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Capillary electrophoretic separation of enantiomers of amino acids and amino acid derivatives using crown ether and cyclodextrin

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The capillary zone electrophoresis using (+)-18-crown-6-tetracarbonic acid as a chiral selector was a suitable method for the enantiomeric separation of racemates of amino acids and of some amino acid derivatives (esters, dipeptides). The influence of the chemical structure of the compounds on the separation was investigated. After optimization of the separation conditions, baseline separations were obtained for most racemates. The addition of acetonitrile and TBAB yielded an improvement of the separation. Improved selectivity was further observed by the application of a cyclodextrin, HP-β-CD, in combination with the crown ether.

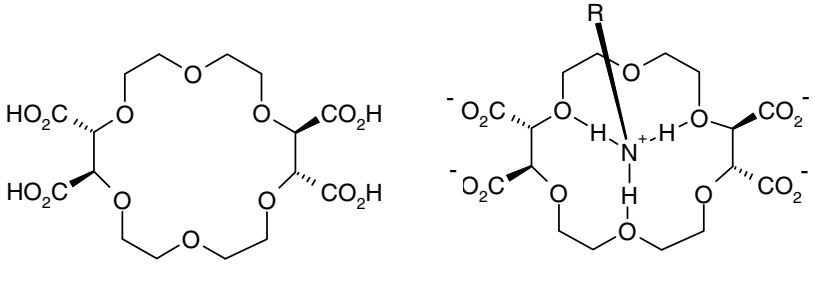
1. Introduction

Enantiomers of racemic drugs normally show different pharmacodynamic and pharmacokinetic properties. Therefore, it becomes more and more important and rational to develop selective synthetic strategies for the one desired enantiomer (Roth et al. 1998). Furthermore, for following the efficiency and purity of a stereoselective synthesis over several steps it is indispensable to analyze the chiral purity of the starting material and of all intermediates. Consequently, the development of efficient analytic methods for this purpose is of high importance. One of these methods is capillary electrophoresis, whose advantages are the high separation performance, the use of small quantities and the simple instrumentation (Nasser et al. 1998). In a preceding paper (Salami et al. 2001) we described the use of micellar electrokinetic chromatography, and in the present paper the possibilities for the separation of enantiomers of some racemic amino acid and dipeptide derivatives by capillary electrophoresis are reported.

Crown ethers, macrocyclic polyethers, form stable and selective complexes with alkali, earth alkali and ammonium cations (Pedersen 1976). Optically active crown ethers were successfully used for the enantiomeric separation in liquid chromatography (Sousa et al. 1978), and crown ethers were used for the separation of enantiomers by capillary electrophoresis (Kuhn et al. 1995). Here, we used a chiral crown ether, (+)-18-crown-6-tetracarbonic acid (a), first synthesized by Lehn et al. (Dietrich et al. 1969). The macrocyclic system of this polyether forms a cavity, which is able to form stable inclusion complexes. The six oxygen atoms of the complex in the ring are approximately planar oriented to the center of the cavity. Ammonium ions or protonated primary amines are complexed using hydrogen bonds with three of these oxygens (b).

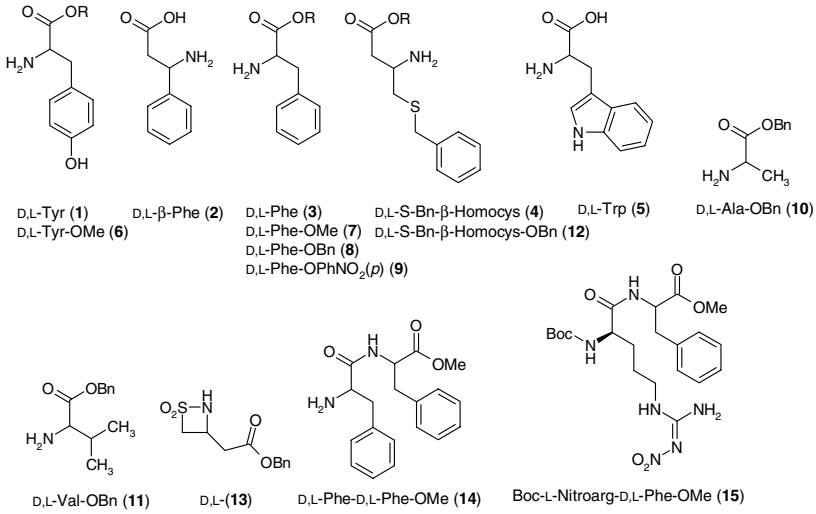
(+)-18-Crown-6-tetracarbonic acid is suitable for the chiral discrimination of amino alcohols, dipeptides (Höhne et al. 1992), amino acids and some drugs with a primary

Scheme 1



(+)-18-Crown-6-tetracarbonic acid (a), complexed with RNH_3^+ (b)

Scheme 2



Compounds **1**–**16** used as analytes

amino group (Schmid et al. 1995). Although the complex formation with the primary amine represents a necessary condition for chiral separation, this is not sufficient for the chiral discrimination of two enantiomers. Additional interactions between the carboxylic groups and other functional groups of the chiral analytes are necessary. In former investigations two recognition mechanisms were proposed. Either, the pairs of carboxylic groups act on both sides of the crown ether as chiral barriers, and thereby divide the interaction area for the guest molecules into two parts. Depending on size and spatial order of the substituents of the guest molecule, diastereoisomeric complexes are formed with different binding constants (Kuhn et al. 1995). Alternatively it was proposed, that the carboxylic acid groups of the crown ether can exhibit electrostatic interaction with polar substituents of the ligands (Kuhn et al. 1992). Both proposals include that the chiral separation is realized by the different binding constants of the complexes influencing the electrophoretic mobility of the enantiomers.

We used amino acids and their derivatives for the synthesis of potential inhibitors of elastase (Venz et al. 2001), saccharin derivatives (Soubh et al. 2002), and chiral β-sultams (Meinzer et al. 2004). For proving the chiral purity of our starting materials and products we had to develop appropriate conditions for the capillary electrophoresis. In this paper we report about the influence of the structure of the compounds, and the influences of different experimental conditions on the chiral separation using (+)-18-crown-6-tetracarbonic acid (18C6H4). The compounds we used for this investigation are given in Scheme 2.

2. Investigations, results and discussion

2.1. Influences of the structure of analytes

The influence of the structure of the enantiomers is clearly demonstrated by the different amino groups. Only a primary amino group can build a sufficiently stable inclusion complex with the crown ether, and therefore, is one condition for the separation. Either secondary nor tertiary amine groups or any other functional groups yield these sufficiently stable inclusion complexes (Hilton et al. 1991). Substance **13**, a cyclic sulfonamide, could not be separated with 18C6H4. An identical result was obtained from experiments with the guanidino derivative **15**. The structure contains two amide functions and a guanidino group

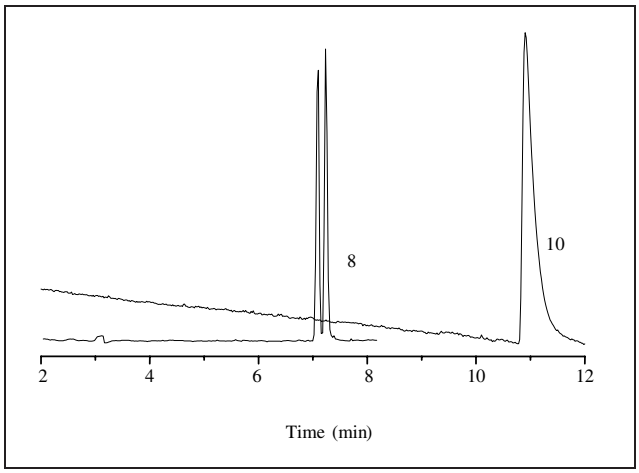


Fig. 1: Separation of **8** and **10**. Buffer: 10 mM Tris/10 mM 18C6H4

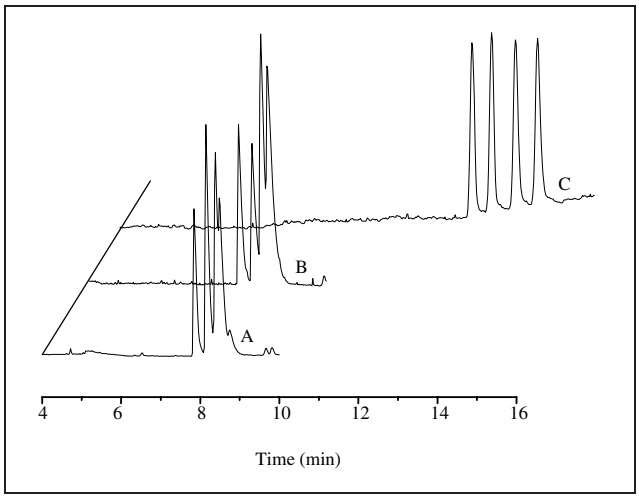


Fig. 2: Separation of the 4 isomers of **14**. Buffer: 10 mM Tris, pH 2.5, 10 mM 18C6H4 (**A**), 10 mM 18C6H4 + 10 mM HP-β-CD (**B**), 10 mM 18C6H4 + 20 mM TBAB (**C**)

acidified by the nitro substituent. At all, **15** is a weak acid, as demonstrated by calculations (Otto 2004), and has no properties of a primary amine. All other compounds bearing a primary amino group fulfill the first condition for a complexation with the crown ether, but, as demonstrated, this complex formation was not sufficient for the separation of the enantiomers. The further interaction between the carboxyl groups of the crown ether and the substituents at the chiral C-atom depended on the nature and mainly the size of these substituents. In accordance with the proposed separation mechanism of a chiral barrier by the carboxyl groups, big substituents yielded better separations as small substituents at the chiral C-atom. For example, substance **8** with a big substituent, benzyl, at the chiral C-atom showed a baseline separation, while compound **10** with a smaller substituent, methyl, in this position was not separated under identical conditions (Fig. 1).
Experiments with the dipeptide **14** demonstrated that dipeptides with a primary amine group and with two chiral centers, in this example separated by three single bonds, were separated, too. Thus, the 4 isomers of **14** were separated by an optimization of the buffer composition (Fig. 2).

2.2. Influence of the crown ether concentration

One of the possibilities to optimize the chiral separation exists in the variation of the concentration of the chiral selector (crown ether). As described (Kuhn 1995), the separation improves with alteration of the crown ether concentration. In agreement with this report, an improvement of the separation of the enantiomers of compound **8** was observed with increasing concentration of 18C6H4 up to a special limit, but with further increase of the concentration the separation factor decreased (30 mM, Fig. 3). A similar behavior was observed during the separation of most other substances. The separation with low concentrations of the crown ether is based on the different complex stabilities of the enantiomers with the crown ether, caused by different interactions between the carboxyl groups and the substituents of the chiral C-atom. Using higher concentrations of the crown ether makes the crown ether available in excess, both enantiomers are completely complexed, and an improvement of the separation is not possible.

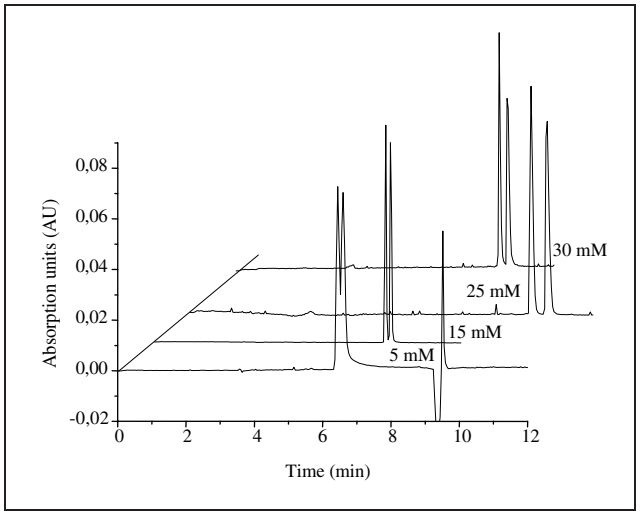


Fig. 3: Separation of the isomers of **8** depending on the crown ether concentration, buffer 10 mM Tris/18C6H4

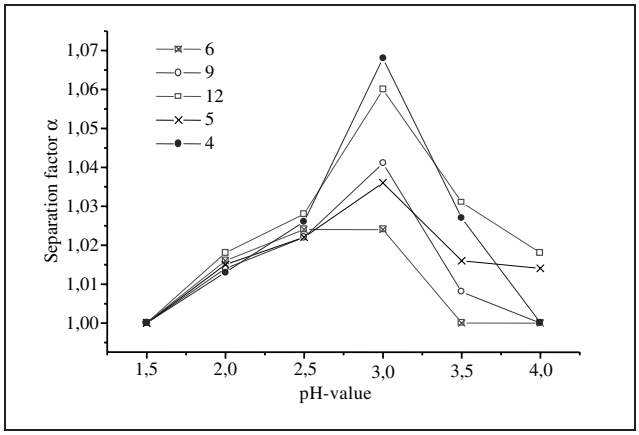


Fig. 4: Dependence of the separation factor α from the pH-value for **4**, **5**, **6**, **9**, and **12**, buffer 10 mM Tris/10 mM 18C6H4

2.3. Influence of the pH-value

The complex formation between the crown ether and the analytes depended strongly on the pH-value of the buffer. With increasing pH-values, the negative charge of the crown ether will increase, caused by the gradual dissociation of the four carboxyl groups, whose pK-values are different: $pK_1 = 2.13$, $pK_2 = 2.84$, $pK_3 = 4.29$, $pK_4 = 4.88$ (Kuhn et al. 1992b). As the number of the carboxylate anions, one to four, is responsible for the stability of the complexes, the separation factor was increased with increasing pH-values. This was demonstrated by experiments with the compounds **4**, **5**, **6**, **9**, and **12** (Fig. 4). The separation factors of these analytes increased with changing the pH-value from 1.5 to 3. At pH 1.5 no separation was observed. This is probably caused by the fact that the carboxyl groups are not dissociated and thereby, cannot effect complex formation. The highest separation factors were reached at pH 3. At this pH-value the deprotonated crown ether is complexed with the analyte, and enables the migration (Kuhn et al. 1995).
With further increase of the pH-values the separation factors decreased again until the analytes were transported with the electroosmotic flow (EOF). This could suggest the explanation that the carboxyl groups are strongly dissociated at pH-values > 3.0, and thereby, show strong interactions with both enantiomers. The complex building constants become similar, and consequently the enantioselectivity goes down.

2.4. Influence of the organic solvents

The addition of an organic solvent to the buffer changes the buffer viscosity η and the zeta potential ζ (Fanali 1991; Yowell et al. 1996) resulting in a reduction of the EOF (Salomom et al. 1991; Schmid et al. 1996). The influence of acetonitrile on the separation factor was determined for compounds **5**, **6**, and **12** (Fig. 5).
An increase of the separation factors with a concentration of acetonitrile up to 20% in the crown ether containing buffer proved to be optimal. Further increase yielded a diminution of the separation factors. By reduction of the EOF by the addition of acetonitrile the migration time was extended, and as a long migration time gives the analytes sufficient time for interaction with the crown ether, separation was improved.
A worsening of the separation with increasing content of acetonitrile in the buffer could have two reasons: With a high concentration of acetonitrile (>20%) the migration

time is shortened, and the stability of the host-guest complex is changed. This stability is lower in solvents having high dielectrical constants as in solvents having small dielectrical constants (Izatt et al. 1985). Consequently, the complex between the analyte and the crown ether becomes very strong in the presence of high contents of acetonitrile, as acetonitrile has a small dielectric constant, $\varepsilon = 37$, compared to that of water, $\varepsilon = 78$ (Riddick et al. 1970). The high stability of the complex leads to diminished enantioselective interactions between the chiral carboxyl groups of the crown ether and the substituents of the chiral C-atom of the analytes. Insufficient separation results.

2.5. Influence of quaternary ammonium salts

By addition of a short-chain tetraalkylammonium reagent like tetrabutylammonium bromide (TBAB) to the buffer, an increase of the ionic strength can be reached. The high ionic strength leads to a weak EOF and consequently to a diminution of the mobility of the analytes (Izatt et al. 1985; Mori et al. 1997). Three compounds, **5**, **6**, and **12**, were used to study the dependence of the separation factors on the TBAB-concentration. With increasing TBAB-concentration from 0 to 50 mM the separation of all three racemates was improved (Fig. 6). This is probably based on different factors. First, the separation is influenced, as the long migration time improves the interaction of the analyte with the crown ether. Second, a hydrophobic interaction could act between the n-butyl groups of TBAB and the substituents of the primary amine (Mori et al. 1997).

2.6. Influence of the combination of crown ether with cyclodextrin

The influence of a cyclodextrin on the chiral recognition in the presence of the crown ether was investigated for five compounds. An improvement of the chiral separation was achieved for three substances, **4**, **6**, and **7**, using HP-β-CD in combination with the crown ether. Of special interest was the result of the separation experiments with **11**. While neither solely with the cyclodextrin, nor solely with the crown ether a separation of **11** could be reached, the combination of both led to a good separation. The best separation was achieved with 10 mM 18C6H4 and 15 mM HP-β-CD (Fig. 7). The reinforcing influence on the chiral separation by the combination of the crown ether with the cyclodextrin is probably caused by the formation of mixed crown ether complexes, and additionally, by the inclusion of the analyte in the cavity of the cyclodextrin.

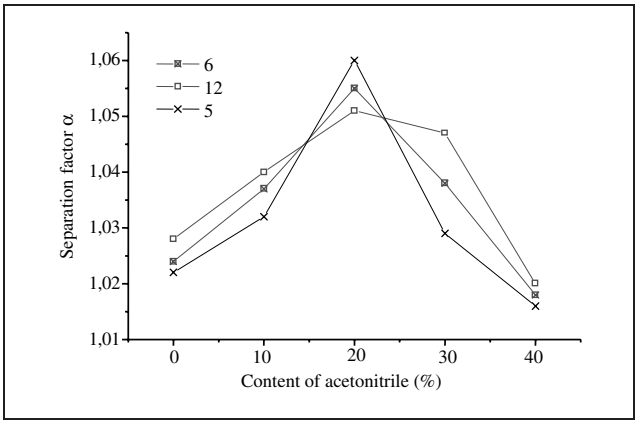


Fig. 5: Dependence of the separation factor of compounds **5**, **6**, and **12** on the content of acetonitrile, buffer: 10 mM Tris/10 mM 18C6H4, pH 2.5/acetonitrile

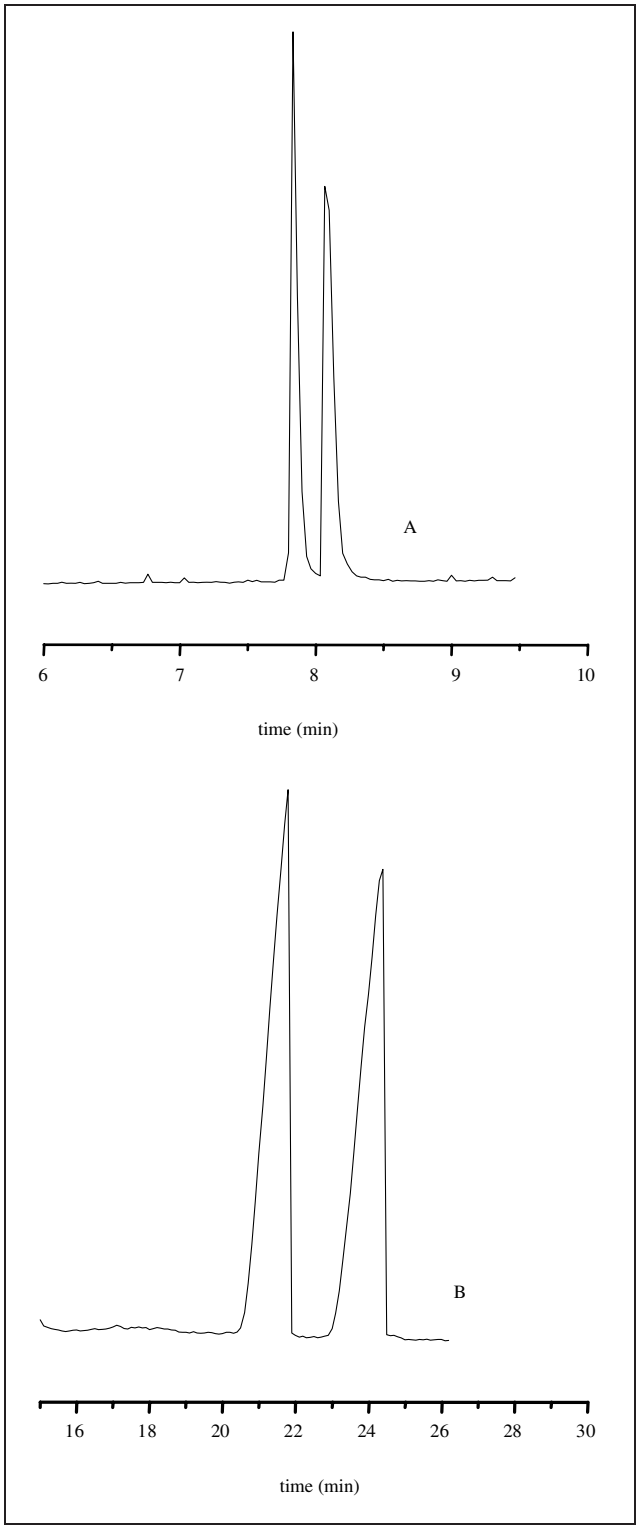


Fig. 6: Separation of **12** without TBAB (**A**), and with 50 mM TBAB (**B**), buffer: 10 mM Tris/10 mM 18C6H4, pH 2.5

3. Experimental

All separations were done on a Beckman P/ACE system 2100 (Beckman Instrument Inc., Fullerton, USA), UV detector. Uncoated capillary length 47 cm (40 cm to the detector) × 50 μm ID. Capillary temperature 25 °C.

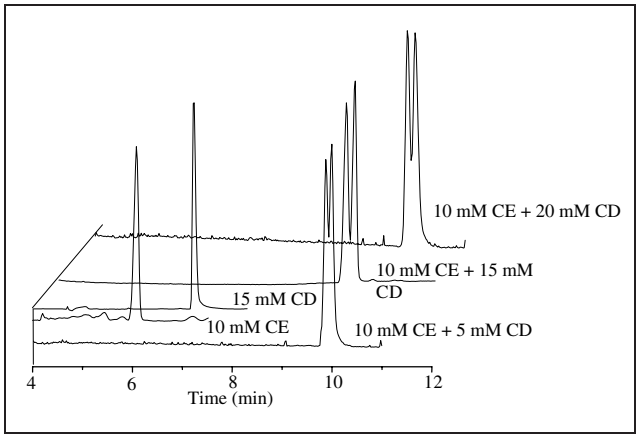


Fig. 7: Separations of **11** with crown ether (CE), cyclodextrin (CD) and mixtures of both, buffer 10 mM Tris, pH 2.5

Injection in the pressure mode for 3 s, with samples in acetonitrile. Applied voltage 20 kV, and detection at 214 nm. Running solution with 10 mM Tris-buffer, 10 mM 18C6H4 (pH 2.5). Data collection with "Gold"-Software (Beckman). (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid (18C6H4), and tris(hydroxymethyl)aminomethane-HCl (Tris) from Fluka AG, Neu-Ulm, Germany; acetonitrile from AppliChem, Darmstadt, Germany; tetrabutylammonium bromide (TBAB) from Ferak Laboratorium GmbH, Berlin, Germany; hydroxypropyl-β-cyclodextrin (HP-β-CD) from Serva, Heidelberg, Germany. Amino acids from Degussa AG, Wolfgang, Germany; amino acid esters prepared by literature procedures (Abu Thaher 1996); D,L-*S*-benzyl-β-homocysteine (**4**), D,L-*S*-benzyl-β-homocysteine benzyl ester (**12**), benzyl D,L-1,1-dioxo-1,2-thiazetidine-3-acetate (**13**), D,L-phenylalanyl-D,L-phenylalanine methyl ester (**14**), and Boc-L-nitroarginyl-D,L-phenylalanine methyl ester (**15**) prepared according literature (Abu Thaher 1996).

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References

Abu Thaher B (1996) Herstellung von (*R*)-Benzyl-[1,1-dioxo-1,2-thiazetidin-3-yl]acetat-Peptiden als Peptidmimetika. PhD Thesis Freiburg i Br.

Dietrich B, Lehn JM, Sauvage JP (1969) Diaza-Polyoxa-Macrocycles et Macrobicycles. Tetrahedron Lett 34: 2885–2886.

Fanali S (1991) Use of cyclodextrins in capillary zone electrophoresis. J Chromatogr 545: 437–444.

Gassmann E, Kuo JE, Zare RN (1985) Electrokinetic separation of chiral compound. Science 230: 813–814.

Hilton M, Armstrong DW (1991) Evaluation of a chiral crown ether LC column for the separation of racemic amines. J Liq Chromatogr 14(1): 9–28.

Höhne E, Krauss G-J, Gübitz G (1992) Capillary zone electrophoresis of the enantiomers of aminoalcohols based on host-guest complexation with a chiral crown ether. J High Resolut Chromatogr 15: 698–700.

Izatt RM, Bradshaw JS, Nielsen SA, Lamb JD, Christensen JJ (1985) Thermodynamic and kinetic data for cation-macrocycle interaction. Chem Rev 85: 271–339.

Kuhn R (1995) Enantiomerentrennung mittels chiralem Kronenether in der Kapillarzonenelektrophorese. GIT Fachz Lab 11: 1031.

Kuhn R, Erni F, Bereuter T, Häusler J (1992a) Chiral recognition and enantiomeric resolution based on host-guest complexation with crown ethers in capillary zone electrophoresis. Anal Chem 64: 2815–2820.

Kuhn R, Stoecklin F, Erni F (1992b) Chiral separations by host-guest complexation with cyclodextrin and crown ether in capillary zone electrophoresis. Chromatographia 33: 32–36.

Kuhn R, Wagner J, Walbroehl Y, Bereuter T (1994) Potential and limitations of an optically active crown ether for chiral separation in capillary zone electrophoresis. Electrophoresis 15: 828–834.

Meinzer A, Breckel A, Abu Thaher B, Manicone N, Otto H-H (2004) Properties and reactions of substituted 1,2-thiazetidine 1,1-dioxides: chiral mono- and bicyclic 1,2-thiazetidine 1,1-dioxides from α-amino acids. Helv Chim Acta 87: 90–105.

Mori Y, Ueno K, Umeda T (1997) Enantiomeric separations of primary amino compounds by non-aqueous capillary zone electrophoresis with a chiral crown ether. J Chromatogr 757: 328–332.

Motellier S, Wainer IW (1990) Direct stereochemical resolution of aspartame stereoisomers and their degradation products by high-performance liquid chromatography on a chiral crown ether based stationary phase. J Chromatogr 516: 365–373.

Nassar AEF, Guarco FJ, Gran DE, Stuart JD, Reuter WM (1998) Optimizing a method for separating chiral compound by capillary electrophoresis. J Chromatogr Sci 36: 19–22.

Otto H-H (2004) calculated with PALLAS 1.2 CompuDrug Ltd: $pK_a = 15.05, 16.74, -2.58, -3.24$ unpublished.

Pedersen C (1967) Cyclic polyethers and their complexes with metal salts. J Am Chem Soc 89: 2495–2496.

Riddick JA, Bunger WB (1970) Organic solvents – Physical properties and methods of purification. Wiley Interscience New York

Roth HJ, Müller CE, Folkers G (1998) Stereochemie & Arzneistoffe. WVG, Stuttgart

Salami M, Otto H-H, Jira T (2001) Chiral separation of amino acid esters by micellar electrokinetic chromatography. Electrophoresis 22: 3291–3296.

Salomon K, Burgi DS, Helmer JC (1991) Separation of seven tricyclic antidepressants using capillary electrophoresis. J Chromatogr 549: 375–385.

Schmid MG, Gübitz G (1995) Capillary zone electrophoretic separation of the enantiomers of dipeptides based on host-guest complexation with a chiral crown ether. J Chromatogr A 709: 81–88.

Schmid MG, Wirnsberger K, Gübitz G (1996) Chiral separation of drug enantiomers by capillary electrophoresis using succinyl-β-cyclodextrin. Pharmazie 51: 852–854.

Soubh L, Besch A, Otto H-H (2002) Synthesis and properties of N-substituted saccharin derivatives. Pharmazie 57: 384–392.

Sousa LR, Sogah GDY, Hoffmann DH, Cram DJ (1978) Host-guest complexation. 12. Total optical resolution of amine and amino ester salts by Chromatography. J Am Chem Soc 100: 4569–4576.

Venz C, Otto H-H (2001) β-Lactam derivatives as enzyme inhibitors: Derivatives of (*E*)-2-[(*RS*)-1-(4-methoxyphenyl)-2-oxo-4-phenyl-3-ylidene]-propionic acid. Pharmazie 56: 686–690.

Yowell GG, Fazio SD, Vivilecchia RV (1996) Enantiomeric separation of *N-tert*-butoxycarbonyl amino acids by capillary electrophoresis using hydroxypropyl-substituted cyclodextrins J Chromatogr A 745: 73–79.