Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1164–1170
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Newly synthesized tetraoxa-diaza crown ether derivatives versus commercialized crown ethers in the separation of positional isomers with capillary electrophoresis

Peter Forlay-Frick [a], Debby Mangelings [a], Tímea Iványi [b], István Lázár [b], Károly Héberger [c], Yvan Vander Heyden [a,*]

[a] *Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel – VUB, Laarbeeklaan 103, 1090 Brussels, Belgium*
[b] *Department of Inorganic and Analytical Chemistry, University of Debrecen, P.O. Box 21, 4010 Debrecen, Hungary*
[c] *Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary*

Received 22 November 2005; accepted 16 February 2006
Available online 3 April 2006

Abstract

Three new tetraoxa-diaza derivatives of 1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane (*R-1*, *R-2* and *R-3*) and three commercially available crown ethers, 18-crown-6 (18C6), (+)-18-crown-6-tetracarboxilic acid (18C6H_4) and 1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane, were investigated to separate the positional isomers of aminophenol, aminobenzoic acid and aminocresol. The running electrolyte, in which the crown ethers were dissolved, was a 50 mM Tris solution adjusted to pH 2.0 with hydrochloric acid. Using 50 mM H_3PO_4 buffer, whose pH was adjusted to 2.0 with Tris, or only hydrochloric acid solution with the same pH, did not allow good separations for the tested components. The effect of the crown ether concentration on the separation of the 11 positional isomers was studied in the concentration range of 10–50 mM. The best separations were achieved using the 18C6 and the 18C6H_4 crown ethers: 9 isomers out of 11 could be separated within one run. The *m*- and *p*-aminophenol isomers could not be separated under the investigated experimental conditions. The newly synthesized tetraoxa-diaza crown ether derivatives were only found suitable for the separation of aminobenzoic acid positional isomers. The macrocyclic ring of the tetraoxa-diaza crown ethers was not able to form a stable inclusion complex with the tested positional isomers. Consequently, the aminophenol and aminocresol isomers were not separated, the isomers migrated with the same or very similar velocities.

Keywords: Capillary electrophoresis; Positional isomers; Crown ethers; Tetraoxa-diaza crown ethers; Aromatic amines

1. Introduction

Positional isomers (e.g. *ortho*-, *meta*- and *para*-aminophenols) are composed of the same elements in the same proportions, but differ in properties due to differences in the arrangement of groups. The separation of positional isomers has become a very important task in the last decades, since these are indispensable raw materials and preservative agents in the pharmaceutical, cosmetic and food industries. For example, *m*-aminophenol, which is a common impurity of aminosalicylic acid, discolors tablets of aminosalycilic acid during oxidation or under influence of irradiation. *p*-Aminobenzoic acid is used in alcoholic lotions and creams as a sunscreen agent. Its potassium salt (potassium-aminobenzoate) has been used in the treatment of various disorders associated with excessive fibrosis, such as scleroderma. However, in some countries the use of *p*-aminobenzoic acid and its ester in cosmetics is prohibited [1].

Over the past 20 years, capillary electrophoresis has become a widely used analytical technique, especially in the field of enantiomer separations [2–8]. This technique is able to separate molecules based on their charge mass distribution. The charged form of molecules, which is necessary for separation, is ensured using buffers. The advantages of capillary electrophoresis (CE) relative to high-performance liquid chromatography are its high efficiency, small sample volume requirement, low solvent consumption and the fact that separation is achieved in a capillary tube without the need for an expensive stationary phase. To improve the separation efficiency, many compounds

* Corresponding author. Tel.: +32 2 477 4734; fax: +32 2 477 4735.
E-mail address: yvanvdh@vub.ac.be (Y. Vander Heyden).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.02.042

can be added to the running electrolyte, such as, for instance, cyclodextrins [2–11] and crown ethers [12–21].

In the last decade, the separation of positional aromatic amine isomers [2,9–11,18–21,24–26] and of different metal ions (alkali, alkaline earth, transition metal and lanthanide cations) [27–35] became an intensively studied field in capillary electrophoresis. The, from chiral separations well-known cyclodextrins [2,9–11], the crown ethers [18–21] and the calixarenes [22,23], amongst others, can be used to separate these isomers. Some examples of separations of positional isomers carried out without additives can also be found in the literatures [24–26].

The tetraoxa-diaza crown ethers belong to the family of macrocyclic compounds, also called macrocycles. Macrocycles is the collective term for chemical compounds, which contain a chemical ring that consists of nine or more atoms, for instance, crown ethers and cyclodextrins. The application of macrocycles is diverse: they are commonly used in organic chemistry, in human therapy (e.g. antibiotics) and in analytical chemistry. In the latter, macrocycles are frequently applied in capillary electrophoresis for the separation of enantiomers, positional isomers and metal ions (alkali, alkaline earth, transition metal and lanthanide cations), and for the preparation of different ion-selective electrodes [36–38].

The applicability of crown ethers for the separation of positional isomers (primary alkylamines) and metal cations can be explained by their structure. Their polyether ring system builds a cavity, which is able to form stable inclusion complexes with protonated primary alkylamines and with different metal ions (particularly alkali and alkaline earth metal cations). For example, in the case of $18C6H_4$, the six oxygen atoms of the ring system are oriented to the center of the cavity. Ammonium or primary alkylamines can be held inside the cavity with three H-bonds in a tripod arrangement. The interactions between the ring and the ligand are necessary for the separation of chiral molecules or positional isomers. For secondary and tertiary amines the host–guest interactions are absent due to the steric hindrance. The carboxylic acid pairs on both sides hinder the ligand to reach the ring and may participate in electrostatic interactions (hydrogen bonds or repulsion forces) with polar substituents of the ligand [13].

The application of tetraoxa-diazo crown ethers as additives for the separation of aromatic amines positional isomers has not been described yet. Nishi et al. [18] used different commercial crown ethers, namely 18-crown-6 (18C6), (+)-18-crown-6-tetracarboxilic acid ($18C6H_4$) and dicyclohexil-18-crown-6, for the separation of aminobenzoic acid, aminophenol and aminocresol positional isomers. Only five of nine tested positional isomers could be separated simultaneously.

In this study, the applicability of six different crown ethers (Fig. 1), 18-crown-6 (18C6), (+)-(18-crown-6)-2,3,11,12-tetracarboxilic acid ($18C6H_4$), 1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane (Kryptofix 22) and three newly synthesized derivatives of Kryptofix 22, namely (2-{16-[(carboxymethyl-carbamoyl)-methyl]-1,4,10,13-tetraoxa-7,16-diazacyclo-octadec-7-yl}-acetylamino)-acetic acid (*R-1*); *N*-[2-(1,4, 10, 13-tetraoxa-7,16-diazacyclo-octadec-7-yl)-propanoyl] glycine (*R-2*); *N*-[(2*R*)-2-(1,4,10,13-tetraoxa-7,16-diazacyclo-octadec-7-yl)-propanoyl] glycine methyl ester (*R-3*) were tested

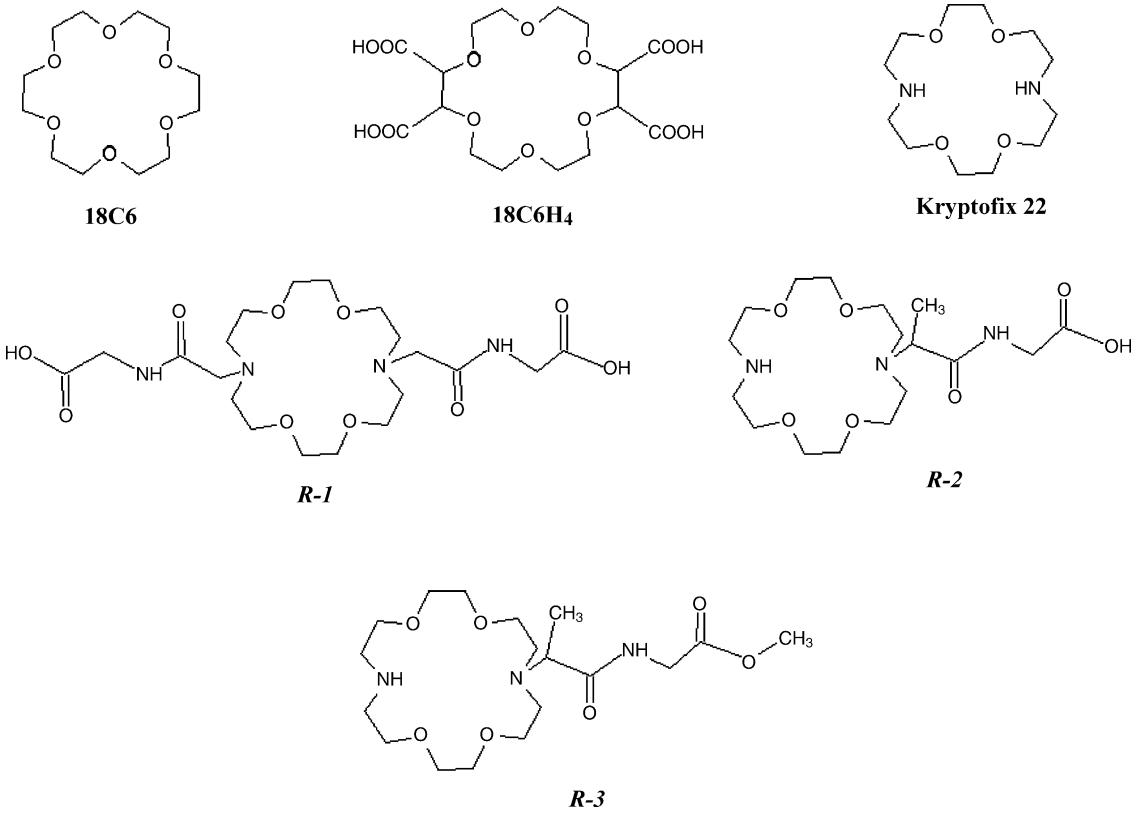

Fig. 1. Chemical structure of 18C6, $18C6H_4$, Kryptofix 22 and the newly synthesized *R-1*, *R-2* and *R-3* crown ethers.

as electrolyte additives for the separation of aminophenol, aminobenzoic acid and aminocresol positional isomers. The abbreviations *R-1*, *R-2* and *R-3* will be used hereafter for the newly synthesized tetraoxa-diaza crown ethers.

The aims of this work were: (a) to develop a new capillary electrophoresis method to separate simultaneously all positional isomers of aminophenol, aminobenzoic acid and aminocresol; (b) to investigate the applicability of the newly synthesized tetraoxa-diaza crown ethers for the separation of these positional isomers; (c) to compare the results obtained using these new tetraoxa-diaza crown ethers with those obtained from the commercially available crown ethers.

2. Materials and methods

2.1. Chemicals

The chemical structure of the used crown ethers is shown in Fig. 1. The 18C6 was supplied from Fluka Chemie (Bornem, Belgium), Kryptofix 22 from Merck (Darmstadt, Germany) and 18C6H$_4$ from Aldrich (Milwaukee, WI, USA). The three new tetraoxa-diaza crown ethers (*R-1*, *R-2* and *R-3*) were synthesized in the laboratory of Inorganic and Analytical Chemistry, University of Debrecen, Hungary. The reaction scheme and a short description of synthesis for *N*-[2-(1,4,10,13-tetraoxa-7,16-diazacycloocta-dec-7-yl)-propanoyl] glycine (*R-2*) can be found in Ref. [17]. A more detailed description of the synthesis of the other two tetraoxa-diaza crown ethers will be published elsewhere.

The tested positional isomers: *o*-aminobenzoic acid (anthranilic acid), *m*-aminobenzoic acid, *p*-aminobenzoic acid, *o*-aminophenol, *m*-aminophenol, 2-amino-*m*-cresol, 4-amino-*m*-cresol, 6-amino-*m*-cresol, 2-amino-*p*-cresol and 5-amino-*p*-cresol were purchased from Aldrich (Steinheim, Germany), while *p*-aminophenol was obtained from Sigma (St. Louis, MO, USA).

Tris(hydroxymethyl)aminomethane (Tris) for the running electrolyte, and 1 M hydrochloric acid, which was used to adjust the pH, were purchased from Merck. The 0.1 and 1 M sodium hydroxide solutions to condition the capillary were obtained from Carlo Erba (Milano, Italy). High purity water for solution preparation was produced in-house by a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Instrumentation and separation conditions

All experiments were performed on a Spectra Phoresis Ultra capillary electrophoresis system (Thermo Separation Products, San Jose, CA, USA) equipped with a UV–vis detector. Separation was carried out in an uncoated fused-silica capillary (Composite Metal Services, Ilkley, UK) of 50 μm internal diameter with 62.2 cm total length (56 cm effective length to the detector). Before first use, the new capillary was rinsed with 1 M NaOH, then with 0.1 M NaOH and finally with Milli-Q water, each for 10 min. Between two different electrolytes, the capillary was rinsed with 0.1 M NaOH and Milli-Q water for 5 min, respectively, and then with the new running electrolyte for 10 min. Before each separation the capillary was rinsed with the running electrolyte for 5 min. All samples were hydrostatically injected using a positive pressure of 0.8 psi (1 psi = 6.9 kPa) for 5 s. The separations were performed at 15 kV with a working temperature of 25 °C. All components were detected at 220 nm. The measurements were performed in triplicate to ensure repeatability.

For the pH adjustment of running electrolytes, an Orion 520A pH meter (Orion, Boston, MA, USA) was used.

Table 1
The pK_a values of the tested positional isomers

Compounds	pK_a (1)		pK_a (2)	
o-Aminophenol	4.74 ± 0.10	NH_2	9.76 ± 0.10	OH
m-Aminophenol	4.30 ± 0.10		10.01 ± 0.10	
p-Aminophenol	5.28 ± 0.10		10.17 ± 0.13	
o-Aminobenzoic acid	2.10 ± 0.10	COOH	4.94 ± 0.10	NH_2
m-Aminobenzoic acid	3.34 ± 0.10		4.75 ± 0.10	
p-Aminobenzoic acid	2.51 ± 0.10		4.86 ± 0.10	
2-Amino-*m*-cresol	4.60 ± 0.10	NH_2	9.87 ± 0.10	OH
4-Amino-*m*-cresol	5.13 ± 0.10		10.34 ± 0.18	
6-Amino-*m*-cresol	5.17 ± 0.10		9.87 ± 0.10	
2-Amino-*p*-cresol	4.86 ± 0.10		10.06 ± 0.18	
5-Amino-*o*-cresol	4.74 ± 0.10		10.36 ± 0.10	

2.3. Electrolytes

The running electrolyte (50 mM Tris solution adjusted to pH 2.0 with hydrochloric acid) was prepared by dissolving tris(hydroxymethyl)aminomethane in Milli-Q water. As the amino groups of the positional isomers have to be protonated during the run, a pH lower than the lowest pK_a value for the amino groups was used (Table 1). The lowest pK_a value was 4.30 for *m*-aminophenol. Therefore the pH of the 50 mM Tris solution was adjusted to 2.0 with hydrochloric acid. The prepared solution could be used for 2 weeks when stored in the refrigerator.

The crown ether containing electrolytes were prepared by dissolving the required amount of crown ethers in the 50 mM Tris–hydrochloric acid electrolyte. The effect of crown ether concentrations on the separation of the positional isomers was tested in the range of 10–50 mM.

2.4. Sample preparations

The sample stock solutions were prepared by dissolving the components in pure methanol, in a concentration of 2 mg/ml. These solutions were stored in the refrigerator and could be used for 2 weeks.

The test solution was prepared by mixing 1.0 ml of the 11 sample stock solutions in a test tube. The final concentration was approximately 0.2 mg/ml for every compound. This test solution was also stored in the refrigerator and used for 2 weeks.

Peak identification was carried out using spiked solutions.

2.5. Calculations

The pK_a values of the acidic and basic (amino) groups for all isomers (Table 1) were calculated by ACD/ChemSketch Software, Version 5.12 (Advanced Chemistry Development, Toronto, Ont., Canada).

3. Results and discussion

Our starting point for method development was based on the paper of Nishi et al. [18]. They tried to separate isomers of aminophenol, of aminobenzoic acid and three aminocresol isomers (4-amino-*m*-cresol, 6-amino-*m*-cresol and 2-amino-*p*-cresol). The best separation was achieved using 20 mM Tris–H_3PO_4 buffer (pH 2.1) containing 10 mM 18C6H_4 crown ether. At these conditions, the *m*- and *p*-aminophenol isomers were not separated nor were 4-amino-*m*-cresol and *o*-aminophenol. The differences between our experimental conditions and those of [18] were the applied electrolyte and the dimension (length and internal diameter) of the capillary. We used a 50 mM Tris–hydrochloric acid electrolyte (pH 2.0) instead of the 20 mM Tris–H_3PO_4 buffer (pH 2.1), and a capillary of 50 μm internal diameter with 56 cm effective length contrary to their 75 μm internal diameter and 37 cm effective length. The difference in pH between our electrolyte and that of [18] was insignificant, while a longer capillary with smaller internal diameter has been used with the aim of improving the separation efficiency. However, only the change of the dimensions of the capillary was not enough to improve the separation efficiency of the system significantly. Accordingly, the change of the electrolyte was needed, too.

The application of different electrolytes without crown ethers was investigated. The best separation of 11 positional isomers, i.e. good baseline separation of seven compounds out of 11, was achieved using 50 mM Tris solution adjusted to pH 2.0 with hydrochloric acid. The application of this electrolyte could be queried, because part of the applied hydrochloric acid was consumed to neutralize Tris. However, experiments showed that this electrolyte gave better results than when applying 50 mM H_3PO_4 buffer, whose pH was adjusted to pH 2.0 with Tris, or only hydrochloric acid solution (pH 2.0). The latter systems did not give adequate separation of the components and resulted in noisy electropherograms. Accordingly, 50 mM Tris–hydrochloric electrolyte was applied for the method development and to test the effect of different crown ethers on the separation efficiency.

3.1. Separation with crown ether free running electrolyte

Using only the running electrolyte (50 mM Tris–HCl electrolyte) without crown ether, 4 of 11 compounds were separated, as shown in Fig. 2. The aminophenol isomers (substances 1, 2 and 3) and most cresol isomers (substances 4, 5, 6, 7) could not be separated. Changing the concentration of running electrolyte in both directions did not improve the separation efficiency. Only the separation of aminobenzoic acid isomers (substances 9, 10 and 11) and one cresol isomer (substance 8) was achieved by this crown ether-free electrolyte under the tested conditions (see Section 2).

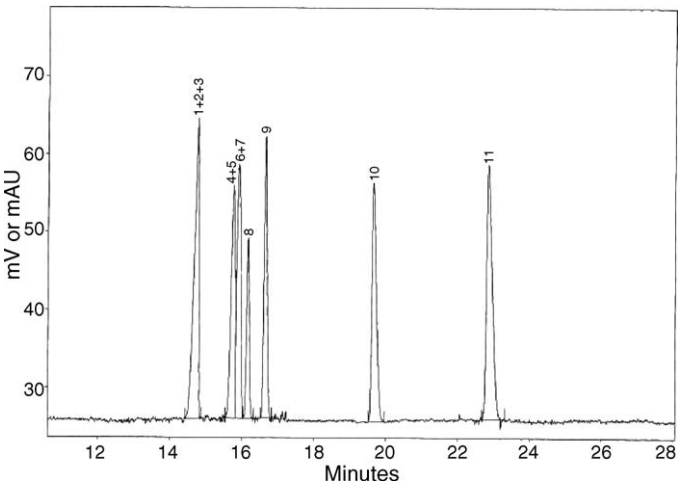

Fig. 2. Electropherogram of 11 positional isomers using 50 mM Tris–HCl electrolyte (pH 2.0). Capillary: 56 cm effective length; temperature: 25 °C; applied voltage: 15 kV; detection: 220 nm. Isomers: (1) *o*-aminophenol; (2) *m*-aminophenol; (3) *p*-aminophenol; (4) 2-amino-*m*-cresol; (5) 5-amino-*o*-cresol; (6) 6-amino-*m*-cresol; (7) 2-amino-*p*-cresol; (8) 4-amino-*m*-cresol; (9) *m*-aminobenzoic acid; (10) *p*-aminobenzoic acid; (11) *o*-aminobenzoic acid.

3.2. Separation with commercially available crown ethers

The 18C6, the 18C6H_4 and the Kryptofix 22 crown ethers belong to the commercially available macrocycles. The Kryptofix 22 is a tetraoxa-diaza crown ether. Its macrocyclic ring contains only four oxygen atoms in contrast to the other two, which have six oxygen atoms. The ability to form hydrogen bonds between the protonated alkylamines and the oxygen atoms of the ring is also possible with Kryptofix 22, similarly to 18C6 and 18C6H_4.

Applying the 18C6 crown ether, the best separation was obtained with 30 mM 18C6 crown ether. Ten peaks were observed as can be seen in Fig. 3. The aminobenzoic acid and

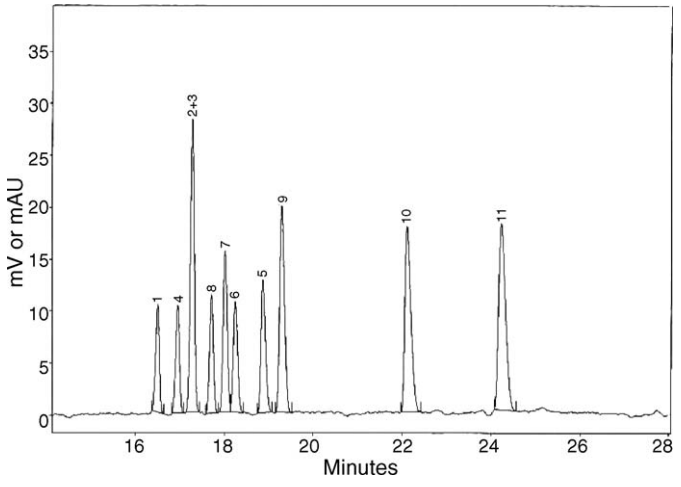

Fig. 3. Electropherogram of 11 positional isomers applying 30 mM 18C6 crown ether in 50 mM Tris–HCl solution (pH 2.0). Same conditions and isomer labels as in Fig. 2 are used.

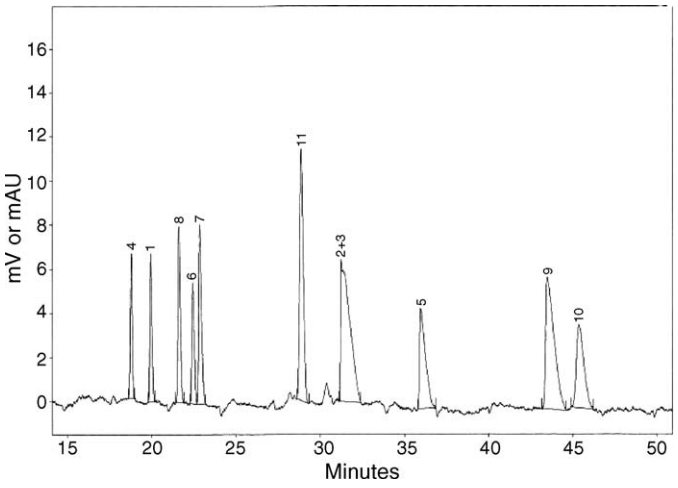

Fig. 4. Separation of 11 positional isomers using 10 mM $18C6H_4$ crown ether in 50 mM Tris–HCl electrolyte (pH 2.0). Same conditions and isomer labels as in Fig. 2 are used.

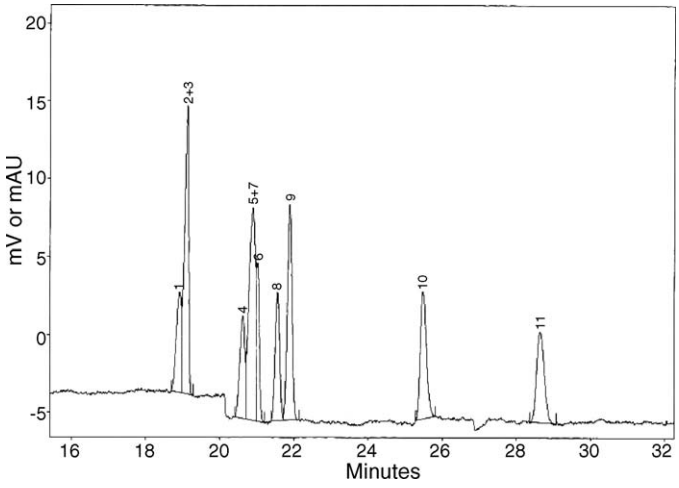

Fig. 5. Electropherogram of 11 positional isomers using 10 mM *R-2* tetraoxa-diaza crown ether in 50 mM Tris–HCl electrolyte (pH 2.0). Same conditions and isomer labels as in Fig. 2 are used.

aminocresol isomers could be baseline separated, although 2-amino-*p*-cresol (substance 7) and 6-amino-*m*-cresol (substance 6) were not completely resolved. The *m*- and *p*-aminophenol isomers co-eluted. Increasing the crown ether concentration did not improve the separation efficiency, while decreasing worsened the separation of the aminocresol isomers. When 18C6 was used in a concentration of 10 or 20 mM, the 4-amino-*m*-cresol, 6-amino-*m*-cresol, 2-amino-*p*-cresol and 5-amino-*o*-cresol isomers were not separated.

Using the $18C6H_4$ crown ether, the best separation was achieved with a concentration of 10 mM. A typical electropherogram is shown in Fig. 4. Nine compounds were separated, similar to 18C6, except for *m*- and *p*-aminophenols (2 + 3) which eluted together. The migration order for aminobenzoic acid and aminophenol positional isomers was *ortho*, *meta* and *para*, although *meta* and *para* migrated together in case of aminophenol. The order reflects well the steric hindrance, which exists for the complexation. A functional group (hydroxyl or carboxylic) next to the amino group in the tested positional isomers hinders the protonated amino groups to get near to the ring enabling it to form three H-bonds with the oxygen atom in a tripodal arrangement. This steric hindrance is the most significant in the case of *ortho*-, then of *meta*-, and finally of *para*-isomers, explaining the obtained migration order. The migration order of the five aminocresol isomers can also be explained by steric hindrance. The carboxylic acid pairs on both sides of the macrocyclic ring of $18C6H_4$ show electrostatic interactions (hydrogen bonds) with the amino group, which contribute to the stable complexation. A lower concentration of $18C6H_4$ crown ether is already sufficient to obtain the same selectivity as with 18C6. When the concentration of $18C6H_4$ was increased, the separation efficiency worsened and the baseline became noisier. Generally, the analysis times were higher and the peak shapes worse (tailing peaks) when using $18C6H_4$ compared to 18C6.

Kryptofix 22 did not have any positive effect on the separation of the isomers in the tested concentration range of 10–50 mM. The same results as with crown ether-free 50 mM Tris–HCl solution (Fig. 2) were obtained. Only the aminobenzoic acid isomers were separated. The reason for the decreased selectivity compared with $18C6H_4$ crown ether is that the two nitrogen atoms in the ring system of Kryptofix 22, which also are possibly charged at the applied pH, prevent the four oxygens to form a stable inclusion complex with the protonated amino group of tested substances.

3.3. Separation with newly synthesized tetraoxa-diaza crown ethers

The *R-1* crown ether did not have any positive effect on the separation of the positional isomers in the tested concentration range of 10–50 mM. The same results as with Tris–HCl electrolyte without crown ether (Fig. 2) was observed. Apparently, the four oxygen atoms of the crown ether ring as well as the substituted side chains on both nitrogen atoms of the ring were not able to form complexes with the ligands, probably again due to the repulsion caused by the positive charges on the nitrogen atoms, explaining the bad separation. Only the aminobenzoic acid isomers were separated by the application of this derivative, similar to previous results (Kryptofix 22).

Using the *R-2* tetraoxa-diaza crown ether derivative in a concentration of 10 mM, we detected nine peaks and thus a (slightly) charged selectivity compared to Kryptofix 22 or *R-1* (Fig. 5). Aminobenzoic acid isomers (substances 9, 10 and 11) could be resolved. Aminophenol isomers (substances 1, 2 and 3) nor aminocresol isomers could clearly be separated. The propanoyl glycine side chain on one of both nitrogen atoms had some effect on the separation of the aminophenol isomers, contrary to the side chains of the *R-1* crown ether. The *o*-aminophenol was partially separated from *m*- and *p*-aminophenol isomers. Using more than 10 mM of *R-2* did not improve the separation and increased baseline noise.

The *R-3* crown ether is a methyl ester derivative of *R-2*. The optimal concentration of *R-3* was found to be 20 mM. With this concentration, we detected 10 peaks as can be seen in Fig. 6.

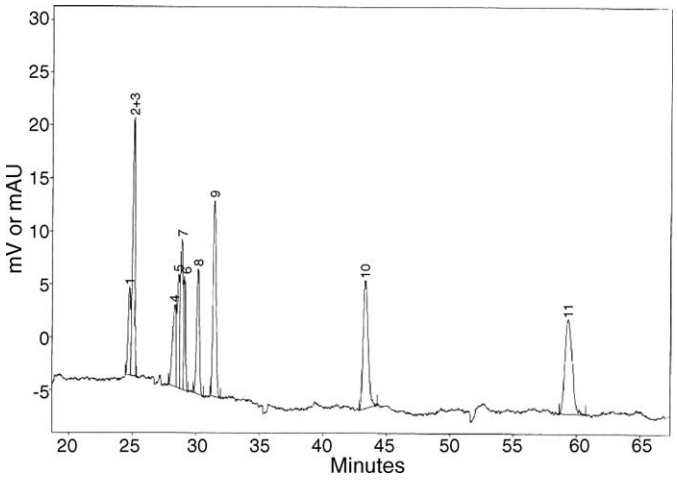

Fig. 6. Separation of 11 positional isomers applying 20 mM *R-3* tetraoxa-diaza crown ether derivative in 50 mM Tris–HCl solution (pH 2.0). Same conditions and isomer labels as in Fig. 2 are used.

Only the aminobenzoic acid isomers (substances 9, 10 and 11) could be resolved. The propanoyl glycine methyl ester side chain had the same effect on the separation efficiency of the aminophenol isomers as the *R-2* derivative and thus resulted in a somewhat improved selectivity compared to the use of *R-1* or Kryptofix 22. Applying more than 20 mM of *R-3*, did not improve the separation efficiency. Moreover, the migration time of the tested components significantly increased, i.e. only one peak could be seen within 70 min. Also the 20 mM electropherogram (Fig. 6) showed a considerably increased analysis time.

4. Conclusions

From the observed results it can be concluded that if the aim is to separate aminobenzoic acid isomers, all crown ethers can be used. However, for this separation they are in fact not needed. If the separation of aminocresol isomers is aimed, only the 18C6 and $18C6H_4$ crown ethers can be applied. Finally, if the aim is to separate the aminophenol isomers, none of the six tested crown ethers results in a baseline resolution.

While the new tetraoxa-diaza crown ether derivatives (*R-1*, *R-2* and *R-3*) had some positive effect in given chiral separations, as was reported earlier, their applicability in the separation of positional isomers seems to be limited. They did not improve the separation efficiency for the tested positional isomers compared to the commercial crown ethers. This can be explained probably by the structure of these molecules, as indicated higher. The macrocyclic ring system containing four oxygen atoms is not able to form stable inclusion complexes with the tested positional isomers, i.e. with the protonated alkylamines probably due to repulsion effects of the nitrogens. Although the substituted side chain(s) on the nitrogen atom(s) of the ring system of these tetraoxa-diaza crown ether derivatives improved the separation efficiency for the tested isomers somewhat (see aminophenol isomers) compared to Kryptofix 22, this was not sufficient to achieve the same separation as with crown ethers having six oxygen atoms and no nitrogens in their ring systems.

It was not possible to separate all 11 aminophenol, aminobenzoic acid and aminocresol positional isomers within one run under any of the tested conditions. The best separation of all tested positional isomers was achieved using the commercially available crown ethers 18-crown-6 (18C6) and 18-crown-6-tetracarboxylic acid ($18C6H_4$). Then, 9 of 11 isomers were separated, while the remaining two, i.e. *m*- and *p*-aminophenol isomers, were co-eluting. Because of the better peak shapes and the shorter analysis time, the application of 18C6 crown ether is to be recommended. The newly developed method using 18C6 crown ether is able to separate simultaneously more aminophenol, aminobenzoic acid and aminocresol positional isomers than the earlier published method [18], where only five of the nine tested isomers were separated.

Acknowledgments

P. Forlay-Frick and T. Iványi express their appreciation to the Flemish Community (BIL 01/23) for financial support. T. Iványi and I. Lázár would like to thank the Hungarian Research Foundation (OTKA T-32100) for financial support. The research of I. Lázár was supported by the Széchényi István Scholarship (SzO 303/2003). The work of K. Héberger was supported by the Hungarian Research Foundation: OTKA T 037684.

References

[1] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, 31st ed., The Pharmaceutical Press, London, 1996.
[2] N.W. Smith, J. Chromatogr. A 652 (1993) 259–262.
[3] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245–276.
[4] N.T. Nguyen, R.W. Siegler, J. Chromatogr. A 735 (1996) 123–150.
[5] C.A. Lucy, R. Brown, K.K.-C. Yeung, J. Chromatogr. A 745 (1996) 9–15.
[6] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 214–226.
[7] C. Perrin, Y. Vander Heyden, M. Maftouh, D.L. Massart, Electrophoresis 22 (2001) 3203–3215.
[8] N. Matthijs, C. Perrin, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Pharm. Biomed. Anal. 27 (2002) 515–529.
[9] H.-C. Chen, W.-H. Ding, J. Chromatogr. A 996 (2003) 205–212.
[10] C.-H. Wu, Y.-S. Lo, H.-C. Nian, Y.-Y. Lin, J. Chromatogr. A 1003 (2003) 179–187.
[11] M.-H. Chen, W.-H. Ding, J. Chromatogr. A 1033 (2004) 167–172.
[12] R. Kuhn, F. Stoecklin, F. Erni, Chromatographia 33 (1992) 32–36.
[13] R. Kuhn, C. Steinmetz, T. Bereuter, P. Haas, F. Erni, J. Chromatogr. A 666 (1994) 367–373.
[14] Y. Walbroehl, J. Wagner, J. Chromatogr. A 680 (1994) 253–261.
[15] P. Castelnovo, C. Albanesi, J. Chromatogr. A 715 (1995) 143–149.
[16] R. Kuhn, D. Riester, B. Fleckenstein, K.H. Wiesmuller, J. Chromatogr. A 716 (1995) 371–379.
[17] T. Iványi, K. Pál, I. Lázár, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 1028 (2004) 325–332.
[18] H. Nishi, K. Nakamura, H. Nakai, T. Sato, J. Chromatogr. A 757 (1997) 225–235.
[19] C.-S. Chiou, J.-S. Shih, Anal. Chim. Acta 360 (1998) 69–76.
[20] Z. Chen, K. Uchiyama, T. Hobo, Electrophoresis 22 (2001) 2136–2142.
[21] M. Mori, H. Tseu, S. Tanaka, J. Chromatogr. A 922 (2001) 399–403.
[22] T. Zhao, X. Hu, J. Cheng, X. Lu, Anal. Chim. Acta 358 (1998) 263–268.
[23] W.-C. Yang, X.-D. Yu, A.-M. Yu, H.-Y. Chen, J. Chromatogr. A 910 (2000) 311–318.
[24] M.W.F. Nielen, J. Chromatogr. 542 (1991) 173–183.

[25] S. Descroix, A. Varenne, N. Goasdoue, J. Abian, M. Carrascal, R. Daniel, P. Gareil, J. Chromatogr. A 987 (2003) 467–476.
[26] X. Guo, Z. Wang, S. Zhou, Talanta 64 (2004) 135–139.
[27] Y. Shi, J.S. Fritz, J. Chromatogr. A 671 (1994) 429–435.
[28] C. Francois, Ph. Morin, M. Dreux, J. Chromatogr. A 706 (1995) 535–553.
[29] C. Francois, Ph. Morin, M. Dreux, J. Chromatogr. A 717 (1995) 393–408.
[30] S.A. Oehrle, J. Chromatogr. A 745 (1996) 87–92.
[31] J. Havel, P. Janoš, P. Jandik, J. Chromatogr. A 745 (1996) 127–134.
[32] A. Tangen, W. Lund, R.B. Frederiksen, J. Chromatogr. A 767 (1997) 311–317.
[33] J.M. Doyle, B.R. McCord, J. Chromatogr. B 714 (1998) 105–111.
[34] X. Cahours, Ph. Morin, M. Dreux, J. Chromatogr. A 810 (1998) 209–220.
[35] F. Tagliaro, G. Manetto, F. Cittadini, D. Marchetti, et al., J. Chromatogr. B 733 (1999) 273–279.
[36] S.D. Alexandratos, C.L. Stine, React. Funct. Polym. 60 (2004) 3–16.
[37] C.-C. Su, M.-C. Chang, L.K. Liu, Anal. Chim. Acta 432 (2001) 261–267.
[38] K. Kobiro, Coordin. Chem. Rev. 148 (1996) 135–149.