

# Resolution of Ephedrine in Supercritical CO<sub>2</sub>: A Novel Technique for the Separation of Chiral Drugs

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**Abstract** □ Racemic ephedrine has been resolved by diastereomeric salt formation with mandelic acid using supercritical CO<sub>2</sub> as precipitating agent. Crystallizations were performed using the Solution Enhanced Dispersion by Supercritical Fluids (SEDS) technique. Temperature was varied between 35 and 75 °C, and pressures ranged from 100 to 350 bar. Resolution, determined by chiral capillary electrophoresis, is described as a function of temperature and density of the supercritical fluid. A comparison of SEDS-produced material with a conventional resolution method shows that SEDS-crystallized material exhibits identical properties to conventionally crystallized material.

## Introduction

Pharmacologically active substances containing chiral centers can exist as pairs of enantiomers, which exhibit the same physical properties but show a different molecular conformation. Since, in general, only one of the enantiomers is biologically active or possesses pharmacological relevance,<sup>1–3</sup> the resolution of enantiomers is of great importance for the pharmaceutical industry.<sup>4–6</sup> Pure enantiomers can be obtained either from chiral starting materials or by asymmetric synthesis.<sup>7</sup> While chiral natural products always exhibit very high enantiomeric purity, only a few natural products show a desired pharmacological activity. By contrast, synthetic drugs are often produced as racemates. It is therefore necessary to have a simple but powerful technique to resolve racemates.

Racemates have been resolved by a variety of methods. If the racemate exists as racemic conglomerate, it will resolve spontaneously when crystallized.<sup>8</sup> In 1850, Pasteur discovered the resolution of sodium ammonium tartrate<sup>9</sup> and was able to separate the enantiomers by sorting them under a microscope. Another method of resolving conglomerates is by seeding a supersaturated solution with crystals of one enantiomer.<sup>10</sup> Additional methods for the resolution of racemates have involved either crystallization by entrainment,<sup>11</sup> or optically active solvents.<sup>12</sup> A detailed description of resolution techniques in terms of thermodynamics is given by Jacques et al.<sup>13</sup>

If the compound of interest is either a carboxylic acid or an organic base, resolution can be achieved by diastereomeric salt formation.<sup>13–16</sup> In many cases a racemate of a chiral carboxylic acid has been resolved using a naturally occurring chiral amine.<sup>17</sup> However, resolution is usually not complete after a single crystallization, and the partially

resolved material has to be recrystallized. A classical example is the resolution of racemic ephedrine with (*R*)-mandelic acid, a procedure reported almost 70 years ago.<sup>18</sup> The authors separated diastereomeric (*R*)-mandelates by crystallization from ethanol, but the initial crop had to be recrystallized several times to achieve a pure product.

Conventional crystallization from organic solvents can often lead to solvent inclusion in the crystal. However, it is known that organic substrates crystallized from supercritical CO<sub>2</sub> produce crystals with solvent levels below 25 ppm.<sup>19</sup> Additionally, supercritical CO<sub>2</sub> has been used to produce a variety of materials of defined crystal size.<sup>20,21</sup> Supercritical CO<sub>2</sub> might therefore provide an alternative method for the resolution of racemates. This possibility was briefly investigated by Fogassy et al.,<sup>22</sup> who precipitated diastereomeric mixtures of several similar chiral carboxylic acids onto glass beads and extracted them with supercritical CO<sub>2</sub>. Although partial resolution was achieved and both enantiomers were recovered, the degree of resolution was poor. Although resolution was explained in terms of acid–base molecular recognition, no attempt was made to exploit the benefits of the supercritical phase.

We present a simple and reliable method to resolve racemic ephedrine with (*R*)-mandelic acid by crystallization from supercritical CO<sub>2</sub>. We show that a high degree of resolution can be achieved within a single crystallization. Furthermore, the product is highly crystalline unlike the conventionally resolved material.

## Experimental Section

**Chemicals**—(1*R*,2*S*)-ephedrine, (1*S*,2*R*)-ephedrine, and (*R*)-mandelic acid had a purity of >99% and were supplied by Aldrich (Gillingham, UK). Methanol was >99.9% and was supplied by BDH Chemicals (Poole, UK). CO<sub>2</sub> was 99.99% and supplied by BOC (Manchester, UK). All chemicals were used without further purification.

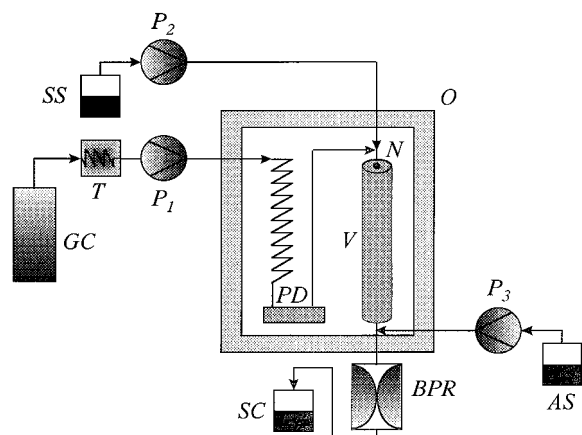
**Equipment**—Experiments were carried out in a SEDS (Solution Enhanced Dispersion by Supercritical Fluids) apparatus for crystallization in supercritical fluids. A scheme of the equipment is given in Figure 1. HPLC pumps P<sub>1–3</sub> (Jasco, model 880) were used to feed CO<sub>2</sub>, solute solution, and additional solvent to the crystallization vessel. Pressure pulses, originating from the pumps, were eliminated by a pulse dampener PD, made from a 1/4 in. stainless steel tube. CO<sub>2</sub> was supplied from a high-pressure cylinder GC, cooled to approximately –10 °C (*T*), and then pumped into the vessel. Simultaneously, solute solution SS was pumped into the crystallization vessel, through a specially designed nozzle N, consisting of two coaxial concentric tubes.<sup>23</sup> The crystallization vessel V (Keystone, 32 mL) was placed inside an oven O (ICI Instruments, TC1900), which controlled the temperature. Pressure was maintained with a backpressure regulator BPR (Jasco, model 880-81). A small additional stream of solvent (0.2 mL·min<sup>-1</sup>) was introduced at the bottom of the crystallization vessel AS to dissolve material soluble in supercritical CO<sub>2</sub>, preventing precipitation in the backpressure regulator. The used solvent was collected SC

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**Figure 1**—Scheme of the SEDS kit with: pumps  $P_i$ , pulse dampener PD, CO<sub>2</sub> cylinder GC, cooler T, solute solution SS, nozzle N, crystallization vessel V, oven O, backpressure regulator BPR, additional solvent AS, solvent collection SC.

after the back pressure regulator BPR. A more detailed description of the equipment and its operating procedure has been given elsewhere.<sup>19,24,25</sup>

**Procedure**—The general experimental procedure was as follows: 0.261 g (0.79 mmol) of (1*R*,2*S*)-ephedrine, 0.261 g (0.79 mmol) of (1*S*,2*R*)-ephedrine, and 0.239 g of (*R*)-mandelic acid (0.79 mmol) were dissolved in 40 mL of methanol (1.93 w/v%) and the solution was pumped at 0.2 mL·min<sup>-1</sup> together with 9 mL·min<sup>-1</sup> CO<sub>2</sub> into the vessel. During the experiment, the solvent dissolved in the supercritical CO<sub>2</sub>, leaving the solute behind. The precipitated solid was collected on a filter plate at the bottom of the vessel. After all solute solution had been fed into the vessel, the apparatus was flushed with CO<sub>2</sub> for 15 min. to remove solvent traces present in the vessel. Temperatures for the crystallization ranged from 35 to 75 °C, and pressures varied between 100 and 350 bar. Temperature during the crystallization was constant to ±0.5 °C, and pressure was constant within 1 bar. Densities of CO<sub>2</sub> were obtained from the IUPAC tables.<sup>26</sup> The small variations in temperature and pressure during any experiment result in a small error in density of the supercritical CO<sub>2</sub>, which is reflected in the error bars.

**Analysis**—Samples were analyzed using capillary electrophoresis (CE), using a Beckman P/ACE 2210 system with a fused silica column 27 cm length and an inner diameter of 50 μm. The buffer consisted of a 25 mM solution of dimethyl-β-cyclodextrin in 100 mM triethanolamine at pH 2.5. A voltage of 10 kV was applied producing a current around 50 μA. Separation temperature was 25 °C at a detection wavelength of 200 nm. Sample concentration was typically around 0.1 mg·mL<sup>-1</sup> in water. Experimental error for the resolution was 0.3 mol %.

Selected samples were investigated by differential scanning calorimetry (DSC) using a Mettler M3 system. A temperature program with a ramp of 2 °C·min<sup>-1</sup> from 80 to 200 °C was used for all samples. Error in melting point ( $T_m$ ) was ±0.5 °C. The enthalpy of fusion ( $\Delta H_f$ ) was determined by integration of the melting peak with an error of ±1.5 kJ·mol<sup>-1</sup>.

## Results and Discussion

**Pure Diastereomers**—The diastereomeric salts (1*S*,2*R*)-ephedrinium-(*R*)-mandelate ((+)-E(-)-MA) and (1*R*,2*S*)-ephedrinium-(*R*)-mandelate ((-)-E(-)-MA) were prepared with the SEDS equipment to obtain reference material for the separation. Experimental parameters for the crystallization of the pure diastereomers are summarized in Table 1. From Table 1 it can be seen that (-)-E(-)-MA was crystallized at the high pressure of 300 bar using methanol as solvent. In contrast, (+)-E(-)-MA was not obtained under these conditions, due to greater solubility in CO<sub>2</sub> than (-)-E(-)-MA. For crystallization of (+)-E(-)-MA, the pressure had to be lowered to 100 bar, and THF used as

**Table 1**—Experimental Parameters for SEDS Crystallizations

|  | (-)-E(-)-MA | (+)-E(-)-MA     | racemate |
|--|-------------|-----------------|----------|
| CO <sub>2</sub> flow, mL·min <sup>-1</sup> | 9           | 9               | 9        |
| solution flow, mL·min <sup>-1</sup>        | 0.2         | 0.2             | 0.2      |
| bottom solvent flow, mL·min <sup>-1</sup>  | 0.2         | 0.2             | 0.2      |
| $p$ , bar                                  | 300         | 100             | 100–350  |
| $T$ , °C                                   | 35          | 35              | 35–75    |
| concentration, w/v%                        | 2.5         | 2.5             | 1.93     |
| solvent                                    | methanol    | tetrahydrofuran | methanol |

**Table 2**—Physicochemical Parameters of Pure Ephedrinium Mandelates

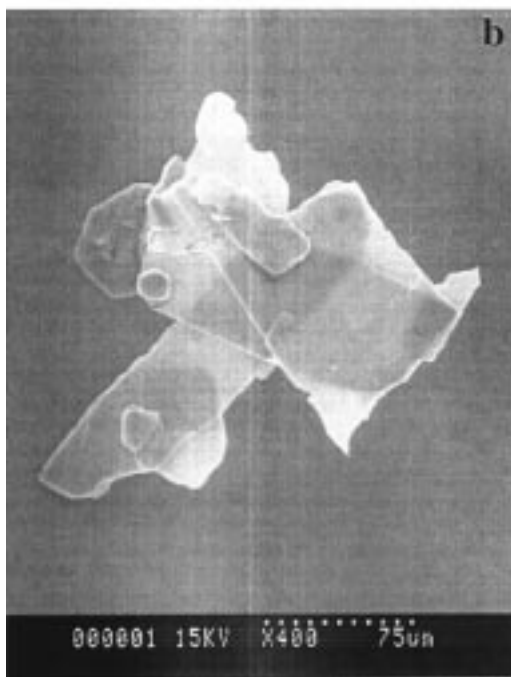
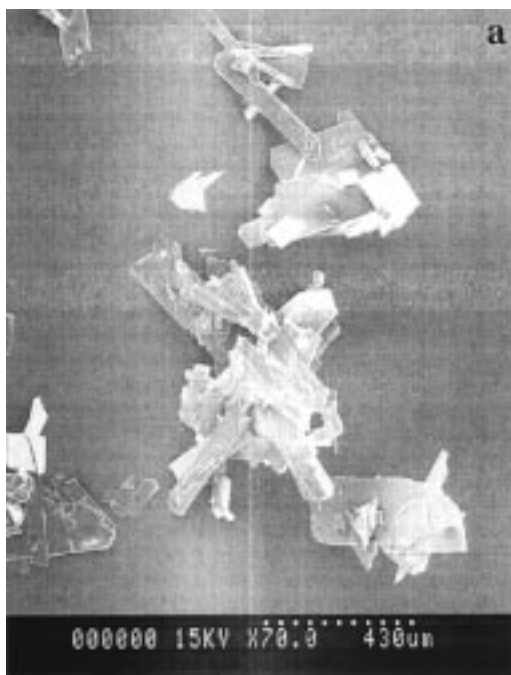
|                                     | (-)-E(-)-MA |                         | (+) -E(-)-MA |                         |
|-------------------------------------|-------------|-------------------------|--------------|-------------------------|
|                                     | SEDS        | "classic" <sup>22</sup> | SEDS         | "classic" <sup>22</sup> |
| $T_m$ , °C                          | 169.1       | 165                     | 108.7        | 110.8                   |
| $\Delta H_f$ , kJ·mol <sup>-1</sup> | 40.3        | 51.9                    | 20.7         | 27.6                    |

solvent, because no precipitation occurred in CO<sub>2</sub> with methanol. (+)-E(-)-MA is less soluble in THF; the same solution concentration is therefore more saturated and precipitates easier when CO<sub>2</sub> is added as an antisolvent.

The differences in crystallization between the diastereomers can be explained by their different solubility in CO<sub>2</sub>. Solubility of a solid in a liquid can be expressed with the enthalpy of fusion and the isobaric heat capacity. Neglecting the influence of the heat capacity and assuming that in the SEDS process both diastereomers are infinitely diluted, differences in solubility between them can solely be related to their heat of fusion. From the melting points and enthalpies of fusion of both salts shown in Table 2, it can be seen that their  $T_m$  differ by more than 50 °C and  $\Delta H_f$  by 20 kJ·mol<sup>-1</sup>.<sup>27</sup> The low  $\Delta H_f$  for (+)-E(-)-MA explains why it is difficult to crystallize from supercritical CO<sub>2</sub>. Table 2 also shows  $T_m$  and  $\Delta H_f$  for the diastereomers produced by SEDS. In comparison to the literature data, the SEDS-produced materials have almost identical melting points. The difference in enthalpy of fusion observed might either be due to small amorphous regions within the SEDS product, or to the minute crystal size of the SEDS material. Supersaturation and subsequent precipitation in the SEDS process occur within milliseconds; therefore, particles can be produced which give rise to the possibility of amorphous regions within the crystal.

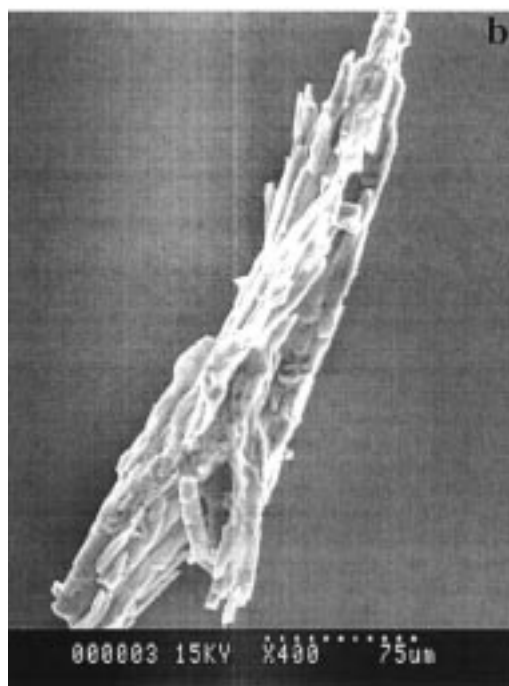
SEM photographs were taken as reference of both diastereomeric salts. From the SEM photograph in Figure 2a it can be seen that (-)-E(-)-MA produced by SEDS is comprised of very thin plates, which were shown to have an average size of 100 × 200 μm. The plates are also translucent, as can be seen from Figure 2b. The SEM photograph in Figure 3a shows the crystal shape of SEDS produced (+)-E(-)-MA. It is distinctively different to the other diastereomer and consists of needlelike structures with an average length of 300 μm. From Figure 3b, it can be seen that individual needle pieces are "grafted" on top of each other.

**Resolution by SEDS**—As discussed above, the difference in enthalpy of fusion between the diastereomers results in a large difference in solubility in supercritical CO<sub>2</sub>. (-)-E(-)-MA possesses the higher enthalpy of fusion and should therefore crystallize preferentially making resolution of the racemate in supercritical CO<sub>2</sub> possible. For the separations, racemic ephedrine and (*R*)-mandelic acid were dissolved in methanol, and the solution was pumped into the crystallization vessel simultaneously with the supercritical CO<sub>2</sub>. Experimental parameters are sum-



**Figure 2**—SEM photograph of pure (1*R*,2*S*)-ephedrinium-(*R*)-mandelate crystallized with SEDS from methanol at 35 °C and 300 bar with a CO<sub>2</sub> flow rate of 9 mL·min<sup>-1</sup> and a solution flow rate of 0.2 mL·min<sup>-1</sup>.

marized in Table 3. Pressure was varied between 100 and 350 bar at a constant temperature of 35 °C, ensuring that a wide range of densities of CO<sub>2</sub> was covered. Additionally, the temperature was changed between 35 and 75 °C keeping the density of the supercritical CO<sub>2</sub> at a constant value. From Table 3 it can be seen that the achieved resolutions range from 89 to 92%, as expected from the large difference in  $\Delta H_f$ . In Figure 4, the achieved resolution is plotted against the density of CO<sub>2</sub>. Bidirectional error bars are given, reflecting the experimental uncertainties. Within experimental error, a linear relationship between resolution and density of CO<sub>2</sub> can clearly be seen. Resolution rises with increasing density of the supercritical fluid at constant temperature. As explained, (+)-E(-)-MA is



**Figure 3**—SEM photograph of pure (1*S*,2*R*)-ephedrinium-(*R*)-mandelate crystallized with SEDS from tetrahydrofuran at 35 °C and 100 bar with a CO<sub>2</sub> flow rate of 9 mL·min<sup>-1</sup> and a solution flow rate of 0.2 mL·min<sup>-1</sup>.

**Table 3**—Parameters for Enantiomeric Resolution of (-)-E(-)-MA with SEDS

| $p$ , bar | $T$ , °C | $\rho_{\text{CO}_2}$ , g·cm <sup>-3</sup> | resolution <sub>(-)-E(-)-MA</sub> , % |
|-----------|----------|---|---------------------------------------|
| 350       | 35       | 0.953                                     | 92.7                                  |
| 300       | 35       | 0.930                                     | 92.3                                  |
| 150       | 35       | 0.816                                     | 91.3                                  |
| 100       | 35       | 0.714                                     | 90.5                                  |
| 150       | 50       | 0.702                                     | 89.5                                  |
| 250       | 75       | 0.713                                     | 87.8                                  |

much more soluble in CO<sub>2</sub> than (-)-E(-)-MA. Thus, at higher pressures the corresponding density of the super-

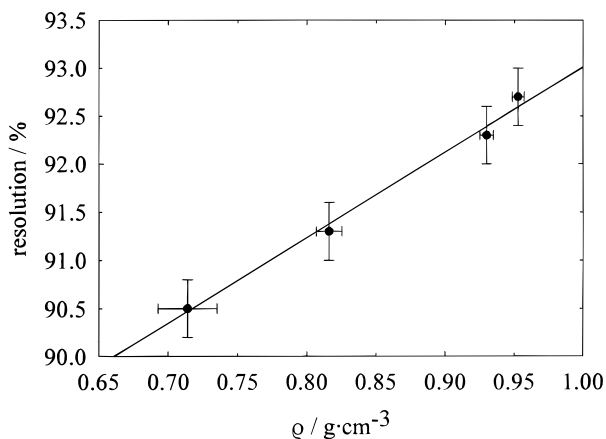


Figure 4—Resolution of SEDS-crystallized ephedrinium-mandelate at a constant density of  $\text{CO}_2$  of  $0.713 \text{ g}\cdot\text{cm}^{-3}$ .

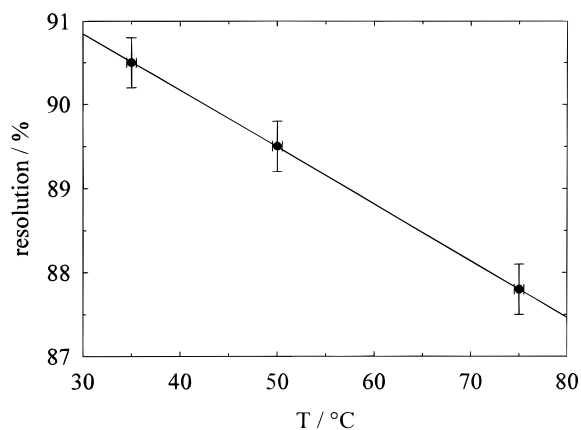


Figure 5—Resolution of SEDS-crystallized ephedrinium-mandelate at a constant temperature of  $35^{\circ}\text{C}$ .

critical  $\text{CO}_2$  is increased supplying more “solvent” in which (+)-E-(−)-MA can be dissolved, resulting in higher enantiomeric excess. As shown, density plays the key role for the resolution, and a higher degree of resolution could therefore be obtained by increasing the density even further. Unfortunately, the density of the supercritical  $\text{CO}_2$  rises approximately logarithmically with pressure and, significantly higher densities can only be achieved by applying practically excessive pressures.

As density is an important factor in the resolution of the ephedrinium mandelates, a second series of crystallizations was performed to investigate the temperature dependence of the resolution. In these experiments, the density of the supercritical  $\text{CO}_2$  was kept virtually constant ( $\pm 1.7\%$ ) at different temperatures, applying a consecutively higher pressure at elevated temperatures. Results are summarized in Table 3. A linear relationship is obtained in Figure 5 for resolution plotted against temperature with resolution decreased from more than 90% to less than 88% as temperature rises from  $35^{\circ}\text{C}$  to  $75^{\circ}\text{C}$ . Bidirectional error bars are included in Figure 5 for the uncertainty in temperature and resolution. The loss in resolution can be explained by changes of  $\Delta H_f$  with temperature for both diastereomers. Although the difference in  $\Delta H_f$  between the diastereomers is large, this difference becomes smaller with increasing temperature, causing less efficient resolution. Furthermore, the methanol solution of the solute is less saturated at elevated temperatures, making it more difficult to crystallize the diastereomers. This results in a lower yield in crystalline product at elevated temperatures.

Table 4—Comparison between SEDS and Conventional Resolution

|                                  |                                | conventional  |  |  |
|----------------------------------|--------------------------------|---|--|--|
|                                  |                                | resolution (−)-E-(−)-MA, %                              | $T_m$ , $^{\circ}\text{C}$                     | $\Delta H_f$ , $\text{kJ}\cdot\text{mol}^{-1}$ |
| crop 1st → 2nd → 3rd             |                                | $88.0 \pm 0.3 \rightarrow 95.3 \pm 0.3 \rightarrow >99$ | 168.9  | 46.2   |
|                                  |                                | SEDS  |  |  |
| $p_1 \rightarrow p_2/\text{bar}$ | resolution (−)-E-(−)-MA, %     | $T_m$ , $^{\circ}\text{C}$                              | $\Delta H_f$ , $\text{kJ}\cdot\text{mol}^{-1}$ |  |
| 100 → 100                        | $90.5 \pm 0.3 \rightarrow >99$ | 167.5   | 41.9   |  |
| 100 → 300                        | $90.5 \pm 0.3 \rightarrow >99$ | 167.4   | 42.2   |  |
| 150 → 150                        | $91.3 \pm 0.3 \rightarrow >99$ | 167.2   | 38.2   |  |
| 300 → 300                        | $92.3 \pm 0.3 \rightarrow >99$ | 168.2   | 42.2   |  |

Table 5—ANOVA Table with  $K = 4$ ,  $N = 12$ ,  $F(3,8)_{0.05} = 4.07^{26}$

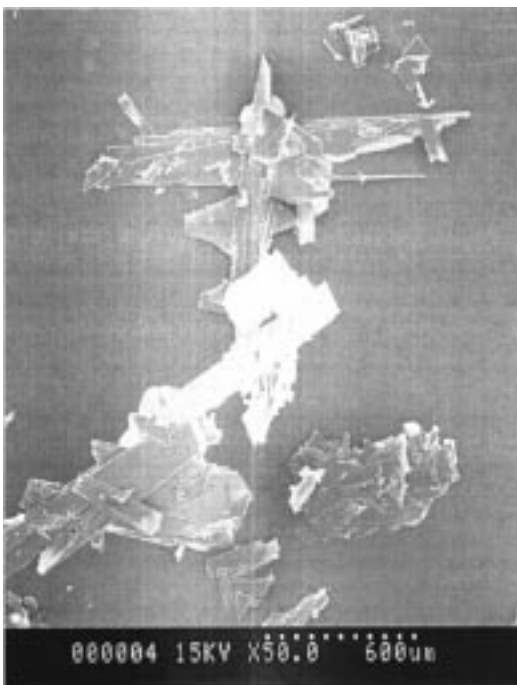
|           | dF | SS   | MS   | F    |
|-----------|----|------|------|------|
| treatment | 3  | 30.4 | 10.1 | 1.46 |
| error     | 8  | 55   | 6.9  |      |
| total     | 11 | 85.4 |      |      |

Chiral separations are an extreme case of impurity removal. Considering one of the enantiomers as an impurity, up to 50% has to be removed to obtain an enantiomerically pure product. Yields for each SEDS crystallization lie between 40 and 45%, meaning that 80–90% of the theoretical yield is obtained. Furthermore, highest yields are achieved at low densities of  $\text{CO}_2$ , because overall solubility of the diastereomeric salts is at a minimum.

**SEDS versus “Classic” Resolution**—In order, to compare the SEDS products with conventionally obtained enantiomers, a classical resolution of ephedrine with mandelic acid was performed according to the method of Manske and Johnson.<sup>18</sup> For this model resolution, racemic ephedrine and (R)-mandelic acid were dissolved in boiling ethanol, and on cooling a diastereomeric salt mixture was precipitated. Part of the initial crop was recrystallized twice from boiling ethanol. The first crop had a resolution of 88%, with a yield of 70%. Consecutive recrystallizations with identical yields increased the resolution to 95.3% and finally after another recrystallization to more than 99% with an overall yield of 34%.

SEDS samples produced at  $35^{\circ}\text{C}$  were recrystallized under identical experimental parameters as for their initial crystallization to determine the efficiency of further purification. The yields for each crystallization was 80% resulting in an overall yield of 64%. Table 4 compares the achieved resolution of the SEDS and classical material and data show that the initial resolution of all SEDS material is higher than for the conventional material. An ANOVA test<sup>28</sup> of the obtained resolutions on a 95% confidence level shows that all samples are significantly different. A summary of the statistical analysis is given in Table 5. Furthermore, after only one recrystallization, the resolution of the SEDS product has risen to more than 99% with no peak of (+)-E-(−)-MA detected by CE. Although the initial resolution of the individual SEDS samples differed by several percent, the recrystallized samples have an identical purity within the error of analysis. Thus it is proposed that also less-resolved material can be resolved to  $>99\%$  after a recrystallization in supercritical  $\text{CO}_2$ .

Figure 6 shows a SEM photograph of ephedrinium mandelate that has been crystallized twice from supercritical  $\text{CO}_2$ . The material exhibits the same very thin plate morphology as pure (−)-E-(−)-MA, as seen in Figure 1. It is known that small amounts of the second enantiomer influence the habit of the crystal<sup>29</sup> and resolution can



**Figure 6**—SEM photograph of SEDS-recrystallized ephedrinium-mandelate from methanol at 35 °C and 300 bar with a CO<sub>2</sub> flow rate of 9 mL·min<sup>-1</sup> and a solution flow rate of 0.2 mL·min<sup>-1</sup>.

therefore be qualitatively monitored by looking at particle shape. If the crystal habit does not resemble that of the desired diastereomer, it can be assumed that the resolution is not complete. In addition to the crystal shape,  $T_m$  and  $\Delta H_f$  are also almost identical to values for pure (-)-E(-)-MA. Small differences in  $T_m$  and  $\Delta H_f$  between the individual samples are likely to result from variations in the HPLC-pumps efficiency over the long run periods of the experiment and not from differences in resolution under different conditions. Table 4 also shows that the SEDS-recrystallized samples exhibit  $T_m$  comparable to conventionally recrystallized material. The difference in  $\Delta H_f$  between SEDS and conventional samples is again due to the smaller crystal size, as explained earlier. In contrast to conventionally obtained (-)-E(-)-MA the material produced by SEDS has the melting point of the pure product after only one recrystallization.

## Conclusions

Crystallization by the SEDS process using supercritical CO<sub>2</sub> has been shown to be a versatile method for the resolution of ephedrine racemates via diastereomeric salt and produce material of high enantiomeric purity. Starting from the racemate, crystals with more than 90% resolution can easily be obtained in one crystallization. Resolution has been shown to be a function of density of the supercritical CO<sub>2</sub> and temperature. At constant temperature resolution increased with increasing density of the supercritical CO<sub>2</sub>, whereas raising the temperature under isopycnic conditions had a detrimental effect on the degree of resolution. Temperature and density effects were explained by the difference of enthalpy of fusion between enantiomers. After one recrystallization, the SEDS product is indistinguishable from material obtained from enantiomerically pure ephedrine samples. Crystals produced with the SEDS technique exhibit enhanced purity and smoother crystal habits compared to conventionally obtained material. Ease

of use and ability to produce highly resolved material make the SEDS process a viable alternative to conventional resolution of chiral compounds.

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