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Determination of binding constants and the influence of methanol on the separation of drug enantiomers in cyclodextrin modified capillary electrophoresis

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Abstract

Binding constants of the optical isomers of phenylalkylamine derivatives and (2,6-di-O-methyl)-β-cyclodextrin were determined using electrophoretic mobility data gained from separations performed by capillary electrophoresis, and assuming a simple equilibrium model. Dependence of the optimum separation conditions and parameters on the binding constants were investigated and the influence of methanol was estimated.

Keywords: Enantiomer separation; Binding constants; Buffer composition; Phenylalkylamines; Drugs

1. Introduction

Chiral analysis of drugs (during their manufacturing, purity test, pharmacokinetic and pharmacodynamic studies and clinical monitoring) has well recognized importance. The need for fast and reliable enantiomer separation methods stimulated an enormously quick development of chiral capillary electrophoresis in the last several years. The results of this intensive work have been published in a vast amount of papers.

Capillary electrophoresis (CE) has been shown to be versatile for chiral separations when used in conjunction with chiral additives, especially various cyclodextrin derivatives [1–5]. Cyclodextrins are cyclic oligosaccharides with the ability to form inclusion complexes with a great variety of guest molecules. The different cavity size of the α, β and γ cyclodextrins and the various physicochemical characteristics of their substituted (charged, uncharged, polymer) derivatives have a considerable impact on chiral resolution, and makes relatively easy to choose the appropriate cyclodextrin derivative to accomplish the desired chiral resolution.

Chiral CE separations are usually performed in free solution mode, where the chiral additives are included in the background electrolyte. The separation is based on the dynamic equilibrium of diastereomer complex formation between the chiral analyte and the chiral selector. The separation can be achieved when the selector–analyte binding constants are different for the two enantiomers and the mobilities of the free and complexed forms differ. Assuming the fast exchange between the free and bound ligands (analytes), Wren and Rowe [6] and Penn et al. [7] proposed a simple model based on the host–guest complexation for optimization of the enantiomer separation conditions. They found, that the optimum concentration of the chiral selector is dependent on the magnitude of the binding constants,

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while the different mobility of the enantiomer pairs is a function of the difference in their complex formation constants. The best selectivities were found in the presence of weak interactions (when the binding constants are low). In their studies, Rawjee and Vigh also included the investigation of the effect of pH on the complex formation, and on the enantiomer separation [8,9].

Application of various additives in the running buffer influences the separation of the optical isomers. The effect of methanol was described by Wren and Rowe [10]. The decrease of the complex formation constants in the presence of methanol, and a shift of the optimum cyclodextrin concentration to higher values were found. The influence of methanol on the separation of tioconazole enantiomers was studied by Penn et al. [7]. In agreement with the previous findings, the binding constants decreased in the presence of methanol, which they explained by the preferred solubility of the free drugs in the running buffer.

In this work our goal was to check the applicability of the equilibrium model on the enantiomer separation and calculate equilibrium constants in fairly complex media. The effect of methanol on the complex formation, as well as the chiral separation was also studied.

2. Experimental

2.1. CE apparatus and separation conditions

A Crystal 300 (ATI, Unicam, Cambridge, UK) capillary electrophoresis system equipped with a variable-wavelength UV absorbance detector set at 190 nm was used. The separations were performed in 70 cm×75 μm I.D. uncoated fused silica capillaries, the length to the detection window was 55 cm. Samples were introduced by electrokinetic injection from 5–10 μM solutions of the test compounds applying 3 kV for 12 s. The applied electric field was 300 V/cm, the separation temperature 21°C. Axxiom 727 software was used for data collection.

20 mM Tris–phosphate pH 2.7 containing 0.5% hydroxypropylmethyl cellulose and various concentration of heptakis (2,6-di-O-methyl)-β-cyclodextrin and methanol was used as running buffer [11].

2.2. Chemicals

The enantiomer pairs of the following standard compounds were tested: amphetamine (A), methamphetamine (MA), deprenyl (D), propargylamphetamine (PA), *para*-fluoro-methamphetamine (*p*FMA), *para*-fluoro-deprenyl (*p*FD), which compounds were kindly provided by Chinoin Pharmaceutical and Chemical Works (Budapest, Hungary), pseudoephedrine (PSE), norephedrine (NE) and ephedrine (E) obtained from Sigma (Budapest, Hungary). All compounds were available as hydrochloride salt and were dissolved in distilled water.

The chiral selector heptakis (2,6-di-O-methyl)-β-cyclodextrin (DIMEB) was purchased from Cyclolab (Budapest, Hungary). Tris(hydroxymethyl)aminomethane (Tris), phosphoric acid and methanol (MeOH) were supplied by Reanal (Budapest, Hungary), while hydroxypropylmethyl cellulose (HPMC, viscosity of 2% aqueous solution: approx. 4000 cP at 25°C) from Sigma.

2.3. Equilibrium model and calculations

In all calculations the simple 1:1 host–guest complex formation was assumed, where the free guest molecule (analyte) is in equilibrium with its complexed form.

$$L_R + C \rightleftharpoons L_RC \quad K_R = [L_RC]/([L_R][C]) \tag{1}$$

$$L_S + C \rightleftharpoons L_SC \quad K_S = [L_SC]/([L_S][C]) \tag{2}$$

where L_R, L_S represent the enantiomers, K_R and K_S are their formation constants with DIMEB (C), respectively. L_R and L_S can have different affinity for the chiral selector, that is $K_R \neq K_S$. The equilibrium is achieved very rapidly for both enantiomers. The electrophoretic mobilities of the enantiomer pairs are equal in the absence of the chiral additive ($\mu_i = \mu_R = \mu_S$). The apparent electrophoretic mobility (μ_a) of a certain analyte is determined by the binding constant and the mobility difference between the free and the bound enantiomer, i.e. μ_a is proportional to the product of mol fraction and the electrophoretic mobility of the free and complexed analyte, respectively:

$$\mu_{aR} = \frac{[L_R]}{[L_R]+[L_RC]}\mu_i + \frac{[L_RC]}{[L_R]+[L_RC]}\mu_{\infty R} \quad (3)$$

where $\mu_{\infty R}$ is the electrophoretic mobility of the L_RC complex.

Wren and Rowe [6] and Penn et al. [7] considered the electrophoretic mobilities of the complexes of an enantiomer pair identical. In our calculation this assumption was not necessary, as we also calculated this value. On the other hand we introduced a kind of "normalized electrophoretic mobility" value for both the free and bound molecules, i.e.:

$\mu_i^* = \mu_i/([L_R] + [L_RC])$, and

$\mu_{\infty R}^* = \mu_{\infty R}/([L_R] + [L_RC])$

These normalized values and Eqs. 1 and 2 were substituted into Eq. 3, respectively:

$$\mu_{aR} = [L_R]\mu_i^* + K_R[L_R][C]\mu_{\infty R}^* \quad (4)$$

and

$$\mu_{aS} = [L_S]\mu_i^* + K_S[L_S][C]\mu_{\infty S}^* \quad (5)$$

By the use of an iterative computer program the equilibrium concentrations of L and C, the binding constants, and the electrophoretic mobility of the host–guest complexes were estimated on the bases of the best fit between the computed and the experimental mobility data. Because of the low buffer pH and the use of additive (pH=2.7, 0.5% HPMC), the electroosmotic flow was very much reduced, and ignored when the apparent electrophoretic mobilities were measured.

The optimum concentrations of the chiral selector were calculated according to Wren and Rowe [6].

$$c_{opt} = \frac{1}{\sqrt{K_R K_S}} \quad (6)$$

The selectivities were estimated using equation, suggested by Penn et al. [7].

$$\alpha = (\sqrt{K_R K_S} + \Delta K/2)/(\sqrt{K_R K_S} - \Delta K/2) \quad (7)$$

where ΔK is the difference of K_R and K_S.

3. Results and discussion

3.1. Mobilities and binding constants

The electrophoretic mobilities of the enantiomer pairs were measured in the 0–48 m*M* concentration range of the chiral selector, (2,6-di-O-methyl)-β-cyclodextrin. Figs. 1 and 2 show the chiral resolution of some of the compounds, as the concentration of the chiral selector was varied in the separation buffer. Application of DIMEB in the buffer decreased the electrophoretic mobility of each test compound. This indicated the inclusion complex formation of separands with the chiral additive, as complexes formed have lower electrophoretic mobilities, than the uncomplexed separands.

Besides the complex formation, the increasing viscosity of the running buffer containing higher concentration of DIMEB also influences the apparent mobility, and this effect has to be taken into correction when the electrophoretic mobilities are estimated. In agreement with the statement of Wren and Rowe [6] we found that the ratio of currents measured during separations at various chiral selector concentration is a good indicator of the ratio of viscosities and can be used to perform the correction. (The viscosity increased by a factor of 1.13 in the 0–24 m*M* concentration range of DIMEB determined by viscosity measurements, while the current ratio was found to be 1.14).

The complex formation constants, selectivity and the optimum concentration of the chiral selector were calculated as it was described above, and are summarized in Table 1.

The difference in mobilities of the enantiomer pairs is shown as a function of the concentration of DIMEB in Fig. 3. The concentrations of DIMEB providing maximum mobility differences are in good agreement with the calculated optimum concentration values.

3.2. The effect of the structure of compounds on chiral discrimination

Comparing the structure of the test compounds and the calculated binding constants, weaker interactions were shown by drugs having hydroxyl group at the α position. This can probably be explained by

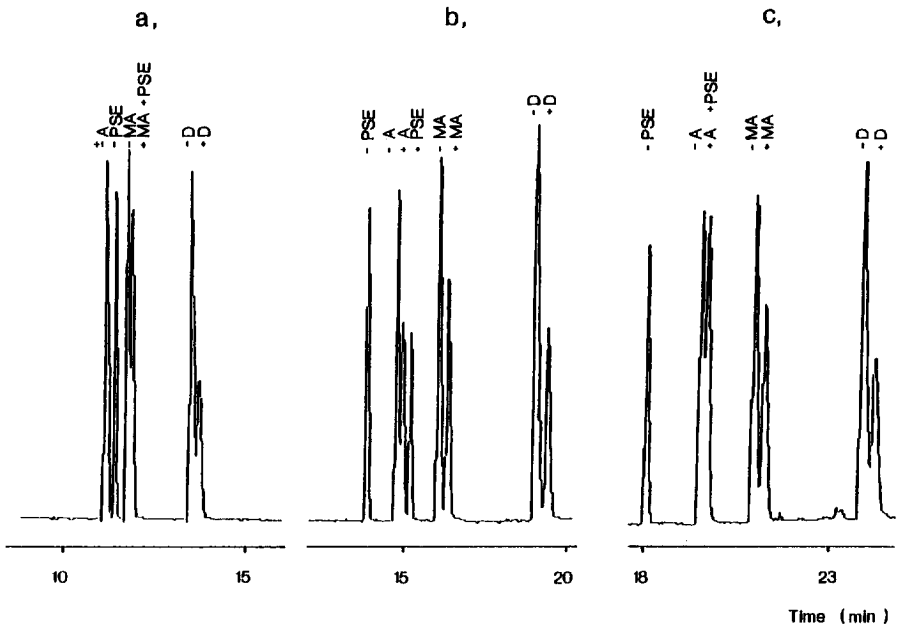


Fig. 1. Separation of enantiomers of amphetamine (A), methamphetamine (MA), pseudoephedrine (PSE) and deprenyl (D) in running buffer containing (a) 3 m*M*, (b) 12 m*M* and (c) 24 m*M* of DIMEB. Other separation conditions are given in Section 2.1.

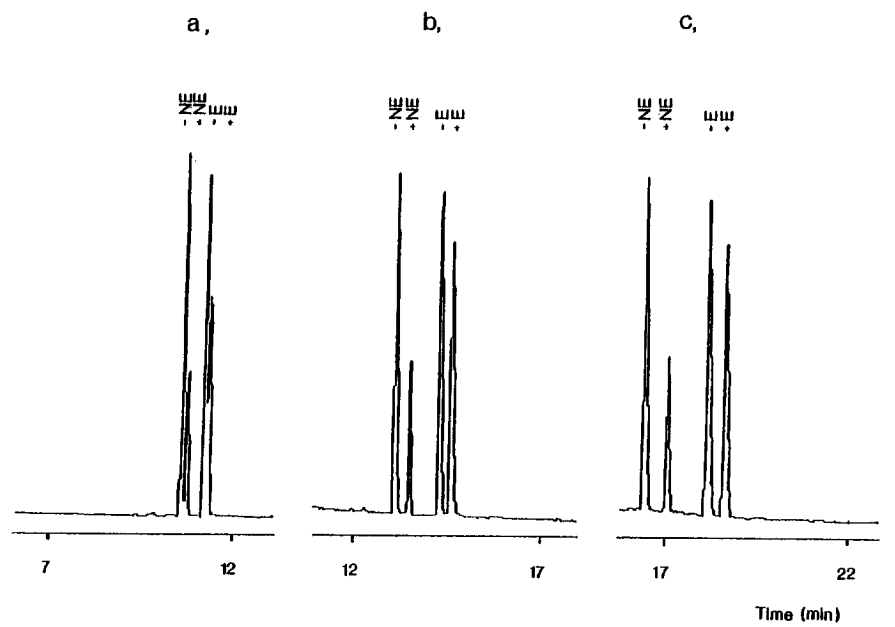
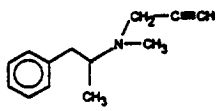
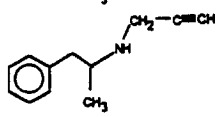
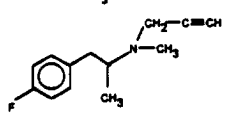
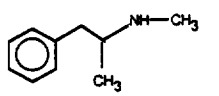
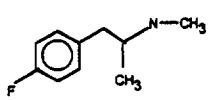
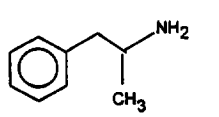
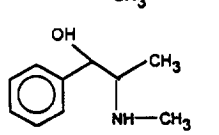
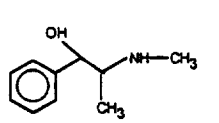
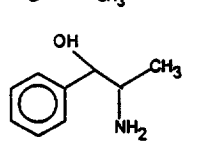


Fig. 2. Separation of enantiomers of norephedrine (NE) and ephedrine (E) in running buffer containing (a) 3 m*M*, (b) 12 m*M* and (c) 24 m*M* of DIMEB. Other separation conditions are given in Section 2.1.

the higher degree of hydration of these compounds, which acts against the inclusion, the optimum fit of the aromatic ring into the hydrophobic cavity. The binding constants of compounds substituted at the nitrogen are higher both in the absence and presence of the α-hydroxyl group, and further increase of the constants were found with the propargyl substitution at this position.

Table 1
Calculated binding constants, selectivity and optimum concentration of chiral selector for phenylalkylamine enantiomers with (2,6-di-O-methyl)-β-cyclodextrin in Tris–phosphate–HPMC buffer pH 2.7, at 21°C

Name	Structure	Binding constants		α	c_{opt} (mmol/dm^3)
		K_1	K_2		
Deprenyl		142.3±4.3	153.3±6.9	1.08	6.8
Propargyl-amphetamine		$K_1 = K_2 = 151.5 \pm 6.6$		–	–
p-Fluoro-deprenyl		123.4±4.5	133.2±5.6	1.08	7.8
Methamphet amine		107.0±3.9	111.9±3.26	1.06	9.1
p-Fluoro-methamphet-amine		98.90±4.1	100.2±3.9	1.02	10.0
Amphet-amine		89.10±1.0	94.5±3.3	1.06	10.9
Ephedrine		57.60±2.5	63.4±3.1	1.10	16.7
Ψ-Ephedrine		53.80±4.0	77.5±4.5	1.45	15.5
Norephedrine		43.80±4.0	52.6±4.1	1.20	20.8

K_1 and K_2 are the binding constants of (−) and (+) enantiomers, respectively. Selectivity (α) and c_{opt} were calculated as given in the Section 2.3.

The fluoro substitution of the aromatic ring at para position (*p*-fluoro-methamphet-amine and *para*-fluoro-deprenyl) apparently reduced the interaction and the measured binding constants. This may be the consequence of the polarization of the aromatic ring.

As for the enantioselectivity is concerned, differential binding of amphetamine enantiomers was observed, which compound is substituted neither at the α-carbon, nor at the nitrogen. The chiral discrimination was found to be the best for compounds substituted by hydroxyl group at α position, probably due to the presence of two chiral centers. The

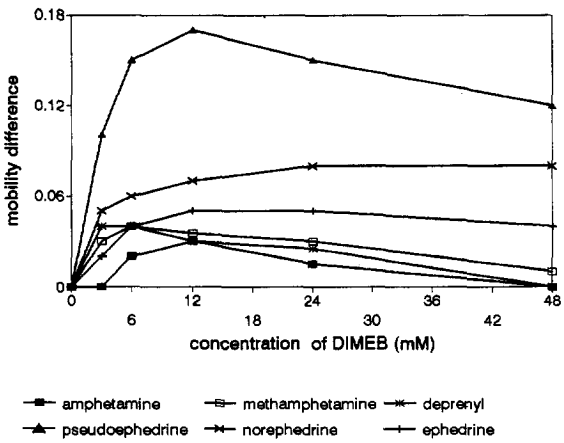


Fig. 3. The mobility difference of enantiomer pairs as a function of the concentration of the chiral selector, DIMEB. The running buffer contained no methanol. Other separation conditions are given in Section 2.1.

importance of the two chiral centers is also supported by the fact that N-substitution alone did not induce an unequivocal change of the chiral discrimination. Its presence resulted in an increase of the chiral recognition in case of pseudo-ephedrine, while a decrease in case of ephedrine, compared to that found with norephedrine. In case of compounds having only one chiral center, chiral resolution could not be achieved with the N-propargyl substituted drug (propargylamphetamine). The methyl substitution at this position was necessary for the different binding and the resolution of the enantiomers.

3.3. The effect of methanol

The binding constants using mobilities of the enantiomers measured when 10% or 20% of methanol were included in the background electrolyte, were also estimated. These data are presented in Table 2.

The complex formation constants of all compounds decreased in the presence of organic solvent, as it was expected. As the binding constants were found reduced, the calculated optimum concentrations of DIMEB in the presence of methanol became higher. This is in good correlation with the experimental data. The maximum mobility difference of the enantiomer pairs measured in running buffer containing 10% methanol shifted into higher DIMEB concentrations as is shown in Fig. 4.

The reduction in binding constants was about 30–40% in 20% compared to 0% of methanol. This reduction is much less in magnitude, than was found for tioconazole by Penn et al. [7]. The selectivities did not show significant change at 10% MeOH, but decreased when the buffer contained 20% MeOH. This can probably be explained by the different characteristics of buffer systems containing low or high mol fraction of the organic solvent [12]. While in low concentration, methanol can be regarded as an organic modifier in a practically aqueous solution, in case of its higher concentration we should consider the buffer as a mixed solvent. So a pronounced solvent effect of methanol can be assumed at its higher concentration. However with the increasing

Table 2
Calculated binding constants, selectivity and optimum concentration of chiral selector for phenylalkylamine enantiomers with (2,6-di-O-methyl)-β-cyclodextrin in Tris–phosphate–HPMC buffer pH 2.7, containing 10% or 20% methanol, at 21°C

Name	Binding constants 10% methanol–water		α	c_{opt} (mmol/dm^3)	Binding constants 20% methanol–water		α	c_{opt} (mmol/dm^3)
	K_1	K_2			K_1	K_2		
Deprenyl	102.7±3.9	111.9±3.2	1.08	9.3	79.3±4.6	85.7±4.2	1.05	12.1
Methamphetamine	78.70±4.7	83.8±4.8	1.06	12.3	61.9±3.1	65.1±3.7	1.05	15.0
Amphetamine	71.20±3.3	78.8±6.2	1.06	9.3	57.2±2.5	57.2±2.5	–	–
Ephedrine	53.20±3.7	56.5±4.1	1.10	18.2	35.2±2.1	36.9±2.4	1.04	27.7
Ψ-Ephedrine	44.60±3.6	62.3±3.6	1.41	18.9	47.3±4.8	58.6±5.0	1.24	19.0
Norephedrine	42.50±2.0	52.2±2.7	1.20	21.2	30.9±2.1	31.6±2.4	1.02	32.0

K_1 and K_2 are the binding constants of (−) and (+) enantiomers, respectively. Selectivity (α) and c_{opt} were calculated as given in the Section 2.3.

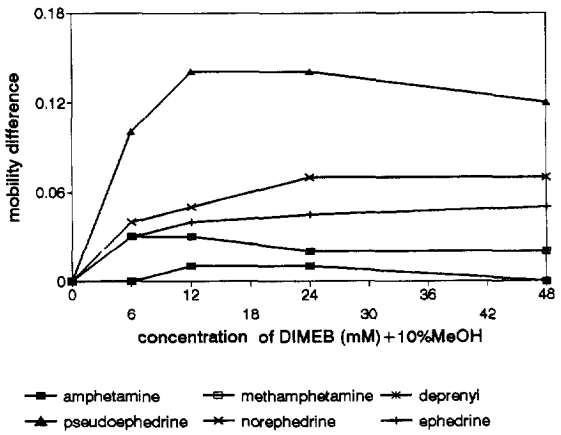


Fig. 4. The mobility difference of enantiomer pairs as a function of the concentration of the chiral selector, DIMEB. The running buffer contained 10% methanol. Other separation conditions are given in Section 2.1.

methanol content, the possibility of competition should also be considered. A 1:1 complex formation of β-cyclodextrin with methanol was supposed and the significance of competition was neglected by Penn et al [7]. According to Buvari et al. [13] methanol forms not only 1:1, but up to 4:1 complexes with β-cyclodextrin. There are no data about the interaction of methanol with DIMEB, but it should be similar. In our studies, a reduction of selectivities found in 20% methanol can also indicate the competition between methanol and the analytes. So, we assume a combination of the two effects of methanol on the complex formation.

4. Conclusions

Chiral capillary electrophoresis separations provide a simple and fast method to determine binding constants of enantiomers with cyclodextrin derivatives even in fairly complex media. A simple model based on 1:1 host–guest complexation is suggested to perform the calculations. The thermodynamic constants can be extrapolated from the estimated values.

The optimum conditions of enantioseparations can be well predicted on the bases of the complex formation constants. Our results support the previous findings and model suggested by Wren and Rowe [6]. The optimum concentration of the chiral selector is inversely proportional to the average of the complex formation constants, while the difference in electrophoretic mobilities of the enantiomer pairs show good correlation with the relative difference of their binding constants.

Compounds having two chiral centers showed the best enantioselectivity with (2,6-di-O-methyl)-β-cyclodextrin among the studied phenylalkylamine derivatives.

The binding constants were found to be decreased in the presence of methanol for all compounds. Both the solvation and competition effects of methanol can have contribution to this change.

Acknowledgments

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References

[1] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[2] S. Fanali, J. Chromatogr., 474 (1989) 441.
[3] S. Fanali and F. Kilár, J. Cap. Electrophoresis, 1 (1994) 72.
[4] B Chankvetadze, G Endresz, D. Bergenthal and G. Blaschke, J. Chromatogr., 717 (1995) 245.
[5] M. Fillet, I. Bechet, P. Chiap, P. Hubert and J. Crommen, J. Chromatogr. A, 717 (1995) 203.
[6] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235.
[7] S.G. Penn, E.T. Bergström, D.M. Goodall and J.S. Loran, Anal. Chem., 66 (1994) 2866.
[8] Y.Y. Rawjee, D.U. Staerk and Gy. Vígh, J. Chromatogr., 635 (1993) 291.
[9] Y.Y. Rawjee, R.L. Williams and Gy. Vígh, J. Chromatogr. A, 652 (1993) 233.
[10] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 609 (1992) 363.
[11] É. Szökő and K. Magyar, J. Chromatogr. A, 709 (1995) 157.
[12] T. Erdey-Grúz, Transport Phenomena in Aqueous Solutions, Akadémiai Kiadó, Budapest, 1974.
[13] Á. Buvári, J. Szejtli and L. Barcza, J. Inclusion Phenomena, 1 (1983) 151.