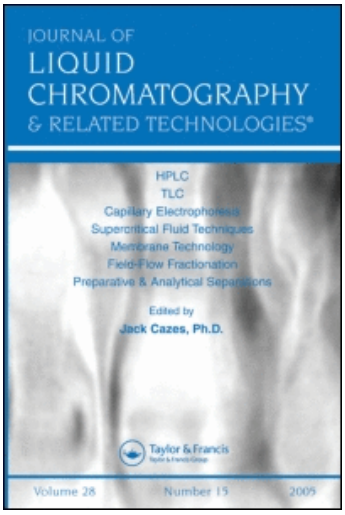

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Studies of the Interactions of Amino Alcohols Using High Performance Liquid Chromatography with Crown Ether Stationary Phases

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Abstract: Separation of amino alcohols on two types of commercially available crown ethers stationary phases (Crownpak CR (+) and Chirosil RCA (+)) is presented. The difference between the two types of crown ether columns is that the Crownpak CR (+) column contains a large non-polar aromatic moiety, while the Chirosil RCA (+) consists of an immobilized crown ether tetracarboxylic acid. The interactions between the amino alcohols and the two stationary phases were characterized by their thermodynamic parameters. The influence of the organic modifier on the retention time and the enantioselectivity was studied using IR experiments. The IR spectra revealed that at a low concentration of organic modifier, Crownpak CR (+) self-interacts through its aromatic moieties, while Chirosil RCA (+) exists in both monomeric and dimeric state. At a higher concentration of organic modifier Crownpak CR (+) exits predominantly as a monomer, while Chirosil RCA (+) exists predominantly as a dimer.

Keywords: Chiral separation, IR spectroscopy, Van't Hoff plots

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INTRODUCTION

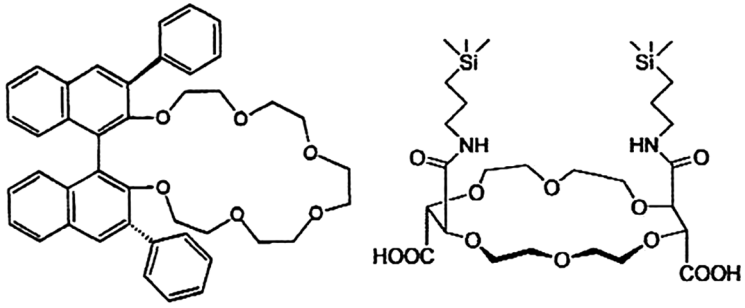
Chromatographic separation of chiral amines is very difficult due to the very polar nature of the amino groups. Because of this, chemical modification of the amino group is often performed in order to achieve enantiomeric separation. Introduction of crown ether stationary phases in chromatography for the purpose of separating chiral amines brings a new dimension to the enantiomeric separation of these compounds.

Crown ethers can be described as cyclic compounds with repeating units of $(-X-C_2H_4-)$, where X is usually an oxygen atom. They were first synthesized by Pederson, for which he received the Nobel Prize in Chemistry in 1987. Crown ethers contain a non-polar exterior (hydrophobic exterior) and polar cavities (hydrophilic cavities).[1,2] The hydrophilic character of the cavity results from the presence of oxygen atoms. As a consequence, the cavity has a strong affinity for cations, predominantly through electrostatic interaction between the cation and the oxygen atom. The strength of these interactions is dependent upon the size and the charge of the guest cation and the size of the cavity of the host crown ether. The stronger interaction is obtained for those cations whose ionic diameters are slightly less than the diameter of the crown ether cavity, thus providing for a tight fit. Crown ethers of the type 18-crown-6 can interact not only with inorganic cations but also with an ammonium group of protonated amines. The inclusion interaction is primarily based on hydrogen bonding between the hydrogen of the ammonium group and the crown ether's oxygen lone-pair electrons.

Cram took advantage of the ability of crown ethers to bind protonated amines in order to perform enantiomeric separation. He investigated structural features which enhance the ability of the host molecule to bind alkylammonium compounds. He discovered that the introduction of bulky groups such as binaphtyl groups on to the exterior of the crown ether could provide additional interaction and produce the separation with protonated amines. The use of these crown ethers was later applied to liquid chromatography by Cram and several of his coworkers.[3] For his work in this field, he shared the Nobel Prize with Pederson and Lehn.

Chromatographic separation of enantiomers was found to be possible for a number of amino esters and amino acids. The separations were performed using mobile phases containing organic solvents. Later it was found that enantiomeric separation of amino bearing compounds could also be performed under aqueous conditions, where the retention of the enantiomer on the column was controlled by the addition of small amounts of organic solvents such as methanol or acetonitrile.

Shimbo et al. used a chiral 18-crown-6 (Figure 1a) dynamically coated on a reversed phase stationary phase.[4,5] This crown ether



(a) (b)

Figure 1. Structure of Crownpak CR (+) (a) and Chirosil RCA (+) (b).

is able to resolve a large number of enantiomeric amines, amino alcohols and amino acids using reversed phase conditions.[4–7] It was found that additives such as perchlorate ion play an important role in chiral separation. This observation is compatible with the theory of chaotropicity.

An anion with high chaotropicity is characterized by high polarizability. Such anions are able to break the structure of water, making it more lipophilic. In the hydration shell of such anions, the water's protons are directed in towards the anion.[8] In a series of anions such as ClO_4^-, CF_3COO^-, NO_3^- and $H_2PO_4^-$ the retention factor of amino alcohols such as cis and trans amino indanol (at a constant pH of 2) increases in the following order: $ClO_4^- > CF_3COO^- > NO_3^- > H_2PO_4^-$.[7] Hyun et al. later developed a variant of this crown ether which was actually chemically anchored on a silica gel matrix. This crown ether successful resolved primary amines as well as amino alcohols.[9]

A different type of crown ether stationary phase is the one derived from 16-crown-6 tetracarboxylic acid, covalently immobilized on silica gel via the reaction between 16-crown-6 tetracarboxylic and amino propyl silica gel. Compared to the previous crown ether, this crown ether is also able to resolved secondary amines.[10–13]

Both types of crown ether columns have been utilized in commercially available columns under the trade names Crownpak CR (+) and Chirosil RCA (+) (Figure 1a and b).

In the present study, we report the enantiomeric separation of aromatic amino alcohols with different structural features with the aim of understanding the nature of the interactions between these analytes and these crown ether stationary phases.

EXPERIMENTAL

Solvents and Reagents

Methanol, Water, Acetonitrile and Methylene Chloride were HPLC grade and were purchased from EMD Chemicals (Gibbstown, NJ). Perchloric acid was purchased from Sigma Aldrich (St. Louis, MO).

HPLC Columns and Chromatographic Conditions

The chiral HPLC columns used in this study, Crownpak CR (+) and Chirosil RCA (+) were purchased from Chiral Technologies, Inc. (West Chester, PA) and Regis Technologies, Inc. (Morton Grove, IL), respectively. The chromatographic conditions used to elute the enantiomers on both crown ether columns consisted of a mixture of water containing 0.1% perchloric acid and acetonitrile. The amount of acetonitrile in the mobile phase was varied according to the condition of the experiments.

Reagents

All the reagents used in the study, (+)-(18-Crown-6)-2,3,11,12-Tetracarboxylic acid, 2-Amino-1-Phenylethanol (S), 2-Amino-1-Phenylethanol (R), 2-Phenyl-Glycinol (S), 2-Phenyl-Glycinol (R), cis-Amino-Indanol (−), cis-Amino-Indanol (+), Naphtylethyl Amine (R), Naphtylethyl Amine (S), (R)-(+)-2-Amino-3-Phenyl-1-Propanol, (S)-(−)-2-Amino-3-Phenyl-1-Propanol, R,R-Amino-Phenyl-Propandiol were purchased from Sigma Aldrich (St. Louis, MO).

The Crownpak CR (+) material was obtained by pumping Methylene Chloride through the column until the baseline came to the same value before crown ether was eluted out of the column. The solution was evaporated using a Büchi R-205 Rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland). The identity of the isolated solid was confirmed by NMR.

Sample Preparation

The sample solutions of all the analytes used in this study were made by dissolving each analyte in methanol at a concentration of 0.2 mg/mL. Five microliters of each solution were injected into the HPLC system. Each sample was injected twice and the results were averaged.

The FTATR-IR spectra were obtained by evaporating solutions of the crown ethers (dissolved in either methanol or methylene chloride) on a Smart Multi Bounce HATR ZnSe 45° sampler. A solution of the mobile phase used in HPLC experiments was added over the film and the spectra were recorded.

Instrumentation

Chromatographic studies were performed on an Agilent 1100 High Performance Liquid chromatograph (Agilent, Palo Alto, CA) equipped with vacuum degasser, quaternary pump, autosampler, thermostated-column oven, and a diode array UV detector. Chromatographic data were acquired with Agilent Chemstation software.

FTATR-IR studies were performed on a Thermo Nicolet 6700 FT-IR (Thermo Fisher Scientific, Waltham, MA) equipped with a Thermo Smart Multi Bounce HATR ZnSe 45° sampler. FT-IR data were acquired with Thermo Omnic Professional Software Suite.

RESULTS AND DISCUSSION

Influence of Temperature on the Chromatographic Parameters

The structures of the compounds used in this study are shown in Figure 2.

The experiments were performed using two types of chiral columns containing the two types of crown ethers (Figure 1a and b). The difference between the two types of crown ether columns is that the Crownpak CR (+) column contains a large non-polar aromatic moiety (Figure 1a) while the Chirosil RCA (+) (Figure 1b) consists of an immobilized crown ether tetracarboxylic acid. The latter is immobilized on a silica gel support through two of its carboxylic functionalities via an amide linkage.[14] Due to these structural features it is expected that the non-polar interaction between the analytes and the Crownpak CR (+) will be much larger than in the case of the Chirosil RCA (+). To study the interactions between the two chiral crown ethers and amino alcohols, 2-Amino-1-Phenylethanol and 2-Phenylglycinol were used as test compounds. Interestingly, while the elution order of the two enantiomers of 2-Phenylglycinol is the same on both columns (*S* enantiomer elutes first), for 2-Amino-1-Phenyl-Ethanol the order of elution is reversed from Crownpak CR (+) where S enantiomer elutes first while on Chirosil RCA (+) R enantiomer elutes first.

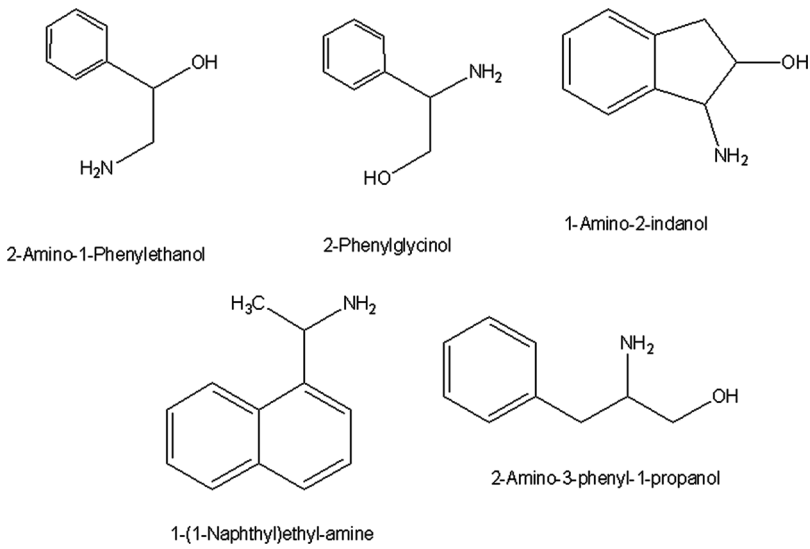


Figure 2. Structure of the compounds used in the study.

To assess the nature of the interactions a temperature study was performed using both stationary phases in the temperature range between 5°C and 50°C. The relationship between the capacity factor (k') and temperature is given by the well-known van't Hoff equation:

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi \tag{1}$$

Equation 1 predicts that a plot of $\ln k$' vs $1/T$ will be a straight line with a slope of $-(\Delta H/R)$ and an intercept of $[(\Delta S/R) + \ln \Phi]$, provided that $\ln \Phi$ is independent of the temperature. If the stationary phase undergoes a change in conformation at a certain temperature, the enthalpy and the entropy of the retention process will change, and the van't Hoff plot will show a change in slope and intercept at the transition temperature. Additionally, the separation factor (α) for a given pair of enantiomers ($\alpha = k_2/k_1$) is a measure of the enantioselectivity and represents the difference in the free energy of interactions of the two enantiomers with the stationary phase. Similiarly, the relationship between α and temperature is given by:

$$\ln \alpha = -\frac{\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \tag{2}$$

In this case, a plot of the $\ln \alpha$ vs $1/T$ will yield a straight line (slope, $-\Delta\Delta H/RT$; intercept, $\Delta\Delta S/R$), provided that the enantioselective interactions do not change over the temperature range studied.

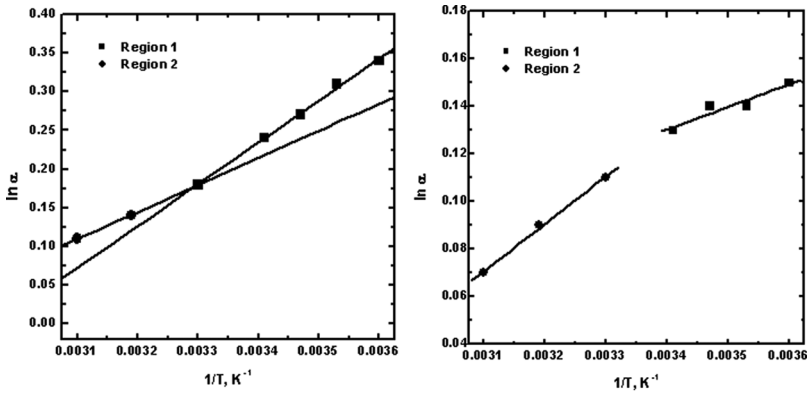


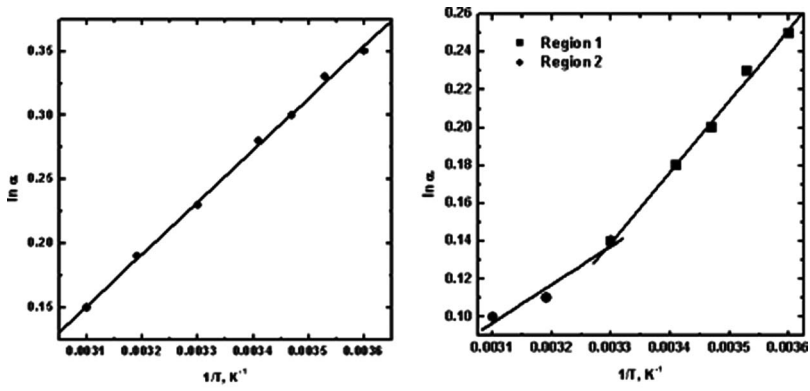
Figure 3. Van't Hoff plot for 2-Phenylglycinol (a) and 2-Amino-1-Phenylethanol (b) on Crownpak CR (+). Mobile phase: 0.1% perchloric acid in water-acetonitrile (95:5, v/v).

The following discussion will present the thermodynamic data corresponding to the influence of temperature on α since it is more pertinent to the enantioselective process. The van't Hoff plots in α for 2-Phenylglycinol and 2-Amino-1-Phenylethanol on Crownpak CR (+) were nonlinear. However, they could be divided into two linear regions – Region 1 from 5°C to 32°C and, Region 2 from 32°C to 50°C (See Figures 3a and b).

The values for $\Delta\Delta H$ (Table 1) for 2-Phenylglycinol in Region 1 are more negative than the values for 2-Amino-1-Phenylethanol, indicating that there are stronger enantioselective interactions with the stationary phase for 2-Phenylglycinol. In addition, $\Delta\Delta S$ for 2-Phenylglycinol is eight times the value of that for 2-Amino-1-Phenyl-Ethanol. The break in the two curves occurs for both compounds at around 28°C, indicating

Table 1. $\Delta\Delta H$ and $\Delta\Delta S$ for 2-phenylglycinol and 2-amino-1-phenylethanol on Crownpak CR (+)

	Region 1		Region 2	
Compound	$\Delta\Delta H$ (cal/mol)	$\Delta\Delta S$ (cal/mol*K)	$\Delta\Delta H$ (cal/mol)	$\Delta\Delta S$ (cal/mol*K)
2-Phenylglycinol	−1100.0	−3.2	−696.4	−2.0
2-Amino-1-Phenylethanol	−190.0	−0.4	−396.1	−1.1



(a) (b)

Figure 4. Van't Hoff plot in a for 2-Amino-1-phenylethanol (a) and 2-Phenylethanol (b) on Chirosil RCA (+). Mobile phase: 0.1% perchloric acid in water-acetonitrile (95:5, v/v).

a possible change in conformation with temperature increase for the Crownpak CR (+) column.

The van't Hoff plot in α for 2-Amino-1-Phenylethanol on Chirosil RCA (+) was linear, while for 2-Phenylglycinol the plot was nonlinear with a break at around 28°C (Figures 4a and b).

The values for $\Delta\Delta H$ and $\Delta\Delta S$ at low temperatures are almost twice as much compared to the higher temperature regions.

Comparative data for 2-Phenylglycinol on this column with the data for the same compound on the Crownpak CR (+) column indicated that in both low and high temperature regions, the values for $\Delta\Delta H$ are more negative on the Crownpak CR (+) than on the Chirosil RCA (+), indicating stronger enantioselective interactions between the compound and the Crownpak column. These differences are due to the contribution of the hydrophobic interaction of the aromatic moiety present on Crownpak CR (+) (Table 2). The reason for the nonlinearity

Table 2. Comparison of $\Delta\Delta H$ and $\Delta\Delta S$ for 2-phenylglycinol on Crownpak CR (+) and Chirosil RCA (+)

	Region 1		Region 2	
Compound	$\Delta\Delta H$ (cal/mol)	$\Delta\Delta S$ (cal/mol*K)	$\Delta\Delta H$ (cal/mol)	$\Delta\Delta S$ (cal/mol*K)
Crownpak (CR+)	−1075.7	−3.2	−596.4	−1.9
Chirosil RCA (+)	−743.1	−2.2	−402.7	−1.1

of 2-Phenylglycinol on the Chirosil RCA (+) is unclear at the present time and is a point of further investigation.

Influence of Acetonitrile Concentration on the Chromatographic Parameters

The relationship between the capacity factor (k') and the concentration of organic modifier in the mobile phase is given by:

$$\ln k' = \ln k_w - S\phi \tag{3}$$

where k'_w is the extrapolated k' for pure aqueous mobile phase, ϕ is the volume fraction of the organic-modifier.

Equation (3) predicts that a plot of $\ln k'$ vs ϕ will be a straight line with the slope of S. S is proportional to the free energy of solute transfer from pure water to pure organic mobile phase and correlates with the molecular surface of the analyte.

The influence of acetonitrile on the enantiomeric separation of the two model compounds was investigated in the range of 0.005–0.05 volume fraction. The enantiomeric separation of 2-Phenylglycinol and 2-Amino-1-Phenylethanol on the Crownpak CR (+) led to nonlinear graphs (Figures 5a and b). They could, however, be divided into two linear regions with a change in slope at approximately 0.015 volume fraction.

The linear region from 0.005 to 0.15 volume fraction (Region A) for both enantiomers of 2-Phenylglycinol has higher S values compared

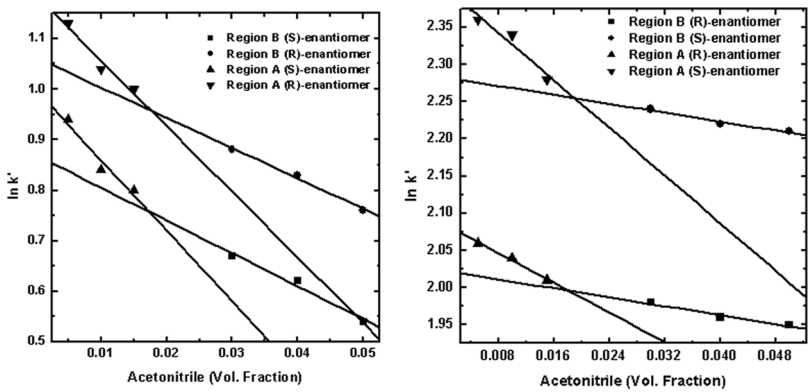


Figure 5. Influence of Acetonitrile concentration on k' of the two enantiomers of 2-Phenylglycinol (a) and 2-Amino-1-Phenylethanol (b) on Crownpak CR (+).

Table 3. Influence of acetonitrile on the slope on Crownpak CR (+)

Compound	Region A		Region B	
	S Enantiomer Slope 1	*R* Enantiomer Slope 2	*S* Enantiomer Slope 1	*R* Enantiomer Slope 2
2-Amino-1-Phenylethanol	−4.5	−4.5	−10.0	−9.0
2-Phenyl-Glycinol	−6.5	−6.0	−14.0	−13.0

to the same region for 2-Amino-1-Phenylethanol. This indicates that the surface available for interaction with the stationary phase is higher for 2-Phenylglycinol than for 2-Amino-1-Phenylethanol. Similarly, in the region between 0.015 and 0.05 volume fraction (Region B), the values for *S* are higher for 2-Phenylglycinol than for 2-Amino-1-Phenylethanol (Table 3).

The variation of the concentration of acetonitrile on the selectivity, α, remained unchanged throughout the entire concentration range studied. This indicates that whatever influence acetonitrile has on the k' of the two enantiomers for the two model compounds, has the same effect on both enantiomers. Thus, the selectivity is unchanged.

The influence of the concentration of acetonitrile on the separation of the two enantiomers for the two model compounds using Chirosil

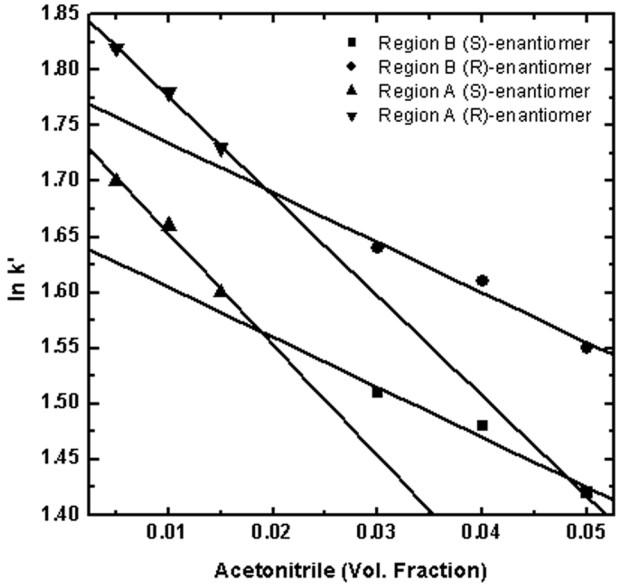


Figure 6. Influence of Acetonitrile concentration on k' of the two enantiomers of 2-Amino-1-phenylethanol on Chirosil RCA (+).

RCA (+) showed a different behavior. While for 2-Phenylglycinol the plot of $\ln k'$ vs. volume fraction of acetonitrile was a straight line ($R^2 > 0.99$), the graph of $\ln k'$ vs. volume fraction of acetonitrile for 2-Amino-1-Phenylethanol was nonlinear (Figure 6).

Similar to the previous case, the data points could be divided into two linear regions, one from 0.005 to 0.015 volume fraction of acetonitrile and another from 0.015 to 0.05. Comparing the S values for 2-Amino-1-Phenylethanol obtained on the Chirosil RCA (+) with the S values for the same compound on the Crownpak CR (+) column, one can observe that the values obtained on the Crownpak CR (+) column are higher than on the Chirosil RCA (+), indicating higher interactions of the compounds with Crownpak CR (+) stationary phase (Table 4).

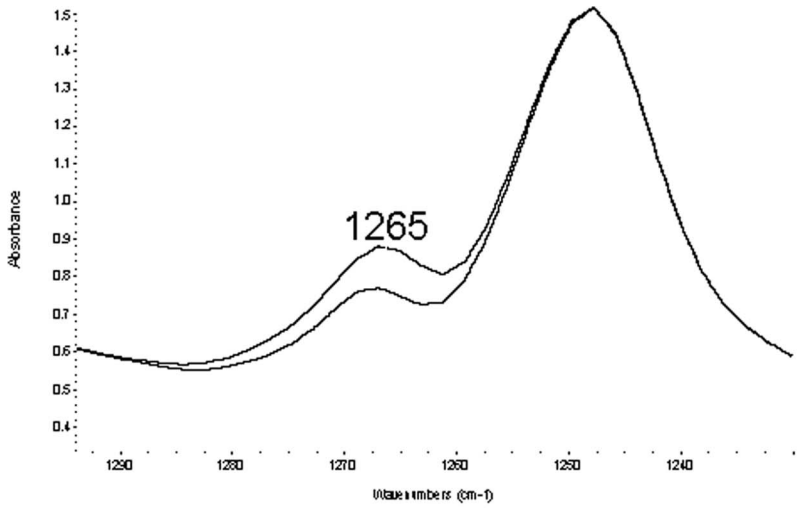
Similarly, the selectivity factor for the two compounds is unaffected by the variation in the concentration of the organic modifier.

In order to ascertain the non-linearity in the plots of $\ln k'$ vs. volume fraction of acetonitrile we pursued FT-ATR infrared spectroscopy experiments. We casted a film of both crown ethers (Crownpak and crown ether tetracarboxylic) on a zinc selenite crystal. A solvent mixture with a composition similar to the composition of the mobile phase used in our experiments was added over the film. The composition of these solvents was similar to the composition used in each linear region of the graph (1% and 4% acetonitrile, respectively, in water containing 0.1% perchloric acid). The two sets of spectra were obtained by subtracting the solvent spectra from the crown ethers spectra. In this way, one can assess the influence of acetonitrile on the structure of the two crown ethers. The two sets of spectra were superimposable except for the region corresponding to the $C = O$ stretching and *Ar-O-C* (where Ar is the aromatic part of Crownpak). An overlay of the two sets of spectra for both types of crown ethers is presented in Figures 7(a) and (b).

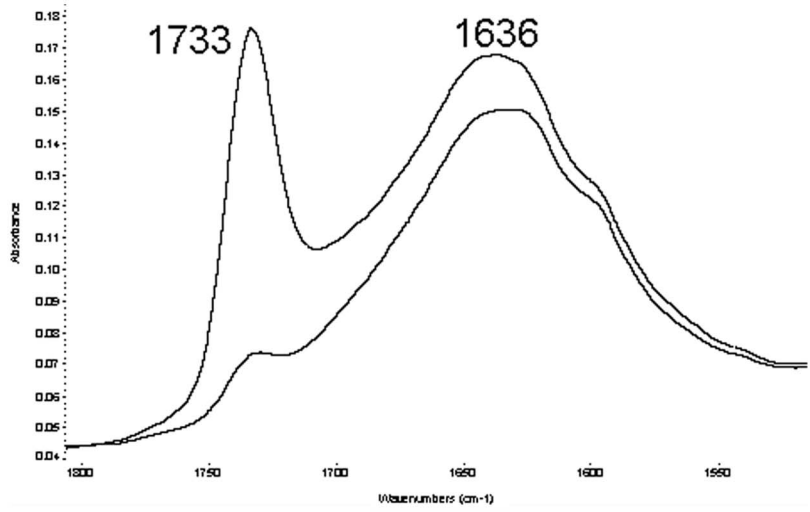
The spectra of Crownpak in the presence of 1% and 4% acetonitrile are almost superimposable, except for the regions at $1265\,cm^{-1}$ corresponding to *Ar-O-C* (Figure 7a).[15] Upon increasing the concentration of acetonitrile, the intensity of these bands decreases.

Table 4. Influence of acetonitrile on the slope on Crownpak (+) and Chirosil RCA (+) for 2-amino-1-phenylethanol

	Region A		Region B	
Column	*S* Enantiomer Slope 1	*R* Enantiomer Slope 2	*S* Enantiomer Slope 1	*R* Enantiomer Slope 2
Crownpak CR (+)	4.5	4.5	10.0	9.0
Chirosil RCA (+)	1.5	1.5	5.0	8.0



(a)



(b)

Figure 7. IR spectra of Crownpak CR (+) in the regions centered at 1265 cm^{-1} (a) (upper trace 1% acetonitrile, lower trace 4% acetonitrile in water containing 0.1% perchloric acid), Crown ether tetracarboxylic in the region from 1733 cm^{-1} to 1636 cm^{-1} (b) (upper trace 1% acetonitrile, lower trace 4% acetonitrile in water containing 0.1% perchloric acid).

To explain these behaviors, one should consider the experimental conditions for these experiments. At a lower concentration of acetonitrile (1%) the hydrophobic interaction between molecules of Crownpak prevails, rendering π stacking of the aromatic part of the crown ether with the neighboring crown ether molecules. This will also lead to an orientation of the dipole moments of this part of the molecule. Due to this phenomenon, the band centered at $1265\,cm^{-1}$ will have higher intensity at 1% acetonitrile compared to 4% acetonitrile. The hydrophobic interaction between the aromatic part of the molecule diminished when the concentration of acetonitrile increases from 1% to 4%, and the dipole moments became disarrayed; as a consequence, the intensity of these bands will decrease. Thus, under these solvent conditions, it is possible to ascertain that the change in slope occurring at approximately 0.015 volume fraction is due to a change in solvation of the aromatic part as well as *C-O-C* bond of the Crownpak molecules.

The crown ether tetracarboxylic acid behaves differently (Figure 7b). Two bands were encountered: a sharp one at $1733\,cm^{-1}$ and a second broad band at $1636\,cm^{-1}$. The band at $1733\,cm^{-1}$, corresponding to the monomeric carboxyl, decreases in intensity upon increasing the concentration of acetonitrile from 1% to 4%. These bands, $1733\,cm^{-1}$ and $1636\,cm^{-1}$ correspond to the monomeric and dimeric carboxyl respectively. To explain this behavior it should be considered that in water, the carboxylic functional groups of crown ether are partially ionized and as a consequence they exist in both monomeric (the band $1733\,cm^{-1}$) and dimeric state (the band $1636\,cm^{-1}$). The ionization of these carboxyls diminishes when the concentration of acetonitrile increases; as a consequence it starts interacting with the neighboring molecules of crown ether tetra carboxylic through hydrogen bonding to form a dimer leading to the broad band at $1636\,cm^{-1}$. Such behavior can explain the change in slope of the two lines which occurs at $\sim$0.015 volume fraction of acetonitrile.

Influence of Structural Variation on the Chromatographic Parameters

The enantiomeric separation on the Chirosil RCA (+) columns is outlined in Table 5.

The separation and the retention are the highest in the case of 2-Amino-1-Phenylethanol, where the amino group is located at the end of the alkyl side chain. As the amino group moves closer to the phenyl ring, the separation and the retention time of the two enantiomers decreases. For cis-Amino-Indanol, the amino group is linked to a five member ring. There is no separation of the two enantiomers, indicating that the rest of the molecule produces steric

Table 5. Influence of structure on k' and selectivity α

Compound	Acetonitrile concentration	k'_1	k'_2	α
Chirosil RCA (+)				
2-Amino-1-Phenylethanol	5.0	6.78	8.68	1.280
2-Phenyl-Glycinol	5.0	2.62	3.06	1.171
Cis Amino Indanol	5.0	2.04	2.04	1.000
Naphtyl Ethyl Amine	5.0	11.34	12.59	1.111
2-Amino-3-Phenyl-1-Propanol	5.0	2.40	2.48	1.034
Crownpak CR (+)				
2-Amino-1-Phenylethanol	5.0	4.15	4.70	1.310
2-Phenyl-Glycinol	5.0	1.71	2.13	1.245
Cis Amino Indanol	5.0	2.25	2.71	1.202
Naphtyl Ethyl Amine	5.0	Did not elute under chromatographic conditions		
2-Amino-3-Phenyl-1-Propanol	5.0	3.44	4.70	1.079

hindrance, which is a factor affecting the enantiomeric separation. For Naphtylethyl-amine, the retention time for the two enantiomers on the column is the highest; however, the selectivity is smaller than that of 2-Amino-1-Phenylethanol. This indicates that for the amino alcohols 2-Phenylglycinol and 2-Amino-1-Phenylethanol the –OH group is also involved in the enantioselectivity. As the sidechain increases, and since the amino group is located in the middle of the side chain, the selectivity and the retention decrease.

The enantiomeric separation on the Crownpak CR (+) column showed trends similar to those of the Chirosil RCA (+) (Table 5). The order of elution, however, of the two enantiomers of 2-Amino-1-Phenylethanol is reversed compared to the Chirosil RCA (+) column. The retention time and the selectivity factor on the Crownpak CR (+) column are larger compared to Chirosil RCA (+), indicating that the non-polar moiety in the Crownpak CR (+) column plays an important role in the enantioselectivity.

CONCLUSIONS

The temperature study with the two model compounds conducted on the Crownpak CR (+) column indicated that there is a possible conformational change of stationary phase occurring around 28°C.

The influence of acetonitrile concentration on the Crownpak CR (+) column also showed a nonlinear behavior, indicating that there are at least two types of interactions governing the retention of the two

enantiomers of the two model compounds. This is due to a change in behavior of the crown ether with the change in concentration of acetonitrile.

The comparison of the two columns indicated that the stronger enantioselective interactions of the model compounds with the Crownpak stationary phases are due to the more negative values for $\Delta\Delta H$ encountered on the Crownpak CR (+) column.

The influence of acetonitrile concentration on the Chirosil RCA (+) column showed a single type of interaction with the stationary phase for 2-Phenylglycinol, while at least two types of interactions were evident for Amino-1-Phenylethanol, due to the break in the plot of $\ln k'$ versus volume fraction of acetonitrile.

The IR spectra of the two types of crown ethers indicated that at low concentrations of acetonitrile the crown ether tetracarboxylic existed as a monomer and dimer, while at higher concentrations of acetonitrile it exits predominantly as a dimer occurring through hydrogen bonds between the carboxyl functional groups. The Crownpak CR (+) self interacts at a low concentration of acetonitrile, while at higher concentration it is solvated. Such behavior affects the retention of the analytes, but does not affect the enantioselectivity.

The influence of structural variation indicated that the position of the amino group on the side chain relative to the aromatic moieties has an important role for retention as well as for enantioselectivity.

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