Enantioseparation of α-Phenylglycine by HPLC on an ODS Column Coated with Chiral Crown Ether

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Key Words
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Summary
Previous work has demonstrated the possibility of creating chiral stationary phases by coating a suitable support material via the mobile phase. The result is a wide variety of chiral phase systems which can be used in liquid chromatography to separate enantiomers by a liquid – solid adsorption mechanism. The present paper demonstrates the potential of this technique for chiral crown ethers incorporating an α-D-mannopyranoside unit using the separation of phenylglycine enantiomers as an example.

Introduction
It has been reported that chromatographic systems with a chiral stationary phase (CSP) physically coated onto a solid support can be successfully used for many enantiomeric separations. Different chiral agents e.g., the mono-octylamide [1] and the ditubyl ester of L-tartaric acid [2], L-aspartylalkylamides [3] and β-cycloextrin derivatives [4–9] have been used for enantiomeric separations in liquid-liquid (LL) and liquid-solid (LS) systems. Another group of chiral agents used in LC systems as the stationary phase are crown ethers [10], which can be immobilized onto the solid support by covalent binding [11, 12], physical adsorption [13–16] or substitution in a polymer matrix [17]. This paper demonstrates the possibility of establishing an enantioselective LS system by multiple injection of a lipophilic chiral crown ether into the eluent stream and thus coating a hydrophobic solid support with a chiral stationary phase (CSP).

Experimental
The chiral crown ether was obtained from methyl 4,6-O-isopropylidene-α-D-mannopyranoside as the chiral start-

Figure 1
Synthesis and structure formulae of the chiral crown ether investigated.
Results and Discussion

Chiral Agent and Its Adsorption on ODS Solid Surface

The chiral crown ether (Figure 1) was synthesized following reaction conditions already fully described [19]. It crystallizes very easily from ethyl acetate and its X-ray data in the uncomplexed form have been published [21, 22]. The protection group in the 4,6-0 position can be readily removed under mild acidic conditions in protic solvents [20]. Previous results concerning the transport of chiral guest species across a liquid membrane using the chiral crown ether (IV) as a carrier have shown the possibility for an enantioselective transport of optically ether unprotected at the 4,6 position [20].

This paper reports the first study on enantioseparation in LS-systems with a chiral crown ether incorporating an α-D-mannopyranoside unit.

The adsorption of the chiral agent onto the ODS solid support can be demonstrated by measuring its capacity factor (k'). Figure 2 shows the dependence of the k' values of the crown ether on the methanol concentration in the mobile phase using a LiChrosorb RP18 column. The solid line connects the experimental data and the dashed line indicates data extrapolated according to the equation:

\[ \log k' = b - a \cdot C_{\text{MeOH}} \]

where: k' is the capacity factor of the crown ether and \( C_{\text{MeOH}} \) the volume concentration of methanol. The retardation and thus adsorption of the chiral agent increases steeply with decreasing methanol concentration and reaches very high values for weak eluents where \( C_{\text{MeOH}} < 10\% \).

The experimental data were obtained at room temperature (about 22 °C) and they have to be seen as rough estimates of the extent of adsorption. However, they show that the retardation of the chiral ether (IV) in the chromatographic system investigated is very strong. This enables the creation of an LSC system with the CSP physically coated onto the hydrophobic solid support. The solid support was coated with chiral agent by injecting 20 × 100 µl of crown ether solution (62.5 mg/ml in CHCl3) in the eluent stream. Chloroform containing unadsorbed chiral ether was displaced from the column by pumping through the eluent; the equilibrium state was reached after passing about 150 column dead volumes.

Chromatographic Separations

The chromatographic chiral system with crown ether (IV) physically coated onto a hydrophobic solid support can be used for the separation of phenylglycine enantiomers. Figure 3a shows the resolution of phenylglycine enantiomers on the CSP with an eluent containing 0.1 mg/ml crown ether (IV) and 0.2 % HClO4 in a 10 % methanol water solution. As can be seen from Figure 3a and Table I the separation factor a is 1.14 but because of the poor peak shapes the resolution only reaches a value of \( R_S < 0.8 \). However, the resolution can be improved adding an ion-pairing agent to the eluent. Figure 3b demonstrates the resolution of α-phenylglycine enantiomers under the same chromatographic conditions as in Figure 3a but with the addition of 0.5 mg/ml potassium 2,4-dimethylcyclohexyl-sulfonate mono-hydrate as ion-pairing agent.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Capacity (k'), selectivity (α) and resolution (R_S) factors for α-phenylglycine enantiomer separations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent:</td>
<td>( \text{H}_2\text{O-\text{MeOH}}/90-10 \text{ v/v} + 0.1 \text{ mg/ml crown ether} )</td>
</tr>
<tr>
<td></td>
<td>( k' )</td>
</tr>
<tr>
<td>A:</td>
<td>0.2 % HClO4</td>
</tr>
<tr>
<td>B:</td>
<td>0.2 % HClO4 + 0.5 mg/ml potassium 2,4-dimethyl-cyclohexylsulfonate</td>
</tr>
</tbody>
</table>

Enantioseparation of α-phenylglycine on an ODS surface physically coated with chiral crown ether. Column (250 x 1 mm I.D.) packed with 5 µm LiChrosorb RP18. Mobile phase: 10 % MeOH, 0.2 % HClO4;

3a without ion pairing agent
3b with 0.5 mg/ml potassium 2,4-dimethyl-cyclohexyl-sulfonate monohydrate as ion-pairing agent. Flow rate 40 µl/min.