Journal of Chromatography, 638 (1993) 85–96
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 807

Enantiomer separation by complexation gas and supercritical fluid chromatography on immobilized polysiloxane-bonded nickel(II) bis[(3-heptafluorobutanoyl)-10-methylene-(1*R*)-camphorate] (Chirasil-nickel)

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(First received September 16th, 1992; revised manuscript received December 10th, 1992)

ABSTRACT

The synthesis of a polysiloxane containing chemically bonded chiral metal complex derived from nickel(II) bis[(3-heptafluorobutanoyl)-10-methylene-(1*R*)-camphorate] (Chirasil-nickel) and its effective immobilization on the inner surface of fused-silica capillaries by thermal treatment is described. As expected, the immobilization properties were found to be dependent on the content of residual reactive Si–R groups in the polymer backbone (R = OCH_3, H). The immobilized Chirasil-nickel stationary phase was employed for the analytical enantiomer separation of coordinating solutes by high-resolution capillary gas and supercritical fluid chromatography. The increase in the relative retention monitored for racemic test solutes after rinsing the columns indicates a slight increase in the effective complex concentration which does not affect the chiral separation factor α. The temperature limit of analysis was raised to 170–180°C for temperature-programmed runs and to 140–150°C under isothermal conditions, thus extending the scope of *complexation gas chromatography* considerably, as demonstrated by various enantiomer separations performed at elevated temperatures. When Chirasil-nickel is immobilized on the inner surface of short, narrow-bore capillaries (50 μm I.D.), it can be employed in *complexation supercritical fluid chromatography*, combining the high solvation strength of supercritical carbon dioxide with the benefit of low operating temperatures, enhancing enantioselectivity.

INTRODUCTION

As a consequence of a steadily refined insight into the relationship between chirality and biological activity, the exact determination of enantiomeric compositions and absolute configurations is an important task not only in research concerned with the synthesis, characterization and use of chiral compounds but also for the evaluation of chiral biologically active compounds (*e.g.*, analysis of chiral pharmaceuticals, flavours, fragrants, pheromones, insecticides and food additives) [1–3]. It is now well appreciated that the separation of enantiomers on chiral, non-racemic stationary phases by capillary chromatography represents a powerful tool for quantitative enantiomer analyses because of the high resolution, speed and sensitivity.

Chiral recognition in chromatography may arise from various types of enantioselective interactions between the solute (selectand) and the chiral, non-racemic moiety (selector), *e.g.*, hydrogen bonding [4], charge transfer [5], dipole–dipole [6], inclusion [7,8] and combinations thereof [9]. *Complexation chromatography* offers a valuable complementary method based on coordination as the formation of the diastereo-

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0021-9673/93/$06.00

meric 1:1 complexes between the lone electron pairs of the selectand and the electronically and coordinatively unsaturated metal-containing selector is due to a rapid and reversible association equilibrium [10,11]. Useful selectors (which were termed Chira-metals) are bis(β-terpeneketoenolates) of manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) [12–14]. The unique molecular architectures displayed by terpeneketoenolates such as β-perfluoroacylated camphor, carvone, menthone and pulegone [15] provide a broad range in the variation of the enantioselectivity involved in the chiral recognition process.

Thus, racemic ethers, ketones, alcohols, acetals, esters and other classes of compounds have been quantitatively separated into enantiomers employing capillary glass of fused-silica columns coated with an 0.1 *m* (*m* = molal) solution of the chiral metal chelate {*e.g.*, nickel(II) bis[(3-heptafluorobutanoyl)-(1*R*)-camphorate] or other related Chira-metals} in an apolar liquid such as squalane or polysiloxanes [2]. As the maximum operation temperature of Chira-metal columns is limited to about 100°C (in some instances to 130°C for a short period of time) [16], it was desirable to decrease the volatility of the metal chelate by incorporating it in a polysiloxane matrix, thus combining the high enantioselectivity of Chira-metals with the thermostability and excellent coating properties of polysiloxanes reminiscent of Chirasil-Val [4], and related stationary phases [17–20], and Chirasil-Dex [21]. Finally, these chiral polysiloxanes can be cross-linked and/or immobilized on the inner surface of glass or fused-silica columns [19,20,22,23], resulting in non-extractable stationary phases containing chemically bonded chiral metal complexes (Chirasil-metal) suitable for on-column injection modes and for the use of mobile phases with higher density as in supercritical fluid chromatography (SFC) [24] and capillary electrophoresis (CE) [25].

In analogy with the total synthesis of Chirasil-Val [4,22] starting from monomers, and earlier attempt to prepare Chirasil-nickel by hydrolysis–equilibration of nickel(II) bis[(3-heptafluorobutanoyl)-(1*R*)-10-(dimethoxymethylsilyl)methylcamphorate] and diethoxymethylvinylsilane in the presence of trimethylsilanol led to a copolymeric stationary phase possessing clearly enhanced thermal stability [26]; however, attempts to cross-link or immobilize this phase by radical induction were not successful as small amounts of radical starters had an adverse effect on the metal chelate leading to a strong decrease in the observed chiral separation factors α [27].

In this paper, we describe a new synthetic pathway to Chirasil-nickel by a different approach through hydrosilylation of 3-heptafluorobutanoyl-(1*S*)-10-methylenecamphor to a preformed polymer containing Si–H functionalities and subsequent formation of the metal chelate which, after coating on the inner surface of fused-silica columns and thermal treatment, shows no loss in enantioselectivity and is stable against common organic solvents and even supercritical carbon dioxide at high densities [28].

EXPERIMENTAL

Materials

(1*S*)-10-Camphorsulphonic acid, polymethylhydrosiloxane, hexachloroplatinic acid and china clay were obtained from Aldrich (Steinheim, Germany). Octamethylcyclotetrasiloxane and hexamethyldisiloxane were obtained from ABCR (Karlsruhe, Germany). All solvents employed were of HPLC grade.

Instrumentation

Carlo Erba Fractovap 2350 and 2150 and Mega HRGC 5300 gas chromatographs (Fisons, Mainz, Germany), equipped with flame ionization detectors (250°C), were used. The carrier gas was nitrogen or preferentially helium (both 99.996%) (Messer-Griesheim, Frankfurt, Germany), used without further purification (caution: hydrogen should not be employed as the carrier gas in *complexation gas chromatography*). The splitting ratio at the injector (250°C) was set to 1:100. In order to avoid overloading conditions, the instruments were set to their highest sensitivity at a tolerable signal-to-noise ratio. For SFC measurements a Carlo Erba SFC3000 instrument (Fisons), equipped with a syringe pump, pneumatic injection valve and flame ioni-

zation detector was used. The splitting ratio was set to about 1:40 and the column flow-rate was regulated by a laboratory-made integral-type restrictor (0.5–0.6 cm/s at 10.0 MPa and 80°C, which is about $5u_{opt}$ under these conditions).

Synthesis of 3-heptafluorobutanoyl-(1S)-10-methylenecamphor (1)

Compound **1** was prepared according to the general procedure of McCreary *et al.* [29] by acylation of 12.1 g (74 mmol) of (1*S*)-10-methylenecamphor [30] with 11 ml (74 mmol) of heptafluorobutanoyl chloride. The crude product was purified by flash-chromatography on 62–200-μm silica [toluene–*n*-hexane (3:1, v/v)], which was extracted with 2,4-pentanedione before use in order to remove traces of iron. The slightly red oil obtained was distilled in a Kugelrohr to yield 10.3 g (38%) of 3-heptafluorobutanoyl-(1*S*)-10-methylenecamphor, b.p. 50–55°C/0.01 mmHg; α_D^{25} +14.4 (0.1 dm; neat); ^{1}H NMR ($CDCl_3$, 400 MHz), δ 11.75 and 2.90 (1H, tautomeric proton), 5.80–5.30 (m, 3H), 2.20–1.20 (m, 5H), 0.93 (s, 1H), 0.89 (s, 1H); mass spectrum, *m/z* 361 (16%), 360 (M^+, 100%), 317 (24%), 191 (19%), 163 (26%); 135 (29%), 108 (22%), 53 (24%).

Synthesis of the dimethylpolysiloxane containing 10% hydromethylsiloxane units (2)

In an atmosphere of nitrogen, 5.0 g (2 mmol polymer, 83 mmol Si–H) of polymethylhydrosiloxane (PMHS), 55.74 g (187 mmol) of octamethylcyclotetrasiloxane (D_4) and 3.45 g (21.3 mmol) of hexamethyldisiloxane (HMDSO) were mixed with 0.7 g of china clay by vigorous stirring, then 0.5 ml of concentrated sulphuric acid was added and the mixture was held at 100°C for 5 days. After cooling, the china clay was removed by suction of the diluted (150 ml of diethyl ether) reaction mixture through a frit. The solution was washed with water until neutral and the organic layer was separated and dried over anhydrous sodium sulphate, filtered and evaporated. The resulting polymer was held under vacuum (10^{-3} mmHg) at 120°C, yielding 52 g (81%) of a clear viscous fluid. The ^{1}H NMR spectrum indicated that the ratio of dimethylsiloxane to methylhydrosiloxane units was 10:1 (9% hydromethylsiloxane units) and titration [31] showed 1.2 mmol of Si–H per gram of prepolymer **2**.

Hydrosilylation of 1 with polysiloxane 2

A 3.0-g amount (1 mmol polymer, 3.6 mmol Si–H) of polysiloxane **2** and 1.3 g (3.6 mmol) of 3-heptafluorobutanoyl-(1*S*)-10-methylenecamphor (**1**) were dissolved in 75 ml of dry toluene in an atmosphere of nitrogen. A few droplets of a *ca.* 0.1% solution of hexachloroplatinic acid in dry tetrahydrofuran were added to the refluxing reaction mixture. After 24 h under reflux, the solvent was evaporated and the crude reaction mixture was heated with 25 ml of anhydrous methanol for about 3 h. The methanolic phase was decanted from the polymer, which was then washed several times with portions of anhydrous methanol. In this way 510 mg of **1** were recovered from the combined methanolic phases. Subsequent removal of low-molecular-mass compounds under vacuum (10^{-3} mmHg; 1 mmHg = 133.322 Pa) at 90°C yielded 3.1 g (72%) of a clear polysiloxane (**3**) with a viscosity similar to that of polymer **2**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.0–0.09 (m), 0.84 (s), 0.94 (s). The ratio of methyl groups in the polysiloxane to methyl groups of the ligand was found to be 20:1, indicating that about half of the Si–H present were consumed to bind the chiral ligand. IR (thin film, cm^{-1}), 2950, 2895, 1725, 1685, 1635, 1405, 1255, 1235, 1100–1010, 855, 790; $[\alpha]_D^{25}$ +22.2 (0.1 dm; $c = 5$, $CHCl_3$).

Preparation of Chirasil-nickel

A 2-g amount of the chiral polymer **3** was dissolved in 30 ml of *n*-heptane and a solution of 220 mg of nickel(II) acetate tetrahydrate (a *ca.* tenfold excess) in 20 ml of methanol was added under an atmosphere of nitrogen. The two-phase reaction mixture formed was vigorously stirred and heated at reflux for 30 min. The solvent was removed quantitatively under vacuum and 50 ml of *n*-pentane were added in order to separate the polymer from excess of nickel(II) acetate. Subsequent filtration and washing with several portions of water, followed by drying over anhydrous sodium sulphate and evaporation of the

solvent, yielded a dark-green polymer with increased viscosity. For removal of low-molecular-mass components Chirasil-nickel was dissolved in a small volume of dichloromethane (5 ml per gram of polymer) and precipitated with a 10–20-fold amount of anhydrous methanol. After three precipitation steps, which were accelerated by centrifugation, the residual solvent was removed at 90°C under vacuum (10^{-3} mm Hg), yielding 1.3 g (65%) Chirasil-nickel, which was stored under nitrogen. IR (thin film, cm^{-1}), 2950, 2895, 1725, 1645, 1635, 1525, 1405, 1255, 1235, 1150–1000, 855, 790; $[\alpha]_D^{25}$ +26.7 (0.1 dm; $c = 5$, $CHCL_3$); atomic absorption spectrometry (AAS) 10.463 mg of Ni per gram of polymer, corresponding to a complex concentration of 0.205 *m*.

Open-tubular columns

Fused-silica tubing of 0.05–0.25 mm I.D. manufactured by Chrompack (Middelburg, Netherlands) was used. Each column was dehydrated at 250°C for 5 h at a very low carrier gas (helium or hydrogen) flow-rate. A solution of Chirasil-nickel in *n*-pentane–dichloromethane (2:1, v/v) (or pure *n*-pentane or diethyl-ether) was prepared and the columns were coated by the static method with a film thickness of 0.25 μm. The coated columns were installed in a GC oven and conditioned by temperature programming at a rate of 1°C/min from 50 to 160°C and held at the latter temperature overnight.

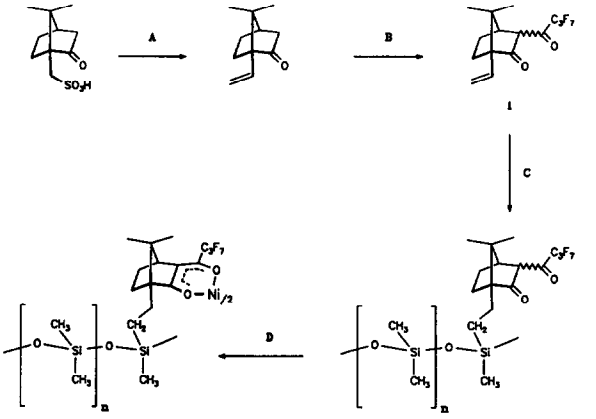
Immobilization

For thermal treatment, the flow-rate of the carrier gas (nitrogen or helium) was adjusted to *ca.* 30 bubbles per minute and the columns were maintained for 24 h at 180°C. When water vapour was added to the carrier gas, it was introduced by passing the carrier gas at room temperature through a vial with distilled water mounted upstream of the injection block [32]. After immobilization the columns were tested and then rinsed with 1 ml of methanol, 15 ml of methylene chloride (≥20 column volumes) and 5 ml of *n*-pentane. The degree of immobilization was calculated from the decrease in k' for *n*-tridecane (C_{13}), measured at 90°C before thermal treatment and after rinsing. The α values and relative retentions with respect to *n*-decane determined for 2-methyltetrahydrofuran, 2-methycyclohexanone and 1-phenylethanol at 90°C gave information about changes in enantioselectivity and in concentration of the chiral selector.

RESULTS AND DISCUSSION

Synthesis

As depicted in Fig. 1, the synthesis of Chirasil-metal in a "polymer-analogous rection" involves the introduction of unsaturation into the terpeneketoenolate ligand of the metal complex according to Matlin *et al.* [33]. We selected 3 - heptafluorobutanoyl-(1*S*)- 10 -methylenecamphor (note the formal change of the descriptor as compared with camphor owing to the priority change in the sequence rule of Cahn, Ingold and Prelog [34]) as a versatile ligand for the preparation of Chirasil-metal as it shows a broad range of enantioselectivity in complexation GC [2]. (1*S*)-10-Methylenecamphor prepared according to Fischer and Opitz [30] was perfluoroacylated according to McCreary *et al.* [29]. Subsequent hexachloroplatinic acid-catalysed hydrosilylation [35] of the unsaturated camphor ketoenolate **1**



A 1. $SOCl_2$, 2. CH_2N_2, 3. > T B 4. LDA, C_3F_7COCl
C 5. H_2PtCl_6, prepolymer 2 D 6. $Ni(OOCCH_3)_2 \times 4\ H_2O$

Fig. 1. Scheme of synthesis of Chirasil-nickel. >T = higher temperature; LDA = lithium diisopropylamide.

with dimethylpolysiloxane containing Si–H groups (**2**) yielded polysiloxane **3**. The polymeric metal complex was obtained in a vigorously stirred two-phase reaction using *n*-heptane as solvent for the chiral polymer **3** and methanol as solvent for the metal(II) acetate. An excess of the metal(II) acetate was used in order to mediate the deprotonation of the β-diketone to afford Chirasil-nickel in nearly quantitative yield [33].

The nickel content of the polymers was determined by atomic absorption spectrometry to be 1.05%, which, taking into account that one metal complex requires two ligands, corresponds to a molality of 0.205 *m* nickel complex (in this calculation the methylene groups of the ligand directly attached to silicon are assumed to be part of the polymer in order to obtain values comparable to Chira-metal stationary phases). The Chirasil-nickel polymer shows an absorption at $\lambda_{max} = 322$ nm in the ultraviolet spectrum characteristic of the $\pi-\pi^*$ transition of the metal–chelate carbonyl groups. In addition to the dominant absorption of the Si–O–Si group at 1000–1100 cm^{-1}, the infrared spectrum of Chirasil-nickel shows bands at 1635, 1645 and 1725 cm^{-1} characteristic of the ketoenolate structure in the metal complex. As it is expected for the paramagnetic nickel(II) ion, all signals of the ligand protons are severly broadened in the ^1H NMR spectrum, showing only two relatively sharp signals of methylsilyl (0–0.1 ppm) and methoxysilyl groups (3.45 ppm) from the polymeric backbone.

Immobilization

For employment in complexation GC, the fused-silica capillaries coated with Chirasil-nickel have to be conditioned at elevated temperature (160°C) overnight to remove strongly coordinating impurities or solvents such as water from the coordination sites of the metal ion. Immobilization and cross-linking of Chirasil-nickel are achieved by thermal treatment at temperatures above 170°C and reduced flow-rates of the carrier gas (nitrogen or helium; *not* hydrogen), which can be seen form Fig. 2, where the degree of immobilization *I*:

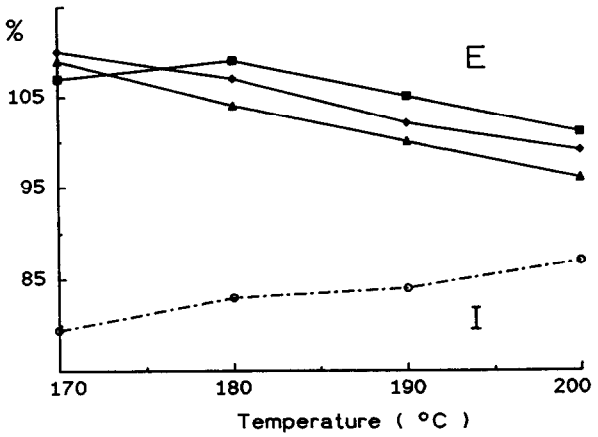


Fig. 2. Degree of immobilization *I* and enantioselectivity factor *E* *vs.* temperature for the thermal treatment (24 h) of Chirasil-nickel. Conditions: 5 m × 0.25 mm I.D. non-deactivated fused-silica columns (film thickness 0.3 μm); chromatographic measurements were performed at 90°C, 0.3 bar N_2. ■ = 2-Methyltetrahydrofuran; ▲ = 2-methylcyclohexanone; ◆ = 1-phenylethanol; ○ = *n*-tridecane.

$$I = 100[k'_{C_{13}}\text{ (after rinsing)}/\ k'_{C_{13}}\text{ (before immobilization)}]$$

and the changes in separation factor α for chiral test solutes as expressed by the enantioselectivity factor *E*:

$$E = 100\{[\alpha\text{(after rinsing)} - 1]/\ [\alpha\text{ (before immobilization)} - 1]\}$$

are shown at different applied temperatures. The low temperature (*ca.* 180°C) needed for the immobilization of Chirasil-nickel is due to the presence of residual reactive sites (SiH, $SiOCH_3$) in the polymetric backbone, well known [36] for the improvement of immobilization during the thermal treatment of polysiloxanes. These reactive sites seem to be mainly methoxysilyl groups as the absorptions of the residual Si–H (IR 2125 cm^{-1}, NMR 4.7 ppm) after hydrosilylation change nearly quantitatively to signals of $Si-OCH_3$ groups (3.45 ppm) when the polymer **3** is refluxed with anhydrous methanol in order to hydrolyse undesired silylenol ethers of the β-diketone.

Compared with former batches of Chirasil-nickel, which were prepared by hydrosilylation

of **1** with a dimethylpolysiloxane containing only 4% hydromethylsiloxane units and showed no appreciable immobilization at temperatures below 200°C, good immobilization properties can be achieved easily by increasing the Si–H content of the prepolymer, thus roughly tripling the number of residual reactive sites in the resulting Chirasil-nickel. The low temperature (*ca.* 180°C) now accessible for the immobilization step does not reduce the enantioselectivity of the chiral stationary phase. The washout procedure even improves the observed enantioselectivity of Chirasil-nickel to values of $E \geq 100\%$ (*cf.*, Fig. 2), which does not depend on the time (12–48 h) or the temperature (140–190°C) employed for conditioning of the columns.

Fig. 3 gives more detailed information about the changes in α, k_2' and relative retention [10]:

$$r_2 = t_2' - t'_{n\text{-decane}}$$

(the subscript 2 refers to the second-eluted enantiomer) when Chirasil-nickel is immobilized at 180°C. Two methods were employed, differing in the use of carrier gas saturated with water vapour (*cf.*, Fig. 3, open squares) for 12 h instead of only heating the columns for 24 h (*cf.*, Fig. 2, closed squares). In both methods the separation factors α do not show significant changes when measured before thermal treatment and after rinsing, but the capacity factors k_2' and relative retentions r_2 of the enantiomers tested increase with a simultaneous decrease in the retention of the *n*-alkane C_{13}. The alkane, whose retention is governed by the loss of stationary phase after rinsing and by any changes in the overall polarity during the immobilization procedure, indicates an increase in polarity during the thermal treatment of Chirasil-nickel in the absence of water. As the retention of the enantiomers, which is strongly influenced by the complex concentration due to the high chemical selectivity found in complexation GC [2], is found to decrease under the same conditions, this must be caused by blocking of the metal complex, *e.g.*, with polar by-products of the immobilization reaction. Rinsing of the column extracts the non-immobilized portions of the chiral stationary phase (15–20%), leading to a reduced retention of the alkane, whereas the k_2'

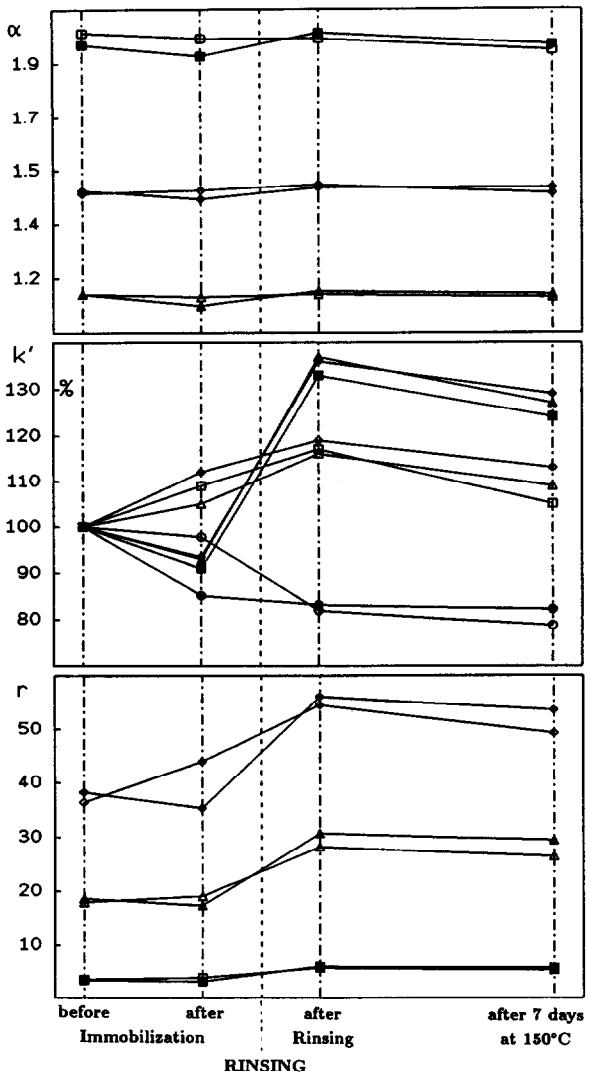


Fig. 3. Changes of α (top), k_2' (middle) and r_2 (bottom) during thermal treatment of Chirasil-nickel at 180°C without water vapour (■, 24 h) and in the presence of water vapour (□, 24 h); other symbols as in Fig. 2. Conditions: 10 m × 0.25 mm I.D. non-deactivated fused-silica column (film thickness 0.3 μm); chromatographic measurements were performed at 90°C, 0.5 bar N_2.

and r_2 values of the enantiomers tested are found to be increased to 130–180%. The increased effective concentration of the metal complex in immobilized Chirasil-nickel thus monitored is the result of both rinsing out mainly achiral parts of the polymeric stationary phase and possibly an increase in the numbers of non-blocked coordi-

nation sites caused by simultaneously washing out of strongly adsorbed molecules with low vapour pressure which may have been introduced as impurities of the solvents used for synthesis and purification/precipitation of the Chirasil-nickel.

It has been found previously that the addition of water vapour to the carrier gas enhances immobilization rates [32,37]. For Chirasil-nickel the same degree of immobilization was already reached in half the time in the presence of water vapour during the thermal treatment. There were no changes in the chiral separation factors α, but surprisingly the water added leads to an increase in the relative retention of the enantiomers during the immobilization procedure (*cf.*, Fig. 3). It is believed that complexed water molecules protect the complexation sites against other polar functionalities in the course of the immobilization reaction. When compared with the immobilization without water, the retention data were found to be always about 10% lower after rinsing of the columns treated with water vapour, indicating that part of the metal complex deteriorates during the contact with water at 180°C.

To summarize, the best conditions for the immobilization of Chirasil-nickel are thermal treatment for 24 h at 180°C with a very low flow-rate of the carrier gas. Although the addition of water vapour reduces the time needed for achieving the same immobilization rate of over 80% without a decrease in enantioselectivity, it leads to slightly lower relative retentions for

TABLE I

ENANTIOMER SEPARATION OF RACEMIC SOLUTES ON CHIRASIL-NICKEL

Solute	Temperature (°C)	Pressure (bar N_2)	k_1 [a]	α [b]	R_s [c]	N (plates/m) [d]
Methyloxirane	70	1.0	15.34	1.04	1.46	430
Ethyloxirane	90	1.0	7.76	1.04	1.38	831
tert.-Butyloxirane	90	1.0	3.29	1.22	6.18	570
(*E*)-*sec.*-Butyloxirane	90	1.0	8.43	1.10	3.27	865
(*Z*)-*sec.*-Butyloxirane			15.69	1.11	3.59	756
2,2-Dimethyl-3-phenyloxirane	75	1.5	22.15	1.07	1.93	680
2-Methyloxetane	130	1.0	5.02	1.29	10.02	915
2-Methyltetrahydrofuran	90	1.0	3.56	2.02	25.40	766
γ-Pentalactone	120	1.2	10.29	1.03	1.20	1027
δ-Hexalactone	140	0.9	10.92	1.02	1.10	1883
Camphor	130	1.0	7.49	1.12	4.59	1120
Fenchone	100	0.7	2.88	1.07	2.50	894
(*E*)-5-Methylhept-2-en-4-one	90	0.8	13.73	1.03	1.60	1427
(*E*)-4-Methylheptan-3-ol	110	0.7	2.47	1.04	1.54	1290
(*Z*)-4-Methylheptan-3-ol			2.64	1.04	1.54	1313
2-Methylhept-2-en-6-ol	120	1.0	6.73	1.10	4.50	1430
6-Methylheptan-2-ol	120	1.0	6.46	1.13	4.54	1099
Octan-2-ol	130	1.0	4.22	1.10	3.75	1190
1-Phenylethanol	130	1.0	3.39	1.21	7.65	1620
1-Phenylpropanol	130	1.0	4.22	1.07	2.92	1083
2-Octyl acetate	130	1.0	2.82	1.07	2.68	1111
1-Phenyl-1-propyl acetate	130	1.0	3.85	1.08	3.28	1123
1-Methyl-1-phenylbutyronitrile	165	0.8	6.56	1.03	1.53	1183
1-Cyanonorbornan-2-ol	160	1.5	14.95	1.07	2.06	763

[a] Capacity factor of the first-eluted enantiomer, measured on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm).

[b] Chiral separation factor.

[c] Resolution $R_s = 1.177\ (\Delta t_R / \Sigma\ w_{0.5})$.

[d] $N = [5.54(t_1'/w_{0.5})^2]L^{-1}$.

the tested enantiomers and was therefore abandoned. In addition, the immobilized Chirasil-nickel obtained by thermal treatment without additional water present exhibits much better long-term stability of all the chromatographic variables investigated. When conditioned after rinsing for 7 days at 150°C and a carrier gas flow-rate usually applied in chromatography (20–30 cm/s nitrogen), the decrease in the relative retention r_2 for the tested racemates was always smaller than 5%, whereas the enantioselectivity factor E decreased by 1–10%, depending on the r_2 value of the solute employed for testing (*cf.*, Fig. 3).

Complexation gas chromatography

Owing to their high chemical selectivity the use of chiral metal complexes for the separation of enantiomers is not that universal when compared with cyclodextrin selectors, but if the separation is successful, the observed enantioselectivity is in most instances higher than

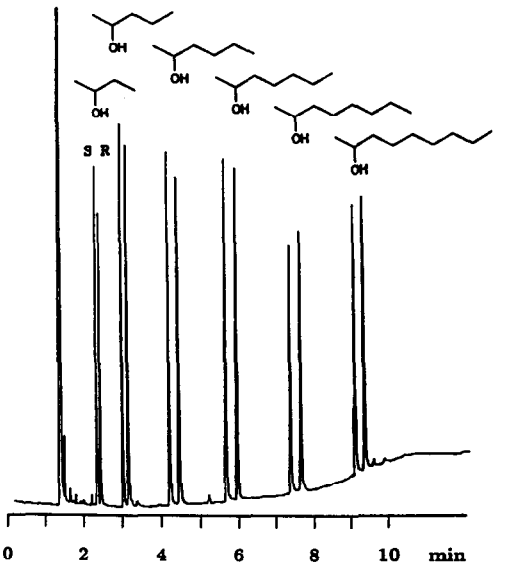


Fig. 5. GC enantiomer separation of homologous 2-alkanols on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: temperature programmed from 125°C to 165°C at 6.0°C/min after an isothermal period of 3 min at 125°C; 1.0 bar He.

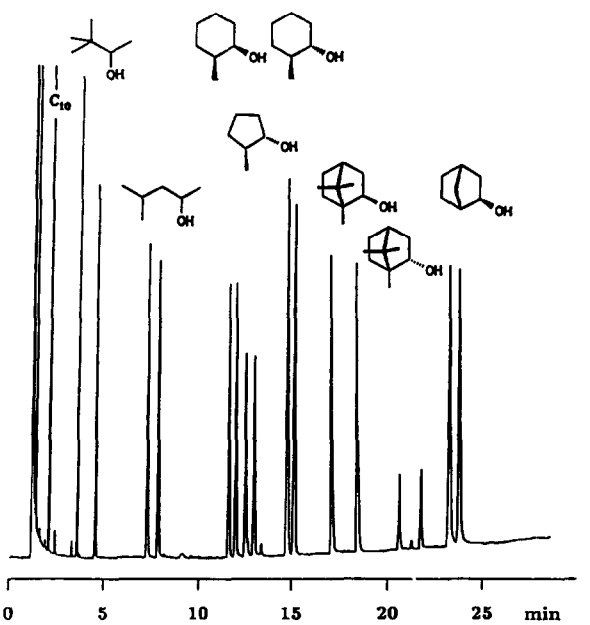


Fig. 4. GC enantiomer separation of acyclic, cyclic and bicyclic alcohols on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: temperature programmed from 100°C to 145°C at 2.0°C/min after an isothermal period of 3 min at 100°C; 1.3 bar He.

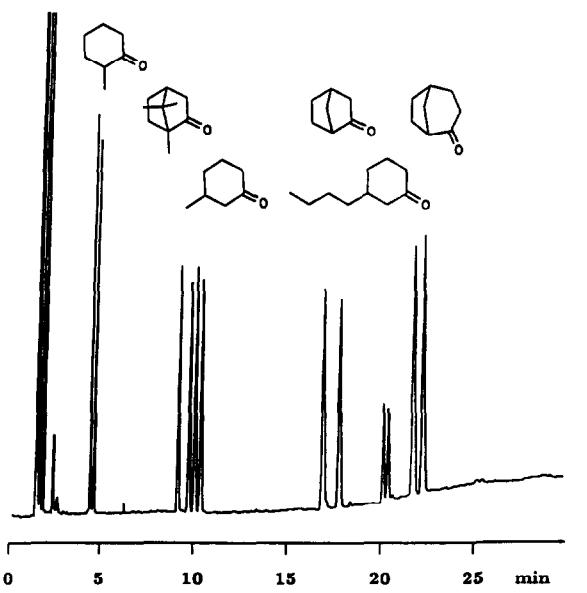


Fig. 6. GC enantiomer separation of cyclic and bicyclic ketones on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: temperature programmed from 130°C to 170°C at 2.0°C/min after an isothermal period of 5 min at 130°C; 1.0 bar N_2.

those found on cyclodextrin columns. Together with the ease of access to both enantiomeric forms of the chiral ligand in the metal complex, which allows the elution order of the separated enantiomers to be reversed, this is of great interest for the tandem-determination of the enantiomeric excess in highly enriched samples [16].

Representative gas chromatograms demonstrating the enantiomer separation of racemates belonging to different classes of compounds obtained on the immobilized Chirasil-nickel are shown in Figs. 4–8 (see also the data in Table I). Although the fused-silica columns were not deactivated prior to coating, cyclic and acyclic alcohols (Figs. 4 and 5) show only slight peak tailing, which is essentially a result of the high complexation strength of alcohols towards the nickel complex, revealing a good coating and separation efficiency. Cyclic and bicyclic ketones (*cf.*, Fig. 6) are separated quantitatively into the enantiomers. Even the enantiomer separation of γ-lactones (*cf.*, Fig. 7), which cannot be readily carried out on Chira-metals [3], is feasible on the Chirasil-nickel stationary phase. For

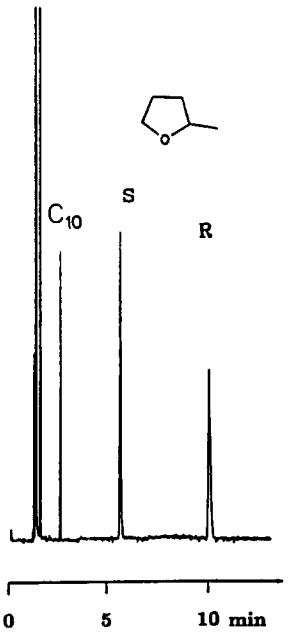


Fig. 8. GC enantiomer separation of 2-methyltetrahydrofuran on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: temperature isothermal at 90°C; 1.0 bar N_2.

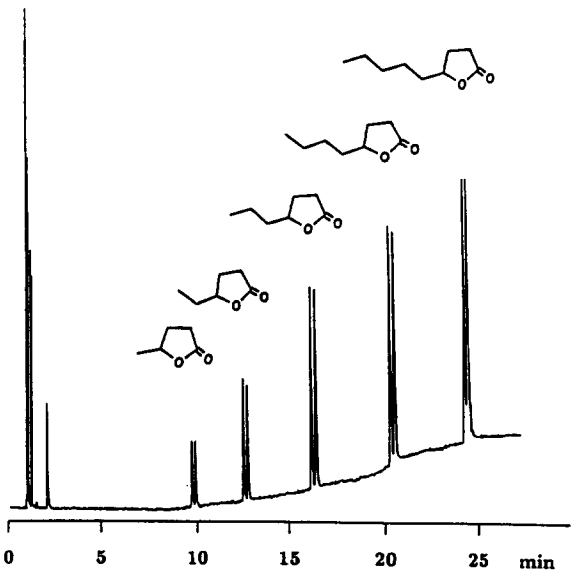


Fig. 7. GC enantiomer separation of homologous γ-lactones on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: temperature programmed from 115°C to 170°C at 2.0°C/min after an isothermal period of 3 min at 115°C; 2.0 bar He.

2-methyltetrahydrofuran a high separation factor ($\alpha = 2.02$ at 90°C; *cf.*, Fig. 8) was found. The configurationally labile enantiomers of homofuran show a temperature-dependent plateau between the resolved antipodes (enantiomerization) [38,39] during chromatography on Chirasil-nickel. The computer-simulated peak profile analysis [40] of chromatograms obtained between 95 and 130°C showed for the first time an example in complexation GC where the metal complex obviously exerted no catalytic effect of the interconversion of homofuran during chromatography as the rate constants found for mobile and stationary phase are very similar to the values obtained independently by polarimetry.

Complexation supercritical fluid chromatography

When coated on the inner surface of small-diameter fused-silica columns, Chirasil-nickel can be employed for *complexation SFC* using carbon dioxide as the mobile phase [28]. It is noteworthy that carbon dioxide, even in the

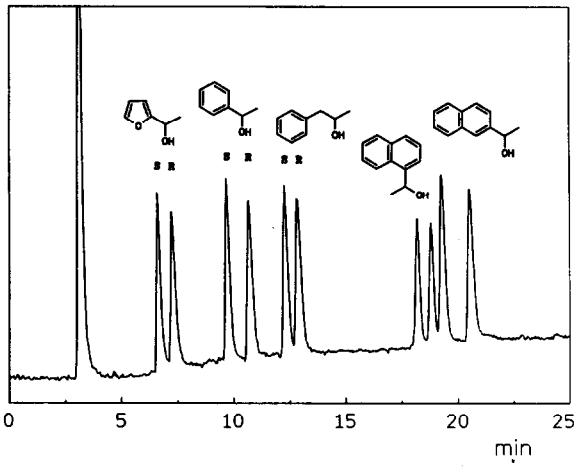


Fig. 9. SFC enantiomer separation of 1-(hetero)aryl functionalized ethanols on a 2.5 m × 0.05 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm). Conditions: CO_2, density programmed from 0.16 g/ml (9.60 MPa) at 0.015 g/ml · min to 0.46 g/ml (23.0 MPa) after an isothermal period of 5 min at 0.16 g/ml; temperature 120°C; flame ionization detection.

supercritical state, does not compete with solute molecules for coordination sites of the nickel ion. A high degree of immobilization is essential for these stationary phases in order to resist the solvation strength of supercritical carbon dioxide. A representative chromatogram is shown in Fig. 9, where the enantiomer separation of underivatized alcohols was achieved on a 2.5 m × 0.05 mm I.D. non-deactivated fused-silica column, coated with immobilized Chirasil-nickel.

Two features render capillary SFC more favourable than capillary GC for the separation of enantiomers. The low volatility of several compounds is not an obstacle in SFC owing to the strongly enhanced solvation power of supercritical mobile phases. As an example, racemic dimethyl 1,1′-binaphthyl-2,2′-dicarboxylate cannot be separated into enantiomers at the high temperature required for elution in complexation GC but can be quantitatively separated by complexation SFC (*cf.*, Fig. 10). More important, however, in the usual enthalpy-controlled domain of enantioselectivity, separation factors α

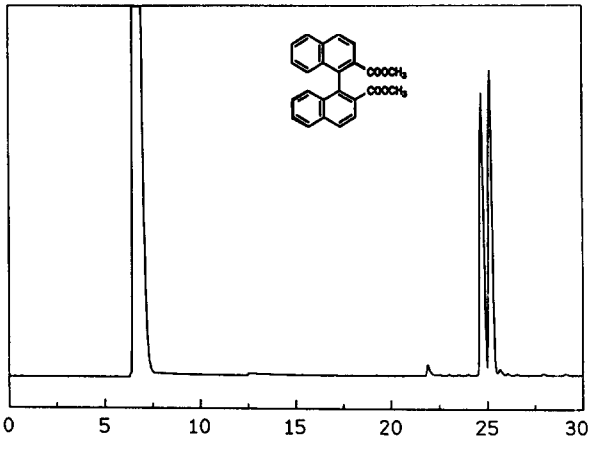


Fig. 10. SFC enantiomer separation of racemic dimethyl-1,1′-binaphthyl-2,2′-dicarboxylate on a 4 m × 0.05 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.40 μm). Conditions: CO_2 from 0.20 g/ml (9.30 MPa) at 0.02 g/ml · min to 0.6 g/ml (23.0 MPa) after an isothermal period of 10 min at 0.20 g/ml; temperature 70°C; flame ionization detection.

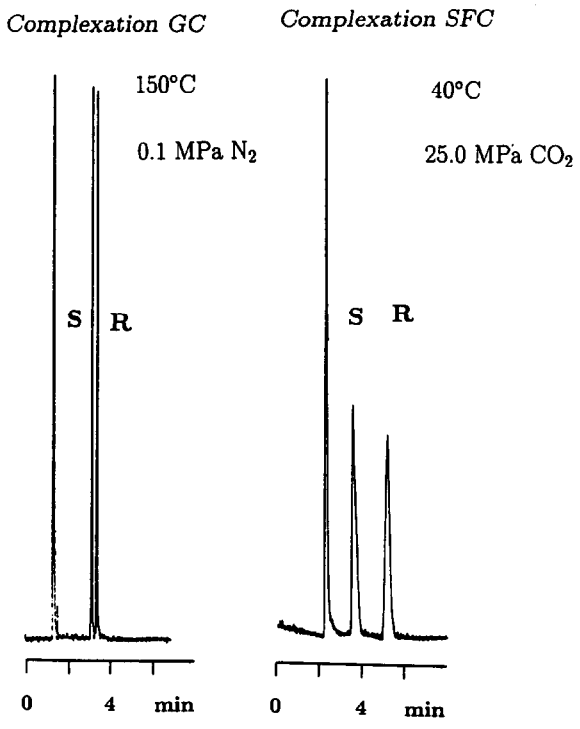


Fig. 11. Comparison of the enantiomer separation of 1-phenylethanol by complexation GC on a 25 m × 0.25 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel (film thickness 0.25 μm; temperature 150°C; 1 bar N_2) and complexation SFC on a 2 m × 0.05 mm I.D. non-deactivated fused-silica column coated with immobilized Chirasil-nickel [film thickness 0.25 μm; temperature 40°C; 0.883 g/ml (25.0 MPa)].

increase on decreasing the temperature of separation. Consequently, it must be the aim of chromatographic enantiomer separation to lower the analysis temperature in order to enhance enantioselectivity.

Fig. 11 shows the enantiomer separation of 1-phenylethanol in complexation GC and complexation SFC achieved under conditions characteristic for each method. When the resolution

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{k_2' + 1}\right)$$

and overall retention times in both methods are adjusted to comparable values ($R_s = 2.15$, being sufficient for the quantitative enantiomer separation), the low analysis temperature accessible in complexation SFC gives $\alpha = 2.38$ (40°C), compared with 1.13 in complexation GC at 150°C.

For rapid elution of the separated enantiomers in SFC, the density of the supercritical carbon dioxide can be increased to 0.883 g/ml [250 atm (1 atm = 101 325 Pa)]. Although SFC cannot compete with GC with regard to efficiency, it is remarkable that here for the first time is an example where the enantioselectivity observed in GC can be improved considerably under SFC conditions by the use of low analysis temperatures, high mobile phase densities and relatively short, narrow-bore capillaries.

This result, which has also been found for other solutes, is in contrast to the results found for Chirasil-Val [41] and Chirasil-Dex [42]. Both chiral stationary phases show a significant decrease in enantioselectivity when the density of supercritical carbon dioxide is increased, which was explained by interactions of carbon dioxide with the selectors. The absence of such interactions reducing enantioselectivity recommends complexation SFC not only for the chromatography of non-volatile chiral molecules but also for an improvement in the enantioselectivity observed in complexation GC. However, a limiting factor that has to be considered is the decrease in the solute diffusion coefficients, especially at low analysis temperatures and high densities of the carbon dioxide. The observed increase in the separation factor α may thus be adversely compensated for by a decrease in efficiency, notably at higher flow-rates of the supercritical mobile phase.

CONCLUSIONS

The use of immobilized Chirasil-nickel as enantioselective stationary phase greatly extends the scope of complexation GC and allows SFC to be employed for the analysis of enantiomers. The synthesis of related Chirasil-metals, *e.g.*, with manganese(II) or zinc(II) ions, carried out in the same manner, will further improve the applicability of this chiral stationary phase in complexation chromatography.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie.

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