EFFECT OF ETHANOL-WATER COMPOSITION ON CLINDAMYCIN HYDROCHLORIDE PSEUDOPOLYMORPHISM

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ABSTRACT

Objective: Formation of clindamycin hydrochloride (clindamycin HCl) in monohydrate-ethanolate from the recrystallization process with ethanol-water (5:2) has been reported a long time ago. However, the effect of ethanol-water compositions into pseudo-polymorphism formation and its stability was not reported yet. This study aimed to investigate the effect of ethanol-water proportion on the formation of clindamycin HCl-monohydrate and its ethanol solvate.

Methods: Clindamycin HCl was recrystallized with the various percentages of ethanol. The fresh and after storage for 24 h at humidity and room temperature (25±2 °C, RH: 70±1%) crystals were characterized by FTIR (Fourier transform infra-red), PXRD (powder x-ray diffractometer), and DTA (differential scanning calorimeter). The study of desolvation/dehydration then was observed with a polarization microscopy-plate heater.

Results: The results showed that monohydrate crystal was obtained from recrystallization in a concentration less than 50% ethanol in water. Next, the ethanolate was produced from the solvent of>70% ethanol. Meanwhile, the 50–70% ethanol produced a hydrate–ethanolate, crystal, which has both hydrate and ethanol in its lattice. This hydrate-ethanolates was unstable, even in ambient temperature.

Conclusion: Concentration of ethanol in water as the solvent will determine the clindamycin HCl pseudo polymorphism, which will back to its original crystal form by the time of storage.

Keywords: Clindamycin HCl, Hydrate, Ethanolate, Stability

INTRODUCTION

Solid active pharmaceutical compounds, including clindamycin HCl, can arrange a pseudo-polymorphism, which is defined as the crystal involving water or other organic solvents in its lattice structure. If the solvent is water, the product is called a hydrate. Meanwhile if with an organic solvent, it is namely solvated. Then both are classified as solvato morph [1, 2]. Tablet manufacturing, which usually involves solvent, drying and compression (mechanical energy) is likely affect the formation or loss of solvate [3]. Likewise, suspension dosage can affect the formation of hydrates. Solvate entrapment can cause changes in the physicochemical properties such as physical stability, compatibility, compressibility, flow rate, solubility, dissolution testing, and bioavailability of solid active pharmaceutical compounds [4, 5].

Clindamycin HCl is a lincomycin class antibiotic that works bacteriostatic, specifically against a wide range of gram-positive aerobic and anaerobic bacteria. Clindamycin is a lincomamide antibiotic that inhibits bacterial protein synthesis and is used for the treatment of anaerobic, streptococcal, and staphylococcal infections. The use of clindamycin is increasing in clinical practice due to its tolerability, efficacy and excellent tissue penetration various studies have shown the association between clindamycin and skin related problems. Clindamycin HCl is a white or nearly white crystalline powder, which is freely soluble in water and in ethanol. Clindamycin HCl can form hydrates and hydrate-solvates [6–8].

The state affects the activity of an antibiotic. Therefore, solids preparations, including hydrates and solvates have to be characterized, as well as their transformation and physical stability, as essential information for pre-formulation of ingredient-related activities. The three-dimensional structure of clindamycin-hydrochloride-hydrate and its hydrate-ethanolate have been reported by Ravikumar and Sridhar [9] (fig. 1).

Fig. 1: Chemical structure of (A) clindamycin HCl; three-dimensional structure of (B) clindamycin HCl monohydrate; (C) clindamycin HCl monohydrate-ethanolate
Solid characterization widely studied to find more information, to support the dosage form formulation. As known, pseudo-polymorphism will affect on drug dissolution and moreover, on bioavailability. This study requires a valid analysis used the instruments, such as: FTIR, DSC/DTA, PXRD, microscope (polarization, scanning electron), etc. Fortunately, some researchers have been reported the successful experiments on solid characterization. Furthermore, some methods of quantification of the amount of crystal changes/transformations also have been developed [1, 2, 9–16].

Nowadays, clindamycin HCl can be found at the market in tablet and liquid suspension. As a substance that has the possibility to change its crystal form, especially pseudo-polyomorphism, the study of effect solvent should be done. The characterization and detection of clindamycin HCl pseudo-polymorphism have been reported [9, 10]. However, so far, the effect of ethanol/water composition on clindamycin HCl-hydrate and ethanolate formation still has not explained details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explained details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12]. This research aimed to explain details yet. This stability also has not been investigated as to niclosamide solvate suspension [12].
Spectrum of clindamycin HCl from ethanol 70%:
(a) fresh; (b) after 24 h of storage

C. Spectrum of clindamycin HCl from ethanol 50%: (a) fresh; (b) after 24 h of storage

Fig. 2: FTIR spectra of clindamycin HCl crystal recrystallized with ethanol/water

Moreover, to make it clearer, the hydrate and ethanolate spectrums observed were listed in table 1 below;

<table>
<thead>
<tr>
<th>Ethanol percentage of solvent for recrystallize</th>
<th>Sample</th>
<th>OH solvate stretching at wave number (cm⁻¹)</th>
<th>OH hydrate stretching at wave number (cm⁻¹)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>95%</td>
<td>A</td>
<td>3563.81</td>
<td>3451.96</td>
<td>ethanolate and hydrate lost after storage</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>3432.67</td>
<td>ethanolate lost, hydrate still remained</td>
</tr>
<tr>
<td>80%</td>
<td>A</td>
<td>3540.67</td>
<td>-</td>
<td>ethanolate lost, hydrate still remained</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>3409.53</td>
<td>both hydrate and ethanolate lost after storage</td>
</tr>
<tr>
<td>70%</td>
<td>A</td>
<td>3517.52</td>
<td>-</td>
<td>both hydrate and ethanolate lost after storage</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>-</td>
<td>both hydrate and ethanolate lost after storage</td>
</tr>
<tr>
<td>60%</td>
<td>A</td>
<td>3544.52</td>
<td>3471.24</td>
<td>no ethanolate, hydrate still remained after storage</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>3432.67</td>
<td>no ethanolate, hydrate still remained after storage</td>
</tr>
<tr>
<td>50%</td>
<td>A</td>
<td>-</td>
<td>3471.24</td>
<td>no ethanolate, hydrate still remained after storage</td>
</tr>
<tr>
<td>40%</td>
<td>A</td>
<td>-</td>
<td>3486.67</td>
<td>no ethanolate, hydrate still remained after storage</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>3440.39</td>
<td>no ethanolate, hydrate still remained after storage</td>
</tr>
</tbody>
</table>

Note: A: the sample before storage, B: the sample after storage in the humidity and room temperature (25±2 °C, RH: 70±1%).

After FTIR experiment, PXRD was used to observe the crystal changes. The diffractogram pattern will distinguish the crystal form produced from the series of recrystallization by ethanol/water. This analysis yielded the results as shown in fig. 3–5. Fig. 3 explains the change of diffractogram: fresh crystal from 95% ethanol/water, after it was stored, then both were compared to its origine crystal, as follows:

**PXRD analysis**

Next, crystallization was done with 70% ethanol/water, yielded diffractogram in fig. 4. This fig. compares the fresh crystal diffractogram with after storage and its origine.

The diffractograms in fig. 5 show the crystal yielded from 50% ethanol/water, the fresh and after storage, compare to its origine clindamycin HCl.
DTA analysis
Differential thermal calorimetry is the analysis to know the internal energy of three-dimensional lattice crystal of the compound. A crystal should have a specific energy which determines its fixed form. As known, all of the transformation need the change of energy. DTA was performed to analyze crystals produced from recrystallization with 40, 50, 60, 70, 80, and 95% ethanol/water. The thermograms, curves yielded from this analysis, are shown in fig. 6.

Afterward, DTA analysis also conducted on the crystals which had been stored at the ambient temperatures along 24 h. The results were shown in fig. 7 as follows:

Dehydration and desolvation observation by polarization microscope
Polarization microscope which completed with a heater plate (Koffler’s hot stage) can show the dehydration and desolvation process. The release of water or ethanol from the crystals will be shown with the droplet around the heated substance. These processes were illustrated in fig. 8 below.

FTIR measurements of the crystals were performed as the first step of hydrate or ethanolate formation. Differ from surface water and solvent, which doesn’t have the specific vibration peak, a
hydrate/ solvate should show a clear spectrum [10–12]. Fresh clindamycin HCl crystal from ethanol 70, 80, and 95% showed infrared peaks at 3517–3563 cm\(^{-1}\) (fig. 2, table 1). It has been reported that ethanolate can be identified by a band at approximately 3500 cm\(^{-1}\), meanwhile the hydrate will show a peak at 3400 to 3490 cm\(^{-1}\) [11]. This indicated that the ethanol solvate crystals were formed in 70% ethanol in water. Nevertheless, this band was lost after 24 h (table 1).

Fig. 8: The releasing of: ethanolate from clindamycin HCl hydrate-ethanolate at a temperature of 60-100 °C (A), hydrate from clindamycin HCl hydrate crystal at a temperature of 80-100 °C (B) (magnification 100-x)

In addition to the solvate peak around 3500 cm\(^{-1}\), a second peak is seen around 3400 cm\(^{-1}\). The recrystallized crystal from 40 and 50% ethanol showed only one peak. The presence of clindamycin HCl’s crystal or hydrate water is indicated by a characteristic OH stretching bands in the in the wave number of 3400-3490 cm\(^{-1}\). Meanwhile, the OH of ethanolate was found at about 3500–3570 cm\(^{-1}\). This data was confirmed with Beckstead (1993) report [10]. With a single exception, this band(s) was also lost after storage for 24 h at room temperature (25±2 °C, RH: 70±1%) as shown in table 1.

The crystals were stored for 24 h, afterward were re-analyzed using FTIR measurement. The results showed that there were not solvate spectrums anymore at wave number 3500-3570 cm\(^{-1}\) region. Table 1 explains that the spectrum of fresh recrystallized clindamycin HCl (A) showed the difference compared to the storage crystal (B). This data indicated that along the storage, the releasing of ethanol/water molecules from the pseudo-polymorphism occurred. It predicted because of the low of energy interaction, which based by a small hydrogen bonding.

All the FTIR data indicated that the crystals from recrystallization with the percentage of ethanol above 70% had formed hydrate-ethanolate. There were seen the existence of a single hydrate and solvate peak (table 1). Then, percentage ethanol of less than 50% will form a hydrate, whereas 60% ethanol produced a mixture of hydrates and solvate-hydrates. This conclusion was based on the presence of single solvate peak and two hydrate peaks. Additionally, hydrate and hydrate-ethanolate lost its water and ethanol after stored for 24 h, which was indicated by the disappearance of the hydrate and solvate spectra in the spectra.

For further analysis, it was used three kinds of samples: recrystallized with 50%, 70%, and 95% ethanol. Recrystallized 70% and 95% was proven have arranged a hydrate-ethanolate, while recrystallized 50% produced the hydrate form. The further characterization was done by PXRD. This work was conducted to investigate the crystal structure changes during storage for 24 h. The recrystallized from 95% ethanol showed loss of solvate after the storage (fig. 3-middle), explained by the change from the pattern, which back to the original diffractogram, similar pattern with its raw material (fig. 3-bottom), especially at the important area at 2θ = 5–25 °.

Recrystallized clindamycin HCl in 70% ethanol also shows solvate hydrate release after storage for 24 h (fig. 4-middle). This fig. Shows the diffractogram after storage was going to a similar pattern with raw material (fig. 4-bottom) with clear decreasing peak at 2θ = 7 °. Recrystallized clindamycin HCl in 50% ethanol (fig. 5-top) also released hydrate after 24 h of storage (fig. 5-middle). The diffractogram indicated, thehydrate released after storage 24 h showed by the similar pattern to the raw material (fig. 5-bottom).

Differential thermal analysis was used to observe the thermic character of clindamycin HCl, which can represent its crystal lattice energy before and after storage. Change of crystal structure will be detected by the change of its pattern resulted. This measurement was done to the crystal yielded from 40–95% ethanol to confirm the types of pseudo polymorphism. Fig. 5 A-G show thermogram of fresh clindamycin HCl crystal from the series concentration of ethanol: 40% (A), 50% (B), 60% (C), 70% (D), 80% (E), 95% (F), compared to the standard (G). This fig. explain the existence of two new curves at a temperature of 60–100 °C. This point of curves indicated as solvates and crystalline water released temperatures, however, the hydrate solvate and hydrate peaks stacked because of the release concurrently. From thermogram data, it has shown that all of the crystal arranged of pseudo-polymorphism.

Furthermore, the diffractogram after storage along 24 h was shown in fig. 7. There was the peak, which marks the solvate hydrate has been lost. At the thermogram of fresh clindamycin HCl from ethanol, 50% is
shown an endothermic curve at temperature 105–125 °C (fig. 7A-a). This curve is indicated the hydrate. Meanwhile in the thermogram of crystal after storage for 24 h, there is no endothermic curve that marks the hydrate is no longer there (fig. 7A-b). It showed that the clindamycin HCl from ethanol 50% experienced dehydration after storage for 24 h at room temperature.

Thermograms in fig. 7B indicated there are not the hydrate and solvate from ethanol 70%, both the fresh (7B-a) and after the storage (7B-b). The losing of solvate or hydrate curves indicates the instability of both ethanolate and hydrate form of clindamycin HCl. The unstable pseudo polymorphism phenomenon has also been reported by Villiers and Mahlatji (2004). The research explains the physical instabilities of niclosamide solvates, which are formed at different carrier suspension [12].

The next analysis was performed to see the hydrate/solvate release from clindamycin recrystallized by 70% ethanol using a polarizing microscope with a heating plate. By heating under a polarized microscope, the loss of solvate/hydrate shown by the bubbles released from clindamycin HCl crystal. The result showed, clindamycin HCl from ethanol 70% had dehydration and desolvation starting from a temperature of 60–100 °C. It was signed with the release of solvent bubbles from the crystal surface (fig. 8A). Similarly, it happened to clindamycin HCl-ethanol 50%, which showed the dehydration was starting from a temperature of 80 °C to 100 °C in fig. 8B.

In general, the pseudo polymorphism phenomenon is the common cases for some drug substances, which will affect on drug dissolution, moreover, will influence the bioavailability. This phenomenon also can occur to clindamycin HCl. Therefore, a good pre-formulary study should be conducted accurately in purposes to improve the best dosage formulation. From the crystal characterization study, it can be reached the planning for the optimal manufacturing process. This must be conducted to support all kinds of dosage form manufacturing since the pseudo polymorphism phenomenon is the common cases for some drug substances, which will affect on drug dissolution, and moreover, will influence the bioavailability. This phenomenon also can occur to clindamycin HCl. Therefore, a good pre-formulary study should be conducted accurately in purposes to improve the best dosage formulation. From the crystal characterization study, it can be reached the planning for the optimal manufacturing process. This must be conducted to support all kinds of dosage form manufacturing since the very simple until the nano-drug formulations. The study can be conducted by severe instruments, which some have been explained before. These solid analyses instruments also have been reported used for the quantitative purpose besides qualitative characterization [1, 2, 9–16].

CONCLUSION

Clindamycin HCl forms pseudo-polymorphism after its recrystallized in the mixtures of ethanol-water, which can be characterized with FTIR, DTA, PXRD, and polarization microscopy. Percentage of ethanol in water will determine the kind of pseudo-polymorphism. The proportion of >70% ethanol in water will form a monohydrate-ethanolate, meanwhile less than 50% of ethanol will produce only a hydrate, then between 50–70% of ethanol will compose the hydrate-ethanolate. However, these pseudo polymorphs of clindamycin HCl were unstable after storage at 24 h in the ambient temperature (25±2 °C, RH: 70±1 %).

CONFLICT OF INTERESTS

Declared none

REFERENCES


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