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Lasalocid adsorbed on porous graphitic carbon as a chiral selector, in capillary liquid chromatography

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Abstract

Lasalocid, which has high affinity for porous graphitic carbon (PGC), has been dynamically adsorbed onto PGC and used in capillary liquid chromatography. Several enantiomers of amines, acids, amino acids and alcohols have been separated on this system and the influence of the solute structure has been studied. For example, 1-(1-naphthyl)ethylamine enantiomers showed an extraordinary separation factor ($\alpha \approx 21$), whereas the enantiomers of the positional isomer 1-(2-naphthyl)ethylamine showed a more moderate separation factor ($\alpha \approx 2$). Decreasing the methanol concentration enhanced the selectivity for 1-(1-naphthyl)ethanol, 1-(2-naphthyl)ethanol, 2,3-dibenzoyltartaric acid and 2,3-di-*p*-toluoyltartaric acid. No change in selectivity could however be found when the chiral selector concentration and ionic strength were varied. The chromatographic system has been stable and the adsorption of Lasalocid has been reproducible. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chiral separations commonly require high capacity columns for determination of chiral purity of less than 0.5% and conventional liquid chromatography (LC) columns have therefore mostly been used. The use of packed capillary liquid chromatography (inner diameter of less than 0.5 mm), see ref. [1] and references therein, offers, however, several advantages compared to conventional LC. Reduced mobile phase consumption, the need for less packing material and higher absolute efficiencies are some of these advantages. In addition, for the separation of enantiomers, the miniaturization of the chromatographic system is of particular interest. The use of capillary columns makes it possible to try new stationary phases, chiral selectors and mobile phases, which are too valuable to use in conventional LC. The possibility of packing longer capillary columns for increased absolute efficiency also offers the chance to separate enantiomers with low separation factors (α).

It is well known that chiral separations may be performed either by using a *direct* or an *indirect* method. In the indirect separation, where the enantiomers are e.g. derivatized to diastereomers and thereafter separated on a non-chiral column, the need for optically pure derivatization reagents becomes a problem. The more straightforward method using direct chiral separation, where the enantiomers are separated either by using a chiral selector in the mobile phase or by immobilization of the selector

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onto the packing material, is therefore recommended. Successful direct separations have been achieved in this way using columns packed with porous graphitic carbon (PGC). Karlsson and Pettersson [2] used *N*-benzyloxycarbonyl-glycyl-L-proline (L-ZGP) in the mobile phase while Heldin et al. [3] dynamically adsorbed (2*R*,3*R*)-di-*cyclo*-hexyltartrate (DCHT) onto the material.

Using PGC as a support provides a hydrophobic, homogeneous and flat surface and a packing material that is stable across the entire pH range, 0–14 [4]. PGC itself has shown selectivity for positional isomers (e.g. *o*-, *m*- and *p*-xylenes), which arises from differences in the ability of the molecules to accommodate themselves to the flat layered surface [5,6]. The hydrophobic character of the PGC material also makes it suitable for dynamically adsorbed chiral selectors [2–4,7].

Lasalocid (or X-537 A), Fig. 1, with its hydrophobic character, should be able to adsorb onto the PGC and therefore be suitable as a chiral selector in capillary LC. To date, no study about the use of Lasalocid as a chiral selector in LC has been published.

Lasalocid is a member of the class of naturally occurring ionophores known as polyether antibiotics. The molecule has been demonstrated to be able to adopt a cyclic arrangement with a hydrophobic outer surface and a central hydrophilic cavity. Lasalocid is produced by the bacterium *Streptomyces lasaliensis* and mediates in the specific transport of cations across biomembranes ([8] and references therein). Westley et al. [9] used Lasalocid as a resolving agent in fractional crystallization. They studied amines containing an asymmetric carbon atom attached directly to the primary amino group and they found that the *R* isomer of the crystalline salts of each enantiomeric pair predominated over the *S* isomer. The interactions involved in the chiral discrimination have been the subject of several investigations (e.g. [9–11]). Moreover, functional modification of Lasalocid and other naturally occurring ionophores has been demonstrated to serve as an interesting new class of chiral receptors [11,12].

Fig. 1. Structure of Lasalocid.

In the present study, Lasalocid has been used as a chiral selector, adsorbed onto PGC, in capillary LC. The separation factors for enantiomers of amines, acids, amino acids and alcohols have been investigated as well as the stability of the columns and the reproducibility of adsorption of the selector onto the support.

2. Experimental

2.1. Column preparation

The columns were prepared by using the slurry method with acetonitrile as the carrier. Fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter (I.D.) of 200 μm [340 μm outer diameter (O.D.)] was used as the column material and Hypercarb (5 μm, batch PGC 129/R1) was utilized as the packing material. The frit was made of a piece of glass fiber paper (Whatman GF/A, W & R Balston, UK). The columns were evaluated before use with 3,5-dihydroxybenzoic acid (dissolved in water) as the test substance and 60% acetonitrile in a total concentration of 0.1 *M* formic acid as the mobile phase.

Lasalocid was dynamically adsorbed onto PGC material by pumping a solution containing 21 mg/l of Lasalocid in 70% methanol (see also Section 2.4) through the PGC-filled column (15 cm×200 μm I.D.). The breakthrough volume, recorded at 308 nm, was used to calculate the amount of selector adsorbed on the support. The volumetric flow-rate used during the adsorption was 1.4 μl/min.

2.2. Instrumentation

The chromatographic system consisted of a liquid chromatographic pump (μPrecision pump; Pharmacia, Uppsala, Sweden), a laboratory-made injection loop [3 μl polyether ether ketone (PEEK) tubing] an ss-4R3 A-EP valve (Nupro, Willoughby, OH, USA), which was used to split the flow (100 μl/min before the column and around 1 μl/min over

the column), and an ultraviolet (UV) detector (μPeak monitor; Pharmacia) with optical fibers (400 μm source and collector; Polymicro Technologies) operating at 220, 254 and 308 nm. An optical cell was created by burning off a segment of the polyimide coating on an empty fused-silica capillary (150 μm I.D.×365 μm O.D.). This empty capillary was then connected to the column by means of a PTFE tube (0.3 mm I.D.×1/16 in. O.D.; 1 in.=2.54 cm) and placed in the detector cell block to which the optical fibers were coupled [13].

2.3. Chemicals and solutes

Acetonitrile (LiChrosolv), methanol (LiChrosolv), phosphoric acid (analytical-reagent grade), sodium dihydrogenphosphate monohydrate (analytical-reagent grade) and formic acid (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany). 3,5-Dihydroxybenzoic acid was purchased from Fluka (Buchs, Switzerland) and Lasalocid sodium salt (97%), from Aldrich (Steinheim, Germany), was used without any further purification. The solutes used in this study were: racemic 1-(1-naphthyl)ethanol (Fluka), racemic 1-(2-naphthyl)ethanol (Fluka), racemic 1-(1-naphthyl)ethylamine (Aldrich), (*R*)-(+)-1-(1-naphthyl)ethylamine (Aldrich), (*S*)-(−)-1-(1-naphthyl)ethylamine (Fluka), (*R*)-(+)-1-(2-naphthyl)ethylamine (Fluka) and (*S*)-(−)-1-(2-naphthyl)ethylamine (Fluka), (*R*)-*N*-benzyl-1-(1-naphthyl)ethylamine hydrochloride (Fluka), (*S*)-*N*-benzyl-1-(1-naphthyl)ethylamine hydrochloride (Fluka), DL-3-(1-naphthyl)alanine (Sigma), L-3-(1-naphthyl)alanine (Sigma), DL-3-(2-naphthyl)alanine (Sigma), (−)-2,3-dibenzoyl-L-tartaric acid monohydrate (Sigma), (+)-2,3-dibenzoyl-D-tartaric acid (Sigma), (−)-2,3-di-*p*-toluoyl-L-tartaric acid monohydrate (Sigma), and (+)-2,3-di-*p*-toluoyl-D-tartaric acid (Sigma).

2.4. Chromatographic conditions

The mobile phases were prepared from methanol, Lasalocid and phosphate buffer pH 2.2, with an ionic strength of 0.1 (unless otherwise stated).

Mobile phase A (Table 1) used during the adsorption of Lasalocid was prepared by dissolving 10.5 mg of the selector in 350 ml of methanol. Thereafter, phosphate buffer was added to a total volume of 500 ml. All mobile phases used in the study (see Table 1) were prepared in a similar way.

The solutes were dissolved in 40% methanol and the disturbance in the base line was used to calculate the dead time (t_0). The columns were equilibrated after every mobile phase change until stable retention factors (k') were obtained. The separation factors for the enantiomers were calculated as $\alpha = k'_2/k'_1$, where k'_1 is the retention factor for the first-eluting enantiomer and k'_2 is the retention factor for the second-eluting enantiomer.

3. Results and discussion

3.1. Amount of chiral selector adsorbed on the column

The selector was adsorbed onto PGC material by pumping a solution containing 21 mg/l of Lasalocid in 70% methanol (mobile phase A, Table 1) at a volumetric flow-rate of 1.4 μl/min. A breakthrough curve was recorded during the adsorption. The time corresponding to the steepest slope of the curve was used as the 'breakthrough time' and that time occurred after about 2900 min. The amount of selector immobilized onto the PGC was then calcu-

Table 1
Mobile phases used in the present study

Mobile phase	Methanol concentration (%)	Selector concentration (mg/l)	Ionic strength of the phosphate buffer, pH 2.2
A	70	21	0.1
B	70	12	0.1
C	40	12	0.1
D	40	12	0.05

lated to be 8×10^{-5} mol/g. This value is in good agreement with the amount of DCHT adsorbed onto PGC [2].

The solubility of Lasalocid in methanol is 1 g/100 ml, while it is practically insoluble in water. In order to coat the surface of the packing material in a reasonable time, a mobile phase with a high methanol content should be used. Furthermore, it is important to consider that the 'coating solution' should not be too different from the mobile phase used for the chiral separations, due to the long equilibration times needed when components in the mobile phase are changed. When the methanol content, the selector concentration and the ionic strength were changed simultaneously, an equilibration time of about ten days was needed. However, when only one parameter (e.g. the selector concentration, mobile phase A and B; Table 1) was changed, the columns were equilibrated more rapidly (a few days). The columns were equilibrated after every mobile phase change until stable retention factors (k') were obtained.

The selectivity factors were found to be reproducible when Lasalocid was adsorbed onto different PGC columns. On three different columns, the enantioselectivities for 3-(2-naphthyl)alanine were 3.9, 4.2 and 4.3. Moreover, after using the different mobile phase compositions, the first mobile phase (A, Table 1) was reused and similar retention and selectivity factors were then achieved. This indicates that there are no memory effects in the results from the different mobile phases.

Furthermore, the stability of a coated column was tested over a period of more than two months using a mobile phase of 70% methanol and 21 mg selector/l (phase A, Table 1). The enantiomers of 3-(1-naphthyl)alanine, 3-(2-naphthyl)alanine and l-(l-naphthyl)ethylamine were injected regularly during this time. The system proved to be very stable. Fig. 2 presents the separation factor of the enantiomers of 3-(1-naphthyl)alanine and the retention factor for the (L)-enantiomer. Likewise the enantiomers of 3-(2-naphthyl)alanine and 1-(1-naphthyl)ethylamine showed stable separation and selectivity factors. These results prove that the amount of selector adsorbed onto the PGC was constant during the period studied.

3.2. Solute structure and achieved stereoselectivity

Fig. 3 shows a chromatogram of a sample containing *rac*-1-(1-naphthyl)ethylamine, the two enantiomers of 1-(2-naphthyl)ethylamine and *rac*-3-(2-naphthyl)alanine. Note that PGC dynamically modified with Lasalocid is well suited to separate the enantiomers of amines and amino acids. As shown in Tables 2 and 3, stereoselectivity was also obtained for different alcohols and acids.

As postulated by Dalgliesh in 1952 [14], at least one of the three interactions in a chiral recognition

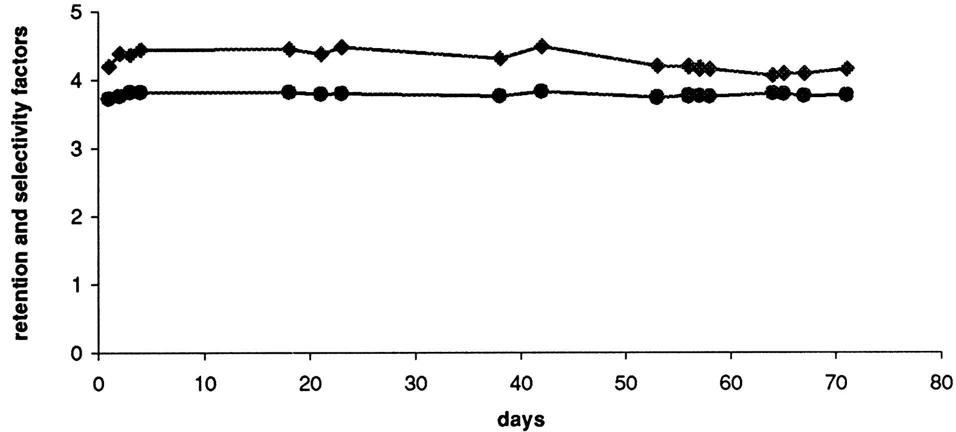

Fig. 2. Stability of the system over a two-month period. Conditions: Mobile phase: 70% methanol, phosphate buffer pH 2.2 (ionic strength, 0.1) and 21 mg Lasalocid/l (mobile phase A, Table 1). UV detection at 220 nm. The separation factor (●) of the enantiomers of 3-(1-naphthyl)alanine and the retention factor (♦) of the L-enantiomer are presented.

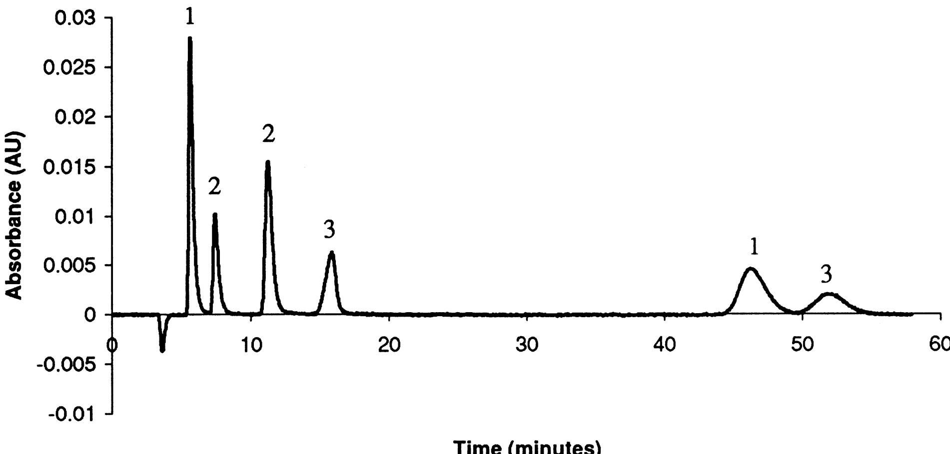

Fig. 3. Separation of enantiomers of two amines and one amino acid. Conditions: Mobile phase: 70% methanol, phosphate buffer pH 2.2 (ionic strength, 0.1) and 21 mg Lasalocid/l (mobile phase A, Table 1). Flow-rate: approximately 1 μl/min. UV detection at 220 nm. Peak identification: (1)=1-(1-naphthyl)ethylamine; (2)=1-(2-naphthyl)ethylamine; (3)=3-(2-naphthyl)alanine.

model has to be stereochemically controlled. It has been emphasized that achiral components, e.g. the surface of the packing material, may contribute to the achieved chiral selectivity [15–18]. In the present study, the position of the ethylamino group on the naphthyl ring proved to be of particular importance (see Table 2). Only a moderate enantioselectivity was achieved for 1-(2-naphthyl)ethylamine compared to the extraordinary selectivity for the enantiomers of 1-(1-naphthyl)ethylamine; *N*-benzyl-1-(1-naphthyl)ethylamine, with a bulky substituent on the amine group, on the other hand, just showed tendencies to separate. Looking at the retention factors reveals that it was only the (*R*)-enantiomer of 1-(1-naphthyl)ethylamine that was strongly retained by the chiral stationary phase. The mechanisms of interaction were difficult to predict as they involved at least the chiral analyte, the chiral selector and probably also the surface of the PGC. Possible points of interaction between Lasalocid and a primary amine in crystallography have been presented by Westley et al. in 1977 [9]. They performed X-ray crystallographic studies of the complex between (*R*)-(+)-1-(4-bromophenyl)ethylamine and Lasalocid and showed that it is most likely that hydrogen bonding occurs from the protonated amine group to the oxygens on the Lasalocid molecule. The difference in bonding strength between the enantiomers was so large that the (*R*)-enantiomer was completely resolved after three recrystallizations. It is likely that, in our study also, there are hydrogen bondings between the amine group and some of the oxygens of the Lasalocid molecule. In our study, the selectivity was also decreased remarkably when the amine group was replaced with an hydroxyl group.

Fairly high stereoselectivities ($\alpha \approx 4$) were obtained for the amino acid derivatives studied, i.e. 3-(1-naphthyl)alanine and 3-(2-naphthyl)alanine. Since the enantiomers of 3-(2-naphthyl)alanine were less retained than the positional isomers, all four isomers could be resolved. The finding that no clear difference in enantioselectivity could be observed indicates a more general mechanism for the analytes without dependence on the substitution of the naphthyl ring.

A few acids were also included in the study. As shown in Table 2, the tartaric acid derivatives just showed tendencies to separate at a methanol concentration of 70%. Dibenzoyltartaric acid was eluted almost with the void volume, whereas the more hydrophobic di-*p*-toluoyltartaric acid was more distributed to the stationary phase and eluted later.

3.3. Influence of selector concentration, organic modifier and ionic strength

To analyze the effect of several parameters that may influence the chiral separation, screening with

Table 2
Influence of selector concentration on retention and enantioselectivity

Structure	Name	k'_1 [a]	$\alpha = k'_2/k'_1$
H_3C, OH	1-(1-naphthyl)ethanol	2.0[b] (2.3)[c]	1.06 (1.04)
OH, H_3C	1-(2-naphthyl)ethanol	1.6 (1.8)	>1.00 (>1.00)
H_3C, NH_2	1-(1-naphthyl)ethylamine	0.6 (0.7)	21.1 (22.5)
NH_2, H_3C	1-(2-naphthyl)ethylamine	1.1 (1.3)	1.99 (2.11)
H_3C, NH	N-benzyl-1-(1-naphthyl)ethylamine	1.0 (1.6)	1.05 (1.04)
H_2N, COOH	3-(1-naphthyl)alanine	4.3 (4.3)	3.77 (3.89)
H_2N, COOH	3-(2-naphthyl)alanine	3.5 (3.6)	3.92 (4.08)
COOH, O, H, O, H, COOH, O	2,3-dibenzoyl-tartaric acid	0.7 (0.8)	>1.00 (>1.00)
COOH, O, H, O, O, H, COOH, CH_3, H_3C	2,3-di-*p*-toluoyl-tartaric acid	4.7 (7.4)	>1.00 (>1.00)

[a] The first-eluting peak corresponded to the (*S*)-forms for 1-(1-naphthyl)ethylamine and 1-(2-naphthyl)ethylamine, while the (*R*)-enantiomer of *N*-benzyl-1-(1-naphthyl)ethylamine eluted first. For 3-(1-naphthyl)alanine, the first-eluting peak was the (L)-enantiomer.
[b] Enantioselectivity at 70% methanol, phosphate buffer pH 2.2 (ionic strength of 0.1) and 21 mg Lasalocid/l. (Mobile phase A, Table 1).
[c] The values shown in parentheses were obtained with 70% methanol, phosphate buffer pH 2.2 (ionic strength of 0.1) and 12 mg Lasalocid/l. (Mobile phase B, Table 1).

Table 3
Influence of ionic strength on retention and enantioselectivity

Structure	Name	k′1[a]	α
	1-(1-naphthyl)ethanol	6.2[b] (5.8)[c]	1.11 (1.11)
	1-(2-naphthyl)ethanol	5.8 (5.3)	1.07 (1.07)
	2,3-dibenzoyl-tartaric acid	4.0 (3.8)	1.10 (1.11)
	2,3-di-*p*-toluoyl-tartaric acid	16.5 (15.1)	1.09 (1.11)

[a]The first-eluting peak of the tartaric acids corresponds to the (−)-enantiomers.
[b]Enantioselectivity at 40% methanol, phosphate buffer pH 2.2 (ionic strength of 0.1) and 12 mg Lasalocid/l. (Mobile phase C, Table 1).
[c]The values shown in parentheses were obtained with 40% methanol, phosphate buffer pH 2.2 (ionic strength of 0.05) and 12 mg Lasalocid/l. (Mobile phase D, Table 1).

different mobile phases was performed. The parameters studied were the selector concentration, the methanol content and the ionic strength of the phosphate buffer.

There are several reasons for using low selector concentration in the mobile phase, when possible. The presence of a selector adds to the costs of the mobile phase and may be of disadvantage for the detection (e.g. having large molar absorptivities). Furthermore, the solubility of the selector in mobile phases of different eluting strengths has to be considered and that makes optimization more tedious. However, the selector has to be present in the mobile phase in order to obtain a stable coating of the achiral support. Preferably, the selector should be highly distributed to the support, even at low concentrations. In other words, if the distribution follows the Langmuir absorption isotherm, it is advantageous that the plateau of the isotherm is reached at low selector concentrations. Not having a completely covered packing material could mean that there is a simultaneous possibility of non-chiral interactions, which may completely ruin the enantioselectivity. In this study, two different concentrations of Lasalocid were compared (21 and 12 mg/l). As presented in Table 2, no clear trends in the retention times and selectivity factors for the solutes could be stated. Assuming a Langmuir adsorption isotherm, these results indicate that the plateau of the isotherm has been reached at both of the concentrations studied.

To retain some early eluting alcohols, the organic modifier content was decreased. Lowering the methanol content, without changing the total ionic strength, (mobile phases B and D, Table 1) gave approximately two-to-four times longer retention times (values in parentheses in Tables 2 and 3). Moreover, the enantioselectivity for 1-(1-naphthyl)ethanol, 2,3-dibenzoyltartaric acid and 2,3-di-*p*-toluoyltartaric acid were enhanced. The chiral interactions between the solutes and stationary phase were hence increased. Nevertheless, the mechanism for chiral recognition is complicated to elucidate,

since it is possible that solvent molecules also take part. Changing the methanol concentration may thus shift all interactions involved in the separation.

When preparing mobile phases it is convenient to mix the organic modifier with a certain amount of buffer solution (e.g. phosphate buffer, pH 2.2). However, using different proportions of the two will give different ionic strengths of the mobile phase. The ionic strength is known to influence the ionic and hydrophobic interactions between two molecules. Thus, in this study, the ionic strength of the buffer was varied between 0.05 and 0.1. As presented in Table 3, the ionic strength was found to have only a minor effect on the retention (5–10% increase using the higher buffer concentration) and no influence on stereoselectivity.

4. Conclusions

It has been shown that Lasalocid dynamically adsorbed onto porous graphitic carbon is a useful technique for chiral separations. Different enantiomers of amines, acids, amino acids and alcohols have been separated and an extraordinary separation factor was obtained for 1-(1-naphthyl)ethylamine. The concentration of organic modifier has been shown to be an important parameter for the enantioselectivity. However, no changes in enantiomeric selectivity were obtained when the ionic strength or selector concentration were modified.

Acknowledgements

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