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Journal of Chromatography A, 694 (1995) 119–128

JOURNAL OF CHROMATOGRAPHY A

# Unified enantioselective capillary chromatography on a Chirasil-DEX stationary phase Advantages of column miniaturization

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**Abstract**

Immobilized Chirasil-DEX (mono-6-O-octamethylenepermethyl-β-cyclodextrin chemically linked to dimethylpolysiloxane) can be employed as a versatile chiral stationary phase in chromatography. The chiral polymer has a long lifetime and is configurationally and thermally stable. The concept of unified enantioselective chromatography has been demonstrated for the enantiomer separation of hexobarbital by gas chromatography, supercritical fluid chromatography, liquid chromatography and capillary electrochromatography on a single open-tubular column (1 m × 50 μm I.D.) coated with Chirasil-DEX. The advantages of miniaturization in contemporary chromatographic enantiomer separation are demonstrated. Chirasil-DEX coated on porous silica is also useful for enantiomer separation in high-performance liquid chromatography.

## 1. Introduction

High-resolution enantiomer separation by chromatography has developed into an indispensable tool in contemporary chiral analysis. While the use of open-tubular columns is common in the GC and capillary electrochromatographic (CEC) modes, packed columns are used in the LC mode and both open-tubular columns and packed columns are employed in supercritical fluid chromatography (SFC). Based on both theoretical considerations and practical experiments, this paper explores the option of a unified approach, reminiscent of that pioneered in achiral separations by Ishii et al. [1], to enantioselective chromatography employing a single open-tubular column coated with a single chiral stationary phase. Attention is focused on miniaturization in modern chromatographic enantiomer analysis.

## 2. Experimental

### 2.1. Materials

Chirasil-DEX was prepared by hydrosilylation of permethylated mono-6-(oct-7-enyl)-β-cyclodextrin as described previously in detail [2]. The analytes were injected as methanolic solutions (ca. 0.1 mg/ml). HPLC-grade solvents (Merck, Darmstadt, Germany) were used. The buffers (20 m*M*), phosphate buffer (pH 7) or borate phosphate buffer (pH 7), were filtered through a

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0021-9673/95/$09.50 
*SSDI* 0021-9673(94)01075-7

0.45-μm pore-size filter (Macherey–Nagel, Düren, Germany).

### 2.2. Preparation of the open-tubular column

Fused-silica tubing (50 μm I.D., 360 μm O.D.; Chrompack International, Middleburg, The Netherlands) was heated at 260°C for 2 h at a low hydrogen flow-rate (0.1 bar inlet pressure). The column was coated without deactivation with a carefully filtered 2.0% solution of Chirasil-DEX in diethyl ether by the static method, yielding a film thickness of ca. 0.25 μm. Immobilization was carried out thermally for 20 h at 190°C in a very slow flow of hydrogen, as described previously [2]. The column was finally rinsed with dichloromethane. As before [3], immobilization was monitored by GC by measuring the retention factors ($k$) of $n$-dodecane and $n$-tridecane and the chiral separation factor ($\alpha$) of 1-phenylethanol. The column efficiency measured by GC was approximately 9000 plates/m for all three test solutes. The column length was 1 m. For UV detection, an optical window, located at a distance of 0.8 m from the injector, was prepared by burning off a section of about 3 mm of the polyimide outer coating.

### 2.3. Preparation of silica gel coated with immobilized Chirasil-DEX

A 4-g amount of porous silica (Nucleosil, 5 μm, 300 Å; Macherey–Nagel, Düren, Germany) was dried by azeotropic distillation with toluene. A slurry of this silica together with 1 g of Chirasil-DEX was prepared with 20 ml of dry dichloromethane in an ultrasonic bath. The solvent was slowly removed in a rotary evaporator, and immobilization was accomplished by heating the coated silica gel at 190°C under high vacuum (0.05 bar) for 20 h. In order to remove non-immobilized Chirasil-DEX, the product was washed with 200 ml each of methanol, dichloromethane and diethyl ether. The degree of immobilization was calculated from the C and H contents, as determined by elemental analysis before and after washing. It was found to be 70–85%, implying that this percentage of the original amount of Chirasil-DEX is deposited on the silica support.

### 2.4. Instrumentation

Carlo Erba VEGA and MEGA gas chromatographs (Fisons, Mainz, Germany), equipped with flame ionization detectors, were used. The carrier gas was hydrogen (99.999%) and the splitting ratio was 1:200. SFC was performed with a Carlo Erba SFC 3000 system as described previously [3]. The mobile phase was carbon dioxide (99.9995%) (Messer Griesheim, Düsseldorf, Germany). CEC was performed with a Kapillar-Elektrophorese System 100 (Grom, Herrenberg, Germany) and a Prince capillary electrophoresis system (Bischoff, Leonberg, Germany), both equipped with an on-column UV detector. A Shimadzu CR-6A Chromatopac integrator (Bischoff) was used for data acquisition. The sample was injected by the hydrostatic method (15 cm, 5s) or the pressure-driven method (40 mbar, 5 s) and detected at 220 nm. The coated column was conditioned with buffer for 1 h (30 kV) and was rinsed between each chromatographic run with water and methanol. Capillary LC was performed with the Prince system mentioned above. The analyte was injected by the pressure-driven method (40 mbar, 5 s) and detected at 220 nm. The operating pressure was 0.2 bar.

For HPLC a Chrompack (Middelburg, The Netherlands) instrument, equipped with a Chrompack Gras pump and a UV var detector, was used. A Rheodyne injection valve with a 20-μl sample loop was employed. Data acquisition was accomplished with a Chrompack control and integration system (PCI). HPLC columns (250 mm × 4.6 mm I.D.) were packed by the conventional slurry method. The column was first purged with methanol, methanol–water (1:1) and water, then conditioned with the operating buffer at a flow-rate of 0.5 ml/min until a stable baseline was observed.

## 3. Theoretical

The chromatographic behaviour of a solute eluted through a coated open-tubular column (Fig. 1) is governed by two principal migrations directed perpendicular to each other, i.e., (i) the

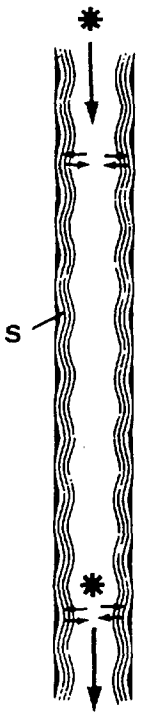


Fig. 1. Schematic view of a chromatographic process. * = Solute; S = stationary (liquid) phase; ↓ = axial migration; ⇄ = transverse migration.

axial movement carrying molecules from the column inlet to the outlet and (ii) the transversal movement necessary to distribute the molecules between the mobile and stationary phases. An important goal in open-tubular chromatography is the search for the highest efficiency as determined by the minimum height equivalent to a theoretical plate, $H_{min}$. Peak broadening caused by diffusion of the solute in the axial movement (i) can be minimized by increasing the average mobile phase velocity, $u$, and by decreasing the diffusion coefficient, $D_m$. Conversely, peak broadening caused by incomplete phase transfer in the transversal movement (ii) can be reduced by decreasing the average mobile phase velocity, $u$, and increasing the diffusion coefficient, $D_m$. Consequently, at a given $D_m$ of the solute, an optimum mobile phase velocity, $u_{opt}$, exists at which the open-tubular column performs at the highest efficiency with the minimum plate height, $H_{min}$. Thus, according to the simplified Golay equation (Eq. 1) [4], with the pressure gradient and diffusion in the stationary phase being neglected, the efficiency of an open-tubular column is governed by the opposing effects of an inverse ($\sim D_m/u$) and a linear ($\sim u/D_m$) relationship of $H$ vs. $u$ (and $1/D_m$).

$$H = \frac{B}{u} + Cu = \frac{2D_m}{u} + \frac{1 + 6k + 11k^2}{96(1 + k)^2} \cdot \frac{d_c^2}{D_m} \cdot u \quad (1)$$

$$u_{opt} = \sqrt{\frac{B}{C}} = \frac{8D_m(1 + k)}{d_c} \cdot \sqrt{\frac{3}{1 + 6k + 11k^2}} \quad (2)$$

$$H_{min} = 2\sqrt{BC} = \frac{d_c}{2(1 + k)} \cdot \sqrt{\frac{1 + 6k + 11k^2}{3}} \quad (3)$$

The mathematical form of Eq. 1 leads to the following conclusions:

(i) if $H$ is plotted against log $u$ (at a constant retention factor $k$), symmetrical curves with nearly identical parabolic shapes result for different chromatographic methods (LC, SFC, GC) (Fig. 2) [5];

(ii) the optimum mobile phase velocity, $u_{opt}$, and the minimum plate height, $H_{min}$, are derived by differentiation of Eq. 1, i.e., $\delta H/\delta u = 0$;

(iii) the optimum mobile phase velocity, $u_{opt}$, is determined by Eq. 2 [6] and depends at a given $k$ on the diffusion coefficient $D_m$ and the column inside diameter $d_c$; consequently, speed of analysis is fast in GC, medium in SFC and slow in LC on the same coated open-tubular column operated at the respective $u_{opt}$;

(iv) the highest efficiency, expressed by the minimum plate height, $H_{min}$, is determined by eq. 3 [6] and increases with decreasing column inside diameter $d_c$, irrespective of the nature of the mobile phase; significantly, $H_{min}$ is equal for a gas, a sub- or supercritical fluid or a liquid (also containing modifiers or present as a buffer system) used as the mobile phase at a given $k$.

In electrodriven systems, i.e., capillary electrophoresis (CE), the $H$ vs. log $u$ curve (Fig. 2, dotted curve) is shifted towards higher efficiency and a favourable optimum mobile phase velocity due to the flat flow profile, as opposed to the parabolic flow profile in pressure-driven systems, e.g., LC.

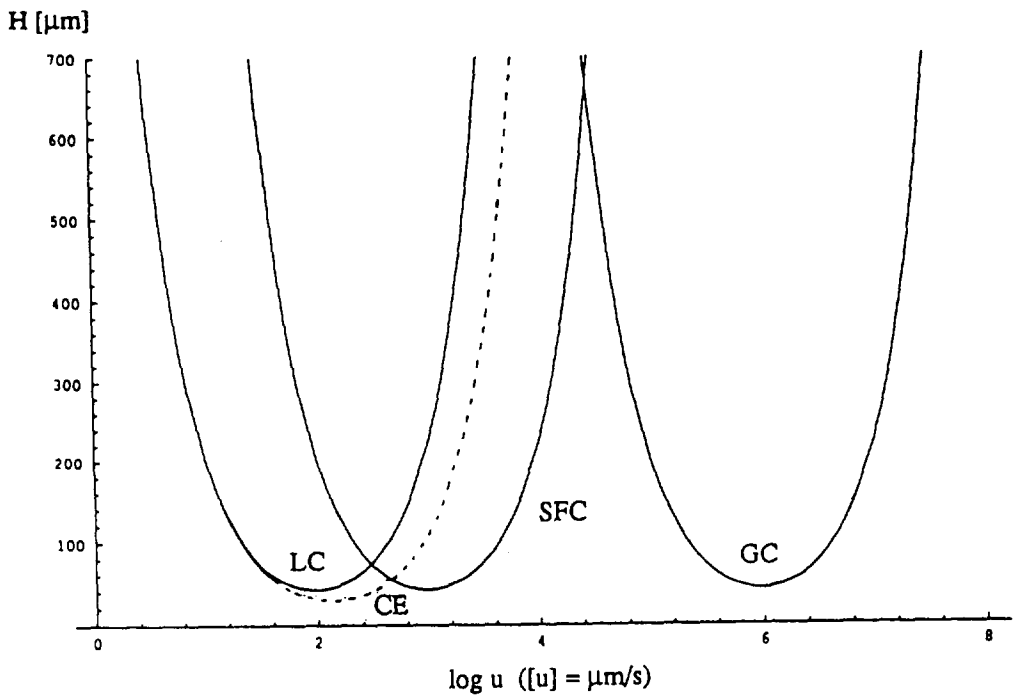


Fig. 2. Height equivalent to a theoretical plate, $H$, versus logarithm of mobile phase velocity, $u$, according to Eq. 1 for a 50 $\mu$m I.D. open-tubular column. $k = 5$; $D_m$ ($\mu m^2\ s^{-1}$) = $10^7$ (GC), $10^4$ (SFC), $10^3$ (LC, CEC); $C_{parabol} = (1 + 6k + 11k^2)/[96(1 + k)^2]$ {note that in CEC, $H_{min}$ and $u_{opt}$ are different owing to the nearly flat flow profile (dotted curve), $C_{flat} = k^2/[16(1 + k)^2]$}.

## 4. Results and discussion

### 4.1. Unified enantioselective open-tubular chromatography

A few years ago, Chirasil-DEX (mono-6-O-octamethylenepermethyl-$\beta$-cyclodextrin chemically linked to dimethylpolysiloxane; cf. Fig. 3) was synthesized and employed as an immobilizable chiral stationary phase for open-tubular GC [7,8]. Chirasil-DEX typically contains 24% (w/w) of permethylated $\beta$-cyclodextrin (i.e., 0.22 molal [2]) in polysiloxane and, thus, statistically one in sixty silicon atoms in the polymer chain carries a CD moiety. Later it was found that Chirasil-DEX is compatible with solvating mobile phases such as sub- and supercritical carbon dioxide at elevated temperature and high pressure (SFC) [3,8–10] and even with buffers over a wide pH range (CEC) [11–14]. Enantiomer separation on a chiral surface by CEC is espe-

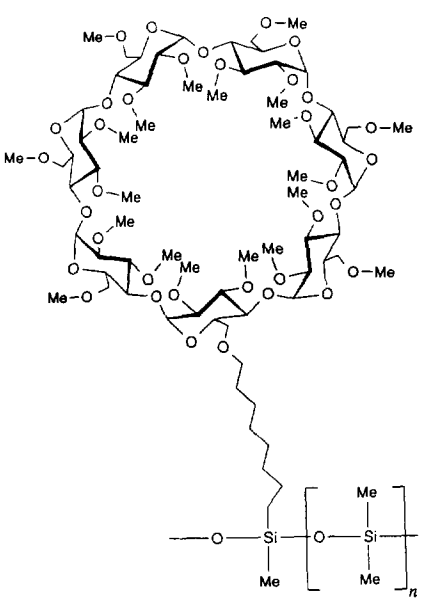


Fig. 3. Representation of Chirasil-DEX (mono-6-O-octamethylenepermethyl-$\beta$-cyclodextrin chemically linked to dimethylpolysiloxane).

cially noteworthy, while this approach was not immediately obvious [11].

Previously, the performance of open-tubular columns coated with Chirasil-DEX by SFC and CEC has always been cross-checked before and during measurements by installing the columns in a GC instrument and by monitoring the retention factors $k$ of test solutes. These investigations prompted us to generalize the principle of unified enantioselective open-tubular chromatography using one column coated with Chirasil-DEX for all important contemporary methods of enantiomer separation available, i.e., open-tubular GC, SFC, LC and CEC. The impetus of this approach arose from the prediction by Eq. 3 that the highest efficiency obtained on a given open-tubular column is independent of the nature of the mobile phase at a given $k$ (see above).

Whereas for SFC and CEC open-tubular column dimensions of 1 m × 50 μm I.D. have previously been used for practical reasons [2,3,8,12–14], larger inside diameters (250 μm) are common in GC [15] and smaller diameters (5–10 μm) are advocated in (achiral) open-tubular LC [16–21]. As a compromise, a 1 m × 50 μm I.D. fused-silica open-tubular column coated with Chirasil-DEX was used. As the result of the different diffusion coefficients $D_m$ in gases and liquids (cf. Fig. 2, caption) analysis times at optimum efficiency in open-tubular LC and CEC are slower by as much as four orders of magnitude than in GC (cf., Fig. 2), thus rendering a unified approach inpracticable in terms of analysis time because the ratio of the mobile phase velocity between GC and CEC is only about 250. However, according to Eqs. 1–3, $H_{min}$ and $u_{opt}$ depend also on the retention factor $k$. Fortunately, it has been observed that, while $k$ may be large in the GC mode [22] and intermediate in the SFC mode [2,3,8], very small values ($k < 1$) are often encountered in the CEC mode for the first-eluting peak [14]. Hence, if the retention factors $k$ observed in the various chromatographic methods involving Chirasil-DEX-coated open-tubular columns are taken into considera-

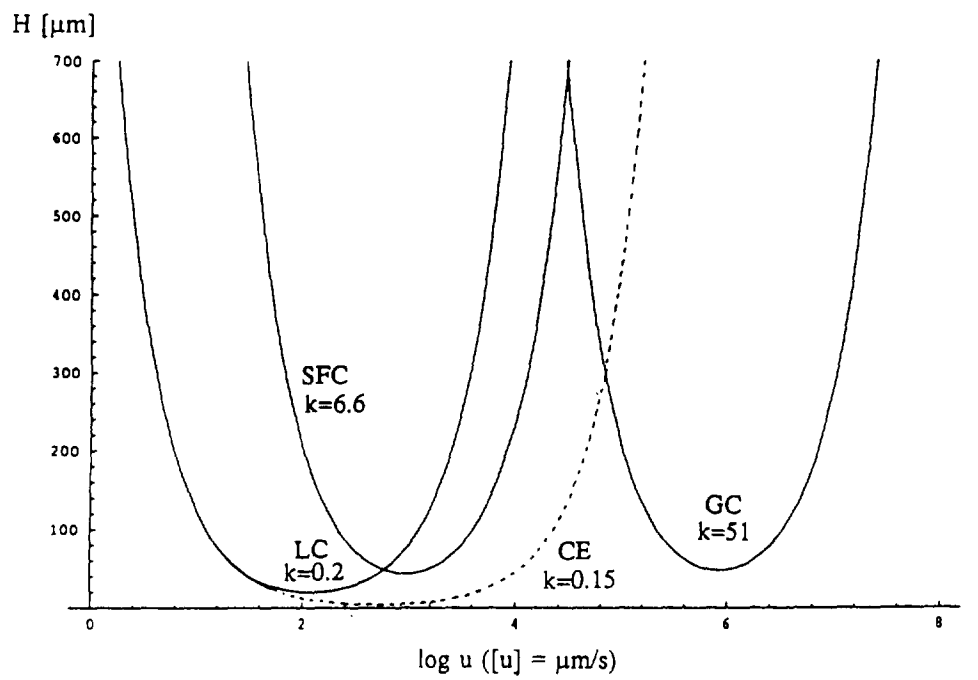


Fig. 4. Height equivalent to a theoretical plate, $H$, versus logarithm of mobile phase velocity, $u$, according to Eq. 1 for a 50 μm I.D. open-tubular column. $k$ Variable; $D_m$ ($\mu m^2 s^{-1}$) = $10^7$ (GC), $10^4$ (SFC), $10^3$ (LC, CEC); $C_{parabol} = (1 + 6k + 11k^2)/[96(1 + k)^2]$ {note that in CEC, $H_{min}$ and $u_{opt}$ are different owing to the nearly flat flow profile (dotted curve), $C_{flat} = k^2/[16(1 + k)^2]$}.

tion, $H$ vs. log $u$ curves as shown in Fig. 4 emerge which are compatible with the unified approach envisaged. For instance, with $k = 0.15$ in the CEC mode the $H$ vs. log $u$ curve becomes very flat and the efficiency is nearly independent of the mobile phase velocity over several orders of magnitude (cf., Fig. 4). In this case, at a flow rate $u$ in the range $u_{opt,CE} < u < u_{opt,GC}$, the GC and CEC curves even intersect with an efficiency reduced by a factor of six compared with $H_{min}$ (GC) (cf., Fig. 4). These considerations led us to probe the goal of unified enantioselective open-tubular chromatography under real experimental conditions. Here we demonstrate for the first time that, e.g., for the chiral solute hexobarbital a unified enantioselective approach is indeed feasible employing the same 1 m × 50 μm I.D. open-tubular column coated with immobilized Chirasil-DEX (film thickness 250 nm) by four independent methods, i.e., open-tubular GC, SFC, CEC and LC (Fig. 5) [23].

Inspection of Fig. 5 merits a number of comments. In the CEC mode the mobile phase is driven by the electroosmotic flow. As the coating of the column wall by the polysiloxane Chirasil-DEX lowers the availability of silanol groups, electroosmosis is reduced and the dead time $t_D$ is increased, rendering the analysis time rather long (20 min). For the sake of comparison, all other separations were performed with the same analysis time (GC, SFC), except in LC (10 min), because shorter analysis times led to unacceptable peak tailing for the second-eluted enantiomer. Apart from the long analysis time, CEC is superior to GC, SFC and LC with respect to all parameters important in enantiomer separation under the practical (non-optimized) conditions applied in Fig. 5:

chiral separation factor $\alpha$: CEC ≈ LC > SFC > GC (at the given temperatures);

peak resolution $R_S$: CEC > SFC ≈ GC > LC;

efficiency $N$ (first peak): CEC > LC > GC > SFC (at the observed retention factors $k$).

The enantiomer separation of hexobarbital by CEC [13,14] on Chirasil-DEX shows interesting features. Thus, $\alpha = 4$ (Fig. 5, right) is the highest value ever observed on Chirasil-DEX. The retention factor $k$ of the first-eluted enantiomer is indeed very low, e.g., $k = 0.15$, thus resulting in further improvements in both $H_{min}$ (efficiency) and $u_{opt}$ (speed of analysis) (Fig. 4). Thus, the first-eluted enantiomer displays a much higher efficiency than the second-eluted peak lying off

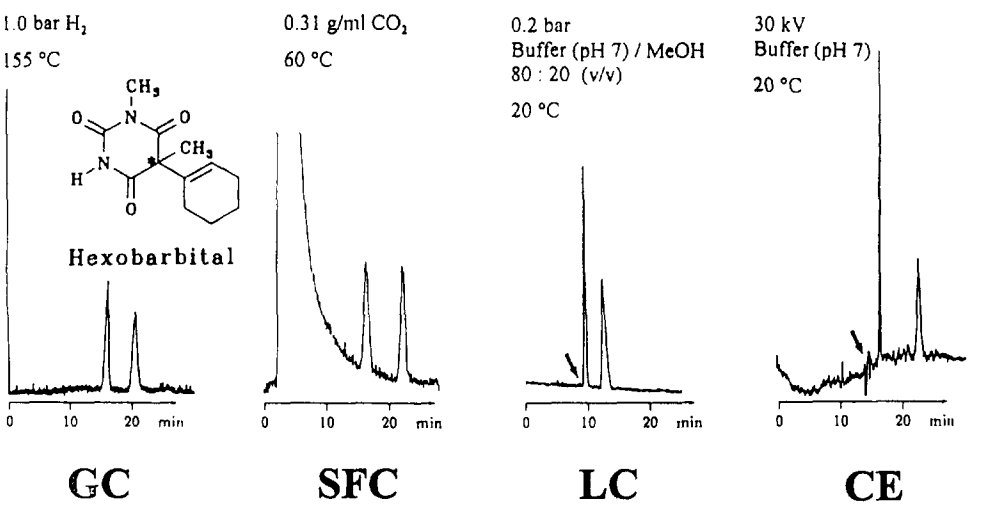


Fig. 5. Enantiomer separation of hexobarbital on a 1 m × 50 μm I.D. fused-silica column coated with Chirasil-DEX (film thickness 250 nm) by GC, SFC, LC and CEC in ca. 20 min. Effective column length in LC and CEC, 85 cm. Buffer, borate–phosphate (pH 7) (the arrow indicates the dead volume).

the $H$ vs. log $u$ optimum in both CEC and LC. When comparing GC and SFC, the gain in enantioselectivity on lowering the separation temperature in SFC is small. As is readily seen from Fig. 4, the unified approach towards enantioselective chromatography is feasible because of the favourable differences in the dead times $t_D$ and retention factors $k$ observed in the four chromatographic methods. In GC, $t_D$ is small and $k$ is large, and therefore the analyte spends most of its time in the stationary phase. In contrast in CEC and LC, $t_D$ is large and $k$ (notably for the first-eluting enantiomer) is very small, and therefore, the analyte spends most of its time in the mobile phase. Overall, these differences lead to similar retention times with acceptable efficiency characteristics. We consider the unified approach described here to be rather a unique than universal approach as it relies on a high separation factor $\alpha$ for the racemic mixture to be resolved.

Hitherto, the unified approach demonstrated here used the same open-tubular column coated with Chirasil-DEX in different chromatographic modes. In the spirit of unified chromatographic approaches advanced by Ishii et al. [1], further endeavours should be directed towards enantioselective separations also by using unified equipment. Thus, enantiomer separation by GC, subcritical-FC, SFC and LC may in principle be carried out with carbon dioxide in the same instrument with continuous transitions between the chromatographic modes by varying the temperature and pressure. CEC and LC have already been performed in this work by using a unified capillary electrophoretic equipment employing an injection system amenable to generating a voltage and pressure gradient. The expected decrease in efficiency due to the change from a flat to a parabolic flow profile when switching from CEC to LC is readily apparent by inspection of Fig. 4. Both electrically and pressure-driven methods may in principle be used in combination. The rapid evolution of instrumental design for small open-tubular column operation and on-column detection is likely to stimulate open-tubular LC investigations for both chiral and achiral separations. The use of open-

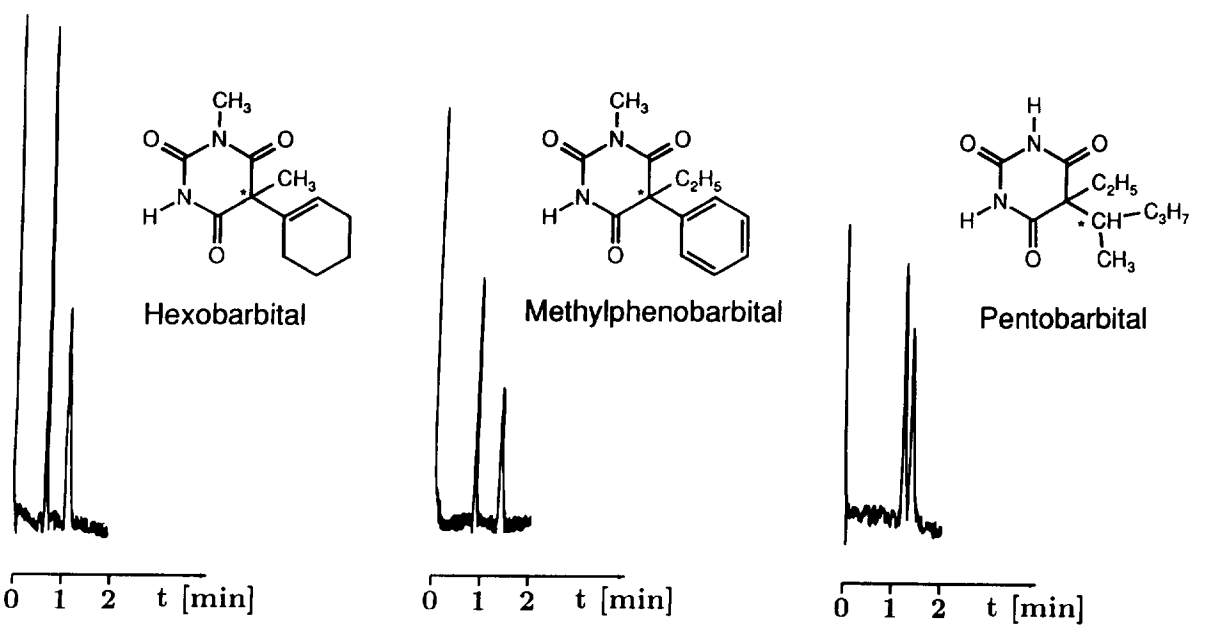


Fig. 6. Rapid enantiomer separation of barbiturates on a 35 cm × 50 μm I.D. fused-silica column coated with Chirasil-DEX (film thickness 60 nm) by GC at 130°C and 1 bar hydrogen.

tubular LC in enantiomer separation demonstrated here for the first time has the following advantages: low flow-rates facilitating coupling techniques (LC–MS); high column permeability and low pressure gradients; highly reduced amounts of chiral stationary phase and mobile phase; low heat capacity facilitating temperature programming; and increased mass sensitivity with concentration-dependent detectors [24].

Supported by theoretical considerations [25,26], it is to be expected that also enantiomer separations by CEC and LC using capillaries coated with Chirasil-DEX will strongly benefit from smaller column diameters. Further investigations are in progress.

### 4.2. Column miniaturization in enantioselective GC

The present investigations led also to the conclusion that column miniaturization is of considerable importance in enantiomer separations by GC. Traditionally, separations have been performed with long (10–25 m) and medium-bore (250 μm I.D.) open-tubular columns, although achiral separations on high-speed narrow-bore open-tubular columns were carried out as early as in 1962 by Desty et al. [27], who resolved fifteen components in few seconds on a 1.2 × 34.5 μm I.D. column. In general, a gain in analysis time [28] by a factor of nine is estimated when changing from a 320 to a 50 μm I.D. column [29]. By using short columns, high inlet pressures are not required. Surprisingly, only a few investigations have demonstrated hitherto the advantages of using short and narrow-bore open-tubular columns for fast enantiomer separations [7,30]. While the advantage of miniaturization in the GC mode is already evident in Fig. 5 (left), further decreases in column length and film thickness of Chirasil-DEX allow enantiomer separations in less than 100 s for,

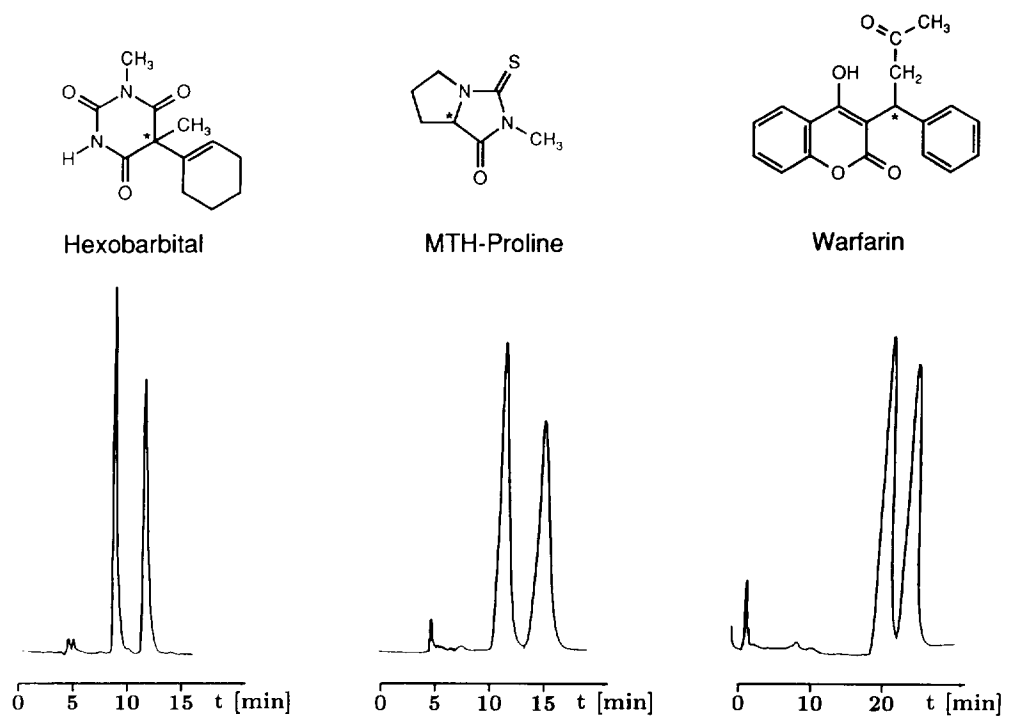


Fig. 7. Separation of enantiomers on a 250 × 4.6 mm I.D. column containing immobilized Chirasil-DEX coated on Nucleosil (300 Å, 5 μm) silica by HPLC. Left, 0.4 ml/min methanol–phosphate buffer (50:50), 240 nm; others, 1 ml/min methanol–phosphate buffer (30:70), 220 nm.

e.g., barbiturates (Fig. 6). The decrease in sample capacity (injection) is partially compensated for by a strong increase in the signal-to-noise ratio via enhanced detection sensitivity resulting from short analysis times leading to very narrow peak widths. Short, narrow-bore columns permit low elution temperatures, which in turn leads to higher separation factors $\alpha$. One disadvantage is the small sample capacity and the tendency towards overloading phenomena in narrow-bore columns [31].

### *4.3. Enantiomer separation on Chirasil-DEX-coated silica by HPLC*

In addition to its use as a versatile stationary phase for open-tubular column chromatography, we have also employed Chirasil-DEX in HPLC by coating it on porous silica. As shown in Fig. 7, this new chiral stationary phase allows the enantiomer separation of several barbiturates by reversed-phase HPLC. The coating of the surface with a non-polar film of Chirasil-DEX represents an interesting alternative to the usual method of direct chemical bonding of cyclodextrins to silica [32–34] and subsequent end-capping of the polar sites on the support. Further systematic investigations, e.g., the role of effective blocking of polar sites at the support and thus reducing mixed retention mechanisms [35] by Chirasil-DEX, are in progress.

## Acknowledgements

Support of this work by the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie is gratefully acknowledged.

## References

[1] D. Ishii, T. Niwa, K. Ohta and T. Takeuchi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 800.
[2] M. Jung and V. Schurig, *J. Microcol. Sep.*, 5 (1993) 11.
[3] M. Jung and V. Schurig, *J. High Resolut. Chromatogr.*, 16 (1993) 215.
[4] M.J.E. Golay, in D. H. Desty (Editor), *Gas Chromatography*, Butterworths, London, 1958, p. 36.
[5] T.L. Chester, in F.J. Yang (Editor), *Microbore Column Chromatography, a Unified Approach to Chromatography*, Marcel Dekker, New York, 1989, p. 369.
[6] G. Guiochon and C.L. Guillemin, *Quantitative Gas Chromatography (Journal of Chromatography Library*, Vol. 42), Elsevier, Amsterdam, 1988, 105.
[7] V. Schurig, D. Schmalzing, U. Mühleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot and J.C. Buyten, *J. High Resolut. Chromatogr.*, 13 (1990) 713.
[8] V. Schurig, D. Schmalzing and M. Schleimer, *Angew, Chem., Int. Ed. Engl.*, 30 (1991) 987.
[9] V. Schurig, Z. Juvancz, G.J. Nicholson and D. Schmalzing, *J. High Resolut. Chromatogr.*, 14 (1991) 58.
[10] D. Schmalzing, G.J. Nicholson, M. Jung and V. Schurig, *J. Microcol. Sep.*, 4 (1992) 23.
[11] S. Mayer and V. Schurig, *J. High Resolut. Chromatogr.*, 15 (1992) 129.
[12] S. Mayer and V. Schurig, *J. Liq. Chromatogr.*, 16 (1993) 915.
[13] S. Mayer, M. Schleimer and V. Schurig, *J. Microcol. Sep.*, 6 (1994) 43.
[14] S. Mayer and V. Schurig, *Electrophoresis*, 15 (1994) 835.
[15] V. Schurig, *J. Chromatogr. A*, 666 (1994) 111.
[16] D. Ishii et al., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 371.
[17] T. Tsuda and G. Nakagawa, *J. Chromatogr.*, 268 (1983) 369.
[18] H. Engelhardt and B. Lillig, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 531.
[19] O. van Berkel, H. Poppe and J.C. Kraak, *Chromatographia*, 24 (1987) 739.
[20] S. Folestad, B. Josefsson and M. Larsson, *J. Chromatogr.*, 391 (1987) 347.
[21] K. Jinno, *Chromatographia*, 25 (1988) 1004.
[22] D. Schmalzing, M. Jung, S. Mayer, J. Rickert and V. Schurig, *J. High Resolut. Chromatogr.*, 16 (1993) 215.
[23] V. Schurig, M. Jung, S. Mayer, S. Negura, H. Grosenick, M. Fluck and A. Glausch, *Analytica 94, Munich, 19–22 April 1994*, abstracts, p. 52.
[24] M. Goto, T. Takeuchi and D. Ishii, *Adv. Chromatogr.*, 30 (1989) 167.
[25] G. Guiochon and H. Colin, in P. Kucera (Editor), *Micro Column High Performance Liquid Chromatography*, Elsevier, Amsterdam, 1984.
[26] J. Vindevogel and P. Sandra, *Electrophoresis*, 15 (1994) 842.
[27] D.H. Desty, A. Goldup and W.T. Swanton, in N. Brenner, et al. (Editors), *Gas Chromatography*, Academic Press, New York, 1962, p. 105.
[28] P. Sandra, *Analusis Mag.* 20 (1992) M27.
[29] A. van Es, in W. Bertsch, H. Frank, W.G. Jennings and P. Sandra (Editors), *Chromatographic Methods*, Hüthig, Heidelberg, 1992.
[30] M. Lindström, *J. High Resolut. Chromatogr.*, 14 (1991) 765.

[31] Z. Juvancz, K. Grolimund and V. Schurig, *J. High Resolut. Chromatogr.*, 16 (1993) 140.
[32] A. Berthod, S. Chang and D.W. Armstrong, *Anal. Chem.*, 64 (1992) 395.
[33] K. Cabrera, D. Lubda and G. Jung, in V. Schurig (Editor), *3rd International Symposium on Chiral Discrimination, Tübingen, 1992*, book of abstractsf, 1992, p. 16.
[34] H. Riering, P. Wollenweber, M. Sieber and A. Neus, in W. Günther, V. Hempel and G. Wulff (Editors), *Instrumentalized Analytical Chemistry and Computer Technology, 1994*, InComBureau, Düsseldorf, 1994, p. 307.
[35] M. Schleimer and W.H. Pirkle, in V. Schurig (Editor), *3rd International Symposium on Chiral Discrimination, Tübingen, 1992*, book of abstracts, 1992, p. 73.