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## Note

# β-Cyclodextrin as a chiral component of the mobile phase for separation of mandelic acid into enantiomers in reversed-phase systems of high-performance liquid chromatography

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Separation of racemates into enantiomers is an essential procedure in organic chemistry, particularly in the total synthesis of natural products. Although this problem has been studied for a long time in many laboratories it has not so far been solved. High-performance liquid chromatography (HPLC) with the use of chiral substances as additives to the stationary phase[1,2] or to the eluent[3,4] is one of the most important, although not well elucidated, methods for racemate separation. β-Cyclodextrin forms diastereomeric inclusion complexes with many chiral organic compounds and has also been successfully used as a component of the stationary phase for racemate separation by HPLC[5,6]. Aqueous solutions of cyclodextrins have proven to be an effective mobile phase in thin-layer chromatographic (TLC) separations of mixtures of isomers[7,8] and in HPLC separations of prostaglandins[9]. No attempts have so far been made to use such a system for the resolution of enantiomers.

The aim of this work was to determine whether β-cyclodextrin may be appropriate as an optically active component of the mobile phase solutions for the resolution of racemates in reversed-phase HPLC systems. The resolution of mandelic acid, both enantiomers of which are readily accessible, has now been attempted.

## EXPERIMENTAL

All reagents were p.a. grade. β-Cyclodextrin (CyD) was supplied by Chinoin, Budapest, Hungary.

Chromatographic measurements were performed using a HPLC unit constructed at the Institute of Physical Chemistry, P.A.N., Warsaw, Poland, equipped with a 5-μl high-pressure injection valve and a Z-shaped detection passage (volume, 8 μl)[10]. Chromatograms were recorded using a Hewlett-Packard 7101 strip chart recorder. For HPLC, use was made of stainless-steel columns (250 × 4 mm I.D.), slurry packed at 435 kg/cm$^2$ by a "balanced weight" technique with 10-μm LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.) as sorbent.

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The mobile phases consisted of aqueous solutions containing various concentrations of CyD and 0.1 *M* phosphate buffer (pH 2.1). All investigated samples were artificial mixtures prepared from pure enantiomers of mandelic acid (MA) dissolved in the mobile phase. The experiments were performed at 22 ± 1°C.

## RESULTS AND DISCUSSION

Fig. 1 illustrates the behaviour of the values of the term $(CyD)_m/(V_0 - V_{obs})$ for (*R*)-(−)- and (*S*)-(+)-mandelic acid as a function of the β-cyclodextrin concentration. The plots enable evaluation of the stability constants of both diastereomeric complexes [(*R*)-(−)-MA–CyD and (*S*)-(+)-MA–CyD], using equation 1, derived by Uekama *et al.*[11]:

$$\frac{(CyD)_m}{V_0 - V_{obs}} = \frac{1}{V_0 - V_c}(CyD)_m + \frac{1}{K_c(V_0 - V_c)} \quad (1)$$

where $(CyD)_m$ is the β-cyclodextrin concentration (moles/l), $V_0$, $V_c$, $V_{obs}$ are the retention volumes of mandelic acid itself, of the inclusion complex of one of its enantiomers [(*R*)-(−)-MA–CyD) or (*S*)-(+)-MA–CyD] and of the corresponding enantiomer observed at a given CyD concentration, respectively, and $K_c$ is the stability constant of a given inclusion complex in the mobile phase:

$$K_c = \frac{(MA\text{–}CyD)_m}{(MA)_m(CyD)_m}$$

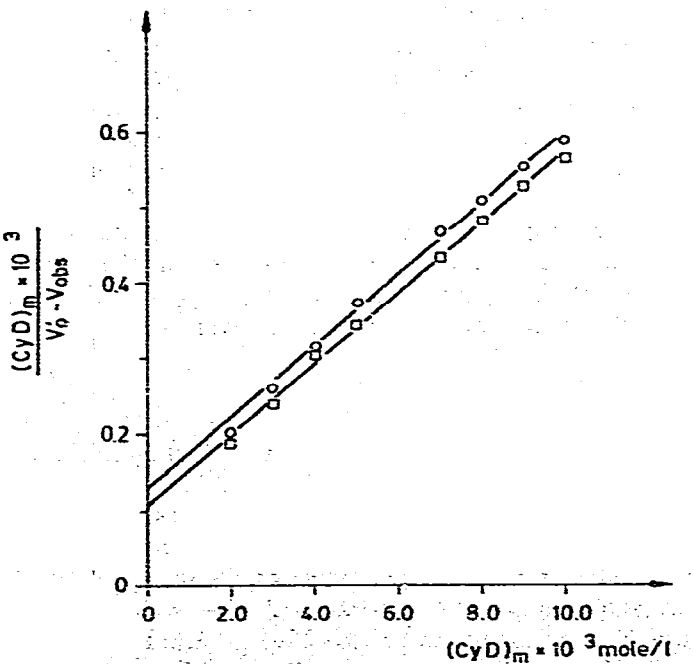


Fig. 1. Values of the term $(CyD)_m/(V_0 - V_{obs})$ for (*R*)-(−)- and (*S*)-(+)-mandelic acid *versus* CyD concentration in the mobile phase solution.

TABLE I

EQUILIBRIUM DISTRIBUTION COEFFICIENT OF MANDELIC ACID ($k$), EQUILIBRIUM DISTRIBUTION COEFFICIENTS OF INCLUSION COMPLEXES FORMED WITH CyD BY ITS ENANTIOMERS ($k_c$) AND THE STABILITY CONSTANTS OF THESE COMPLEXES ($K_c$)

| *Compound* | $k$ | $k_c$ | $K_c$ |
|---|---|---|---|
| (*R*)-(−)-MA | 19.2 | 5.9 | 460 |
| (*S*)-(+)-MA | 19.2 | 6.3 | 410 |

Table I shows the equilibrium distribution coefficient ($k$) of mandelic acid, determined in 0.1 *M* aqueous phosphate buffer of pH 2.1, the equilibrium distribution coefficients ($k_c$) of inclusion complexes formed with CyD by its enantiomers determined in $1.2 \cdot 10^{-2}$ *M* CyD in the same buffer and the stability constants ($K_c$) of these complexes evaluated from the plots shown in Fig. 1 and from equation 1[11].

Equation 1, originally derived for ion-exchange sorbents[11] was adapted by us for the reversed-phase system on the assumption that only neutral organic species are adsorbed onto the stationary phase. This assumption is based on our findings that the capacity factors of MA and of its inclusion complexes are approximately zero in the mobile phase solution at pH $>$ p$K_a$ + 2, since the p$K_a$ of MA amounts to 3.4 at pH $>$ 5.4.

Two chromatograms for two consecutive injections of the mixture of MA enantiomers are shown in Fig. 2. It is evident that the enantiomers were eluted at different rates (selectivity factor $\alpha = k_1/k_2 = 1.05$).

Similar experiments were successfully performed using phenylalanine as an example of an amino acid.

The ability of $\beta$-cyclodextrin to form inclusion complexes with various organic compounds of acidic, basic and neutral character, together with knowledge of the

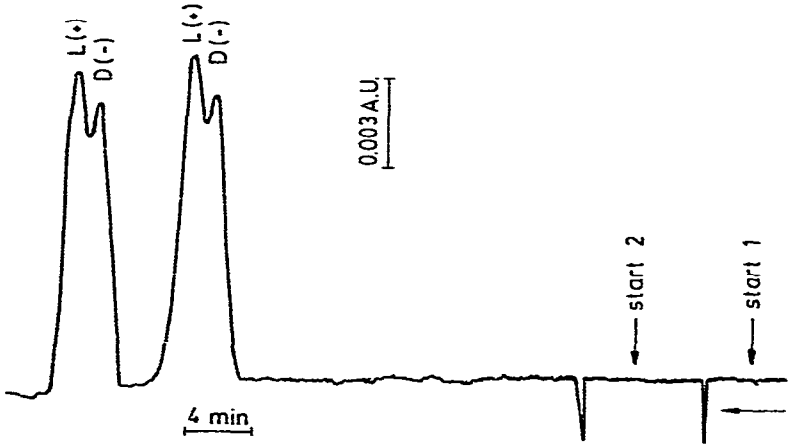


Fig. 2. Elution curves for two consecutive injections of the mixture of $2.10 \cdot 10^{-4}$ *M* mandelic acid enantiomers. Mobile phase composition, $5 \cdot 10^{-3}$ *M* CyD in aqueous phosphate buffer of pH 2.1 (0.1 *M*); flow-rate, 0.6 ml min$^{-1}$.

kinetics of the inclusion processes, may be of great importance in solving the problem of chromatographic racemate resolution. Attempts at improving the separative power in our system are under way.

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