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# Coupled column chromatography in chiral separations

# I. Enantiomeric separation on swollen microcrystalline cellulose triacetate columns after a preseparation on a non-chiral alkylsilica column[a]

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## ABSTRACT

A two-dimensional chromatographic system was evaluated that combines a column packed with swollen crystalline cellulose triacetate for optical resolution of enantiomers with a non-chiral column for the preseparation of these enantiomers from other analytes or impurities. This two-dimensional approach greatly enhances the low peak capacities usually obtained with swollen microcrystalline cellulose triacetate columns. In this way determination of enantiomers can be achieved also in more complex samples and the significance of the purity determination of the enantiomeric peaks can be improved. The coupled column system was evaluated with respect to the compatability of the mobile phases and the possibilities of achieving peak compression at the top of the second column.

## INTRODUCTION

In the last 10 years, the separation of enantiomers has attracted great interest for a wide spectrum of compounds. Chiral stationary phases based on cellulose derivatives have been applied for this purpose in many instances[1–3]. Microcrystalline cellulose triacetate (CTA) and cellulose tribenzoate (CTB) can be used directly in a swollen state as packing materials with small particle sizes (down to 7–10 $\mu$m) in high-performance liquid chromatographic (HPLC) columns[4–7]. These adsorbents provide high enantioselectivity for a great variety of substances, including those with only one polar functional group. This feature and a surprisingly high loadability without loss of performance are the main advantages of these materials[5,6,8,9]. Their main drawback, however, is the very large peak dispersion for most types of analytes.

[a] Dedicated to Professor J.F.K. Huber on the occasion of his 65th birthday.

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Recent papers[8,9] discussed certain stategies for improving the efficiency of chiral separations by means of swollen crystalline cellulose triacetate (swcrCTA) packings. In spite of significant improvements in peak dispersion on optimizing the chromatographic conditions, the plate height values still remain high for many types of substances. The resulting lack of theoretical plates can be overcome in many instances by the high enantioselectivity obtainable by swcrCTA adsorbents to give good enantiomeric resolution. The low peak capacity of a swcrCTA column, however, which results from the low plate number, remains a problem. A low peak capacity implies that one has only a small chance of finding separation conditions where the enantiomers will be sufficiently separated from each other and from other compounds present. Even with well separated and seemingly pure enantiomeric peaks one needs a certain peak capacity to ensure and to demonstrate that no trace peak is hidden underneath the analyte peak. This is also important for more or less pure chiral substances, because high accuracy and precision are usually required for the determination of the enantiomeric excess.

An effective technique for enhancing the peak capacity is the use of a multi-column set-up with column switching[10]. The whole potential of on-line column switching techniques can only be utilized if the two following conditions are met: the mobile phase carryover from the first column should not cause a severe and detrimental deconditioning of the second column, and proper selection of the mobile phases should make it possible to achieve a peak compression effect in the second column[11]. In our case the question also has to be discussed of whether the carryover severely affects or disturbs the swelling state of the swcrCTA material.

In this work the possibilities and limitations of column switching in a coupled system consisting of a non-chiral alkylsilica column operated in a reversed-phase system and a subsequent chiral swcrCTA column were examined with respect to the above criteria: compatability of the mobile phases and possibilities for obtaining peak compression effects.

Conclusions drawn for this column-switching system may also be useful for the optimization of off-line preseparations, especially when considering deconditioning of the CTA column by the injected solvent and the determination of the maximum allowable injection volume.

## EXPERIMENTAL

### *Instrumentation*

Chromatographic experiments were carried out with an HPLC pump (Model L-6200 intelligent pump, Merck–Hitachi, Tokyo, Japan), a syringe-valve injector (Model 7161, Rheodyne, Cotati, U.S.A.) equipped with a 20-$\mu$l loop, a column oven (Model 655A-52, Merck–Hitachi), two switching valves (Model 7000, Rheodyne) and a UV detector (Model L-4000, Merck–Hitachi) connected to an integrator (Model D-2000 chromato-integrator, Merck–Hitachi).

### *Columns*

*Non-chiral column.* Prepacked stainless-steel columns (250 mm × 4.0 mm I.D.) filled with chemically bonded octylsilica material, particle diameter 5 $\mu$m (LiChrosorb RP-8; E. Merck, Darmstadt, F.R.G.).

*Chiral column.* Prepacked columns (250 mm × 4.0 and 10.0 mm I.D.) filled with swollen crystalline cellulose triacetate, mean particle diameter 10 μm (Hibar; E. Merck).

*Reagents and samples*

Organic solvents were obtained from E. Merck: methanol of LiChrosolv quality and absolute ethanol of analytical-reagent grade. Water was distilled twice from a quartz apparatus and additionally purified by passing it through an RP-8 column before eluent preparation. Alcohol–water mixtures were partially premixed and degassed in an ultrasonic bath.

Pure standard solutions of the racemic test analytes were used for the evaluation of the peak compression effect. Solutions of less pure standard analytes were employed to demonstrate the separation power of the column-switching set-up.

*Chromatographic procedure*

A schematic diagram of the two-column set-up is shown in Fig. 1 and a general chromatographic elution and column switching protocol is given in Table I. The procedure starts with the injection of the sample onto the non-chiral alkylsilica column and the elution on this first column with ethanol–water mixtures suitable for the entire separation problem. In this step, the racemic analytes are separated from matrix constituents and impurities. A small fraction of the eluate which contains the chiral analyte is transferred to the CTA column. After the transfer of this fraction to the second column, the elution on the first column is finished. Thereafter, column 1 is switched off and the capillary bypass is used. At the same time the eluent composition is changed to an ethanol–water mixture suitable for the separation on the CTA column, and the capillaries are rinsed. After rinsing, the CTA column is switched on and the analyte stored in this column is eluted. The temperature of the first column was ambient and of the CTA column 50°C. UV detection at 254 nm was used. Mobile phase void volumes of the CTA columns were calculated by assuming a packing porosity of 0.71[8].

## RESULTS AND DISCUSSION

*Peak capacities*

The peak capacity, $n_{(R_s)}$, *i.e.*, the maximum number of peaks that can be separated in a chromatogram with a desired resolution, $R_s$, in a given time, in the ideal

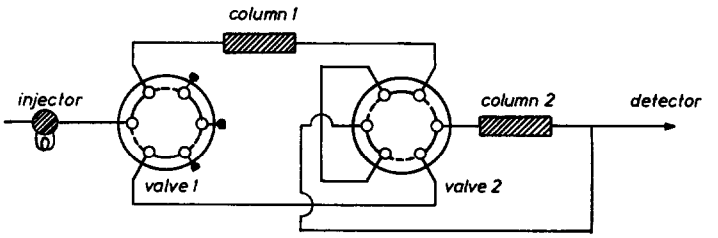


Fig. 1. Schematic diagram of the coupled column chromatographic system used: column 1, alkylsilica (OS or ODS); column 2, swollen crystalline cellulose triacetate (CTA). Valves: rotor position A, solid lines; rotor position B, broken lines.

TABLE I

GENERAL ELUTION AND COLUMN-SWITCHING PROGRAMME

| *Time interval*[a] | *Position of valve* | | *Column* | *Eluent*[b] | *Flow-rate*[c] | *Comment* |
|---|---|---|---|---|---|---|
| | *1* | *2* | | | | |
| 0–*t*1 | A | A | RP | 1 | 1 | Preseparation of the sample |
| *t*1–*t*2 | A | B | RP + CTA | 1 | 2[d] | Transfer of the racemic analyte |
| *t*2–*t*3 | A | A | RP | 1 | 1 | End of RP elution |
| *t*3–*t*4 | B | B | Capillaries | 2 | 1 or higher | Change of eluent<br>Rinsing of capillaries |
| *t*4–*t*5 | B | A | CTA | 2 | 2 | Chiral separation |

[a] *t*1 = Beginning of analyte transfer, *e.g.*, at the beginning of the appearance of the racemic analyte; *t*2 = end of transfer; *t*3 = end of the chromatogram on the RP column; *t*3–*t*4 = time for eluent change and rinsing the capillaries (3–5 min); *t*4 = beginning of elution of CTA column; *t*5 = end of elution of CTA column, end of chromatogram.

[b] Eluent 1, eluent optimized for separation in column 1; eluent 2, eluent optimized for separation in column 2.

[c] Flow-rates 1 and 2: optimum flow-rates for columns 1 and 2, respectively.

[d] If the pressure of both columns is too high, a lower flow-rate can be selected.

case that all possible peak positions can be utilized, is calculated here by means of the equation.

$$n_{(R_s)} = \log(1 + k'_n)/\log(1 + R_s/\sqrt{\bar{N}}) \quad (1)$$

where $k'_n$ is the capacity factor of the last eluted peak, $n$, and $\bar{N}$ is the mean plate number. For the desired resolution of subsequently eluting peaks, $R_s$, we chose a value of 6[a].

Assuming for a typical octylsilica (OS) column of length 25 cm an average plate number of 9000 and choosing a $k'_n$ value of 20 as the upper limiting value for the practical use, the peak capacity value is *ca.* 50.

The plate numbers achievable of swcrCTA columns depend very much on the chemical structures of the analytes. Assuming $\bar{N}$ to be 900, which is obtained with a column of length 25 cm under favourable conditions, and $k'_n$ to be 5, the maximum number of separable peaks is about ten. When looking for average values in typical chiral separations by means of swcrCTA adsorbents, an $\bar{N}$ value of about 600 seems to be more realistic[8,9]. In addition, a lower value should be assumed for $k'_n$, *e.g.*, 3, as in practice very few analytes are eluted with $k' > 3$ when using commonly applied mobile phases (low capacity factors are also convenient in order to keep the analysis time acceptably short). With these more realistic assumptions, a peak capacity of *ca.* 6 is obtained. This is a very low value compared with that obtainable with other chromatographic support materials.

[a] The resolution, $R_s$, is defined here by $R_s = (V_{R_j} - V_{R_i})/\sigma_{V_i}$), where $V_R$ are retention volumes and $\sigma_{V_i}$ is the volume standard deviation of the first-eluted peak. For peaks of equal height, $R_s$ values of 6 mean just baseline separation. The numerical values of $R_s$ calculated according to this definition are about four times larger than would be obtained by using the definition given by Snyder and Kirkland[12].

A peak capacity value of 6 may be sufficient for the separation of two enantiomers. It is, however, too low, to allow separation also from impurities or to demonstrate their absence with confidence. The large increase in peak capacity is the main reason for introducing a two-column set-up for this type of separation. In principle and under certain conditions, peak capacity values can be obtained in two-column systems which are as high as the product of the peak capacity values of the individual columns. These conditions are met if peak dispersion resulting from the first column is negligible with respect to the overall dispersion or is overcome by peak compression.

*Peak compression at the top of the second column*

Peak-sharpening effects generally can be obtained by enhancing the elution power of the eluents. These effects are well known from gradient elution. In coupled column chromatography, those column combinations are especially useful, where at a given eluent composition the analyte is retained more strongly on the second column than on the first: $k_{i_{co}}^{col.2} > k_{i_{co}}^{col.1}$ (co represents carryover). In such a case one may apply a step gradient after the transfer of the analyte onto column 2 by using a stronger eluent for the second column than for the first at the time of transfer (*i.e.*, during the carryover). The peak compression effect which is obtained on applying a step gradient at the top of the second column can be quantified by the peak compression factor, *pcf*. This factor can be evaluated to a certain approximation by the equation[13]

$$pcf_i = \sigma_{V_i}(\text{before compr.})/\sigma_{V_i}(\text{after compr.}) = k'^{col.2}_{i_{co}}/k'^{col.2}_{i_{el.2}} \quad (2)$$

where el.2 indicates the eluent used for the second column.

Table II shows that, with water–ethanol eluents and with the same eluent composition, all analytes investigated are in fact retained more strongly by the CTA than by the OS adsorbent used. A strong step gradient can therefore be applied at the top of column 2.

The influence of the eluent composition on the retention is illustrated in Fig. 2 for ethanol–water mixtures. These data are required when applying eqn. 2. With the alkylsilica phases, the capacity factors of the given analytes show a monotonic decrease with increasing alcohol concentration. This is a typical and well known behaviour for non-ionic solutes at not too high alcohol concentrations. There one may even assume an approximately linear correlation between ln $k'$ and the volume fraction of the alcohol in the aqueous mobile phase.

With CTA columns the dependence of $k'$ on the ethanol concentration is not monotonic in the region of ethanol concentrations usually applied with this adsorbent. The decrease in retention with increasing ethanol concentration follows the opposite trend at high concentrations of ethanol.

The observed behaviour is due to the special adsorption mechanism on swcrCTA adsorbents[14]. The strength of the competitive effect of solvent components is significantly influenced by the steric size of the solvent molecules. Hence the competitive effect of water on this adsorbent is stronger than that of ethanol. This effect acts in the direction of reducing the capacity factor values with increasing concentration of water. On the other hand, the smaller solvation power of water compared with ethanol for many analytes causes the activity coefficients of the

TABLE II

CAPACITY FACTORS WITH OCTYLSILICA AND swcrCTA STATIONARY PHASES AS A FUNCTION OF THE CONCENTRATION OF ETHANOL IN WATER–ETHANOL MOBILE PHASES

| *Compound* | | *Octylsilica* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *80%*[a] | *72%* | *64%* | *40%* | *24%* | | |
| Phenyloxazolone | | | 0.03 | 0.09 | 0.50 | 2.69 | | |
| Phenyldioxolone | | 0.07 | 0.09 | 0.14 | 1.00 | 3.72 | | |
| Hexobarbital | | 0.08 | 0.11 | 0.20 | 1.59 | | | |
| Spirobiindanone | | 0.11 | 0.19 | | 3.88 | | | |
| Methaqualone | | 0.13 | 0.28 | 0.44 | 4.50 | | | |
| Tröger's base | | | 1.26 | 2.27 | 4.77 | | | |
| | | *swcrCTA* | | | | | | |
| | | *96%*[a] | *86.4%* | *80%* | *76.8%* | *72%* | *60%* | *40%* |
| Phenyloxazolone | I[b] | 0.54 | 0.34 | 0.23 | | 0.30 | 0.56 | 1.87 |
| | II[b] | 0.88 | 0.57 | 0.43 | | 0.70 | 0.89 | 2.71 |
| Phenyldioxolone | I | 1.84 | | | 1.66 | | | |
| | II | 3.47 | | | 3.66 | | | |
| Hexobarbital | I | 0.83 | 0.60 | 0.59 | | 0.85 | 1.42 | |
| | II | 1.21 | 0.99 | 1.02 | | 1.22 | 2.50 | |
| Spirobiindanone | I | 0.95 | 0.67 | | 1.08 | | | |
| | II | 2.03 | 1.80 | | 3.14 | | | |
| Methaqualone | I | 0.27 | 0.18 | | 0.29 | | | |
| | II | 0.42 | 0.29 | | 0.44 | | | |
| Tröger's base | I | 1.10 | 0.74 | | 1.24 | | | |
| | II | 2.31 | 1.67 | | 2.99 | | | |

[a] Ethanol concentration in mobile phase (%, v/v).
[b] I and II indicate first and second enantiomer, respectively.

analytes in the mobile phase, and hence the capacity factors, to increase with increasing concentration of water. The superposition of both effects produces the capacity factor dependence given in Fig. 2.

By use of eqn. 2 and the data from Fig. 2, one can evaluate the conditions under which and the extent to which peak compression can be obtained. As a rule, peak compression can simply be obtained in most instances by choosing a higher alcohol concentration in eluent 2 than in the carryover, with the exception of ethanol concentrations higher than 75–80% (v/v).

*Exploitable peak compression effect*

To evaluate the optimum eluent compositions for eluent 2, one must consider the optimum separation conditions for the racemic analytes on the CTA column together with the peak compression potential at the top of this column given under these conditions. One should consider that the peak compression factor at the top of column 2 is not required to be the maximum attainable. It is sufficient if the peak variance in the carryover is reduced to such an extent that it does not affect the resolution to more

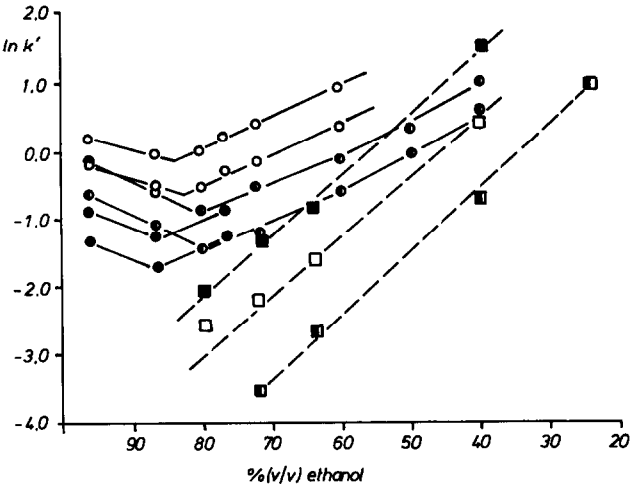


Fig. 2. Logarithm of the capacity factors of test analytes on swcrCTA and octylsilica adsorbents as a function of the volume fraction of ethanol in ethanol–water mobile phases. Temperature, 50°C. Analytes: hexobarbital (open symbols), 5-phenyltetrahydrooxazol-2-one (half filled symbols) and methaqualone (full symbols). Circles, swcrCTA adsorbent; squares, octylsilica adsorbent.

than a few percent. The maximum exploitable compression factor therefore obviously depends on the peak variance in the carryover and the variance resulting during elution in column 2.

The influence of the peak variance in the carryover on the total resolution is evaluated by means of eqn. 3, assuming that more or less the whole analyte peak is transfered to column 2:

$$\frac{R_s^{(1+2)}}{R_s^{(2)}} = \left[1 + \left(\frac{\sigma_V^{col.1}}{\sigma_V^{col.2}}\right)^2\right]^{-1/2} \tag{3}$$

where $R_s^{(2)}$ means the resolution obtained by use of column 2 and $R_s^{(1+2)}$ that obtained by the use of a combination of both column; $\sigma_V$ is the standard deviation of the eluted peaks in volume units.

For reasonable capacity factors and plate numbers in the first (OS) and second (CTA) columns, and considering typical inside diameters of the columns (4 mm for the OS and 4 and 10 mm for the CTA column), the relative decrease in enantiomeric resolution due to the first column is shown in Table III, under the assumption that peak compression is not performed. Conversely, these data illustrate the maximum effect on the peak widths in the final chromatograms that can be expected from peak compression at the top of the column under the optimum conditions. For columns with equal diameter, the decrease in resolution is sometimes significant and may be up to 30% for compounds weakly retained on the CTA column. Here, the application of peak compression will result in a decisive improvement. If one combines the OS column of 4 mm I.D. with the chiral column of 10 mm I.D., the decrease in resolution is very small and in fact negligible. In this instance, a peak compression at the top of column 2 will produce no observable effect on the final chromatogram.

TABLE III

CALCULATED PEAK PROFILE STANDARD DEVIATIONS, $\sigma_V$, OF THE COLUMNS USED AND CALCULATED DECREASE IN CHROMATOGRAPHIC RESOLUTION ON USING TWO-COLUMN CHROMATOGRAPHY WITHOUT PEAK COMPRESSION

| *Column 1 ($N$ = 8000,[a] I.D. = 4 mm, $V_0$ = 2.4 ml)* | | *Column 2 ($N$ = 600)[b]* | | | | |
|---|---|---|---|---|---|---|
| | | *I.D. = 4 mm, $V_0$ = 2.4 ml* | | | *I.D. = 10 mm, $V_0$ = 15 ml* | |
| $k'$ | $\sigma_V$ (μl) | $k'$ | $\sigma_V$ (μl) | $R_s^{(1+2)}/R_s^{(2)}$ | $\sigma_V$ (μl) | $R_s^{(1+2)}/R_s^{(2)}$ |
| 3 | 107 | 0.5 | 147 | 0.808 | 919 | 0.993 |
| | | 1.0 | 196 | 0.877 | 1225 | 0.996 |
| | | 1.5 | 255 | 0.916 | 1531 | 0.998 |
| 5 | 161 | 0.5 | 147 | 0.674 | 919 | 0.985 |
| | | 1.0 | 196 | 0.772 | 1225 | 0.991 |
| | | 1.5 | 255 | 0.835 | 1531 | 0.995 |

[a] Typical value for RP columns.
[b] Typical value for swcrCTA columns.

Chromatograms of 5-phenyltetrahydrooxazol-2-one, hexobarbital and Tröger's base obtained by the OS–CTA coupled column system are shown in Figs. 3–5. In each instance, the eluents for the first column were selected such that reasonable capacity factors are obtained on the OS column and a too high concentration of water is avoided (*cf.*, discussion on the compatibility of mobile phases). The eluents for the second column were selected such that the requirements for enantiomeric resolution and short analysis time were satisfied[14]. The separation of the enantiomers obtained with the coupled column system is seen to be as good as that given by a single CTA column.

Table IV shows the capacity factor, enantioselectivity and peak dispersion data corresponding to these chromatograms together with the data obtained by single CTA chromatography. Experimental peak compression factors, determined from peak widths in the chromatograms according to eqn. 4, and the maximum obtainable compression factors at the top of column 2, as calculated from capacity factor data according to eqn. 2, are also given.

$$pcf^{exp} = \left[\frac{(\sigma_V^{col.1})^2 + (\sigma_V^{col.2})^2 + (\sigma_V^{ex})^2}{(\sigma_V^{col.1+col.2})^2 + (\sigma_V^{ex})^2}\right]^{1/2} \quad (4)$$

where the superscript ex indicates contributions from the injector, detector, capillaries and switching valves. $(\sigma_V^{col.1})^2$ has to be substituted by the peak variance in the carryover if only parts of the analyte are transferred to column 2.

First, and most important for practical applications, one can see in Table IV that the final peak widths ($\sigma_V$ data) remain essentially unchanged in all instances, whether using a single CTA column or a column-switching system. Because of the higher elution strength of eluent 2 with respect to the carryover, peak compression can be expected to take place at the top of the CTA column in all instances (*cf.*, $pcf^{max}$ data). It

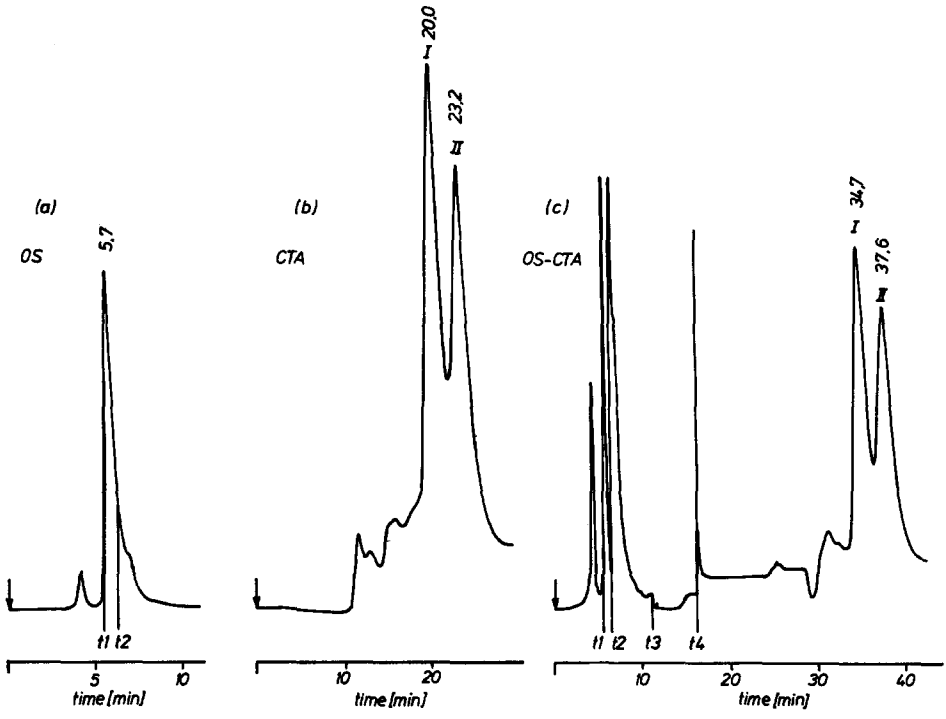


Fig. 3. Chromatograms of 5-phenyltetrahydrooxazol-2-one obtained in single column systems and in the octylsilica–swerCTA two-column system including column switching. Separated enantiomers are indicated by I and II. (a) OS column only; (b) $CTA_{(II)}$ column only; (c) OS-$CTA_{(II)}$ with column switching. Column dimensions: OS, 250 × 4 mm I.D.; $CTA_{(II)}$, 250 × 10 mm I.D. Temperature: column 1, ambient; column 2, 50°C. Eluent 1, ethanol–water (40:60); eluent 2, ethanol–methanol–water (67.2:20:12.8). Flow-rate 1, 0.5 ml/min; flow-rate 2, 1.0 ml/min. Times of events: $t1$ = 5.5; $t2$ = 6.5; $t3$ = 11.0; $t4$ = 16.0; $t5$ = 42.0 min.

is obvious that a significant peak compression effect in the final chromatograms (see $pcf^{exp}$ data) can be observed only in instances where the small-diameter CTA column was employed. Otherwise the effect vanishes because of the dispersion in the wide-diameter column.

From Table IV a decrease in the capacity factors on the CTA adsorbent by the column switching procedure can be seen. It is probably due to a slightly higher temperature in the CTA column when operated in the column-switching mode, which results from different working temperatures in columns 1 and 2 in our set-up. The increase in enantioselectivity is probably caused by the high water content in the carryover. Increasing enantioselectivity with increasing water concentration in the eluent can be observed for many analytes with CTA adsorbents[7,14]. The higher water contents in the carryover thus produces a useful side effect in this type of column-switching system.

*General compatibility of the mobile phases*

The use of coupled column chromatography requires that the carryover from the first column is fully compatible with the stationary phase and the eluent used in the

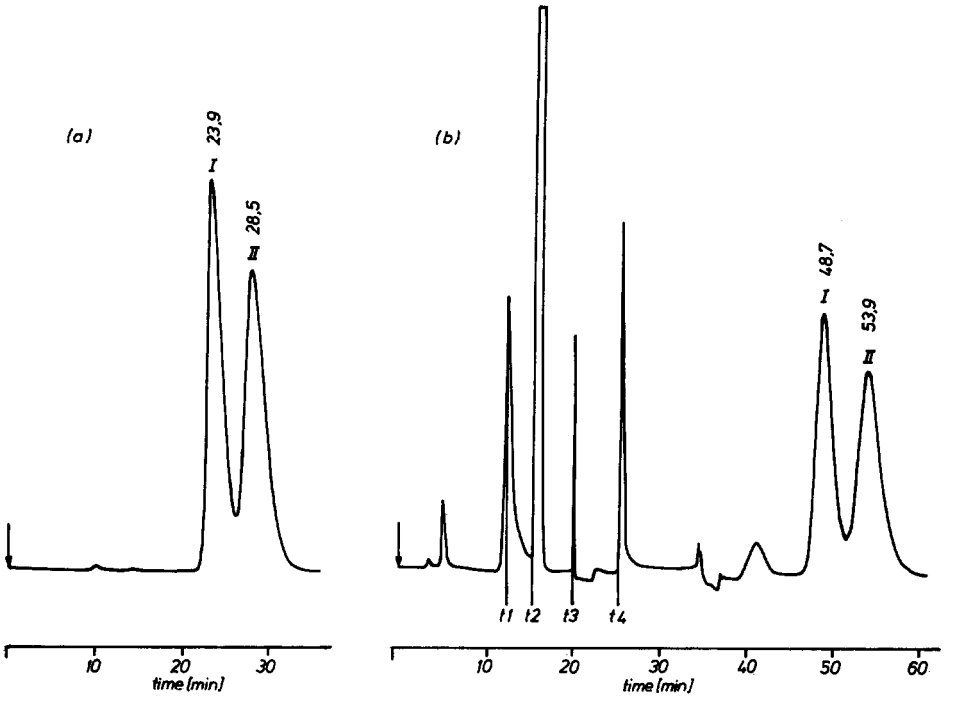


Fig. 4. Chromatograms of hexobarbital obtained from a single CTA column and in the octylsilica–swcrCTA two-column system including column switching. (a) $CTA_{(l)}$ column only; (b) OS-$CTA_{(l)}$ with column switching. Column dimensions: OS, 250 × 4 mm I.D.; $CTA_{(l)}$ 250 × 4 mm I.D. Temperature: column 1, ambient; column 2, 50°C. Eluent 1, ethanol–water (40:60); eluent 2, ethanol–methanol–water (67.2:20:12.8). Flow-rate 1, 0.5 ml/min; flow-rate 2, 0.16 ml/min. Times of events: $t1$ = 12.25; $t2$ = 15.20; $t3$ = 20.0; $t4$ = 25.0; $t5$ = 61.0 min.

second column. This means, first, that for our column-switching system, solvents which may solvate the CTA adsorbent (chloroalkanes, THF, ketones) must be excluded from the carryover.

Second, with swcrCTA stationary phases, an additional constraint results from the swollen state of the packed bed. Previous investigations[14] have shown that changes in the eluent composition probably also affect the swelling state of the CTA packing. It is therefore necessary to prevent an irreversible change in the swelling state of the bed by the eluent carryover. With regard to the proposed two-column combination, too high a water concentration in the carryover may be problematic for the CTA column. In our experience, the packing of the column is not disturbed, at least up to a concentrations of 76% (v/v) water in the ethanolic mobile phase, although temporary changes in the swelling state are very likely. The critical content of water at which breakdown of the packing occurs has not been determined. Up to 76% of water all the changes in the swelling state did not cause problems with the stability of the packed bed. Plate-number data indicate that these changes are fully reversible on returning to the initial eluent composition that was used for the packing of the column [in this instance ethanol–water (96:4)].

TABLE IV

CAPACITY FACTOR ($k'$), ENANTIOSELECTIVITY ($\alpha$) AND PEAK STANDARD DEVIATION ($\sigma_V$) DATA OF ANALYTES IN SINGLE-COLUMN AND COUPLED-COLUMN CHROMATOGRAPHY, AND PEAK COMPRESSION FACTORS (*pcf*) DERIVED THEREFROM

$CTA_{(I)}$, column with I.D. 4 mm, flow-rate = 0.16 ml/min; $CTA_{(II)}$, I.D. 10 mm, flow-rate = 1.0 ml/min; OS, octylsilica, flow-rate = 0.5 ml/min. Other chromatographic conditions as given in Figs. 3–5; co indicates the carryover; $pcf^{exp}$, experimental peak compression factor, determined according to eqn. 4; $pcf^{max}$, maximum obtainable peak compression factor at the top of column 2, evaluated according to eqn. 2. Enantioselectivity coefficients in the coupled-column system are calculated according to $\alpha = (t_{R_2}^{OS\text{–}CTA} - t_4 - t_{R_0}^{CTA})/(t_{R_1}^{OS\text{–}CTA} - t_4 - t_{R_0}^{CTA})$, $t_{R_1}$ and $t_{R_2}$ being the retention times of the two enantiomers, $t_4$ the time of starting the elution on the CTA column (*cf.*, Table I) and $t_{R_0}$ the elution time of the system peak of the eluent component methanol (*i.e.*, 14 min in both columns at the flow-rates applied).

| *Compound* | *Parameter* | $CTA_{(I)}$ | $OS\text{–}CTA_{(I)}$ | $CTA_{(II)}$ | $OS\text{–}CTA_{(II)}$ |
|---|---|---|---|---|---|
| 5-Phenyltetrahydrooxazol-2-one[a] | $k'^{CTA}_{1\,el.2}$ | 0.65[b] | 0.31[b] | 0.43 | 0.33 |
| | $k'^{CTA}_{2\,el.2}$ | 0.85[b] | 0.48[b] | 0.66 | 0.53 |
| | $\alpha$ | 1.31[b] | 1.52[b] | 1.55 | 1.61 |
| | $(\sigma_V)_1$ ($\mu$l) | 86[b] | 96[b] | 680 | 680 |
| | $(\sigma_V)_2$ ($\mu$l) | 106[b] | 102[b] | 760 | 760 |
| | $(\sigma_V)^{co}$ ($\mu$l) | | 500 | | 130 |
| | $pcf_1^{exp}$ | | 5.3 | | 1.02 |
| | $pcf_2^{exp}$ | | 5.0 | | 1.01 |
| | $k'^{CTA}_{1\,el.1}$ | | | 1.87[c] | |
| | $k'^{CTA}_{2\,el.1}$ | | | 2.71[c] | |
| | $pcf_1^{max}$ | | 6.0[d] | | 4.3 |
| | $pcf_2^{max}$ | | 5.6[d] | | 4.1 |
| Hexobarbital[a] | $k'^{CTA}_{1\,el.2}$ | 0.71 | 0.66 | 0.67 | 0.55 |
| | $k'^{CTA}_{2\,el.2}$ | 1.04 | 1.03 | 1.05 | 0.90 |
| | $\alpha$ | 1.46 | 1.56 | 1.57 | 1.62 |
| | $(\sigma_V)_1$ ($\mu$l) | 131 | 140 | 880 | 810 |
| | $(\sigma_V)_2$ ($\mu$l) | 173 | 182 | 1200 | 1090 |
| | $(\sigma_V)^{co}$ ($\mu$l) | | 500 | | 300 |
| | $pcf_1^{exp}$ | | 3.7 | | 1.15 |
| | $pcf_2^{exp}$ | | 2.9 | | 1.00 |
| | $k'^{CTA}_{1\,el.1}$ | | | 3.6[c] | |
| | $k'^{CTA}_{2\,el.1}$ | | | 6.0[c] | |
| | $pcf_1^{max}$ | | | 5.4 | |
| | $pcf_2^{max}$ | | | 5.7 | |
| Tröger's base[e] | $k'^{CTA}_{1\,el.2}$ | | | 1.13 | 0.96 |
| | $k'^{CTA}_{2\,el.2}$ | | | 2.41 | 2.23 |
| | $\alpha$ | | | 2.14 | 2.33 |
| | $(\sigma_V)_1$ ($\mu$l) | | | 1410 | 1420 |
| | $(\sigma_V)_2$ ($\mu$l) | | | 2960 | 2800 |
| | $(\sigma_V)^{co}$ ($\mu$l) | | | | 310 |
| | $pcf_1^{exp}$ | | | | 1.02 |
| | $pcf_2^{exp}$ | | | | 1.06 |
| | $k'^{CTA}_{1\,el.1}$ | | | 1.5[c] | |
| | $k'^{CTA}_{2\,el.1}$ | | | 3.6[c] | |
| | $pcf_1^{max}$ | | | 1.3 | |
| | $pcf_2^{max}$ | | | 1.5 | |

[a] Eluent 1, ethanol–water (40:60); eluent 2, ethanol–methanol–water (67.2:20:12.8).
[b] Flow-rate, 0.08 ml/min; temperature of CTA column, 60°C.
[c] Obtained by extrapolation (Fig. 2).
[d] Calculated from the $k'^{CTA}$ data given under $OS\text{–}CTA_{(I)}$.
[e] Eluent 1, ethanol–water (72:28); eluent 2, ethanol–water (96:4).

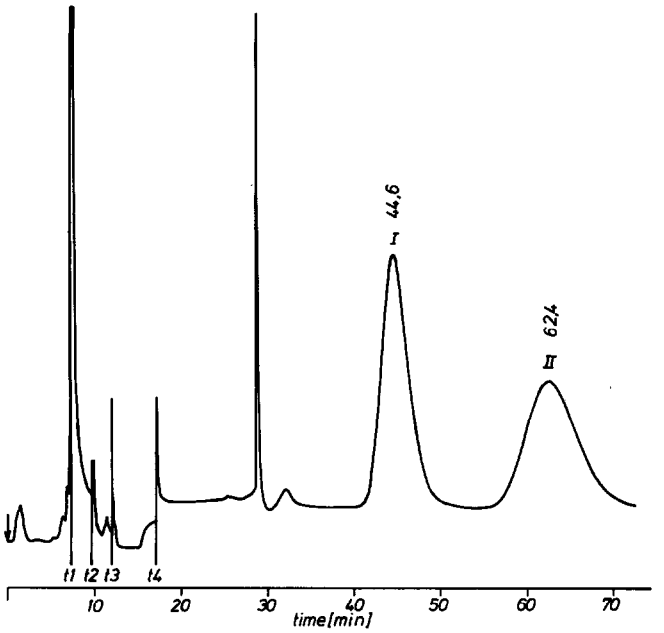


Fig. 5. Chromatogram of Tröger's base obtained with the octylsilica–swcrCTA two-column system with column switching. Column 1, octylsilica; column 2, swcrCTA$_{(II)}$. Column dimensions: OS, 250 × 4 mm I.D.; CTA$_{(II)}$, 250 × 10 mm I.D. Temperature: column 1, ambient; column 2, 50°C. Eluent 1, ethanol–water (72:28); eluent 2, ethanol–water (96:4). Flow-rate 1, 0.5 ml/min; flow-rate 2, 1.0 ml/min. Times of events: $t1$ = 7.0; $t2$ = 9.50; $t3$ = 12.0; $t4$ = 17.0; $t5$ = 72.0 min.

## CONCLUSION

For optical purity control and enantiomeric excess determination, a high peak capacity is required in order to exclude the possibility with sufficiently high significance that small impurity peaks interfere with the peaks of the enantiomers. Even for apparently pure substances one needs a minimum peak capacity to make purity evaluation possible at a certain level of accuracy and precision. To increase the low peak capacity of swcrCTA columns, a two-column set-up with column switching can be used which combines a non-enantioselective separation step on an alkylsilica column with an enantioselective separation step on the swcrCTA column. In order to take advantage of the maximum achievable peak capacity in a column-switching system, the variance of the transferred peak must be negligible with respect to the variance originating from the second column, or has to be made negligible by peak compression.

Owing to the strong peak dispersion in swcrCTA columns, the peak broadening produced by the alkylsilica columns can often be neglected. This is obvious when using CTA columns of 25 cm × 10 mm I.D., as they are commercially available, and OS columns of 4 mm I.D. Using columns of equal diameter, peak compression is worthwhile. Peak compression may also be of interest for semi-preparative separations, for which swcrCTA adsorbents are especially suitable. Overloading effects

expected in column 1 (OS) when using swcrCTA columns for semi-preparative purposes can be compensated for in this way.

Peak compression at the top of the CTA column can be achieved in most instances simply by choosing a higher concentration of alcohol in eluent 2 than in the carryover, when applying mixed water–alcohol mobile phases. This strategy may not be successful at very high concentrations of ethanol. Compatibility of the mobile phases for both columns is given for ethanol–water mixtures with a water concentration of least up to 75% (v/v) in the carryover. Up to this value, the CTA packing has been proved to be stable. The exact limit at which the packing breaks down has not been determined. For not very hydrophilic analytes, the given concentration range should be sufficient for successful reversed-phase chromatography. Obviously, with respect to the solubility of the CTA material, chloroalkanes, ketones of THF must not be present in the carryover.

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