly examined.

2. Experimental

Me-β-CD (average degree of substitution 1.8) and HE-β-CD (molar substitution 0.6) were supplied by Wacker (Munich, Germany). *Heptakis*(2,3-di-O-acetyl)β-cyclodextrin was generously donated by H. Mallwitz and U. Holzgrabe of the Pharmazeutisches Institut der Universität Bonn (Germany). Propranolol and its derivatives (1–5) were kindly provided by Dr. G. Bedford of Zeneca, Macclesfield, UK.

A Biorad HPE CE system was used with a BioRad 20 cm × 25 μm I.D. coated capillary. Samples were loaded by electromigration and separated at room temperature at a constant current of 5 μA. Data were recorded at the analyte λ_{max} values (λ_{288} for compounds 1–3 and λ_{254} for compounds 4 and 5) with the Biorad 800 HRLC detector (0.005 = AUFS), version 2.30. Samples of compounds 1–5 were prepared by dissolution in methanol–50 m*M* potassium dihydrogen phosphate pH 3.0 (30:70, v/v) at about 0.5 mg/ml. Buffers were prepared from potassium dihydrogen phosphate (50 m*M*) using freshly distilled and filtered water and then adjusted to the appropriate pH with orthophosphoric acid before the addition of methanol. The required amount of CD was then added to the buffers, which, along with the sample solutions, was then filtered through a 0.2-μm filter (Whatman, Maidstone, UK) and centrifuged at 11 400 *g* for 5 min before use.

Enantiomeric resolution values determined by FSCE have been calculated according to Eq. 1; [9]

$$R_s = 1.177(t_2 - t_1)/(Wa_{1/2} + Wb_{1/2}) \quad (1)$$

where t_1 and t_2 are the migration times for peaks 1 and 2, whilst $Wa_{1/2}$ and $Wb_{1/2}$ are the widths of peaks 1 and 2 respectively, at half peak height. It is appreciated that the experimentally determined resolution values include a factor for column efficiency as well as selectivity, however all the data reported here has been obtained using the same column and all the analytes were examined under a range of identical operating conditions under which changes in resolution values are mainly due to changes in selectivity values.

Coated capillary columns, such as used in this work, are able to minimize EOF, which can prove detrimental to resolution [10]. Apparent

electrophoretic mobilities (μ_{ep}) can be calculated according to Eq. 2; [2]

$$\mu_{ep} = lL/Vt_m \quad (2)$$

where l is the length to the detector, L is the total capillary length, V is the operating voltage, and t_m is the migration time of a neutral marker.

Electroendosmotic flow (EOF) measurements were attempted according to recommendations from the manufacturers Biorad. A neutral marker, in our case acetone, was loaded at the outlet reservoir in a running buffer to acetone ratio of 20:1. The polarity was set from negative to positive and the time taken for the neutral marker to pass from the outlet reservoir to the detector window was measured (a distance of 4.6 cm). The λ_{max} was 264 nm, the maximum for acetone in an aqueous acid [11].

3. Results and discussion

The aqueous/buffer mixture was chosen to ensure a stable pH (3.0) at which analytes 1–5 possessed a positive charge (pK_a's of 1–5 are around 9 ± 0.5) and thus migrated towards the cathode. Attempts to measure the EOF under these conditions were successful only in that they gave a maximum possible value of $8 \cdot 10^{-6}$ cm^2 V^{-1} s^{-1}. This is very low and compares favourably with Wren and Rowe [2], who ignored EOF at the higher value of $0.04 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}.

3.1. Effect of heptakis(2.3-di-O-Acetyl)β-cyclodextrin (Ac-β-CD)

With Ac-β-CD only 1 and 2 were enantiomerically resolved under these experimental conditions (Table 1). Acetylation increases the hydrophobicity of the CD and changes the hydrogen bonding ability of the CD which often results in low enantioselectivity [12]. However Yamashoji et al. [13] have shown that Ac-β-CD can be used to give improved chiral resolution of DL-alanine β-naphthylamide in CE when compared to β-cyclodextrin.

With only the two most hydrophobic compounds (1 and 2) showing any enantio-recognition (Fig. 2) and possessing the longest migration times, it would appear that analyte hydrophobicity is a major factor in the determination of resolution values and complex formation constants when using this CD derivative in FSCE. There are few published incidents were Ac-β-CD has provided enhanced separation over the underivatized β-CD [14]. Previous work in this laboratory has in fact demonstrated the ability of Ac-β-CD to provide enhanced enantio-selectivity for a series of phenethylamines in FSCE, when compared to results achieved using β-CD [15]. All five analytes however showed longer migration times as the concentration of Ac-β-CD was increased, indicating that they underwent

Table 1
Migration times (t_m, min) for compounds 1–5 in the presence of increasing amounts (mM) of *heptakis*(2,3-di-O-acetyl)β-cyclodextrin (Ac-β-CD). Resolution values (R_s) in parentheses. FSCE conditions: methanol–50 mM KH_2PO_4, 30:70, pH 3. Constant current of 5 μA.

Analyte	0.0 mM	4 mM	7.4 mM	15 mM	25 mM	35 mM[a]
1	6.84	10.82	10.88	11.56, 11.65	11.78, 11.89	8.48
	(0)	(0)	(0)	(<0.1)	(0.26)	(0)
2	6.94	11.07	11.35, 11.39	12.27, 12.41	12.39, 12.56	8.98
	(0)	(0)	(<0.1)	(0.63)	(0.81)	(0)
3	6.32	10.58	10.67	11.29	11.37	8.24
	(0)	(0)	(0)	(0)	(0)	(0)
4	4.30	9.82	10.02	10.34	10.39	6.53
	(0)	(0)	(0)	(0)	(0)	(0)
5	5.59	10.27	10.43	10.86	11.05	7.51
	(0)	(0)	(0)	(0)	(0)	(0)

[a] 4 M urea was necessary to enable dissolution of this amount of cyclodextrin.

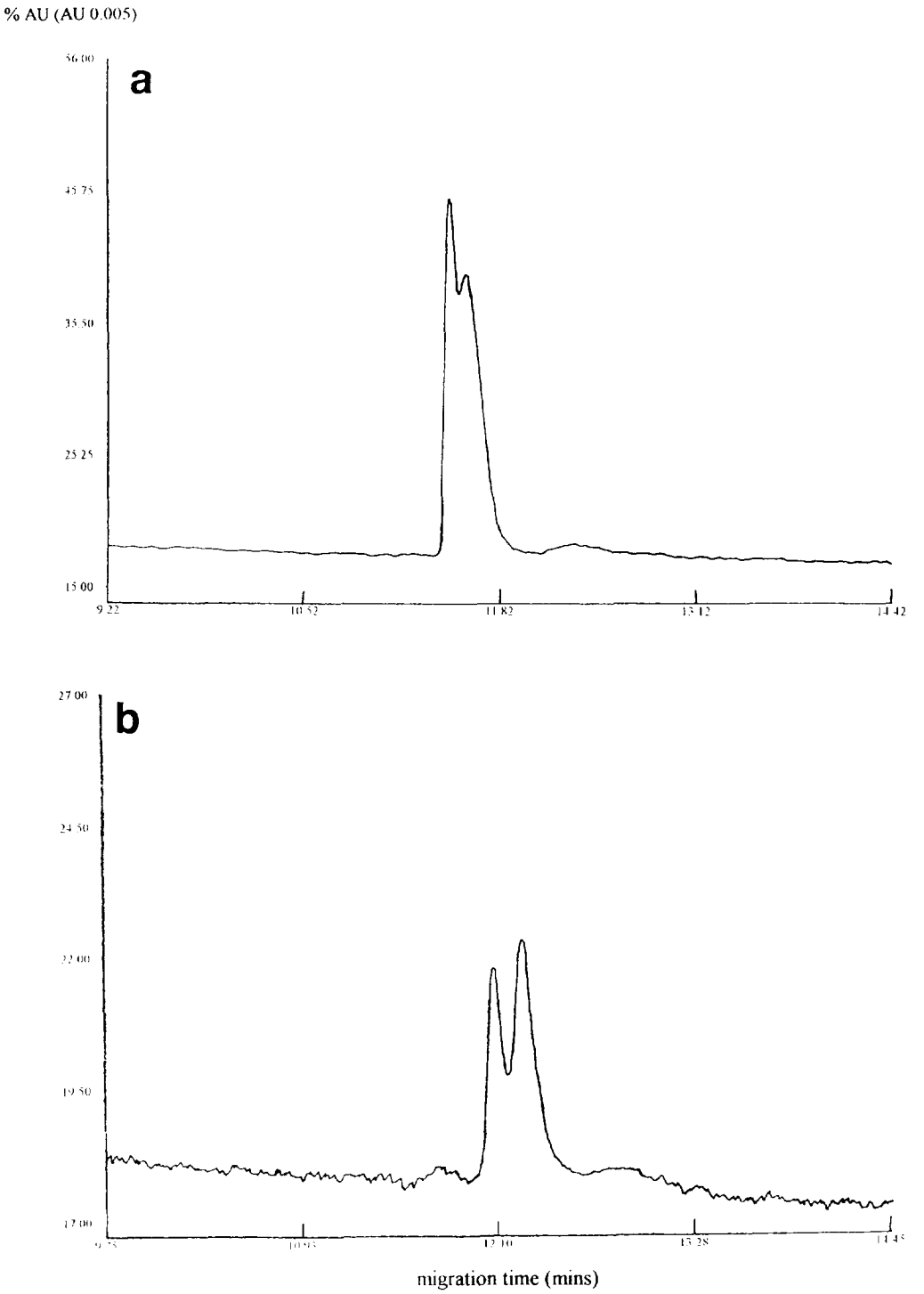

Fig. 2. Electropherograms for compounds 1 (a) and 2 (b). FSCE conditions: methanol–50 m*M* KH_2PO_4, 30:70, pH 3, containing 25 m*M* Ac-β-CD. Constant current of 5 μA.

complexation to some extent. These migration times were longer in the presence of Ac-β-CD than Me-β-CD up to the 15 m*M* level (suggesting higher formation constants), but shorter than the migration times seen with HE-β-CD (suggesting lower formation constants). Yet enantio-resolution was much higher for all the analytes with HE-β-CD and also Me-β-CD, except in the case of 2. Therefore the ability of a molecule to form an inclusion complex with a CD does not cause enantio-discrimination per se [16], although it is deemed to be a necessary pre-requisite in most instances [17].

Due to the insoluble nature of Ac-β-CD it was necessary to use 4 *M* urea to solubilise 35 m*M* of this CD in the buffer. This resulted in lower migration times than would have been expected if urea was absent, and also in the removal of all signs of enantio-recognition for 1 and 2. The strongly polar urea molecules may have significantly reduced any possible hydrogen-bonding of the CD with 1 and 2, thus negating resolution and shortening the migration times by increasing the amount of time 1 and 2 spent as the faster moving, free analytes.

3.2. Effect of methyl-β-cyclodextrin (Me-β-CD)

Methylated β-cyclodextrins have been extensively employed in CE chiral separations, often demonstrating enhanced resolutions over the parent macrocycle [18–22]. Wren and Rowe stated that in the presence of Me-β-CD, hydrophobicity would be the major force driving complexation in a series of β-blockers [2]. More hydrophobic solutes would require the presence of less CD to achieve their individual optimum resolution values. This hypothesis is partly supported by our results.

Compounds 1 and 2 each gave maximum resolution values at 25 m*M* Me-β-CD, with the more hydrophobic 2 having a higher resolution value of 0.73, compared with compound 1 which is less non-polar and gave a lower resolution of 0.54 (see Table 2). Compound 3 is less hydrophobic than either 1 or 2 and gave a lower maximum resolution of 0.32 at the highest Me-β-CD concentration of 35 m*M* (see Fig. 3). Similar in behaviour was compound 5 which is less hydrophobic than 1, 2 or 3 and also gave its maximum resolution at 35 m*M*. However the resolution of 5 (1.54) was higher than observed with the other more non-polar analytes. Also, the least hydrophobic of the compounds, 4, unexpectedly showed its maximum resolution of 0.68 at only 25 m*M* Me-β-CD, when it would have been thought to require a higher concentration than the other compounds, based on the simple model advanced by Wren and Rowe [2].

Different structural features of the five analytes therefore seem to be important not only in the actual complexation process but also in the

Table 2
Migration times (t_m, min) for compounds 1–5 in the presence of increasing amounts (m*M*) of methyl-β-cyclodextrin (Me-β-CD). Resolution values (R_s) in parentheses. FSCE conditions: methanol–50 m*M* KH_2PO_4 30:70, pH 3. Constant current of 5 μA.

Analyte	0.0 m*M*	4 m*M*	7.4 m*M*	15 m*M*	25 m*M*	35 m*M*
1	6.84	7.84	8.39, 8.48	9.37, 9.51	11.07, 11.23	11.87, 12.05
	(0)	(0)	(0.22)	(0.26)	(0.54)	(0.48)
2	6.94	8.12	9.37, 9.42	10.12, 12.23	14.20, 14.39	14.34, 14.45
	(0)	(0)	(0.17)	(0.27)	(0.73)	(0.31)
3	6.32	7.50	7.82, 7.88	9.57, 9.68	13.44, 13.62	14.07, 14.18
	(0)	(0)	(0.16)	(0.20)	(0.30)	(0.32)
4	4.30	6.50	6.57, 6.63	8.39, 8.53	10.90, 11.14	11.58, 11.82
	(0)	(0)	(<0.1)	(0.21)	(0.68)	(0.53)
5	5.59	7.00	7.90, 7.99	9.08, 9.26	11.51, 11.78	13.87, 14.23
	(0)	(0)	(0.63)	(0.73)	(1.32)	(1.54)

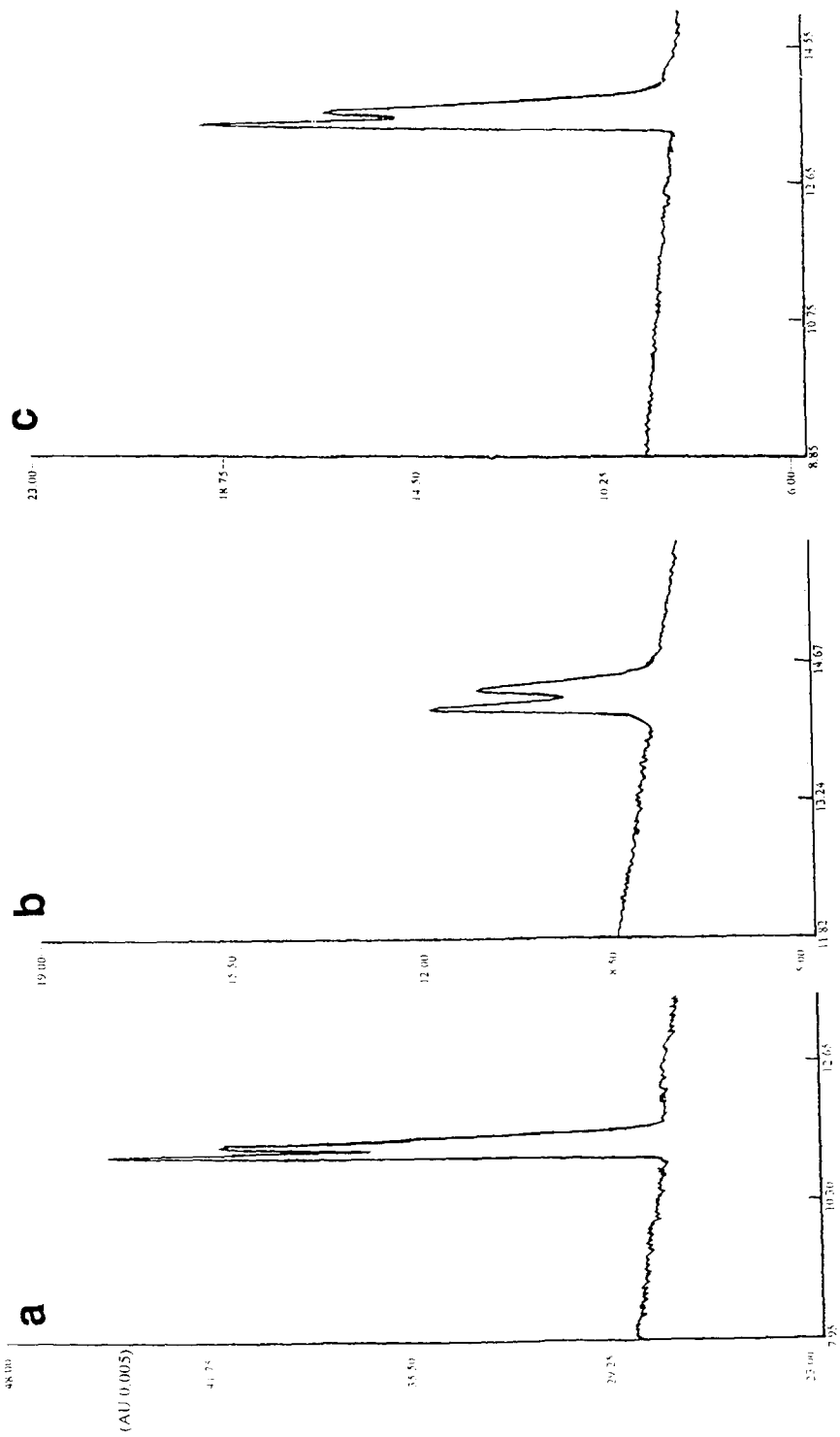

Fig. 3. Electropherograms for compounds 1 (a), 2 (b) and 3 (c). FSCE conditions: methanol–50 m*M* KH_2PO_4, 30:70, pH 3, containing Me-β-CD at 25 m*M*, 25 m*M* and 35 m*M*, respectively. Constant current of 5 μA.

overall enantio-recognition mechanism. Compounds 4 and 5 gave higher maximum resolution values than 1 and 2 respectively, suggesting that the presence of the naphthyl moiety proved detrimental to resolution by perhaps holding the stereogenic centre too far from the CD rim to permit optimum hydrogen-bonding with the CD hydroxyl groups.

The bulky *tert.*-butyl R group promoted enantio-recognition in comparison to the *iso*-propyl R group with maximum resolution values of $2 > 1$ and $5 > 4$. This may be related to the proposed hydrophobicity effect, with the more non-polar compounds having a stronger association with the Me-β-CD and hence also displaying longer migration times. Chang et al. [23] found that *tert.*-butyl derivatives of amino acids were well resolved on a bonded hydroxypropyl-β-CD column in HPLC, clearly supporting the idea that this group can be beneficial for chiral discrimination. However, by virtue of the insignificant chemical shifts demonstrated by the *iso*-propyl protons of propranolol, NMR spectroscopy has demonstrated that this R group (see compound 1, Fig. 1) is not incorporated into the β-CD cavity [24]. Therefore the R groups must interact with the modified CD rim to influence enantio-recognition, perhaps via some steric and/or hydrophobic effect, which alters the analyte's position in the CD cavity and by doing so, holds the analyte hydroxyl and amine groups in a more favourable orientation to encourage chiral recognition.

Methylation is known to not only make the parent β-CD more hydrophobic [25] but also to increase the cavity depth and enhance the flexibility of the macrocycle due to a reduction in the number of possible hydrogen bonds between the O(2) and O(3) hydroxyl groups [26]. These changes may explain the improved analyte enantio-recognition seen with Me-β-CD by allowing a "better fit" of the analyte (in this case those compounds with R = *tert.*-butyl) with the more hydrophobic and conformationally flexible Me-β-CD molecule.

3.3. Effect of hydroxyethyl-β-cyclodextrin (HE-β-CD)

The enantiomers of propranolol have been resolved using HE-β-CD in CE [27], although in the same work the authors were unable to achieve a similar degree of separation with Me-β-CD. Other work has indicated that HE-β-CD generally gave poorer chiral separations than both methylated β-cyclodextrins [20] and even β-cyclodextrin itself [21]. In this work HE-β-CD gave longer migration times for all five compounds than did Me-β-CD or Ac-β-CD, suggesting that the analytes formed stronger inclusion complexes with this CD derivative under the experimental conditions used here. Strongly related to this is the fact that the highest resolution values for all the analytes occured with this CD (Table 3). The extended hydrogen-bonding sites

Table 3
Migration times (t_m, min) for compounds 1–5 in the presence of increasing amounts (m*M*) of hydroxyethyl-β-cyclodextrin (HE-β-CD). Resolution values (R_s) in parentheses. FSCE conditions: methanol–50 m*M* KH_2PO_4 30:70, pH 3. Constant current of 5 μA.

Analyte	0.0 m*M*	4 m*M*	7.4 m*M*	15 m*M*	25 m*M*	35 m*M*
1	6.84	12.48, 12.56	12.64, 12.78	14.39, 14.63	15.84, 16.12	16.06, 16.36
	(0)	(<0.1)	(0.44)	(0.74)	(0.83)	(0.74)
2	6.94	12.42, 12.54	12.53, 12.71	13.85, 14.14	15.34, 15.68	16.00, 16.39
	(0)	(0.66)	(1.08)	(1.31)	(1.51)	(1.67)
3	6.32	11.19	11.99, 12.07	13.86, 14.01	15.61, 15.80	16.63, 16.84
	(0)	(0)	(<0.1)	(0.62)	(0.69)	(0.77)
4	4.30	10.10	10.64, 10.72	12.19, 12.41	13.57, 13.84	13.78, 14.04
	(0)	(0)	(<0.1)	(0.94)	(1.03)	(0.29)
5	5.59	10.52	10.90, 11.01	13.08, 13.32	14.45, 14.77	15.53, 15.90
	(0)	(0)	(<0.1)	(1.12)	(1.30)	(1.54)

on the HE-β-CD chains may be responsible for these observations, by allowing a closer spatial interaction with the hydrogen donor-acceptor sites near the asymmetric centre on the lengthy analyte alkyl chain. HE-β-CD was also the only CD to provide any enantio-recognition at the 4 m*M* level, where it partially resolved 1 and 2, with the more non-polar 2 having a higher resolution value of 0.66.

However, HE-β-CD caused a reversal in the migration order of 1 and 2 when compared to the results obtained with Me-β-CD and Ac-β-CD. Thus 2 had faster migration times than 1 over the entire CD concentration range, yet still maintained higher enantio-resolution values than 1. Evidently structural changes to the rim of the CD cavity are responsible for the behaviour seen here, although the exact mechanism is not as yet understood. NMR studies are in progress to help to clarify the situation.

A second difference noted with HE-β-CD was that the presence of the analyte naphthyl moiety had a more varied effect on enantio-resolution. Although the maximum resolution value of 4 was still greater than that of 1 (as with Me-β-CD), 5 had a lower maximum resolution than 2 which was not the case with Me-β-CD. The most hydrophobic of the solutes, i.e. 2, therefore gave the highest individual maximum resolution with HE-β-CD (see Fig. 4), whereas the less non-polar 5 displayed the largest resolution with Me-β-CD. In both these cases neither 2 nor 5 had the longest migration times with HE-β-CD and Me-β-CD, respectively.

^{1}H NMR investigations have indicated that the naphthyl moiety of propranolol (1) penetrates into the β-CD cavity, forming complexes with a 1:1 stochiometry, whilst its alkyl chain lies directly over the rim of the CD cavity [24]. This allows the hydroxyl and amine groups of 1 to hydrogen-bond with the secondary hydroxyl groups of the β-CD molecule [28]. It is therefore not surprising that both pairs 1/4 and 2/5 should have portrayed different enantio-resolution values as respectively, they differ only in their R′ groups, i.e. that part of the structure which is responsible for inclusion into the CD cavity and which thus plays a crucial role by holding the molecule in the correct orientation for optimum enantio-recognition.

As with Me-β-CD, the existence of a *tert.*-butyl group on the analytes promoted enantio-recognition in the presence of HE-β-CD, with resolution values of $2 > 1$ and $5 > 4$ at all CD concentrations.

HE-β-CD has additional hydrogen-bonding sites on its modified rim, accounting perhaps for it displaying the highest maximum resolution values observed in this work. The ability of many modified CDs to improve on enantio-recognition values seen with the parent CDs has been described before in many other FSCE applications [8,16,25,29].

3.4. Effect of β-cyclodextrin (β-CD)

It was not possible to fully investigate the effects of β-CD under the buffer conditions used with the other CDs due to its very low solubility in 30% methanol (0.64 g/100 ml) [30]. Therefore only a 4 m*M* β-CD buffer was prepared (methanol–50 m*M* KH_2PO_4, 30:70, v/v, pH 3). No enantio-resolution was observed for any of the analytes at this CD concentration (unlike HE-β-CD which resolved 1 and 2) and the migration times were faster here than with the other three CD derivatives, suggesting the possibility of lower CD analyte formation constants.

A method developed by Fanali [8] was briefly tested to observe how the structural differences of the five analytes affected their ability to be resolved using a urea solubilized β-CD (40 m*M*) methanol–phosphate (30:70) buffer (Table 4). Optical resolution values were of the order $2 > 1 > 5 > 3 > 4$. As seen with the CD derivatives, the presence of a *tert.*-butyl group helped promote resolution with $2 > 1$ and $5 > 4$. When R′ = naphthyl, enantio-resolutions increased with $1 > 4$ and $2 > 5$, which is directly opposite to the results obtained with Me-β-CD and also in contrast to the resolution order seen for 1 and 4 with HE-β-CD. It would seem therefore that under these conditions β-CD favours the recognition of compounds 1 and 2, the bulkiest and most hydrophobic of the five analytes.

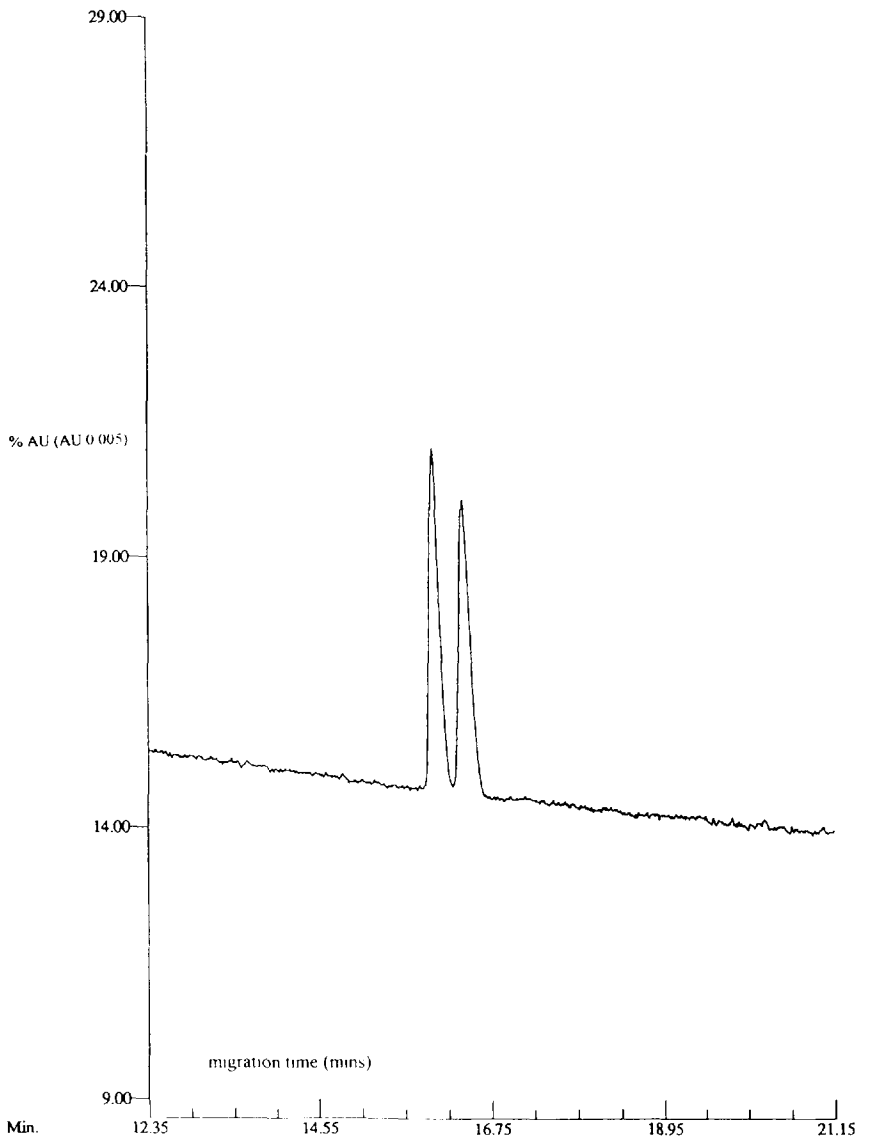

Fig. 4. Electropherogram for compound 2 FSCE conditions: methanol–50 m*M* KH_2PO_4, 30:70, pH 3, containing 35 m*M* HE-β-CD. Constant current of 5 μA.

3.5. Effect of methanol on resolution and migration times

It was decided to examine what effect the removal of methanol would have on the analyte resolutions and migration times achieved in the presence of the CD derivatives. Limited by the relatively insoluble Ac-β-CD, the 7.4 m*M* level was chosen as the maximum CD concentration in the absence of organic modifier. Me-β-CD and HE-β-CD concentrations were thus kept equal to this level to allow for a direct comparison of results.

With both HE-β-CD and Ac-β-CD the removal of methanol had a detrimental effect on resolution. None was observed for any of the

Table 4
Resolution values (R_s) and migration times (t_m, min) for compounds 1–5 in the presence of β-cyclodextrin. FSCE conditions: methanol–50 m*M* KH_2PO_4 30:70, pH 2.5, 4 *M* urea. Constant current of 5 μA.

1	1.05	12.06, 12.23
2	1.31	10.80, 11.03
3	0.28	10.43, 10.54
4	<0.1	8.76, 8.87
5	0.63	9.86, 10.04

analytes with HE-β-CD in the absence of methanol, whereas they all formally displayed some resolution when methanol was present. Ac-β-CD (at 7.4 m*M*) had only previously resolved 2 when methanol was present. This slight enantio-resolution was removed when methanol was absent. With Me-β-CD the effect on resolutions seemed more variable. Enantio-resolutions of 1, 2 and 3 increased when no methanol was present, whilst those of 4 and 5 decreased. Migration times increased in every case when methanol was removed.

As stated by Wren and Rowe [9], organic solvents such as methanol are believed to change the apparent mobility difference between the analyte enantiomers in FSCE, which in turn can increase or decrease the observed enantioselectivity depending on the CD type and concentration. This finding is mirrored in these results where 30% methanol was shown to have a beneficial effect on resolution with HE-β-CD and Ac-β-CD, yet it produced both higher and lower resolutions using Me-β-CD, dependent on the analyte structure (and hence its CD complex formation constant). Solvation can radically alter both the geometry and relative stabilities of various cyclodextrin–analyte conformations [17], so it is not unexpected that enantio-resolution values and migration times should be affected when methanol is added to the buffer.

4. Conclusions

Of the three CD derivatives used, HE-β-CD gave the largest resolutions for all five analytes and was the only CD to show any enantio-selectivity at the 4 m*M* level. Increased flexibility of the Me-β-CD macrocycle and additional hydrogen bonding sites on the HE-β-CD rim may have been responsible for the resolution values seen with these CD derivatives. Ac-β-CD, the most hydrophobic of the CDs, showed the poorest enantio-selectivities, resolving only 1 and 2 to a small extent. This was related to a change in the hydrogen-bonding ability of Ac-β-CD [13]. In the presence of urea, β-CD (as used by Fanali [8]) showed some enantio-selectivity to all five analytes and even gave the largest resolution value seen for 1 (1.05).

The presence of a *tert.*-butyl group on the analyte alkyl chain (which is not believed to enter the CD cavity) enhanced resolutions with all the CDs used here (except in the case of 4 and 5 with Ac-β-CD) viz. maximum resolution values were of the order $2 > 1$ and $5 > 4$. The analyte positioning in the CD cavity and around the CD rim may have been altered by the *tert.*-butyl group due to some steric and/or hydrophobic effect, which in turn led to an increase in the observed enantio-selectivity.

When only the R′ group differed (i.e., for 1 and 4, and 2 and 5), resolution values either increased or decreased depending on the CD used. This R′ group is believed to enter the CD cavity [24]. If the cavity dimensions are altered by changes to the CD rim [22,26] then it would not be unexpected that enantio-resolution values should also change, perhaps as the analyte experiences a better or poorer "fit" with the CD cavity.

The presence of methanol was seen to have a varied effect on enantio-resolutions which was linked to the findings of Wren and Rowe [9] who stated that organic solvents altered the apparent mobility difference between enantiomers. Such a change could then alter the resolution values depending on the type and concentration of CD being used.

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