Enantiomeric Separations by Capillary Electrophoresis: Theoretical Method to Determine Optimum Chiral Selector Concentration

Carlina Lancioni, Sonia Keunchkarian, Cecilia B. Castells, Leonardo G. Gagliardi*

Laboratorio de Investigación y Desarrollo de Métodos Analíticos, LIDMA, Facultad de Ciencias Exactas (Universidad Nacional de La Plata, CIC-PBA, CONICET), Buenos Aires, Argentina

**Corresponding author: leogagliardi@quimica.unlp.edu.ar*

Tel: (+54) 221 - 4226979, Fax: (+54) 221 – 4216947

Highlights

- A theoretical approach to optimize BGE chiral selector concentration is presented.
- The method was applied to the separation of 4 racemic pharmaceutical drugs mixture.
- Chiral separations were achieved with 2HPBCD as BGE additive.
- The best separation is obtained at the optimum concentration found by the method.

Abstract

A method to optimize the ligand concentration [S] in the background electrolyte of capillary electrophoresis separations is presented. It is based on the use of a model which predicts apparent electrophoretic mobilities as a function of ligand concentration (expressed as p[S] = -log[S]). This model is employed to compose the expression of a recently proposed criterion to qualify separations in electrophoresis. Two strategies to find the optimum p[S], leading to the best separation of all compounds, are explained: 1.- a graphical method using a windows map depicting the single separation criteria between all possible combination of compounds by pairs, and 2.- an analytical method where an extended multicriterion optimization function is composed and optimum p[S] is found by mathematical maximization. The procedure is applied to a hard-to-separate model system: enantiomeric separations of racemic mixtures. 2-Hydroxypropyl-β-cyclodextrin was chosen as a model ligand, and four pharmaceutical drugs as model analytes. In order to demonstrate the performance of the procedure, results of electrophoretic separations obtained at p[S] found as optimum are compared with separations obtained at p[S] values slightly higher and lower than the optimum.

Keywords

CAPILLARY ELECTROPHORESIS; CYCLODEXTRIN; CHIRAL SEPARATION; SEPARATION OPTIMIZATION; MULTICRITERION OPTIMIZATION FUNCTION.

1. Introduction

The quantitative determination of the enantiomeric forms of chiral compounds is an area of increasing interest in separation science. The usual procedure to achieve enantiomeric separation in techniques such as liquid chromatography (LC), gas chromatography or thin-layer chromatography, involves the interaction with a chiral compound or "selector" in stationary or mobile phase. Capillary electrophoresis (CE) is a liquid phase separation technique, which offers the alternative of being operated under several modes depending on the sample properties [1–6]. Since it does not necessary involve typical parabolic flow profiles of pressure driven techniques, higher efficiencies can be achieved. This particular feature makes CE suitable for the analysis of chiral compounds in which selectivities are usually low. A huge amount of reports on chiral separation methods can be found, and the simplest approach seems to be the addition of a chiral selector in the background electrolyte (BGE). A variety of chiral selectors including bile acids [7,8], chiral surfactants [9], or cyclodextrins [10–12] have been employed. All of them have also been used in methods implemented in LC.

In method development, the chiral selector concentration must be optimized as any other variable influencing the separation. The simplest procedure consists of searching for the best enantioseparation of a single chiral compound. However, this procedure might become more complex when the aim is to simultaneously optimize the enantioseparation involving many compounds. In certain extent, the increase of chiral selector concentration can improve separation of some pairs of enantiomers; nevertheless it is not a general behavior. Some separations can improve as the chiral selector content is raised while this concentration is within a low range; although the behavior is the opposite when the selector is within a range of high concentrations. The chiral separation for a given enantiomeric pair can be the best

possible at certain concentration, however, when dealing with simultaneous separation of many racemic compounds, the optimum selector concentration for each pair might differ and, as the chiral selector concentration is changed, the separation of some pairs would improve while the separation of others would be worsened. Therefore, an optimization criterion must be applied in order to find a compromise for the simultaneous separation of all enantiomers.

In most cases, the optimization of the separation is done on empirical basis. Experimental results at different conditions are evaluated until a reasonable and/or sufficient resolution is obtained. This procedure is cost and time consuming, and conditions found are not strictly the optimum.

In this work we propose a theoretical approach to optimize the simultaneous separation of enantiomeric pairs of a group of compounds as a function of the chiral selector concentration. Cyclodextrins (CDs) are well known host molecules consisting of (1,4)-linked D-(+)-glucopyranose units. Native CDs are chiral and non-racemic cyclic oligosaccharides that exhibit different complexation affinities with enantiomeric guests. Its cavity is able to host hydrophobic molecules (or hydrophobic parts of them) while, in contrast, the external part is sufficiently hydrophilic to impart certain solubility in water and polar solvents. In many cases, their binding and chiral recognition ability is improved after derivatization of the hydroxyl groups with different functional groups. We propose to use 2-Hydroxypropyl-β-cyclodextrin (2-HP-β-CD) as a model ligand, and a group of pharmaceutical drugs under both enantiomeric forms of its racemic mixtures as model analytes. The chosen analytes are: pindolol, propranolol, oxprenolol and homatropine methylbromide. Chemical structures indicating the asymmetric center (*) are shown in Figure 1. The first three compounds belong to β-blockers which are widely used for treatments of cardiovascular and non-cardiovascular disorders.

Homatropine methylbromide is an anticholinergic used to relieve gastrointestinal spasms and abdominal cramps.

After applying the proposed method to find the optimum chiral selector concentration value, further experiments at higher and lower concentrations than the optimum value were done to demonstrate the validity of the model.

2. **Experimental**

2.1. Instrumentation and software

Separations were carried out on a Lumex Capel 105M CE system, equipped with UV detector (Lumex Ltd., St. Petersburg, Russia). pH measurements were performed using an Accumet Research AR25 potentiometer (Fischer Scientific, New Hampshire, USA) and a Schott Blueline 11-pH glass combination electrode (SI Analytics GmbH, Mainz, Germany).

Data fittings and plots were donde with *OtiPlot v0.9.8.9* or *SciDavis v1.D009* with compositions and format conversions based on *Impress (LibreOffice5)* and *GIMP 2.8.16*, while calculations were done with *Calc v5.1.6.2 (LibreOffice 5)*.

2.2. Reagents and Materials

A MilliQ® water purification system (Millipore, Bedford, MA, USA) was used to provide deionized water. Methanol was HPLC grade (Sintorgan, Buenos Aires, Argentina). Chemical reagents used as BGE components were analytical grade or better. A H_3PO_4/ NaH_2PO_4 100 mM, pH 2.50 stock buffer solution was prepared by adding the required quantity of 5.0 M phosphoric acid (standardized by titration as usual), adding the required volume of a stock solution of sodium hydroxide 1M until the desired pH is reached. Finally ultrapure water is added up to the final volume. The reported pH is the value measured in the final stock of buffer solution.

2-Hydroxypropyl-β-cyclodextrin (average molecular weight 1460) was obtained from Sigma-Aldrich GmbH (Steinheim, Germany). BGE solutions were prepared by dissolving the cyclodextrin in the H_3PO_4/ NaH_2PO_4 100 mM, pH 2.50 buffer solution. Racemic analytes: oxprenolol, pindolol and propranolol were purchased from Sigma (Steinheim, Germany); and

homatropine methylbromide was obtained from USPC Inc. (Maryland, USA). Individual solutions of each model analyte were prepared by dissolving the solid in a 50:50 v/v methanol/water mixture at an approximate concentration of 0.5 mg/mL. Finally, a standard mixture solution containing the four pairs of enantiomers was prepared by mixing equal volumes of each individual solution. All solutions were degassed by immersion in an ultrasonic bath, filtered through a 0.22 μm membrane before use and stored at 4 °C.

Fused-silica capillaries (50 μm inner diameter) purchased from Polymicro Technologies (Phoenix, AZ, USA), were cut at a total length of 60 cm, and an effective length of 50 cm.

2.3. Procedures

New capillaries were activated for the first use by subsequently flushing at 1000 bars: 1 M NaOH (20 min), water (10 min), 0.1 M HCl (5 min), water (5 min), and BGE (20 min). Between runs, capillaries were preconditioned by flushing 0.1 M NaOH (1 min), water (1 min) and BGE (2 min).

Analytes were hydrodynamically injected by applying 30 mbar during 2 sec. Separations were performed at 25°C, UV detection at 214 nm, using a potential of 25 kV.

3. Theory

3.1. Mathematical modeling

Considering the following binding equilibria for each enantiomeric form of a racemic compound and assuming a 1:1 stoichiometric ratio:

$$S + A_1 \overset{K_1}{\leftrightarrow} SA_1 \qquad K_{SA_1} = \frac{[SA_1]}{[S][A_1]} \tag{1}$$

$$S + A_2 \overset{K_2}{\leftrightarrow} SA_2 \qquad K_{SA_2} = \frac{[SA_2]}{[S][A_2]} \tag{2}$$

Where A_1 and A_2 are the two enantiomers of the considered compound; S is a chiral selector; SA_1 and SA_2 are the inclusion complexes, with binding constants K_{SA1} and K_{SA2}, respectively. Apparent electrophoretic mobility of any analyte distributed under two different species, - *such as free and complexed forms* - is a linearly weighted function of the mobilities of each form [13,14]. This can be mathematically expressed as:

$$\mu_{app\ A_i} = \frac{[A_i]\mu_{A_i}}{[A_i]+[SA_i]} + \frac{[SA_i]\mu_{SA_i}}{[A_i]+[SA_i]} \qquad i = 1,2 \tag{3}$$

Where μ_{Ai} and μ_{SAi} denote the electrophoretic mobilities of free and complexed forms, respectively. Considering Equations 1 and 2, this expression can be rearranged to:

$$\mu_{app\ A_i} = \frac{\mu_{A_i}}{1+10^{-(p[S]+pK_{SA_i})}} + \frac{\mu_{SA_i}10^{-(p[S]+pK_{SA_i})}}{1+10^{-(p[S]+pK_{SA_i})}} \qquad i = 1,2 \tag{4}$$

Where "*p*" indicates, as usual, the mathematical operation of applying negative logarithm. Acquiring experimental data of apparent electrophoretic mobilities at different chiral selector concentrations, μ_{A1}, μ_{A2}, and K_{SA1} or μ_{SA1}, μ_{SA2}, and K_{SA2} can be obtained as parameters of

the non-linear fitting of the obtained data set to equation 4. It is worth to be mentioned that a treatment must be done to the experimental data values in order to obtain a set of correct values. The dissolution of different amounts of cyclodextrin implies not only a variation of its concentration, but also a change of the BGE viscosity in which the analytes migrate under free or complexed forms. The correction of the experimental migrations provides values in pure water; hence the proper aforementioned parameters can be obtained [15].

3.2. Optimization

Equation 4 allows the prediction of apparent mobilities as a function of chiral selector concentration if the electrophoretic mobilities and the equilibrium constants are known. Therefore, it makes possible to predict by extension the behavior of more than one compound and, consequently, the optimization of their separation. Recently, a criterion to qualify the separation of many compounds in electrodriven separations with a single scalar was proposed [16]. The criterion, whose elementary form was called t'_{ij}, considers the separation of compounds between them and also the separation of each one from neutral compounds, always present in real samples. The expression of this parameter is as follows:

$$t'_{ij} = [\mu_{app(i)}\mu_{app(j)}(\mu_{app(i)} - \mu_{app(j)})]^2 \qquad (5)$$

At higher t'_{ij} values, better separations are obtained between analytes and from neutral compounds. Therefore, mathematical maximization of t'_{ij} function can be used to find the optimum condition of the chosen variables. The key is to know the dependencies of all the involved μ_{app} on the variable (or variables) desired to be optimized.

The criterion proposed in equation 5, can be applied to separate a single pair; however, it can be extended for optimizing the separation of multicomponent samples on the base of two different strategies.

The first one consists of composing a single scalar function which qualifies simultaneously the separation of all the analytes between them, and from the neutral compounds. The general expression proposed for this function is:

$$T' = \left[\left(\mu_{app(1)}\mu_{app(2)}\mu_{app(3)} \dots \mu_{app(n)}\right)\left(\left(\mu_{app(1)} - \mu_{app(2)}\right)\left(\mu_{app(1)} - \mu_{app(3)}\right) \dots \left(\mu_{app(1)} - \mu_{app(n)}\right) \dots \left(\mu_{app(n-1)} - \mu_{app(n)}\right)\right)\right]^2$$

$$= \left[\left(\prod_{i=1}^{n} \mu_{app(i)}\right)\left(\prod_{(i,j)(j<i)}^{n(n-1)} \Delta\mu_{app(i,j)}\right)\right]^2 \quad (6)$$

The aim of this work is to optimize only one variable -the chiral selector concentration-, but the intrinsic criteria of the *T'*-parameter are the separations between many peaks taken by pairs, therefore, it constitutes a multicriterion optimization function (MCOF). The scalar value of MCOF does not have a physical meaning, but characterizes properly the separation of all compounds. As well as for t'_{ij}, the key is to know the dependencies of all the involved μ_{app} on the variable (or variables) desired to be optimized. For MCOF is also valid that at higher *T'* values, better separations, thus mathematical maximization of *T'*-function can be also applied to find the optimum condition of the chosen variable(s). Maximization can be achieved by means of three different methods: The formal procedure consists of obtaining the derivative expression and make it equal to zero. As an alternative, a method based on exploration of the function, graphically or analytically can be used. The third method, which can be considered an intermediate between the previously mentioned, and the selected to be used in this work, consists of estimating the values of the derivatives as slopes between consecutive points of the variable (i.e. ΔTij/Δp[S]), searching for the point where it becomes zero. The only caution that must be considered is that successive points of the variables must be close enough making the

discrete slopes similar to the real derivative values. Considering that pK used to compose the complete T function can not be determined with an accuracy better than ±0.01 we calculate slopes between points with Δp[S] steps 10-folds lower –i.e. ΔpS=0.001. This procedure was chosen because it is simple, valid, and can be achieved by means of accessible software, although, any other maximization procedure can also be used.

The second optimization strategy consists of composing the t'_{ij} expressions for all possible combinations of compounds taken by pairs in order to depict a windows map (WM) and apply an optimization method similar to that proposed in early years by Purnell et al. [17]. The authors proposed the selectivity factors as criterion to qualify separations in gas chromatography and optimize the relevant variables. The same authors used later the WM for optimizing NMR lanthanide shifts as a function of chelate concentration using [M]/[S] ratios, where [M] is the free metal concentration and [S] the chelate concentration, proving that the concept of WM can be applied to optimize experimental variables in a wide diversity of fields [18]. WM has been lately applied to LC [19] and CE [20] separations by using resolution (*Rs*) as optimization criterion. However, since *Rs* dependencies with any variable are very hard to predict in CE, its application requires the acquisition of a huge number of experimental data with small step changes of the optimization variable for having an acceptable accuracy by interpolation. This approach supposes a very little or no-advantage over a trial-and-error method. This main drawback requires to search a different criterion based on equations whose predictions can result in accurate and useful information from a reduced number of experiments and, if feasible, covering a wide range of variables. The t'_{ij} criterion fulfills these requirements and also allows the use the WM strategy. In this strategy all the predicted lines for t'_{ij} of all pairs must be depicted versus the optimization variable. The pair with worst separation at every value of the variable is that with lower t'_{ij} among all possible pairs.

Therefore, the lower t'_{ij} values delimit upper and lower areas in the WM, obtaining a complete visualization of the separation parameters along the whole range of the studied variable and facilitating the search of the optimum condition. In this work we predict μ_{app} of model analytes using as variable the ligand concentration, given as p[S] form, accordingly to equation 4, to compose all the t'_{ij} and T' parameters in order to prove the wide validity and robustness of both optimization strategies, and to suggest good chances to apply them for optimizing other variables, or even to optimize multiple variables simultaneously.

4. Results and discussion

In a previous work, electrophoretic mobilities of selected model analytes were experimentally determined at 25 °C in presence of different concentrations of 2-HP-β-CD as chiral selector. Experimental data were corrected to obtain the apparent mobilities in pure water, and finally each series was fitted to equation 4 to obtain the parameters gathered in Table 1 *["Determination of Binding Constants by Affinity Capillary Electrophoresis" C. Lancioni, S. Keunchkarian, L.G. Gagliardi - submitted for publication]*. The dependencies of μ_{app} with p[S] for all the model analytes are depicted in Figure 2. For each racemic analyte, the same color for solid and dotted lines were used to describe the individual behavior of each enantiomer. Equation 4 applied to all sample components can be used to compose t'_{ij}-function whose maximization, either by WM or by MCOF strategies will lead to find the [S] value that allows the best overall separation. In Figure 3 the WM is built by drawing with gray lines all t'_{ij} between all possible pairs considering the 8 analytes – i.e. the two enantiomeric forms of the four model drugs- as a function of p[S]. This combination leads to 28 possible t'_{ij}, although attention must be focused only on the lower t'_{ij} lines, corresponding to the critical pair of analytes at each p[S] condition. These lower t'_{ij} lines establish boundary zones delimiting two areas which can be painted in black (lower zone) or white (upper zone). In the WM, two wide black windows can be seen: one of them in the p[S] range from 1.05 to 2.85 ([S] = 1.4 - 89.0 mM), delimited on the top by propranolol enantiomers (t'_{78}) and oxprenolol enantiomers (t'_{34}), but interrupted by two sharp edges touching zero at p[S] ≈ 2.10 and 2.20 that corresponds to the inversion in migration order of each pindolol and oxprenolol enantiomers, which can be easily observed in Figure 2 around the mentioned p[S] values. Then, these two sharp edges of Figure 3 are not two but an overlapping of four t'_{ij} values.

The second window is located within the p[S] range from 3.05 to 5.00 ([S] = 0.01 – 0.89 mM). However, t'_{ij} parameter is larger in the maximum of the first window at a p[S] value of 1.75 ([S] = 17.78 mM). This value, $p[S]_{opt}$, corresponds to the chiral selector concentration that yields the best separation of all the analytes accordingly to this procedure. In a previous work the known relationship between apparent mobility and pH was used to compose t'_{ij} and T' for a family of harmala alkaloids with the aim of optimizing the acidity condition of the BGE based on both strategies [16]. Pero-Gascón et al. have recently reported a successful optimization composing the qualification parameters t'_{ij} and T' using another known model to predict μ_{app} as a function of the square root of its charge [21]. This method to determine optimum p[S] value is simple and useful [17,22–24].

Alternatively, $p[S]_{opt}$ can be obtained by composing and maximizing MCOF accordingly with equation 6. In Figure 4 a plot of T' vs. p[S] is shown and the $p[S]_{opt}$ value (equal to 1.75) obtained by T' maximization is indicated. While the application of WM strategy leads to focus on the critical pair, and the use of MCOF qualifies the separation between all peaks and, therefore, the $p[S]_{opt}$ values obtained with both procedures can be slightly different, although in our case they match very well.

Figure 5A shows the electropherogram obtained for the separation of a racemic mixture of the four studied model analytes with a BGE containing $[S]_{opt}$. Despite the fact that not all pairs are baseline separated, the overall resolution is satisfactory and, accordingly to the model it is the best possible in terms of chiral selector concentration.

In order to experimentally verify if the [S] found with the proposed strategies (WM and MCOF) is really the optimum for this separation system, electrophoretic runs were done with BGE containing higher and lower [S] than $[S]_{opt}$.

A BGE with 50 mM of 2-HP-β-CD (p[S] = 1.30, $p[S]<p[S]_{opt}$) was firstly prepared and electropherogram obtained is shown in Figure 5B. A comprehensive interpretation of the results must be done in view of the behaviors shown in Figure 2, and WM of Figure 3, where p[S]=1.30 was intentionally indicated with vertical dashed lines.

The separation is clearly worse than that obtained at $p[S]_{opt}$. The critical pair (pair with lower resolution) is composed of peaks identified as 7 and 8. These two peaks with longer migration times correspond to propranolol enantiomers, in agreement with the WM of Figure 3, where the boundary line of the left side of the window is limited by t'_{ij} of these two enantiomers, while consistently, it can also be seen in Figure 2 that the smallest difference between μ_{app} at this p[S] value corresponds to this pair with lower mobilities.

Another BGE containing 10 mM of 2-HP-β-CD, (p[S] = 2.00, $p[S]>p[S]_{opt}$) was prepared, and the electropherogram is shown in Figure 5C. It is clear again that the separation is worse than that in Figure 5A obtained at $p[S]_{opt}$. Electropherogram 5C shows peaks 3, 4, 5 and 6 heavily overlapped , and it is not clear which is the critical pair, although the boundary line on the WM of Figure 3 at p[S] =2.00 indicates that the critical pair is 3-4, corresponding to oxprenolol enantiomers.

Conclusions

A predictive method to optimize ligand concentration in capillary electrophoresis separations was presented. Two alternative strategies to carry it out this optimization were explained: one graphical based on the use of windows map (WM), and another analytical based on mathematical maximization of a composed multicriterion optimization function (MCOF). The powerful of the procedures were demonstrated by optimizing the simultaneous separation of the enantiomeric pairs of four pharmaceutical drugs used as model analytes as a function of the chiral selector concentration. The selected analytes were pindolol, propranolol, oxprenolol and homatropine methylbromide, and the chiral selector chosen as model was 2-HP-β-CD. Procedures for composing the WM and the MCOF were explained and plots were shown. In order to demonstrate the accuracy of the optimization procedures, electrophoretic separation of all enantiomers at the optimum p[S] value was shown. It was also compared with the separation obtained at p[S] values higher and lower than the optimum in which separations were worse, and the critical pairs at p[S] values higher and lower agree with that predicted by the WM, demonstrating extreme consistency. The criterion t'_{ij} used under the strategy of WM or either MCOF can be considered as consolidated methods for the optimization of single variables, and as starting point to advance over the optimization of multiple variables.

Acknowledgements

Authors gratefully acknowledge financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP 2012 Nº0244), Agencia Nacional de Promoción Científica y Técnica (ANPCyT PICT 2014 Nº3597 and PICT 2016 Nº0553) and Universidad Nacional de La Plata (UNLP 11X/696, 11X/698 and 11N/790). C. L. acknowledges CONICET for the PhD fellowship.

References

[1] X. Wang, Y. Hu, F. Qu, R.U. Khan, Multiple modes of capillary electrophoresis applied in peptide nucleic acid related study, J. Chromatogr. A. (2017). doi:10.1016/j.chroma.2017.04.038.

[2] P.I. Koczka, R. Bodor, M. Masár, A. Gáspar, Application of isotachophoresis in commercial capillary electrophoresis instrument using C4D and UV detection, Electrophoresis. 37 (2016) 2384–2392. doi:10.1002/elps.201600194.

[3] Z. Malá, P. Gebauer, P. Bocek, Analytical capillary isotachophoresis after 50 years of development: Recent progress 2014–2016, Electrophoresis. 38 (2017) 9–19. doi:10.1002/elps.201600289.

[4] D. Gollapudi, D.L. Wycuff, R.M. Schwartz, J.W. Cooper, K. Cheng, Development of high-throughput and high sensitivity capillary gel electrophoresis platform method for Western , Eastern , and Venezuelan equine encephalitis (WEVEE) virus like particles (VLPs) purity determination and characterization, Electrophoresis. 38 (2017) 2610–2621. doi:10.1002/elps.201700217.

[5] M. Gregus, H. Roberg-larsen, E. Lundanes, F. Foret, P. Kuban, S. Ray, Non-aqueous capillary electrophoretic separation of cholesterol and 25-hydroxycholesterol after derivatization with Girard P reagent, Elsevier Ireland Ltd, 2017. doi:10.1016/j.chemphyslip.2017.05.014.

[6] X. Xiao, Y. Zhang, J. Wu, L. Jia, Poly(norepinephrine)-coated open tubular column for the separation of proteins and recombination human erythropoietin by capillary electrochromatography, J. Sep. Sci. (2017) 1–9. doi:10.1002/jssc.201700720.

[7] A. Bielejewska, K. Duszczyk, A. Kwaterczak, D. Sybilska, Comparative study on the enantiomer separation of 1,1’ -binaphthyl-2,2’ diyl hydrogenphosfate ands 1,1’--bi-2-

naphtol by liquid chromatography and capillary electrophoresis using single and combined chiral selector systems, J. Chromatogr. A. 977 (2002) 225–237.

[8] A. Iuliano, G. Félix, Exhaustively substituted bile acids as chiral selectors for enantioselective chromatography Aim , use and perspectives, J. Chromatogr. A. 1031 (2004) 187–195. doi:10.1016/j.chroma.2003.10.135.

[9] X. Wang, J. Hou, M. Jann, Y. Yi, S.A. Shamsi, Development of a chiral micellar electrokinetic chromatography–tandem mass spectrometry assay for simultaneous analysis of warfarin and hydroxywarfarin metabolites: Application to the analysis of patients serum samples, J. Chromatogr. A. 1271 (2013) 207–216. doi:10.1016/j.chroma.2012.11.046.

[10] A. Gogolashvili, E. Tatunashvili, L. Chankvetadze, T. Sohajda, J. Szeman, A. Salgado, B. Chankvetadze, Separation of enilconazole enantiomers in capillary electrophoresis with cyclodextrin-type chiral selectors and investigation of structure of selector-selectand complexes by using nuclear magnetic resonance spectroscopy, Electrophoresis. 38 (2017) 1851–1859. doi:10.1002/elps.201700078.

[11] Z. Szabó, G. Tóth, G. Völgyi, B. Komjáti, G. Hancu, L. Szente, T. Sohajda, S. Béni, D. Muntean, B. Noszál, Chiral separation of asenapine enantiomers by capillary electrophoresis and characterization of cyclodextrin complexes by NMR spectroscopy, mass spectrometry and molecular modeling, J. Pharm. Biomed. Anal. 117 (2016) 398–404. doi:10.1016/j.jpba.2015.09.022.

[12] J.M. Saz, M.L. Marina, Recent advances on the use of cyclodextrins in the chiral analysis of drugs by capillary electrophoresis, J. Chromatogr. A. 1467 (2016) 79–94. doi:10.1016/j.chroma.2016.08.029.

[13] S.A. C Wren, Z. Pharmaceuticals, Chiral separation in capillary electrophoresis,

Electrophoresis. 16 (1995) 2127–2131. doi:https://doi.org/10.1002/elps.11501601343.

[14] V. Solínová, H. Mikysková, M.M. Kaiser, Z. Janeba, V. Kasicka, Hana Mikyskov a Estimation of apparent binding constant of complexes of selected acyclic nucleoside phosphonates with B-cyclodextrin by affinity capillary electrophoresis, Electrophoresis. 37 (2016) 239–247. doi:10.1002/elps.201500337.

[15] P. Dubský, M. Ördögová, M. Malý, M. Riesová, CEval: All-in-one software for data processing and statistical evaluations in affinity capillary electrophoresis, J. Chromatogr. 1445 (2016) 158–165. doi:10.1016/j.chroma.2016.04.004.

[16] M. Tascon, F. Benavente, C.B. Castells, L.G. Gagliardi, Quality criterion to optimize separations in capillary electrophoresis: Application to the analysis of harmala alkaloids, J. Chromatogr. A. 1460 (2016) 190–196. doi:https://doi.org/10.1016/j.chroma.2016.07.032.

[17] R.J. Laub, J.H. Purnell, Quantitative optimization of system variables for chromatographic separations. The column temperature, J. Chromatogr. A. 161 (1978) 49–57. doi:https://doi.org/10.1016/S0021-9673(01)85211-0.

[18] R.J. Laub, A. Pelter, J.H. Purnell, Application of the Window Analysis Optimization Method to Lanthanide Shift Nuclear Magnetic Resonance Spectra, Anal. Chem. 51 (1979) 1878–1880. doi:https://doi.org/10.1021/ac50047a070.

[19] S.N. Deming, M.L.H. Turoff, Optimization of Reverse-Phase Liquid Chromatographic Separation of Weak Organic Acids, Anal. Chem. 50 (1978) 546–548. doi:https://doi.org/10.1021/ac50026a003.

[20] Y.F. Yik, S.F.Y. Li, Resolution optimization in micellar electrokinetic chromatography: Use of overlapping resolution mapping scheme in the analysis of dinitrophenyl derivatized amino acids, Chromatographia. 35 (1993) 560–566.

doi:https://doi.org/10.1007/BF02267917.

[21] R. Peró-Gascón, F. Benavente, J. Barbosa, V. Sanz-Nebot, Determination of acidity constants and prediction of electrophoretic separation of amyloid beta peptides, J. Chromatogr. A. (2017). doi:10.1016/j.chroma.2017.05.069.

[22] S.N. Walters, F. H.; Deming, Window diagrams versus the sequential simplex method: Wich is correct?, Anal. Chim. Acta. 167 (1985) 361–363. doi:https://doi.org/10.1016/S0003-2670(00)84439-6.

[23] R.J. Laub, J.H. Purnell, Criteria for the use of mixed solvents in gas-liquid chromatography, J. Chromatogr. 112 (1975) 71–79. doi:https://doi.org/10.1016/S0021-9673(00)99943-6.

Figure Captions

Figure 1. Chemical structures of: (a) pindolol, (b) propranolol, (c) oxprenolol and (d) homatropine methylbromide. Symbol * indicates the assymetric center.

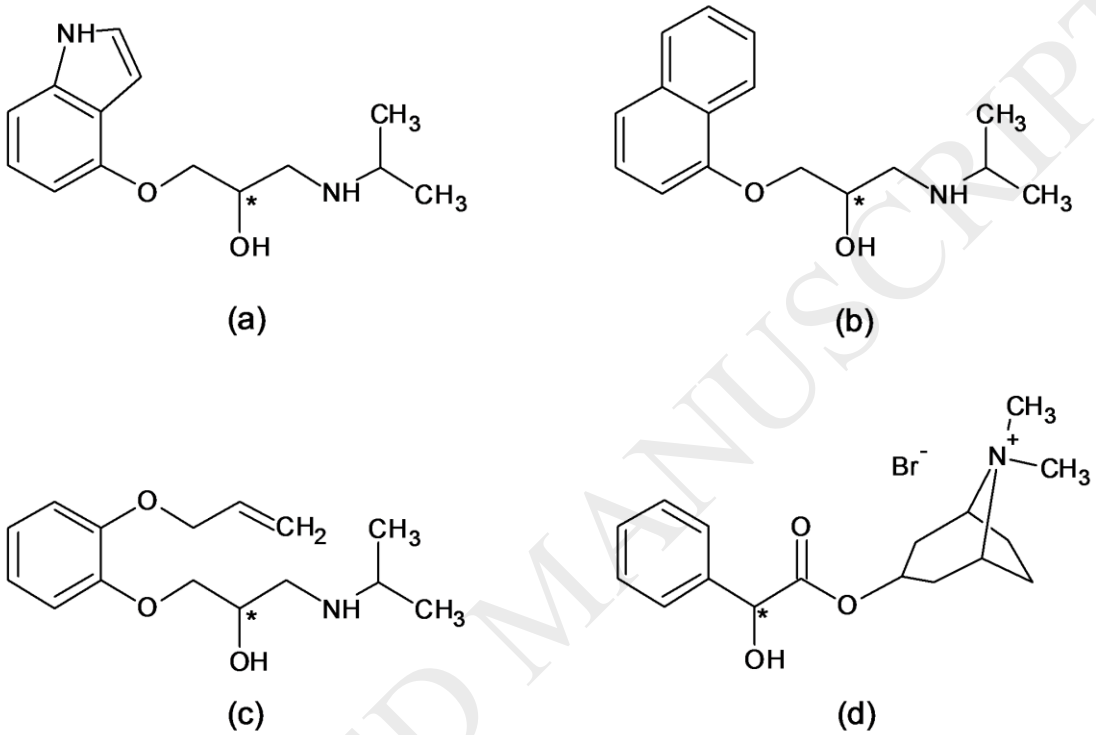


Figure 2. Plots of apparent mobilities vs. p[S] for pindolol, propranolol, oxprenolol and homatropine methylbromide enantiomers. Dashed vertical lines indicate p[S] values experimentally studied.

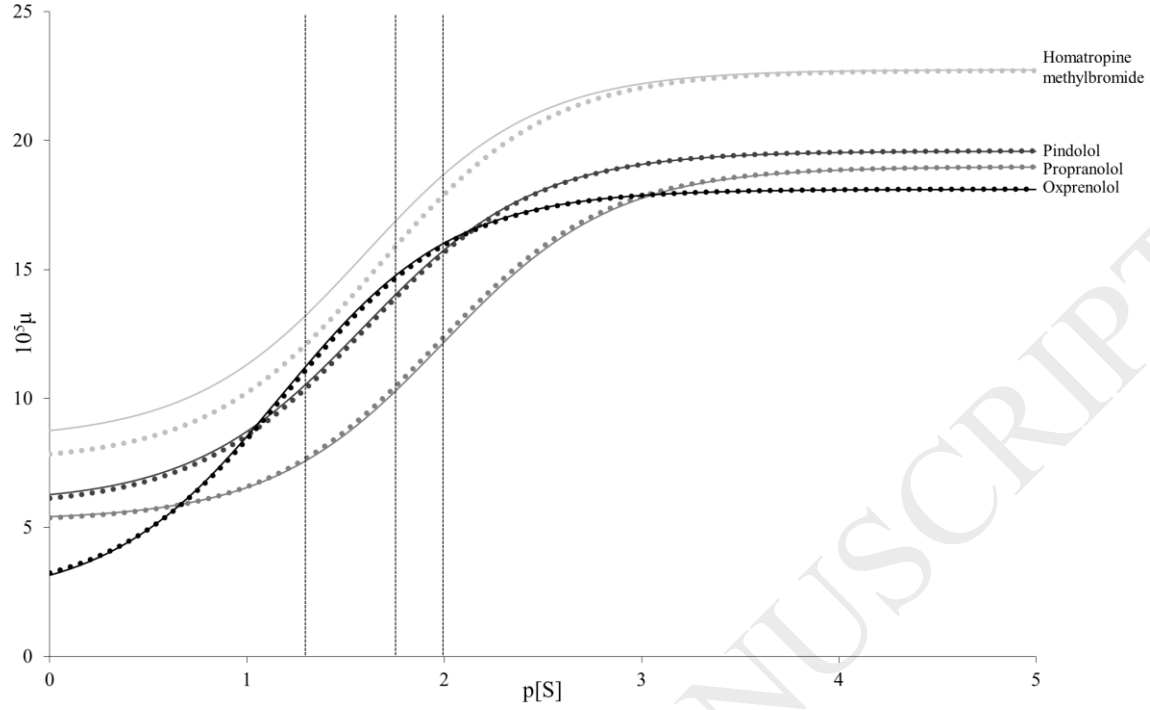


Figure 3. Plots of t'_{ij} parameter for model analytes as a function of p[S] for enantiomers of (1,2) homatropine methylbromide, (3,4) oxprenolol, (5,6) pindolol and (7,8) propranolol. Dashed vertical lines indicate p[S] values experimentally studied.

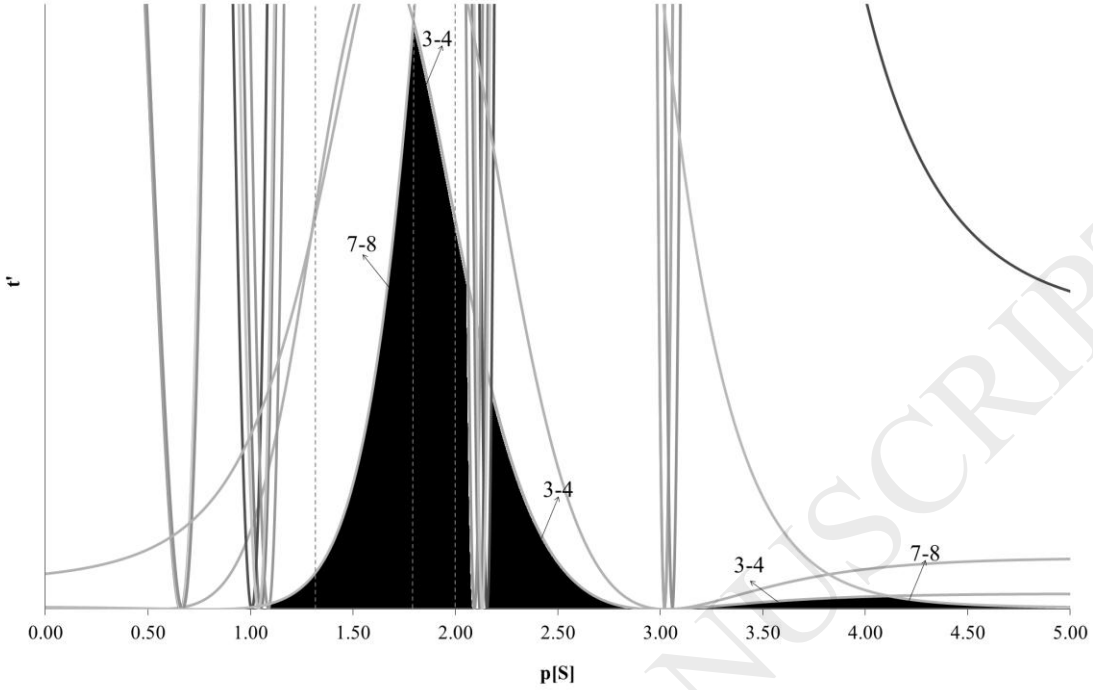


Figure 4. Multicriterion optimization function, *T'*, as a function of p[S], predicted for the separation of the four pairs of enantiomers selected as model analytes.

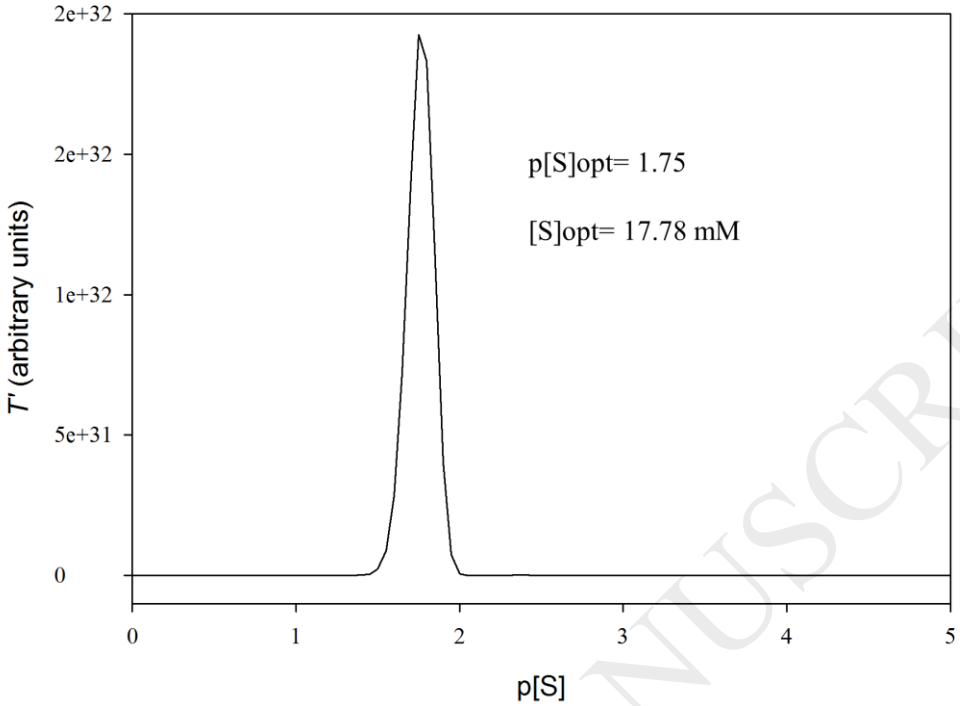
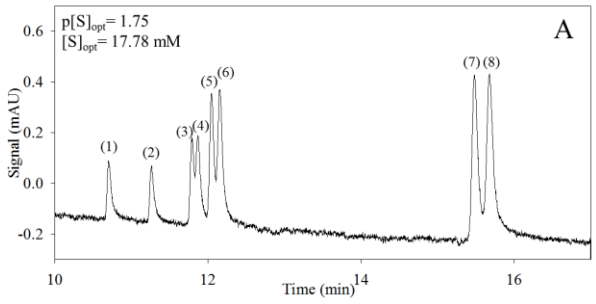
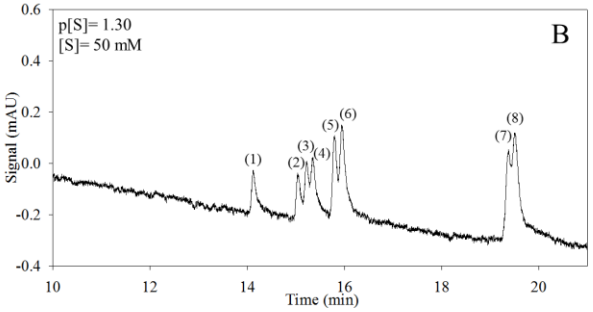
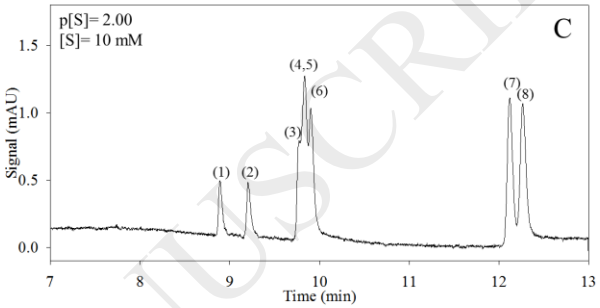


Figure 5. Electropherograms of a racemic mixture of (1,2) homatropine methylbromide, (3,4) oxprenolol, (5,6) pindolol and (7,8) propranolol. Temperature 25°C; voltage 25 kV; capillary, 50 µm i.d., 60 cm total length, 50 cm detector length; UV detection at 214 nm; hydrodynamic injection, 30 mbar during 2 seconds. Buffer 100 mM H_3PO_4/NaH_2PO_4, pH = 2.5. A) $p[S]_{opt}$ = 1.75 ([2-HP-β-CD] = 17.78 mM), B) p[S] = 1.30 ([2-HP-β-CD] = 50.00 mM), C) p[S] = 2.00 ([2-HP-β-CD] = 10.00 mM.







Tables

Table 1. Binding constants and electrophoretic mobilities ($cm^2V^{-1}s^{-1}$) of free and complexed model analytes with 2-Hydroxypropyl-β-cyclodextrin at 25°C.

Model analyte	$10^4\mu_{A1}$	$10^4\mu_{A2}$	$10^4\mu_{SA1}$	$10^4\mu_{SA2}$	K_{SA1}	K_{SA2}
Homatropine methylbromide	2.210	2.214	2.564	3.125	27.82	36.58
Oxprenolol	1.858	1.857	2.152	2.300	19.51	20.00
Pindolol	1.934	1.937	0.570	0.647	27.43	28.58
Propranolol	1.874	1.875	1.422	1.626	61.69	66.11