Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Effect of Organic Cosolvents on Enantio-Enrichments via Cyclodextrin-Based Precipitations: An Examination of Production Efficiency

Eve Y. Zhou[a]; Gary L. Bertrand[a]; Daniel W. Armstrong[a]
[a] DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MISSOURI-ROLLA, MISSOURI

To cite this Article Zhou, Eve Y. , Bertrand, Gary L. and Armstrong, Daniel W.(1995) 'Effect of Organic Cosolvents on Enantio-Enrichments via Cyclodextrin-Based Precipitations: An Examination of Production Efficiency', Separation Science and Technology, 30: 11, 2259 — 2276
To link to this Article: DOI: 10.1080/01496399508013111
URL: http://dx.doi.org/10.1080/01496399508013111

SEPARATION SCIENCE AND TECHNOLOGY, 30(11), pp. 2259–2276, 1995

Effect of Organic Cosolvents on Enantio-Enrichments via Cyclodextrin-Based Precipitations: An Examination of Production Efficiency

EVE Y. ZHOU, GARY L. BERTRAND,
and DANIEL W. ARMSTRONG*
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF MISSOURI–ROLLA
ROLLA, MISSOURI 65401

ABSTRACT

Crystallization or precipitation of cyclodextrins in the presence of racemates has proven to be a useful method to enrich enantiomers. The technique is commonly performed in neat aqueous solutions which limits its utility in resolving many hydrophobic compounds with poor water solubility. In this work we demonstrate that it is possible to enhance the enantiomeric enrichment of (±)-3-dimethylaminopropiophenone using organic cosolvents via β-cyclodextrin precipitation. In addition, an equation that allows the calculation of the production efficiency of an enantiomeric enrichment is proposed and utilized. Water plus various organic solvents, including methanol, ethanol, isopropanol, 1-propanol, *tert*-butanol, and acetonitrile, are studied as cosolvents. Several factors are investigated in terms of their effect on the enantiomeric enrichment including a) molar ratio of β-cyclodextrin to analyte, b) solubility of the analyte, c) solubility of β-cyclodextrin, d) solution pH, and e) amount of analyte coprecipitated with β-cyclodextrin. The production efficiency of enantioselective crystallization in cosolvents is evaluated. The use of appropriate organic cosolvents appear to increase the usefulness of cyclodextrin coprecipitations for the production of enantiomerically enriched compounds.

INTRODUCTION

Although a large number of enantiomers can be resolved at the analytical level, preparative and process-scale chiral separations remain chal-

* To whom correspondence should be addressed.

lenging and often difficult. When a high-performance liquid chromatography (HPLC) enantiomeric separation is scaled up from the analytical level, it often suffers from drawbacks such as insufficient efficiency, tedious operation, poor capacity, and high cost of chiral stationary phases. Investigation and development of new effective and efficient methods for large-scale enantiomeric separations are of interest in many areas (1).

Cyclodextrins are naturally occurring optically active molecules. They can interact with many racemic compounds when dissolved in aqueous solution, thereby forming diastereomeric inclusion complexes of different stabilities. Cyclodextrins and their derivatives have proven to be very useful as chiral stationary phases for the separation of enantiomers by HPLC (2–6), GC (6–16), and TLC (17, 19); or as chiral mobile phase additives in HPLC (2, 6, 18), TLC (2, 6, 19–21), and CE (22–29). Cyclodextrins also have been used to enrich enantiomers in conjunction with other separation techniques including membrane methods (30), foam flotation (31), solid–liquid phase adsorption (32), and crystallization (33, 34). There are several advantages in using cyclodextrin-based crystallization procedures to resolve bulk racemates over the more classical crystallization approaches (33, 34). For example, cyclodextrins complex with neutral as well as ionizable molecules. The classic method of making diastereomeric salts requires that the racemic molecule have an acid or base functionality. Otherwise, derivatization is necessary. The use of cyclodextrin as the chiral selector eliminates the need for derivatization or charged functional groups. Naturally occurring alkaloids are commonly used as chiral selectors in classic crystallization-based separation methods. These and other resolving agents often are highly toxic. They also tend to have strong absorbance in the UV spectral region and often fluoresce as well. Cyclodextrins, on the other hand, are nontoxic, non-UV-absorbing, easily recyclable, and biodegradable.

It is well known that β-cyclodextrin has low solubility (1.85 g/100 mL) (35) in water at ambient temperatures. Various methods have been used to increase the solubility, including the addition of urea (19–21, 35), metal salts (36, 37), and small amounts of common organic solvents, such as ethanol, isopropanol, acetonitrile, tetrahydrofuran, and dimethylsulfoxide (35, 38, 39). Enantioselective crystallization with cyclodextrins is most commonly carried out in neat aqueous solutions (33, 34). This limits its usefulness in resolving a large number of hydrophobic compounds which have poor solubility in aqueous solutions. Addition of organic solvents to the aqueous medium is often essential for increasing the solubility of a compound, but it is often considered as deleterious in precipitation or crystallization-based enantioseparations due to the fact that organic solvents can possibly alter or perturb the cyclodextrin inclusion complexation process (6, 35). Solution studies have shown that the addition of

miscible organic modifiers to water can affect a number of properties associated with cyclodextrin inclusion complex formation (e.g., chromatographic selectivity and retention, fluorescence intensity, binding constants, stoichiometry, etc.). For example, both the retention and selectivity in reversed-phase HPLC and TLC on bonded cyclodextrin stationary phases are influenced by addition of methanol and acetonitrile as mobile phase additives (2, 3, 18, 40). The selectivity in reversed-phase HPLC with β-cyclodextrin as the chiral mobile phase additive is also affected by organic modifier (38, 41). The fluorescence intensities of aqueous cyclodextrin:guest complexes can be enhanced by introducing organic cosolvents into the aqueous medium (42–45). In these cases, organic additives are thought to either modify the cyclodextrin cavity to give guests "a tighter fit" [e.g., acting as a space regulator (43)] or participate in the inclusion complex with the guest to form ternary complexes which are more stable than the binary complexes (35). The binding constants of most compounds to cyclodextrins, as well as their stoichiometry, can be altered by cosolvents (46).

The structures of cyclodextrin inclusion complexes can differ significantly in the crystalline state from the solution state. In solution, the organic guest molecules reside largely in the cavity but are in dynamic equilibrium with the bulk solution as well as with other cyclodextrin molecules. In the crystalline state, the guest molecules can be accommodated not only in the cyclodextrins' cavity but also in the intermolecular cavities formed by the crystal lattice or sandwiched between cyclodextrin molecules (35). A number of diastereomeric crystallizations have been accomplished in neat aqueous solutions (33, 34). However, the effect of organic cosolvents on enantiomeric enrichment via cyclodextrin precipitation has not been examined to our knowledge.

The goal of this study is to develop a viable large-scale chiral separation method for a hydrophobic compound, 3-dimethylaminopropiophenone (3-DAP), which is the precursor of an analgesic drug Oxyphene (*N*-dimethyl-2-methyl-3-hydroxyl-3,4-diphenylbutylamine). Another objective is to use this hydrophobic racemate as a model compound to evaluate the various effects of organic cosolvents on enantiomeric enrichment via crystallization with β-cyclodextrin. Finally, equations describing the production efficiency of the enriched product are proposed and evaluated.

EXPERIMENTAL

Materials

Both bulk β-cyclodextrin and the 25 cm (4.6 cm i.d.) β-cyclodextrin-bonded phase column (Cyclobond I) were obtained from Advanced Separation Technologies, Inc. (Whippany, New Jersey). All solvents were pur-

chased from Fisher Scientific (Pittsburgh, Pennsylvania). (±)-3-Dimethylaminopropiophenone was provided by Mallinckrodt Inc. (St. Louis, Missouri). Disposable syringe filters (0.45 μ) were purchased from Alltech Associates, Inc. (Deerfield, Illinois).

Apparatus

A HPLC unit consisting of a Shimadzu model LC-6A solvent delivery module, a SPD-6A UV detector, and a CR2AX Chromatopac recorder was used for all data analysis. The wavelength was set at 254 nm, and the mobile phase flow rate was 1 mL/min. The mobile phase was 1% triethylammonium acetate buffer (pH 4.1). Up to 20 μL of sample was injected, and the HPLC column containing β-cyclodextrin stationary phase was operated at ambient temperature.

Procedure

For all experiments, 113 mg β-cyclodextrin was placed in a vial containing 1 mL distilled water. This produces a heterogeneous solution at room temperature. However the excess cyclodextrin will dissolve at elevated temperature, producing a ~0.1 M solution. Sufficient racemic analyte, (±)-3-dimethylaminopropiophenone, was weighed so that the desired molar ratio of analyte to β-cyclodextrin was obtained. The vial was heated on a hot plate until cyclodextrin was completely dissolved. The organic cosolvent was added at this point when applicable. The vial was then capped and allowed to cool to room temperature before being stored at 4°C for 15–30 hours. The mother liquid was withdrawn by a syringe and filtered through a disposable syringe filter prior to examination for enantiomeric purity.

The dissolution test of (±)-3-dimethylaminopropiophenone was done by titration. The desired organic solvent was slowly added drop by drop with continuous agitation till a clear solution was formed.

RESULTS AND DISCUSSION

Factors Affecting Enantio-Enrichments

Many parameters are known to affect enantioselective precipitations or crystallizations with cyclodextrins in aqueous solution, including the size of the cyclodextrin cavity, pH, the molar ratio of host to guest, etc. (33). The optical enrichment of 3-dimethylaminopropiophenone was affected by these experimental conditions in ways very similar to the previously reported enrichments of dansyl amino acids (33). In particular, enrichment can only be achieved in the presence of β-cyclodextrin. No enantioselectivity was observed with either α- or γ-cyclodextrin. The solution pH also

affected enantioselectivity. A maximum enantiomeric enrichment was observed at pH 8 as shown in Table 1. The fact that the enantioselectivity for many ionizable racemates is pH dependent is not surprising and has been documented previously (3, 4, 33). The free base, (±)-3-dimethylaminopropiophenone, is hydrophobic and cannot be readily dissolved in water. However, it becomes increasingly soluble with the addition of miscible organic modifiers. As can be seen from Table 2, 0.3% (w/v) of added analyte forms homogeneous hydro-organic solutions as long as least 20–30% (by volume) of a miscible organic modifier is present. As noted in other investigations, the formation of a cyclodextrin:analyte complex may be an important requirement in a crystallization-based separation technique (6, 35). Complexation, however, does not necessary result in an enantiomeric enrichment unless it also is enantioselective. Furthermore, it might be possible that some stereospecific adsorption outside of the cyclodextrin cavities or on the crystal surface contributes to an enantiomeric enrichment. While it is clear that added organic solvents increase the solubility of the analyte (thereby making it more available to the cyclodextrin), they also compete with the analyte for the cyclodextrin cavity (i.e., inclusion complex formation). Therefore it is not clear a priori whether the presence of organic cosolvents will help or hurt an enantioselective precipitation process.

Figure 1(A) shows an optimum 42% enantiomeric excess for 3-DAP (equivalent to a enantiomeric ratio of 71:29) in the mother liquid with a

TABLE 1
Effect of pH on Enantiomeric Excess[a]

pH	% Enantiomeric excess[b]
3	12.2
6	15.6
8	34.4
9	24.4
10	6.4

[a] A 2:1 molar ratio of β-CD:3-DAP was used for all experiments. The pH was adjusted using acetic acid or triethylamine.

[b] Enantiomeric excess (Ee) is defined as follows: $Ee = [(C_1 - C_2)/(C_1 + C_2)] \times 100\%$, where C_1 is the enantiomer present in higher concentration and C_2 is the enantiomer present in lower concentration.

TABLE 2
Comparison Studies of Dissolution of 3-Dimethylaminopropiophenone (3-DAP) in Cosolvents[a]

Cosolvent % (v/v)		Methanol	Ethanol	1-Propanol	2-Propanol	*t*-Butanol	Acetonitrile
2.98 mg of	10	–	–	–	–	–	Colloid
3-DAP in	20	+	+	+	+	–	Colloid
1.0 mL	≥30	+	+	+	+	+	+
H_2O							
2.98 mg of	10	–	–	–	–	–	–
3-DAP in	20	–	–	–	–	–	Colloid
1.0 mL	30	–	–	–	Colloid	–	Colloid
H_2O	40	–	–	+	+	+	+
	50	Colloid	Colloid	+	+	+	+
	≥60	+	+	+	+	+	+

[a] The (–) symbol refers to a mixture (heterogeneous solution) in which 3-DAP is not dissolved. The (+) symbol means that there is a clear homogeneous solution (i.e., the 3-DAP is completely dissolved). All experiments were performed at ambient temperatures.

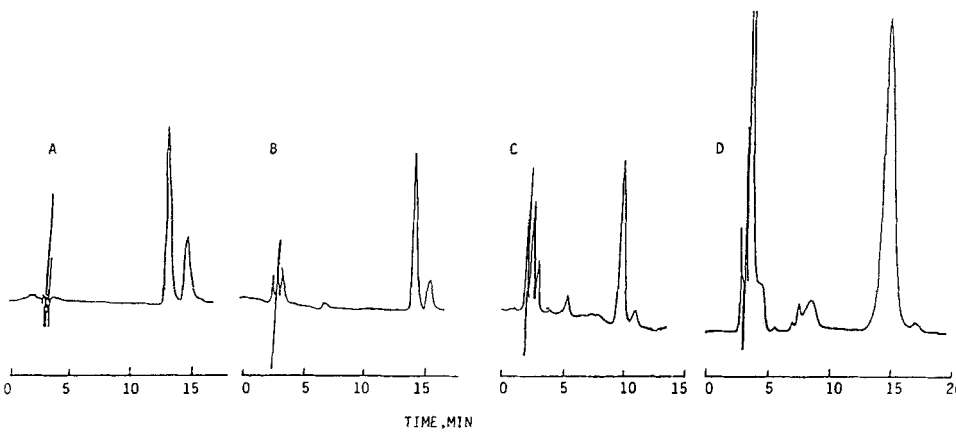

FIG. 1 HPLC chromatograms of 3-dimethylaminopropiophenone showing the enantiomeric enrichment in the mother liquid after crystallization in (A) 0.1 M β-cyclodextrin neat aqueous solution with a 2:1 molar ratio of cyclodextrin:analyte, 22 hours of crystallization at 4°C; (B) 50% (v/v) isopropanol cosolvent, and the rest of the conditions are identical to (A); (C) 50% (v/v) isopropanol cosolvent with a molar ratio of 8:1, and the rest of the conditions are the same as for (A); and (D) recrystallization by mixing 1 mL of the mother liquid from (B) with 1 mL of 0.1 M β-cyclodextrin aqueous solution plus 0.5 mL of isopropanol for 20 hours. The HPLC conditions are indicated in the Experimental Section.

single step crystallization from aqueous solution. Figure 1(B) shows a 62% enantiomeric excess (enantiomeric ratio of 81:19) in the mother liquid when a cosolvent of 50% (by volume) isopropanol was used, but where all other experimental conditions were identical to those of Fig. 1(A). As can be seen, the enantiomeric excess was enhanced in the presence of the organic cosolvent. Hence it would be beneficial to examine and better understand the effects of miscible organic solvents on the enantiomeric excesses produced upon precipitation of cyclodextrin in the presence of a racemate. Interestingly, the molar ratio of cyclodextrin to analyte that produced the maximum enrichment was found to be different in aqueous solutions versus hydro-organic solutions, as shown in Figs. 2 and 1(C). Enantiomeric enrichments were obtained at molar ratios between 1:1 to 8:1 (cyclodextrin:3-DAP). In aqueous solution, enrichment was most pronounced at a ratio of two cyclodextrins to one 3-DAP (Fig. 2, Curve A). However, unlike the results in neat aqueous solution, enrichment was

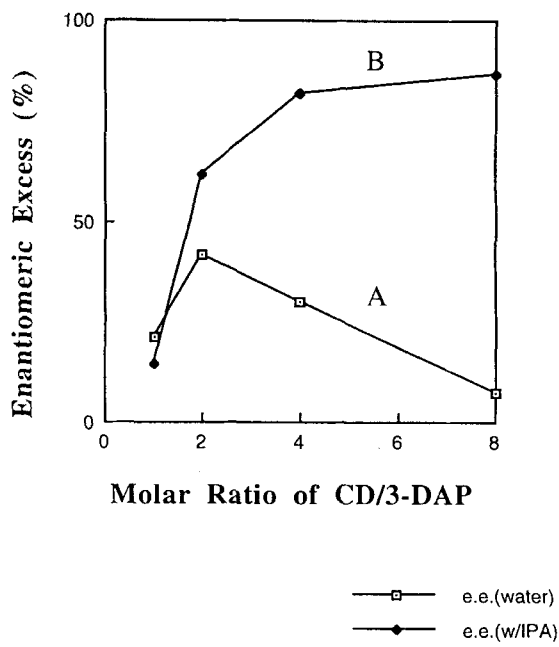

FIG. 2 Plots showing the enantiomeric excess as a function of molar ratio of β-cyclodextrin:analyte in (A) neat aqueous solution and (B) 50% (v/v) isopropanol cosolvent. The concentration of β-cyclodextrin solution was kept at 0.1 M, crystallization time was 22 hours, and the temperature was 4°C.

found to increase with the increasing molar ratio in the presence of organic cosolvents (Fig. 2, Curve B). As a result, the enantiomeric enrichment of (±)-3-dimethylaminopropiophenone can be significantly improved by simply using a higher molar ratio of β-cyclodextrin to analyte in hydro-organic solvents. As shown in Fig. 1(C), an even greater enantiomeric excess (87%) can be obtained with a single step precipitation with a cyclodextrin: analyte ratio of 8:1, and using 50% isopropanol as a cosolvent.

In order to obtain a better understanding of β-cyclodextrin-mediated enantioselective precipitations in the presence of organic modifiers, a variety of organic solvents were investigated including methanol, ethanol, 1-propanol, *tert*-butanol, and acetonitrile. The first thing that should be considered is the effect of these organic modifiers on the solubility of β-cyclodextrin. The solubility behavior of β-cyclodextrin in hydro-organic solvents has been studied by several groups previously (38, 39). The most thorough investigation was done by Chatjigakis and coworkers, in which the solubility of β-cyclodextrin in hydro-organic solvent mixtures was measured at a variety of volume ratios from neat water to high concentrations of the organic modifier. According to their results, the solubility of β-cyclodextrin increased with the increasing volume percentage of organic solvent, reaching a maximum at around 20–30% of the organic solvent. The solubility then decreased and eventually dropped to zero at high percentages of organic solvents such as acetonitrile and all alcohols studied, except methanol. Addition of methanol to the aqueous solution depressed the solubility of β-cyclodextrin regardless of the amount introduced (39).

We examined the effects of organic modifiers on the enantiomeric enrichment in cyclodextrin-based crystallizations or precipitations. Figures 3 through 6 show both the solubility of β-cyclodextrin (Curve A) and the enantiomeric excesses of (±)-3-dimethylaminopropiophenone in the mother liquor (Curve B) as a function of the volume percentage of various organic solvents. It is interesting that the shapes of the curves for enantiomeric enrichments appear to be similar to the β-cyclodextrin solubility curves (Figs. 3–6). For instance, with acetonitrile cosolvents (Fig. 3), both the solubility of β-cyclodextrin and the enantiomeric enrichment increased to a maximum value at ~20% (by volume) organic additive, then decreased thereafter. Similar trends were observed with ethanol (Fig. 4) and isopropanol (Fig. 5) cosolvents. When using methanol as a cosolvent (Fig. 6), the enantiomeric excess decreased over the entire range of concentration as did the solubility of β-cyclodextrin. Although the solubility data were measured at ambient temperatures which differs from the actual temperatures of the crystallization or precipitation process (4°C), this temperature difference does not alter the shapes of the solubility curves significantly, according to the experimental measurements done by Chatjigakis et al.

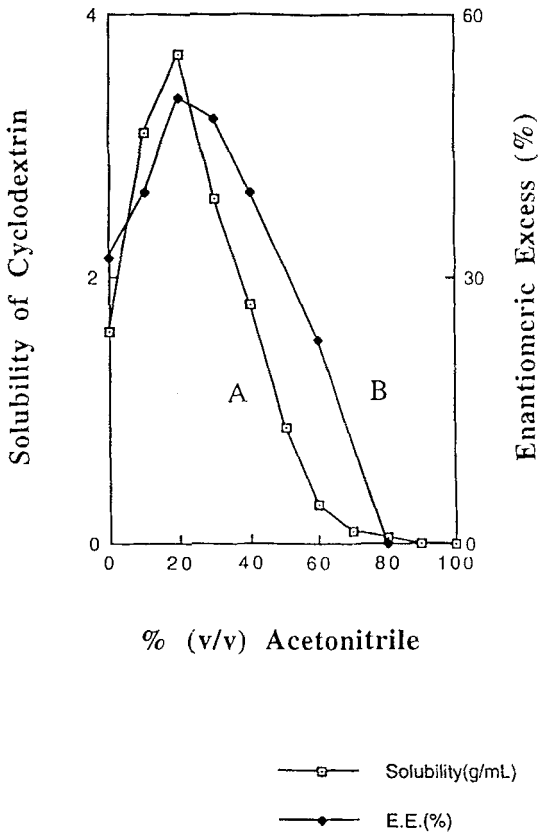

FIG. 3 Plots showing (A) solubility of β-cyclodextrin and (B) enantiomeric excess of 3-DAP as a function of volume percentage of isopropanol in aqueous solution at a molar ratio of 4:1, crystallization time of 16 hours, and a temperature of 4°C. The solubility data are from Ref. 39.

(39), Jozwiakowski and Connors (47), and discussed by Szejtli (35). The correlation between the enantiomeric enrichment and β-cyclodextrin solubility suggests that the enrichments in the presence of organic cosolvents are a function of the amount of β-cyclodextrin in solution. The increased solubility of β-cyclodextrin improves the enrichment while decreased solubility reduces the enrichment. It is possible that the effectiveness of an enantioselective crystallization may be predicted, in part, via a β-cyclodextrin solubility factor. In addition, both 1-propanol and *tert*-butanol cosolvents were studied, although no previous solubility data were avail-

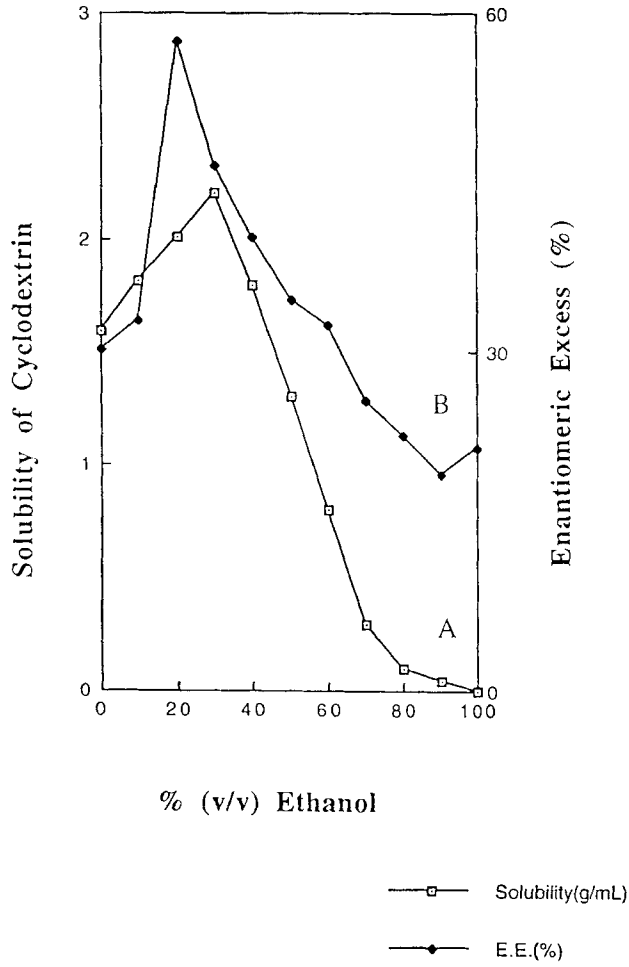

FIG. 4 Plots showing (A) solubility of β-cyclodextrin and (B) enantiomeric excess of 3-DAP as a function of volume percentage of ethanol in aqueous solution at a molar ratio of 4:1, a crystallization time of 15 hours, and a temperature of 4°C. The solubility data are from Ref. 39.

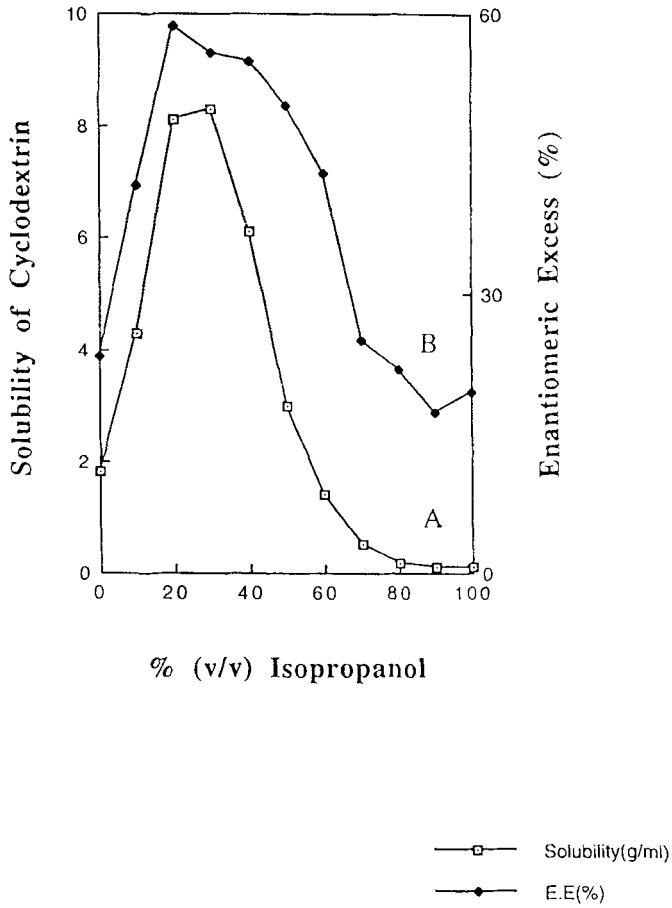

FIG. 5 Plots showing (A) solubility of β-cyclodextrin and (B) enantiomeric excess of 3-DAP as a function of volume percentage of acetonitrile in aqueous solution at a molar ratio of 4:1, a crystallization time of 20 hours, and a temperature of 4°C. The solubility data are from Ref. 39.

able in these cases (Figs. 7 and 8). Their enantiomeric enrichment curves suggest that these two alcohols resemble ethanol or isopropanol, but not methanol, in their behavior as cosolvents.

Evaluation of Production Efficiency

The efficiency of crystallization or precipitation with hydro-organic solvents was considered. The yield of purified enantiomers is an important

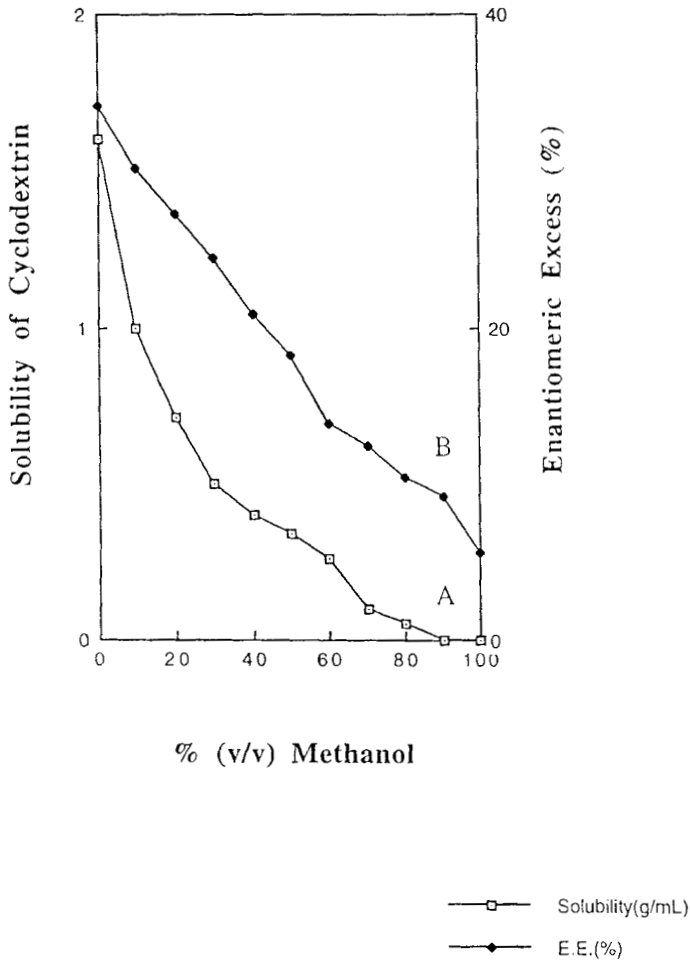

FIG. 6 Plots showing (A) solubility of β-cyclodextrin and (B) enantiomeric excess of 3-DAP as a function of volume percentage of methanol in aqueous solution at a molar ratio of 4:1, a crystallization time of 20 hours, and a temperature of 4°C. The solubility data are from Ref. 39.

issue for large-scale separations. The production efficiency measures the enantiomeric enrichment per unit of chiral separation agent. If a quantity of separation agent (N_z moles) is added to a solution containing the racemate at an initial amount of N_i (the initial moles of enantiomer $N_{iR} = N_{iS} = 0.5N_i$), then after precipitation the solution contains the remaining

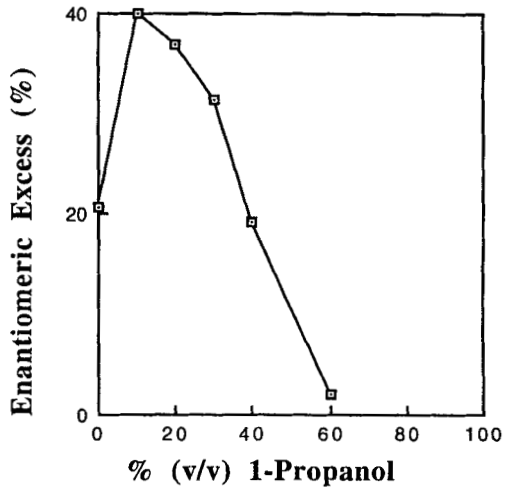

FIG. 7 Plot showing the enantiomeric excess of 3-DAP as a function of volume percentage of 1-propanol in aqueous solution at a molar ratio of 4:1, a crystallization time of 27 hours, and a temperature of 4°C.

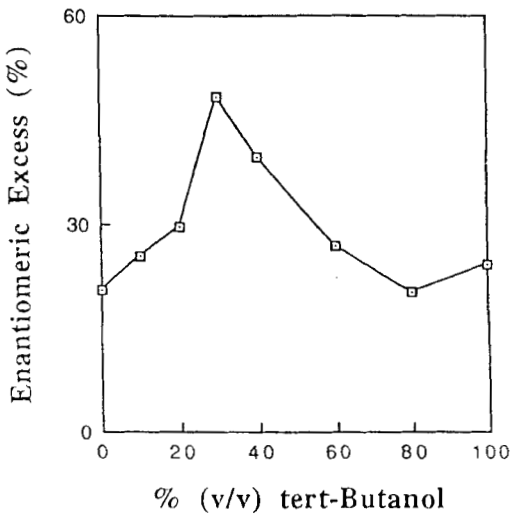

FIG. 8 Plot showing the enantiomeric excess of 3-DAP as a function of volume percentage of *tert*-butanol in aqueous solution at a molar ratio of 4:1, a crystallization time of 25 hours, and a temperature of 4°C.

dissolved enantiomers at concentrations of N_R and N_S (assume for the purpose of this discussion that $N_R > N_S$). Further purification of either the solution phase or the precipitate phase is likely to be limited to one pure component and the racemate. The maximum efficiency is then related to the excess moles of either component in a phase divided by the number of moles of agent required to effect this separation. We suggest that the production efficiency (ϵ) can be calculated with the following equation:

$$\epsilon = (N_R - N_S)_{solution}/N_z = (N_S - N_R)_{precipitate}/N_z. \quad (1)$$

In the mother liquid, the enantiomeric excess (Ee) can be expressed as:

$$(Ee)_{solution} = [(N_R - N_S)_{solution}/N_{solution}] \times 100\% \quad (2)$$

or since $N_{solution} = N_i - N_{precipitate}$,

$$(Ee)_{solution} = [(N_R - N_S)_{solution}/(N_i - N_{precipitate})] \times 100\% \quad (3)$$

As can be seen from Eq. (3), the enantiomeric excess in the mother liquid is determined by the excess moles of enantiomer in the mother liquid and the total moles of analyte in the precipitate phase. It is possible to increase the enantiomeric excess in the mother liquid by increasing the amount of analyte in the precipitate phase without changing the enantioselectivity (which is indicated by the difference: $N_R - N_S$).

Figure 9 shows both the production efficiency profile (Curve A) and the total moles of analyte (Curve B) in the precipitate phase with an ethanol cosolvent (at different volume percentages). Two things should be noted here: 1) the production efficiency (Fig. 9, Curve A) and the enantiomeric enrichment (Fig. 4, Curve B) are affected very differently by added organic solvents as their curves are roughly opposite to one another, and 2) the production efficiency curve is also roughly opposite to the moles of analyte curve (Fig. 9). The only logical explanation for this is that the enhanced enrichments at certain organic modifier concentrations must result from a greater removal of analyte from the mother liquid (a larger $N_{precipitate}$ value in Eq. 3) and not from any improvement in the enantioselectivity (reflected by the difference between N_R and N_S in Eqs. 1 and 3). If improved enantioselectivity was the cause of the enhanced enrichment, the efficiency would be seen to increase as well (from Eq. 1). As can be seen from Fig. 9, the production efficiency decreased when the amount of analyte in the precipitate phase increased. In fact, it appears that the enantioselectivity was somewhat depressed with added cosolvent. This is because, as indicated in Eq. (1), $(N_R - N_S)$ must become smaller if the efficiency decreases with increasing $N_{precipitate}$. Similar efficiency profiles and moles of analyte curves were observed with all cosolvents studied.

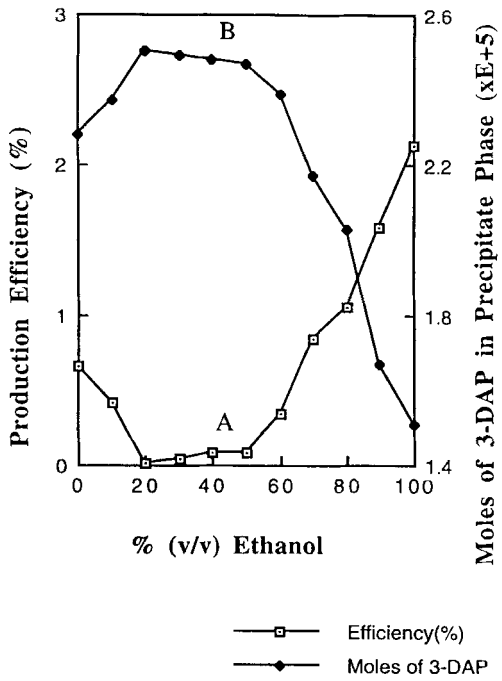

FIG. 9 Plots showing (A) the production efficiency and (B) the total concentration of 3-DAP (mg/mL) in mother liquid as a function of the volume percentage of ethanol additive in aqueous solution.

Figure 10 shows the efficiency (Curve A) and the enantiomeric excess (Curve B) as a function of molar ratio of β-cyclodextrin to analyte in 50% (v/v) isopropanol cosolvent (using a constant β-cyclodextrin solution concentration of 113 mg/mL and different amounts of racemate). The production efficiency reached a peak value at the molar ratio of 2 and decreased dramatically thereafter (Fig. 10). In this case the enantiomeric enrichment increases with the increasing molar ratio, which contributes positively to the production efficiency, but the increasing amount of cyclodextrin (relative to analyte) reduces the production efficiency. Obviously there is a trade-off between production efficiency and the degree of enrichment in this precipitation system.

Besides their efficacy in enhancing enantiomeric enrichments, there is another less obvious advantage to using hydro-organic solvents. Mixed solvent systems will be needed if recrystallization or reprecipitation is necessary for further purification. Since enantiomeric enrichment in neat

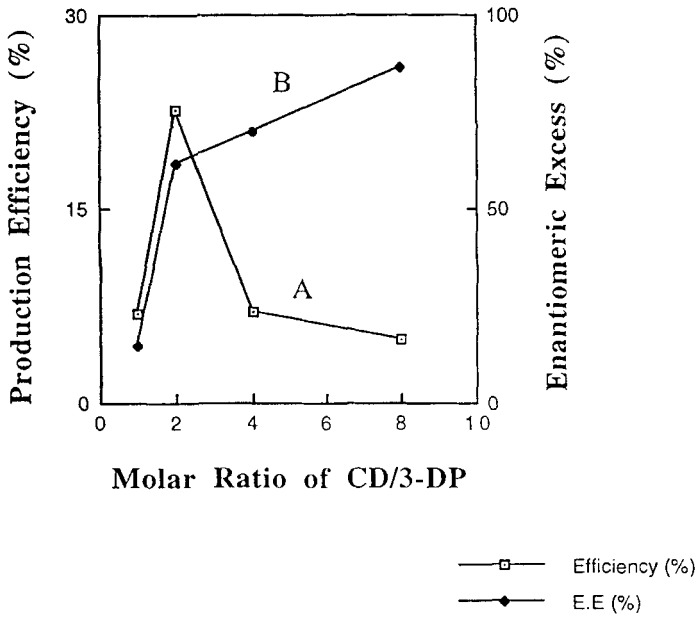

FIG. 10 Plots showing (A) the production efficiency and (B) the enantiomeric excess as a function of molar ratio of β-cyclodextrin:3-DAP in isopropanol cosolvent. The experimental conditions are described in Fig. 1(B).

water is most pronounced at molar ratios near 2:1 cyclodextrin to analyte (Fig. 2, Curve A), the exact concentration of both the analyte and cyclodextrin must be known before fresh chiral separation agent can be added to maintain the efficient separation. This becomes much less a problem in hydro-organic solvent mixtures since any molar ratio of cyclodextrin to analyte greater than 2:1 gives a high degree of enrichment (Fig. 2, Curve B; Figs. 1, Curves B, C, and D). For example, more than 99% of one enantiomer (an enantiomeric ratio of 99.3:0.7) was obtained after a second precipitation in an isopropanol cosolvent (Fig. 1, Curve D).

Every racemate will have certain characteristics (e.g., different pK_as, hydrophobicities, functional groups, etc.) that must be accounted for by varying the experimental conditions. In some cases cyclodextrins may not be the best chiral precipitating agents. However, regardless of the precipitating agent used, the mathematical and experimental approach described herein should be useful in understanding and optimizing the separation process. The equations for production efficiency and enantiomeric excess are highly useful in understanding and optimizing the com-

peting factors that affect and control the production of enriched enantiomers in a precipitation-based separation.

ACKNOWLEDGMENTS

Support of this work by the Biotechnology Research and Development Corporation and by the National Institute of Health (Grant NIH BMT 2R01 GM36292-04) is gratefully acknowledged.

REFERENCES

1. S. C. Stinson, *Chem. Eng. News*, *71*, 38 (1993).
2. M. L. Hilton and D. W. Armstrong, "Contribution of Cyclodextrins and Derivatives to Liquid Chromatography," in *New Trends in Cyclodextrins and Derivatives* (D. Duchene, Ed.), Editions DE Sante, Paris, 1991, p. 517.
3. S. M. Han and D. W. Armstrong, *Chiral Separations by HPLC, Applications to Pharmaceutical Compounds* (A. M. Krstulovic, Ed.), Ellis Horwood Limited, Chichester, UK, 1989, p. 208.
4. T. J. Ward and D. W. Armstrong, *J. Liq. Chromatogr.*, *9*(2,3), 407 (1986).
5. D. W. Armstrong, *Ibid.*, *7*(S-2), 353–376 (1984).
6. W. L. Hinze, *Sep. Purif. Methods*, *10*(2), 159–237 (1981).
7. A. Berthod, W. Y. Li, and D. W. Armstrong, *Carbohydr. Res.*, *201*, 175 (1990).
8. D. W. Armstrong, W. Y. Li, C. D. Chang, and J. Pitha, *Anal. Chem.*, *62*, 914 (1990).
9. D. W. Armstrong, W. Y. Li, A. M. Stalcup, H. V. Secor, R. R. Izac, and J. I. Seeman, *Anal. Chem. Acta*, *234*, 365 (1990).
10. W. A. König, S. Lutz, O. Mischnich-Lubbecke, B. Brassat, and G. Wenz, *J. Chromatogr.*, *447*, 193 (1988).
11. W. A. König, S. Lutz, G. Wenz, C. Gorgen, C. Newmann, A. Gabler, and W. Boland, *Angew. Chem.*, *101*, 351 (1989).
12. V. Schurig, A. Ossig, and R. Link, *Ibid.*, *101*, 197 (1989).
13. V. Schuring and H.-P. Novotny, *J. Chromatogr.*, *441*, 155 (1988).
14. W. Li, H. L. Jin, and D. W. Armstrong, *Ibid.*, *509*, 303 (1990).
15. D. W. Armstrong, Y. Tang, T. Ward, and M. Nichols, *Anal. Chem.*, *65*, 1114 (1993).
16. Y. Tang, Y. Zhou, and D. W. Armstrong, *J. Chromatogr. A*, *666*, 147 (1994).
17. A. Alak and D. W. Armstrong, *Anal. Chem.*, *58*, 582 (1986).
18. M. Pawlowska and J. Zukowski, *Mikrochim. Acta*, *III*, 55–69 (1990).
19. S. M. Han and D. W. Armstrong, in *Planar Chromatography in the Life Sciences* (J. C. Touchstone, Ed.), Wiley, New York, 1990, p. 81.
20. J. D. Duncan, *J. Liq. Chromatogr.*, *13*(14), 2737 (1990).
21. J. D. Duncan and D. W. Armstrong, *J. Planar Chromatogr.*, *4*, 204 (1991).
22. K. Otsuka and S. Terabe, *J. Chromatogr.*, *515*, 221 (1990).
23. H. Nishi, T. Fukuyana, and S. Terabe, *Ibid.*, *553*, 503 (1991).
24. S. Fanali, *Ibid.*, *545*, 437 (1991).
25. A. Shibukawa, D. K. Lloyd, and I. W. Wainer, *Chromatographia*, *35*, 419 (1993).
26. J. Snopek, H. Soinic, M. Novatny, E. Smolkova-Keulemansova, and I. Jelinek, *J. Chromatogr.*, *559*, 215 (1991).
27. A. Guttman, A. Paulua, A. S. Cohen, N. Grinberg, and B. K. Karger, *Ibid.*, *448*, 41 (1988).

28. S. Mayer and V. Schurig, *J. High Resolution Chromatogr., 15*, 129 (1992).
29. S. Mayer and V. Schurig, *J. Liq. Chromatogr., 16*, 915 (1993).
30. D. W. Armstrong and H. Jin, *Anal. Chem., 59*, 2237 (1987).
31. D. W. Armstrong, E. Y. Zhou, S. Chen, K. Le, and Y. Tang, *Ibid., 66*, 4278 (1994).
32. D. W. Armstrong, E. Y. Zhou, and S. Chen, Unpublished Results.
33. H. Jin, A. Stalcup, and D. W. Armstrong, *Chirality, 1*, 137 (1989).
34. M. Mikolajozyk and J. Drabowicz, *J. Am. Chem. Soc., 100*, 2510 (1978).
35. J. Szejtli, *Cyclodextrins & Their Inclusion Complexes*, Akademic Kiado, Budapest, 1982, pp. 1–185.
36. A. Buvari and I. Barcza, *J. Incl. Phenom. Mol. Recognit. Chem., 7*, 379 1989.
37. A. W. Coleman and I. Nicolis, *Ibid., 13*(2), 139 (1992).
38. M. Taghvaei and G. H. Stewart, *Anal. Chem., 63*, 1902 (1991).
39. A. Chatjigakis, C. Donze, and A. W. Coleman, *Ibid., 64*, 1632 (1992).
40. D. W. Armstrong, G. L. Bertrand, K. D. Ward, and T. J. Ward, *Ibid., 62*, 332 (1990).
41. J. Zukowski, D. Sybilska, and J. Jurezak, *Ibid., 57*, 2215 (1985).
42. G. Nelson, G. Patonay, and I. M. Warner, *Ibid., 60*, 274 (1988).
43. A. Ueno, K. Takahashi, Y. Hino, and T. Osa, *J. Chem. Soc., Chem. Commun.*, p. 194 (1981).
44. K. Kano, I, Takenoshita, and T. Ogawa, *Chem. Soc. Jpn., Chem. Lett.*, p. 321 (1982).
45. C. D. Tran and J. H. Fendler, *J. Phys. Chem., 88*, 2167 (1984).
46. V. C. Anigbogu, A. M. de la Peña, T. T. Ndou, and I. M. Warner, *Anal. Chem., 64*, 484 (1992).
47. M. J. Jozwiakowski and K. A. Connors, *Carbohydr. Res., 143*, 51 (1985).

Received by editor October 13, 1994