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Molecular interactions of hydrated co-amorphous systems of prilocaine and lidocaine

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ABSTRACT

It is generally accepted that water as a plasticizer can decrease the glass transition temperatures (T_g's) of amorphous drugs and drug excipient systems. However, previous studies suggest that water, as an anti-plasticizer, can increase the T_g of co-amorphous systems of prilocaine (PRL) and lidocaine (LID). In order to investigate the intermolecular interactions between water and co-amorphous PRL-LID systems, Fourier transform infrared spectroscopy (FTIR) and principal component analysis (PCA) were conducted. Water was found to bind with the carbonyl groups of PRL and LID molecularly evenly in the hydrated co-amorphous PRL-LID systems. Furthermore, the physical stability of hydrated co-amorphous PRL-LID systems was improved due to the anti-plasticizing effect of water, compared with the anhydrous samples. The preference of water to interact with the carbonyl groups of PRL and LID as binding sites could be associated with the anti-plasticizing effect of water on the co-amorphous PRL-LID systems.

1. Introduction

Co-amorphous systems are developed as a formulation strategy to improve the physical stability of pure amorphous drugs, based on the formation of a homogeneous amorphous phase by mixing a drug and a low molecular weight co-former at molecular level (Löbmann et al., 2011; Umerska et al., 2021). The co-former may form defined intermolecular interactions with the drug, such as hydrogen or ionic bonds, contributing to the stabilization of co-amorphous systems (Han et al., 2011; Umerska et al., 2021). In a previous study, drug-drug co-amorphous systems consisting of prilocaine (PRL) and lidocaine (LID) were prepared in order to inhibit the crystallization of the individual amorphous components (Xu et al., 2022). Molecular interactions between PRL and LID were indirectly observed via the deviations between the experimental T_g's and the theoretical T_g based on the Gordon-Taylor equation (Xu et al., 2022), however no information about the detailed interaction pattern on a molecular level was gained at this time. The systems of PRL and LID were investigated thoroughly with regard to their eutectic behavior (Nyqvist-Mayer et al., 1985, 1986). Molecular interactions between PRL and LID in the supercooled liquid phase were investigated by Fourier transform infrared spectroscopy (FTIR) at a molar ratio of PRL of 0.5 (Brodin et al., 1984). However, it was found that FTIR was unable to identify molecular interactions between PRL and LID, due to the spectral complexity (Brodin et al., 1984). Thus, limited structural information was provided about molecular interactions between PRL and LID in the amorphous systems.

Water is generally regarded as an effective plasticizer for amorphous drugs and co-amorphous systems. It decreases the glass transition temperatures (T_g's) and results in physical instabilities (Royall et al., 1999; Sovago et al., 2016). However, recent studies suggest that water can increase the T_g of PRL and LID, thus acting as an anti-plasticizer on them (Ruiz et al., 2017; Xu et al., 2022). Furthermore, an anti-plasticizing effect of water is still maintained in co-amorphous systems of PRL-LID at all mixing ratios and nicotinamide-PRL systems at high PRL concentrations (Xu et al., 2022; Xu et al., 2023). The nature of the anti-plasticizing effect of water on PRL was ascribed to the preferential targeting of water to the carbonyl group of PRL, which is visible in FTIR by the shift of the carbonyl group of PRL upon hydration (Ruiz et al., 2017). In contrast, the elucidation of intermolecular interactions of water on co-amorphous systems with PRL and LID has not been investigated. To understand the nature of an anti-plasticizing effect of water in amorphous systems, valuable knowledge can be gained from a detailed investigation of molecular interactions between water and co-amorphous PRL-LID systems. Understanding the mechanism of anti-
plasticization may have potential applications in the stabilization of co-amorphous systems in the presence of water.

The aim of the study was to investigate the molecular interactions between water and co-amorphous PRL-LID systems. Ruiz et al. was able to use FTIR to describe the hydrogen bonding pattern between water and the carbonyl group of amorphous PRL (Ruiz et al., 2017). In co-amorphous systems, the interactions between water and co-amorphous PRL-LID systems would be more difficult to recognize, as the molecular interactions between PRL and LID also affect the spectra. Due to the spectral complexity of co-amorphous systems, principle component analysis (PCA) of FTIR spectra was employed to investigate the molecular interactions of anhydrous and hydrated systems, as this approach has previously been shown to be beneficial (Di et al., 2021; Liu et al., 2020). Quantum chemical calculations were conducted to probe the structural features and visualize the molecular interactions of co-amorphous systems.

2. Materials and methods

2.1. Materials

Pramocaine (PRL, MW = 220.31 g/mol) was purchased from Fluorochem Ltd. (Hadfield, U.K.). Lidocaine (LID, MW = 234.34 g/mol) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The chemical structures of PRL and LID are shown in Fig. 1. Water (18.2 MΩ) was freshly prepared using a Milli-Q water system from ELGA LabWater (High Wycombe, U.K.).

2.2. Methods

2.2.1. Sample preparation

Anhydrous co-amorphous PRL-LID systems (approximately 10 mg) at molar ratios of PRL from 0.3 to 0.9 and pure PRL were prepared by melting crystalline samples on a hot plate followed by quench-cooling using non-hermetically sealed pans. Due to fast recrystallization of amorphous LID (Blaabjerg et al., 2016), it was not possible to prepare co-amorphous PRL-LID samples with a molar ratio of PRL below 0.3.

For hydrated co-amorphous PRL-LID systems and pure PRL, a drop of water was added to the anhydrous crystalline mixtures of PRL and LID in a DSC pan, followed by water evaporation monitored on a microbalance until the desired total mass was reached. The molar ratio of water to the co-amorphous PRL-LID systems and pure PRL was XH2O = 50 %. This molar ratio maximizes the anti-plasticizing effect of water on co-amorphous PRL-LID systems (Fig. 2). The changes of peaks could be explained by a dilution effect on PRL correlated with increasing LID concentration.

2.2.2. Spectra collection

Due to the low Tg of the co-amorphous PRL-LID systems and pure PRL in the range from 213 K to 219 K (Xu et al., 2022), the supercooled liquid phase was used to investigate the interactions in the amorphous systems. No signs of crystallinity were observed for any of the samples. The nature of hydrogen bonding in the supercooled liquid phase can be correlated with that in the amorphous phase (Kalra et al., 2017). FTIR spectroscopic analyses was performed at ambient temperatures using a MB3000 FTIR spectrometer (ABB Ltd, Zurich, Switzerland) in attenuated total reflectance (ATR) mode. The spectra of anhydrous and hydrated samples were recorded at a wave number range from 400 to 4000 cm⁻¹ with 16 scans at 4 cm⁻¹ resolution.

2.2.3. Physical stability

Physical stability of the samples was investigated using X-ray powder diffraction (XRPD). Measurements were performed with X’Pert PANalytical PRO X-ray diffractometer (PANalytical, Almelo, The Netherlands) with Cu Kα radiation (λ = 1.541 87 Å). The co-amorphous PRL-LID samples were stored over freshly activated silica gel at 5 °C, i.e. at dry conditions. The anhydrous samples were placed without lid, while the hydrated samples were hermetically closed to ensure a stable water content. All samples were analyzed until recrystallization was observed.

2.2.4. Multivariate data analysis

Standard normal variate (SNV) transformation in the spectral range of 1400–1800 cm⁻¹ and 3100–3500 cm⁻¹ was performed on the obtained FTIR data, followed by PCA using Simca 17.0 (Sartorius, Göttingen, Germany).

2.2.5. Quantum chemical calculations on structural models

One hundred starting structural models of anhydrous and hydrated co-amorphous PRL-LID systems were randomly constructed with PRL, LID and water molecules using Molclus program (Lx, 2023), by changing the orientations and distances among the components. These structural models were optimized at the PM6-DH + level using MOPAC program to get five structural models with low energy (Stewart, 2016). The obtained five structural models were further optimized using density functional theory (DFT) at the B3LYP-D3(BJ)/6-31G(d) level, where D3 (BJ) represents Grimme’s empirical dispersion correction (Goerigk and Grimme, 2011). Based on the optimized structures, the bonding energies were calculated at B3LYP-D3(BJ)/6-31+G(d,p) level to find the lowest energy structural models (Burns et al., 2014). All DFT calculations were performed using GAUSSIAN 09 (Gaussian, Inc., Wallingford, USA).

3. Results and discussion

3.1. FTIR spectra of anhydrous and hydrated co-amorphous PRL-LID systems

3.1.1. Interactions between PRL and LID

Based on the chemical structures of PRL and LID, the wavenumber ranges of 1400–1800 cm⁻¹ and 3100–3500 cm⁻¹ were chosen to analyze the interactions between the carbonyl, amine and aromatic groups of PRL and LID. As shown in Fig. 2, the bands of the amide C=O (1682 cm⁻¹), amide N–H (1519 cm⁻¹), amine N–H (3296 cm⁻¹), and aromatic C=C (1587 and 1452 cm⁻¹) groups of PRL were visible in all the spectra of anhydrous co-amorphous PRL-LID systems and anhydrous PRL. With increasing LID concentration, the amide N–H group of LID at 1492 cm⁻¹ appeared, and the intensity of peaks of PRL was decreased. No shifts of investigated peaks were observed in the anhydrous co-amorphous systems (Fig. 2). The changes of peaks could be explained by a dilution effect on PRL correlated with increasing LID concentration. Overall, it was not possible to obtain information from the FTIR spectra with regard to the interactions between PRL and LID.

3.1.2. Interactions between water and co-amorphous PRL-LID systems

A comparison between anhydrous and hydrated samples of individual chemical groups is shown in Fig. 3. An obvious shift was observed in the position of the amide C=O group from 1682 cm⁻¹ to 1677 cm⁻¹.
upon hydration (Fig. 3A), while no effect of water on the amine (Fig. 3B) and aromatic (Fig. 3C) groups can be observed. Ruiz et al. noted that the shift of amide C=O group of PRL upon hydration could be due to the hydrogen bonding of water to the carbonyl group of PRL (Ruiz et al., 2017). However, the amide C=O group of LID cannot be observed in the FTIR spectra of hydrated co-amorphous PRL-LID systems. This is attributed to an overlap with the amide C=O group of PRL. FTIR spectra cannot give a clear indication of the hydrogen bonding site of water, and it remains unresolved whether the carbonyl group of either PRL or LID is involved in hydrogen bonding with water in co-amorphous PRL-LID systems.

3.2. Multivariate data analysis of FTIR spectra

As visualization on the FTIR spectra did not result in a clear description of the hydrogen bonding pattern, PCA was used to gain insights in the interactions of co-amorphous PRL-LID systems. Molecular interactions that contribute to spectral differences were evaluated by the PCA scores and the corresponding loading plots of the first two principal components (PC-1 and PC-2).

3.2.1. PCA scores and PC-1 loading plots

The PCA scores based on the measured FTIR spectra of anhydrous and hydrated PRL-LID systems are shown in Fig. 4. Two principal components explained >99 % of the variation in the spectra. The first principal component (PC-1) accounted for more than 98 % of the total variance. The score values of PC-1 decreased with an increasing molar ratio of PRL in the anhydrous and hydrated co-amorphous PRL-LID systems. The second principal component (PC-2) explained around 1 %
of the variation. A maximum for the score values of PC-2 was seen at a molar ratio of 0.6 and 0.7 in the respective anhydrous and hydrated systems, compared with the other mixing ratios.

In order to understand the pattern of the score plots, the loading plots of anhydrous and hydrated samples were examined. The PC-1 loading plots are shown in Fig. 5 together with the FTIR spectra of anhydrous PRL and co-amorphous PRL-LID with a molar ratio of PRL of 0.3. The loading plot of PC-1 of hydrated co-amorphous PRL-LID systems showed a strong resemblance with that of anhydrous co-amorphous PRL-LID systems. This supports the suggestion that water distributed molecularly evenly between PRL and LID in the co-amorphous PRL-LID systems, consistent with a previous study (Xu et al., 2022). The amide N–H group (1492 cm\(^{-1}\)) of LID was a main contributor to a positive loading, while negative loadings were mainly related with the amide N–H (1519 cm\(^{-1}\)) and aromatic C–C (1587 and 1452 cm\(^{-1}\)) groups of PRL.

The PC-1 loading plots of co-amorphous PRL-LID systems are in agreement with the PC-1 scores with increasing molar ratios of LID. Therefore, it is inferred that the differences in the compositions in anhydrous and hydrated co-amorphous PRL-LID systems are mainly described by PC-1.

### 3.2.2. PC-2 loading plots

Despite the limited explained variance, previous studies showed that PC-2 could be correlated to the interactions in co-amorphous systems (Di et al., 2021; Liu et al., 2020). For anhydrous co-amorphous PRL-LID systems, the PC-2 loading is dominated by the positive contribution of amide N–H groups of PRL (1519 cm\(^{-1}\)) and LID (1492 cm\(^{-1}\)) (Fig. 6). Due to the electrostatic repulsive forces between amide N–H groups, intermolecular interactions between N–H moieties of the amide groups of PRL and LID are not expected to notably happen (Wojnarowska et al., 2018). At a molar ratio of PRL of 0.6, the amide N–H groups of both PRL and LID contribute prominently to the loading of PC-2, indicating...
changes in the electron distribution that are not captured in PC-1, and are thus beyond the pure concentration differences. Consequently, these changes are attributed to changes in the interaction pattern between the two amide groups. In addition, the positive loadings, attributed to the aromatic C–C (1587 and 1452 cm$^{-1}$) and amine N–H (3296 cm$^{-1}$) groups of PRL, indicated that these groups were involved in the interactions of anhydrous co-amorphous PRL-LID systems. These molecular interactions were previously investigated by deviations between the experimental $T_g$s and the theoretical $T_g$s based on the Gordon-Taylor equation (Xu et al., 2022). There it was shown that the strongest deviation was also observed at a molar ratio of PRL of 0.6.

It is worth mentioning that the contribution of the amide C=O group of PRL in the loading plot of PC-2 of anhydrous PRL-LID was different from that of LID (Fig. 7A). When looking at the hydrated system, the differences between these two amide C=O groups decreased, and the signal shifted to a lower wavenumber in the hydrated systems compared with the anhydrous systems. This indicates that water binds evenly in the hydrated systems, thus decreasing the structural differences of the amide C–O groups of PRL and LID, respectively, upon hydration. Comparing hydrated and anhydrous systems, similar contributions were observed for the amine N–H (Fig. 7B) and aromatic C=C (Fig. 7C) groups of PRL in the PC-2 loading plots. This indicates that the amine N–H and aromatic C=C groups of PRL interacted similarly in the hydrated and in the anhydrous systems. Overall, the amide C=O groups of PRL and LID were both involved in the interactions with water.

3.3. Quantum calculations on structural models

The PC-2 scores of the FTIR data indicated a maximum of mutual interactions at a molar ratio of PRL of 0.6 in the anhydrous systems. The PC-2 loading plot further indicated that the amide groups of PRL and LID, as well as aromatic and amine groups of PRL were involved in interactions within the anhydrous PRL-LID systems. In order to investigate the molecular interactions with a different approach, quantum calculations were performed. The structural models of PRL and LID were constructed with a molar ratio of PRL of 0.6 to show the molecular interactions of anhydrous co-amorphous PRL-LID systems. The optimized five structural models with their corresponding binding energies are shown in Fig. S1. The structural model with the lowest binding energy is displayed in Fig. 8A. This can be assumed to be the most reasonable structural model of anhydrous PRL-LID. It was found that the interactions between PRL and LID were formed via hydrogen bonding between $N_1$H of PRL and $O_2$ of LID, and π-π interactions between the aromatic groups of PRL and LID. Moreover, molecular interactions within PRL molecules also stabilized the anhydrous systems. Hydrogen bonding was observed between the amine groups (N1H and N2H) and the amide C=O group of PRL. This possible structural model of anhydrous PRL-LID gives the indication that diverse molecular interactions with amide C=O, amine N–H and aromatic C=C groups of PRL and LID may occur depending on the involved molecules.

For the hydrated co-amorphous PRL-LID systems, the PC-2 scores indicated that molecular interactions were maximized at a molar ratio of PRL of 0.7. The PC-2 loading plots indicated that water only interacted with the amide C–O groups of PRL and LID. A molar ratio of PRL of 0.7 of co-amorphous PRL-LID systems was chosen to investigate the molecular interactions with one water molecule. The optimized five structural models of hydrated co-amorphous PRL-LID with their corresponding binding energies are shown in Fig. S2. The structural model

![Fig. 7. Comparison of PC-2 loading plots between anhydrous and hydrated co-amorphous PRL-LID systems of amide C=O (A), amine N–H (B) and aromatic C=C (C) groups.](image-url)
with the lowest binding energy is displayed in Fig. 8B. The water molecule preferred to interact with the amide C–O groups of PRL and LID. The preference of binding sites of water rationalized the observed FTIR spectral trends, indicating that the amide C–O groups of both PRL and LID were involved into the interactions with water.

Although the nature of the plasticizing effect of water can be complex (Alonso-González et al., 2021), it is believed to be due to the hydroxyl groups of water molecules forming hydrogen bonds with the amorphous drug molecules, breaking the original intermolecular hydrogen bond network of the anhydrous drug (Newman and Zografi, 2019). This leads to an increase in molecular mobility and thus a decrease in the Tg of the drug (Esmaeili et al., 2019; Zhang et al., 2006). It could therefore have been expected that the amide C–O and amine N–H groups of PRL and LID in the co-amorphous systems form a hydrogen bond network with the hydroxyl group of water, leading to water-plasticization. However, the preference of water to interact with the amide C–O groups of PRL and LID, restricted the amount of hydrogen bonds formed between the amine groups of the drugs and water. Thus, water did not form an extensive hydrogen bond network, leading to anti-plasticization of the co-amorphous PRL-LID systems.

3.4. Physical stability

The physical stability of anhydrous and hydrated co-amorphous PRL-LID samples was investigated upon storage at 5 °C under dry conditions due to the low Tg values (Xu et al., 2022). The results are shown in Table 1. The majority of samples was still stable after 3 months of storage. Recrystallization was more prone to occur in the anhydrous samples where recrystallization was observed at molar ratios of PRL of 0.3, 0.7 and 0.9. This could be associated with the lower appearance of molecular interactions between PRL and LID compared with that at a molar ratio of PRL of 0.6, consistent with the spectroscopic investigations. In contrast, for hydrated samples, recrystallization only occurred for co-amorphous PRL-LID samples with a molar ratio of 0.3. The higher physical stability in hydrated PRL-LID systems was consistent with the increased Tg of co-amorphous PRL-LID systems upon hydration. The improved stability might be due to the intermolecular interactions between water and co-amorphous PRL-LID systems.

<table>
<thead>
<tr>
<th>Molar ratios of PRL</th>
<th>Stability</th>
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<tbody>
<tr>
<td>Anhydrous PRL-LID systems</td>
<td>0.3: &lt;1 day</td>
</tr>
<tr>
<td></td>
<td>0.7: &lt;1 month</td>
</tr>
<tr>
<td></td>
<td>0.9: &lt;3 weeks</td>
</tr>
<tr>
<td></td>
<td>0.4, 0.5, 0.6, 0.8: &gt;3 months</td>
</tr>
<tr>
<td>Hydrated PRL-LID systems</td>
<td>0.3: &lt;1 week</td>
</tr>
<tr>
<td></td>
<td>0.4, 0.5, 0.6, 0.7, 0.8, 0.9: &gt;3 months</td>
</tr>
</tbody>
</table>

of PRL and LID were involved in the interactions between water and co-amorphous PRL-LID systems, according to spectroscopic investigations and quantum chemical simulations. In addition, the physical stability of hydrated co-amorphous PRL-LID systems was improved compared with the anhydrous systems. This is consistent with the anti-plasticizing effect of water on co-amorphous systems. In conclusion, the anti-plasticizing effect of water on co-amorphous PRL-LID systems can be attributed to molecular interactions of water with the carbonyl groups of both PRL and LID.

CRediT authorship contribution statement

Xiaoyue Xu: Writing – original draft, Investigation, Formal analysis, Conceptualization. Thomas Rades: Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization. Holger Grohganz: Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.123807.

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