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Systematic approach to treatment of enantiomeric separations in capillary electrophoresis and liquid chromatography II. A study of the enantiomeric separation of fluoxetine and norfluoxetine

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Abstract

A systematic approach to enantiomeric separations in capillary electrophoresis (CE) and liquid chromatography (LC) with chiral mobile phase additives (MPA) or a chiral stationary phase (CSP) is used in the study of fluoxetine and norfluoxetine with cyclodextrins as chiral selectors. Binding constants and selectivities are determined under the same experimental conditions (mobile phase, buffer composition). Good agreement is found between results from the three techniques. The role of the buffer salt is investigated by comparison of binding constants obtained with triethylammonium and sodium acetate buffers.

Investigation of the effects of derivatisation of the selector in CE and LC with MPA demonstrates the appropriate choice of cyclodextrin type for use in LC. By studying the influence of organic modifier content on separation parameters, CE can predict a useful solvent working range for a CSP.

1. Introduction

The different pharmacological behaviour of drug enantiomers has become increasingly important due to the fact that they are often readily discriminated by biological systems and may have different toxicological, pharmacokinetic and pharmacodynamic profiles. The growing awareness in this field has resulted in an increased demand for suitable methods to determine the stereoisomeric composition of a drug. Generally enantiomers are only distinguishable in a chiral environment, thus the separation methods are based on interaction of enantiomeric solutes with an optically-active compound used as a chiral selector.

In liquid chromatography (LC) the direct separation and determination of the enantiomers is performed using either a chiral stationary phase (CSP) or by the addition of a chiral mobile phase additive (MPA) [1,2]. There has recently been a dramatic growth in the use of capillary electrophoresis (CE) with chiral MPAs for the separation and analysis of enantiomers [3,4]. Benefits of CE include speed of separations, high efficiencies and resolution, low running costs

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relative to LC, and robust assays for bulk drugs [5].

We have recently initiated development of a systematic approach to the treatment of enantiomeric separations which brings together information from CE and LC using the same selector as MPA or CSP [6]. By formulating theory and applying this to systems where analytes, selector and buffer composition are the same, our aim is to link the two key separation techniques of CE and LC. This should allow the substantial body of knowledge in reversed-phase LC to be used in a rational way to guide method development in CE. In addition, rapid and inexpensive CE experiments could be used in method development for LC, which remains the method of choice for analyses where low detection limits are required, and for preparative enantioseparations.

In our previous paper (Part I, [6]), equations are given for the optimum determination of binding constants and selectivity for selector–selectand complexes involving enantiomeric analytes in CE and LC with MPAs. LC separations of enantiomers with CSPs and MPAs are categorised in one of four ways according to the nature of the stationary and mobile phase equilibria involved. A quantitative comparison is given of CE and LC separations of dansyl derivatives of glutamate and leucine with β-cyclodextrin (β-CD) as selector. In the present paper we apply this general treatment to the separations of enantiomers of fluoxetine and norfluoxetine, for which LC separations using a β-CD CSP have previously been optimised [7].

Fluoxetine is an important antidepressant drug for the treatment of unipolar mental depression. Both fluoxetine (FL) {(±)-N-methyl-3-phenyl-[α,α,α-trifluoro-*p*-tolyl-oxyl]-propylamine hydrochloride} and its N-desmethylated metabolite norfluoxetine (NR) (Fig. 1) enhance serotoninergic neurotransmission through selective inhibition of presynaptic serotonin reuptake [8]. The enantiomers of FL exhibit their selective 5-hydroxytryptamine (5-HT) uptake inhibition with about equal potencies [9]. However, many differences have been reported in the literature regarding their pharmacological properties, as well as those of NR [9,10], necessitating the development of new analytical methods permitting the chiral separation of these compounds.

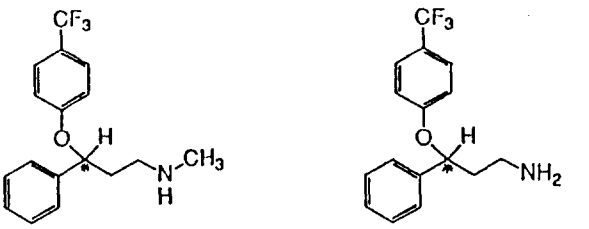

Fig. 1. Structures of fluoxetine and norfluoxetine.

The aims of this paper are (i) to study binding with selector in the mobile phase under the conditions used for the optimised CSP separation, (ii) to systematically investigate the role of the mobile phase, salt concentration and solvent composition, and (iii) to compare binding and separations with native and derivatised β-CDs.

We did not set out to achieve the best possible chiral separation of FL and NR in both CE and LC, but rather to investigate the ability to form links between the two techniques. Optimisation of the separation of FL enantiomers by CE has been previously investigated by Soini et al. [11], who found that a buffer containing 10 m*M* methyl-β-cyclodextrin, 30 m*M* Tris, pH 2.8, 0.1% methylhydroxycellulose gave excellent results.

2. Theory

Whilst a full explanation of the theory can be found in our previous paper [6], a brief summary is given here. Building on the treatment given by Sybilska et al. [12], we catagorise four cases for chiral discrimination in LC.

Case 1. Chiral mobile phase additives and achiral stationary phase. All discrimination in the mobile phase. *Case 2.* Chiral mobile phase additives partially bound to achiral stationary phase. Discrimination in both mobile and stationary phase. *Case 3.* Dynamically coated chiral stationary phase. All discrimination in the

stationary phase. *Case 4.* Covalently bonded chiral stationary phase. All discrimination in the stationary phase.

Case 1 is analogous to CE with mobile phase additives, and therefore an identical rational separation strategy applies for optimising the selector concentration in the two techniques. In this case, k', the capacity factor versus C, the concentration of the free selector, is a binding curve analogous to the CE binding curve of mobility, μ, vs. C, with equations for the curves [6,13]

CE:

$$KC = \frac{\mu_0 - \mu}{\mu - \mu_\infty}$$

LC:

$$KC = \frac{k'_0 - k'}{k' - k'_\infty}$$

When using chiral MPAs in CE or a case 1 LC chiral separation, optimum mobility or retention time difference occurs when $\bar{K}C = 1$, where $\bar{K}$ is the average binding constant defined as $(K_1 K_2)^{1/2}$. In all our papers selectivity, α, is given by the ratio of binding constants for the enantiomers, K_2/K_1, in order to have the same definition when using CSPs and MPAs. Some authors in the CE field have equated chiral selectivity, α, to the mobility ratio μ_1/μ_2 [14]. This is inappropriate for a thermodynamic analysis, since α from this definition is a concentration-dependent quantity.

The non-linear least squares method we use for data fitting [13,15] is superior to methods previously adopted [12] in that by using the primary data all the points are weighted equally, without excess emphasis being placed on points at high selector concentration as is the case when using inverse data methods [16]. This is particularly beneficial when dealing with very strongly bound analytes. Cases 3 and 4 assume that all binding to the stationary phase occurs at identical chiral selector sites. The ratio of the capacity factors for the enantiomers, k'_2/k'_1, is equal to K_2/K_1. Individual binding constants cannot be obtained directly from LC without knowledge of phase ratios. Case 2 is intermediate between case 1 and cases 3 and 4.

3. Experimental

3.1. Capillary electrophoresis

Methyl-β-cyclodextrin was a gift from Wacker Chemicals (Halifax, UK), sulphobutyl-β-cyclodextrin was a gift from Jones Chromatography (Hengoed, UK). Fluoxetine and norfluoxetine were gifts from Eli Lilly, and were in the form of hydrochloride salts. All other materials were from Aldrich (Gillingham, UK).

Capillary electrophoresis experiments were carried out on an automated CE system (P/ACE 2100, Beckman, High Wycombe, UK), thermostatted at 25°C. The fused-silica separation capillary had an internal diameter of 50 μm, a total length of 57 cm and a length from inlet to detector of 50 cm. A voltage of 20 kV was normally used for the separation, with on-capillary UV detection at 230 nm. The run buffer (unless otherwise stated) was prepared by titrating a 1% (w/w) triethylamine solution with dropwise addition of glacial acetic acid, until the desired pH was achieved. Organic modifiers were added as required, and cyclodextrin added in varying amounts. Samples were dissolved in run buffer at the concentrations stated and prepared fresh each day. The samples were loaded with a 1 s pressure injection (corresponding to 1 nl). Each experiment was run in duplicate, with mesityl oxide as the neutral marker. Relative viscosity was determined from the ratio of the currents I, and I_0 at [cyclodextrin] = 0 mM, with and without cyclodextrin in the run buffer; $I_0/I = \eta/\eta_0$ [13].

3.2. Liquid chromatography

All solvents were of HPLC grade and were purchased from Tech-Line (Athens, Greece). Triethylamine and glacial acetic acid were of analytical grade and were purchased from Aldrich. The LC system consisted of a pump (Waters, Model 501), an injector (Rheodyne,

Model 7125) with a 5-μl loop and a spectrophotofluorimeter detector (Perkin Elmer, Model LS30) with an 8-μl flow cell. Detection was accomplished at an excitation wavelength of 235 nm and an emission wavelength of 315 nm. The chromatograms were obtained using an integrator (Hewlett Packard, Model HP3394A). For the MPA work a phenyl column (100 × 4.6 mm I.D., Hellamco, Athens, Greece) was used. For the CSP work a Cyclobond I column (Spherisorb-phenyl S5, 250 × 4.6 mm I.D., Advanced Separation Technologies) was used, with a flow-rate of 0.4 ml min^{-1} (10% acetonitrile mobile phase composition) and 0.8 ml min^{-1} (20% acetonitrile mobile phase composition). When not in use both these columns were stored in methanol. The void volume of each column was determined by injecting 5 μl of pure methanol. All experiments were performed at room temperature. The mobile phase was prepared as for the CE experiments, filtered and degassed under vacuum using a Millipore system. The stock solutions of FL and NR were prepared at 1 mg ml^{-1} and kept in amber-coloured bottles in a refrigerator and made fresh each week. Working standard solutions of 1 μg ml^{-1} were prepared fresh every day in mobile phase. Typically a volume of 5 μl of each solution was injected.

4. Results and discussion

4.1. Comparison of binding constant data from CE and LC

Previous experiments [7] optimised a set of conditions for the simultaneous LC separation of FL and NR enantiomers using a Cyclobond I CSP with a 1% (w/v) triethylammonium acetate ($Et_3NH^+Ac^-$) buffer, pH 5.5, containing 10% acetonitrile (MeCN). This stationary phase is composed of β-CD bonded on 5 μm spherical silica beads. The tether to the β-CD is an ether linkage between a surface silanol group and one or two of the primary hydroxyl groups on the β-CD [17]. An LC separation using β-CD as a MPA was investigated using the same buffer conditions and an achiral reversed-phase phenyl LC column. By varying the β-CD concentration it is possible to obtain a plot of k' vs. [β-CD], and hence to obtain a binding constant for the FL–β-CD complex as discussed in the theory section. A CE experiment was carried out again using identical buffer conditions, with β-CD as a MPA, and a plot of mobility, μ, vs. [β-CD] gave the binding constant for the FL–β-CD complex.

The separation of FL in $Et_3NH^+Ac^-$ buffer with 10% MeCN is shown in Fig. 2, using LC

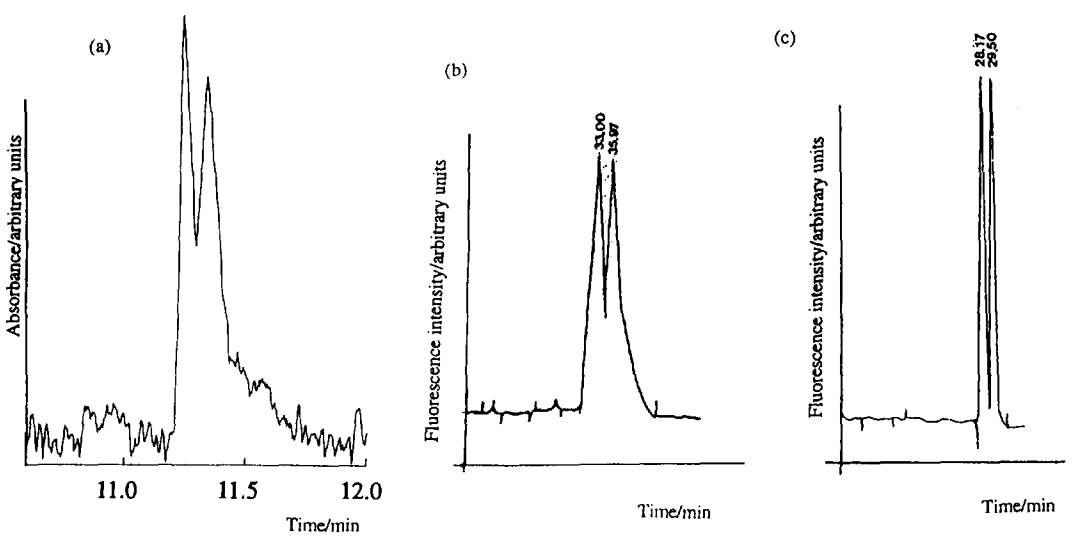

Fig. 2. Separations of fluoxetine enantiomers using (a) CE with 1 m*M* β-CD added to buffer, (b) LC with 1 m*M* β-CD added to mobile phase and (c) LC with a Cyclobond I CSP. Buffer conditions 1% triethylammonium acetate, pH 5.5, 10% acetonitrile. Other conditions as described in Experimental section.

Table 1
Comparison of enantiomeric separation parameters for fluoxetine and norfluoxetine with β-CD using (i) LC with CSP, (ii) LC with MPA and (iii) CE

Technique	Parameter	Fluoxetine	Norfluoxetine
(i) LC with CSP	k'_S	18.9	16.2
	k'_R	20.1	17.1
	α	1.06	1.05
	R_s	0.87	0.98
(ii) LC with MPA	K_S	$1147 \pm 51\ M^{-1}$	$1206 \pm 37\ M^{-1}$ [a]
	K_R	$1268 \pm 46\ M^{-1}$	
	α	1.11	–
	R_s	0.57	–
(iii) CE	K_S	$1078 \pm 61\ M^{-1}$	$991 \pm 26\ M^{-1}$
	K_R	$1144 \pm 65\ M^{-1}$	$1043 \pm 27\ M^{-1}$
	α	1.06	1.05
	R_s	0.62	0.68

Buffer composition: pH 5.5, 1% triethylammonium acetate, 10% MeCN.
[a] Unresolved.

with CSP, LC with β-CD as MPA, and CE with β-CD as buffer additive. Table 1 compares binding constant, selectivity and resolution values from all three methods. As mentioned in the Theory section the binding constants obtained by CE and LC with a MPA should be identical. The binding constants from LC with MPA and CE were found to be in good agreement, with differences outside the error limits probably attributable to temperature differences. The LC experiments were carried out at ambient temperature (25–27°C), and the CE experiments were carried out using an instrument setting of 25.0°C.

Excellent agreement is seen between selectively measured by CE and LC with CSP, $\alpha = 1.06$ for FL and 1.05 for NR respectively. This implies that there is no hindrance to binding to the CSP arising from steric effects of the tether to the surface silanol groups. Resolution in the NR case was insufficient to provide selectivity data. Table 1 shows that highest resolution is obtained using LC with the CSP.

Assignments of peaks were made by spiking with an excess of one enantiomer. In CE, the first migrating enantiomer was confirmed as the (+)-(*S*)-enantiomer, this being the weaker binding of the two. In LC with CSP the (+)-(*S*)-enantiomer was also confirmed as the first eluting enantiomer, this being the least retained on the column, and hence having the lowest value of k'. In LC with MPA the (+)-(*S*)-enantiomer has the higher k' values, since the β-CD pulls more of the stronger binding (−)-(*R*)-enantiomer into the mobile phase.

4.2. Effect of LC buffer additives in CE separations

When transferring buffer conditions between LC and CE care must be taken to understand the nature of the buffer solutions. In LC the use of $Et_3NH^+Ac^-$ at a relatively high concentration of 0.1% to 1% is necessary in order to give a dynamic coating to unreacted silanol groups and thus avoid peak broadening and tailing [18]. Previous work using cyclohexanol has shown that large organic additives in the buffer may compete for the cyclodextrin cavity [13]. This complexation will cause the apparent binding constant to decrease, and hence the optimum [β-CD] to achieve chiral resolution to be increased. Therefore the role of the LC buffer in the CE experiment was investigated by using sodium acetate (Na^+Ac^-) in place of $Et_3NH^+Ac^-$.

The 1% $Et_3NH^+Ac^-$ buffer used in the LC experiments has an ionic composition of 100 m*M* and its action as a buffer at pH 5.5 is due to the

presence of CH_3COOH and CH_3COO^-, and not due to the Et_3NH^+ cation: the pK_a of Et_3NH^+ is 10.8. Many CE experiments use concentrations of buffer very much less than 100 m*M*. The CE chiral separation of FL was repeated with 10% MeCN using a 50-m*M* Na^+Ac^- buffer at pH 5.5 as a replacement for the $Et_3NH^+Ac^-$ buffer. The electroosmotic flow (EOF) in the absence of β-CD was $0.99 \cdot 10^{-8}$ $m^2V^{-1}s^{-1}$ for the 100-m*M* $Et_3NH^+Ac^-$ buffer, and $3.08 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} for the 50-m*M* Na^+Ac^- buffer. Several groups [19,20] have also observed a decrease in EOF with $Et_3NH^+Ac^-$ buffer systems. 10 m*M* $Et_3NH^+Ac^-$ in a water–methanol (85:15) system has been reported to reverse the EOF [20]. This was attributed to the Et_3NH^+ cation binding to the capillary wall and changing the sign of the charge at the Stern layer. Whilst we observe a substantial decrease in the EOF in the 100-m*M* $Et_3NH^+Ac^-$ water–acetonitrile (90:10) buffer, no flow reversal was found. The dependence of EOF magnitude and direction on the Et_3NH^+ concentration, organic modifier type and concentration is therefore complex. Since a high ionic concentration helps to reduce electromigration dispersion and the low EOF and positive coating of the walls is beneficial for resolution and peak shape of positively charged analytes, the 100-m*M* $Et_3NH^+Ac^-$ buffer should in principle be better than the 50-m*M* Na^+Ac^- buffer for separation of the cationic species FL and NR. Fig. 3 shows the comparison of the CE chiral separation of FL in the $Et_3NH^+Ac^-$ buffer and the Na^+Ac^- buffer, both containing 1 m*M* β-CD. The migration time of FL is longer in the 100 m*M* $Et_3NH^+Ac^-$ buffer, the resolution is greater, whilst the peak shape is poorer.

Calculation of the binding constants in the sodium acetate buffer system for FL gave $\bar{K} = 760 \pm 95\ M^{-1}$ with $\alpha = 1.04$, and $\bar{K}$ for NR equal to $840 \pm 105\ M^{-1}$ with $\alpha = 1.02$. If the buffer in the CE capillary was not interacting with the analyte–selector complex we should expect to see identical binding constants and selectivity, with changes limited to EOF and resolution [13]. This is clearly not the case; comparison of data in Table 2 shows that the values of the binding

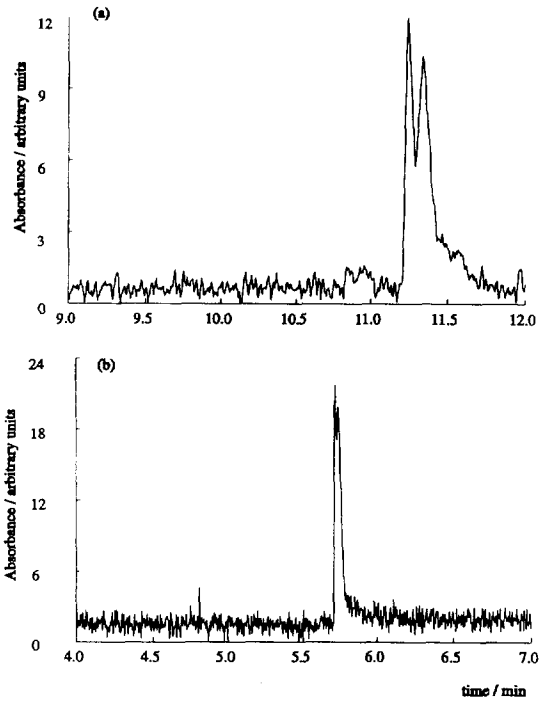

Fig. 3. Separation of FL enantiomers by CE in (a) 1% triethylammonium acetate buffer, pH 5.5, 1 m*M* β-CD, 10% acetonitrile, (b) 50 m*M* sodium acetate buffer, pH 5.5, 1 m*M* β-CD, 10% acetonitrile. Other conditions as described in Experimental section.

constants in the Na^+Ac^- buffer are 20–30% less than in the $Et_3NH^+Ac^-$ buffer. α values are also systematically lower. Li and Lloyd [20] report the formation of a ternary complex between amino acids, $Et_3NH^+Ac^-$, and β-CD. The differences measured here in binding constant and selectivity may also be due to ternary complex formation, and further experiments are underway to clarify the nature of any possible binding of $Et_3NH^+Ac^-$ to β-CD.

Differences in ionic strength between the two buffers cannot account for the observed differences in binding constant. Correction to $\bar{K}$ for non-ideality using Debye–Hückel theory gives values of the average thermodynamic equilibrium constant, K^0, for the enantiomers [13,21]. When this is done there is still a substantial difference between K^0 values derived from the

Table 2
Comparison of binding constant data in triethylammonium acetate and sodium acetate buffer solution, with correction for non-ideality

	Fluoxetine		Norfluoxetine	
	Na^+Ac^- (50 mM)	$Et_3N^+Ac^-$ (100 mM)	Na^+Ac^- (50 mM)	$Et_3N^+Ac^-$ (100 mM)
$\bar{K}(M^{-1})$	760	1111	840	1017
K^0	794	1182	878	1082

two buffers, and this applies to both FL and NR. Table 2 shows the measured values of $\bar{K}$ and the corrected values of K^0 for both FL and NR.

4.3. Effect of derivatised cyclodextrins in CE and LC

Derivatised cyclodextrins are often used to enhance selectivity of chiral separations using CE. We have investigated the variation in binding constant with the use of methylated-β-CD (Me-β-CD) and hydroxypropyl-β-CD (HP-β-CD). Table 3 shows the variation in binding constant for FL with chiral selector, in CE and LC with MPA. As in previous sections identical conditions for buffer composition were used in CE and LC. Binding constants from the two techniques are again seen to be in good agreement. FL–HP-β-CD is seen to have a very low binding constant in comparison with FL–β-CD. Since hydroxypropylation introduces bulky groups at the cavity rim, these might sterically interfere with FL inclusion into the cavity. The binding constants with Me-β-CD are about 10% lower than with the native β-CD. The most significant difference between these two cyclodextrins however, lies in the selectivity, α, which is considerably lower for Me-β-CD than the native β-CD and HP-β-CD, and thus leads to worse resolution [13].

The use of charged cyclodextrins in chiral separations by CE can expand the resolution window in comparison with neutral cyclodextrins, and can also strengthen binding when the cyclodextrin and analyte have opposite charges [22]. We investigated the use of sulphobutyl-β-CD, a negatively charged cyclodextrin, for the separation of the positive charged FL. The electropherogram in Fig. 4 shows baseline separation of the enantiomers, obtained using reverse polarity, under identical buffer conditions as in

Table 3
Variation of binding constant with chiral selector in CE and LC with MPA

Selector	$K_S(M^{-1})$	$K_R(M^{-1})$	α	R_s
CE				
HP-β-CD	307 ± 14	326 ± 15	1.06	0.59
Me-β-CD	995 ± 20	1019 ± 21	1.02	0.32
β-CD	1078 ± 65	1144 ± 65	1.06	0.62
SB-β-CD	–	–	–	1.71
LC with MPA				
HP-β-CD	363 ± 27	398 ± 30	1.10	0.41

Conditions: pH 5.5, 1% $Et_3N^+Ac^-$ buffer, 10% MeCN.

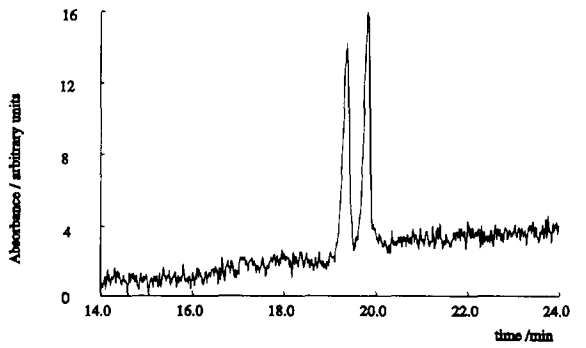

Fig. 4. Separation of fluoxetine enantiomers by CE, using 7.5 m*M* sulphobutyl-β-cyclodextrin as a buffer additive. Other conditions as described in Experimental section.

Table 3. A resolution of 1.71 was obtained, the highest value for all the selectors investigated.

4.4. Effect of organic modifiers in CE and LC

The effect of variation of MeCN concentration was studied using the three techniques. Data for FL is given in Table 4 for the binding constants, capacity factors, selectivity and resolution obtained using 20% MeCN. Satisfactory agreement between binding constants from LC with MPA and CE is seen. Upon addition of organic modifier there is a decrease in binding constant. This is similar to observations with another cationic species, tioconazole [13]. Theory suggests that the action of the organic modifier is to increase the affinity of the analyte for the mobile phase, with no change to selectivity. Comparison of data for LC with MPA and CE in Tables 1 and 4 shows that this is the case. Using the LC with a CSP resolution is essentially lost in going from 10% to 20% acetonitrile. Although two peaks could be observed, the separation between the peak maxima was insufficient to give meaningful values of R_s and α. The higher flow-rate used with 20% acetonitrile may in part explain the loss of resolution, since R_s has previously been shown to decrease with increasing flow-rate in inclusion chromatography on a Cyclobond I column [7]. The decrease in the binding constant with increase in organic modifier concentration is graphically demonstrated in Fig. 5 where the binding curves at 10% and 20% MeCN are overlaid. These solution phase results would suggest, following the ideas in the Theory section, that k' should decrease by a factor of 7.5 and the capacity factor should be insufficient to give any chiral resolution when comparing K in 10% and 20% acetonitrile. Whilst k' does not appear to scale linearly with binding constant, information from CE can still be used as a guide for method development in LC.

Table 4
Comparison of enantiomeric separation parameters for fluoxetine and norfluoxetine with β-CD using (i) LC with CSP, (ii) LC with MPA and (iii) CE

Technique	Parameter	Fluoxetine
(i) LC with CSP	k'_S	13.24
	k'_R	13.58
	α	–
	R_s	<0.5
(ii) LC with MPA	K_S	$124 \pm 4\ M^{-1}$
	K_R	$135 \pm 4\ M^{-1}$
	α	1.09
	R_s	0.52
(iii) CE	K_S	$143 \pm 7\ M^{-1}$
	K_R	$152 \pm 8\ M^{-1}$
	α	1.06
	R_s	0.68

Buffer composition: pH 5.5, 1% triethylammonium acetate, 20% MeCN.

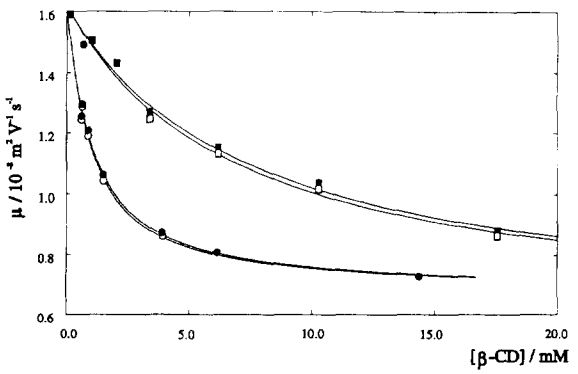

Fig. 5. Binding curves from CE for fluoxetine enantiomers comparing 90:10 (circles) and 80:20 (squares) water–acetonitrile buffer, where open symbols are the (*R*)-enantiomers and closed symbols are the (*S*)-enantiomers. Conditions as described in Experimental section.

5. Conclusion

The same binding processes are shown to occur in CE and LC, and binding constants and selectivities measured with cyclodextrin selectors in both CE and LC with mobile phase additives are shown to be in good agreement. Using an aqueous triethylammonium acetate buffer–acetonitrile (90:10) as solvent, chiral resolution was obtained using both mobile phase additive and chiral stationary phase techniques. Increasing the organic modifier content to 20% decreased binding constants as expected, and gave insufficient resolution using the CSP. A benefit of CE can be seen here, in the prediction of a useful solvent working range for a CSP. A range of solvent compositions could be screened quickly using CE, without the need to extensively use the CSP column, and thus extend column life time.

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References

[1] S.G. Allenmark, *Chromatographic Enantioseparations*, Ellis Horwood, Chichester, 1988.
[2] D. Sybilska and J. Zukowski, in A.M. Krstulovic (Editor), *Chiral separations by HPLC –Applications to Pharmaceutical Compounds*, Ellis Horwood, Chichester, 1989, Ch 7.
[3] M.M. Rogan, K.D. Altria and D.M. Goodall, *Chirality*, 6 (1994) 25.
[4] S. Terabe, K. Otsuka and S. Nishi, *J. Chromatogr. A*, 666 (1994) 295.
[5] K.D. Altria, R.C. Harden, M. Hart, J. Hevizi, P.A. Hailey, J.V. Makwana and M.J. Portsmouth, *J. Chromatogr.*, 641 (1993) 147.
[6] S.G. Penn, G. Liu, E.T. Bergström, D.M. Goodall and J.S. Loran, *J. Chromatogr. A*, 680 (1994) 147.
[7] S. Piperaki and M. Parissi-Poulou, *Chirality*, 5 (1993) 258.
[8] R.F. Bergström, L. Lemberger, N.A. Farid and R.L. Wolen, *Br. J. Psychiat.*, 153 (1988) 47.
[9] D.T. Wong, R.W. Fuller and D.W. Robertson, *Acta Pharm. Nord.*, 2 (1990) 171.
[10] R.W. Fuller, D.T. Wong and D.W. Robertson, *Med. Res. Rev.*, 11 (1990) 17.
[11] H. Soini, M. Riekkola and M.V. Novotny, *J. Chromatogr.*, 608 (1992) 265.
[12] D. Sybilska, J. Zukowski and J. Bojarski, *J. Liq. Chromatogr.*, 9 (1986) 591.
[13] S.G. Penn, E.T. Bergström, D.M. Goodall and J.S. Loran, *Anal. Chem.*, 66 (1994) 2866.
[14] S.A.C. Wren and R.C. Rowe, *J. Chromatogr.*, 603 (1992) 235.
[15] S.G. Penn, E.T. Bergström, G. Liu, I. Knights, A. Ruddick and D.M. Goodall, *J. Phys. Chem.*, in press.
[16] W.B. Person, *J. Am. Chem. Soc.*, 87 (1965) 167.
[17] R.D. Armstrong, T.J. Ward, N. Pattabiraman, C. Benz and D.W. Armstrong, *J. Chromatogr.*, 414 (1987) 192.
[18] T.J. Ward and D.W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407.
[19] I. Bechet, P. Paques, M. Fillet, P. Hubert and J. Crommen, *Electrophoresis*, 15 (1994) 818.
[20] S. Li and D.K. Lloyd, *J. Chromatogr. A*, 666 (1994) 321.
[21] M.A. Survay, D.M. Goodall, S.A.C. Wren and R.C. Rowe, *J. Chromatogr. A*, in press.
[22] B. Chankvetadze, G. Endresz and G. Blaschke, *Electrophoresis*, 9 (1994) 407.