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Rational approach to the selection of conditions for diastereomeric resolution of chiral amines by diacid resolving agents

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Abstract—This paper presents an experimental and modelling approach to understanding the key conditions for diastereomeric resolutions of chiral amines by diacid resolving agents. The model is used to calculate resolution yields and enantiomeric excesses on the basis of acid–base chemical equilibria, formation of diastereomeric salts and their solubilities. Model parameters were determined experimentally and used to model predicted process variables, which were then compared to experimental resolution data as a function of the resolving agents/amine molar ratio. Based on this model, suggestions for the rational design of diastereomeric resolutions are made, highlighting the benefits of employing molar ratios higher than 1.5.

1. Introduction

Diastereomeric resolution is a primary technique employed for chiral separations and has been applied across quite different scales, from milligrams to several tons, yielding enantiomerically enriched products with high yields.[1] This method is often considered the most straightforward, economical and easiest to perform on a large scale.[2,3] In a diastereomeric resolution, a chiral resolving agent reacts with either the racemic acid or base to form two diastereomeric salts, which have at least one different physical property, usually solubility,[4] allowing for their separation by differential precipitation/crystallisation of one of the diastereomers. The crystallisation can be kinetic or thermodynamically controlled. In the first case, the separation of diastereomeric salts relies on the rate of crystal formation, while in the latter it relies on the difference in their solubilities. The present work is focussed on the latter case.

The main manipulated parameters for diastereomeric resolution are resolving agent, molar ratio of resolving agent to racemic substrate, racemic substrate concentration, resolution solvent and resolution temperature. Several methods for the selection of the appropriate resolution system employing theoretical techniques have been attempted: computer-assisted models, examination of the crystal structure of diastereomer salts, study of energy differences between diastereomer salts and empirical correlations.[5–8] However, the resolution system is still essentially selected on the basis of experimental trial and error.[5,6] Fredga et al.[9] were the first to try to systematise this experimental approach, using the one equivalent method to screen, at the 1 ml scale, different resolving agents, resolution solvents and racemic substrate concentrations (in a range from 0.1 to 1.0 M). Simultaneous addition of several members of a family of resolving agents to a racemic substrate has also been proposed[5] for rapid screening in a procedure known as the 'Dutch Method'. This type of strategy is sustainable as a design of experiment (DOI) approach, but not ideal when a degree of mechanistic understanding is desired. For given resolution conditions, resolution yield (Y) and enantiomeric excess (ee) can be calculated from the eutectic composition, avoiding the need for obtaining the complete ternary phase system, and so determination of eutectic points can also be used for the selection of the resolution system.[8–11]

During selection of the resolving agent, it is important to note that the main advantage of the diastereomeric resolution is the relatively low costs of the process, which are

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mainly associated with the resolving agent costs. Looking at the commonly available resolving agents, tartaric acid and its derivatives are the most popular choice for resolving chiral bases, accounting, for 1368 resolutions of chiral bases reported by Kozma.[12] Some 33% of these were performed by tartaric acid and 25% by two of its derivatives, di-*O*,*O*′-toluyl-tartaric acid (DTTA) and di-*O*,*O*′-*p*-benzyl-tartaric acid (DBTA). These values reflect the consequences of tartaric acid being easily isolated from nature at a relatively low cost.

It is interesting to note that these popular resolution agents are diacids, and so can form neutral and acidic diastereomeric salts, comprising of two or one molecule of amine for each molecule of the diacid, respectively. Also, in principle, the same molecule of neutral salt can combine both amine enantiomers in the same salt, herein after referred as mixed enantiomer neutral salt. Obviously, the formation of this salt would decrease ee. Nevertheless, statistically, these diacids have found more use than monoacids; even the popular mandelic acid is only used in 8% of the reported resolutions.[12]

Most of the resolutions described in the literature are performed at a ratio between acid resolving agent and amine of one molar equivalent, half molar equivalent (Marckwald method) or at half molar equivalent in the presence of an achiral acid (Pope and Peachey method). Resolutions yielding solids with 100% ee at the first crystallisation are rare in the academic literature, and it is usual to find reports involving four to five consecutive re-crystallisations.[13] The effect on resolutions of resolving agent diacid/amine molar ratios (Γ) values lower than one,[13,14] has already been studied; however, no studies have systematically reported the effect of excess resolving agent, which for dicarboxylic resolving agents favours the formation of acidic diastereomeric salts. In parallel, a mathematical model describing the effect of pH and Γ, when monoacids were used as resolving agents, was previously proposed.[15] Nevertheless, no mathematical model has yet been presented describing resolutions of racemic amines by a diacid resolving agents.

Herein we report a model to calculate ee, Y, and mother liquor pH for resolutions of chiral amines by a diacid for $0.2 < \Gamma < 2.0$. Experiments are used to determine model parameters, and then the models are used to predict the ee and Y values. These are compared to experimental data for ee and Y obtained for two chiral amines over a range of molar ratio Γ. Two models are considered, which neglect and include, respectively, the acid–base equilibria. A strategy for the design of diastereomeric resolutions is then proposed. This involves measuring the solubilities of the four pure diastereomeric salts (two acid and two neutral salts) in different resolution solvent systems at given temperatures. Using these values as model inputs, it is possible to select Γ and amine concentration at which the resolution should be performed. This approach is not entirely a priori, since it relies on the knowledge of the diastereomer salts solubility limits, and the main disadvantage is the need for small amounts of the pure enantiomers for preparation of the four diastereomers. However, once these enantiomers are isolated on a preparative scale (through consecutive diastereomeric resolutions, more sophisticated resolution agents or expensive resolution techniques such as chiral chromatography), the proposed approach can easily be applied to design a diastereomeric resolution at industrial scale.

2. Results and discussion

2.1. Model results

The resolutions of two different racemic amines, PEA and PPI2, with (+)-DTTA and (−)-DTTA as resolving agents, respectively, were used to test the mathematical model (Fig. 1). PEA is a primary small aromatic amine (MW 121 g mol^{-1}) used as a chemical building block, with only one stereogenic centre. PPI2 is a piperidine of a relatively large size (MW 224 g mol^{-1}), supplied as a racemate comprising two enantiomers. In spite of the two stereogenic centres in each enantiomer, in this paper they are only designated as (*R*)-PPI and (*S*)-PPI enantiomers. The (*S*)-PPI enantiomer, that is, (3*S*,4*R*)-enantiomer of the piperidine, is used as precursor for synthesis of biologically active compounds and, is therefore of pharmaceutical interest. The experimental parameters employed as inputs for calculations in both model systems are summarised in Table 1.

Two models were developed as described in the model details section. The main difference between these models is that model II accounts for the existence of acid–base equilibria, whereas the simpler model I neglects their existence. Figure 2a–d shows values calculated using the two mathematical models for the formation of the four diastereo-

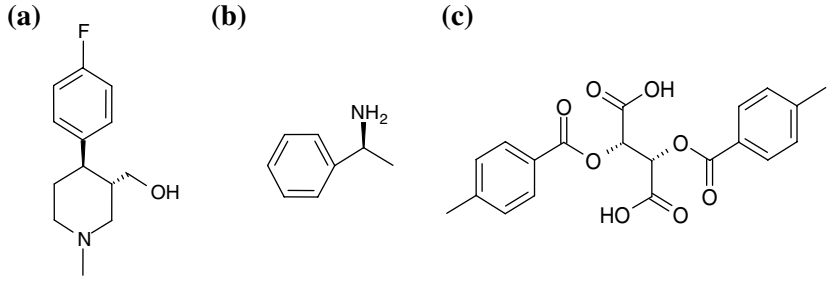

Figure 1. Chemical structures of chiral amines and resolving diacid agent used in this work. (a) (3*S*,4*R*)-Enantiomer of the piperidine of pharmaceutical interest (*S*-PPI). (b) (*S*)-Phenyl ethyl amine (*S*)-PEA. (c) (2*S*,3*S*)-Di-*O*,*O*′-*p*-toluyl-D-tartaric acid (+)-DTTA.

Table 1. Model parameters

Entry	Amine	PEA	PPI2
1	Ka_1 (M^{-1})	$10^{-5.2}$	$10^{-5.2}$
2	Ka_2 (M^{-1})	$10^{-6.6}$	$10^{-6.6}$
3	Ka_{amine} (M^{-1})	$10^{-8.4}$	$10^{-7.5}$
4	$Kd_{AS} = Kd_{AR}$ (M)	10^{5}	10^{5}
5	$Kd_{AS2} = Kd_{AR2}$ (M)	10^{10}	10^{10}
6	Ks^{AR} (mM)	2.8	361.0
7	Ks^{AS} (mM)	4.7	12.0
8	Ks^{AR2} (mM)	387.3	14.0
9	Ks^{AS2} (mM)	28.3	11.0
10	$[Amine]_0$ (mM)	176.0	280.0

meric salts as a function of Γ employed. For both models, the calculated results (Fig. 2a and b) show that for $\Gamma < 0.5$ (half equivalent method), only the neutral diastereomeric salts are formed. Under this condition, virtually all the resolving agent is consumed by the formation of diastereomeric salts ($AS2_t = 2{\cdot}\Gamma{\cdot}S_0$ and $AR2_t = 2{\cdot}\Gamma{\cdot}R_0$), whereas a significant part of the amine is left unreacted in solution ($S_{t,ML} = S_0{\cdot}(1 - 2{\cdot}\Gamma)$ or $R_{t,ML} = R_0{\cdot}(1 - 2{\cdot}\Gamma)$). For $0.5 < \Gamma < 1.5$, a mixture of neutral and acid salts is formed. Finally, for $\Gamma > 1.5$ only the acidic salts (AS and AR) are formed, with virtually all the amine consumed by the formation of these salts, and the resolving agent added in excess is left unreacted in solution. Notice that the calculated profiles are equal for the two neutral salts and for the two acidic salts. This is a direct consequence of the assumption that there is no chiral recognition in solution (Assumption 5, see Section 4).

A higher solubility limit (Ks) was found for the (*R*)-neutral salt in the case of PEA (Table 1, entry 8) and for the (*R*)-acid salt in the case of PPI2 (Table 1, entry 6). Therefore it is expected that the resolution of PEA is based on the differential solubility of neutral salts (Ks^{AR2} and Ks^{AS2}), whereas the resolution of PPI2 is based on the differential solubility of acidic salts (Ks^{AR} and Ks^{AS}). The calculated ee's shown in Figure 2c and d illustrate this, with the highest predicted ee's at $\Gamma < 0.5$ in the case of PEA (neutral salt formation), and above $\Gamma > 1.5$ in the case of PPI2 (acidic salt formation).

The calculated data in Figure 2a and b show a significant impact of the acid–base equilibria for the region of acidic

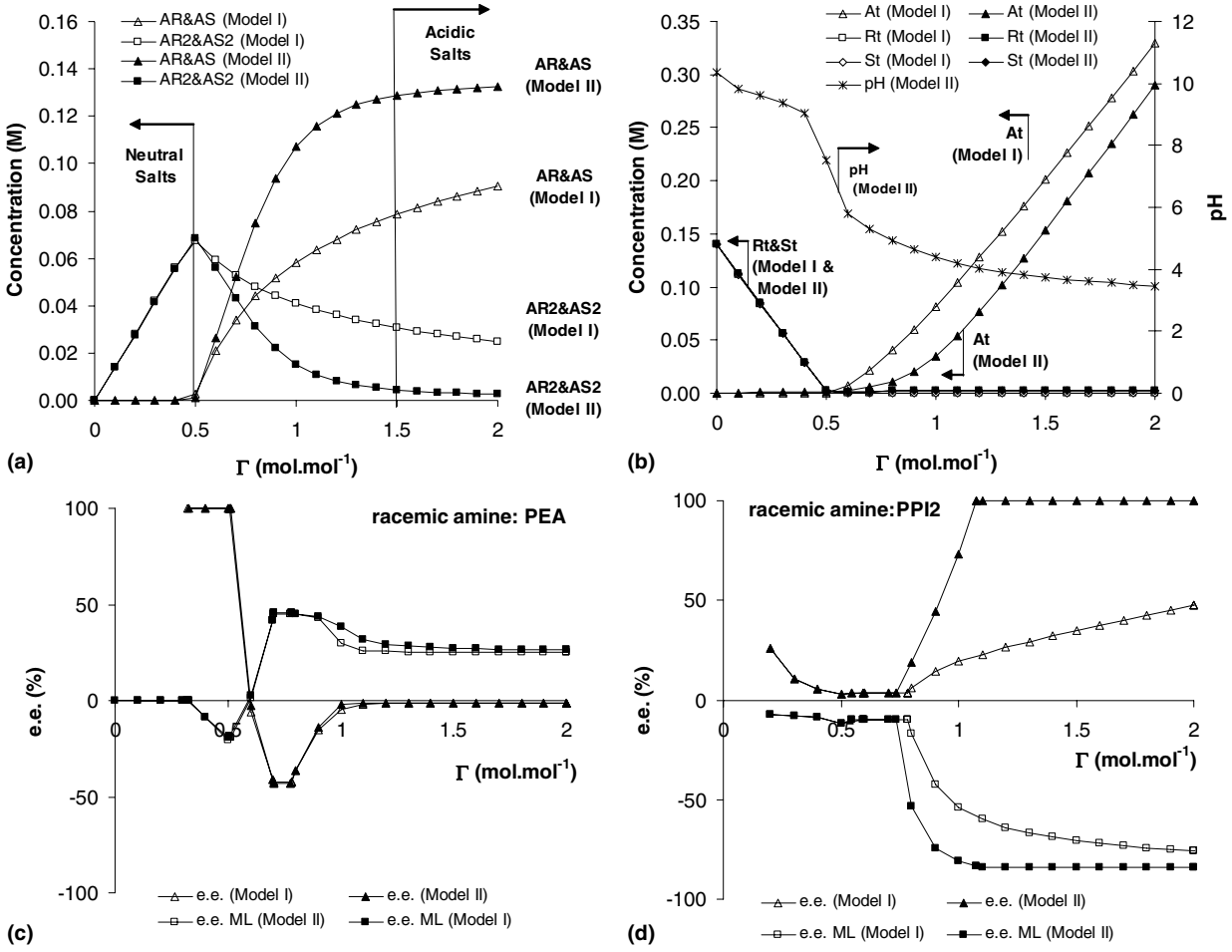

Figure 2. Model results comparison. Models I and II, respectively, neglect and account for acid–base equilibrium. Results in (a) and (b) were calculated for $[amine]_0 = 280$ mM, but neglecting the precipitation of any salt, that is, a value of zero was fixed for W_{AS}, W_{AR}, W_{AS2}, W_{AR2}. Results in (c) and (d) were calculated using input parameters given in Table 1 for the PEA and PPI2 amine–DTTA systems, respectively.

salt formation ($\Gamma > 1.5$), but suggest that it is not so important for the region where neutral salt formation takes place ($\Gamma < 0.5$). To follow this further, specific calculations for PEA and PPI2 are made in Figure 2c and d. Accounting for, or neglecting, acid–base equilibria does not affect the predicted ee's for a resolution based on the preferential precipitation of one of the neutral salts, as shown in Figure 2c for the case of PEA. However, considerably different ee's are predicted by the different models (Fig. 2d) for the case of PPI2, where resolution is based on the difference in solubilities of the acidic diastereomeric salts. Consequently, it is concluded that the acid–base equilibrium of racemic amine and resolving diacid agent should be taken into account, and from here onwards, model II will be employed in all calculations.

Resolutions of both amines were performed according to the resolution protocols described at constant racemic amine concentrations, with varying amounts of resolving agent added to vary Γ. For both systems, the ee's of both solid and mother liquor was measured, as well as the Y and mother liquor pH. The experimental and calculated results obtained are plotted in Figure 3a–d. These show a good relationship between predicted and experimentally measured values. Figure 3a shows a good match between ee for PEA except at $\Gamma < 0.5$; Figure 3c shows that for PPI2, ee is well-predicted across the range of Γ, while Figure 3b and d shows that the model predictions for Y and pH both compare very well with the experiment for both amines.

PPI2 resolutions were performed in acetone–water 97:3 wt % at 5 °C with amine concentrations of 280 mM. The maximum ee for these resolutions were found for $\Gamma > 1.5$. Under these conditions, (*R*)-acidic salt was formed at concentrations (140 mM) lower than its solubility limit ($Ks^{AR} = 361$ mM) and so it remains completely dissolved in the mother liquor. The (*S*)-acidic salt has a low solubility limit ($Ks^{AS} = 12$ mM), and hence, precipitation was observed. For $0.2 < \Gamma < 0.75$, the (*R*)-PPI and (*S*)-PPI neutral

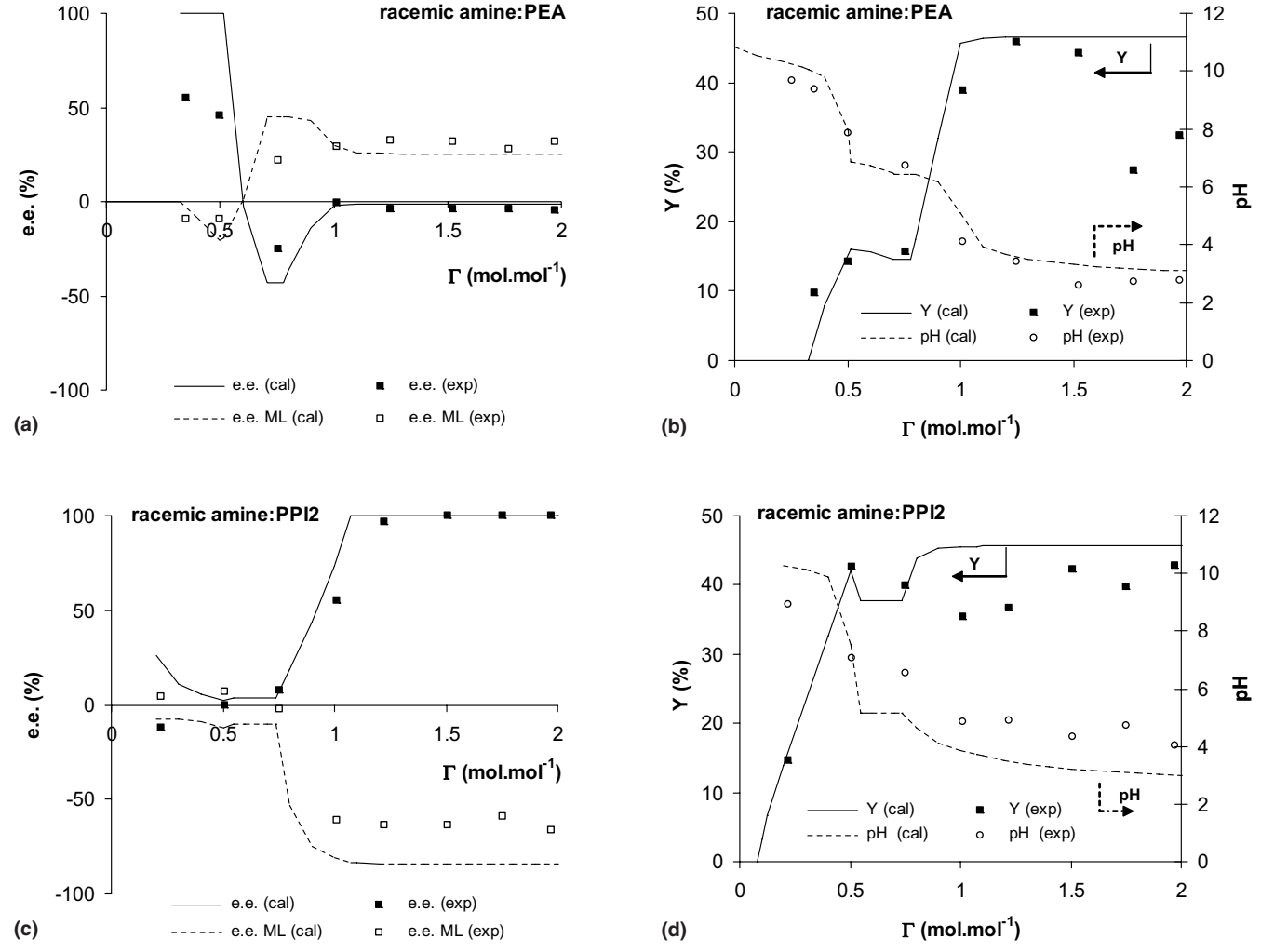

Figure 3. Comparison between experimental and modelling results. (a) and (b) show results for PEA and (c) and (d) for PPI2. Calculated results were obtained using model II and input parameters given in Table 1. (a) and (c) show the ee for (*S*)-enantiomer, of the obtained solids, and of the mother liquor (ee ML). (b) and (d) show the resolution yield, calculated as the percentage of (*S*)-amine obtained in the solid over the total racemic amine fed to the resolution $[amine]_0$.

salts are formed (Fig. 2a), and due to the low solubility of these salts ($Ks^{AR2} = 14$ mM and $Ks^{AS2} = 11$ mM), both precipitate, yielding a solid with negligible ee (Fig. 2d).

PEA resolution was performed in isopropanol–water 50:50 wt % at 22 °C and with an amine concentration of 176 mM. The maximum experimental ee observed was 55.2% at $\Gamma = 0.35$, whereas the model predicts an ee of 100%. The theoretical value is consistent with a high solubility for the (*R*)-PEA neutral salt ($Ks^{AR2} = 387.3$ mM) and a lower solubility for the (*S*)-PEA salt ($Ks^{AS2} = 28.3$ mM). The divergence of theoretical and experimental values might be due to the formation of the mixed enantiomer neutral salt, which was not accounted for in the model. At $\Gamma > 1$, both (*R*)-PEA and (*S*)-PEA acid salts are formed at concentrations significantly higher than their solubility limits ($Ks^{AR} = 2.8$ mM and $Ks^{AS} = 4.7$ mM), and therefore, both salts precipitate, yielding a solid with negligible ee.

In the case of PEA, it is also interesting to note that theoretical and experimental results show a slight negative ee between $0.5 < \Gamma < 1.0$. This result is the consequence of an inversion in the order of solubility for the (*S*)- and (*R*)-salts, when both neutral and acidic PEA salts are formed. That is, in this model system the more soluble salt of the neutral salts is, by far, the neutral (*R*)-salt, however the opposite situation occurs for the acidic salts, in which the (*S*)-salt is more soluble than the (*R*)-salt. For this region in Γ, the four salts are formed (Fig. 2a) and the inversion of the ee observed is a consequence of the balance between the formation and different solubilities of these four species. Moreover, for the same range of Γ values, the calculated and experimental Y follows the same trend, which once again is ruled by the diastereomer salt solubility.

2.2. Rational design of resolutions

The above comparison between model calculations and experimental data shows that the model predictions are reasonably accurate for the two model systems presented. This suggests that this model approach can be used for design of other resolutions.

Notice that a transition between high and low ee was found for both neutral (PEA, Fig. 3a) and acidic salt (PPI2, Fig. 3c) based resolutions. Examination of species profiles (Fig. 2a) shows that the highest variety of diastereomeric salts is found for $0.5 < \Gamma < 1.5$. For this range of Γ values, all the diastereomeric salt solubility limits have to be accounted for, increasing the complexity of the resolutions performed under such conditions and seldom yielding highly enantiopure products. Therefore, more enantiopure products are obtained at $\Gamma < 0.5$ or $\Gamma > 1.5$. This observation contradicts the usual practice, reported in the literature, of screening resolution conditions at one mole equivalent ($\Gamma = 1.0$).

A second important argument to be made is that, depending on the solubility limits, more efficient resolutions can be achieved when they are based on the formation of acidic salts, rather than neutral, and thus $\Gamma > 1.5$ should be preferentially employed. This suggestion is based on two different observations; the first is related to the potential formation of mixed enantiomer neutral salts, resulting in poor ee. The second point is related to Y (Fig. 3b and d); as a consequence of a low usage of resolving agent, more free amine is left in solution (Fig. 2b), leading to a lower Y in a neutral salt (PEA) based resolution than in an acidic salt based resolutions (PPI2). Once again, this suggestion differs from the strategy suggested in the literature, where the majority reported work employs $\Gamma \leqslant 1$, usually claiming that this procedure leads to savings on resolving agent. We advocate that molar ratios of greater than 1.5 be employed, and suggest that the increased use of resolving agents is countered by development of facile techniques for recovery and re-use resolving agents within a process.

Therefore, the proposed approach for designing diastereomeric resolutions consists of focusing on the measurement of the solubility for the four enantiopure diastereomer salts in different solvent systems and at different temperatures, as follows:

1. **Obtain the pure enantiomers** through alternative resolution techniques or with consecutive diastereomeric resolutions.
2. **Select potential resolution solvent systems.** Methanol, ethanol, isopropanol, ethylacetate and acetone and their respective water mixtures are the most commonly employed and therefore the suggested candidates for solvent systems.
3. **Estimate the solubilities of the four pure diastereomeric salts** (Ks^{AS}, Ks^{AR}, Ks^{AR2} and Ks^{AR2}) in different solvents and at different temperatures. For example, the solubility curves for the salts in different solvent systems can be obtained by adding several concentrations of pure enantiomer and resolving agent at $\Gamma < 0.2$ and $\Gamma > 1.5$ (respectively, for neutral and acidic salt formation).
4. **Temperature profiles** over time, consist of an initially higher temperature (sometimes at solvent boiling temperature) with the aim of dissolving all the material in solution, followed by a decrease in temperature, until precipitation is observed and finally, a plateau at a temperature low enough to ensure that equilibrium has been achieved.
5. **Compare the solubilities** between the two acidic salts and the two neutral salts in the different solvent systems and establish which resolution solvent system and type of salt, neutral or acidic, provides the larger difference in solubilities between the (*R*)- and (*S*)-diastereomeric salts.
6. **Select the concentration of the racemic base** (i.e., racemic base in resolution solvent) to be employed in order to ensure that a crystalline solid with 100% ee is obtained. This concentration should be such that the more soluble salt remains entirely dissolved in the mother liquor, but the less soluble salt is present in quantities high enough to saturate the liquid solution and yield a solid product. In other words for a resolution based on acidic salt formation, the concentration of racemic amine fed should be lower than twice the more soluble acidic salt

solubility. Similarly, for a resolution based on a neutral salt formation, this value should be lower than $2/\Gamma$ times the more soluble neutral salt solubility, since for $\Gamma < 0.5$ only a fraction of the amine fed is used to form the diastereomeric salts.

Once amine concentration is set at a value low enough to avoid the precipitation of the more soluble salt, the precipitate of the less soluble salt has an ee 100%, leaving all of the more soluble salt dissolved in the mother liquor. Therefore, under such conditions, the amount of precipitated salt and Y can be calculated on the basis of amine concentration fed and the solubility of the less soluble salt. A final question, for which a quantitative answer is important, is how large should the difference between the solubility of the two different diastereomeric salts be to make a resolution efficient? The answer has already been tackled by Kozma[12] for resolutions with a chiral monoacid. Since the proposed approach in this paper targets ee = 100% in the first resolution, we will illustrate the dependence of Y on the ratio of the solubilities of the (*R*)- and (*S*)-diastereomer salts in Figure 4. The first result illustrated in Figure 4 refers to a resolution based on the formation of acidic salts, in which the potential formation of mixed enantiomer neutral salts is not an issue, and shows that efficient resolutions, with Y's around 40%, can be achieved for ratios of solubility higher than 5. This value corresponds to 80% of the maximum theoretical Y at a value of 50%. In the case of PPI2, a ratio $Ks^{AR}/Ks^{AS} = 30$ was found in acetone–water 97:3 wt %. Figure 4 also illustrates the calculated Y's for a resolution based on the formation of neutral salts, neglecting the formation of mixed enantiomer neutral salt. In such cases, part of the amine was assumed to remain unreacted and, therefore, the maximum theoretical yield is lower than 50%. Higher amounts of unreacted amine remain in the mother liquor for lower Γ values employed, leading to lower amounts of diastereomer salts formed and available to precipitate, and so also to lower Y's. Maximum theoretical Y values of 25% and 17.5% were calculated for Γ of 0.5 and 0.35, respectively. Once again, a value corresponding to 80% of these maximum theoretical Y is calculated at diastereomeric salt solubility ratio of 5. For PEA, a ratio of $Ks^{AR2}/Ks^{AS2} = 14$ was found in isopropanol–water 50:50 wt %.

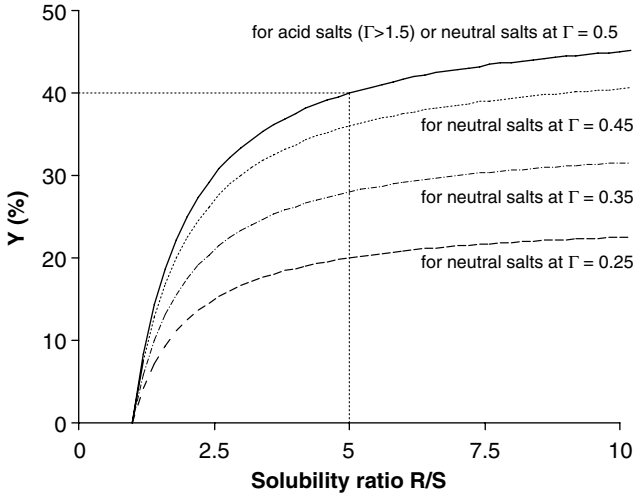

Figure 4. Calculation of resolution yield as a function of the solubility ratio of (i) acid ($\Gamma > 1.5$) and (ii) neutral ($\Gamma = 0.5$ and 0.35) diastereomeric salts. In this ideal limiting case the concentration of racemic mixture fed to the resolution is set to the maximum value that allows all (*R*)-amine is kept in solution as free enantiomer or diastereomeric salt. Thus the molar concentration of racemic mixture is exactly (i) 2 times the solubility of the more soluble acid diastereomeric salt or (ii) $2/\Gamma$ times the solubility of the more soluble neutral diastereomeric salt. Notice that to obtain 100% ee, the concentration of racemic mixture fed to resolution has to be less than this value.

3. Conclusions

Two mathematical models describing diastereomeric resolution of racemic amines by a chiral diacid resolving agent have been presented. The models predict ee and Y, and for the more complex model that takes into account the acid-base equilibria and pH. By comparing the predicted results, the importance of taking the acid base equilibria into account in the model was shown. The model predicted results for the values of Y, and ee in the crystals and in the mother liquors compared well with the experimental results. The model predicts that for $\Gamma < 0.5$, neutral salts are preferentially formed and for $\Gamma > 1.5$ acidic salts are preferentially formed. Furthermore a mixture of acidic and neutral salts can be found for $0.5 < \Gamma < 1.5$. On this basis it can be concluded that diastereomeric resolutions should be performed either at $\Gamma < 0.5$ or $\Gamma > 1.5$, based on neutral or acidic salt formation, respectively. It is suggested that the decision of which molar ratio to employ, as well as the selection of solvent system, should be based on the determination of the solubilities of the enantiopure diastereomer salts. It was concluded that to perform an efficient resolution, a solubility ratio (solubility of the more soluble diastereomer divided by solubility of the less soluble diastereomer) of higher than 5 was required. Concentration of the racemic amine fed to resolution should be calculated so that the more soluble salt remains entirely dissolved in the mother liquor. We speculate that the difference between experimental and theoretical ee for the PEA system is due to the formation of a mixed enantiomer neutral salt. This, together with yield considerations, suggest that where possible, the diastereomeric resolutions should be based on the formation of acidic salts at $\Gamma > 1.5$.

4. Model details

4.1. Model equations, mathematical model assumptions, inputs and outputs

Two mathematical models are used in this work (Figs. 5 and 6). These assume that

1. Only four diastereomeric salts are formed, two acidic and two neutral, that is, there is no formation of 'mixed enantiomer' ASR neutral salt.
2. The unreacted amine and diacid resolving agent are completely soluble in the mother liquor at the resolution temperatures.

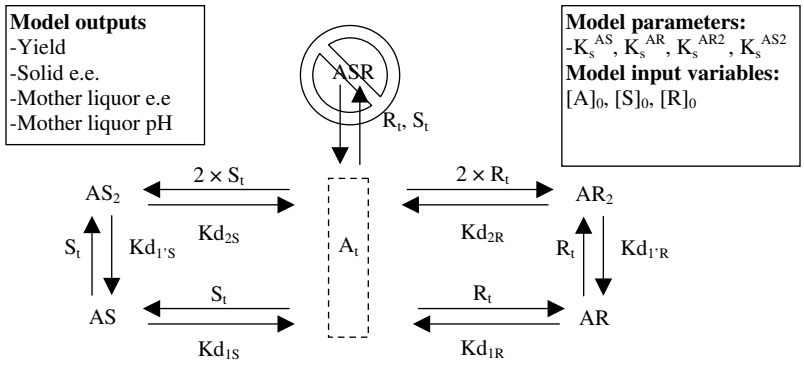

Figure 5. Equilibria considered in model I, which neglects acid–base equilibrium.

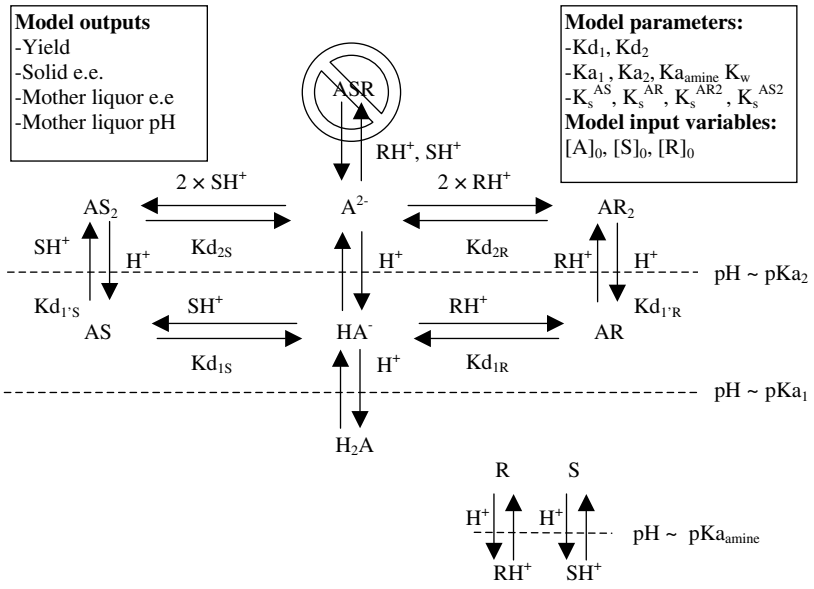

Figure 6. Equilibria considered in model II, which takes into account acid–base equilibrium.

3. Precipitation/crystallisation of the four diastereomeric salts follows ideal behaviour and is thermodynamically controlled, following the solubility limits of the respective diastereomeric salts (Ks^{AS}, Ks^{AR}, Ks^{AR2} and Ks^{AR2}).
4. The two proposed models differ in the following assumptions:
   (a) For model I (the simpler model) the acid–base equilibrium of the diacid resolving agent and amine enantiomers is neglected, and the diastereomeric equilibrium constants are defined on the basis of the total amount of reactants dissolved in the mother liquor (Eqs. 8a–11a).
   (b) For model II (the more complex model), acid–base equilibrium are taken into account and is assumed that the formation of diastereomeric salts occurs through ionic bonds between the charged carboxylate groups of the diacid resolving agent and ammonium group of the amine. Therefore, the equilibrium constants for diastereomeric salt formation are based only on the ionic forms of the reactants (Eqs. 8b–11b). Moreover, the acid–base equilibria for the amine and the diacid resolving agent, with the respective acid dissociation constants, are also taken into account through Ka_{amine}, Ka_1 and Ka_2.
5. There is no chiral recognition in solution.[16] Therefore, the same value is assigned to equilibrium constants of reactions in the mother liquid independently of the enantiomer considered, that is, $Kd_{1S} = Kd_{1R} = Kd_1$, $Kd_{1'S} = Kd_{1'R} = Kd_{1'}$ and $Kd_{2S} = Kd_{2R} = Kd_2$.
6. Diastereomeric salt formation tends towards irreversibility and the values of the equilibrium constants were selected accordingly.
7. The Gibbs free energy of bond formation between the free resolving agent and the first amine (acidic salt formation), and the acidic salt and the second amine (formation of the neutral salt) is the same, hence they have the same formation enthalpy and constants, that is, $Kd_1 = Kd_{1'}$ and $f = 1$. Thus Kd_2 is equal to $(Kd_1)^2$.

Assumptions 5 and 6 are supported by experimental data published elsewhere.[16] Briefly, this data was obtained by nanofiltering different solutions comprising different molar ratios of diacid resolving agent and racemic PEA, at concentrations below the solubility limits of the diastereomeric salts. The molecular weight cut off of the membrane employed was selected in order to permeate only the unreacted amine, whilst the resolving agent diastereomeric salts were retained. The ee of the permeated solutions was found to be near zero, which confirms Assumption 5. Moreover, analyses of the amine in the permeate showed that (i) the formation of the diastereomeric salts is practically irreversible, confirming Assumption 6, and (ii) at molar ratios between 0.2 and 1.0, neutral salts are formed preferentially to acidic salts, confirming the species profiles obtained in Figure 2a. To expand this data, a similar study was undertaken for the PPI2 model system as part of the present work and similar results were observed (Fig. 7). Therefore values for diastereomeric salts formation constants (Kd_1, Kd_2) have been selected at values high enough to simulate irreversible behaviour. Evaluation of the effects of different values for these constants on the species profiles have been calculated and the results of the models are shown in Figure 8. Values in the range of 10^2–10^8 M^{-1} were previously estimated for the formation of diastereomeric salts formed by mono-acids resolving agents.[15]

Figure 8a shows that for increasing values of Kd_1, above 10^4 M^{-1} (with $f = 1$, that is Kd_2 10^8 M^{-2}), there is no significant effect on the calculated diastereomeric salt profiles. Therefore further increases in the values of these constants no longer changes the final outcome of the model results in terms of salt formation. Figure 8b considers the case when the formation of the acidic salt is more energetically favourable than the binding of the second amine so that $f < 1$. Again, no significant effects are observed on the final calculated species profile. This is the scenario that has physical meaning, since it is always energetically easier to protonate A^{2-} than AH^- ($1/Ka_2 > 1/Ka_1$). Nevertheless, the model calculations for a hypothetical case in which the formation of the neutral salt is highly favoured over

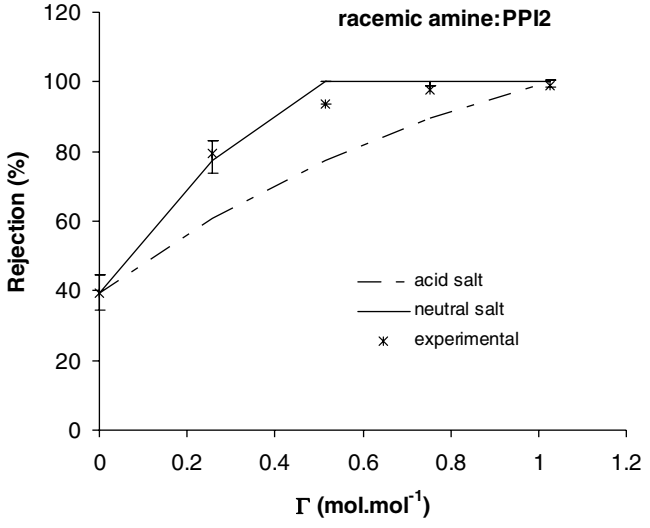

Figure 7. Comparison of experimental and theoretical rejections of PPI2 amine as function of DTTA/amine molar ratios. Rejection = (1 − Cp/Cr) × 100, with Cp and Cr as the PPI2 concentrations on the permeate and retentate, respectively. Detailed experimental techniques can be found elsewhere.[16]

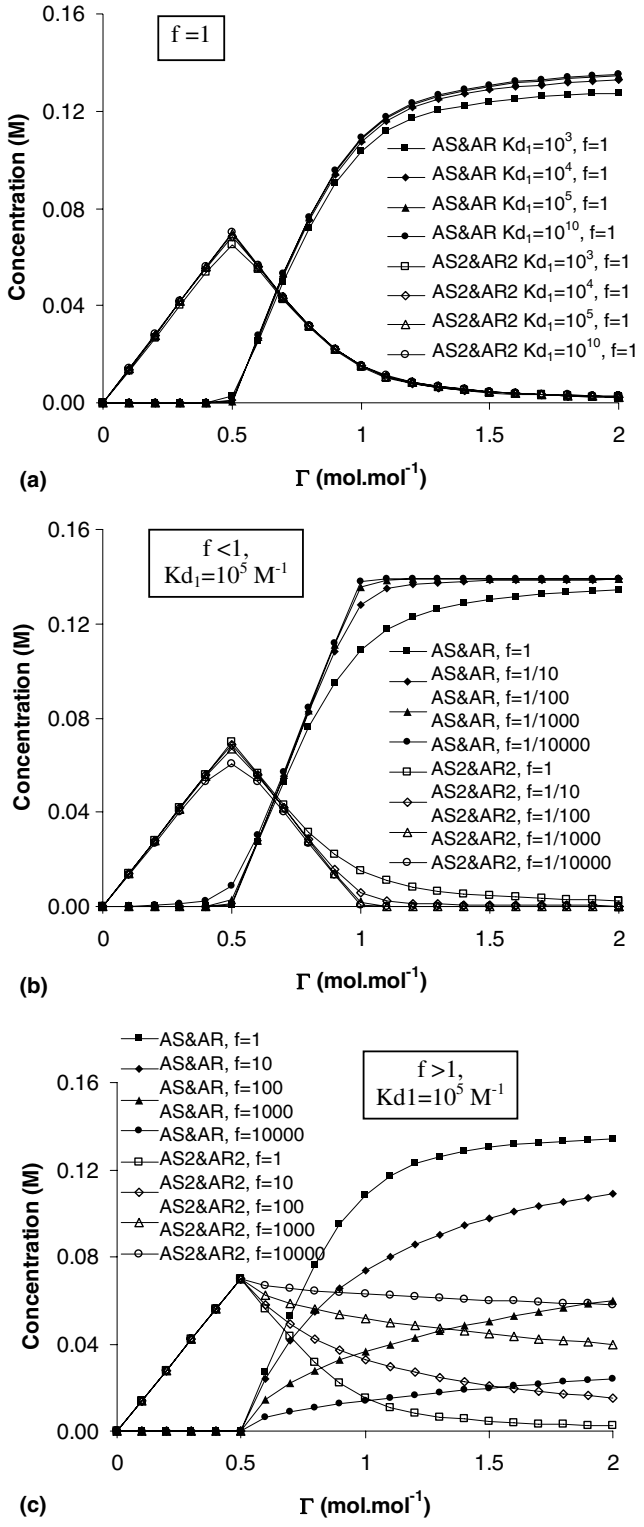

Figure 8. Selection of the diastereomeric formation constant values. $Kd_1 = Kd_{1S} = Kd_{1R}$, $Kd_{1'} = Kd_{1'S} = Kd_{1'R}$, $f = Kd_{1S}/Kd_{1'S} = Kd_{1R}/Kd_{1'R}$.

the acidic salt, are also shown in Figure 8c. A significant effect on the species profile, especially for $\Gamma > 0.5$, would be observed for this improbable case.

The selected values for Kd seem to describe the observed phenomena well. However, these constants can be independently measured in further studies. The solubility limits of the four diastereomeric salts (Ks^{AS}, Ks^{AR}, Ks^{AR2} and Ks^{AS2}) and acid–base constants (Ka_1, Ka_2, Ka_{PPI2}, Ka_{PEA}) have been measured in independent experiments for both model systems following the protocol described in Section 5.

4.2. Model equations

Two models are used in this paper: model I, a simpler model, which neglects the acid–base equilibria, and model II, which takes into account the acid–base equilibria between the amine enantiomers (*R*,*S*) and the diacid resolving agent (A). Eqs. 1–3 are total molar balances for each of the three species which account for their existence as free species in the mother liquor, or as dissolved or solid diastereomeric salts.

$$[A]_0 = [A]_{t,ML} + [AS]_t + [AR]_t + [AS2]_t + [AR2]_t \quad (1)$$

$$[R]_0 = [R]_{t,ML} + [AR]_t + 2 \times [AR2]_t \quad (2)$$

$$[S]_0 = [S]_{t,ML} + [AS]_t + 2 \times [AS2]_t \quad (3)$$

Both models assume that the free amines are completely soluble (Assumption 2) in the mother liquor, and that the formation of solids by each of the diastereomeric salts at the resolution temperature follows the respective solubility limits (Assumption 3). This imposes the following conditions for each diastereomeric salt:

$$\text{if } [AR]_t < Ks^{AR} \quad \text{then } W_{AR} = 0 \quad \text{else } W_{AR} = [AR]_t - Ks^{AR} \quad (4)$$

$$\text{if } [AS]_t < Ks^{AS} \quad \text{then } W_{AS} = 0 \quad \text{else } W_{AS} = [AS]_t - Ks^{AS} \quad (5)$$

$$\text{if } [AR2]_t < Ks^{AR2} \quad \text{then } W_{AR2} = 0 \quad \text{else } W_{AR2} = [AR2]_t - Ks^{AR2} \quad (6)$$

$$\text{if } [AS2]_t < Ks^{AS2} \quad \text{then } W_{AS2} = 0 \quad \text{else } W_{AS2} = [AS2]_t - Ks^{AS2} \quad (7)$$

Diastereomeric salt formation is assumed to occur only in solution, according to the following equilibrium constants (Assumption 4):

$$Kd_{1R} = \frac{[AR]_t - W_{AR}}{[A]_{t,ML} \cdot [R]_{t,ML}} = \frac{[AR]_{ML}}{[A]_{t,ML} \cdot [R]_{t,ML}} \quad (8a)$$

$$Kd_{1S} = \frac{[AS]_t - W_{AS}}{[A]_{t,ML} \cdot [S]_{t,ML}} = \frac{[AS]_{ML}}{[A]_{t,ML} \cdot [S]_{t,ML}} \quad (9a)$$

$$Kd_{2R} = \frac{[AR2]_t - W_{AR2}}{[A]_{t,ML} \cdot [R]^2_{t,ML}} = \frac{[AR2]_{ML}}{[A]_{t,ML} \cdot [R]^2_{t,ML}} \quad (10a)$$

$$Kd_{2S} = \frac{[AS2]_t - W_{AS2}}{[A]_{t,ML} \cdot [S]^2_{t,ML}} = \frac{[AS2]_{ML}}{[A]_{t,ML} \cdot [S]^2_{t,ML}} \quad (11a)$$

Also

$$Kd_{1'R} = \frac{[AR2]_{ML}}{[AR]_{ML} \cdot [R]_{t,ML}} \quad \text{and} \quad Kd_{2R} = Kd_{1R} \times Kd_{1'R} = \frac{[AR]_{ML}}{[A]_{t,ML} \cdot [R]_{t,ML}} \frac{[AR2]_{ML}}{[AR]_{ML} \cdot [R]_{t,ML}}$$

Similarly,

$$Kd_{1'S} = \frac{[AS2]_{ML}}{[AS]_{ML} \cdot [S]_{t,ML}} \quad \text{and} \quad Kd_{2S} = Kd_{1S} \times Kd_{1'S} = \frac{[AS]_{ML}}{[A]_{t,ML} \cdot [S]_{t,ML}} \frac{[AS2]_{ML}}{[AS]_{ML} \cdot [S]_{t,ML}}$$

Since we assume no chiral recognition in solution, *S* and *R* constants have the same value, that is, $Kd_{2S} = Kd_{2R}$, $Kd_{1S} = Kd_{1R}$ and $Kd_{1'S} = Kd_{1'R}$ (Assumption 5). It is also assumed that the equilibrium for the formation of diastereomer salts tends to irreversibility (Assumption 6) and therefore high values for the Kd constants were used as model inputs. From Assumption 7 $Kd_1 = Kd_{1'}$ and so $Kd_2 = (Kd_1)^2$.

Model II considers for Brönsted acid–base equilibria between the amine and diacid resolving agent. These species can exist in neutral or ionic forms, and therefore their concentrations in solution have to be described as the following species:

$$[A]_{t,ML} = [A^{2-}]_{ML} + [AH^-]_{ML} + [H_2A]_{ML}$$

$$[R]_{t,ML} = [R]_{ML} + [RH^+]_{ML}$$

$$[S]_{t,ML} = [S]_{ML} + [SH^+]_{ML}$$

Moreover, Eqs. 12–17 have to be added to the model, to take into account the acid–base equilibriums and solution electroneutrality:

$$Ka_1 = \frac{[AH^-]_{ML} \cdot [H^+]_{ML}}{[H_2A]_{ML}} \quad (12)$$

$$Ka_2 = \frac{[A^{2-}]_{ML} \cdot [H^+]_{ML}}{[HA^-]_{ML}} \quad (13)$$

$$Ka_{Amine} = \frac{[R]_{ML} \cdot [H^+]_{ML}}{[RH^+]_{ML}} \quad (14)$$

$$Ka_{Amine} = \frac{[S]_{ML} \cdot [H^+]_{ML}}{[SH^+]_{ML}} \quad (15)$$

$$[H^+]_{ML} + [SH^+]_{ML} + [RH^+]_{ML} = [HA^-]_{ML} + 2 \times [A^{2-}]_{ML} + [OH^-]_{ML} \quad (16)$$

$$[H^+]_{ML} \cdot [OH^-]_{ML} = Kw \quad (17)$$

Model II also assumes that the formation of diastereomeric salts occurs through the reaction ionic forms of reactants (Assumption 4) and so Eqs. 8a–11a of the simpler model are replaced by Eqs. 8b–11b

$$Kd_{1R} = \frac{[AR]_t - W_{AR}}{[HA^-]_{ML} \cdot [RH^+]_{ML}} \quad (8b)$$

$$Kd_{1S} = \frac{[AS]_t - W_{AS}}{[HA^-]_{ML} \cdot [SH^+]_{ML}} \quad (9b)$$

$$\mathrm{Kd}_{2R} = \frac{[\mathrm{AS2}]_t - W_{\mathrm{AS2}}}{[\mathrm{A}^{2-}]_{\mathrm{ML}} \cdot [\mathrm{RH}^+]^2_{\mathrm{ML}}} \quad (10b)$$

$$\mathrm{Kd}_{2S} = \frac{[\mathrm{AS2}]_t - W_{\mathrm{AS2}}}{[\mathrm{A}^{2-}]_{\mathrm{ML}} \cdot [\mathrm{SH}^+]^2_{\mathrm{ML}}} \quad (11b)$$

Also $\mathrm{Kd}_{2R} = \mathrm{Kd}_{1R} \times \mathrm{Kd}_{1'R}$ and $\mathrm{Kd}_{2S} = \mathrm{Kd}_{1S} \times \mathrm{Kd}_{1'S}$.

Assumptions 5–7 also hold for the more complex model and therefore the same values for the Kd constants are used in both models. Additionally, model II assumes the same acid–base equilibrium constant (K_{amine}) for both amine enantiomers (Assumption 6).

Each model is a system of algebraic equations and was solved using gPROMs, a commercially available mathematical package from Process Systems Enterprise Ltd (UK). The last four equations (Eqs. 18–21) are used to calculate the model outputs.

$$\text{Yield } (\%) = \frac{W_{\mathrm{AS}} + 2 \times W_{\mathrm{AS2}}}{[\mathrm{S}]_0 + [\mathrm{R}]_0} \times 100 \quad (18)$$

$$\text{ee } (\%) = \frac{(W_{\mathrm{AS}} + 2 \times W_{\mathrm{AS2}}) - (W_{\mathrm{AR}} + 2 \times W_{\mathrm{AR2}})}{(W_{\mathrm{AS}} + 2 \times W_{\mathrm{AS2}}) + (W_{\mathrm{AR}} + 2 \times W_{\mathrm{AR2}})} \times 100 \quad (19)$$

$$\mathrm{pH} = -\mathrm{Log}[\mathrm{H}^+] \quad (20)$$

$$\text{ee ML } (\%) = \frac{([\mathrm{S}]_{t,\mathrm{ML}} + [\mathrm{AS}]_{\mathrm{ML}} + 2 \times [\mathrm{AS2}]_{\mathrm{ML}}) - ([\mathrm{R}]_{t,\mathrm{ML}} + [\mathrm{AR}]_{\mathrm{ML}} + 2 \times [\mathrm{AR2}]_{\mathrm{ML}})}{([\mathrm{S}]_{t,\mathrm{ML}} + [\mathrm{AS}]_{\mathrm{ML}} + 2 \times [\mathrm{AS2}]_{\mathrm{ML}}) + ([\mathrm{R}]_{t,\mathrm{ML}} + [\mathrm{AR}]_{\mathrm{ML}} + 2 \times [\mathrm{AR2}]_{\mathrm{ML}})} \times 100 \quad (21)$$

5. Experimental

5.1. Materials

Solvents (HPLC grade) were obtained from Aldrich–Sigma UK. Pure (*R*)- and (*S*)-enantiomers and a racemic mixture of PEA were also supplied by Aldrich–Sigma UK. (+)-Di-*O*,*O*′-*p*-toluyl-D-tartaric acid and (−)-di-*O*,*O*′-*p*-toluyl-L-tartaric acid, that is, (+)-DTTA and (−)-DTTA, were supplied by Fluka UK. Racemic PPI2 and pure *S*-PPI enantiomer were supplied by GSK Ltd. *R*-PPI was obtained throughout resolution of racemic PPI2 with (+)-DTTA according to the resolution protocol described below.

5.2. Resolution protocols

5.2.1. PEA. Isopropanol–water 50:50 wt % homogeneous solution was employed as resolution solvent. Racemic PEA and (+)-DTTA were dissolved in separate 2.5 ml aliquots of this solvent at 55 °C. The concentration of racemic PEA in 2.5 ml was fixed at a value of 352 mM. The amount of (+)-DTTA added was varied according to the final Γ value sought. The two clear 2.5 ml solutions were pre-heated at 55 °C and added together at this temperature; the resulting 5 ml solution was also clear. The solution was stirred for 1 h at 55 °C, and then allowed to cool down until 40 °C. After stirring for a further hour at this temperature, the solution was allowed to cool down until 25 °C and stirred for further 24 h. The resulting solid was separated by vacuum filtration, washed with resolution solvent and dried at room temperature for 24 h, and weighed. This crystallisation protocol is a modification of the method previously reported for the resolution of 2-amino-1-phenylethanol.[3]

5.2.2. PPI2. Acetone–water 97:3 wt % homogeneous solution was employed as resolution solvent for PPI2. Resolution was performed by mixing 10 ml of a solution of 560 mM PPI2 in this solvent with an equal volume of (−)-DTTA solution in the same solvent. Again, the amounts of (−)-DTTA varied according with the Γ value sought. The two solutions were pre-heated at 40 °C and added together. The resulting solution was stirred at 40 °C for half an hour, and then allowed to cool down under stirring at room temperature for a half hour, and finally aged for 1 h at 4 °C. The crystals obtained were filtered under vacuum and washed with resolution solvent.

5.3. Analysis

PEA and PPI2 amine concentrations and ee analysis were carried out by HPLC. For PEA, a Unicam Crystal 200 HPLC was used, with the mobile phase comprising 90% hexane–10% isopropanol–0.1% ethanolamine and flowing at a rate of 0.75 ml min^{-1} for 20 min per analysis through a Daicel OD-H chiral (5 mm, 250 mm × 0.46 mm) column, with a UV detector wavelength adjusted to 254 nm. In the case of PPI2 analyses, a Gilson 712 HPLC was used, with the mobile phase comprising 95% acetronitrile–5% methanol–0.02% trifluoroacetic acid–0.01% ammonia and flowing at a rate of 0.70 ml min^{-1} through a Cyclobond I 2000 AC (5 mm, 250 mm × 0.46 mm) column, with the UV detector wavelength adjusted to 265 nm. For analysis, an aliquot of mother liquor or solid was dissolved in NaOH (2 M) and extracted into mobile phase or DCM, respectively for PEA or PPI analysis. Calibrations have been prepared accordingly.

Experimentally, ee's and *Y*'s were estimated as

$$\text{ee} = \frac{\text{mol } S_{\mathrm{W}} - \text{mol } R_{\mathrm{W}}}{\text{mol } S_{\mathrm{W}} + \text{mol } R_{\mathrm{W}}} \times 100 \quad (22)$$

$$\text{ee ML} = \frac{\text{mol } S_{\mathrm{ML}} - \text{mol } R_{\mathrm{ML}}}{\text{mol } S_{\mathrm{ML}} + \text{mol } R_{\mathrm{ML}}} \times 100 \quad (23)$$

$$\text{Yield} = \frac{\text{mol } S_{\mathrm{W}}}{\text{mol } S_0 + \text{mol } R_0} \times 100 \quad (24)$$

Analyses of DTTA were also carried out using a 712 Gilson HPLC, but with a Phenomex Luna C18 (2)

(50 × 2.0 mm, 3 μm) column. Two independent solutions were employed as mobile phases, water and ACN, both containing 0.1% of TFA, at a flowrate of 0.50 ml min^{-1}. For these analyses, a solvent gradient from water to ACN solution in 20 min was employed, followed by a plateau of 5 min for the ACN solution. The UV detector wavelength was adjusted to 259 nm. For analysis, an aliquot of mother liquor or solid was dissolved in HCl (5 M) and extracted into mobile phase or DCM. Calibrations were prepared accordingly.

5.4. Measurement of solubility and p*K*a for amines

Pure diastereomeric salts of the amines were prepared by dissolving pure enantiomers of the amine and DTTA in the resolution solvent at Γ of 0.2 or 2.0 for the neutral and acidic salts, respectively. The isolated solids of the pure diastereomeric salts were added in excess to the resolution solvent at the final resolution temperature (5 or 25 °C for PPI2 or PEA resolutions, respectively), ensuring that a saturated solution is obtained by observing solid precipitate. The solutions were left stirring over 24 h and centrifuged thereafter. A known amount of the resulting liquid phase was dried at room temperature over 24 h and the net mass measured as the dissolved salt in the respective sample. The solubility limit was calculated from the mass ratio of dissolved crystals, the liquid phase left to dry, the solvent density, diastereomer stoichiometry and molecular weight of amine and DTTA. The acid dissociation constants Ka_1, Ka_2 and Ka_{PPI2} and Ka_{PEA} were measured in the resolution solvents by titration with triethylamine or trifluroacetic acid for the diacid resolving agent, DTTA, or the amines, respectively.

6. Nomenclature

A	chiral diacid resolving agent (M)
A^{2-}	di anion chiral diacid resolving agent (M)
AR	pure acidic (*R*)-diastereomeric salt (M)
AR2	pure neutral (*R*)-diastereomeric salt (M)
ARS	mixed enantiomer neutral diastereomer salt (M)
AS	pure acidic (*S*)-diastereomeric salt (M)
AS2	pure neutral (*S*)-diastereomeric salt (M)
C	amine concentration (M)
Cp	amine concentration in the permeate (M)
Cr	amine concentration in the retentate (M)
(+)-DTTA	(+)-di-*O*,*O*′-*p*-toluyl-D-tartaric acid
(−)-DTTA	(−)-di-*O*,*O*′-*p*-toluyl-L-tartaric acid
ee	(solid) enantiomeric excess (%)
ee ML	mother liquor enantiomeric excess (%)
f	$Kd_{1S}/Kd_{1'S} = Kd_{1R}/Kd_{1'R}$
H^+	hydrogenium cation (M)
H_2A	neutral chiral diacid resolving agent (M)
HA^-	mono-anion chiral diacid resolving agent (M)
Ka_1	first disassociation acid constant of the chiral diacid resolving agent (M)
Ka_2	second disassociation acid constant of the chiral diacid resolving agent (M)
Ka_{amine}	disassociation acid constant of the amine (M)
Kd_1	formation constant for acidic diastereomeric salt (M^{-1})
Kd_{1S}	formation constant for the (*S*)-acidic diastereomeric salt (M^{-1})
Kd_{1R}	formation constant for the (*R*)-acidic diastereomeric salt (M^{-1})
$Kd_{1'}$	formation constant for the neutral salt from the acidic salt (M^{-1})
$Kd_{1'S}$	formation constant for the (*S*)-neutral salt from the (*S*)-acidic salt (M^{-1})
$Kd_{1'R}$	formation constant for the (*R*)-neutral salt from the (*R*)-acidic salt (M^{-1})
Kd_2	overall formation constant for neutral diastereomeric salt (M^{-2})
Kd_{2S}	overall formation constant for the (*S*)-acidic diastereomeric salt (M^{-2})
Kd_{2R}	overall formation constant for the (*R*)-acidic diastereomeric salt (M^{-2})
Ks^{AR}	solubility limit of (*R*)-acidic salt in the resolution solvent (M)
Ks^{AS}	solubility limit of (*S*)-acidic salt in the resolution solvent (M)
Ks^{AR2}	solubility limit of (*R*)-neutral salt in the resolution solvent (M)
Ks^{AS2}	solubility limit of (*S*)-neutral salt in the resolution solvent (M)
Kw	autoionisation constant of water (M^2)
OH^-	hydroxide anion (M)
PEA	α-phenyl ethyl amine
(*S*)-PEA	(*S*)-phenyl ethyl amine
(*R*)-PEA	(*R*)-phenyl ethyl amine
PPI2	racemic piperidine of pharmaceutical interest
R	(*R*)-enantiomer (M)
RH^+	ionic (*R*)-amine enantiomer (M)
S-PPI	(3*S*,4*R*) enantiomer of the piperidine of pharmaceutical interest
S	(*S*)-enantiomer (M)
SH^+	ionic (*S*)-amine enantiomer (M)
R-PPI	(3*R*,4*S*) enantiomer of the piperidine of pharmaceutical interest
W	weight of solid obtained by mother liquor volume (M)
Y	resolution yield (%)
Γ	resolving agent/amine resolution ratio (mol mol^{-1})
Subscripts and superscripts	
0	fed to the resolution
t	total
ML	mother liquor
W	solid product

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