EFFECTS OF THERMAL ENERGY, MECHANICAL ENERGY, AND SOLVENT ON CIPROFLOXACIN HYDROCHLORIDE MONOHYDRATE PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

Ciprofloxacin HCl is available in the form of anhydrate and monohydrate. Water content can affect the hydrolysis, hygroscopicity, bond, and crystal properties of an active substance. This study was aimed to observe the physicochemical phenomena that occur in ciprofloxacin HCl monohydrate because of thermal energy, mechanical energy, and solvent. Ciprofloxacin HCl monohydrate from two different producers were given treatments by thermal energy, mechanical energy, and solvent. Afterward, the ingredients were characterized using XRD, DSC, and SEM. Other than that, crystal habit, modulus of elasticity, and dissolution were assessed. The results of characterization before and after treatment were compared to observe any changes. Ciprofloxacin HCl monohydrate from two different producers showed similarities in physicochemical properties. Manufacture treatment, which includes thermal energy, mechanical energy, and solvent, affected the physicochemical characteristics of ciprofloxacin HCl monohydrate. The effect of thermal energy and solvent can change ciprofloxacin from hydrate to anhydrate, thus changed its dissolution level. Meanwhile, mechanical effect by grinding and compression can decrease crystallinity and change the particle size of ciprofloxacin HCl monohydrate, thus change its dissolution level. The results of this study showed that the process of manufacturing can change the physicochemical characteristics of ciprofloxacin HCl monohydrate.

Keywords: Ciprofloxacin HCl Monohydrate, Thermal Energy, Mechanical Energy, Solvent, Physicochemical property

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INTRODUCTION

In the process of manufacturing pharmaceutical preparations, the quality of active pharmaceutical ingredient (API) highly affects the stability, effectivity, and safety of the drug. The unique molecular condition of each solid provides different physicochemical properties.^{1,2} API has been widely reported to be in the form of hydrate, which is a crystal solid containing a water molecule inside the crystal lattice. Based on its structure, hydrate crystal can be classified into isolated site hydrates, channel hydrates, and ion coordinated hydrates. Other than that, according to the amount of water molecule in a crystal lattice, hydrate can be classified into several classes, i.e. hemi-, mono-, tri-, theta-, penta-, and hexahydrate. Ciprofloxacin hydrochloride is available in the form of anhydrate and monohydrate.³ Ciprofloxacin HCl monohydrate (Fig.-1) is the salt form of ciprofloxacin. This substance has the chemical name of monohydrochloride monohydrate salt from 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, monohydrochloride, monohydrate. Ciprofloxacin HCl monohydrate is a second-generation fluoroquinolone class antibiotic used in the medication of gram-negative bacterial infection and is included in BCS (Biopharmaceutics Classification System) class IV.

In every stage of the preparation manufacturing process, water can interact with API. The amount of water in API can be affected by RH and a certain temperature, thus increasing the affinity between active substance and a water molecule. The water content will affect hygroscopicity, bond, and crystal

properties.⁴ This study characterized ciprofloxacin HCl monohydrate API from two different sources. This study was aimed to observe physicochemical phenomena that occur in ciprofloxacin HCl monohydrate caused by thermal energy, mechanical energy, and solvent from each ingredient. This study has not yet been studied, therefore new information regarding the physicochemical characteristics and possibilities in each ciprofloxacin hydrochloride monohydrate API can be put into consideration in determining parameters that need to be controlled during tablet manufacturing process.

Fig.-1: Structure of Ciprofloxacin Hydrochloride Monohydrate⁴⁵

As a consideration, several processes during pharmaceutical active ingredient manufacturing such as heat^{6,7}, grinding and compressing process are manufacturing steps that involve mechanical energy^{8,9} and solvent⁶ as the cause of solid transformation. Other than polymorph transformation, a solid transformation that can be caused by solvent include amorphous transition to crystalline, hydrate substance formation or solvate and substance derivatization, e.g. transformation of theophylline anhydrate to theophylline monohydrate in phosphate buffer solvent with pH 6 or co-crystal formation of carbamazepine-saccharin using solvent evaporation technique.¹⁰

The solid transformation causes a difference in the physicochemical characteristics of the resulting solid. This was since every solid has its physicochemical characteristics. Several characteristics that can change include solubility¹¹, dissolution rate and bioavailability¹²⁻¹⁴, hygroscopicity¹⁰, chemical stability and reactivity¹⁵, mechanical property.^{10,16} Unwanted solid transformation can be prevented during the manufacturing process and vice versa. If the solid transformation is wanted, then something should be done during the manufacturing process to initiate the process of transformation as expected.¹⁷

EXPERIMENTAL

Materials

Ciprofloxacin hydrochloride monohydrate was purchased from Aarti Drugs Limited (Mumbai, India) and Chemo S.A (Lugano, Switzerland), water, ethanol from Merck (Darmstadt, Germany), and hydrochloride acid was purchased from J.T Baker (NJ, USA).

Methods

Effect of Thermal Energy

A certain amount of sample was heated in a heat chamber (Binder, Germany) for one hour at 40, 110, and 180°C. Heating results were characterized according to DSC and PXRD.

Effect of Mechanical Energy

Effect of Grinding On Ciprofloxacin Hydrochloride Monohydrate

A certain amount of sample was ground using Retsch RM 200 mortar grinder (Retsch, Germany) at 100 rpm speed for 180 minutes. The results of grinding were characterized according to DSC, PXRD, SEM and dissolution.

Effect of Compression on Ciprofloxacin Hydrochloride Monohydrate

A certain amount of sample was sifted using 140 µm mesh. The sample was scaled at 500 mg and was inserted into a die with 13 mm diameter, then compressed using a hydraulic press (Perkin Elmer, MA,

USA) for 7 seconds. The compression force used was 4.9, 19.6, and 24.5 kN. The tablet was then characterized, including SEM, DSC, PXRD and dissolution.

Effect of Solvent

A certain amount of sample was dissolved in a solvent and evaporated at room temperature until the crystal formed. The solvent used was water and 96% ethanol. The crystal was characterized by crystal habit, DSC, PXRD and dissolution.

Characterization of Solids

X-ray Diffraction (PXRD)

PXRD data were collected using Bruker D8 Advance X-ray diffractometer (Madison, WI). A certain amount of sample was placed in a sample holder and was spread evenly. The analysis was performed using a 2θ angle range of $5-45^\circ$. The condition of diffractometer measurement was as follows: $K\alpha$ X-ray source; Cu target; 30.0 mA current; 25°C temperature; 10.4018 deg/minute scan speed; 40.0 kV voltage.

Differential Scanning Calorimetry (DSC)

The DSC studies were performed on a LINSEIS PT-1600 (Robbinsville, NJ), calibrated for cell constant and temperature using indium. Approximately 6-8 mg of sample was scaled and placed into aluminum pan. DSC instrument was arranged at $28-555^\circ\text{C}$ with $10^\circ\text{C}/\text{minute}$ of heating speed.

Polarization Microscope

The habit of the sample was investigated by polarization microscope BX-50 (Olympus, Tokyo, Japan). The sample was dispersed in paraffin liquid on object glass and was covered with a cover glass. The sample was observed using a polarization microscope with 400x magnification. The image was captured using AnalySIS GetIT software which was connected to the ocular lens of the microscope.

Scanning Electron Microscope (SEM)

The sample was placed on a sample holder and was layered with gold. The sample was observed in various magnification using a HITACHI SU3500 scanning electron microscope (Tokyo, Japan).

Modulus of Elasticity Study

A certain amount of sample was sifted using a $140\ \mu\text{m}$ mesh. The sample was scaled at 500 mg and was inserted into a die with 13 mm diameter, then was compressed using a hydraulic press (Perkin Elmer, MA, USA) for 7 seconds. Compression force used were 4.9; 9.8; 14.7; 19.6; 24.5; and 29.4 kN. The hardness and dimension of the tablet were measured. Afterward, tensile strength was measured, and the modulus of elasticity curve was made.

Dissolution Study

The sample was sifted using $140\ \mu\text{m}$ mesh and a certain amount of it was inserted into dissolution jar containing 900 mL of 0.01 N HCl. Dissolution was performed using the Hanson SR8Plus dissolution tool (Hanson Research, CA, USA) apparatus type II (paddle), 50 rpm mixing speed at 37°C . Ten mL of sample was taken at an interval of 5, 10, 15, 20, 30, 45 and 60 minutes and was substituted by 10 mL of new dissolution medium. The sample solution was filtered using filter paper (steriLUX $0.45\ \mu\text{m}$). The filtrate was analyzed using UV spectrophotometer $\lambda\ 276\ \text{nm}$.^{4,18}

RESULTS AND DISCUSSION

Effect of Thermal Energy

Figure-2 showed that both ingredients, ciprofloxacin HCl monohydrates, showed similar diffractograms. According to Bragg law, when an X-ray is fired on crystalline material of ciprofloxacin HCl, then it will produce a unique diffraction pattern. This pattern is resulted according to the atomic structure of the said crystal. The crystallinity of ingredient A was 82.8% and B 82.2%. Both ingredients showed no difference before treatment. Heating treatment at 40°C did not show any changes in both samples while starting from

110 and 180°C, both samples showed changes in diffraction peak pattern. This was due to heat energy produced at 40°C is not enough to induce transformation in the manufacturing process of ciprofloxacin HCl monohydrate. Meanwhile, 110 and 180°C temperature caused ciprofloxacin HCl monohydrate to change into anhydrate form, which was shown by a new peak at $2\theta \pm 12$ and 16° and the loss of peak at 2θ in 21° .¹⁹

Fig.-2: X-ray Diffraction Pattern of Ciprofloxacin HCl Monohydrate After Thermal Energy

Fig.-3: DSC of Ciprofloxacin HCl Monohydrate After Thermal Energy

Figure-3 showed the DSC thermogram curve from ciprofloxacin HCl monohydrate. DSC is thermal analysis methods are a well-established technique in research. The result of calorimetric measurements is a DSC curve shown as the temperature/time dependence on the heat flux (per time unit).²⁰ Ingredient A showed the first endothermic curve at 143.41°C ($\Delta H = -1.21$ J/g) which was suspected to be caused by dehydration. The exothermic curve followed at 180°C ($\Delta H = +9.88$ J/g) which showed crystallization. The second endothermic curve at 318.82°C ($\Delta H = -1.70$ J/g) showed the melting temperature of the crystal formed accompanied by decomposition. Dehydration occurred at ± 142 -144°C as shown on DSC thermogram. The thermogram curve of samples A and B at 40°C showed no changes. Meanwhile, endothermic curve shift to the right occurred at 110 and 180°C. Without exothermic curve at 180°C, shifting occurred at 300°C with lower endothermic curve. At 180°C heating, thermogram of sample showed exothermic curve, which showed the formation of new crystal structure without water or anhydrides. At 180°C, ciprofloxacin HCl monohydrate can release its hydrate water to create ciprofloxacin HCl anhydrate. According to Kakkar *et al.*,²¹ ciprofloxacin HCl monohydrate is categorized as channel hydrates. Therefore, water molecules can easily bond and detached from ciprofloxacin HCl monohydrate without damaging its crystal structure.

Effect of Mechanical Energy

Effect of Grinding

Based on the crystal habit of ciprofloxacin HCl monohydrate observed using SEM as depicted on Fig.-4, both ingredients had both acicular (needle) shape, formed from prismatic crystal which grew lengthways, thus looking like a needle. Crystal habit A was sharper and narrower compared to crystal habit B. Fig.-4 showed grinding result for 180 minutes which caused decrease particle size, accompanied by damage to crystal structure and decrease crystallinity. Fig.-4A₁ and B₁ depicted crystal habit after 60 minutes of grinding which showed no changes in habit shape, which means that only the sharp ends became flat. Fig.-4A₂ and B₂, the result of grinding for 120 minutes, showed changes in agglomeration shape of long rod shape. Meanwhile, grinding for 180 minutes showed a flatter and wider A and B was dominated by needle shape and were more stable during grinding.

Fig.-4: Photomicrograph of SEM for Ciprofloxacin HCl Monohydrate Before Grinding (A And B) And After Grinding (A₁ And B₁) For 60 Minutes, (A₂ And B₂) For 120 Minutes, And (A₃ And B₃) For 180 Minutes

According to Fig.-5, the longer the grinding process, the wider the intensity of ciprofloxacin HCl monohydrate peaks, which indicated a decrease in crystallinity. Decrease crystallinity with the addition of grinding period can be seen in Table-1. This was caused by the looseness of the lattice plane of ciprofloxacin HCl monohydrate. In this case, higher mechanical energy was needed to damage crystal structure above its critical limit, thus creating amorphous shape.²²

Table-1: Crystallinity Percentage of Ciprofloxacin HCl Monohydrate After Grinding

Sample	Crystallinity (%)						
	0'	30'	60'	90'	120'	150'	180'
A	82.8	81.8	81.2	80.4	79.0	77.6	76.8
B	82.2	81.2	80.8	80.5	79.2	78.4	75.7

Fig.-5: X-ray Diffraction Pattern of Ciprofloxacin HCl Monohydrate After Grinding

Fig.-6: DSC of Ciprofloxacin HCl Monohydrate After Grinding

Figure-6 showed a thermogram identical to heating results at 110 and 180°C, which showed an endothermic curve which was an indicator of shifting crystallization to 300°C. In the first endothermic curve, shifting occurred to 162°C. Longer duration of grinding can decrease crystallinity, which can also be seen from the endothermic curve that shifted to the left, in line with increasing grinding duration.

Dissolution result, according to Noyes-Whitney equation, showed that the lower the particle size, the higher the surface area, thus the higher the dissolution rate. Decrease crystallinity by grinding can also increase the dissolution rate and higher free energy. Therefore, it is unstable thermodynamically.²³ This was following the dissolution result in Fig.-7. There was a difference in dissolution rate between baseline ciprofloxacin HCl powder and the result of 180 minutes grinding to ingredient A and B. This was caused by smaller particle size and decrease crystallinity.

Effect of Compression Force

The relatively high interparticle force caused ciprofloxacin HCl monohydrate to have poor flow property. On the other hand, ciprofloxacin HCl monohydrate is relatively easy to compress. Both ingredients showed similar modulus of elasticity curve (Fig.-8). Modulus of elasticity measurement was performed, as an extrapolation from compactness measurement of ingredients when compressed. Ciprofloxacin HCl monohydrate can be compressed using 4.9 kN pressure and reached maximum tensile strength at 19.6 kN pressure. Pressure administration from 4.9 to 19.6 kN showed that ciprofloxacin HCl monohydrate was in

the plastic and flowing phase. When given higher force, ciprofloxacin HCl monohydrate underwent fatigue and fracture phase.

Fig.-7: Dissolution Profile of Ciprofloxacin HCl Monohydrate After Grinding

Ciprofloxacin HCl monohydrate powder was pressed using several compression forces, then its physicochemical properties were observed. The pressure was chosen according to the elasticity curve, i.e. 4.9; 19.6; and 24.5 kN. These three numbers were chosen because they represented critical pressure in elastic modulus curve changes of ciprofloxacin HCl monohydrate.

Fig.-8: Elastic Modulus Curve of Ciprofloxacin Hydrochloride HCl Monohydrate

Table-2: Crystallinity Percentage of Ciprofloxacin HCl Monohydrate After Compression

Sample	Crystallinity (%)			
	0 kN	4.9 kN	19.6 kN	24.5 kN
A	82.8	78.4	74.2	73.9
B	82.6	78.1	74.3	73.8

XRD diffraction pattern in Fig.-9 and Table-2 showed that ciprofloxacin HCl monohydrate powder after the compression process led to decreased crystallinity in certain peaks of ciprofloxacin HCl monohydrate at 2θ 15, 17, and 18.5° . This decrease of crystallinity during the compression process was caused by a weakened crystallinity degree.

DSC thermogram in Fig.-10 depicted A and B after the compression process in three different compression force variations. In line with the thermogram after grinding and heating at 110 and 180°C , compression results also cause endothermic change to 160°C and exothermic to 300°C . The exothermic

peak at 450°C cannot confirm the occurring physics event. Decrease crystallinity can also be seen by decrease endothermic peak at around 300°C, as decrease melting temperature in line with decrease crystallinity. When the crystallinity of a substance increases, then it's melting point will also increase because its crystal lattices are more organized, thus harder to melt.

Fig.-9: X-Ray Diffraction Patterns of Ciprofloxacin HCl Monohydrate after Compression Force

Fig.-10: Thermogram of Ciprofloxacin HCl Monohydrate Before and After Compression

SEM results analysis depicted in Fig.-11 and Fig.-12 showed that compression caused sintering to ciprofloxacin HCl monohydrate tablets. This was due to the fusion process, where two or more ciprofloxacin HCl monohydrate particles fused into one. Smaller particles joining to become bigger caused decreased surface area of ciprofloxacin HCl monohydrate particles.²⁴

The dissolution result in Fig.-13 showed a decrease in the dissolution rate of the compressed powder. This was because the compression force of 4.9-9.8 kN showed elastic-plastic condition, and 14.7-19.6 kN showed plastic condition where particles are bigger in size. Meanwhile, forces at 24.5-29.4 kN resulted in fracture conditions, decrease particle size, decrease interparticle bond and increase dissolution rate.

Effect of Solvent

There were two types of solvent, i.e. water and ethanol as representatives that can dissolve ciprofloxacin. XRD results can be seen in Fig.-14a which showed that recrystallization results in water solvent produced no changes in diffractogram. Meanwhile, the recrystallization result using ethanol showed new peaks from ciprofloxacin HCl monohydrate at 2θ 8.3, 12.1, 14.8, and 16.4°. The formation of this new diffraction pattern indicated changes of ciprofloxacin HCl from hydrate to anhydrate. Meanwhile, a thermogram from the solvent can be seen in Fig.-14b, which was similar to the result of thermal treatment

at 110 and 180°C. Recrystallization result using water also showed endothermic shifting to the right. Sample A showed endothermic shifting to 154.20°C and sample B to 155.02°C. Meanwhile, the result of the thermogram using ethanol also showed endothermic shifting to the right, with sample A to 155°C and sample B to 155.86°C.

Fig.-11: SEM Photomicrograph Of Ciprofloxacin HCl Monohydrate A. (a), (b), and (c) for Fragment of Tablet, (d), (e), and (f) for Surface of Tablet

Fig.-12: SEM Photomicrograph Of Ciprofloxacin HCl Monohydrate B. (a), (b), And (c) For Fragment of Tablet, (d), (e), And (f) For Surface of Tablet

Fig.-13: Dissolution Rate of Ciprofloxacin HCl Monohydrate A And B After Compression

Fig.-14: (a) X-ray Diffraction Patterns and, (b) DSC Thermogram of Ciprofloxacin HCl Monohydrate After Recrystallization

Fig.-15 and 16 showed crystal habits of recrystallization result using water in ingredients A and B which was analyzed using polarization microscope, some of them were shaped like squares and needles. Meanwhile, using 96% ethanol, the crystal was still in the form of disorganized needles. This was due to differences in solvent polarity during recrystallization, thus produce changes in polar crystal planes and causing different crystal habits. Water solvent was also polar, where crystal polar planes grew larger compared to non-polar planes. Therefore, in acid and polar media, recrystallization results from water solvent showed higher dissolution.

Fig.-15: Crystal Habit of Ciprofloxacin HCl Monohydrate A Using (a) Distilled Water and (b) 96% Ethanol As Solvent

Fig.-16: Crystal Habit of Ciprofloxacin HCl Monohydrate B Using (a) Distilled Water And (b) 96% Ethanol as Solvent.

The dissolution result also showed that a higher dissolution of water recrystallization was caused by hydrate form of ciprofloxacin, while recrystallization using ethanol decreased dissolution because of the resulted ciprofloxacin was anhydrate, which can be seen in Fig.-17.

Fig.-17: (a) Dissolution Profile of Ciprofloxacin HCl Monohydrate A, (b) Dissolution Profile of Ciprofloxacin HCl Monohydrate B, Both Before And After Recrystallization

CONCLUSION

Ciprofloxacin HCl monohydrate ingredient from two different producers showed similarity in physicochemical properties. Manufacturing treatment, which consists of thermal energy, mechanical energy, and solvent affected physicochemical characteristics of ciprofloxacin HCl monohydrate API. Thermal energy and solvent can change ciprofloxacin from hydrate to anhydrate, thus changing its dissolution rate. Meanwhile, mechanical effect by grinding and compressing can decrease crystallinity and change the particle size of ciprofloxacin HCl monohydrate, thus changing its dissolution rate. These results showed that the manufacturing process can change the physicochemical characteristics of ciprofloxacin HCl monohydrate.

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