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Separability of diastereomer salt pairs of 1-phenylethylamine with enantiomeric 2-substituted phenylacetic acids by fractional crystallization, and its relation to physical and phase properties

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Abstract—The separations of three pairs of the title diastereomer salts by crystallization have been investigated, as examples of the 'classical' resolution of enantiomers via conversion to diastereomers. All three fractional crystallizations occurred relatively slowly, and appeared to be thermodynamically controlled with the outcomes corresponding with the key features of the phase diagrams. In one case, $X = CH_3$, the salts–solvent ternaries exhibited eutonic behaviour, and the direction of isomeric enrichment changed markedly on passing through the eutonic composition. These salts also formed solid solutions on crystallization, but high separation factors were nevertheless recorded. In another example, $X = OH$, the ternaries indicated near-ideal solubility behaviour of the salt mixtures, and the separations by crystallization again corresponded. The stability and solubility ordering of the diastereomer pair $X = CH_3$ in the crystallization temperature range 5–50 °C were determined by the temperature-dependent entropic contribution to the free energy. Our results show that the use of simple surrogate parameters, such as the difference in the enthalpies of formation of the two salts, cannot be used as a reliable guide to their separability by crystallization. More rapid crystallizations are likely to be additionally influenced by kinetic factors, and their investigation is planned in future work.

1. Introduction

The so-called 'classical' method of resolving racemates via adduction with a single-enantiomer resolving agent followed by fractional crystallization of the two diastereomer products, originally attributed to Pasteur,[1] has taken on considerable industrial significance for the preparation and sourcing of enantiomerically pure chemical compounds.[2,3] Most commonly, the enantiomers are converted to a diastereomer pair via an acid–base reaction such as that shown in Scheme 1.

$(RS)\text{-A}^-\text{H}^+$	+	$(R)\text{-B}$	$\rightarrow$	$[(R)\text{-A}^-.(R)\text{-BH}^+]$ + $[(S)\text{-A}^-.(R)\text{-BH}^+]$
Enantiomer acids (Mixture)		Resolving agent (Single enantiomer)		Diastereomers (Different physical properties)

Scheme 1.

In spite of their widespread application in research and industry, there has been very little progress to date in developing a systematic understanding of these processes, in terms of relating the efficacy of the resolution to the properties of the salt pair and the crystallization parameters. The usual approach to resolving agent selection and process development is an experimental screening of alternatives,[4] a trial-and-error procedure that may at best be based on prior art.[3] However, it has been suggested[5] that the separability of the diastereomer salt pairs may be related to their physical properties, such as structural characteristics and

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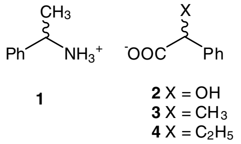
solubility data. A study of the resolution of a series of cyclic organophosphoric acids using ephedrine bases[6] has shown correlations between resolution efficiency and the differences in the enthalpies of formation of the diastereomer pairs, albeit over a very limited series of structurally similar compounds. This work also reported qualitative relationships between crystal packing arrangements and solubility, while a more recent study has interpreted a successful resolution in terms of significant differences in structure motifs exhibited by each diastereomer in the solid state.[7]

Herein, we report a detailed investigation of the relationships between the separations achieved by fractional crystallization and the physical and phase properties of the series of title compounds in Scheme 2. These were originally selected because (i) there were indications in the literature that the separation and phase behaviour of the three salt pairs were likely to be different,[8,9] (ii) crystal structures were available[10] and (iii) the simplicity of the molecules and the rigidity imparted by the phenyl groups should facilitate molecular modelling calculations of the structures and their state functions. The latter have been carried out with a view to developing methods for the ab initio prediction of the structures and state properties of individual diastereomer salts, and are reported elsewhere.[11–13]



Scheme 2.

2. Results and discussion

2.1. Phase equilibria

The solubilities of the six individual diastereomer salts in Scheme 2 in ethanol were measured between 5 and 60 °C, by combining (*R*)-1-phenylethylamine **1** with the corresponding acid enantiomer. The salts **1.3** exhibit the conservative pattern of increasing solubility with temperature as shown in Figure 1, with no phase transitions detected across the temperature range investigated. Regression of these data gave the enthalpies and entropies of dissolution which are shown in Table 1, with a very good fit of the data to the model equation ($R^2 \sim 0.998$). The difference between the calculated enthalpies of dissolution [$\Delta H_{\text{diss}}(RS) - \Delta H_{\text{diss}}(RR)$] of -4.99 kJ mol^{-1} agrees well with the corresponding value obtained for the dissolution of the diastereomers in water by solution calorimetry,[14] -3.88 kJ mol^{-1}.

Figure 1 shows that (*R*)-**1**·(*S*)-**3** is less soluble than (*R*)-**1**·(*R*)-**3** at temperatures above 10 °C, which corresponds to a lower free energy of formation of the crystalline *RS* solid. However, both the solubility curve fits and solution calorimetry[14] indicate that the enthalpy of formation of the (*RR*)-form is the lower value. The (*RS*)-form is thus entropically stabilized, with the entropy contribution to the free energy determining the relative stability and solubility of the diastereomer pair in this temperature region. The suggested use of the differences in the enthalpies of formation of the solid as indicators of both relative solubility and the propensity to separate[5,6] will therefore give the wrong ordering in this case.

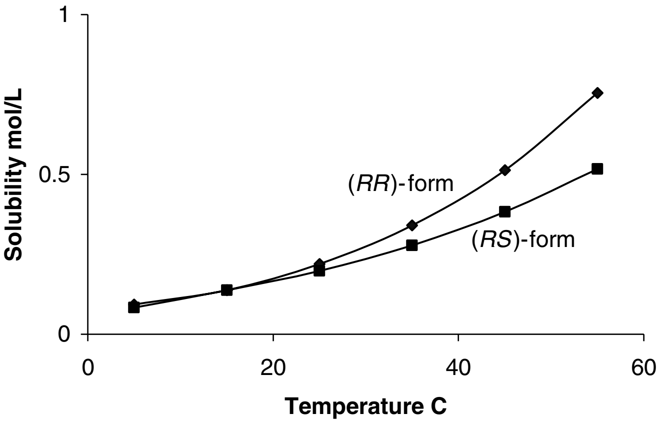


Figure 1. Solubility versus temperature for individual diastereomer **1.3** salts.

Table 1. Enthalpies and entropies of dissolution of salts **1.3** in ethanol from solubility measurements

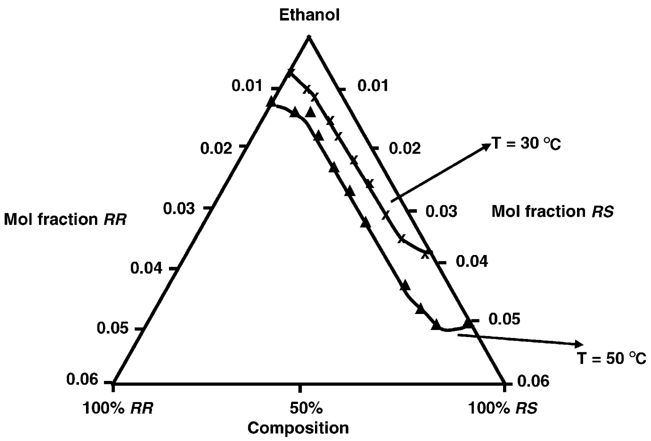
Diastereomer	ΔH_{diss} (kJ mol^{-1})	ΔS_{diss} (J mol^{-1} K^{-1})
(*RS*)-**1**	27.54	66.62
(*RR*)-**1**	32.53	89.14

For both the salt pairs **1.2** and **1.4**, the (*RR*)-forms are considerably less soluble than the (*RS*)-forms across the temperature range investigated. The solubility–temperature profiles for all of these salts appear almost linear within this range, and some measured values are given in Table 2. The forms of these curves do not lend themselves to regression analysis to obtain state functions over the limited temperature range investigated.

Ternary phase relationships for the three diastereomeric systems (*RS*)-salt–(*RR*)-salt–ethanol have been measured at 30 and 50 °C, and are shown in Figures 2a, 3a and 4a, respectively. For the mandelate salts **1.2** (Fig. 2a), the curves indicate a near-ideal pattern of solubility behaviour of the salt mixtures, as is often the case where the solubilities of two dissolved species differ markedly. In such cases, the effect of the less soluble species on the solvent activity is very small, giving rise to near-ideal behaviour of the mixed system. However, the solubility curves in Figure 2a do show some curvature close to the ethanol–(*RS*)-salt axis, indicating that there may be slightly increased total solubility where small quantities of the (*RR*)-salt are present. The tie-line data in the table accompanying Figure 2a, obtained by analyzing the solids recovered at equilibrium with the solution concentrations shown in the diagram, are all composed of mixtures of the (*RR*)- and (*RS*)-salts, and indicate that the equilibrium solid phases are probably solid solutions.[15]

Table 2. Measured solubilities of individual diastereomer salts **1.2** and **1.4**

Diastereomer	Temperature (°C)	Solubility (mol L^{-1})	Diastereomer	Temperature (°C)	Solubility (mol L^{-1})
(*RR*)-**2**	5	0.160	(*RS*)-**2**	5	0.451
	35	0.260		45	1.182
(*RR*)-**3**	12.7	0.075	(*RS*)-**3**	12.7	0.331
	49.6	0.123		49.6	0.729



Solid – solution equilibrium data (mole fractions) for points on solubility curve (selection):

Temperature °C	Point on solubility curve (solution composition)		Tie-line intercept on *RR*-*RS* axis (solid composition)
	RR	*RS*	*RR*
30	0.0041	0.0051	0.890
	0.0047	0.0066	0.866
	0.0047	0.0137	0.714
	0.0044	0.0191	0.657
	0.0036	0.0282	0.558
	0.0033	0.0329	0.270
50	0.0085	0.0024	0.933
	0.0069	0.0064	0.902
	0.0084	0.0152	0.805
	0.0084	0.0200	0.736
	0.0082	0.0249	0.661
	0.0081	0.0383	0.566

Figure 2a. Isothermal ternary solution equilibria for **1.2** diastereomer salts at 30 and 50 °C.

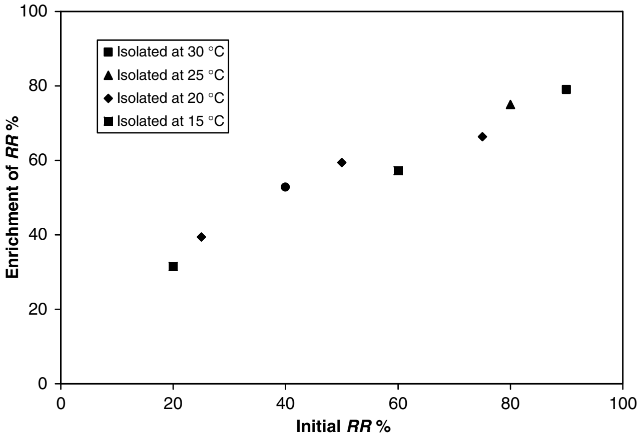


Figure 2b. Measured separations by cooling crystallization of **1.2** diastereomer salts.

Table 3. Comparison of the compositions of bulk crystallized products from separation measurements of salts **1**–**3** with individual crystal compositions

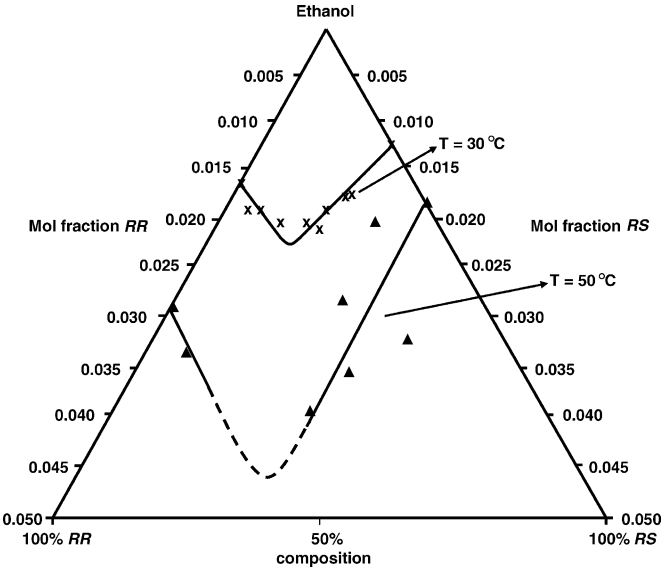
Initial *R*:*S* ratio	Isolation temperature (°C)	*R*:*S* ratio of bulk crystallized product (Fig. 3b)	*R*:*S* ratio of selected individual crystals
75/25	20	89.9:10.1	86.3:13.7
			91.5:8.5
			90.6:9.4
			89.1:10.9
			90.1:9.9
			90.2:9.8
25/75	20	4.5:95.5	1.9:98.1
			3.2:96.8
			4.4:95.6

Figure 3a for salts **1.3** shows a eutonic corresponding to *RR*:*RS* ratios around 70:30 at 30 °C. At 50 °C, very few data points were obtained in the near vicinity of this composition, but the data on either side of it are consistent with such a feature at the higher temperature. As in the previous case, the tie-line data indicate solid solutions in the equilibrated solids. To investigate further whether solid solutions form in this case, individual single crystals were sampled from crystallized products from separability measurements (see below), and their compositions were determined. These compositions were compared with the bulked data from the corresponding separation measurement in Table 3. The compositions of the individual crystals correspond quite closely to the bulk compositions, which indicates that the crystallized product comprises of a solid solution, with the composition of each individual crystal roughly equal.

The ternary diagram for salts **1.4** (Fig. 4a) exhibits a eutonic point at low *RR*:*RS* ratios (around 15:85) at 50°. The eutonic is still detectable at 30 °C, but is very much less marked than at 50 °C.

2.2. Separability measurements

Results for the separation of the three above diastereomer pairs in single cooling crystallizations are shown in Figures 2b, 3b and 4b, respectively. The abscissae show the *RR*:*RS* ratios at the start of the experiments. Typically, solutions saturated with the starting salt mixtures have been prepared at 50 °C using solubility data from the phase diagrams, and then cooled at a constant rate until either crystals appeared or the required final temperature as shown in Figures 2b, 3b, 4b was attained. In some cases where no crystals had appeared on attaining the final temperature, the supersaturated solution was maintained at this temperature until crystals formed, as detailed below. The ordinates show the isomeric 'enrichments' of the crys-



Solid – solution equilibrium data (mole fractions) for points on solubility curve (selection):

Temperature °C	Point on solubility curve (solution composition)		Tie-line intercept on *RR-RS* axis (solid composition)
	RR	*RS*	*RR*
30	0.0162	0.0027	0.973
	0.0087	0.0092	0.148
	0.0059	0.0103	0.044
	0.0052	0.0100	0.041
50	0.0322	0.0044	0.985
	0.0189	0.0165	0.386
	0.0153	0.0188	0.204
	0.0110	0.0143	0.181
	0.0088	0.0219	0.134
	0.0051	0.0138	0.114

Figure 3a. Isothermal ternary solution equilibria for **1.3** diastereomer salts at 30 and 50 °C.

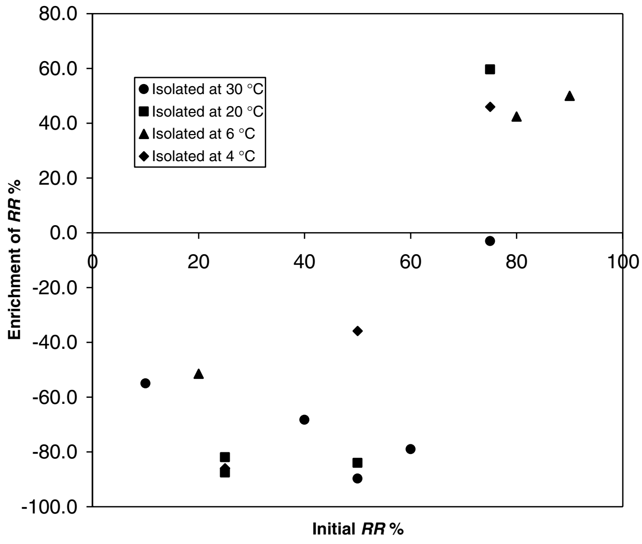
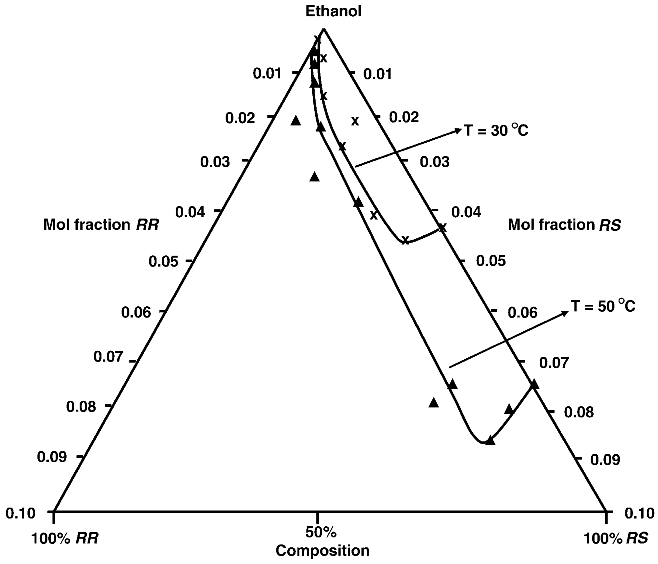


Figure 3b. Measured separations by cooling crystallization of **1.3** diastereomer salts.



Solid – solution equilibrium data (mole fractions) for points on solubility curve (selection):

Temperature °C	Point on solubility curve (solution composition)		Tie-line intercept on *RR-RS* axis (solid composition)
	RR	*RS*	*RR*
30	0.0051	0.0017	0.767
	0.0041	0.0146	0.0
	0.0090	0.0362	0.0
50	0.0034	0.0014	0.968
	0.0089	0.0121	0.914
	0.0155	0.0158	0.882
	0.0097	0.0273	0.433
	0.0159	0.0569	0.681
	0.0139	0.0726	0.403
	0.0102	0.0709	0.333

Figure 4a. Isothermal ternary solution equilibria for **1.4** diastereomer salts at 30 and 50 °C.

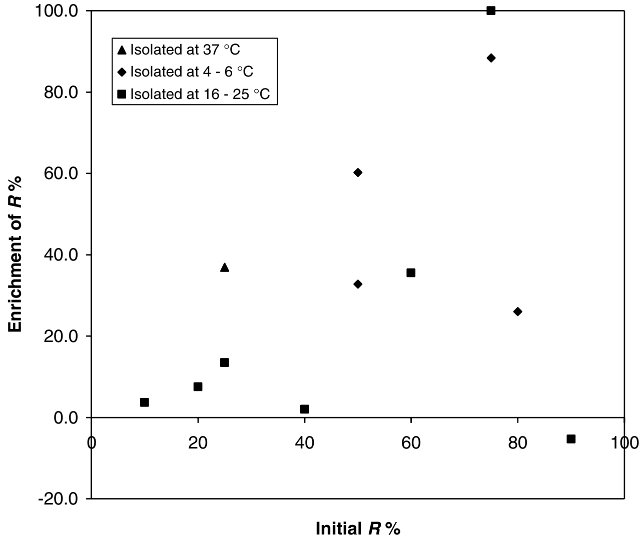


Figure 4b. Measured separations by cooling crystallization of **1.4** diastereomer salts.

tal products for each experiment. The enrichment expresses the extent to which the compositions of the crystallized solids approach a pure single enantiomer (100%) from the initial *RR*:*RS* ratio (0%), with the sign convention that enrichments in *RR* are designated positive and those in *RS* negative. For cases where the (*RR*)-salt is enriched in the crystallized product:

$$\text{Enrichment }\% = \frac{RR\%\text{ in product crystals} - RR\%\text{ at start of experiment}}{100\%\ RR - RR\%\text{ at start of experiment}} \times 100$$

and where the *RS*-salt is enriched:

$$\text{Enrichment }\% = \frac{-RS\%\text{ in product crystals} - RS\%\text{ at start of experiment}}{100\%\ RS - RS\%\text{ at start of experiment}} \times 100$$

Results for the salts **1.2** are shown in Figure 2b. This is the simplest case, where the phase diagram (Fig. 2a), shows a near-ideal pattern of solution behaviour for the salt mixtures, and the (*RR*)-form is markedly less soluble than the (*RS*)-form. All crystallizations show enrichment in the (*RR*)-form, as would be expected from the simple axiom that the less soluble salt will separate and crystallize more readily. The relative enrichment also increases with the starting *RR*:*RS* ratio, indicating the propensity of the less soluble (*RR*)-form to separate from solution. This is a favourable condition for effective resolution, since purification may be increased by repeated crystallizations. Although crystallization was not rapid by commonly accepted standards, it occurred more readily than in salt systems **1.3** and **1.4**, in particular at high initial *RR*:*RS* ratios. Crystallized solid recoveries were in the range 32–45% of the total salt used in each case.

A similar separability diagram for salts **1.3**, expressed as isomeric enrichments, is shown in Figure 3b. Here, a very different pattern of behaviour occurs, with a sharp reversal in the direction of enrichment around an initial *RR*:*RS* ratio of 70:30. With starting *RR*:*RS* compositions below 70%, the (*RS*)-diastereomer is enriched in the crystallized product, while the (*RR*)-isomer is enriched where the starting composition exceeds 70% of the (*RR*)-form. The (total) crystallized solid recoveries obtained at the various isolation temperatures used are given in Table 4. Salts **1.3** crystallized much less readily than salts **1.2**, and crystallizations could be carried through to the required isolation temperature without an excessive build-up of solid. The suspensions were held at the final temperature after cooling for periods varying from 24 h to 30 days, and no systematic variations of either yield or enrichment as a function of this time interval were observed.

Table 4. Total crystallized solid recoveries of salts **1.3** at different isolation temperatures (Fig. 3b)

Isolation temperature (°C)	Mean solid recovery (%)	Standard deviation (abs.%)
30	15.6	2.3
20	26.0	6.2
6	60.8	4.5
4	46.9	5.2

Initial temperature 50 °C, cooling rate 0.5 K min^{-1}.

The change in direction of enrichment in Figure 3b is entirely consistent with the eutonic behaviour exhibited in the ternary phase diagram (Fig. 3a). The existence of the eutonic as a composition of maximum total solubility implies that, in any fractional crystallization controlled by solubility considerations, the less soluble single salts on either side of the eutonic will preferentially separate from the solution. The reversal in Figure 3b occurs at an initial *RR*:*RS* ratio of 70:30, where Figure 3a shows eutonics at both 30 and 50 °C.

The enrichments in Figure 3b do not show significant, systematic variations with isolation temperature, although Table 4 indicates that the recoveries generally exhibited an increase with decreasing temperature, as would be expected with the corresponding decrease in salt solubilities shown in Figure 1. The similarity in the solubilities of the two salts below 20 °C suggests that the numerical values of the enrichments on either side of the 70:30 divide should also be similar, as is the case. Generally, the enrichments appear slightly more extreme at the higher isolation temperatures, with smaller solid yields, although we cannot demonstrate that the effect is experimentally significant. Conventionally, enrichment tends to decrease with increasing solid separation because the solution becomes progressively depleted in the preferentially separating component. However, the variation in enrichment with solid yield and isolation temperature, under equilibrium control, will depend most significantly on the difference between the eutonic and single salt solubilities at the prevailing (isolation) temperature.

The corresponding separability data for the salts **1.4** are shown in Figure 4b. Except for a single point at initial *RR*:*RS* = 90:10, showing slight (5%) enrichment of the (*RS*)-isomer (which may include measurement error because of the small amounts of salts employed in this case), all crystallizations showed enrichment of the (*RR*)-form in the solid. While the scatter of data is quite extensive, there appears to be a general increase in the (*RR*)-enrichment with increasing initial *RR*:*RS* ratio, as is shown by salts **1.2** in Figure 2b, and probably for the same reason that the (*RR*)-salt is considerably less soluble than the (*RS*)-salt across the temperature range employed (Table 2). The crystallized solid recoveries for these salts were also much more variable, showing neither the approximately constant value determined by the isolation temperature as with salts **1.2**, nor the systematic variation with isolation temperature as with salts **1.3** (Table 4). There appears to be no correlation between the extent of isomer enrichment and the crystallized solid recovery.

While the ternary phase diagram for salts **1.4** (Fig. 4a), shows a eutonic at 50 °C, the feature is much less apparent at 30 °C, and the data of Leclercq and Jacques[9] show no evidence of it at 10 °C. If this feature were significant to the fractional crystallization of these salts, a reversal in the direction of enrichment similar to that observed with salts **1.3** may be anticipated for very low *RR*:*RS* starting ratios. However, measurements conducted under these conditions consistently showed a low level of (*RR*)-enrichment, with no indication of a reversal in direction. This pattern of behaviour correlates with the phase behaviour at 30 °C, close to the isolation point, where the eutonic is much less marked. Thus, the reduction or disappearance of the eutonic at the lower temperature effectively nullifies the effect of reversing the direction of enrichment.

We have measured the separations of the three diastereomer salt pairs in Figure 1 by fractional crystallization, and have correlated these separations with the phase equilibrium behaviour of the salt pairs in solution. For one of the salt pairs **1.3**, the ternary diagram shows a eutonic, and we have determined that the direction of isomeric enrichment changes markedly as the composition of salts in the crystallizing solution passes through this eutonic point. This is consistent with a thermodynamically controlled fractional crystallization, where a less soluble component will be driven to separate from solution in preference to a more soluble component, in this case the eutonic.

Salts **1.2** and **1.4** both showed the (*RR*)-form separating preferentially from solution, in accordance with solubility measurements that showed the (*RR*)-forms of both salts to be considerably less soluble than their (*RS*)-counterparts across the range of temperature employed. Although the phase diagram for salts **1.4** exhibited a eutonic at 50 °C at *RR*:*RS* ratios around 15:85, this is not particularly well marked at 30 °C, and the solubility enhancement brought about by the eutonic appears insufficient to drive enrichment of the (*RS*)-isomer in the crystallized product at lower initial *RR*:*RS* ratios. All separations reported in this paper are thus consistent with the ternary phase behaviour at the lower temperature, 30 °C, close to the isolation temperatures. No differences in this pattern of behaviour were observed where lower isolation temperatures down to 3–5 °C were employed, and the phase equilibrium data for salts **1.3** and **1.4** at 10 °C reported by Leclercq and Jacques[9] show no qualitative differences in the ternary behaviour to that recorded here at 30 °C.

While the above results establish the important role of the phase equilibrium behaviour in solution in determining the outcome of fractional crystallization as a separation method, there are many other behaviour aspects, relating to both equilibrium and nonequilibrium conditions, that can influence the efficacy of separation, and these need to be investigated further. All of the crystallizations reported here occurred relatively slowly, taking place over periods of hours rather than minutes, with nucleation occurring under fairly extreme conditions of supercooling and supersaturation. In general, such conditions are undesirable in industrial practice, and crystallizations that occur more rapidly and can be carried out at moderate supersaturations are preferred. In such rapid crystallizations, nucleation and growth kinetics may play a critical role in determining the outcome of the separation, by controlling the relative rates at which product crystals may be formed. More rapid crystallizations of this type will be the subject of future research. Other factors that can impede separation include the formation of solid solutions, double and other multiple salts, and difficulties in obtaining crystallized salts from solution. These have been briefly discussed by Leclercq and Jacques,[9] albeit without offering any separability data or drawing any definitive conclusions.

We attempted to prepare pure crystal samples of all the salts in Scheme 2, both to examine the effects of solid-state structural differences on separability, and also as structure references for our modelling work, details of which we have reported elsewhere.[12] For salts **1.2** and **1.4**, crystals of the (*RS*)-forms were much more difficult to prepare than the corresponding (*RR*)-forms, due to a tendency of the (*RS*)-salts to separate from solution as powders and small, poorly formed crystals. The solids obtained in the separation measurements with salts **1.2** and **1.4**, with enrichment of the (*RR*)-forms, were also of very poor crystalline quality, and it was not possible to obtain further information beyond simple chemical analysis by examining the solid products.

Parameters relating to the individual diastereomer components, such as differences in the enthalpies of formation of the crystalline solids, have been proposed as indicators of separability as has previously been suggested.[5,6] Predictive methods based on this approach assume initially that the solution behaviour of the diastereomer pair will be approximately ideal. Here, salts **1.2** most closely meet this condition. However, the solubilities of the two salts in this case differ widely across the entire temperature range of the experiment, and it would be possible, at least in principle, to predict the outcome from a simple consideration of the single-salt solubility data. Generally, this pattern of near-ideal solution behaviour will be more commonly realized in cases where the two solubilities differ by a considerable margin.

However, salts **1.3** give a very clear example where such correlations and predictions will not lead to the correct answer. Firstly, consideration of the difference in the enthalpies of formation as a predictor of separation behaviour will give the wrong ordering of the solubilities of the two compounds, which is dominated by the effect of the entropic contribution. Secondly, such methods take no account of the formation of eutonics, which, our results show, completely determine the direction in which enrichment via crystallization will occur. The effects of eutonics and eutectics on diastereomer separation has been discussed recently,[16,17] with the general impression given that eutonic formation favours separability. These discussions also postulate eutonic behaviour as an alternative to less favourable behaviour patterns, such as solid solutions,[17] implying that the two types of behaviour are mutually exclusive. However, both appear to be exhibited by salts **1.3**. Furthermore, the high numerical enrichments in Figure 3b combined with the lack of evidence that enrichment level falls off drastically with increased solids recovery, suggest that a separation process based on multiple recrystallizations is likely to yield a highly-enriched product in spite of the solid solution behaviour.

The salts system **1.4** exhibits phase characteristics common with both salts **1.2** and **1.3**. In common with salts **1.2**, the (*RR*)-form is much less soluble than the (*RS*)-form across the temperature range, and the ternary solubilities at 30 °C more closely resemble the ideal pattern of behaviour than the eutonic. However, at 50 °C the ternary shows a eutonic at an *RR*:*RS* ratio around 15:85. We find a small enrichment in the (*RR*)-isomer on starting from initial *RR*:*RS* ratios in excess of this value (Fig. 4b). We have also found it very difficult to prepare a good quality crystal of the (*RS*)-form of this salt, as with the corresponding salt (*R*)-**1**·(*S*)-**2**.[12] The separation behaviour of salts **1.4** thus resembles that of salts **1.2**, probably for the same underlying reasons.

It has been suggested[16] that the separability of a diastereomer pair can be predicted from an analysis of its binary melting behaviour, on the grounds that a eutectic melt composition will be congruent with a solution eutonic, and the detection of the former by differential scanning calorimetry will give information on the likely performance of a fractional crystallization. In theory, this can only be true if a number of conditions apply: both diastereomer components must be stable up to their melting points, and the interactions of the solutes with the solvent must be similar. Also, in cases where this principle is applicable, the eutonic composition must remain invariant with temperature, and must also remain the same for all solvents from which crystallization is to be attempted. For salts **1.4**, the eutonic at higher temperature implies that a melt eutectic would be detected, if it were possible to carry out a measurement at the melting points. Our experience with these salts suggests that they are not particularly stable as solids at elevated temperatures, so it would probably be difficult to carry out such a measurement. However, at 30 °C and below, the eutonic effectively disappears, and an investigation of such high temperature behaviour would not correctly predict the separations obtained by fractional crystallization under these conditions.

There are literature reports that multiple salts may form in the mandelate system **1.2**. Two of these involve racemic compositions of the base with single[18] or mixed[19] enantiomer acid moieties, and the possibility of their formation has been eliminated from our experiments by the use of a single base enantiomer, as has the complex conglomerate behaviour that has recently been reported in the quaternary system of **1.3**.[20] The other two multiple salts of **1.2** consist of adducts of each of the two diastereomers with two free acid moieties.[21,22] Single-crystal and powder X-ray diffraction analysis shows no evidence of these forms in our crystallized products.[12] HPLC analysis has shown that all the salts employed in this study were effectively dissociated into their ions in ethanolic solutions.

3. Conclusions

We have shown that the fractional crystallizations of the model diastereomer salts in Scheme 2 follow behaviour patterns predicted from the measured equilibrium data. All of these crystallizations take place relatively slowly, conditions which generally favour the observed thermodynamic control of the outcomes.

Salts **1.3** exhibit a marked eutonic in their salts–solvent ternary phase diagram, and the direction of isomeric enrichment changes abruptly on passing through the eutonic composition. The dominant effect of such eutonics in determining the separation behaviour means that no predictions may be made from the state functions of the individual salts in these cases. This salt pair also appears to crystallize as solid solutions, but high separation factors were nevertheless recorded. For salts **1.2**, the corresponding ternary exhibits near-ideal solubility behaviour for mixtures with no eutonic, and no reversal in the direction of enrichment occurs. In this case, however, the solubilities of the two salts differ considerably, while the results of the fractional crystallization may be inferred directly from the solubilities of the individual salts.

The solubilities (and stabilities) of the two diastereomer salts **1.3** in the working temperature range 5–50 °C are differentiated by the temperature-dependent entropic contribution. Solution calorimetry and solubility measurements have revealed that the (*RS*)-salt is thermodynamically more stable at temperatures exceeding 5 °C, although its enthalpy of formation is approximately 4 $\mathrm{kJ\,mol^{-1}}$ higher than that of the corresponding (*RR*)-salt.

Our experimental findings indicate that the use of simple surrogate parameters, such as the difference in the enthalpies of formation of the two salts, cannot be used as a reliable guide to their separability by crystallization. A more complete understanding of the phase equilibrium behaviour of the salts–solvent ternaries is required for such predictions to be made. More rapid crystallizations are likely to be more strongly influenced by kinetic factors, causing the separations to deviate from the predictions of thermodynamics. We propose to examine examples that crystallize more rapidly in future work.

4. Experimental

(*R*)-1-Phenylethylamine (99+%) and the six enantiomer acids in Scheme 2 were obtained from Lancaster Synthesis and Alfa Aesar; the acids were sourced as both single enantiomers (97–99%) and as racemates (98+%). These were used without further purification. The ethanol solvent was of HPLC grade. Individual diastereomer salts were prepared by mixing stoichiometric quantities of base and acid in ethanol solution and evaporating to dryness at room temperature in an open vessel.

The solubilities of individual diastereomer salts were measured by contacting 10 mL aliquots of solvent with excess solid salt at a particular temperature in a water bath for periods of 1 week, with gentle stirring. Solution samples were extracted using a pre-heated, filtered syringe, diluted and analyzed by HPLC using a 100 mm C18 column. For the ternary equilibrium measurements, salt mixtures were prepared by mixing base with acid mixtures of various compositions prepared by combining single enantiomers and racemates, and evaporating as above. The excess solid thus obtained was then contacted with 8 mL aliquots of solvent (ethanol), and held for 7–10 days in sealed vessels in a water bath with gentle agitation. The contents of the vessels were then filtered rapidly at temperature, and the quantities of solid and filtrate recovered were determined. Filtrates were evaporated to dryness and the recovered solids weighed. The solids recovered both on the filter paper and from the filtrate were analyzed as below for the acid enantiomer ratios. Mass balances on the recovered materials revealed that up to 0.5 mL of solvent was lost during the recovery procedures, probably on the filter paper. A check was made between the measured acid enantiomer ratios in the filtrate, and the corresponding ratios calculated from the mass balance using the recoveries and enantiomer ratios of the filtered solid. Generally the calculated and measured values of these ratios agreed and were better than 95%.

For the measurements of separation by fractional crystallization, solutions (10 mL) were made up using (*R*)-1-phenylethylamine **1** and acid enantiomer mixtures in various ratios to concentrations corresponding to the solubility limits given by the 50 °C solubility curves in the phase diagrams (Figs. 2a, 3a and 4a). The temperature of the solutions was raised to 55 °C prior to the start of each crystallization, to ensure complete dissolution at the outset. The solution aliquots were placed in jacketed vessels fitted with a thermostat, and were cooled at a constant 0.5 K min^{-1} until the reported isolation temperature was attained. The vessels were held at the isolation temperature until no further solid appeared by visual inspection to separate from solution. Where the solution remained clear on attaining the final temperature, the vessel was maintained at this temperature for periods of 24 h–30 days. Isolation was carried out by rapid filtration and assaying the solid and solution products as above. Checks of the measured and calculated enantiomer ratios of the filtrates, as above, showed agreement and were better than 95%.

The acid enantiomer ratios were determined by chiral HPLC, using a 250 mm length Regis Whelk-01® reverse-phase column. For acids **3** and **4** (Scheme 2), an eluent of 45:45:10 hexane/dichloromethane/IPA with a HOAc/NH_4OAc buffer (0.02%) was initially employed at a flowrate of 1.5 mL min^{-1} [RTs: (*R*)-**3** 8.3 min, (*S*)-**3** 9.5 min, (*R*)-**4** 6.3 min, (*S*)-**4** 5.6 min]. However, difficulties with the precipitation of the buffer solids led to the eluent being modified to 98:02 hexane/IPA with 0.1% acetic acid at the same flowrate [RTs: (*R*)-**3** 6.1 min, (*S*)-**3** 7.5 min, (*R*)-**4** 6.2 min, (*S*)-**4** 5.5 min]. For acids **2**, an eluent of 95:5 water/ethanol with 0.1% TFA was employed [RTs: (*R*)-**2** 6.8 min, (*S*)-**2** 8.1 min]. In all cases, the salts were completely dissociated in ethanol solution, with the (*R*)-1-phenylethylamine base moiety eluting at RT > 12 min.

For the individual crystal assays in Table 3, single crystals were selected and sampled from the filtered, crystallized product from the separation experiment, and examined initially under a polarising microscope to check that they were not agglomerates or multiple crystals. They were dissolved in a small quantity (~0.5 mL) of ethanol and assayed for the acid isomer ratio using the HPLC methods above.

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References

1. Pasteur, L. *Compt. Rend. Acad. Sci.* **1853**, *37*, 162; *Leçons de Chimie Professées en 1860*. Soc. Chim.: De Paris, Paris, 1861.
2. Rekoske, J. E. *AIChE. J.* **2001**, *47*, 2.
3. *CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation*; Kozma, D., Ed.; CRC Press: Boca Rason, Florida, ISBN 0-8493-0019-3, 2002.
4. Jacques, J.; Collet, A.; Wilen, W. H. *Enantiomers, Racemates and Resolutions*; John Wiley and Sons, 1981; Reissue **1994** with corrections, Krieger Publishing, Malabar, Florida.
5. Collet, A. *Compr. Supramol. Chem.* **1996**, *10*, 113.
6. Leusen, F. J. J.; Noordik, J. H.; Karfunkel, H. R. *Tetrahedron* **1993**, *49*, 5377.
7. Kinbara, K.; Kobayashi, Y.; Saigo, K. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1767.
8. Brianso, M.-C. *Acta Crystallogr.* **1981**, *B37*, 618.
9. Leclercq, M.; Jacques, J. *Bull. Soc. Chim. Fr.* **1975**, 2052.
10. Cambridge Crystallographic Data Centre. See also Brianso, M.-C. *Acta Crystallogr.* **1976**, *B32*, 3040; *Acta Crystallogr.* **1978**, *B34*, 679; *Acta Crystallogr.* **1981**, *B37*, 740; Larsen, S.; Lopez de Diego, H. *Acta Crystallogr.* **1993**, *B49*, 303; Lopez de Diego, H. *Acta Chem. Scand.* **1994**, *48*, 306.
11. Karamertzanis, P. G.; Price, S. L. *J. Phys. Chem. B* **2005**, *109*, 17134–17150.
12. Anandamanoharan, P.; Karamertzanis, P. G.; Cains, P. W.; Hulme, A. T.; Vickers, M.; Tocher, D. A. *Crystallogr. Growth Design*, in preparation.
13. Karamertzanis, P. G.; Price, S. L., in preparation.
14. Yff, B.; Menken, L.; Royall, P. G.; Cains, P. W. *Thermochim. Acta*, in preparation.
15. Collet, A. *Angew. Chem., Int. Ed.* **1998**, *27*, 3239–3241.
16. Dyer, U. C.; Henderson, D. A.; Mitchell, M. B. *Org. Proc. Res. Dev.* **1999**, *3*, 161.
17. Marchand, P.; Lefebvre, L.; Querniard, F.; Cardinaël, P.; Perez, G.; Counioux, J.-J.; Coquerel, G. *Tetrahedron: Asymmetry* **2004**, *15*, 2455.
18. Lopez de Diego, H. *Acta Crystallogr.* **1994**, *C50*, 1995.
19. Lopez de Diego, H. *Acta Crystallogr.* **1995**, *C51*, 935.
20. Dufour, F.; Gervais, C.; Petit, M.-N.; Perez, G.; Coquerel, G. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2022–2036.
21. Lopez de Diego, H. *Acta Crystallogr.* **1995**, *C51*, 253.
22. Larsen, S.; Lopez de Diego, H. *J. Chem. Soc., Perkin Trans. 2* **1993**, 469.