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Application of Automation and Thermal Analysis to Resolving Agent Selection

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Abstract:

The use of differential scanning calorimetry as a means to identify diastereoisomeric salts with a clear eutectic composition that is needed for effective resolution is described and forms the basis of a resolving agent screening process. Automated salt synthesis using an Advanced ChemTech robot is also described. Rapid data analysis is facilitated using an in-house software package, Resolution Companion, which also enables identification of the optimum crystallization conditions following a trial crystallization experiment.

Introduction

The limited availability of novel homochiral molecules is a significant constraint in drug design which can lead to the synthesis of less than ideal alternatives to the intended molecule. The availability of homochiral molecules also has an impact on the speed of development; this is most dramatic at the early stages of synthesis scale-up, when material is required immediately to progress key toxicological and clinical studies. One series of technologies which can rapidly supply homochiral compounds is the separation of racemates, and of those the technique of crystallization of diastereoisomeric salts is extremely effective—principally because it is simple to operate and it affords both enantiomers. Recently, the scientific rationale behind the process has become more widely understood,[1] and the technique is not regarded with the same mysticism as it once was. However, until now the main limitation of diastereoisomeric salt crystallization has been the time taken in screening for the appropriate salt.

In this paper we describe the application of automation to the screening process and the subsequent rapid analysis using differential scanning calorimetry (DSC), a largely ignored technique but one which fits perfectly with an initial screening process. Another part of the screening process involves converting the data obtained from DSC and initial crystallization trials into defining the optimum crystallization conditions. To facilitate this, we have designed and written the user-friendly software, Resolution Companion, which we are willing to share with interested parties.

Background

Thermodynamic Description of Classical Resolution. To effect a successful classical resolution, the crystalline diastereoisomeric salt pair should, in general, be of the eutectic type. A binary (melting) phase diagram of the form depicted in Figure 1 characterizes such systems. The liquidus

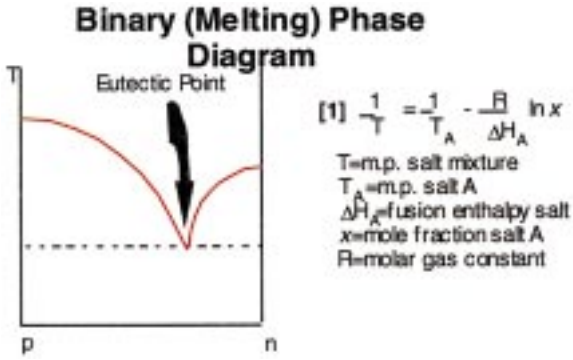

Figure 1.

curve[2] for each diasteroisomer can be accurately described by the Schroeder−van Laar equation (1, in Figure 1), which is of a form identical to that of the thermodynamic expression for the depression of freezing point of an ideal solid. The composition at which the liquidus curves for the *n* and *p* salts meet is the eutectic and corresponds to the composition of minimum melting point.

To understand crystallization from a solution, the ternary system comprising *n* salt, *p* salt, and solvent requires consideration.[3] Figure 2 depicts a prism, triangular in cross section, with limiting phase diagrams for the binary systems *n*-salt/*p*-salt (the melting phase diagram already described), *n*-salt/solvent, and *p*-salt/solvent on the three major faces. The surface generated within the prism is the ternary phase diagram of the system, which describes the temperature dependence of the eutectic composition. For simplicity, the ternary phase diagram is usually represented at a defined temperature T_0 which corresponds to the cross section of the prism at this temperature (Figure 2).

The eutectic composition now relates to the point of maximum solubility, and empirically it is often found that the solubility ratio of the *n* and *p* salts is essentially constant and, to a large extent, independent of temperature.[4] As the temperature is increased, less solvent is required for dissolution, and at the temperature corresponding to melting of the binary eutectic, no solvent is required to form a single phase; i.e., the eutectic points in the binary and ternary phase diagrams converge to the same limit (Figure 3).

The line linking the triangle apex (pure solvent) to the eutectic composition at the base of the triangle is the eutectic

(1) Wilen, S. H.; Collet, A.; Jacques J. *Enantiomers, Racemates and Resolutions*; Wiley and Sons: New York, 1981.

(2) The liquidus curve defines the interface between regions of solid + liquid and only liquid in the phase diagram.

(3) According to ref 1, the term *n* salt refers to the salt pair for which the sign of optical rotation for each component of the salt is different. Conversly the components of a *p* salt possess the same sign for their optical rotations.

(4) For example, see: Leclerque, M.; Jacques, J. *Bull. Soc. Chim. Fr.* **1975**, 2052. Van der Haest, X.; Wynberg, H.; Leusen, F. J. J.; Bruggink, A. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 523.

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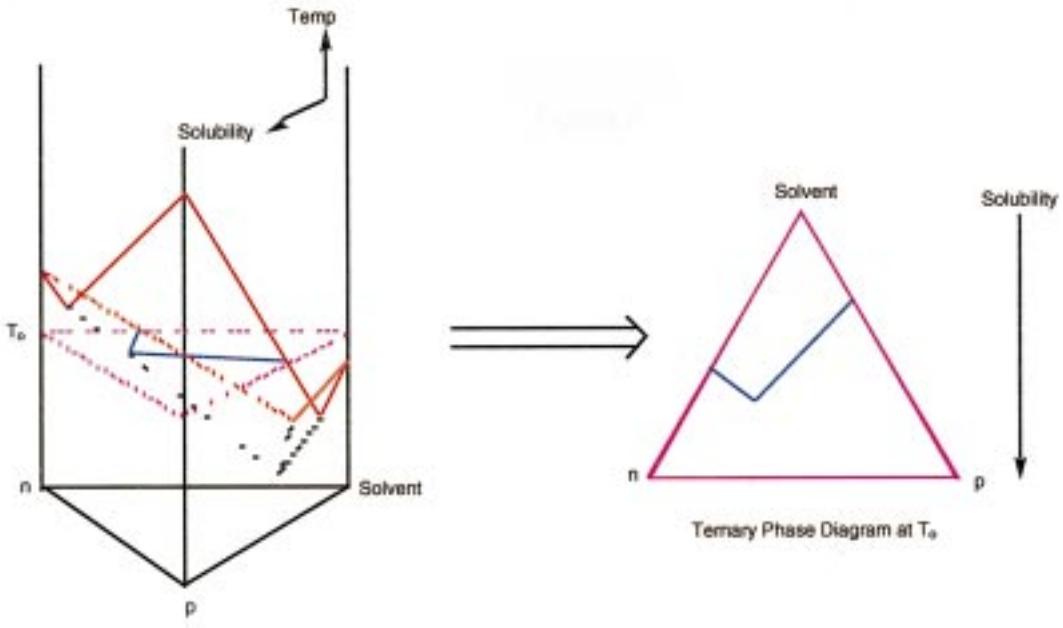

Figure 2.

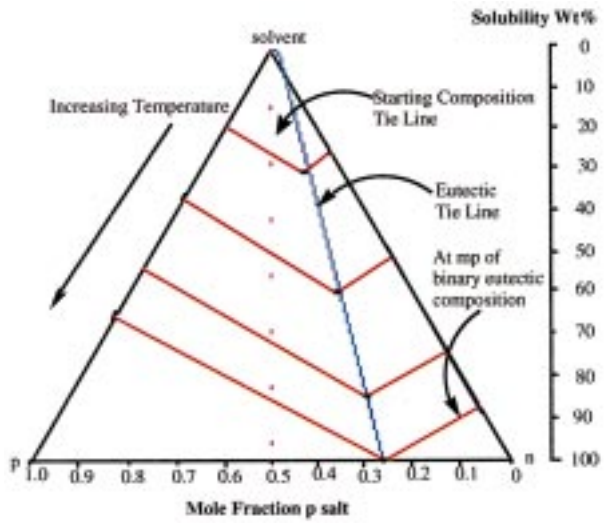

Figure 3.

tie line. The ratio of *n*-salt:*p*-salt at any point on this line is constant (and identical to the binary eutectic composition), whilst the relative amount of solvent increases as the apex is approached.

The maximum yield of diastereoisomerically pure salt obtainable from crystallization of the racemic mixture is given by the ratio *SE′*/*pE′* (Figure 4), an application of the lever rule. The salt concentration required to achieve this optimum is given by the intersection of the line *p*−*E* and the starting composition tie line. Taken together, it can thus be concluded that the resolution efficiency depends solely upon the position of the binary eutectic point and is independent of the nature of the solvent. Solvent variation merely varies the position of the eutectic point on the eutectic tie line and hence alters the concentration at which the optimum efficiency is achieved; the maximum yield of diastereoisomerically pure salt remains unchanged. Thus, the efficacy of potential resolving agents can be judged from

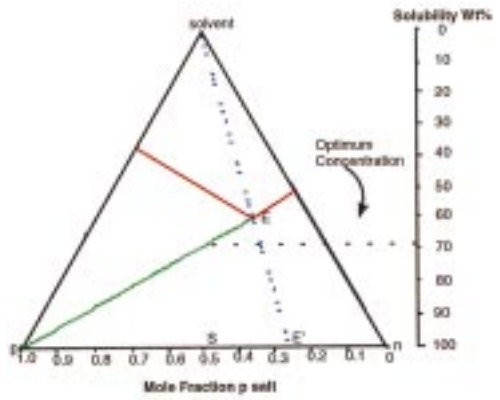

Figure 4.

construction of the simpler binary (melting) phase diagram. To construct the binary phase diagram, both of the diastereoisomerically pure salts are required, such that accurate melting point values can be obtained using DSC for a number of samples of differing composition.[5]

Use of DSC for Resolving Agent Screening. A basis for screening potential resolving agents has been described by Collet and co-workers[1] and further developed by Acs and co-workers,[6] which relies upon obtaining the DSC thermogram of only the 1:1 diastereoisomeric salt mixture, thus precluding the need to initially have pure samples of each of the diastereoisomeric salts. If the system is of the required eutectic type, then it will be characterized by two melting endotherms, the first connected with melting of the eutectic composition and the second with melting of the remaining diastereoisomeric salt. Moreover, it was shown that the Schroeder−van Laar equation (eq 1, Figure 1) could be

(5) For a recent example, see: Ebbers, E. J.; Plum, B. J. M.; Ariaans, G. J.A.; Kaptein, B.; Broxterman, Q. B.; Bruggink, A.; Zwanenburg, B. *Tetrahedron: Asymmetry* **1997**, *8*, 4047. Ebbers, E. J.; Ariaans, G. J. A.; Zwanenburg, B.; Bruggink, A. *Tetrahedron: Asymmetry* **1998**, *9*, 2745−2753.

(6) Kozma, M.; Pokol, G.; Acs, M. *J. Chem. Soc., Perkin Trans. 2* **1992**, 435.

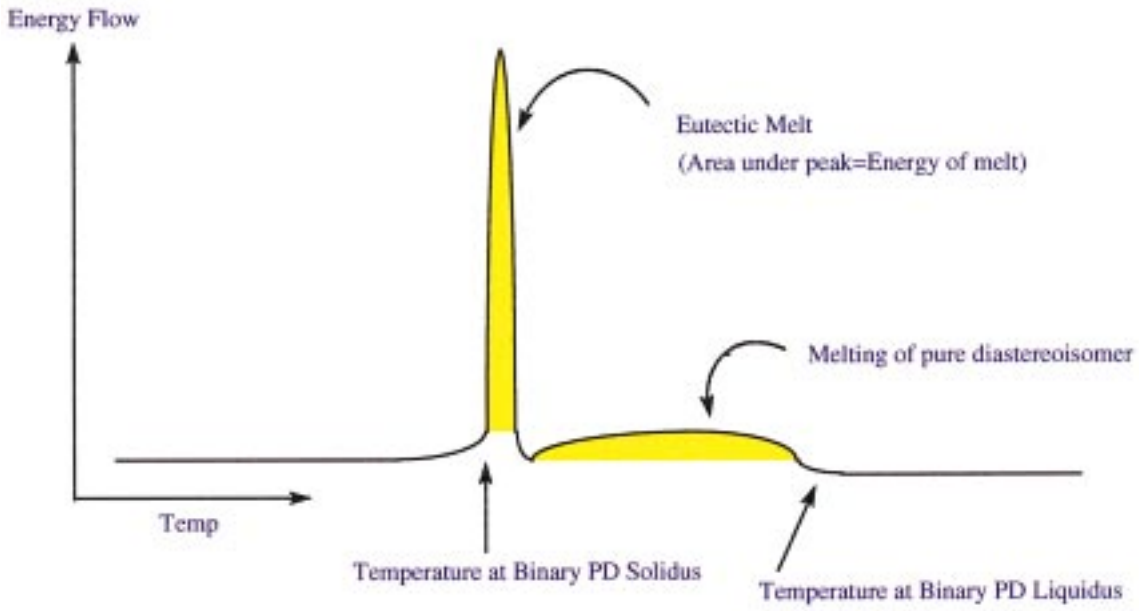

Figure 5.

manipulated such that the eutectic composition and resolution efficiency are roughly determined from quantities directly measurable in the DSC thermogram (Figure 5).

Results

To simplify the procedure and remove the need for graphical data handling, a software package, Resolution Companion, was developed in-house. This package enables (i) the construction of binary phase diagrams using the Schroeder−van Laar equation, (ii) the rapid analysis of data from DSC thermograms, and (iii) the construction of ternary phase diagrams for evaluation of optimal solution concentrations. Note that only the position of the ternary eutectic point is important for this application; construction of the liquidus curves is not necessary. Further details of this package are available in the Supporting Information.

The method was used to identify a suitable resolving agent for *N*-acetyl *o*-methyl phenylalanine (**2**). Fifteen commercially available chiral bases were screened, as detailed in the Experimental Section, and the solid salts analysed by DSC. The results are tabulated in Table 1.

Entries 5 and 6 possessed two melting endotherms and hence were potential resolving agents. The remaining four solids either were amorphous (no melting endotherm) or displayed a complex DSC thermogram, and thus were ignored. The required thermal data were recorded from the DSC spectra; some overlap of the peaks is inevitable, so the peak areas are only approximate.[8] The values are tabulated in Table 2, along with the predicted eutectic composition and resolution yield (by using Resolution Companion).

(1*R*,2*S*)-2-Amino-1,2-diphenylethanol was selected as the resolving agent since it possessed the more favorable binary eutectic point. The next stage was to perform some trial crystallizations, with the aim of effecting a partial resolution. This was achieved by taking 200 mg of racemic **2** with 1 equiv of the resolving agent and slowly adding the solvent at a temperature close to its boiling point until dissolution just occurred. After cooling, the precipitated salt was collected, and its weight and optical purity were recorded. The mother liquor was concentrated, and the weight and optical purity of this salt were also recorded (Table 3).

Use of 2:1 ethanol/water achieved the desired partial resolution (entry 2), affording a 76% diastereomeric excess of the (*S*)-salt, as judged by chiral HPLC. Note that the solubility of the salt in ethanol alone was too low (entry 1) and that use of a significant excess of solvent afforded essentially diastereoisomerically pure material (entry 3). The composition of the mother liquor for entry 2 is, by definition (assuming equilibrium crystallization), the eutectic composition and comprised a 0.27:0.73 ratio of (*R*):(*S*) salts, as determined by chiral HPLC. Hence, the binary eutectic composition is 0.27, and since 210 mg of salt was dissolved

Table 1

entry	chiral base	amount (mg)	outcome
1	(−)-ephedrine	15	oil
2	(+)-Ψ-ephedrine	15	oil
3	(+)-norephedrine	14	solid
4	(−)-*N*-methyl ephedrine	16	oil
5	(+)-2-phenylethylamine	11	solid
6	(1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol	19	solid
7	quinine	29	oil
8	cinchonidine	27	solid
9	quinidine	29	oil
10	(+)-dehydroabietylamine	26	solid
11	(*R*)-1-amino-2-propanol	7	oil
12	(−)-*cis*-myrtanylamine	14	oil
13	(*R*)-(−)-2-amino-1-butanol	8	oil
14	(*S*)-1-naphthylethylamine	16	solid
15	L-(+)-*threo*-2-amino-1-phenyl-1,3-propanediol	15	oil

(7) ACT refers to the Advanced ChemTech synthesis robot, which is primarily used for solid-phase synthesis.
(8) Greater accuracy can be achieved using peak-fitting software, for example PeakFit (Jandel Scientific Software).

Table 2.

chiral base	eutectic T_{onset} (°C)	salt T_{end} (°C)	eutectic Q (J/g)	salt Q (J/g)	binary eutectic composition	resolution yield (0.5 max)
(+)-2-phenylethylamine	177	193	74	30	0.33	0.24
(1*R*,2*S*)-2-amino-1,2-diphenylethanol	172	186	63	78	0.22	0.35

Table 3.

			crystallized salt		salt in liquor		
entry	solvent	volume (mL)	weight (mg)	de	weight (mg)	de	comment
1	ethanol	>10					solubility too low
2	2:1 ethanol/water	5	142	76	210	45	
3	2:1 ethanol/water	11	100	93	282	26	dilute conditions

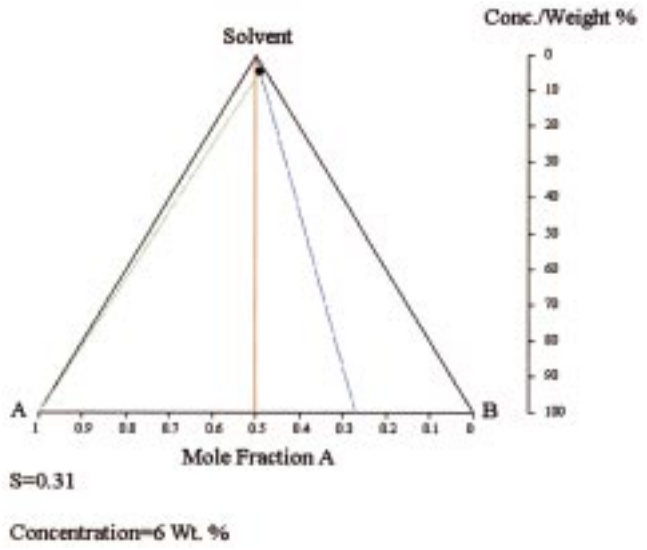

Figure 6.

Table 4

entry	scale	salt de (%)	salt yield (%)
1	trial	95	36
2	preparative	97	30

in 5 mL (4.3 g) of solvent, the eutectic solubility is 4.66 wt %. The optimum concentration for which diastereoisomerically pure material could be obtained at maximum yield was calculated to be 6 wt % (Figure 6) by use of Resolution Companion. The results for the final trial and subsequent preparative crystallization are presented in Table 4. Note the good agreement for the eutectic composition and crystallization yield when compared to the DSC prediction.

Automated Salt Synthesis. The 15 commercially available chiral bases (Table 1) were made up into ethanolic solutions (0.09 M). Using the ACT[7] robot, 400 μL of each base was transferred into wells 1−15 of a 96-well microtitre plate. A solution of (±)-*N*-acetyl *o*-methyl phenylalanine (0.09 M) was made up in ethanol, and 400 μL of the solution was dispensed into each of wells 1−15.

The plate was then shaken for 5 min, and the ethanol was evaporated off in a vacuum centrifuge. Seven solids were observed, which included entries 5 and 6. DSC analysis showed endotherms corresponding to solvent loss but no melting endotherm at higher temperatures, suggesting that the samples were amorphous.

The amorphous nature of the solids may have been due to the technique of evaporation used. The run was therefore repeated, this time using a vacuum oven at 50 °C to evaporate the ethanol. Again, seven solids were observed, including entries 5 and 6. DSC analysis showed two melting endotherms for both of these entries. The remaining solids were shown to be amorphous or gave complex thermograms and were ignored.

Automation showed good correlation with the nonautomated experiment and is therefore suitable for future screening of resolving agents.

Summary and Future Perspectives

The use of DSC to aid in the selection of a resolving agent has been demonstrated and forms the basis of an automated screening procedure. It is planned to extend the library of chiral bases (currently standing at 27) and also to prepare an analogous library of chiral acids. These libraries will be stored as microtitre plates that may be rapidly retrieved whenever a resolution problem presents itself. It is also planned to integrate automated DSC analysis into the process. The software package Resolution Companion may be obtained from the authors on a "shareware" basis.

The method has some limitations. A failure to crystallise under multiwell evaporation does not that imply crystallisation will not occur under other conditions. Polymorphism, degradation, and signal overlap can complicate DSC analysis. Solvate formation can markedly alter the phase diagram.

It is our intention in the future to submit a full paper with many more worked examples to validate further this screening procedure.

Experimental Section

DSC thermograms were recorded and integrated using a Perkin-Elmer thermal analysis System. Sample size was approximately 3 mg, and runs were performed at a heating rate of 5 K/min in closed pans containing a pinhole in the lid. Optical purity determinations were achieved by means

of a Kontron chiral HPLC system, equipped with a Chiralpak AD column. The eluent was 15% IPA/1% TFA/84% hexane at a flow rate of 1 mL/min.

Salt Screening. A stock solution containing 1 g of (±)-*N*-acetyl *o*-methyl phenylalanine (**2**) in ethanol, made up to a total volume of 50 mL, was prepared. One milliliter of this solution (containing 0.09 mmol of acid) was added to 1 equiv of the respective base (Table 1), dissolved in 1−2 mL of ethanol in a 10-mL round-bottom flask. The resulting solution was then concentrated in vacuo. If the resulting salt was a solid, then it was dried at 50 °C in vacuo overnight and subjected to DSC thermal analysis.

Trial Crystallizations. A 25-mL two-necked round-bottom flask, equipped with a reflux condenser and rubber septum, was charged with **2** (200 mg, 0.9 mmol) and (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol (196 mg,0.9 mmol) and approximately 0.5 mL of solvent. The mixture was heated with stirring to 75 °C and solvent added via syringe in 0.5-mL portions until dissolution was complete. Heating was removed, and the flask was left to cool to ambient temperature. After 1.5 h, the precipitated solid was collected and dried at 74 °C in vacuo for 5 h. The mother liquor was concentrated in vacuo and dried in the same fashion. The amount and optical purity of the so-formed salts are reported in the tables.

Preparative Crystallization. A 10-L round-bottom flask equipped with reflux condenser and overhead stirrer was charged with **2** (53 g, 239 mmol) and (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol (51.2 g, 240 mmol). A 2:1 ethanol/water mixture (1990 mL, 6 wt % solution) was then added. The mixture was heated with stirring to 80 °C until dissolution was complete (ca. 1 h). Heating was then removed, and the flask was left to cool to ambient temperature. After continued overnight stirring, the precipitated solid was collected and dried at 60 °C in vacuo for 5 h. The yield and optical purity are reported in Table 4.

Acknowledgment

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Supporting Information Available

Description of the Resolution Companion software package. This material is available free of charge via the Internet at http://pubs.acs.org.

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