Investigation of The Behaviour of Pregabalin Enantiomers

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Keywords
Resolution, Resolution by formation of diastereomers, Optimization, Enantiomeric purity, Diastereomeric salt replenishment.

ABSTRACT
The behavior of pregabalin enantiomers obtained by resolution of the free γ-amino acid, racemic pregabalin (PGA) was investigated in the process of the resolution via diastereomeric salt formation. Various resolution methods, purification possibilities of the enantiomeric mixtures, the effect of the achiral compound, the crystallization time of the diastereomeric salt, and the effect of the solvent on the resolution were studied. Summarizing our experimental results, we can establish that the resolution of pregabalin is affected by kinetic control, and significant enantiomeric enrichment can be reached with the replenishment of the diastereomeric salt.

Introduction
There is a growing interest both scientifically and industrially in the economical separation of chiral, enantiomerically pure compounds. Most of the pharmacologically active compounds are chiral, so the pharmaceutical industry has to prepare the isomer with a more favorable therapeutic effect from the racemic compound formed during the synthesis. In accordance with the FDA's 1992 regulation, when preparing a racemic drug, the pharmacokinetic and pharmacodynamic effects of the enantiomers must also be investigated in order to detect different indications or possible toxic side effects [1].

For this reason, research into new resolving agents and the development of efficient resolution procedures are well founded. Resolution of free amino acids is a major challenge for researchers. In our research, instead of a known method for the separation of free γ-amino acid (PGA), we investigated the possibility of developing a similar procedure to the conventional ones. In addition, different resolution by formation of diastereomers of racemic mandelic acid and racemic ortho-chloro mandelic acid (2-Cl-MA) were investigated [2,3]. We also aimed to compare different resolving agent mixtures to improve resolvability.

Studying the resolution of pregabalin according to the patent [4], it was considered, that it would be advisable to replace one mol of (S)-mandelic acid with another aromatic achiral carboxylic acid due to the high material demand of the reaction (Figure 1) [5]. We have chosen related molecular structured achiral additions with the same chemical character such as benzoic acid (BA), salicylic acid (SA) and methoxyphenylacetic acid (MPA).

Separation of enantiomers of racemic amino acids often requires several steps, especially in the case of protection of the acidic or basic group, because in this case coupling and then removing the protecting group must perform two unnecessary steps. However, it is increasingly common to separate free amino acids (without transitional protecting groups) by fractional crystallization of diastereomeric salts with a chiral acid or base, followed by decomposition of the diastereomers to give the enantiomer (enantiomeric mixture). In this case, the most efficient separation of the diastereomeric salts (ee → 100%, T → 100%, F → 1.0) is, of course, as a function of the solvent, the achiral reagents, the molar ratios and the crystallization time. However, no matter how effective the separation of diastereomeric salts may be, the amphoteric amino acid enantiomers should also be separated most efficiently and easily from the obtained salts. These listed possibilities were investigated in the experimental separations of
physiologically active enantiomer from the racemic compound (S)-Pregabalin ((S)-PGA).

(S)-PGA

\[
\begin{align*}
\text{NH}_2 \\
\text{COOH}
\end{align*}
\]

Figure 1: The biologically active enantiomer of pregabalin.

Resolution methods for racemic mixtures and enantioselective synthetic methods are widely discussed in the literature [6-12]. For the resolution of racemic PGA, it was known [4] to decompose the diastereomeric salt obtained from IPA solvent with 1.5 molecular equivalents of (S)-mandelic acid ((S)-MA) to the crystallizing (S)-PGA enantiomer obtained by boiling and cooling with tetrahydrofuran solvent.

According to another method [13], the racemic PGA is resolved in butanol with (R,R)-tartaric acid ((R,R)-TA) and the (S)-PGA is obtained from the diastereomeric salt by crystallization after boiling in isopropyl alcohol. With these methods, the enantiomeric separation efficiency is 0.7.

\[
\begin{align*}
\text{OH} & \quad \text{COOH} \\
\text{OH} & \quad \text{Cl} \\
\text{Cl} & \quad \text{OH} \\
\text{HO} & \quad \text{COOH}
\end{align*}
\]

(S)-(+) MA (S)-(+) 2-CMA (R)-(+) 2-CMA (2R,3R)-(+) TA

Figure 2: Applied resolving agents.

Compared to the racemic PGA, these resolving agents or mixtures were used in smaller proportions as equivalents, but with achiral acids, the amount of total acid was added to the equivalent of the racemic compound. Thus, in addition to hydrochloric acid, benzoic acid (BA), salicylic acid (SA), and 2-methoxyphenylacetic acid (MPA) were used.

\[
\begin{align*}
\text{NH}_3 + 0.6 \text{ mol acid} & \Rightarrow 5 \times \text{IPA, 1 hour} \Rightarrow (S)-\text{PGA} + (S)-\text{MA} \\
\text{NH}_3 + 0.4 \text{ mol acid} & \Rightarrow 5 \times \text{IPA, 1 hour} \Rightarrow (S)-\text{PGA} + (S)-\text{MA} \\
\text{NH}_3 + \text{MPA} & \Rightarrow 5 \times \text{IPA, 1 hour} \Rightarrow (S)-\text{PGA} + (R)-\text{MA}
\end{align*}
\]

Figure 3: Application of achiral additions - Pope and Peachy's method.

After the separation of diastereomers crystallized from free (unprotected) racemic amino acids, the further reaction step is the separation of enantiomers from the resolving agent, which is often the case for amphoteric amino acids. E.g. the (S)-PGA * (S)-MA salt or the (S)-PGA * (R,R)-TA salt is decomposed by boiling in solvent which (S)-PGA precipitates out of solution as a salt, but the resolving agent remains in the solution and the crystalline precipitation does not dissolve back. Thus, the (S)-PGA * (S)-MA salt is boiled with tetrahydrofuran, while the crude (S)-PGA * (R,R)-TA salt is recrystallized and then decomposed with N,N-diisopropylamine boiled in a 4:1 mixture of isopropyl alcohol and water to achieve proper separation of (S)-PGA. To prepare one mol of (S)-(+) PGA with this method, 2.62 moles of racemic PGA, 5.43 moles of (S)-(+) MA, 51 times the amount of isopropyl alcohol, and 16.3 times the amount of tetrahydrofuran were used.

However, we used a much simpler method to precipitate the (S)-PGA enantiomer from the former diastereomers by adding aqueous ammonia.

\[
\begin{align*}
(S)-\text{PGA} * (S)-\text{MA} + \text{NH}_3 & \Rightarrow \text{H}_2\text{O} \Rightarrow (S)-\text{PGA} \text{ crystal} + (S)-\text{MA} \text{NH}_3 \text{ mother liquor} \\
(S)-\text{PGA} * (R,R)-\text{TA} + \text{NH}_3 & \Rightarrow \text{H}_2\text{O} \Rightarrow (S)-\text{PGA} \text{ crystal} + (R,R)-\text{TA} \text{NH}_3 \text{ mother liquor}
\end{align*}
\]

Figure 4: Decomposition of diastereomeric salts.

Similarly good results were obtained for these resolutions as for the resolution according to the patent [4], where 1.5 mol of (S)-MA was used. Next, we examined how the solvent change affects resolution. Further working with the same achiral additions, the reactions were performed first in a mixture of 5 times the amount of isopropyl alcohol and twice the amount of water and then in twice the amount of water.

\[
\begin{align*}
5 \times \text{IPA} + 0.6 \text{ mol acid} + 0.4 \text{ mol acid} & \Rightarrow 2 \times \text{H}_2\text{O} \Rightarrow (S)-\text{PGA} + (S)-\text{MA} \\
5 \times \text{IPA} + 0.6 \text{ mol acid} & \Rightarrow 2 \times \text{H}_2\text{O} \Rightarrow (S)-\text{PGA} + (R)-\text{MA}
\end{align*}
\]

Figure 5: Resolution of racemic pregabalin using achiral additives – in a mixture of isopropyl alcohol and water and in water.

When benzoic acid and salicylic acid were used, the pure enantiomer was isolated without repeated resolution and without recrystallization of the diastereomeric salt. We have found that (S)-mandelic acid in 0.6 molar equivalents provides better separation than half equivalent method.

In case of using methoxyphenylacetic acid as an achiral compound, interestingly, higher enantiomeric purity can be achieved in water than in a mixture of isopropyl alcohol and water. Compared to the original 5637767 U.S. Pat. preparation of the enantiomer of (S)-pregabalin by reducing the amount of isopropyl alcohol to
one tenth, using 0.6 molecular equivalents instead of 1.5 molar equivalents of (S)-mandelic acid, supplemented with 0.4 molecular equivalents of achiral aromatic carboxylic acid, we were able to make the preparation of the pure (S)-pregabalin enantiomer significantly more economical.

**Conditions Affecting Resolution**

After achieving successful resolutions by using achiral aromatic carboxylic acids as an additive, it was hypothesized that hydrochloric acid could also be a beneficial achiral acid (Figure 6). In addition, the effects of diastereomeric crystallization time, solvent amount, pH, and ultrasound on the result of the resolution were investigated in parallel.

![Figure 6: Resolution of pregabalin with (S)-mandelic acid using HCl – general formula.](image)

Leaving the diastereomer to crystallize for two days gave a racemic mixture using half molecular equivalent of (S)-mandelic acid. If the diastereomeric crystals were filtered after waiting 10 minutes, a very modest result was obtained, while if they were immediately filtered, the enantiomeric purity obtained from the diastereomeric salt was 84.4%, and the yield was 94.7%. Based on our experiments, it can be concluded that the final result of pregabalin resolution is influenced by kinetic control (Table 1). We found that ultrasound aided the precipitation of the diastereomeric salt and in the case of resolutions favored by kinetic control, the composition of the diastereomeric salt was stabilized by ultrasound to prevent the formation of thermodynamic control. A very pure enantiomer can be obtained by filtration and decomposition of the rapidly precipitating diastereomeric salt. Furthermore, we found that the ideal pH for resolution was 6.

![Table 1: Kinetic control study.](image)

<table>
<thead>
<tr>
<th>Experimental results from crystalline precipitation</th>
<th>(S)-MS [mol]</th>
<th>time</th>
<th>ee [%]</th>
<th>Y [%]</th>
<th>F [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-MS [mol]</td>
<td>time</td>
<td>ee [%]</td>
<td>Y [%]</td>
<td>F [%]</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>&lt;1 minute</td>
<td>84</td>
<td>94</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>10 minutes</td>
<td>84</td>
<td>6</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>2 days</td>
<td>-</td>
<td>64</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Value calculated from the ratio of the specific rotation of the enantiomeric mixture and the pure enantiomer measured under the same conditions. Yield was calculated to the total amount of the corresponding enantiomer, i.e. half the amount of the racemic compound. c Resolubility or F-factor, can be calculated from the multiplication of yield and enantiomeric purity.

**Use of Mixtures of Resolving Agents**

Dutch researchers have recognized that in some cases better separation can be achieved by reacting a racemic compound with a mixture of resolving agents (especially those with a related molecular structure) than by using them separately. Many times, these resolving agents alone are unsuitable for diastereomeric salt formation, but the interaction of structurally similar resolving agents promotes diastereomeric salt formation, thereby significantly improving resolvability [14-16]. However, the more structurally similar the members of the mixtures, the more synergistic effect prevails [17].

Based on all this, we also tried to resolve pregabalin with different compositions of resolving agent mixtures. We also tested what results we would achieve by changing these ratios.

Table 2 shows that for (S)-mandelic acid, (S)-2-chloromandelic acid, a 9:1 ratio was found to be most effective when used in half-equivalent amounts, but gave more modest results than resolution with pure (S)-mandelic acid. An enantiomeric purity of 93.2% was also obtained with a 9:1 mixture of (R)-3-chloromandelic acid and (R)-2-chloromandelic acid, in which case (R)-pregabalin was precipitated in the diastereomeric salt.

![Table 2: Resolution of pregabalin with resolving agent mixtures in different proportions.](image)

<table>
<thead>
<tr>
<th>[R]</th>
<th>[(S)]</th>
<th>from the crystalline precipitation</th>
<th>from the mother liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>93.2%</td>
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<td>85,6%</td>
<td>28,2%</td>
</tr>
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**Multiple Replacement Of The Precipitated Diastereomeric Salt – Optimization**

The result of the last resolution shown in Table 2 is already an optimized result obtained by replacing multiple times the resolving agent (S)-2-chloromandelic acid and (2R,3R)-tartaric acid equivalent to the precipitated diastereomer in the mother liquor (Figure 7).

![Figure 7: Replacement of the diastereomeric salt.](image)
Conclusion
Summarizing our experience in the study of the behavior of pregabalin enantiomers, we found that kinetic control prevails during the resolution. If diastereomeric salts were crystallized for a longer time, the purity of the enantiomeric mixture reduced. The process according to U.S. Pat. No. 5,637,767 has been carried out with similar results in mixture of isopropyl alcohol and water, and in water only, and by modifying the mentioned process with 0.6 molar equivalents of (S)-mandelic acid and 0.4 molar equivalents of achiral aromatic related molecular structured carboxylic acid (benzoic acid, salicylic acid, methoxyphenylacetic acid) instead of 1.5 molar equivalents of (S)-mandelic acid. Resolving agent mixtures (Dutch resolution) of different composition and proportions were studied and also significant enantiomeric enrichment was achieved by replacing the diastereomeric salt, optimizing the Dutch resolution result.

Experimental
Resolution with half equivalent amount of (S)-(+) -mandelic acid
To a mixture of 0.8 g (5 mmol) of racemic PGA, 0.39 g (2.5 mmol) of (S)-(+) -MA was added 1.5 cm³ of water and 0.21 cm³ of HCl, which was heated until dissolved. After cooling, the crystalline precipitate was filtered off after standing for 20 minutes. The diastereomeric salt thus obtained was suspended in 0.8 cm³ of water and 0.8 cm³ of NH₄OH was added.

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<td>(S)-(+) -PGA</td>
<td>0.22 g (1.45 mmol)</td>
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<tr>
<td>(R)-(+) -PGA</td>
<td>0.18 g (1.125 mmol)</td>
<td>-11.1, ee: 11%</td>
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Resolution with a mixture of (S)-(+) -mandelic acid and (S)-(+) -selectial acid
The combined 0.39 g (2.5 mmol) of racemic PGA, 0.21 g (1.125 mmol) of (S)-(+) -CMA and 0.02 g (0.125 mmol) of (R)-TA was treated with 0.105 mL of 37% hydrochloric acid and 0.75 mL of water and heated until dissolved. After cooling, the crystalline precipitate was filtered off after standing for 10 minutes. Equivalent amounts of racemic PGA, (S)-(+) -CMA and (R)-TA relative to the weight of the diastereomer were added to the mother liquor to replace the precipitated and filtered diastereomer. I repeated the process twice, the results are shown in Figure 7 and Table 2.

Resolution with a mixture of (S)-(+) -mandelic acid and (2R, 3R)-(+) -tartaric acid
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References

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