# DISPLACEMENT CHROMATOGRAPHY ON CYCLODEXTRIN–SILICAS

## II. SEPARATION OF *cis–trans* ISOMERS IN THE REVERSED-PHASE MODE ON α-CYCLODEXTRIN–SILICA

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SUMMARY

The feasibility of preparative separations of *cis–trans* isomers by displacement chromatography on analytical-scale α-cyclodextrin–silica columns operated in the reversed-phase mode is demonstrated by using the isomers of 3-hexen-1-ol as model substrates and *n*-alkanols as displacers. The importance of matching the size of the cyclodextrin cavity and the solutes is shown. The crucial role of the displacer (both type and concentration) in the success of the displacement chromatographic separation is demonstrated.

INTRODUCTION

Cyclodextrins are capable of forming inclusion complexes with guest molecules[1–3]. As the cyclodextrin cavity has a well defined size and geometry, the stability of the inclusion complex depends on the snugness of the fit and the strength of the intermolecular interactions that develop between the polar functional groups of the guest solute and the secondary and primary hydroxyl groups of the cyclodextrin[2–4]. Cyclodextrins can be used to effect the separation of positional and geometric isomers, *cis–trans* isomers and ciral solutes provided that the solutes can penetrate the cyclodextrin cavity.

The analytical separations of positional and geometric isomers[4–9] and enantiomers[10–13] have been reviewed recently and are not dealt with in his paper. The *cis* and *trans* isomers of stilbene[14], six prostaglandins[15], cyclic nitrosamines[16], acyclic nitrosamines[17], cyclohexane derivatives[18] and tamoxifen[19] were successfully separated on β-cyclodextrin-silica columns using aqueous methanol and acetonitrile eluents. Generally, the *trans* isomers elute first. The bulkier and/or more hydrophobic substituents lead to longer solute retention and different separation selectivity.

Compared with other silica-based high-performance liquid chromatographic (HPLC) stationary phases, most cyclodextrin–silicas have low loading capacities and show relatively low selectivity factors. Hence they are more suited for analytical separations than for preparative work. However, in Part 1[20] we showed that the preparative chromatographic limitations of cyclodextrin–silicas can be circumvented if the columns are used in the self-focusing displacement chromatographic mode.

Horváth et al.[21] used modern HPLC hardware to achieve efficient non-linear chromatographic separations by displacement chromatography[22,23]. Several good recent reviews discuss the principles and use of displacement chromatography in detail (e.g., ref. 24). A number of research groups are actively pursuing the theoretical and practical aspects of displacement chromatography[25-28].

We showed in Part 1[20] that good preparative separations of positional and geometric isomers can be effected on analytical $\beta$-cyclodextrin–silica columns even when the elution-mode separation selectivities are as low as 1.05–1.09. Sample loadings as high 60 mg were achieved on 4.6 mm I.D. analytical columns. In this paper we show that the preparative displacement chromatographic separation of cis and trans isomers is also possible on cyclodextrin–silicas in the reversed-phase mode.

## EXPERIMENTAL

A multifunctional displacement chromatograph, assembled from commercially available components as described in Part 1[20], was used. The instrument can be operated in (i) the elution mode to determine the capacity factors of the solutes and to analyse the fractions collected during the preparative separations, (ii) the frontal mode to determine the adsorption isotherms of the displacers and the solutes and (iii) the displacement mode to carry out the preparative separations. The experimental procedure was the same as described previously[20].

Both $\alpha$-cyclodextrin–silica (Cyclobond III, 5 $\mu$m) and $\beta$-cyclodextrin–silica (Cyclobond I, 5 $\mu$m) were obtained from ASTEC (Whippany, NJ, U.S.A.) and slurry-packed in our laboratory into 4.6 mm I.D. stainless-steel analytical columns. The cis and trans isomers of 3-hexen-1-ol (Aldrich, Milwaukee, WI, U.S.A.) were used as test solutes and 1-hexanol and 1-heptanol (Aldrich) as displacers. All chemicals were used without further purification. All eluents, carrier solutions and displacer solutions were prepared from HPLC-grade methanol (Mallinckrodt, St. Louis, MO, U.S.A.) and Milli-Q water (Millipore, Bedford, MA, U.S.A.).

## RESULTS

In order to develop a displacement chromatographic separation, the elution-mode retention behavior of the solutes has to be studied first. This information is then used to select the composition of the carrier solution. The log $k'$ vs. methanol concentration curves of cis- and trans-3-hexen-1-ol are shown in Fig. 1. As in ordinary reversed-phase chromatography, an almost linear relationship is obtained. However, for a successful displacement chromatographic separation, the solutes have to be sufficiently retained in the carrier solution ($k' > 10$). It can be seen in Fig. 1 that the $k'$ value of the less retained cis isomer is only 1.9, even in the weakest eluent, pure water. The weak retention probably results from the $\beta$-cyclodextrin cavity being too large for the 3-hexene-1-ol isomers and a tight fit cannot be achieved. Therefore, the retention studies were repeated on an $\alpha$-cyclodextrin-silica column.

The log $k'$ vs. methanol concentration curves for cis- and trans-3-hexen-1-ol are shown in Fig. 2. Again, good linear retention behavior can be observed. Sufficient (although by no means large) retention can be achieved with pure water as the carrier solvent.
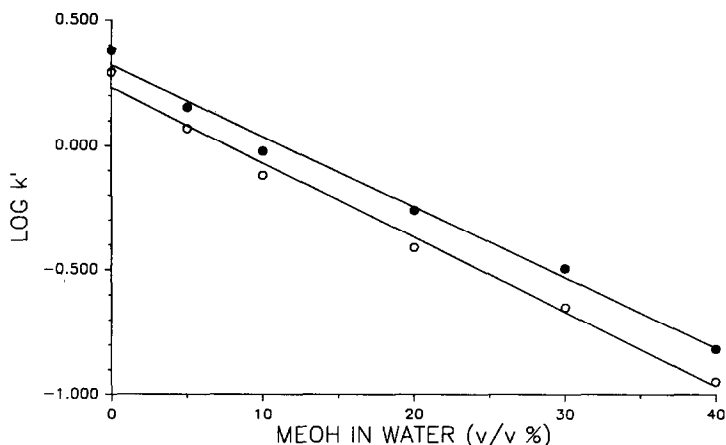
Fig. 1. Retention of (●) *cis*- and (○) *trans*-3-hexen-1-ol on β-cyclodextrin–silica as a function of the methanol (MEOH) concentration of the eluent.

Once the composition of the carrier solution has been determined, a suitable displacer has to be selected for the separation. Components that have a structure similar to that of the solute are generally good displacers on cyclodextrin–silicas, provided that they are slightly more hydrophobic than the solutes and/or have slightly stronger intermolecular interactions with cyclodextrin than do the solutes. Therefore, 1-hexanol and 1-heptanol were selected as potential displacers. Their retention curves are also shown in Fig. 2. Both *n*-alkanols are more retained than the *cis–trans* solute pair. The elution mode, infinite dilution separation selectivity factors for the *cis–trans* pair and the *n*-hexanol–*trans* isomer solute pair are approximately the same. Thus, at least in principle, both 1-hexanol and 1-heptanol are good displacers, because the *cis* and *trans* isomers can be separated from each other and then, owing to the identical
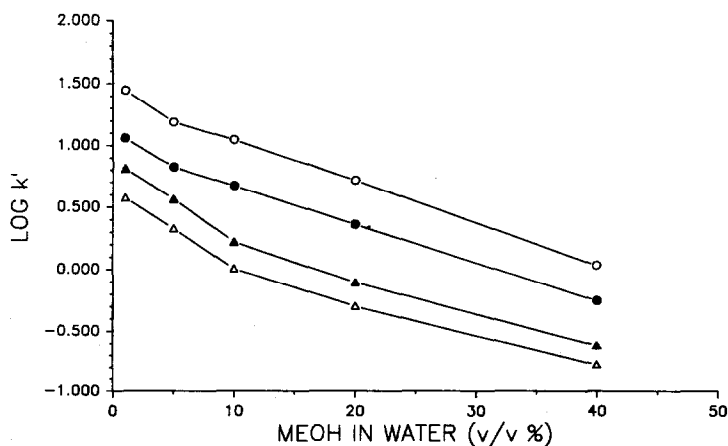


Fig. 2. Retention of (●) 1-hexanol, (○) 1-heptanol and (△) *cis*- and (▲) *trans*-3-hexen-1-ol on α-cyclodextrin–silica as a function of the methanol (MEOH) concentration of the eluent.

α values the *trans* isomer can also be separated from the displacer. To test the validity of this assumption, the adsorption isotherms of the solutes and of both potential displacers were determined.

The adsorption isotherms of the solutes, *cis*- and *trans*-3-hexen-1-ol and the potential *n*-alkanol displacers are show in Fig. 3. Both 1-hexanol and 1-heptanol are more strongly adsorbed than the *cis–trans* solute pair, indicating that either of them could be used as a displacer. The isotherms of 1-hexanol and *trans*-3-hexen-1-ol are much closer to each other than those of *cis*- and *trans*-3-hexen-1-ol. This shows that the selectivities of the separation at infinite dilution (Fig. 2) and at higher concentrations (Fig. 3) are different.
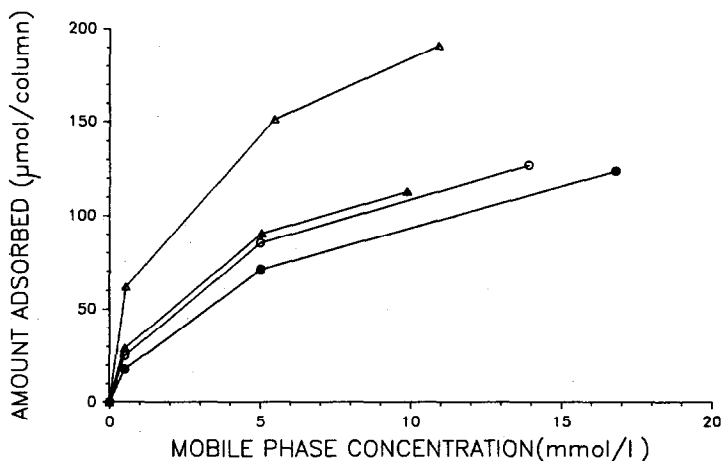


Fig. 3. Adsorption isotherms of (▲) 1-hexanol, (△) 1-heptanol and (●) *cis*- and (○) *trans*-3-hexen-1-ol on α-cyclodextrin–silica from pure water as carrier.

Once the adsorption isotherms are known, the actual displacement chromatographic separations can be designed. First, an 11.8 m*M* solution of 1-heptanol in water (saturated 1-heptanol solution) was tried as a displacer. The dispacement chromatogram of a sample containing 3 mg of the *cis* and 2.9 mg of the *trans* isomer is shown in Fig. 4. There are two steps and the plateau of the displacer in the chromatogram. Fractions of 300 μl were collected for further analysis. By analyzing the individual fractions, the reconstructed displacement chromatogram could be obtained. The reconstructed displacement chromatogram in Fig. 5 reveals that the first band is pure *cis* isomer and the second is pure *trans* isomer. When the yields corresponding to the 99% purity level are calculated they turn out to be 94.5% for the first component and 75.6% for the second. It is likely that, with smaller fraction sizes, an even higher yield could have been obtained, especially for the first component.

In order to obtain more concentrated fractions, a less steep displacer operational line has to be selected. However, as the displacer used in Fig. 4, 1-heptanol, is already at its saturation concentration, the less strongly adsorbed 1-hexanol has to be selected as the new displacer. An operational line 25% less steep than in Fig. 4 can be obtained by
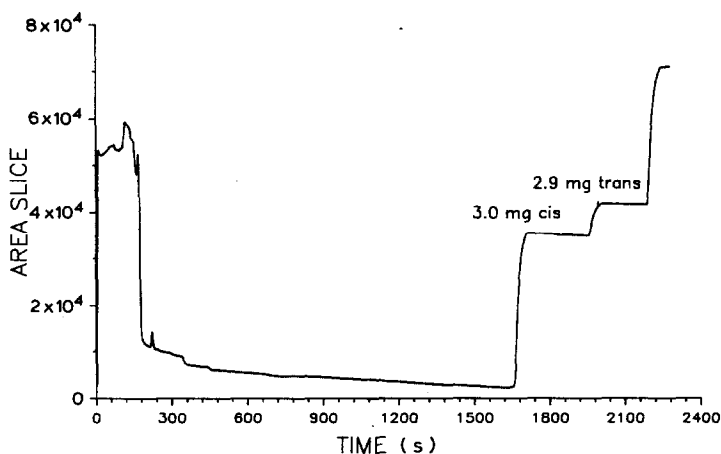
Fig. 4. Displacement chromatogram of a 6-mg sample of 3-hexen-1-ol on two 250 mm × 4.6 mm I.D. analytical α-cyclodextrin columns, with 11.9 m$M$ 1-heptanol in pure water as displacer, at 1.0 ml/min and 30°C.

using 8.1 m$M$ 1-hexanol solution as the displacer. The displacement chromatogram of 6 mg of a *cis–trans* isomer sample is shown in Fig. 6. There is a large elution-type peak at the beginning of the chromatogram, followed by three steps and the plateau of the displacer. The reconstructed displacement chromatogram derived from the analysis of the collected fraction is shown in Fig. 7. The first elution-type peak corresponds to pure *cis* isomer and so does the first plateau. The second plateau contains a mixture of the *cis* and *trans* isomers and their ratio is constant throughout the zone, indicating that the higher separation speed afforded by the new operational line cannot effect a complete separation of the same sample load that was well resolved in Fig. 4. The third plateau again contains the pure *trans* isomer.
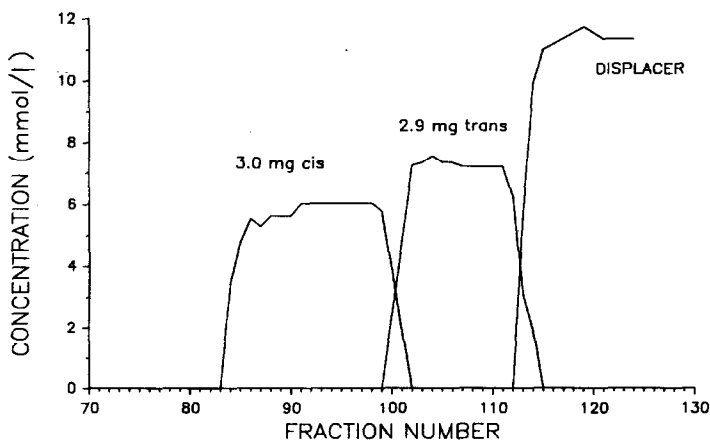


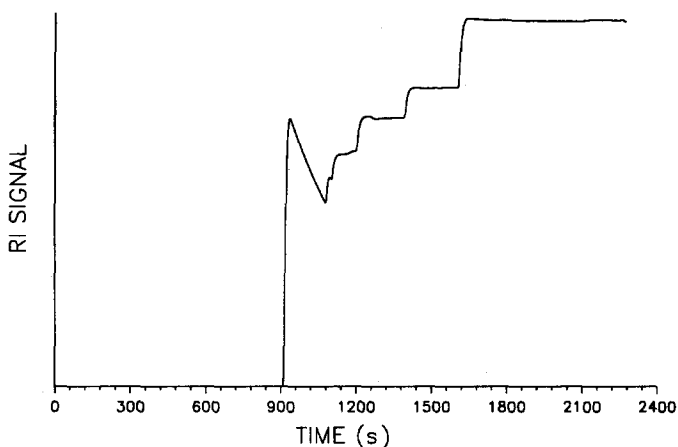Fig. 5. Reconstructed displacement chromatogram of the separation shown in Fig. 4. Fraction size, 300 $\mu$l.

Fig. 6. Displacement chromatogram of a 6 mg sample of 3-hexen-1-ol on two 250 mm × 4.6 mm I.D. analytical α-cyclodextrin columns, with 8.1 m$M$ 1-hexanol in pure water as displacer, at 1.0 ml/min and 30°C. RI = Refractive index.


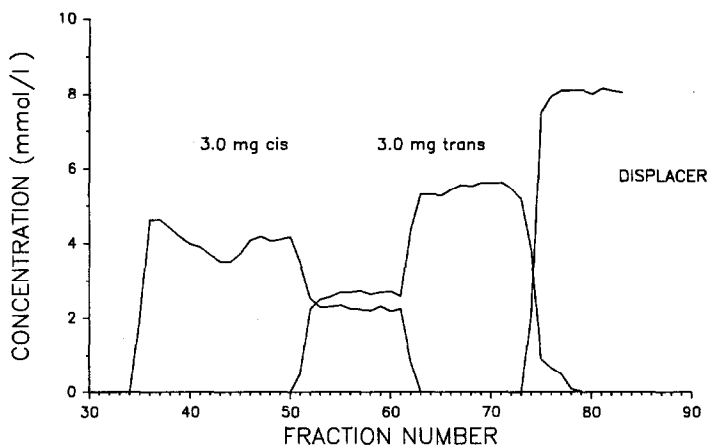
Fig. 7. Reconstructed displacement chromatogram of the separation shown in Fig. 6. Fraction size, 300 µl.

CONCLUSIONS

Based on elution-mode retention studies and adsorption isotherm measurements, a reversed-phase displacement chromatographic system has been developed for the separation of cis- and trans-3-hexen-1-ol. High purity and good yield could be achieved by using structurally related n-alkanols as displacers. The type and concentration of the displacer plays a major role in the success and quality of the displacement chromatographic separation.

ACKNOWLEDGEMENTS

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