Enantioseparations by subcritical fluid chromatography at cryogenic temperatures

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Abstract

Two chiral stationary phases, the Whelk-O 1 and its polysiloxane-borne analog, the polyWhelk-O, have been used to separate enantiomers using subcritical carbon dioxide at cryogenic temperatures. As the temperature is decreased, greater enantioselectivity and resolution is obtained, usually without significant loss in column efficiency. Indeed, an increase in chromatographic efficiency with the reduction of temperature has been observed in several instances. The enantiomers of several compounds that show low enantioselectivity at room temperature have been cleanly separated at temperatures as low as −47°C. Additionally, the enantiomers of atropisomers having low configurational stability at ambient temperature have been fully separated under similar cryogenic conditions. The ability to retain good chromatographic efficiency at low temperature is attributed to the rapid adsorption–desorption kinetics of this type of brush-type stationary phase. © 1997 Elsevier Science B.V.

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1. Introduction

The selector used in the Whelk-O 1 chiral stationary phase was originally designed to differentiate between the enantiomers of profens [1]. This chiral stationary phase (CSP) and its polysiloxane-based analog, the polyWhelk-O, have been used to separate the enantiomers of many different classes of compounds by high-performance liquid chromatography (HPLC) and by supercritical fluid chromatography (SFC) [2–7]. The chiral recognition processes responsible for many of these enantioseparations are relatively well understood. Consequently, for enantiomers in which a hydrogen bond acceptor and an aromatic moiety are near to and flanking the stereogenic center, one can often accurately anticipate when enantioseparation will be possible and in

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which order the enantiomers will elute from these CSPs. Although these CSPs are broadly applicable, they will not (and, indeed, no CSP will) separate the enantiomers of all chiral compounds.

When one encounters a difficultly in the resolvable analyte, one possible recourse is to reduce the temperature of the column [8]. Lowering column temperature almost always increases retention and enantioselectivity, whereas an increase in efficiency is rather unusual [9,10]. Accordingly, many CSPs lose chromatographic efficiency at subambient temperatures and suffer a loss in resolution despite the increase in enantioselectivity owing to slow adsorption–desorption kinetics. Such mass transfer characteristics seem to be more of a problem for CSPs which are either polymeric or which seem to function as "chiral cavities". Brush-type CSPs seem, in general, to have the better mass transfer characteristics.

The rapid mass transfer kinetics of the Whelk-O 1 CSP allow it to be used under HPLC conditions to separate the enantiomers of rapidly interconverting compounds at cryogenic temperatures [11,12]. This CSP has also been used in subcritical fluid chromatography (SubFC) at −10°C [6]. Owing to the reduced viscosity of subcritical carbon dioxide relative to that of most HPLC mobile phases, it seemed likely that both investigated CSPs would retain chromatographic efficiency at cryogenic temperatures when suitably modified carbon dioxide is used as a mobile phase. To test this hypothesis, several racemates, the enantiomers of which, at ambient temperature, either cannot be separated at all or which separate rather poorly, were chosen for this study. Initially studied were several secondary alcohols and ketones, compounds **1–8**, which show low enantioselectivities on these CSPs at room temperature. Subsequently studied were the enantiomers of several atropisomeric arylnaphthalenes, compounds **9–13**, that can not be separated at room temperature because the enantiomers are interconverting too rapidly.

2. Experimental

Chromatography was carried out on a Hewlett Packard 1050 supercritical fluid chromatograph equipped with an HP 1050 diode-array detector using the commercially available (3S,4R)-Whelk-O 1 and (3S,4R)-polyWhelk-O columns (250 mm×4.6 mm) as CSPs. The mobile phase consisted of carbon dioxide modified with different amounts of methanol. All chromatographic experiments were conducted at a nominal flow rate of 2.0 ml/min and a back pressure of 200 bar was employed. For cryogenic SubFC, the mobile phase was precooled by passage through a coil of 1/16″ stainless steel tubing immersed in the dry-ice–2-propanol bath which also contained the column. The column was equilibrated at the chosen temperature for 10 min. The temperature was measured with a calibrated thermocouple and held constant through the run.

3. Results and discussion
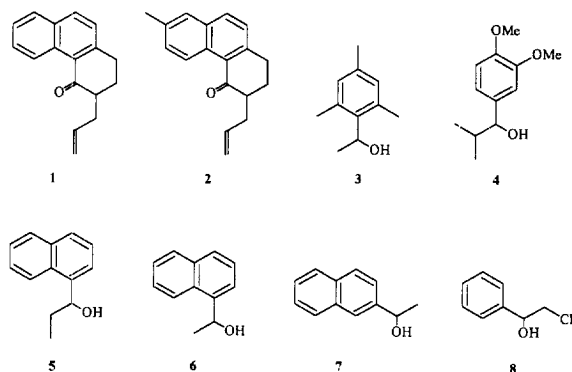
The alcohols and ketones selected to study the use

Fig. 1. Structure of the analytes **1–8**.

of the Whelk-O 1 and the polyWhelk-O in SubFC at cryogenic temperatures are depicted in Fig. 1.

None of the analyte enantiomers were fully separated at room temperature on either of the CSPs using different amounts of methanol as the modifier in SubFC. The influence of the temperature on the enantioseparation of **7** using the polyWhelk-O in SubFC is shown in Fig. 2.

As the temperature is reduced, enantioselectivity and resolution are significantly improved and the enantiomers are well separated at −41.0°C. Had the mobile phase not been sufficiently precooled to minimize radial and axial temperature gradients, these would have tended to cause peak broadening or distortion [13–15], the magnitude of these effects being greater at the lower temperatures. Cryogenic temperatures are not necessarily detrimental to column efficiency. Interestingly, for compounds **3**, **7**, and **8** column efficiency, i.e. the number of theoretical plates, reaches a local maximum at −23.0°C on the polyWhelk-O CSP. At −41.0°C, column efficiency is still better than at 0.0°C (Table 1).

Although the influence of the temperature on the efficiency is analyte-dependent, we obtained similar effects, i.e. an increase of enantioselectivity and resolution as well as relatively high chromatographic efficiency at low temperatures with all analytes studied. Accordingly, the enantiomers of analytes **1–8** were fully separated on the polyWhelk-O by SubFC under cryogenic conditions.

A decrease of the temperature of the mobile phase causes an increase in density and a decrease in diffusivity. This tends to slow the rate of mass transfer. On the other hand, the amount and relative
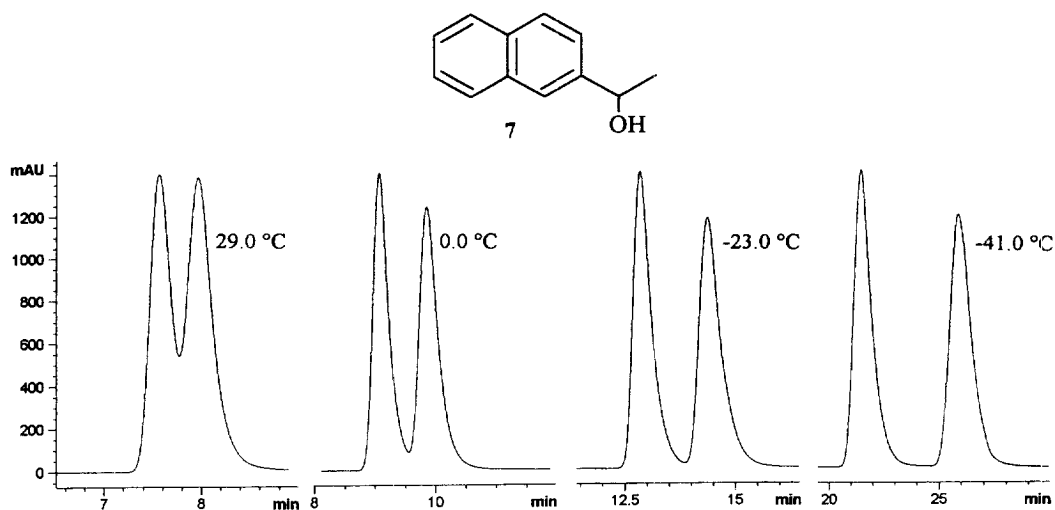
Fig. 2. Influence of the temperature on the enantioseparations of 7 on the polyWhelk-O CSP by SubFC. Operating conditions: see Table 1.

proportions of methanol–carbon dioxide mobile phase components "dissolved" in the CSP are expected to be temperature-dependent and might thus change the physical and chemical properties of the CSP [16–18]. It seems reasonable that greater solvation of the polyWhelk-O CSP at low temperatures would lead to swelling of the polysiloxane which would increase diffusivity in the stationary phase. These two opposing effects on column efficiency may be responsible for the maximization of efficiency which is sometimes observed at particular cryogenic temperatures.

As is shown for 3 in Figs. 3 and 4 we observed similar influences of temperature on enantioselectivity and resolution with the Whelk-O 1. For thermodynamic reasons, selectivity and retention increase steadily at reduced temperatures. Since increased solvation of the CSP might reduce the extent to which neighboring selectors interact with each other (or with residual silanol groups) this might also increase selectivity. This de facto increase in phase ratio could also increase retention. The significant increase in resolution at low temperatures results from increased selectivity and retention factors combined with relatively high chromatographic efficiency.

Resolution as a function of selectivity, efficiency and retention depends on the chemical and physical properties of the mobile and the stationary phase. Therefore, resolution changes with the nature and

amount of the modifier. Accordingly, the use of different amounts of the modifier, i.e. 2% methanol in carbon dioxide using the polyWhelk-O or 10% methanol using the Whelk-O 1, does not allow a detailed comparison of both CSPs. However, we found a similar influence of the temperature on the chromatographic performance of the Whelk-O 1 (Table 2).

Under the applied chromatographic conditions, the Whelk-O 1 provided less enantioselectivity but higher column efficiency. Using the same amount of modifier, all analytes were strikingly less retained on the polyWhelk-O. Especially at cryogenic temperatures, the polyWhelk-O proved to be superior in resolution and analysis time.

The enantiomers of a variety of stereolabile compounds have been separated by SubFC [7] and HPLC [11,12,19–24] at low temperatures. We used the polyWhelk-O to separate the enantiomers of axially chiral lignans (Fig. 5). As a consequence of their low energy barrier to rotation about the chiral axis, all atropisomeric lignans undergo rapid enantiomerization at room temperature and enantioseparations require cryogenic conditions [25]. Again, the polyWhelk-O provided sufficient selectivity and column efficiency to separate the enantiomers by cryogenic SubFC. We were able to separate the enantiomers of the configurationally most stable arylnaphthalene 9 at 0.0°C using 5% methanol as the modifier in carbon dioxide. Enantiomerization of the atropisomers 10–

Table 1
Chromatographic data for the enantioseparations of 1-8 on the polyWhelk-O at different temperatures

Entry	α	k_1	R_s	N
$T=29.0°C$				
1	1.04	3.63	/	/
2	1.03	6.14	/	/
3	1.21	0.63	1.41	6000
4	1.05	2.71	/	/
5	1.07	2.14	0.91	5700
6	1.07	2.51	0.92	5100
7	1.07	2.48	0.91	5500
8	1.07	2.44	0.85	4500
$T=0.0°C$				
1	1.09	5.33	1.32	4900
2	1.09	9.80	1.31	4500
3	1.32	0.79	2.35	5500
4	1.09	3.43	0.80	2300
5	1.12	3.45	1.60	4600
6	1.11	3.88	1.58	5900
7	1.11	3.69	1.03	2700
8	1.11	3.69	1.07	2500
$T=-23.0°C$				
1	1.15	7.47	2.01	4000
2	1.14	14.91	1.87	2900
3	1.42	1.02	4.01	6700
4	1.12	4.05	1.34	3200
5	1.15	5.62	2.08	4400
6	1.14	5.29	1.87	4300
7	1.14	5.17	1.86	4500
8	1.14	5.27	1.98	5100
$T=-41.0°C$				
1	1.22	11.31	2.68	3200
2	1.19	27.35	2.17	3300
3	1.58	1.41	5.07	5500
4	1.20	5.20	1.66	1900
5	1.27	9.66	2.93	3000
6	1.25	8.28	3.14	3600
7	1.23	8.85	2.88	3700
8	1.26	8.64	3.00	3700

α=enantioselectivity, k_1=retention factor of the less retained enantiomer, R_s=resolution, N=number of theoretical plates calculated for the less retained enantiomer, / =no data available. Operating conditions: Mobile phase: 2% methanol in carbon dioxide; flow rate: 2 ml/min; back pressure: 200 bar; UV detection at 254 nm.

12 is significantly faster and the enantioseparations require cryogenic temperatures, i.e. -29.5, -47.5 and $-42.0°C$ (Table 3). Owing to insufficient configurational stability or insufficient chiral recogni-
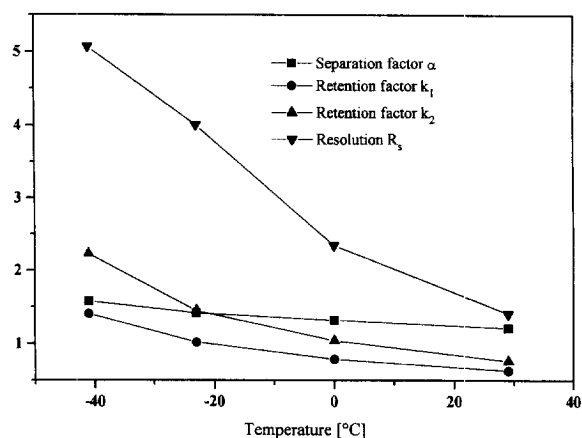

Fig. 3. Chromatographic data for the separation of the enantiomers of 3 using the polyWhelk-O in SubFC at different temperatures. Operating conditions: see Table 1.

tion, we were not able to resolve the enantiomers of 13 even at $-50.0°C$.

In as much as compounds 1-12 were successfully resolved into the enantiomers under cryogenic conditions, the chromatographic data presented in this study show that use of cryogenic temperatures in conjunction with SubFC can be helpful in optimizing enantioseparation conditions, provided the CSP exhibits sufficiently rapid mass transfer kinetics. While the kinetics of mass transfer are also analyte depen-
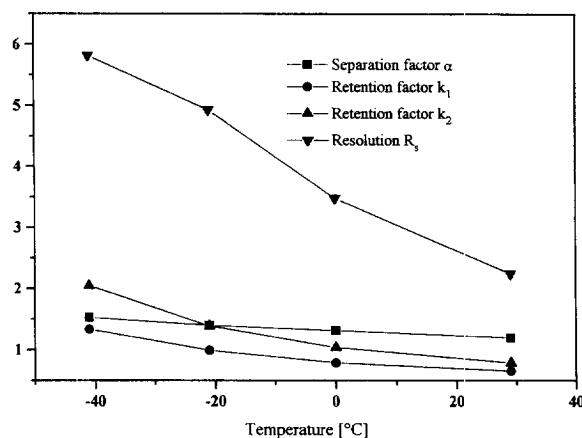

Fig. 4. Chromatographic data for the separation of the enantiomers of 3 using the Whelk-O 1 in SubFC at different temperatures. Operating conditions: see Table 2.

Table 2
Chromatographic data for the enantioseparations of 1–8 on the Whelk-O 1 at different temperatures

Entry	α	k_1	R_s	N
T=29.0°C				
1	1.00	3.70	/	/
2	1.02	5.62	/	/
3	1.20	0.66	2.25	12 000
4	1.05	1.80	/	/
5	1.05	2.12	0.82	14 000
6	1.03	2.17	/	/
7	1.04	2.14	/	/
8	1.03	2.12	/	/
T=0.0°C				
1	1.03	6.11	/	/
2	1.02	9.94	/	/
3	1.32	0.79	3.48	10 000
4	1.07	2.29	0.91	4900
5	1.08	3.22	1.67	15 000
6	1.05	3.00	0.92	13 000
7	1.05	3.01	0.59	5000
8	1.05	2.92	0.98	12 000
T=−21.0°C				
1	1.07	9.64	1.65	14 000
2	1.06	15.51	1.23	8000
3	1.40	0.99	4.93	11 000
4	1.09	2.96	0.95	3000
5	1.09	4.74	1.20	4000
6	1.06	4.32	0.80	4900
7	1.06	4.51	1.20	10 000
8	1.06	4.35	1.31	13 000
T=−41.0°C				
1	1.14	15.65	1.98	4000
2	1.12	31.22	1.79	4000
3	1.53	1.34	5.82	7800
4	1.12	3.94	1.56	4000
5	1.11	7.71	1.92	6900
6	1.08	6.73	1.45	7600
7	1.09	6.61	1.42	5900
8	1.08	6.87	1.44	7000

α=enantioselectivity, k_1=retention factor of the less retained enantiomer, R_s=resolution, N=number of theoretical plates calculated for the less retained enantiomer, / =no data available. Operating conditions: Mobile phase: 10% methanol in carbon dioxide; flow rate: 2 ml/min; back pressure: 200 bar; UV detection at 254 nm.

dent, the Whelk-O 1 and the polyWhelk-O CSPs are clearly capable of meeting the stationary phase's share of the kinetic requirements for high chromatographic efficiency at low temperatures.
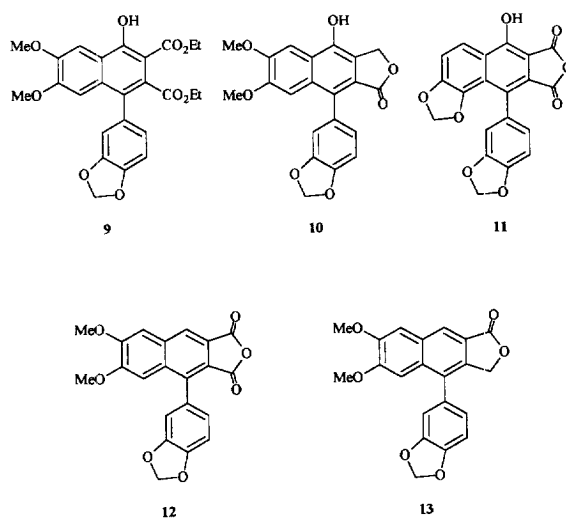

Fig. 5. Structure of the investigated arylnaphthalenes 9–13.

Table 3
SubFC enantioseparations of lignans 9–12 on the polyWhelk-O at different temperatures

Entry	*T*, % modifier	α	k_1	R_s
9	0.0°C, 5% MeOH	1.44	2.16	4.11
10	−29.5°C, 15% MeOH	1.30	7.30	2.29
11	−47.5°C, 3% MeOH	1.28	2.25	1.26
12	−42.0°C, 5% MeOH	1.26	2.74	1.26

Operating conditions: Mobile phase: carbon dioxide containing various amounts of methanol; flow rate: 2 ml/min; back pressure: 200 bar; UV detection at 254 nm.

Acknowledgements

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