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Solid-State Transformations of Fluoroquinolones Induced by Mechano- and Tribo-Chemical Processes

A dissertation submitted for the degree of

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by

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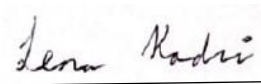
Under the supervision of Prof. Lidia Tajber

2025

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university. A small proportion of the work described in this thesis was carried out by others, and this is duly acknowledged in the text wherever relevant. I declare that all other work is entirely my own.

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Lena Kadri

Summary

This study investigated mechanically induced solid-state transformations of levofloxacin hemihydrate (LVXh). In addition, a kinetic understanding of ball milled (BMed) LVXh was established for the first time. Furthermore, a comparison of the ball milling (BMing) and spray drying (SDing) processes was performed and the results showed that partial or complete amorphization can be achieved, respectively, implying that the outcome is mechanism and process dependent. Additionally, the interconversion pathways of LVXh, LVX gamma (LVX γ), and LVX amorphous (LVXam) as a result of milling LVXh in the solid-state have been uncovered for the first time.

This research disclosed the possibility of achieving amorphization of LVXh using a polymer excipient, Eudragit[®] L100 (EL100). The mechanism of LVXh:EL100 system formation was discovered for the first time, disclosing that in the first step the drug undergoes amorphization, and the second step consists of a reaction between the amorphized drug and the polymer, ultimately, leading to polymer ionization. Upon performing a comparative investigation, it was discovered that variability in the product outcome of the LVXh:EL100 system existed, depending on the mill used. Furthermore, in-situ measurements disclosed that each of the crystallographic planes of LVXh amorphized at the same time, implying no weak crystallographic plane(s). Thermal treatment demonstrated the identification of the anhydrous/dehydrate form of LVXh, known as anhydrous LVX γ . According to the literature, this behavior is comparable to LVXh when milled on its own, however, it is now reported for the first time in the presence of a polymer.

This study demonstrated that BMing ciprofloxacin (CIP) with acidic or neutral polymers can result in complete or partial amorphization, and an investigation of crystallinity by altering the type of mill and parameters used was performed. An investigation of the CIP amorphous polymeric samples showed that proton transfer to the CIP carboxylate group occurred. This was already known in the literature and attributed to the presence of a carboxylate group from an acidic polymer or amino acid. However, the discovery was made for the first time that proton transfer can partially occur upon the addition of neutral polymers. Moreover, it was reported for the first time that the amorphous phase within a semi-crystalline polymeric system can be more or less stable depending on the neutral polymer added.

The research conducted demonstrated that the addition of a second polymer has the ability to increase or decrease the degree of crystallinity of the original binary system. Changes to the hydro-phobicity or -philicity of amorphous polymeric systems occurred upon the addition of a second polymer. Furthermore, the addition of a second acidic polymer may have the ability to interfere with interactions involving the protonated carboxylate group of CIP, unlike the addition of a neutral polymer.

The investigation into the binary and ternary systems confirmed improvements to the solubility of CIP, particularly in aqueous solution and fasted state simulated intestinal fluid (FaSSIF) buffer. In addition, the CIP sample containing EL100 and Hypromellose (HPMC) (CIP:EL100:HPMC) demonstrated superior results against bacterial strains compared to the other binary and ternary systems tested and CIP alone. Nonetheless, the mechanisms of antibiotic resistance in the CIP-resistant strains examined were unchanged by the systems designed.

These findings are valuable as they can be transferred and/or applied to current and future fluoroquinolone drugs with similar molecular structures in the hope of developing a greater understanding of mechanically induced solid-state, amorphization, and salt/proton transformations. The discoveries obtained from this research can help to fill the gaps in mechano- and tribo-chemistry and contribute towards making the pharmaceutical industry and the environment greener. Above all else, this project includes valuable results and discoveries that can help enhance the properties of fluoroquinolone drugs, to ultimately improve patients' health across the globe.

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Abbreviations and Symbols

AGJ	artificial gastric juice
AMR	antimicrobial resistance
APIs	active pharmaceutical ingredients
APS	amorphous polymer salt
ASD	amorphous solid dispersion
[B]	concentration of the free base
[BH⁺]_s	concentration of the protonated amorphous salt
BCS	biopharmaceutics classification system
BES	besifloxacin
BMed	ball milled
BMing	ball milling
CH₃COOH	acetic acid
CH₃COONa	sodium acetate
CIFs	crystallographic information files
CIP	ciprofloxacin
CLCs	critical loading conditions
cm	centimeter
CPMAS	cross polarization magnetic angle spinning
CSD	Cambridge Structural Database
DCS	developability classification system
DPI	dry powder inhalation
DSC	differential scanning calorimetry
DVS	dynamic vapor sorption
EL100	Eudragit [®] L100
EMs	eutectic mixtures
ENRO	enrofloxacin
ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , and <i>Enterobacter</i> species

EUCAST	European Committee on Antibiotic Susceptibility Testing
FaSSIF	fasted state simulated intestinal fluid
FaSSIF	fasted state simulated intestinal fluid
FDA	Food and Drug Administration
FeSSIF	fed state simulated intestinal fluid
FeSSIF	fed state simulated intestinal fluid
FTIR	Fourier-transform infrared spectroscopy
GAT	gatifloxacin
GIT	gastrointestinal tract
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
HPMC	hypromellose
HPMCAS	hypromellose Acetate Succinate
HPMCP	hypromellose phthalyl
Hz	hertz
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous
KH₂PO₄	monopotassium phosphate
KolVA64	Kollidon [®] VA64
LAG	liquid assisted grinding
LVX	levofloxacin
LVXh	levofloxacin hemihydrate
LVXm	levofloxacin monohydrate
LVXα	levofloxacin alpha
LVXγ	levofloxacin gamma
M	molar
MHB	Mueller Hinton Broth
MIC	minimum inhibitory concentration
MICs	minimum inhibitory concentrations
mL	milliliter
mM	millimolar

MM400	mixer mill
mol	mole, Avogadro constant number (6.02×10^{23})
M_w	molecular weight
MXF	moxifloxacin
NaCl	sodium chloride
NaH₂PO₄·2H₂O	sodium phosphate monobasic dihydrate
NaH₂PO₄·H₂O	sodium phosphate monobasic monohydrate
NaOH	sodium hydroxide
NCEs	new chemical entities
NH₄H₂PO₄	ammonium dihydrogen phosphate
nm	nanometer
NNMR	nitrogen nuclear magnetic resonance
NOR	norfloxacin
OFX	ofloxacin
PAA	polyacrylic acid
PBS	phosphate buffered saline
pH	measurement of the acidity or basicity of a solution
pK_a	negative base -10 logarithm of the acid dissociation constant (K _a) of a solution
PM100	planetary mill
PMs	physical mixtures
ppm	parts per million
PXRD	powder x-ray diffraction
PVP	polyvinylpyrrolidone
rDCS	refined developability classification system
RH	relative humidity
Rpm	revolutions per minute
s	salt as the saturation species at a pH below pH _{max}
SDDS	supersaturating drug delivery system
SDed	spray dried
SDing	spray drying

SSNMR	solid-state nuclear magnetic resonance
S_T	total solubility
T_g	glass transition temperature
TGA	thermogravimetric analysis
TSE	twin screw extruder
USP	United States Pharmacopoeia
UTIs	urinary tract infections
WHO	World Health Organization
ZrO₂	zirconium oxide
ΔH_{Cryst}	enthalpy of crystallization
2θ	2 theta (diffraction angle)
μm	micron/micrometer
α	degree of transformation
α	alpha
β	beta
γ	gamma
δ	delta
i	times powder has undergone CLCs
m	impacts
Ø	diameter of a circle
V	total volume of powder
v	total volume of powder affected by CLCs
v*	subvolumes
v/v	volume per volume
w	weight fractions
w/w	weight per weight
κ	rate constant
K	ratio between v and V
λ	wavelength
ρ	true densities
χ	volume fraction of powder

%	percent
°	degrees
°C	degrees Celsius

Chapter 1.0 General Introduction

Chapter 1.0 General Introduction

1.1 Solubility

Poor solubility of newly developed active pharmaceutical ingredients (API) is a major challenge for the pharmaceutical industry. Approximately 40% of newly developed drugs are poorly water-soluble or lipophilic in nature, and around 70–90% of all new chemical entities (NCEs)/drug molecules have poor aqueous solubility.¹⁻³ Therefore, it is essential to enhance the solubility of poorly soluble APIs, to allow more drug to be absorbed into the system, and ultimately, to improve the bioavailability of the drug. The most common form of drug administration is via the oral route due to patient compliance, ease of administration, reduced transportation costs, and manufacturing scalability.⁴

1.1.1 Bioavailability

Bioavailability is defined by the fraction percent of the orally administered drug that reaches the systemic circulation and arrives at the site of drug action to produce a therapeutical effect.⁵ Absolute bioavailability is the actual percentage of the administered dose that reaches the general circulation.⁶ Drugs administered via intravenous (IV) injection⁷ are assumed to be 100% bioavailable. Relative bioavailability is the comparison of two formulations or two routes of administration of the same formulation, independent of an IV administration.⁶ The route of administration and the drug dose can significantly impact both the rate and extent of bioavailability. Oral drugs must pass through the gastrointestinal tract (GIT), before being absorbed through the intestinal wall, and into the circulatory system.

1.1.2 The Biopharmaceutics Classification System

The Biopharmaceutics classification system (BCS) is used to classify drug substances into four groups based on aqueous solubility and intestinal membrane permeability (**Figure 1.1**).⁸ This is in accordance with the Food and Drug Administration (FDA), World Health Organization (WHO), and European Medicines Agency.^{9, 10} The BCS classification system is based on parameters that affect absorption such as solubility, dissolution rate, and permeability. The definition of solubility in the BCS is the quantity of a substance that can be dissolved in a given amount of solvent. Permeability is the ability of a drug to pass across a biological membrane. A drug is highly permeable if it has an absorption of $\geq 85\%$.^{9, 11, 12} Dissolution is the process in which a substance forms a solution. An API may be classified as rapidly dissolving

if $\geq 85\%$ of the dose dissolves in ≤ 900 mL of buffer within 30 minutes, using United States Pharmacopoeia (USP) apparatus I at 100 rpm or apparatus II at 50 rpm.⁹

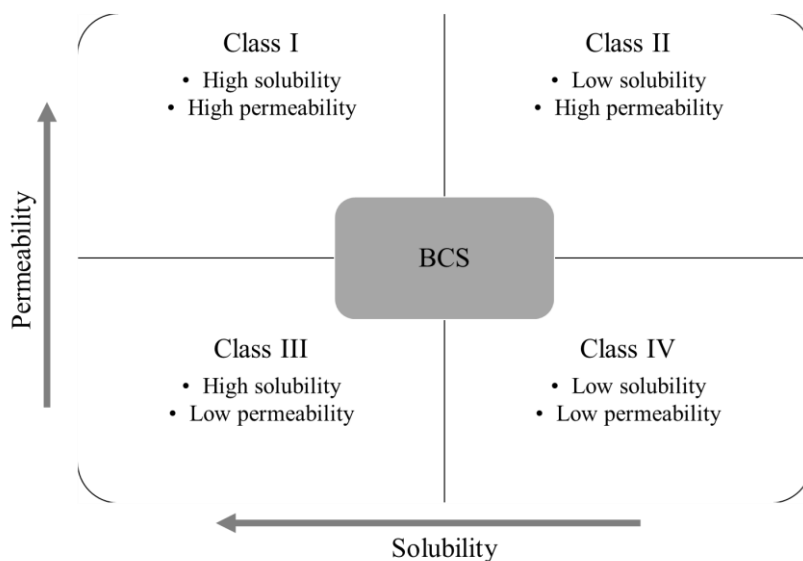


Figure 1.1 The biopharmaceutics classification system based on solubility and permeability.⁸

1.1.3 The Developability Classification System

A modified classification system was introduced in 2010 by Butler *et al.* known as the Developability Classification System (DCS),¹³ and a refined Developability Classification System (rDCS) has since been proposed.¹⁴ These systems are used as a tool for evaluating the developability of APIs and predicting the critical factors to enhance *in vivo* performance.¹³ Both systems evaluate the probability of dissolution rate limitations and target particle size.¹⁵ Overall, these systems were designed to further develop the understanding of NCE to determine the suitability for oral delivery and to assist the BCS as a preliminary tool.^{14, 16}

1.2 Structure of a Drug

The most common form for drugs to exist is in the crystalline form due to its superior stability. The term crystalline describes a structure in which the structural units, known as unit cells, are regularly repeated in three-dimensional space. The transformation from the crystalline to the amorphous form of a drug may transpire during the pharmaceutical processing of a drug,¹⁷ and/or upon the addition of coformers/excipients.¹⁸⁻²⁰

1.2.1 Amorphous Form

The solubility of poorly soluble drugs can be enhanced by disrupting the crystal lattice by means of amorphization.²¹ The amorphous form of a drug is the most energetic form in the solid-state due to its disordered makeup and absence of long-range order.²² In consequence, less energy is required to enter the solution, and thus, the amorphous form of the drug is known to be more soluble than the crystalline form.²³

1.2.2 Limitations of the Amorphous Form

The amorphous form of the API shows poor physical stability since the amorphous API has a higher Gibbs free energy. The thermodynamic instability of this form may lead to the relaxation, nucleation, and crystal growth of the API.^{24, 25} Thus, the main challenge with amorphous drugs is the crystallization of the amorphous form to its most stable, crystalline form.

1.2.3 Overcoming Limitations of the Amorphous Form

Fortunately, the use of polymer(s) can generate an anti-plasticizing effect and increase the overall glass transition temperature (T_g) of the system produced, thereby reducing the thermodynamic driving force for crystallization.²⁶⁻²⁸ Furthermore, specific drug-polymer interactions, such as hydrogen bond interactions, can form between the API and the polymer. A combination of these outcomes can reduce the molecular mobility of the API and enhance the stability of the amorphous drug.²⁹⁻³¹ The generation of specific drug-polymer interactions has been observed for multiple drugs such as ciprofloxacin (CIP),¹⁹ enrofloxacin (ENRO),²⁰ ketoconazole,³² sulfamethoxazole,³³ trimethoprim,³³ and clofazimine³⁴ with acidic or neutral polymers that resulted in the formation of ionic, hydrogen, and/or dipole-dipole interactions/bonds.

1.3 Amorphous Solid Dispersions

An ASD is a term used to describe a system where the API is in the amorphous form and is distributed within a polymer matrix in the solid phase. Improved solubility of drugs can be achieved by generating an ASD that is in a high energy state as less energy is required to break the bonds due to its disordered manner compared to its ordered, crystalline counterpart. These high-energy forms can generate the supersaturation of the drug.³⁵ Supersaturation is when the drug concentration exceeds the equilibrium solubility in the same medium.³⁶ If supersaturation is achieved, the free drug concentration and the thermodynamic activity of the API will be increased beyond its solubility limit at the absorptive site, ultimately, leading to an increase in the driving force for transportation across and into the biological membrane.^{37, 38} Enhancing drug solubility is heavily dependent on the stability of the induced supersaturated state.³⁹

1.3.1 Generations of Solid Dispersions

Many different carriers are utilized to form solid dispersions whereby the API and carrier can be in the amorphous or crystalline phase. Thus, based on the carriers chosen, different generations or classifications can be used to categorize formulations such as first, second, and third generation (**Figure 1.2**). Solid dispersions were first reported in the literature by Sekiguchi and Obi in 1961, whereby they discovered that the formulation of eutectic mixtures (EMs) improved the rate of drug release and the bioavailability of poorly water-soluble drugs.^{40, 41} EMs describe two crystalline components, usually a poorly soluble drug and a hydrophilic carrier, that have a lower melting point than the individual components.⁴² Levy and Kanig further explored solid dispersion systems through molecular dispersions, using mannitol as the carrier.^{43, 44} Solid dispersions using urea and sugars increased the dissolution and solubility of an API.

Second generation solid dispersions were introduced and used amorphous polymeric carriers.⁴⁵ Third generation solid dispersions can be described as using surfactants, a mixture of surfactants and polymers, or a mixture of polymers. Surfactants can improve the solubility and dissolution properties of an API by reducing the surface tension between a liquid and a solid.^{27, 28} The stability of solid dispersion can be enhanced by surfactants by preventing nucleation and agglomeration.⁴⁶ Polymers can also improve the stability of solid dispersions by increasing the Tg and by producing an anti-plasticizing effect.⁴⁷⁻⁴⁹ Thus, this generation aimed to further

improve the solubility, dissolution, and stability of the first and second generation solid dispersions.

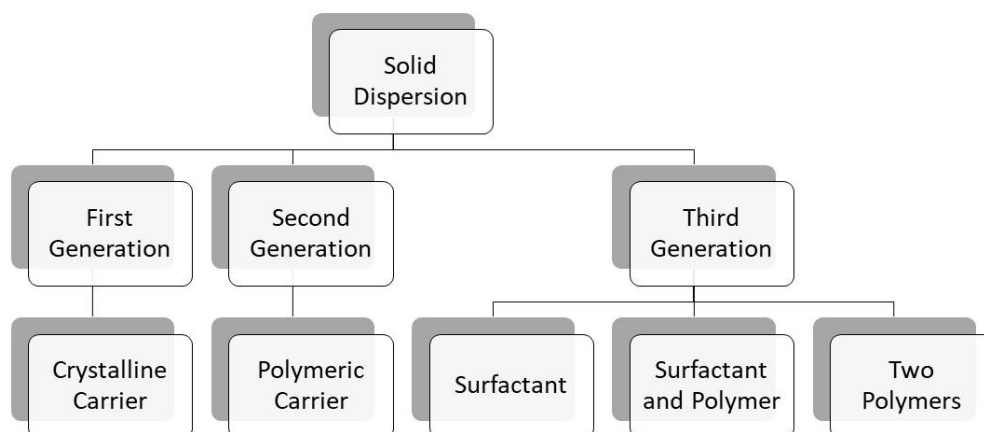


Figure 1.2 First, Second, and Third Generation of Solid Dispersions.

1.3.2 Limitations of Amorphous Solid Dispersions

In order to reestablish the thermodynamic equilibrium solubility^{35, 39} of supersaturating drug delivery systems (SDDS), precipitation can occur and reduce the free drug concentration, and thus, the absorption ability. This poses a limitation for the enhancement of the solubility of drugs. In addition, the solid-state stability of ASDs remains an issue in the present day as the amorphous form often crystallizes to its most stable crystalline form.

1.3.3 Overcoming Limitations of Amorphous Solid Dispersions

Certain polymers can prevent precipitation of APIs from occurring, highlighting the importance of optimal polymer selection. Additionally, the stability of ASDs may be further developed by the inclusion of an ionic interaction, often between the API and polymer, resulting in the formation of an amorphous polymer salt (APS) due to the occurrence of a proton transfer.^{19, 20} Salts offer further stability due to their strong ionic interactions¹⁸⁻²⁰ and can enhance the solubility of acidic and basic drugs.¹⁹ APS is a promising approach for improving the solubility and stability of drugs as it provides a solution that uses a combination of ionization and amorphization.²⁰ Research has been conducted by Mesallati *et al.*^{19, 20, 50} and Paluch *et al.*⁵¹ in order to investigate the preparation of ASDs, APSs, and amorphous salts of fluoroquinolone drugs. Furthermore, over the years, research has shown that a combination of two polymers to form ternary ASDs can significantly enhance the stability of the drug compared to binary

systems by providing additional interactions between the drug and polymers.⁵² A study was performed by Prasad *et al.* that showed that ternary ASDs had superior stability compared to binary ASDs of indomethacin.⁵³ Furthermore, studies suggest that the use of polymers with different solubilities in aqueous media can offer advantages by differing in hydro-phobic/philic properties. For example, an enteric polymer that is insoluble in acidic aqueous media and is more hydrophobic, combined with an amphiphilic polymer with both hydrophobic and hydrophilic properties may provide enhancements to the formulations.⁵⁴⁻⁵⁶ Thus, binary and ternary ASDs/APSs were of interest for this study.

1.3.4 Other Strategies to Improve Solubility of Drugs

Multiple strategies have been utilized in the pursuit of improving the solubility of drugs. Drug dispersion in carriers such as ASDs/APSs has already been discussed in **Section 1.3**. However, other dispersions exist such as eutectic mixtures which include the mixture of two incompatible components that generate a new entity with a low melting point. The Modification of the crystal structure can be achieved through crystal engineering, or the formation of hydrates/solvates, polymorphs, or co-crystallization (co-crystals). Co-crystals are created by forming intermolecular interactions such as π - π stacking, and hydrogen bond interactions to connect two or more different molecular units, without changing the chemical structure of the APIs. Chemical Modifications can be employed to form crystalline or amorphous salts. Additionally, adjusting the pH can alter the charge of the drug to neutral (isoelectric point, IEP), which can influence the precipitation behavior of an API. Particle size reduction can improve the dissolution of the drug by increasing the surface area, which in turn improves the interaction of particles and the solvent. Nanosuspension involves reducing API particle sizes to 10–1000 nm and stabilizing the nanoparticles using surfactants^{57, 58} to enhance the solubility of an API. The surfactants prevent agglomeration and maintain the stability of the nanosuspension. Prodrug formation involves chemically modifying an API from its active form to an inactive molecule that is temporarily linked to a carrier, known as promoiety. This modification assists in the transportation of the API across biological barriers. Once the prodrug reaches its target site, the promoiety is cleaved, thereby activating the drug and allowing it to exert its therapeutic effects. This strategy can enhance the APIs bioavailability and improve its delivery to specific tissues or cells.⁵⁹ Complexes can be generated by connecting two or more molecules to form one entity by the formation of weak molecules, such as hydrogen bonds, hydrophobic interactions, and London forces. Cyclodextrins which are externally hydrophilic and internally hydrophobic, can

be used to form an inclusion complex. In this process, a guest molecule, such as an API is encapsulated within the host cavity of the cyclodextrin. This interaction can enhance the solubility and stability of the API, improving its delivery and bioavailability in pharmaceutical formulations.^{57, 58}

1.4 Mechanical Energy

1.4.1 Milling

Milling is a pharmaceutical process that utilizes mechanical energy to reduce the size of coarse particles to finer particles. According to the Noyes–Whitney equation, these particles often offer improved dissolution rate due to their reduced size, and thus, larger surface area. In addition, milled particles result in altered surface roughness and shape, which can also affect wettability and dissolution.^{60, 61} Milling is often one of many processing steps involved in the manufacture of formulated drug products. However, mechanical energy from the mill can lead to crystal defects and disordered crystal structures, resulting in partial or complete amorphization. The resulting product is described as “mechanically activated”, introducing the terms “mechanochemistry” and “tribochemistry”.⁶² Multiple mills exist such as ball, cutter/hammer, roller,^{61, 63} jaw crusher, rotor, knife, mortar, and disc mills.⁶³ Ball mills will be discussed in this work with a particular focus on the Retsch mixer mill MM400 and the Retsch planetary ball mill PM100.

1.4.2 Traditional Uses of Mechanochemical Energy

Mechanochemistry was traditionally carried out by manual grinding by use of a mortar and pestle and can be dated back to the Stone Age. Mechanochemistry advanced in the eighteenth-hundreds.^{64, 65} However, it was not until the 1960s that the processing of powders gained greater traction, leading to further discoveries of mechanically activated solids resulting in amorphous and nanostructured phases in the 1980s.⁶⁶⁻⁶⁹ Although chemical transformations were often successful, the final product could experience variability due to changes of force by human factors, and exposure to diverse and inconsistent environmental conditions due to the open nature of the mortar.⁷⁰⁻⁷² Fortunately, it is now possible to achieve the same desired outcome by automated processes such as ball milling. This allows longer durations of milling to be implemented and a reduction in the exposure of human and environmental factors.⁷³

1.4.3 Mechanochemistry

Numerous forms of energy can initiate and generate chemical reactions such as photochemistry, thermochemistry, and electrochemistry using light, heat, or electricity.⁷⁴ A less known area of chemistry, known as mechanochemistry, can also initiate and generate chemical reactions by using mechanical energy. Mechanochemistry is a term used to describe chemical reactions stimulated by mechanical forces produced at room temperature by the mechanical

process.^{70, 75-77} It is also defined by the International Union of Pure and Applied Chemistry (IUPAC) as a “chemical reaction that is induced by the direct absorption of mechanical energy”.⁷⁸ Mechanical force generates energy that can initiate the breakage of molecules through various forces such as impact, shear, friction, stress, and strain. Impact force is the leading force for “mechanochemistry”, in comparison to “tribochemistry” which is governed by shear and friction forces, yet still includes impact forces.⁷⁹⁻⁸¹ Chemical reactions can occur due to the efficacious rupture of these bonds, permitting reactions to supervene.^{75, 77} Despite the limited knowledge in this field, compared to other areas mentioned, this area of chemistry is growing at a fast rate and was recognized by IUPAC in 2019 as one of the top ten chemical innovations that sought to change the world.⁸²

1.4.4 Tribochemistry

The expansion of knowledge in the field of mechanochemistry has shifted a focus toward terminology for ease of classification of reactions produced by different primary mechanical forces. In the case where shear and friction forces are more dominant than impact forces, a more recent term “tribochemistry” has been used to describe this type of mechanical energy.⁷⁹⁻⁸¹ This means that traditional uses of grinding using a mortar and pestle that are dominated by shear and friction forces, yet still include impact forces (just to a lesser extent than “mechanochemistry”), now fall under the classification of “tribochemistry”.

1.4.5 Mechanochemical Processes

Multiple mechanochemical processes are available that can mechanically activate a drug, disrupt non-covalent interactions, and rupture bonds. This may result in a change in the molecular geometry of a drug, disruption to the crystal lattice, and/or allow chemical reactions to transpire.⁸³ The most common mechanical process movements comprise vibration, oscillation, spinning, and/or stirring. This is caused by mechanical forces which may include impact, shear, and/or friction, which can result in stress and strain on the drug (**Figure 1.3**).⁷¹

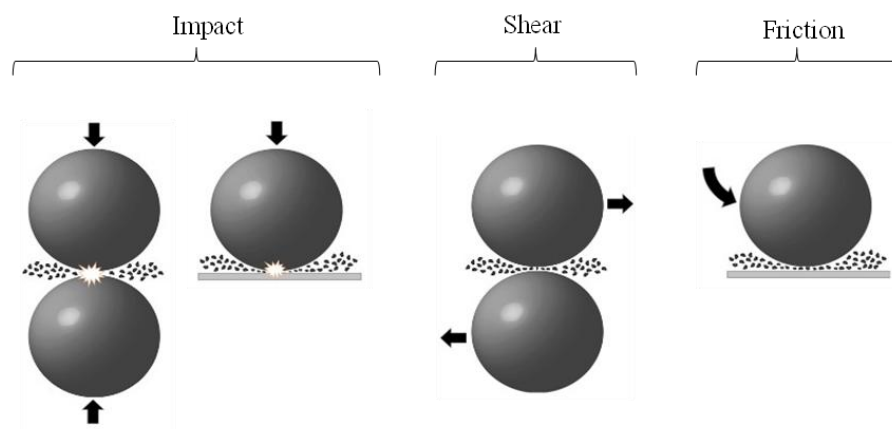


Figure 1.3 Mechanical impact, shear, and friction forces produced during ball milling.⁸¹

1.4.5.1 Tumbler Ball Mill

The tumbler ball mill was the first industrially used mill inspired by the process of manual grinding using a mortar and pestle and operating upon rotation of a cylindrical drum. As the drum rotates, the material and balls rise and fall, undergoing low impact collisions. This mill depends on gravity and requires a large drum size to operate effectively (**Figure 1.4**). Automatic mills were further developed to overcome the limitations of the tumbler mill by increasing the energy of impact forces and/or allowing the process to run at a laboratory scale. Vibrational mills such as the mixer mill, and oscillatory mills such as the planetary ball mill were introduced.⁶⁴

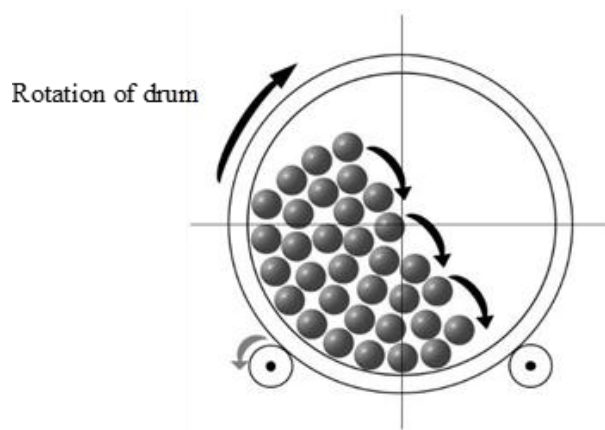


Figure 1.4 Vertical cross section of a tumbler ball mill displaying the internal and external motion of the balls and the drum.⁸¹

1.4.5.2 Retsch Mixer Mill MM400

The Retsch mixer mill MM400 undergoes vibration and horizontal radial oscillations, facilitating the movement of the jar and the ball(s) around and against the rounded ends and sides of the jar (**Figure 1.5**). The sample is therefore exposed to ball-to-ball and ball-to-wall interactions. This facilitates the application of extreme forces such as impact, shear, and friction. The primary impact forces at play permit the Retsch mixer mill MM400 to be classified under the term mechanochemistry.⁷⁹⁻⁸¹

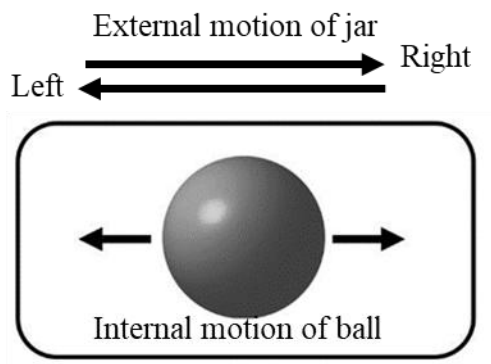


Figure 1.5 Vertical cross section of a Retsch Mixer Mill MM400 displaying the internal motion of the ball and the external motion of the milling jar.⁸¹

1.4.5.3 Retsch Planetary Ball Mill PM100

The Retsch planetary ball mill PM100 undergoes oscillating movements of the sun wheel and the grinding jar, both moving in opposing directions (**Figure 1.6**). While the sun wheel moves clockwise and completes one full rotation, the grinding jar moves counterclockwise, completing two full rotations, thus, moving in a ratio of 1:-2. The opposing directions of the sun wheel and grinding jar result in varying ball and jar speeds.⁸⁴ The primary mechanical forces at play are shear and friction. This results in the Retsch planetary ball mill PM100 being classified under the term tribochemistry.⁷⁹⁻⁸¹

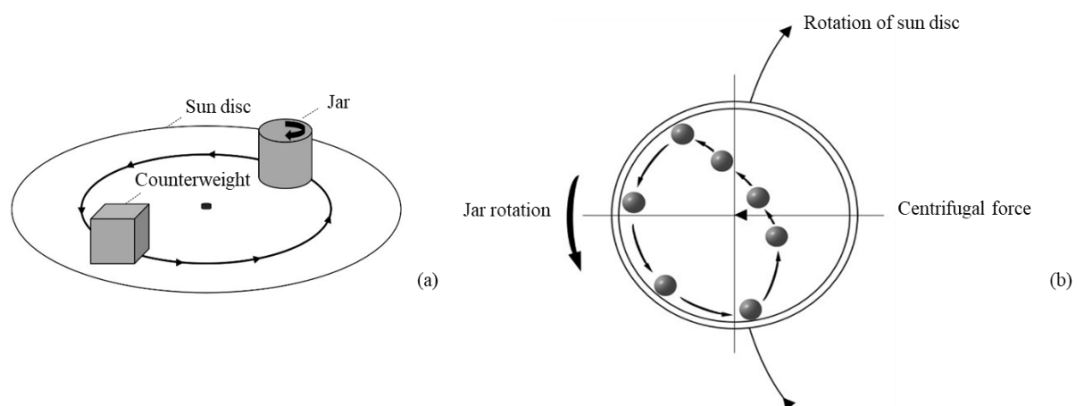


Figure 1.6 An illustration of a Retsch planetary ball mill PM100 displaying (a) the operations of a planetary ball mill and (b) the horizontal cross section of a ball mill jar and the internal motion of the balls.⁸¹

1.4.5.4 Extrusion

The process of extrusion, such as a twin-screw extruder (TSE), has been recognized as a method to scale up mechano- and tribo-chemical reactions. A TSE usually contains a hopper that feeds the material into a temperature-controlled barrel comprising two rotating screws that mix and transport the material along the barrel to the output where the product is collected (**Figure 1.7**). The material is exposed to mechanical forces, shear, and friction which result in stress and strain.^{85, 86}

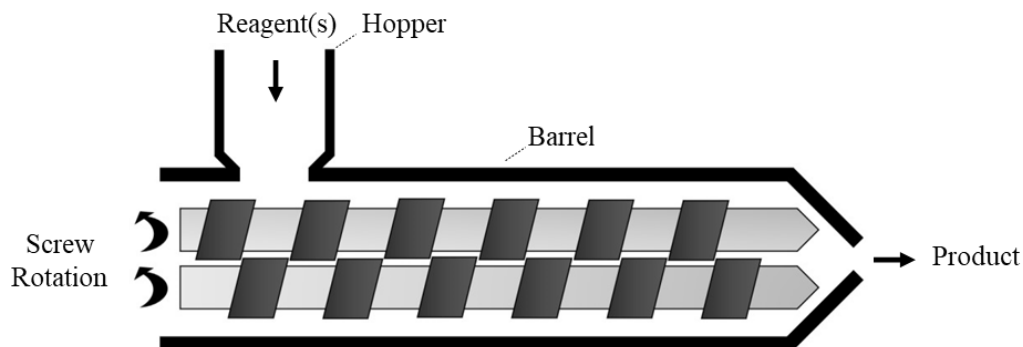


Figure 1.7 Vertical cross section of a twin screw extrusion device.⁸¹

1.4.5.5 Summary of Mills Utilized

The two ball mills that will be discussed in this thesis are the Retsch mixer mill MM400 and the Retsch planetary ball mill PM100. Both mills mentioned operate differently, and thus, can be classified as applying mechanochemistry and tribochemistry, respectively, due to their contrasting operative mechanisms.

The Retsch mixer mill MM400 facilitates transformations by the generation of forces produced by impact, shear, friction, resulting in stress and strain, with the leading force being impact. In contrast, the Retsch planetary ball mill PM100 predominantly produces shear and friction forces, resulting in stress and strain.^{79, 81} Particles can be impacted differently depending on the mechanical actions it is exposed to such as impact, shear, and friction forces. This could potentially produce a different mechanism of action depending on the chosen mechanical process.^{87, 88}

Mechano- and tribo-chemistry are two terms that will be used throughout this thesis to differentiate between the two different Retsch mills used i.e., a MM400 and a PM100. Mechano- and tribo-chemistry offer many advantages such as preventing the interference of undesirable reactions with the solvent, implementing green chemistry, and improving human health by means of eliminating or reducing hazardous solvents.^{89, 90}

1.5 Mechanical Activation

It is understood that mechanical activation promotes new phases and chemical transformations.⁹¹ The process of crystal breakage is considered a significant factor at the beginning of mechanical treatment and is believed to become less important as the process continues. The energy supplied by mechanical action is accumulated at the molecular level as crystal defects near or at the crystal surface.⁹² Crystal defects can give rise to elastic deformation, plastic deformation, stress relaxation, and structural distortions.^{62,93} These changes can expedite mechanical activation, and thus, reactivity. The point of fracture results in the formation and/or the destruction of chemical bonds (**Figure 1.8**).^{94,95} Changes to the mechanism of stress relaxation can be the source of prolonged mechanical reaction induction periods.⁹² Although defects promote the occurrence of mechanical activation and reactions, particle size reduction alone without the inclusion of defects has been mentioned as a potential facilitator in transformations using mechanochemistry.⁹⁶ Particle size reduction may not necessarily lead to an increased reaction rate,⁹¹ however, it is known to facilitate the reaction through increased surface area.⁹⁷

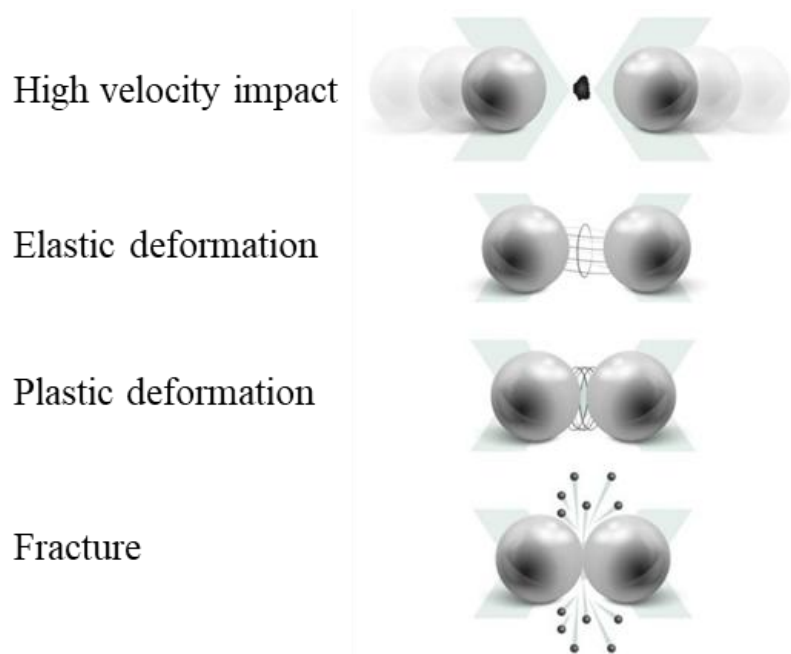


Figure 1.8 Mechanical treatment and the collision of balls when exposed to high energy ball milling.⁸¹

1.5.1 Parameters

1.5.1.1 Energy Input

A high energy input is important to overcome the activation barrier and successfully start a mechanically activated reaction.^{98, 99} The Retsch mixer mill MM400 uses frequency energy inputs most commonly between 15-30 Hz, with the maximum impact hypothesized at 25 Hz.¹⁰⁰ The Retsch planetary ball mill PM100 uses rotations per minute (rpm) with a variable speed between 100-650 rpm that can input sufficient energy into the system.^{19, 84}

1.5.1.2 Balls Used for Milling

The milling vessel and balls can be made of various materials such as stainless steel, hardened steel, zirconium oxide (ZrO₂), silicon nitride, or agate.⁷¹ Stainless steel and ZrO₂ were used in this work. It is known that the hardness and weight of the ball(s) will affect the energy transferred by impacts leading to faster reactions.¹⁰¹ The advantages of using additional balls are now well understood. This can be attributed to the overall increase in mass, which results in stronger and more impactful energy transfers, as well as a larger surface area for impact that affects more particles, rather than simply increasing the number of collisions.^{92, 102} It has also been demonstrated that the use of lighter milling balls reduces the reaction rate. Thus, highlighting again the importance of energy transfer from a single collision rather than the number of collisions.¹⁰² However, the conclusions made regarding the impact of balls on kinetics have to be treated separately from other aspects, as studies show that the use of smaller and more milling balls can lead to yield optimization, for example.¹⁰³ Thus, ball selectivity should depend on the desired outcome.

Interestingly, the sample impacted by the collision between the jar wall and ball or between balls is complete before the collision itself reaches completion.¹⁰² In addition, the diameter of the ball is not seen to have as much of an impact on energy transfer. This shows that although more surfaces would be covered with a larger diameter, and thus, more samples exposed to mechanical action, it is perhaps the longer duration of impacts leading to more intense critical loading conditions (CLCs) that make all the difference.¹⁰¹ CLC is a mechanical term used to describe the generation of sufficient mechanical stress that results in mechanical deformation as a preliminary step for the initiation of phase transformation.¹⁰⁴ Nevertheless, studies have demonstrated that a larger surface area resulting from increased diameters significantly affects phase transformations, as more particles are exposed to mechanical forces.^{100, 105} Additionally, larger balls have greater surface areas, which enhance mixing and

ultimately contribute to improved homogeneity. This improved homogeneity ensures that all particles are better exposed to CLCs and facilitates particle size reduction.¹⁰⁰

1.5.2 Impact of Heat

Mechanical energy can result in two outcomes, work, and heat: The energy is absorbed by the chemical species (work), and the remainder which is not absorbed is released as heat.¹⁰⁶ It is the absorbed energy that is the driving force behind mechano- and tribo-chemistry reactions and transformations. Upon phase transitions, additional energy is released in the system as heat and is thought to have indirect effects on the reaction.⁷⁹ A correlation has been established between the reaction kinetics and the temperature inside the jar. The heat released from the system can raise the final temperature, which in turn enhances friction capabilities by increasing the thermal energy at the contact points between surfaces.¹⁰⁷ While temperature increases are observed during milling, particularly during sigmoidal phases, studies indicate that the changes are subtle and primarily attributed to other factors,⁹¹ such as the energy exerted by the mechanical process, rather than the exothermic reactions occurring within the jar. Experiments using liquid nitrogen have been conducted to remove heat from the system, allowing researchers to investigate the impact of temperature on product formation and reaction kinetics. The results obtained demonstrated that the reaction still occurred and produced the same product, just at a slower rate compared to when heat was present. While product formation does not depend on heat, this indicates the significant role that heat plays in influencing the kinetics of a mechanical process. Specifically, heat affects the rate of reaction, thereby impacting overall kinetics.⁹¹

1.5.3 Impact of Water

Water can be released during milling as a by-product of various chemical reactions, particularly those involving organic materials or hydrated compounds, as well as from moisture present in the starting materials. During the milling process, the heat generated can lead to the evaporation of water, which may then escape from the system. This released water may reduce temperatures by absorbing heat from the reaction or limiting friction during the milling process.^{107, 108} Although released water has been investigated as a potential catalyst, this hypothesis was not substantiated, highlighting that water as a by-product does not cause the sigmoidal kinetic shape observed.⁹¹ Nevertheless, water could also play a role in increasing the reaction rate by aiding in diffusion.¹⁰⁸ Water released as a by-product can act as a lubricant, which helps reduce friction between particles. This lubrication facilitates smoother interactions among particles, allowing them to slide past each other more easily. As a result, it can improve

dielectric permeability and polarizability by creating a more uniform environment for the distribution of electric fields. This enhanced interaction can lead to better mixing and more effective reactions within the milling process. Additionally, the form in which water exists in the system has not been reported to impact the reaction, but rather the reaction is affected by the quantity of water produced or added.⁹² Ultimately, regardless of the minor temperature and friction changes during the ball milling (BMing) process, once the heat of the system remains below the T_g of the chemical species, it will not hinder transformations such as amorphization.¹⁰⁹ Chemical alterations can only occur in the presence of local mechanical stress that is intense enough to overcome CLCs.¹⁰⁴ Temperature and water release do not influence the rate-determining step, which is crucial for the reaction's progress. Instead, the most significant factors are the increased frequency of reactive interactions between particles¹⁰⁸ and the accumulation of energy.⁹²

1.5.4 Product Formation

When multiple phases occur as a result of milling, it is possible for two different phases with optimal orientations to collide with each other and overcome the energy activation (E_a) barrier facilitating the formation of a third phase.¹⁰⁴ However, challenges may arise in accessing untouched parts of the chemical species that did not undergo CLCs or did not have sufficient CLCs to overcome the E_a barrier needed for a successful transformation to transcend.¹¹⁰ Once the product is formed, the harder it becomes for the starting material to be exposed to CLCs to allow for chemical reactions to take place.¹⁰⁴ However, it must be noted that other factors may also contribute to the remaining presence of the starting material and the absence of complete product formation. The important factors for activation and reactivity such as impact, contact, CLCs, energy, reduced particle size, and crystal defects are known. However, the most critical factor for product formation remains unknown.¹⁰⁰ Therefore, the lack of one or more of these factors may contribute to the presence of unconverted drug. As a reaction continues, the speed of formation of the product decreases. This is thought to be related to the increase in product formation, and thus, the reduction in the probability of the starting material to experience the mechanical action and CLCs needed to undergo phase transitions or transformations.¹⁰¹ It is also known that when a reaction reaches completion, a steady state is obtained and the final product will not change as long as the parameters remain the same.⁹² More research on the impact of mechanical action on chemical species should be conducted in order to further understand the kinetics behind product formation.

1.5.5 Reproducibility of Transformations

It is known that mechanical activation can cause chemical species to exist in non-equilibrium states, facilitating the formation of phase transformations.⁸³ and thus, metastable states of existence.¹¹¹ Changes and reactivity of these particles occur due to the direct result of mechanical action which leads to highly excited, short-lived states followed by fast relaxation of particles.¹¹² Although kinetic limitations exist for solid-state reactions and the most stable form may not be produced, the mechanical process will always yield the same result, once the same parameters, material(s), and amounts are used. The process of mechano- or tribo-chemistry is therefore extremely unique, as regardless of the stability of a transformation, it can always be reproduced, and thus, allow the potential discovery of new phases.¹¹³

1.6 Development of Quinolone Drugs

The first quinolone drug, nalidixic acid, was approved in the 1960s for the treatment of urinary tract infections (UTIs) caused by gram-negative bacteria. However, the spectrum of activity was limited and it could not treat the wider variety of infections caused by gram-positive and gram-negative bacteria.¹¹⁴ Furthermore, extreme bacterial resistance developed causing a reduction in the effectiveness of the drug, and thus, new quinolone drugs with improved properties became a much-needed focus for patients' health. Thus, the development of quinolones led to the classification of such drugs into four generations according to their spectrum of activity.¹¹⁵ The first generation of quinolones was active against almost all gram-negative organisms, except for *Pseudomonas* species.¹¹⁶ All further generations are classified as fluoroquinolones, active against a wide range of gram-positive and gram-negative bacteria and commonly used for respiratory and urinary tract infections.

The second generation of quinolones was able to inhibit all gram-negative organisms, including *Pseudomonas* species by the inclusion of a fluorine (F) atom, piperazine ring, and/or cyclopropyl group.¹¹⁷ Developments into the second generation continued and an alkylated piperazine group was added to the structure, making it the first modification to inhibit gram-positive bacteria.¹¹⁸ The third generation was formed upon further functional group additions, making it more active against gram-positive bacteria, including penicillin-sensitive and penicillin-resistant *Streptococcus pneumoniae* and atypical pathogens i.e., neither gram-positive nor gram-negative. Lastly, the fourth generation added an extra nitrogen (N) atom and showed activity against anaerobic organisms^{115, 119} i.e., organisms that do not require oxygen for growth. Fluoroquinolone drugs have been extensively used to treat patients across the globe due to their wide spectrum of activity and greater potency. Fluoroquinolone drugs such as CIP, levofloxacin (LVX), moxifloxacin (MXF), and ofloxacin (OFX) are included in the WHO list of essential medicines.¹²⁰ However, the extensive use of these antibiotics has led to the emergence of bacterial resistance.¹²¹ The mechanism of action of quinolone and fluoroquinolone drugs involves preventing the transcription and replication of DNA by inhibiting the synthesis required to achieve such outcomes.¹²² Furthermore, limitations of poor solubility within the quinolone and fluoroquinolone family exist. Thus, one of the main focuses of this thesis was to solve the issue of poor solubility of the fluoroquinolone drug CIP.

1.6.1 Chosen Fluoroquinolone Drugs

1.6.1.1 Levofloxacin

LVX is a third-generation fluoroquinolone drug (**Figure 1.9**).¹²³ LVX fights against Gram-positive and Gram-negative bacteria, and atypical respiratory pathogens.¹²⁴ It also works against penicillin which is susceptible and resistant to *Streptococcus pneumoniae*.¹²⁴⁻¹²⁶ LVX contains ionizable functional groups. These consist of a carboxylic acid group and a basic piperanyzyl group.^{124, 127} LVX's solubility profile depends on the pH environment. The outcome of LVX solubility studies resulted in solubility values within the range of 32-91 mg/mL when in a pH environment of 1.2-6.8 at body temperature, i.e., 37 °C.¹²⁴ LVX is a BCS class I drug due to its high solubility and permeability properties. LVX also demonstrates rapid dissolution rates.¹²⁴

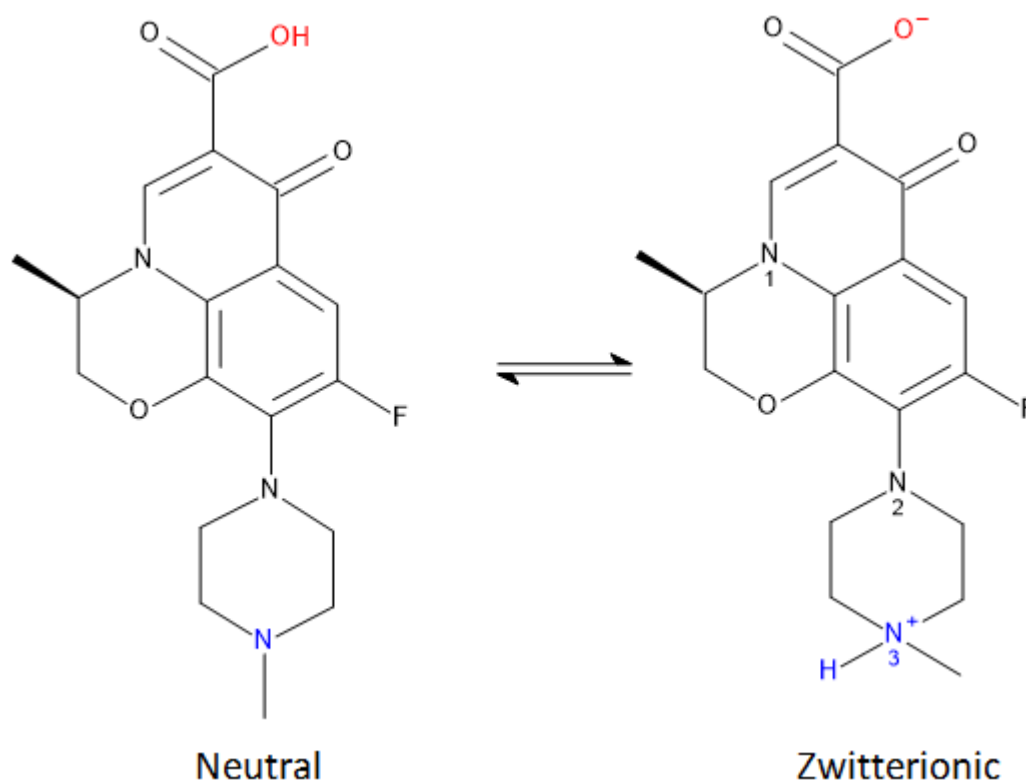


Figure 1.9 Structure of neutral and zwitterionic levofloxacin.

1.6.1.2 Ciprofloxacin

CIP is a second-generation fluoroquinolone antibiotic. CIP is an important drug as it has a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria and is listed by the WHO as an essential medicine.¹²⁸ Furthermore, CIP is a poorly soluble BCS

Class IV drug due to its possession of an overall neutral charge and strong intermolecular interactions, resulting in a solubility of 0.06 and 0.08 mg/mL in water at 25 and 37 °C, respectively.¹²⁹ CIP is poorly soluble in common solvents such as ethanol, methanol, propanol, acetone, and chloroform.¹³⁰ CIP has both an acidic and basic group and can ionize depending on the pH.^{19, 50, 131} The pK_a of the CIP carboxylic acid group is approximately 6.2 and the secondary amine group is approximately 8.6, respectively.¹³² Although CIP is commercially available in the ionized/zwitterionic form due to its improved stability in the solid-state as a result of the intermolecular bonds from the charged groups,¹³³ the drug can exist in equilibrium in the unionized/neutral or zwitterionic form in neutral media (**Figure 1.10**).¹³⁴

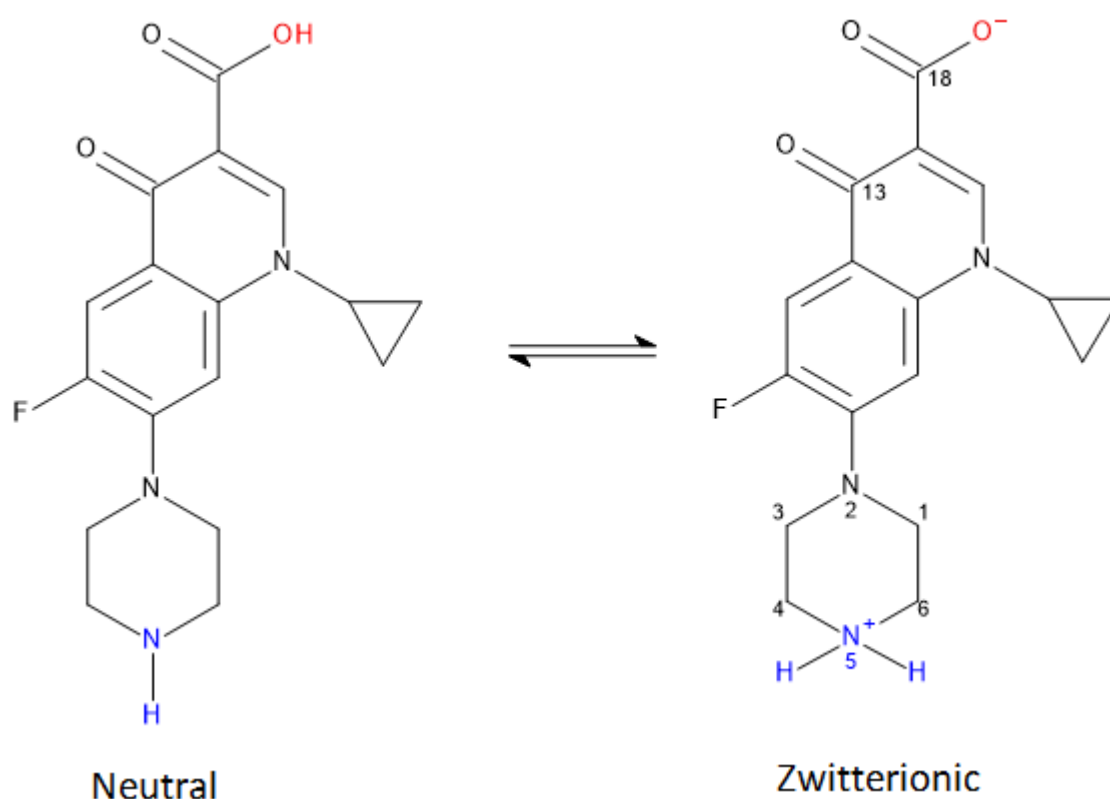


Figure 1.10 Structure of neutral and zwitterionic ciprofloxacin.

1.7 Downstream Processing of Fluoroquinolone Drugs

1.7.1 Milling of Fluoroquinolone Drugs

The milling process was initially designed to enhance homogeneity and reduce the particle size. However, further research has revealed that milling can also allow polymorphic transformation and amorphization to occur when high amounts of energy are inputted into the system.¹³⁵ Research shows that multiple fluoroquinolones have been ball milled (BMed) in order to improve the properties of the drugs such as solubility, dissolution rate, particle size, homogeneity of two cohesive drug powders, and to reduce side effects.^{19, 20, 50, 136-141} In addition, three reports of fluoroquinolone drugs, norfloxacin (NOR),¹⁴² ENRO,¹⁴³ and CIP,¹⁴⁰ have been published in the literature whereby they have been used as model organic pollutants in an effort to reverse the damages caused to natural waters and soils around the world as a result of these pollutants escaping and residing in our environment. Nevertheless, only NOR underwent bioremediation using the BMing process.^{140, 143} NOR, CIP, and ENRO, were investigated in a separate study via BMing, with the aim to further enhance the crystal engineering understanding of fluoroquinolone drugs and their properties for future potential pharmaceutical materials. These fluoroquinolones were mixed with acid cofomers and processed by liquid-assisted grinding (LAG) to produce new molecular salts, ternary molecular ionic cocrystals, viscoelastic materials, and supramolecular gels.¹³⁶

The production of nanocrystals of besifloxacin (BES) was investigated using Povacoat[®] as a stabilizer by the process of wet bead milling. This approach aimed to improve BES's low solubility in water, making it more effective for treating bacterial conjunctivitis, a highly contagious condition often associated with resistant bacteria. The studies demonstrated improved dissolution, stability, and antibacterial efficacy.¹³⁷ In a separate study, dry powder inhalation (DPI) drugs, CIP and colistin, were co-jet milled in an attempt to overcome the challenge of heterogenous, random agglomeration formation as a result of mixing two powders. The results demonstrated the possibility for homogeneity to be achieved with two drugs of comparable fine particle fraction and deposition behavior for inhalation formulations by jet-milling. Agglomeration continues to pose challenges due to the adhesion of small and large particles. Achieving uniformity and minimizing agglomeration between the two powders is an ongoing issue, particularly given the limited control over particle size in processes such as jet milling.¹³⁹

Another study was carried out whereby CIP hydrochloride monohydrate (CIP HClm) was BMed to reduce its particle size before spray drying (SDing), for the generation of antibiotic inhalation powders to treat patients with cystic fibrosis. CIP crystalline phase remained unchanged during the BMing process. LVXh was used in this study, however, the drug was not BMed but rather SDed due to its high solubility. The research demonstrated improvements in the dissolution performance of CIP formulations compared to unprocessed CIP. Additionally, the CIP formulations prepared using the BMing process were absent from solvents, in contrast to LVXh prepared by the SDing method whereby solvents were used and remained in the sample (1.3-3.5%). This demonstrates the importance of eliminating or at least limiting the use of solvents where possible, and the advantages of solvent-free BMing.¹⁴¹ A separate investigation was carried out on gatifloxacin (GAT) whereby the amorphous form was achieved by milling and melt quenching for comparative purposes. The milled amorphous form resulted in a reduced rate of crystal growth and enhanced stability compared to the sample produced by melt quenching.¹³⁸

CIP was BMed and cryomilled by Mesallati *et al.* to investigate the amorphization capabilities of the drug. The intensity of the Bragg peaks in PXRD was reduced after 4 hours of milling, however, further BMing durations did not result in further reductions to the crystalline Bragg peaks. CIP was also cryomilled at 0 °C to prevent any possibility of crystallization occurring by ensuring the Tg of CIP, 80-100 °C, was never reached. The intensity of the Bragg peaks was reduced compared to ball milling at room temperature and partial amorphization was achieved. However, four sharp peaks still remained, indicating the strength of the crystalline lattice.⁵⁰ This research demonstrated the inability to fully amorphize CIP on its own, even when BMed for up to 6 hours at room temperature.⁵⁰ However, GAT was investigated and found to amorphize on its own, after dehydration, when BMed for up to 3 hours using a centrifugal ball mill at 300 rpm.¹³⁸

Mesallati *et al.* BMed CIP with amino acids and successful amorphization was accomplished to produce amorphous material. Furthermore, the Tg was also increased for these formulations when compared to the starting materials and demonstrated promising long-term stability. In addition, the solubility was improved when compared to the physical mixtures. The only observed disadvantage was the potential for some formulations to reduce the permeability when compared to unprocessed CIP.¹⁸

Another study of CIP by Mesallati *et al.* demonstrated improved properties by APS formation using acidic polymers. Solubility enhancements were achieved for all formulations and the permeability did not decrease.¹⁹ as seen in previous studies mentioned.^{18, 19} The stability of formulations was shown to recrystallize after several days of exposure to accelerated conditions, however, amorphization was maintained at 90% relative humidity (RH) at 25 °C. Lastly, antibiotic efficacy was maintained and improvements were observed for several samples in the MIC and MBC studies compared to unprocessed CIP.¹⁹ ENRO and acidic polymers were BMed and the production of ASDs/APSs with higher Tgs, solubility, and dissolution values was achieved.²⁰

1.7.2 Polymer Selection

Polymer selection is a critical factor in the design of optimal ASDs/APSs for physical stability, *in vitro* and *in vivo* performances of a drug. The Tg, molecular weight, and viscosity of polymers are important factors to consider when designing formulations for ASDs as they affect the stability, and *in vitro* performance of the ASDs. Furthermore, the selection of an ionizable polymer is necessary for the formation of APSs due to the facilitation of proton transfer, and thus, improved molecular interactions through ionic bonding, and ultimately, enhanced stability.¹⁹ As previously discussed in **Section 1.3**, it is necessary to choose a polymer(s) with a high Tg to prevent nucleation, crystal growth, and crystallization, and thus, overcome stability issues. Full miscibility and prevention of phase separation are important factors for intermolecular interactions between the API and the polymer(s) and depend heavily on the polymer selection and compatibility with the API.¹⁴⁴

Polymer selection is important for enhancing the dissolution and supersaturation of a drug with the aim of enhancing the absorption of the drug, and thus, the bioavailability. This can be described by the “spring and parachute” effect whereby “spring” represents the fast dissolution that generates the supersaturated drug concentration in the solution, and “parachute” signifies the conservation of supersaturation. Rapid crystallization of the amorphous drug in **Table 1.1** has been widely explored in research for the development of ASD and APS formations.^{19, 20} Hydroxypropylmethylcellulose acetate succinate (HPMCAS) and hypromellose (HPMC) are amongst the selected polymers used for ASDs that are on the market today as FDA approved APIs.¹⁴⁵ Polymers with high degradation temperatures are desirable as they enable the use of a wider range of processing techniques. Permeability studies and *in vivo* studies are important studies to consider for a more accurate indication of enhanced bioavailability.

To summarize, polymer selection is an important factor for optimal performance of an ASD/APS with the aim of improving the solubility and stability of the poorly soluble drug, with the overall aim of enhancing the bioavailability. The main attributes for optimal ASD/APS systems that were focused on for this study include improvement to the solubility of the poorly soluble drug, CIP, the stability of the ASDs/APSs in the solid-state, and the stability of the ASDs/APSs in supersaturated conditions in solution.

1.7.2.1 Cellulosic Polymers

Cellulose polymer occurs naturally from plants and is the most prevalent type of biopolymer in the world. Cellulose derived polymers are modified to improve the solubility in water. The cellulose polymers discussed in this thesis comprise HPMC and HPMCAS (**Table 1.1**). They are amongst the most popular polymers for successful ASDs, followed by polyvinylpyrrolidone (PVP), as seen by the commercial ASD products on the market.¹⁴⁵ HPMC has been reported to exhibit miscibility with a wide range of APIs due to the existence of its hydrophilic hydroxyl groups and lipophilic methoxy groups,¹⁴⁶ and can lead to improved stability.¹⁴⁷ HPMC grades vary by viscosity and molecular weight. Thus, the lowest viscosity and molecular weight were chosen for these studies to improve the performance of ASDs¹⁴⁸ Acetic acid anhydride and succinic acid anhydride esterification aids in the adaption of HPMC into HPMCAS.¹⁴⁹

HPMCAS is a weakly hygroscopic polymer that demonstrates superior physical stability^{146, 150} and inhibition of crystallization during supersaturation compared to ASDs containing polymers such as PVP and polyacrylic acid (PAA) which are more hygroscopic.^{151, 152} HPMCAS is highly effective in inhibiting drug crystallization from supersaturated solutions and is categorized by grades L, M, or H. The different grades contain the same viscosity value, and thus, are distinguished by the percentage of acetyl (COCH_3 : 5-9 (L), 7-11 (M), 10-14 (H) %, respectively) and succinyl ($\text{COCH}_2\text{CH}_2\text{COOH}$: 14-18 (L), 10-14 (M), 4-8 (H) %, respectively) substituents present.¹⁵³ These substituents are recognized for enhancing drug-polymer interactions. The acetate group has been reported to form a hydrogen bond with an amine group, and the succinate group to form molecular interactions with carbonyl and amine groups.¹⁵⁴ Grades L and/or H were selected for the most part of the studies in **Chapters 4, 5, and 6** to represent the lowest and highest percentage of the substituents for comparative purposes.

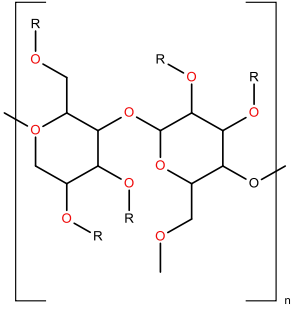
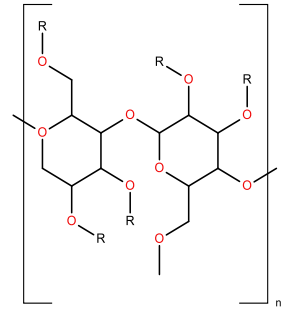
Hypromellose phthalyl (HPMCP) contains a carboxybenzoyl (phthalyl) group. The phthalyl group in its undissociated/unionized form is hydrophobic in nature, and thus, exhibits low solubility at certain pH environments e.g., in water. The phthalyl group is known to ionize in weak acids and neutral medium and therefore becomes water-soluble. Thus, the pH at which HPMCP becomes soluble can be controlled by adjusting the phthalyl content. This polymer can exist as either HP-55 or HP-50 and comprise different pH-solubilities and molecular weights. HP-55 was chosen as it has a higher phthalyl content, and lower molecular weight and viscosity, all of which are desirable properties for the optimal formulation design of ASDs/APSs.¹⁵⁵

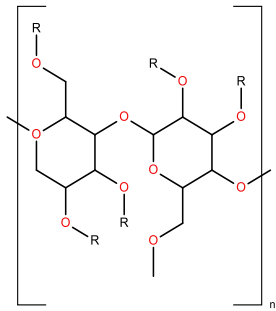
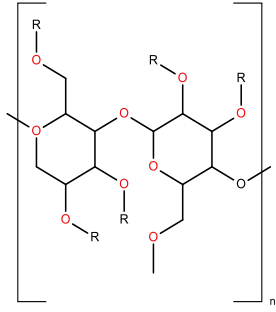
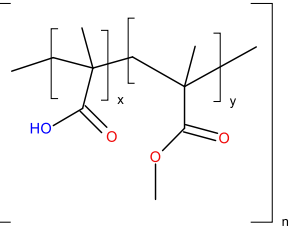
1.7.2.2 Non-Cellulose Derived Polymers

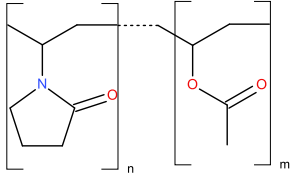
Eudragit® L100 (EL100) and Kollidon® VA64 (KolVA64) are non cellulose derived polymers that were chosen for investigation in this thesis (**Table 1.1**). Eudragit® is a synthetic group of polymers that are available as anionic, cationic, or neutral copolymers that exhibit pH-dependent water solubility. Different varieties of the polymer target different parts of the GIT, such as the small intestine, colon, and large intestine.¹⁵⁶ EL100 is an anionic methacrylic acid and methyl methacrylate 1:1 copolymer that can form hydrogen bonds with APIs^{19, 20, 144} and achieve enhanced physical stability.¹⁴⁴ The hydrogen bond is likely to be between the amine group of the API and the carbonyl group of the polymer.¹⁴⁴

KolVA64 (Copovidone) is a vinylpyrrolidone and vinyl acetate copolymer in a ratio of 6:4 by mass that is soluble in all hydrophilic solvents such as water and alcohols. It is hygroscopic in nature; however, it is less hygroscopic than Kollidon® 30 (povidone) for example. Nevertheless, KolVA64 is often mixed with less hygroscopic polymers such as cellulose derived polymers.¹⁵⁷

Table 1.1 List of polymeric excipients and their important properties for polymer selection.^{145, 149, 156, 158}

Polymer abbreviation	Chemical name	Structure	Tg (°C)	Degradation Temperature (°C)	Average molecular weight (g/mol)	Solubility
HPMC	Cellulose	 R = H, CH₃, CH₂CH(CH₃)OH	126	244	20,000	Soluble in water
HPMCAS-LG	Cellulose, 2-hydroxypropylmethyl ether, acetate hydrogen butanedioate	 R = COCH₃, COCH₂CH₂COOH, H, CH₃, CH₂CH(CH₃)OH, CH₂CH(CH₃)OCOCH₃, CH₂CH(CH₃)OCOCH₂CH₂COOH, H	119	204	55,000–93,000	pH solubility ≥ pH 5.5

HPMCAS-HG	Cellulose, 2-hydroxypropylmethyl ether, acetate hydrogen butanedioate	 R = COCH₃, COCH₂CH₂COOH, H, CH₃, CH₂CH(CH₃)OH, CH₂CH(CH₃)OCOCH₃, CH₂CH(CH₃)OCOCH₂CH₂COO H	122	204	55,000–93,000	pH solubility ≥ pH 6.5
HPMCP-HP55	Cellulose, hydrogen 1,2-benzenedicarboxylate, 2-hydroxypropyl methyl ether	 R = H, CH₃, CH₂CH(CH₃)OH, COC₆H₄COOH	133	194	78,000	pH solubility ≥ pH 5.5
EL100	Poly (methacrylic acid, ethyl acrylate)		130	176	≈ 125,000	pH solubility ≥ pH 6.0

KoIVA64	Acetic acid ethenyl ester, polymer with 1-ethenyl-2-pyrrolidinone		106	292	45,000–70,000	Soluble in water
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1.8 Project Aims

Mechano- and tribo-chemistry is growing at a fast rate, however, more scientific contributions need to be made in order to better understand this type of chemistry and processing so that it can be implemented into the future of pharmaceuticals. This project aims to advance the understanding of the kinetic (**Chapter 2**), mechanistic (**Chapter 3**), and solid-state (**Chapters 2-5**) behavior of BMed fluoroquinolone drug(s) (LVXh and/or CIP), both in the presence and absence of polymer(s), using mechano- and tribo-chemistry techniques. Furthermore, the poor solubility of APIs is a huge challenge that continues to face the pharmaceutical industry today. Amorphization is a known solution to this problem, although it presents its own challenges, such as poor stability. Therefore, binary and ternary amorphous polymeric systems were generated to improve the solubility of CIP and the poor stability of the amorphous form (**Chapter 4 and Chapter 5**). The enhancement of the solubility of CIP, the stability of the systems after solid-state solubility studies, and the antibacterial activity of the systems were investigated (**Chapter 6**).

In **Chapter 2**, the focus was on understanding the mechanisms behind solid-state transformations of LVXh induced by the ball milling process whilst also investigating the feasibility of obtaining an amorphous form of LVX. The spray drying process was used as a comparative mechanical process. An additional aim was to investigate BMing process variables such as process duration, different numbers of balls, and varying the diameter of such balls in the pursuit of obtaining amorphous LVX. This chapter focused on generating an understanding of the interconversion pathway of LVXh to LVXam, as a result of milling LVXh in the solid-state. The objective of **Chapter 3** was to investigate LVXh as an amorphous binary system using EL100 while utilizing the BMing process. Further aims of this chapter were to compare the samples produced using different mills (a Retsch mixer mill MM400, a Retsch planetary ball mill PM100, and a Fritsch vibration mill), to investigate the degree of crystallinity of LVXh, and to perform a scale up reaction. Lastly, this chapter focused on determining the mechanism of formation of LVXh:EL100 binary amorphous system.

The primary aim of **Chapter 4** was to investigate amorphous and semi-crystalline polymeric systems of CIP using acidic and neutral polymers to build on previous discoveries made by Mesallati *et al.*¹⁹ An additional objective of this chapter was to investigate the use of the MM400 and the PM100 mills, and to examine the parameters of the MM400 in the pursuit of generating amorphous polymeric systems with reduced processing times. This chapter

focused on the investigation of drug-polymer interactions with CIP. In addition, this chapter provides the findings necessary to proceed to the formulation of ternary systems of CIP, discussed in **Chapter 5**.

Chapter 5 aimed to investigate ternary amorphous polymeric systems using either two acidic polymers or an acidic and neutral polymer, using the milling process (MM400 and PM100). Another objective of this chapter was to explore the increase or decrease in the degree of crystallinity to the original binary system upon the addition of a third component and/or varying the percentage (w/w) of the second polymer added. A final aim was to effectively rank, in order of superiority, the addition of the polymers and/or API to disclose the optimal addition of components for decreased levels of crystallinity.

The focus of **Chapter 6** was to investigate the solubility of the binary and ternary amorphous polymeric systems compared to CIP using aqueous, FaSSIF, and FeSSIF buffers. An additional aim was to examine whether crystallization occurred in the solid-state after solubility studies of the samples, using PXRD analysis to assess the crystallization. Furthermore, an additional objective of this chapter was to determine the minimal inhibitory concentration (MIC) of the systems compared to CIP for multiple strains of bacteria, including several ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).

Chapter 7 is a general discussion, and conclusions of the research conducted, and the main findings made from each of the research chapters (**Chapters 2-6**). Lastly, this chapter discusses the potential future direction that could be implemented to build on this area of research.

Chapter 2.0 Mechanochemically Induced Solid-State Transformations of Levofloxacin

Chapter 2.0 Mechanochemically Induced Solid-State Transformations of Levofloxacin

2.1 Introduction

Mechanochemistry is the area of science that addresses the chemical reactions initiated and driven by the application of mechanical forces to chemical systems involving at least one solid phase.^{66, 77, 111} Mechanochemistry has been utilized since the early 19th century.¹⁵⁹⁻¹⁶² However, it was not until the 1960s that the processing of powders gained much traction, leading to further discoveries of mechanically activated solids resulting in amorphous and nanostructured phases in the 1980s.^{66, 69, 163-166} More recently, the well-known ability of mechanical forces to induce transformations in solids has attracted renewed attention from wide sectors of inorganic and organic synthetic chemistry, becoming the most rapidly growing area of chemical sciences.^{167, 168} Thus, significant emphasis has been placed on the mechanochemical synthesis of fine chemicals and pharmaceuticals.¹⁶⁹⁻¹⁷²

Solid-state transformations of APIs have been reported upon milling as a form of mechanical treatment. This was the case for sulfathiazole whereby polymorphism was detected as a direct result of mechanical activation by the process of milling.¹⁷³ Sulfathiazole polymorphic forms I and III also demonstrated the capability to further transform to the amorphous phase upon further processing. Following recrystallization, depending on the polymorph, the return to either the pure polymorphic form or a mixture of polymorphs was plausible.¹⁷⁴ Further examples of pharmaceutical materials exhibiting polymorphic transformations on mechanical treatment include chloramphenicol palmitate,¹⁷⁵ mannitol,¹⁷⁶ and sorbitol.¹⁷⁷

Fluoroquinolone drugs are broad-spectrum antimicrobial agents, active against gram-positive and negative bacteria.¹⁷⁸ Many fluoroquinolone drugs are included on the WHO list of essential medicines. For oral solid dosage forms, CIP is listed as a watch group antibiotic due to its high probability of developing bacterial resistance.¹²⁰ Additionally, it is classified as one of the most critically important antimicrobials for human medicine.¹⁷⁹ MXF and LVX are listed essential antituberculosis medicines. For solutions, OFX is listed as part of the essential ophthalmological preparations for anti-infective agents. In addition, OFX and CIP are listed as essential ear, nose, and throat medicines.¹²⁰

Research shows that multiple fluoroquinolones have been ball BMed in order to improve properties of the drugs such as solubility, and dissolution rates, reduce particle size, enhance crystal engineering,¹⁸⁰ and detect new polymorphic forms.¹³⁷ Examples include CIP^{18, 19} and ENRO,²⁰ which were BMed with amino acids and acidic polymers to produce amorphous binary and ternary systems for solubility^{19, 20} and dissolution enhancement.²⁰ NOR, CIP, and ENRO were investigated via BMing to enhance the crystal engineering understanding of fluoroquinolone drugs. These fluoroquinolones were mixed with acid coformers and prepared by LAG to produce new molecular salts, ternary molecular ionic cocrystals, viscoelastic materials, and supramolecular gels.¹⁸⁰ BES was BMed to increase its low solubility in water to improve the treatment of bacterial conjunctivitis. Nanocrystals were produced via wet bead milling to enhance dissolution, with the promising potential of improving bioavailability. Another investigation was carried out on GAT whereby the amorphous form was achieved by milling and melt quenching to better understand the characteristics of various amorphous forms of GAT with multiple hydrate and anhydrous polymorphs being reported in their crystalline form.¹³⁷ Also, CIP hydrochloride monohydrate was BMed to reduce its particle size before spray drying to produce antibiotic inhalation powders.¹⁴¹

SDing is another method, which can be employed to alter the physicochemical properties of drugs. This method relies on the evaporative removal of solvent from droplets produced by atomization of a liquid feed comprising the API. The first drugs that were processed by SDing were phenylbutazone¹⁸¹ and thiazide diuretics.¹⁸² The antibiotic aztreonam was spray dried (SDed) without the use of an excipient to simplify the SDing process and to reduce side effects caused by excipient addition.¹⁸³ However, excipients are known to improve the properties of micronized APIs such as solubility,¹⁸⁴ flowability¹⁸⁵ surface roughness, and particle dispersibility.¹⁸³

LVX (**Figure 1.9**) was chosen for this research as it is a more complex fluoroquinolone drug in comparison to CIP and ENRO in terms of its solid-state forms. This complexity can be explained by the ability of LVX to exist in many different crystal forms.¹⁸⁶⁻¹⁹⁰ LVX can crystallize into two hydrate forms i.e. hemihydrate and monohydrate.¹⁸⁹ Five water-free forms identified as amorphous, gamma (γ), delta (δ), beta (β), and alpha (α) have been identified through the dehydration and heating of the known hydrate form, LVXh, in its solid phase.¹⁸⁶⁻¹⁹⁰ Moreover, levofloxacin monohydrate (LVXm) has only been reported to form anhydrous α following heated conditions in solution.¹⁸⁶⁻¹⁹⁰ Nevertheless, the crystal structure of the β and δ

forms remain unidentified, while the structures of the hemihydrate, monohydrate, γ ,¹⁸⁷ and α ¹⁸⁸ forms are known.¹⁸⁷ The interconversion pathways of the various forms of LVX can be observed in **Figure 2.1**.¹⁸⁶⁻¹⁹⁰

The first detection of the presence of anhydrous forms was reported after DSC analysis of LVXh whereby a broad endotherm between 20-70 °C indicated the loss of water, followed by the melting of anhydrous phases γ , β , α at temperatures 225.4-234.1 °C.^{186, 189} However, the further detection of the water-free crystal structures was achieved through high-temperature PXRD analysis of anhydrous/dehydrate LVX gamma (LVX γ) (150 °C) from LVXh, and anhydrous/dehydrate LVX alpha (LVX α) (190 °C) from LVXm,¹⁸⁷ or by desolvation of LVXh to form LVX γ and LVX α .¹⁸⁸ The detection of partial amorphization from LVXh was hypothesized to be a result of dehydration and was only detected via DSC analysis.¹⁸⁹ LVX is administered orally in its hemihydrate form (LVXh), due to its superior stability.¹⁸⁸ The anhydrous/dehydrated forms associated with LVXh and LVXm are unstable at room temperature, thus their presence may adversely impact the stability of LVX powder.

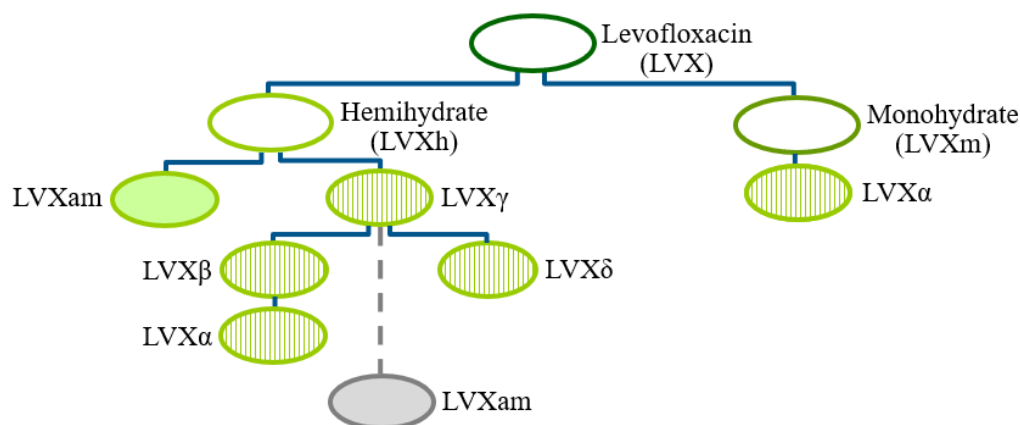


Figure 2.1 Interconversion pathways of LVX to hydrates (open symbol), amorphous (filled symbol), and anhydrous/dehydrated forms (striped symbol).^{186, 189} The broken line and grey symbol indicate a new pathway to amorphous LVX discovered in this work.

In this work, the utmost focus is on understanding the mechanisms behind solid-state transformations induced by a mechanochemical process as well as spray drying due to the possibility of solid conversions considering the ubiquity of these secondary pharmaceutical processes. The feasibility of obtaining an amorphous form of LVX was investigated using both processes. However, a more in-depth investigation was performed for BMed samples to gain insights into the influence of BMed duration and the impact of utilizing different numbers of balls and varying the diameter of such balls. Thus, process variables were examined in the

pursuit of obtaining amorphous LVX. The processed forms were characterized for their solid-state properties using thermal analysis and powder X-ray diffraction, and the kinetics of the transformations were also determined. The physical stability of the systems was established. This study is novel as LVX has never been BMed before, nor has it been SDed without the use of an excipient to successfully achieve amorphization. Furthermore, this chapter will discuss the new discoveries made regarding the interconversion pathways of LVXh, LVX γ , and LVXam, as a result of milling LVXh rather than using the previous dehydration and desolvation methods for discovering solid-state forms.

2.2 Experimental Section

2.2.1 Materials

LVXh was obtained from Merck (UK). Deionized water (14.0 M Ω ·cm) prepared using an Elix 3 connected to a Synergy UV system (Millipore, UK) was used in all experiments. Potassium bromide FTIR grade, $\geq 99\%$ trace metals basis, was purchased from Merck (UK).

2.2.2 Methods

2.2.2.1 Milling using Retsch Planetary Ball Mill PM100

LVXh was BMed using a Retsch planetary ball mill PM 100 (Germany) at 400 rpm. The samples were milled for a total duration of 4 hours at timepoint measurements taken every 1, 3, 5, 7, 9, and 10 minutes, and again at 0.5, 1, 2, 3, and 4 hours. 50 mL stainless steel grinding jar was used, and 2 g of sample was added to the jar. Three stainless steel milling balls with a diameter of 1.5 cm and weight of 14 g each (**Method 1**) and eight balls with a diameter of 1.2 cm and weight of 7 g each were used for comparison purposes. Ball milling was conducted at room temperature for a maximum of 30 minutes at a time. A 15 minute break was implemented after every 30 minutes of milling. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if necessary. Approximately 2 mg of sample were removed for analysis via PXRD (**Section 2.2.2.3**) at each timepoint required for the Rietveld analysis. Around 15 mg of the sample was removed for DSC analysis (**Section 2.2.2.4**) after 0.5, 1, 2, 3, and 4 hours of ball milling. Approximately 5 mg of the sample was removed for FTIR analysis (**Section 2.2.2.7**) and 15 mg for DVS analysis (**Section 2.2.2.8**) after 4 hours of ball milling.

2.2.2.2 Milling using Retsch Mixer Mill MM400

LVXh was BMed using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s). A 50 mL stainless steel grinding jar was used. A sample of 2 g and three stainless steel milling balls with a diameter of 1.5 cm and weight of 14 g each were added to jar. This condition will be referred to as **MM400_Cond1**. Ball milling was conducted at room temperature with maximum intervals of 99 minutes. A 15 minute break was implemented after every 99 minutes of milling. The samples were milled for a total duration of 4 hours at timepoint measurements taken every minute from 1 to 10 minutes, and again at 0.5, 1, 2, 3, and 4 hours. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if

necessary. Around 2 mg of sample were removed for analysis via PXRD (**Section 2.2.2.3**) at each timepoint required for the Rietveld analysis. Around 15 mg of the sample was removed for DSC analysis (**Section 2.2.2.4**) at timepoints 0.5, 1, 2, 3, and 4 hours of ball milling.

A sample of 2 g and five stainless steel milling balls with a diameter of 1.2 cm and weight of 7 g each were added to the jar. Ball milling was conducted at room temperature with maximum intervals of 99 minutes. A 15 minute break was implemented after every 99 minutes of milling. The samples were milled for a total duration of 2 hours at timepoints taken at 3 minutes, 0.5, 1, and 2 hours. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if necessary. Around 2 mg of sample were removed for analysis via PXRD (**Section 2.2.2.3**) at each timepoint for the Rietveld analysis.

LVXh was BMed using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s25 mL ZrO₂ jar was used, and 0.08 g of sample was added to jar. One ZrO₂ ball of 1.2 cm and a weight of 5 g was used. This condition will be referred to as **MM400_Cond2**. Ball milling was conducted at room temperature with maximum intervals of 99 minutes. A 15 minute break was implemented after every 99 minutes of milling. The sample was milled for a total duration of 4 hours at timepoint measurements taken every minute from 1 to 10 minutes, and again at 12, 15, 20, 30, 50, 60, 70, 90, and 2, 3, and 4 hours. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if necessary. Around 2 mg of the sample was removed for analysis via PXRD (**Section 2.2.2.3**) at each timepoint for the Rietveld analysis. Around 15 mg of the sample was removed for analysis via DSC (**Section 2.2.2.4**) at timepoints 0.5, 1, 2, 3, and 4 hours of ball milling. An additional 15 mg of sample was removed for analysis via DVS (**Section 2.2.2.8**) after ball milling for a total of 4 hours.

2.2.2.2 Spray drying

SDing was performed with Prof. Lidia Tajber (Trinity College Dublin, Ireland). A Büchi B-290 mini spray dryer (Switzerland) equipped with a nozzle fitted with a 1.5 mm cap and 0.7 mm tip was selected to spray dry LVX. The conditions of the pump speed were 20% (7.5 mL/min) and the aspirator 90%. Nitrogen (with a pressure of 7 bar) and air were mixed to form the desired drying gas. Water was chosen as a solvent to produce pure SDed LVX. An excess of LVXh was added to 1 L of water and stirred overnight at 200 rpm using a Stuart (UK) hotplate

stirrer SB162. The next day, the liquid was filtered under vacuum to remove any undissolved LVX. The filtered solution was then SDed using an inlet temperature of 120 °C.

2.2.2.3 Powder X-Ray Diffraction (PXRD)

PXRD was implemented at room temperature using a benchtop Rigaku MiniflexII X-ray diffractometer (Japan) and a Haskris cooler (USA). The samples were scanned from 5 to 40° 2 θ . The settings were as follows, step width 0.05, scan rate 0.05° per second, and 1 second signal collection time per step. The tube (Cu, 1 kW normal focus) output voltage and current operated at 30 kV and 15 mA, respectively.

2.2.2.4 Differential Scanning Calorimetry (DSC)

DSC was executed using a Mettler Toledo DSC (Switzerland), which was attached to a RP-100 LabPlant refrigerated cooling system (UK). The equipment was calibrated using an indium standard. Nitrogen was used as the purge gas. Approximately 3 to 5 mg samples were analyzed. 40 μ L sealed aluminum pans were used and the lids were manually pierced with three holes. Samples were heated from 25 °C to 250 °C at 10 °C/min.

2.2.2.5 Modulated Temperature Differential Scanning Calorimetry (MTDSC)

DSC was carried out using a Q200 Differential Scanning Calorimeter (TA Instruments, UK). Nitrogen was used as the purge gas (50 mL/min). Approximately 1 to 2 mg samples were analyzed. Sealed aluminum hermetic pans were used, and lids were manually pierced with three holes. The samples were equilibrated at -40 °C and the temperature modulated by $\pm$ 0.80 °C every 60 seconds, followed by an isothermal hold of 2 minutes. Samples were ramped at 5 °C/min to 200 °C. Results were analyzed using the TA Universal Analysis software (TA Instruments, UK).

2.2.2.6 Rietveld Refinement

The Rietveld refinement was performed, and data was analyzed primarily by Dr. Maria Carta (University of Cagliari, Italy) with involvement from Dr. Giulio Lampronti (University of Cambridge, United Kingdom) and Prof. Fancesco Delogu (University of Cagliari, Italy). The Rietveld refinement on the samples collected as described above was used to analyze PXRD patterns quantitatively. The method uses a set of mathematical functions to describe parametrically the PXRD patterns of pure crystalline phases, or their mixtures, based on their crystalline structure and microstructure while accounting for specimen and instrumental factors.^{191, 192} For pure crystalline substances, peak intensity, width, and position provide insight into structural and microstructural features. In the case of mixtures, the Rietveld refinement

enables the quantification of the weight fraction of each crystalline phase by dividing the area under the XRD profile of each crystalline phase by the total area under the overall XRD profile. In the presence of amorphous phases, the Rietveld method can be still used provided that the specific contribution to the diffracted intensity of the amorphous is suitably taken into account.¹⁹³

The Rietveld analysis was performed using TOPAS 6 software. Initially, Crystallographic Information Files (CIFs) were downloaded from the Cambridge Structural Database (CSD) and used to generate an initial function aimed at describing the experimental data. The obtained function was then matched to the experimental data by refining parameters such as crystallite size peak intensity and background shape. Once a satisfactory match was obtained, the relative amount of crystalline and amorphous phases was estimated.

2.2.2.7 Solid-State Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted using a Spectrum One FTIR spectrometer (PerkinElmer, USA) supplied with Spectrum Software version 6.1. The parameters selected were a spectral range of 450–4000 cm^{-1} , resolution of 4 cm^{-1} , scan number of 10, and scan speed of 0.2 cm/s . KBr disks were created by direct compression using a manual hydraulic press (P&T Precision Hydraulics Ltd, Ireland). Unprocessed LVXh, BMed LVXh, and LVXam were first mixed with dried KBr using a spatula in a ratio of 1:30 for LVXh and 1:167 for LVXam. A pressure of approximately 10 bar for 1 minute was applied to the sample to form the KBr discs. Deconvolution of the carbonyl region of the spectra (1800–1650 cm^{-1}) was conducted to separate overlapping bands. Following subtraction of the baseline, Lorentz peak fitting was carried out on the spectra using Origin 2018 software.

2.2.2.8 Dynamic Vapor Sorption (DVS)

DVS was used to investigate 4 hour BMed sample of LVXh and SDed LVXam by use of an Advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., UK). The temperature of analysis was set to 25.0 ± 0.1 °C. An amount of approximately 15 mg of sample was inserted into the stainless steel mesh sample carrier and positioned in the instrument. The sample was equilibrated at 0% RH, and a continual mass was attained ($\text{dm/dt} \leq 0.002$ mg/min). The reference mass was documented, and the sorption (0-90% RH) and desorption (90-0% RH) program was applied in intervals of 10% RH. At each interval, the sample mass was equilibrated ($\text{dm/dt} \leq 0.002$ mg/min for approximately 10 min) or if the maximum equilibration time was exceeded before the RH was altered. The maximum

equilibration times were either 5 hour (**Method 1**), 8 hour (**Method 2**), or 12 hour (**Method 3**) for the SDed sample, and 5 hour (**Method 1**) for BMed powders. An isotherm was constructed from the complete sorption and desorption profile. The samples were analyzed via PXRD at the end of the sorption-desorption cycle.

2.3 Results and Discussion

2.3.1 Ball Milling of Levofloxacin

The mechanical processing of solids by the action of ball milling relies on the mechanical contact from the milling tools. The mechanical energy facilitates the collision of surfaces and unconstrained dynamic compaction. Individual powder particles experience a mix of compression and shear forces, which rely heavily on the compaction rate. In turn, this depends on the mechanical action of the ball mill, such that the movement of the jar plays a major role, i.e., oscillatory or vibrational. The Retsch planetary ball mill PM100 and the Retsch mixer mill MM400 can be expected to give rise to different milling dynamics. In the case of the PM100, shear/frictional processes govern the mechanochemical outcome.⁷⁹ This tends to result in a lower rate of mechanical energy transfer to the powders, and thus, a lower milling intensity, ultimately resulting in an increase in process duration. For the MM400, milling dynamics are usually dominated by impact, facilitating higher milling intensities, and thus, reduced processing durations.^{79, 194} Both milling methods were used and compared in this work.

2.3.2 Powder X-Ray Diffraction

Initially, the processing of LVXh by a Retsch planetary ball mill PM100 was attempted. Unprocessed LVXh depicts sharp Bragg peaks, confirming the crystalline nature of the material (**Figure 2.2**).¹⁹⁵ The most intense LVXh peak is situated at $9.8^\circ 2\theta$ (peak 2). However, once LVXh was BMed for 0.5 hours or more, the most intense peaks are visible at positions $6.7^\circ 2\theta$ and $26.5^\circ 2\theta$ (peaks 1 and 6). After 4 hours of BMing, the peak at approx. $6.7^\circ 2\theta$ (peak 1) splits into two peaks. This second peak is evident throughout the ball milling process; however, it only becomes split after 4 hours of BMing. Peaks marked as 2, 4, 5, and 6 are similar to their original starting peaks when compared to unprocessed LVXh. The result of BMing LVXh and exposing the molecule to friction, stress, and strain may result in an alteration of the crystal structure of LVXh, visible as slight alterations of the diffractograms. **Figure 2.2** illustrates the reduction of Bragg peak intensity alongside peak broadening, which increased with the duration of BMing. This may indicate crystallite size reduction and the formation of crystallite lattice microstrains.^{177, 195} It is evident that despite BMing for 4 hours, complete abolishment of the diffraction peaks cannot be achieved.

A comparison of different ball quantities and diameters was performed. A slight reduction in intensities of Bragg peaks was observed for the powder milled using three 1.5 cm balls compared to that processed using eight 1.2 cm balls (**Figure 2.2**). This observation may

be a result of greater void volume within the jar facilitating efficient movement of the balls. In contrast to the latter, the reduction of void volume may have an impact on the mixing ability, which could in turn limit the production of friction and shear forces, and ultimately, lead to more intense crystalline Bragg peaks.

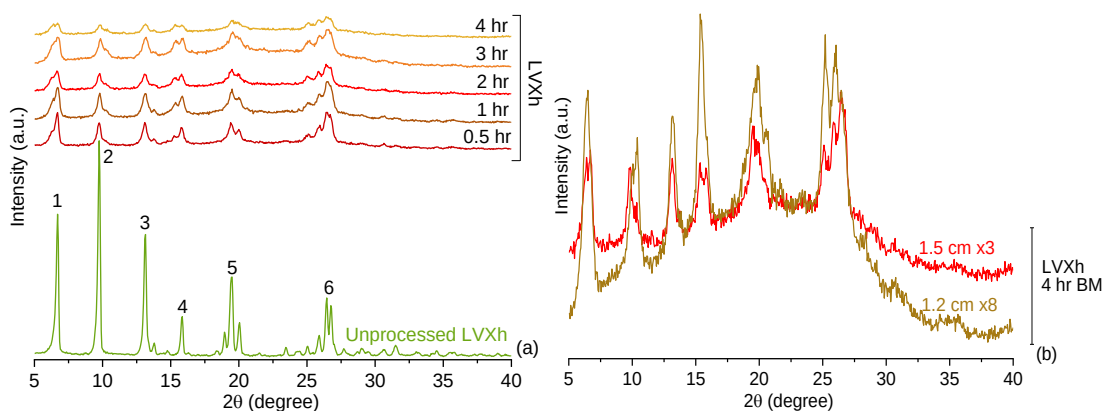


Figure 2.2 PXRD analysis of powders subjected to milling using the Retsch planetary ball mill PM100 and 50 mL stainless steel jar of (a) unprocessed LVXh and LVXh subject to milling between 0-4 hours using three 1.5 cm balls and (b) LVXh subject to milling for 4 hours using eight 1.2 cm and LVXh subject to milling for 4 hours using three 1.5 cm balls at 400 rpm.

Subsequently, two conditions using a Retsch mixer mill MM400 were evaluated: MM400_Cond1 and MM400_Cond2 (**Section 2.2.2.1.2**). **Figure 2.3** shows that the Bragg peaks were still present in the materials following BMing. Similarly, the pattern observed in **Figure 2.2**, peaks 1 and 6 remained the most prominent peaks after 0.5 hours or more of processing using the MM400_Cond_1. In the case of the MM400_Cond_2, peak 1 remained the most prominent, while peak 6 showed a slight reduction. Variation in peak splitting is shown in both **Figure 2.2** and **Figure 2.3**, and slight distinctions are observable between the three conditions i.e., PM100, MM400_Cond1, and MM400_Cond2. This may be due to the contrasting mechanical action of the two mills which produce different primary forces.

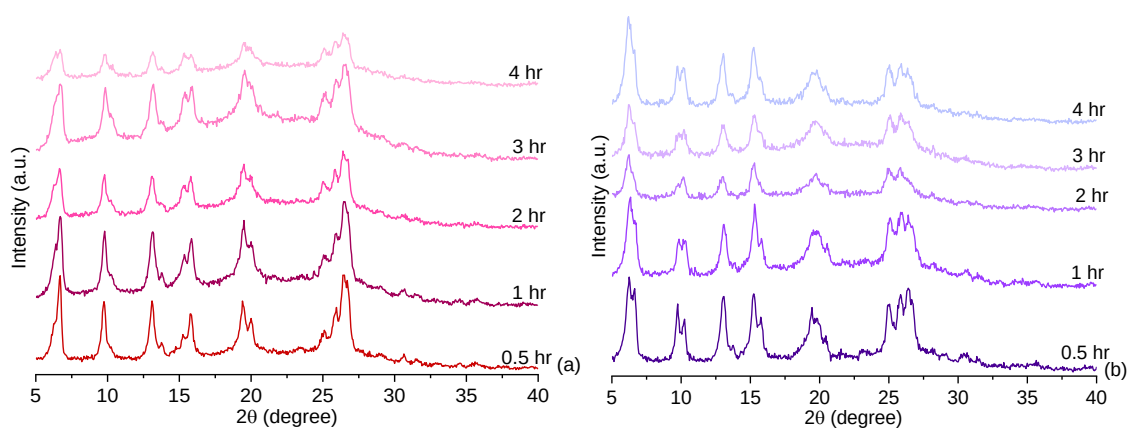


Figure 2.3 PXRD analysis of LVXh subject to milling between 0.5-4 hours at 25 Hz using the Retsch mixer mill MM400 (a) 50 mL stainless steel jar and three 1.5 cm balls (MM400_Cond_1) and (b) 25 mL ZrO₂ jar and one 1.2 cm ball (MM400_Cond_2).

2.3.3 Thermal Analysis

DSC analysis of unprocessed LVXh and processed LVXh subject to milling using the PM100, the MM400_Cond_1, and the MM400_Cond_2 was examined (**Figure 2.4**). Thermograms of the PM100 processed samples displayed changes with respect to the dehydration as well as the melting peaks. The DSC data for unprocessed LVXh depicts a dehydration peak at 74.8 °C. When LVXh was BMed for 0.5-4 hours the dehydration peak slightly shifted to a lower temperature. After 0.5 hours of BMing the peak temperature decreased to 73.5 °C, while after 4 hours of milling, it was at 70.3 °C, thus continually decreasing as BMing continued. This may indicate the disruption of the crystal lattice, such that the crystalline water can be liberated. This is in alignment with research, which suggests that water molecules can be released without destroying the crystal lattice by breakage of the hydrogen bonds that connect the water molecules to the piperazine rings.¹⁸⁶ Thermograms of the MM400 Cond_1 processed samples display divergence from not only unprocessed LVXh but also from that of the PM100 and the MM400 Cond_2 concerning the dehydration peak which demonstrated incomplete removal of crystalline water. Interestingly, after BMing for 0.5 hours, the temperature increased from 74.8 to 81.7 °C suggesting stabilization of anhydrous/dehydrate, likely from the remaining H₂O known to facilitate hydrogen bonding.¹⁸⁶ Nevertheless, upon further processing, the temperature continually decreased, however, it remained above the starting temperature of 74.8, until a processing time of 3 hours, where it decreased to 72.3 °C. Despite the reduction in temperature, further processing to 4 hours led to a temperature rise of 76.7 °C, surpassing yet again, the temperature of unprocessed LVXh. The reduction and increase of the dehydration peak temperature may suggest the removal and reentry of crystalline water. The dehydration

peaks of processed samples for thermograms of the MM400 Cond_2 display similarities to that of a PM100 as a result of their consistent and continual decrease in peak temperature from 74.0 to 65.9 °C for 0.5 and 4 hours of processing. As mentioned previously, this reduction in temperature may indicate the release of crystalline water.

The exotherm peaks were examined for all methods upon processing of LVXh. Unprocessed LVXh powder did not display an exotherm after the dehydration peak, however, following BMing, an exothermic peak of crystallization appeared for almost all samples, this may indicate amorphous phase formation. It is evident that the exothermic peaks of processed PM100 samples slightly increased in temperature from 92.4 to 94.7 °C as the BMing duration increased from 0.5 to 4 hours of milling, respectively. This may suggest a subtle increase in stability of the proposed amorphous form upon further BMing. Similar to the PM100 samples, the MM400 Cond_1 processed sample of ≥ 1 hour displayed an exothermic peak of crystallization as a direct result of sample processing. In comparison, the temperature increased from 92.2 to 93.8 °C for 1 and 4 hours of milling. Lastly, the MM400 Cond_2 showed the appearance of an exothermic peak of crystallization for all samples. However, the exotherm remained quite consistent and only demonstrated a minute increase in temperature, from 94.4 to 94.7 °C.

Unprocessed LVXh undergoes melting/decomposition at onset values of 221.6 and 231.9 °C, which were previously assigned to the melting of LVX γ and LVX β .^{186, 189} Processed LVXh by the PM100, the MM400 Cond_1, and the MM400 Cond_2 experienced melting/decomposition between approximately 221-240 °C, in addition to altered thermal events unique to processed LVXh.

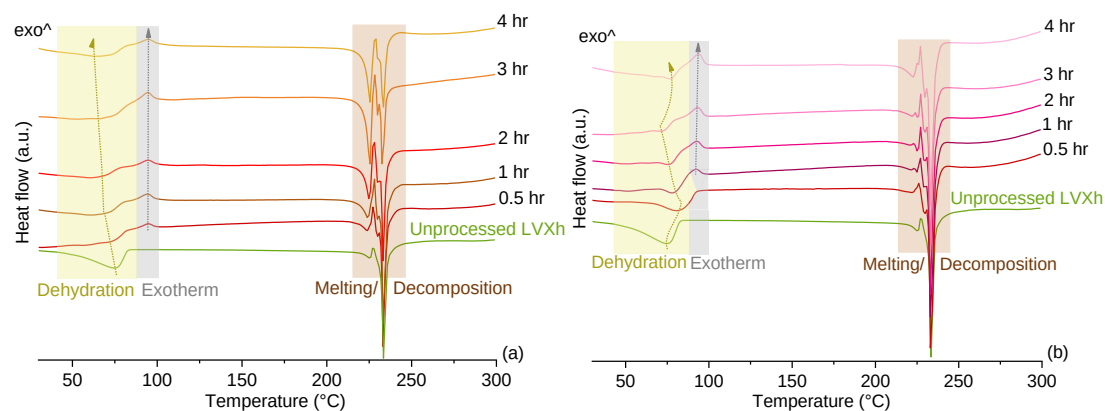


Figure 2.4 DSC analysis of unprocessed LVXh and LVXh subject to milling between 0.5-4 hours using a (a) PM100, 50 mL stainless steel jar and three 1.5 cm balls at 400 rpm (b) MM400, 50 mL stainless steel jar and three 1.5 cm balls (MM400_Cond_1) (c) MM400, 25 mL ZrO₂ jar and one 1.2 cm ball (MM400_Cond_2).

2.3.4 Generation of Amorphous LVX by Spray Drying

To date, fluoroquinolone drugs have never been SDed without the use of an excipient, except for CIP. CIP was previously SDed from a saturated solution made in water, which resulted in an amorphous powder. Unfortunately, CIP is practically insoluble in water, thus the drug concentration in the solution was very low and the process was deemed inefficient. The same group attempted spray drying of CIP from ethanol/water 9:1 (v/v), but the powder exhibited a small degree of crystallinity.⁵⁰

LVX has never been SDed on its own to produce the amorphous form, until now. Previous studies showed that LVX has been SDed to achieve complete or almost complete amorphization using metal chlorides,¹⁹⁶ leucine,¹⁹⁷ N-acetylcysteine,¹⁹⁸ lysozyme,¹⁹⁹ and a proteolytic enzyme.²⁰⁰ LVX was SDed from a solution made in water and this led to the formation of LVX that was completely X-ray amorphous. This is evident by the absence of sharp Bragg peaks in the PXRD, and instead, the formation of a halo (**Figure 2.5**). It is therefore clear that a pure amorphous phase of LVX can be obtained using a bottom-up approach, but not through the top-downmilling method. This behavior is similar to that of CIP, where an amorphous phase was only obtained by SDing, but not BMing.⁵⁰

Thermal analysis of LVXam showed that its Tg was around 80 °C and the Tg event was followed by two exothermic events, most likely of crystallization, with onset values of 102.9 °C and 106.2 °C (**Figure 2.5**). The instantaneous crystallization following the Tg represents nucleation and crystal growth and may indicate a lack of stability.²⁰¹ This is similar to amorphous CIP, which demonstrated a crystallization peak at 121°C, following the Tg at 86.7 °C.⁵⁰ The Tg observed for LVXam is of a lower temperature than that of CIP suggesting that the amorphous phase of LVX might be less stable than that of CIP.

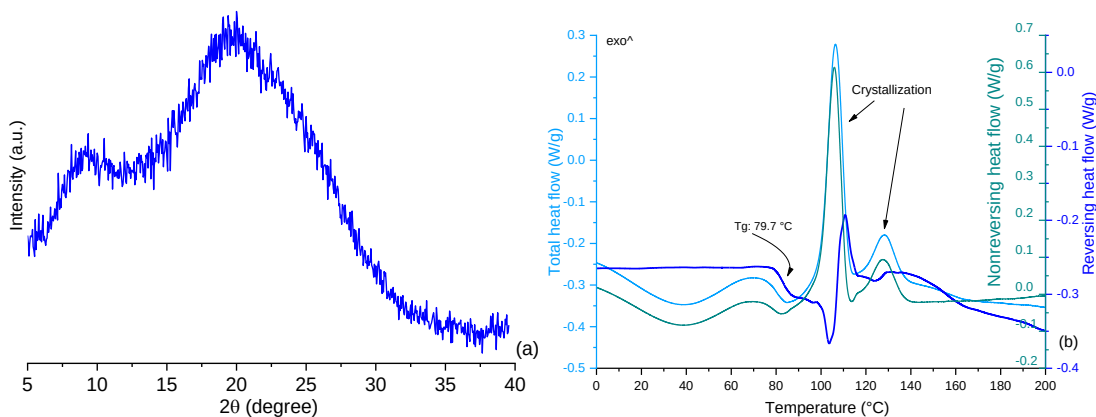


Figure 2.5 (a) PXRD analysis of spray dried LVXam and (b) MTDSC analysis of spray dried LVXam.

2.3.5 Infrared Analysis

IR analysis was conducted for unprocessed and processed LVX (**Figure 2.6**). Unprocessed LVXh depicts an OH bonding band at 3257 cm^{-1} . BMed LVXh prompts slight OH stretching at 3265 cm^{-1} and a slightly more pronounced peak.²⁰² These changes could be due to H bonding interactions with OH. Moreover, LVXh SDed to produce LVXam not only engages in the stretching of OH but participates in broadening and shifting to 3459 cm^{-1} as a likely result of H-bonding interactions with OH.²⁰³ This broadening stretches from $3280\text{--}3649\text{ cm}^{-1}$ as opposed to the usual OH band of unprocessed LVXh stretching from $3169\text{--}3325\text{ cm}^{-1}$ and BMed LVXh stretching from $3171\text{--}3353\text{ cm}^{-1}$. Unprocessed LVXh and BMed LVXh exhibit stretching of CH_3 at 2938 and 2975 cm^{-1} ,²⁰² with a subtle shift to 2935 and 2978 cm^{-1} for LVXam. Unprocessed LVXh carbonyl $\text{C}=\text{O}$ in COOH was observed at 1724 cm^{-1} .²⁰⁴ BMed LVXh and LVXam carbonyl $\text{C}=\text{O}$ in COOH were detected at 1727 cm^{-1} . Unprocessed LVXh, BMed LVXh, and LVXam cyclic ketone $\text{C}=\text{O}$ group appear at 1623 cm^{-1} .²⁰⁵ The absence of peak shifting and/or broadening confirms that the carbonyl on the cyclic ketone $\text{C}=\text{O}$ group is not involved in any H bonding interactions. The $\text{C}=\text{C}$ stretching bands for unprocessed LVXh, BMed LVXh, and LVXam remain between $1413\text{--}1545\text{ cm}^{-1}$, with no signs of shifting, suggesting the absence of additional H bonding interactions. However, slight changes are depicted for SDed LVXam when compared to LVXh BMed and unprocessed LVXh whereby the disappearance of a small peak at 1492 cm^{-1} is evident, which may indicate LVXam intermolecular bonding in contrast to crystalline LVXh. Unprocessed LVXh and BMed LVXh display C-N bond at 1092 cm^{-1} .²⁰² In contrast, SDed LVXam shows the appearance of C-N stretching at 1095 cm^{-1} . In summary, the hydroxyl group OH on the carboxylic acid group underwent the most drastic shift and broadening of all reported LVXh groups and bonds. The observations made are consistent with diffractogram and thermogram findings in that the confirmation of partial or complete disruption of LVXh crystal lattice was evident.

Since slight shifts were observed for the $\text{C}=\text{O}$ group of the carboxylic moiety, a more detailed investigation was conducted by applying a Lorentz function peak split to separate the overlapping signals of the bands. The data showed that the aggregate carbonyl stretching band is composed of two bands, at approximately 1725 and 1708 cm^{-1} for unprocessed LVXh, 1727 and 1706 cm^{-1} for the BMed sample, and 1730 and 1711 cm^{-1} for the SDed sample (**Figure A.2.1**), suggesting that the $\text{C}=\text{O}$ moiety in SDed LVXam was affected by the processing. This is further confirmed by the ratio of the peak area of the band at a higher wavelength to that of a

lower wavelength being 2.58, 3.11, and 0.78 for the unprocessed LVXh, BMed LVXh, and SDed LVX, respectively. This could reflect the different engagement of the carbonyl group in hydrogen bonds and/or the formation of the zwitterion form of LVX (**Figure 1.9**), which was previously observed for CIP, another fluoroquinolone drug.⁵⁰

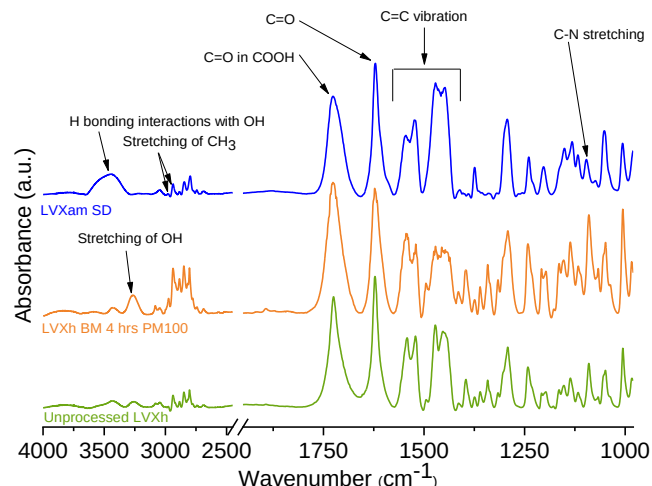


Figure 2.6 FTIR of unprocessed LVXh and LVXh subject to milling for 4 hours using a PM100, and LVXam after SDing.

2.3.6 Kinetic Modeling of the Mechanically Induced Phase Transformations of LVXh

The Rietveld method was used to analyze the different sets of PXRD data obtained from LVXh processed using a MM400 and a PM100 (**Figures A.2.2-A.2.4**). An example of this approach is presented in **Figure 2.7** and it is evident that the experimental data points and the Rietveld fit profiles incomparably overlap. This signifies an accurate estimate of the relative quantity of different phases, which exist upon mechanical processing of LVXh, i.e., LVXh, LVXhy, and LVXam.

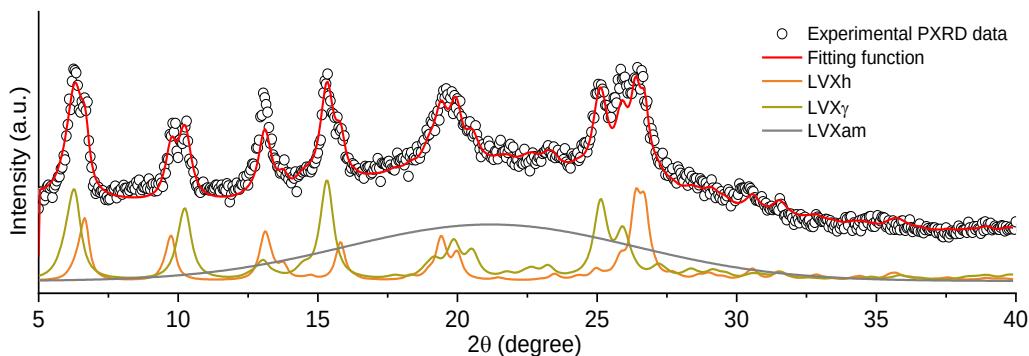


Figure 2.7 Experimental PXRD pattern and PXRD profiles obtained by the Rietveld method. Data refer to LVXh powder BMed for 5 min at 25 Hz using a MM400 ball mill, a 25 mL ZrO₂ jar, and one 1.2 cm ball. The experimental points (dots) and overall the Rietveld profile (red solid line), as well as the Rietveld profiles for LVXh (orange solid line), LVX γ (gold solid line), and LVXam (grey solid line) are shown.

The quantitative PXRD analysis reveals that irrespective of the mechanical processing conditions, ball milling induces, first, the formation of the γ phase and then, the appearance of an amorphous phase. Although the identified LVX γ and LVXam phases are mixtures, a new method of forming these polymorphs from LVXh has been established. Previous studies described the formation of LVX γ by dehydration of LVXh above 70 °C under nitrogen conditions.^{186, 189} Further investigations succeeded in producing pure LVX γ from LVXh at 150 °C in synthetic air.¹⁸⁷ Multiple LVXh solvates were found to convert to LVX γ by desolvation i.e., heating and solvent removal.¹⁸⁸

Although multiple water-free phases exist for LVX (**Figure 2.1**), they vary in conformation orientation of the piperazine ring, which influences the ease and/or mechanism of dehydrate rehydration, making some water-free forms slightly more or less unstable than others.¹³ LVX γ although unstable at ambient conditions, may be the more stable anhydrous/dehydrate form due to the presence of unchanneled voids surrounded by rigid methyl groups, which in combination prevent water from re-entering without the complete collapse of the crystal structure first.¹⁸⁷ This is in contrast to water-free LVX α which can directly rehydrate without the collapse of the crystal structure. Additionally, the pure form of LVX α has yet to be formed from the dehydration of LVXh¹⁸⁸ (**Figure 2.1**) making it a less likely candidate to form under milling conditions. The necessity for LVX γ to collapse before rehydrating may explain the transition from LVX γ to LVXam. According to the literature, it is also possible for LVX γ to convert to LVX δ if cooled below 54 °C whilst remaining absent of any moisture.¹⁸⁷ LVX δ did not transpire in this study as the reaction proceeded in a closed vessel and was only opened for sample removal. The likelihood of the escape of any free water molecules was thus limited. In any case, the reentrance of moisture would be possible upon brief exposure to the atmospheric conditions if water was to escape, confirming that moisture free conditions were not provided.

To interpret the kinetic evidence and gain a deeper insight into the observed transformations, a simple kinetic model was implemented. The model was based on several assumptions, which considered, at least to a first approximation, the statistical nature of the mechanical processing by ball milling and the stress conditions experienced by the powder during individual impacts. It was assumed that, during individual impacts, the mechanical loading exceeds a certain threshold in a set of small sub-volumes v^* that are located in the volume of compressed powder. The total volume of powder affected by CLCs, v , during individual impacts, is the sum of the volumes v^* . If the powder inside the jar is effectively stirred

and the volumes v^* are stochastically involved in any given impact, the statistics of v^* affected by CLCs can be described analytically with κ being the ratio between v and the total volume of powder, V , inside the jar. The volume fraction of powder, $\chi_i(m)$, that has undergone CLCs i times after m impacts can be expressed as **Equation 2.1**:

Equation 2.1 The volume fraction of powder that has undergone CLCs.

$$\chi_i(m) = \left[\frac{(\kappa m)^i}{i!} \right] \exp(-\kappa m) \quad \text{Eqn. (2.1)}$$

Equation 2.1 represents the probability that CLCs affect a total volume v under the hypothesis that impacts always involve the same fraction of powder, κ . Although it describes how volumes v^* undergo CLCs, it does not consider the possible changes induced by CLCs in volumes v^* . In this regard, it is reasonable to expect that specific values α_i of the degree of transformation in volumes v^* after i CLCs can be associated with volume fractions $\chi_i(m)$. Accordingly, the total degree of transformation, $\alpha(m)$, can be written as **Equation 2.2**:

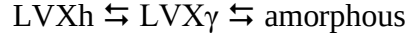
Equation 2.2 The total degree of transformation.

$$\alpha(m) = \sum_{i=1}^{\infty} \chi_i(m) \alpha_i \quad \text{Eqn. (2.2)}$$

which can be regarded as a weighted average of the transformation degree over the different volume fractions $\chi_i(m)$.

Equation 2.2 relates to the volume v effectively involved in CLCs to the degree of transformation. In the absence of more detailed information, it is reasonable to keep the assumptions that allow obtaining a transformation kinetics in line with experimental evidence, as simple as possible. Thus, it can be noted that **Equation 2.2** results in an exponential decrease of the initial LVXh phase if it is hypothesized that the degree of transformation from LVXh to LVX γ in individual volumes v^* changes abruptly from 0 to 1 as the volumes undergo a single CLC. In other words, this hypothesis implies that the entire volume v is involved in the formation of LVX γ after the first CLC. In such volume, the transformation is complete. Following on from the first impact the powder inside the jar comprises a volume fraction of LVXh $\chi_{LVXh}(1)$, equal to $1 - \kappa_1$ and a volume fraction of intermediate LVX γ $\chi_\gamma(1)$, equal to κ_1 . Upon the second impact, CLCs can involve both the residual LVXh phase and the newly formed LVX γ . It may be expected that LVXh keeps transforming into LVX γ with a rate constant equal to κ_1 . However, it is reasonable to hypothesize that the intermediate LVX γ can, on the one hand, partially reverse the transformation and form LVXh with a rate constant equal to κ_{-1} and, on the other, give rise to the amorphous phase with a rate constant equal to κ_2 . Accordingly,

after the second impact, the volume fractions of LVXh, $\chi_{LVXh}(2)$, LVX γ anhydrous phase, $\chi_{\gamma}(2)$, and amorphous phase, $\chi_{am}(2)$, are equal to $1 - \kappa_1 - \kappa_1(1 - \kappa_1) + \kappa_1 \kappa_{-1}$, $\kappa_1 + \kappa_1(1 - \kappa_1) - \kappa_1 \kappa_{-1} - \kappa_1 \kappa_2$ and $\kappa_1 \kappa_2$, respectively. With the assumption that the intermediate LVX γ and amorphous phases can partially reverse their transformation, it is possible to write the following kinetic scheme:



Since the observed transformations occur in relatively long-time intervals, this denotes that the rate constants are much smaller than 1. Thus, the following kinetic equations can be defined (**Equation 2.3**, **Equation 2.4**, **Equation 2.5**):

Equation 2.3 *The fraction of LVXh as a function of milling time.*

$$\alpha_{LVXh}(t) = a + (1 - a)\exp(-\kappa_1 t) \quad \text{Eqn. (2.3)}$$

Equation 2.4 *The fraction of LVX γ as a function of milling time.*

$$\alpha_{\gamma}(t) = (1 - a)[1 - \exp(-\kappa_1 t)] - b(1 - a)[1 - (1 + \kappa_2 t)]\exp(-\kappa_2 t) \quad \text{Eqn. (2.4)}$$

Equation 2.5 *The fraction of LVXam as a function of milling time.*

$$\alpha_{am}(t) = b(1 - a)[1 - (1 + \kappa_2 t)]\exp(-\kappa_2 t) \quad \text{Eqn. (2.5)}$$

where κ_1 and κ_2 can be regarded as rate constants, while a and b account for the coexistence of three solid phases. Specifically, a represents the fraction of LVXh attained at the end of the transformation, and $b(1 - a)$ represents the final fraction of the amorphous phase.²⁰⁶ **Equation 2.3**, **Equation 2.4**, and **Equation 2.5** can be best fitted to experimental data. The obtained kinetic curves are shown in **Figure 2.8**. It was depicted that the phase transformation indeed has a consecutive character, so LVX γ must be regarded as an intermediate between the starting phase, which is LVXh, and the final amorphous material (LVXam).

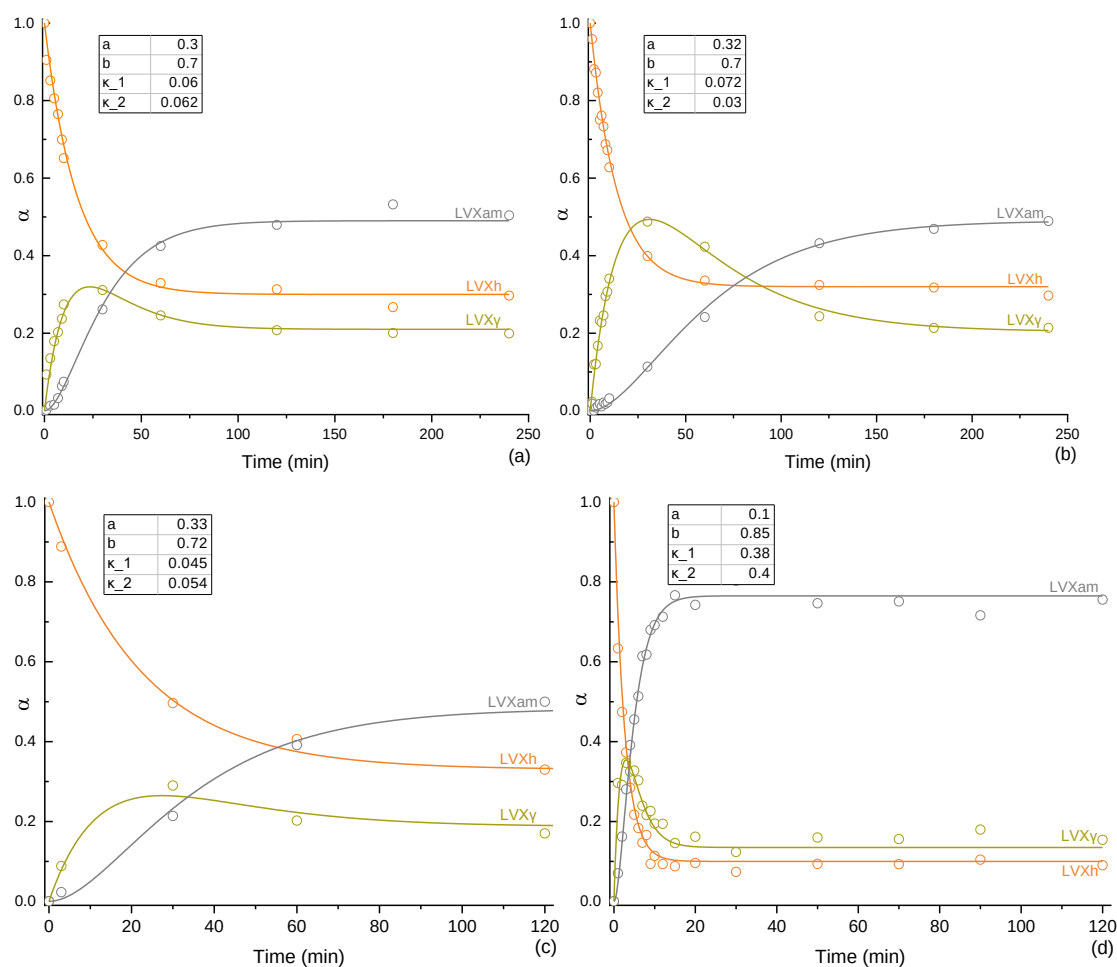


Figure 2.8 The fraction (α) of LVXh (orange), LVX γ (gold), and LVXam (grey) as a function of milling time, t . Experiments were carried out with a (a) PM100 ball mill and 50 mL stainless steel jar using three 1.5 cm balls at 400 rpm, (b) MM400 ball mill operated at 25 Hz with the 50 mL stainless steel jar and three 1.5 cm balls (MM400_Cond_1) and (c) MM400 ball mill operated at 25 Hz with the 50 mL stainless steel jar and five 1.2 cm balls. (d) MM400 ball mill and 25mL ZrO₂ jar using one 1.2 cm ball at 25 Hz (MM400_Cond_2). Included are fitting parameters a , b , κ_1 , and κ_2 . The unit for κ_1 and κ_2 is min^{-1} .

Using a PM100, LVX γ achieved α maximum of 0.32 at approximately 25 minutes of milling before reducing to $\alpha = 0.21$ at the end of milling. The amount of LVXam at the end of the process (2 hours) was approximately $\alpha = 50$. The quantities of LVX γ and LVXam remained constant at steady state at approximately 105 minutes of milling (**Figure 2.8a**). For the MM400_Cond1 the LVX γ phase achieved α maximum of 0.49 at approximately 25 minutes of milling before reducing to $\alpha = 0.21$. Similar to a PM100, LVXam obtained α maximum = 0.49 (**Figure 2.8b**). Increasing the number of balls from three to five did not affect the quantities of LVX γ and LVXam considerably (**Figure 2.8c**) and the steady state was achieved after around 100 minutes. For the MM400 Cond_2 the LVX γ phase achieved α maximum = 0.35 at approx.

3 minutes of milling before reducing to $\alpha = 0.14$. These conditions gave the greatest amount of LVXam, $\alpha = 0.77$. The levels of LVX γ and LVXam remained constant upon reaching steady state at approx. 20 minutes of milling (**Figure 2.8d**).

To summarize, maximum quantities of LVX γ phase were reached for the PM100 and the MM400 Cond_1 after 25 minutes of milling and after 3 minutes of milling for the MM400 Cond_2. Additionally, around 50% conversion to LVXam was achieved using the PM100 and the MM400 Cond_1 compared to the MM400 Cond_2 where around 80% was amorphized. The degree of disorder appears to be in agreement with PXRD data presented in **Section 2.3.2 (Figure 2.8)**. The best fitted values and final fractions of LVXh, LVX γ , and LVXam for the PM100 and the MM400_Cond1 are very similar, while slight differences in κ_1 and κ_2 values are observed (**Figure 2.8**). This suggests that the mechanochemical transformation was relatively insensitive to changes in the mechanical action of the mill or the frequency of impacts. This contrasts with the final fractions and rate of transformation of LVXh, LVX γ , and LVXam for the MM400_Cond2, whereby the rate of transformation was remarkably faster. However, it should be noted that a smaller amount of powder (0.1 g), was used in experiments with the ZrO₂ jar, while 2 g was used when a stainless-steel jar was employed.

The ability to quantify polymorphs of APIs is extremely important for optimal polymorph selection for the formulation. Methods such as IR, Raman, and solid-state nuclear magnetic resonance (SSNMR) are commonly used as a quantitative method for polymorphs,^{207, 208} including amorphous forms.²⁰⁹ However, in more recent years the Rietveld refinement has been used for this purpose in isolation or in combination with more traditional methods described due to its accurate analysis²⁰⁷ and efficient procedure. The Rietveld refinement was utilized as a quantitative method for analyzing the stability, transformations, kinetics, and crystal domain size alterations of suspensions of mebendazole in the solid-state.²⁰⁷ Venlafaxine hydrochloride²⁰⁸ and carbamazepine^{210, 211} were also investigated for quantification, identification,^{208, 210, 211} and purity analysis of polymorphs detected.²⁰⁸ In addition, this approach has also been used to quantify the crystallinity of recrystallized amorphous solid dispersions of fenofibrate and ketoconazole.²⁰⁹ The accuracy of the results obtained, the ability to detect and quantify the different forms simultaneously using conventional PXRD data, and the speed of analysis make the Rietveld refinement an advantageous quantification method for phase transformation in the solid-state, as shown in this work.

2.3.7 Physical Stability of Processed LVX

DVS was used to analyze LVXh after 4 hours of ball milling using the PM100, the MM400 Cond_2, and SDed LVXam. These analyses were carried out to evaluate the effect of moisture on the solid-state stability of the BMed sample in comparison to the SDed sample (**Figure 2.9**). The results depict a sample mass increase when RH increases for BMed LVXh. There was evidence of partial amorphization and/or crystal lattice defects as the LVXh sample milled for 4 hours crystallized at 70% RH during the analysis and overall, the powder sorbed around 4% (w/w) of moisture at 90% RH. In contrast, the desorption plot illustrates a rapid decrease in mass (%), which highlights the quick weight loss and dehydration of the sample. The detection of amorphization is in agreement with the exothermic crystallization peak obtained for BMed samples (**Figure 2.4**) and the amorphization confirmation utilizing the Rietveld analysis (**Figure 2.7**). The sample BMed by the PM100 lost approx. 2% (w/w) moisture between 90-10% RH (desorption) over 300 minutes, followed by another loss of 2% (w/w) moisture between 10-0% RH over 450 minutes. The sample in BMed by the MM400 lost approx. 2.5% (w/w) moisture between 90-10% RH over 300 minutes, and subsequently showed further loss of approx. 1.5% (w/w) moisture between 10-0% RH over 200 minutes.

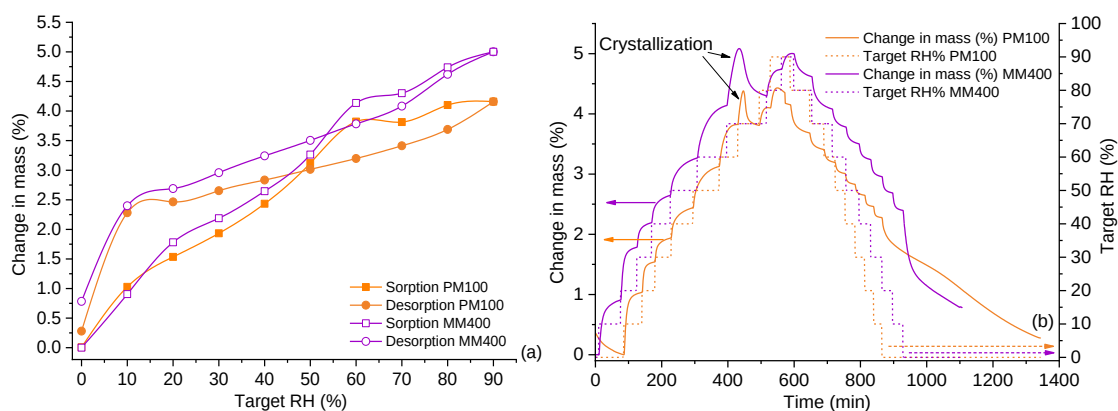


Figure 2.9 DVS plots of LVXh BMed for 4 hours (a) isotherm plots of LVXh sample BMed at 400 rpm using a PM100 and BMed at 25 Hz using the MM400_Cond_2 (b) mass plot over time of LVXh sample BMed at 400 rpm using a PM100 and BMed at 25 Hz using the MM400_Cond_2.

The LVXam powder obtained by SDing sorbed more moisture than the semi-crystalline BMed LVXh powder with sorption reaching $\geq 5\%$ (w/w) of moisture at around 60-70% RH for methods 1, 2 and 3 (**Figure 2.10**). The ability to sorb large amounts of moisture is in alignment with the characteristics of amorphous materials, which are known to sorb more moisture compared to crystalline or semi-crystalline materials. The sample subjected to DVS methods 1 and 2 lost 1% (w/w) moisture between 90-10% RH (desorption) over 200 minutes, and method

3 over 150 minutes. The moisture loss is comparable for all methods, however, the sample analyzed using method 3 differs in the duration of moisture loss. The mass/kinetic plots for samples ran using methods 1 and 2 show that the majority of the sample crystallized at 70% RH, with residual amounts crystallizing at 80% RH. Method 3 facilitated a longer drying cycle of 12 hours which allowed the sample to completely crystallize after 3 hours of exposure at 70% RH.

Thus, both amorphous phases generated by BMing and SDing crystallize at the same % RH (**Figure 2.9** and **Figure 2.10**), revealing their similar capabilities to withstand crystallization under moisture conditions. Additionally, the crystallization peaks obtained for the SDed sample (**Figure 2.10**) are substantially larger compared to those shown for the BMed samples (**Figure 2.9**). This is as expected since the starting material of the SDed sample was completely amorphous (**Figure 2.5**) in contrast to the BMed sample which was semi-crystalline in nature (**Figure 2.2** and **Figure 2.3**). DVS and post-DVS PXRD analyses showed that all processed samples (BMed and SDed) crystallized to the most stable hemihydrate form, matching the 2.5% water weight of LVXh.

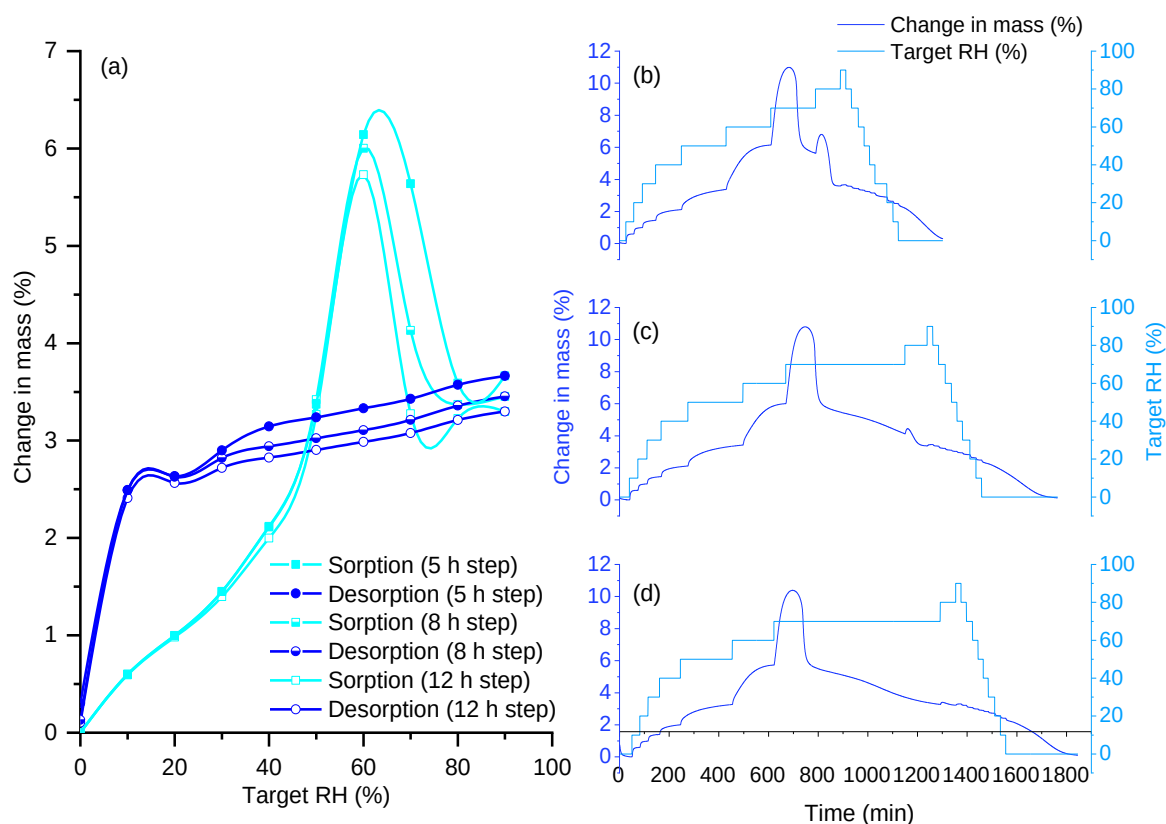


Figure 2.10 DVS plots of SDed LVXam (a) isotherm plot using method 1 (5h step), 2 (8h step) and 3 (12 h step) (b) mass plot over time using method 1 (c) mass plot over time using method 2 (d) mass plot over time using method 3.

2.4 Conclusions

Solid-state transformations of LVXh were examined primarily on the milling process, utilizing spray drying as a comparative method. The solid-state changes were detected using PXRD, the Rietveld analysis, DSC, FTIR, and DVS. Different mechanical action and parameters led to a reduction in crystallinity, as evidenced by the changes observed in the diffractograms of LVXh upon processing using a PM100 and a MM400, with the MM400 demonstrating the greatest Bragg peak reductions for both methods i.e., MM400 Cond_1 and MM400 Cond_2. Thermograms of the PM100 and the MM400 Cond_2 suggested the disruption of the crystal lattice, facilitating the liberation of the water. The thermograms of samples subjected to milling revealed an exothermic peak of crystallization. The presence of anhydrous/dehydrate form and the event of crystallization were related to LVX γ and LVXam, respectively, by the Rietveld method. The mechanism discovered, established that LVXh first transforms to the anhydrous/dehydrate LVX form γ (LVX γ) as an intermediate phase, before transpiring to LVXam. This is particularly interesting since LVXam was previously reported to form directly from LVXh upon dehydration. Additionally, LVX γ was only ever obtained by dehydration/heating and desolvation methods, according to the literature. Thus, a new method of forming polymorphs by the process of milling was developed, and the mechanism by which LVX γ plays an essential role in achieving amorphization was revealed. The Rietveld method revealed that the PM100 and the MM400 Cond_1 had longer rates of transformation of LVX γ and powders contained more LVX γ form compared to that processed by the MM400 Cond_2. The use of the MM400 Cond_2 resulted in approximately 80% conversion of LVXh to LVXam compared to other methods. Despite incomplete amorphization upon utilization of the milling process, complete amorphization of LVX was achieved by spray drying from water. The comparison of BMing and SDing revealed that the outcome of amorphization is both mechanism and process dependent. The pure phase of LVXam was reported for the first time in the literature, as having a glass transition of around 80 °C. DVS showed that both amorphous phases generated by BMing and SDing crystallize at 70 % RH, revealing their similar capabilities to withstand crystallization at moisture conditions. These findings are valuable as the new discoveries obtained may be transferred and/or applied to current and future fluoroquinolone drugs with similar molecular structures. In addition, these discoveries contribute towards a greater understanding of mechano- and tribo-chemistry and in turn can help to fill the gaps of solid-state mechanochemistry as the future of pharmaceuticals moves toward a greener world.

Chapter 3.0 Mechanochemical Comparison of Ball Milling Processes for Levofloxacin Amorphous Polymeric Systems

Chapter 3.0 Mechanochemical Comparison of Ball Milling Processes for Levofloxacin Amorphous Polymeric Systems

3.1 Introduction

Mechanochemistry describes the chemical reactions stimulated by energy and mechanical forces produced at room temperature by the mechanical process.^{76, 77, 159, 212} Mechanical force initiates the breakage of molecules due to friction, stress, and strain. Subsequently, chemical reactions can occur due to the efficacious rupture of these bonds, permitting reactions to supervene.^{77, 212} Mechanochemistry offers many advantages such as manufacturing solid dispersions without the use of solvents, tackling the issue of poorly soluble drugs as no solvent is required, preventing the interference of undesirable reactions with the solvent, and implementing green chemistry and improving human health by means of eliminating hazardous solvents.^{89, 90}

Mechanochemistry was conventionally carried out by manual grinding by use of a mortar and pestle. Although chemical transformations were often successful, the final product may experience variability due to changes of force by human factors, and exposure to diverse and inconsistent environmental conditions due to the open nature of the mortar.^{71, 159, 169} Fortunately, it is now possible to use automated processes such as BMing. This allows longer durations of BMing to be implemented and a reduction in the exposure to human and environmental factors.⁶⁶ The milling process was initially designed to enhance homogeneity and reduce the particle size of a material. However, further research has led to the discovery that milling can also allow polymorphic transformation and/or amorphization of APIs to occur when high amounts of energy are introduced into the system.¹³⁵

Many studies have been published on ASDs. ASDs are systems that contain an amorphous API in a polymer matrix. In most instances, polymers are used to stabilize the amorphous material by increasing the T_g of the amorphous mixture. This results in reduced mobility, increased stability, and thus, crystallization prevention of the API from its amorphous form to its more stable crystalline form due to an anti-plasticizing effect.⁴⁷⁻⁴⁹ The intimate mixing, at the molecular level, allows new interactions to take place between the drug and polymer.²¹³ However, in most recent studies by Mesallati *et al.* it has been found that not only can acidic polymers improve the stability of an amorphous system, but these polymers can also facilitate the phase transformation of fluoroquinolone drugs. These findings led to enhanced

solubility of CIP and ENRO using EL100, HPMCAS-L, and HPMCAS-M, and antibacterial activity using HPMCAS.^{19, 20}

In a study previously performed by this group, LVXh, a complex fluoroquinolone drug was milled to establish a mechanistic understanding of solid-state transformations using different ball mills, and to investigate the feasibility of amorphization using these processes, in addition to the SDing process. Despite the use of different mills, and thus, mechanical and tribochemical forces, BMing displayed the inability to completely amorphize LVXh. Nevertheless, it was revealed that partial amorphization was in fact possible by primarily converting LVXh to anhydrous LVX γ form, which subsequently transformed into LVXam. The mechanism of LVXam formation was also shown to be reversible, with LVX γ remaining as the intermediate step in both forward and backward reactions. Additionally, BMing and SDing processes were compared, and unique mechanisms of product formation, depending on the chosen process were confirmed (**Chapter 2**). Following on from this study, it became clear that LVX had never been milled using an excipient, until now.

One study to date successfully showed the conversion of LVX to amorphous LVX using a neutral polymer (and sugar) to produce hydrogels using heated mixing.²¹⁴ Additionally, other studies have been performed whereby LVX was converted to either an amorphous or co-amorphous form of the drug using SDing,^{198, 215-217} freeze drying,^{217, 218} solvent evaporation/casting,^{202, 219, 220} electrospinning,^{221, 222} and precipitation.²²³ The cofomers used to carry out these studies consisted of either amino acids, aromatic acids, neutral polymers with sugar, acidic polymers, amino acids, enzymes, dicarboxylic acid/dicarboxylic fatty acid, or cyclodextrins. Studies by Mesallati *et al.* demonstrated the possibility of amorphizing CIP and ENRO with the use of an acidic polymer.^{19, 20} Thus, the milling of LVXh (**Figure 1.9**) using an acidic polymer, EL100 (**Figure 3.1**) for the purpose of achieving amorphization is discussed in this chapter.

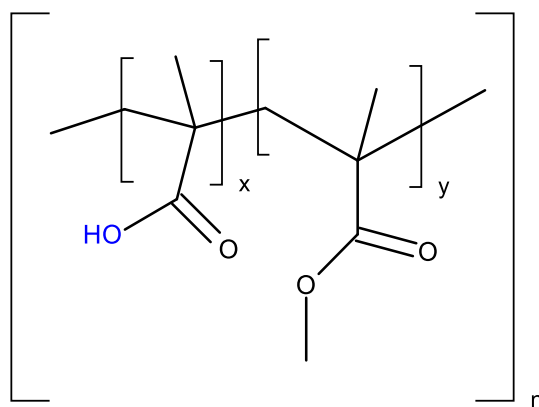


Figure 3.1 Structural formula of EL100.

The objective of this chapter was to prepare LVX:EL100 amorphous binary systems while utilizing the BMing process. Additional aims of this chapter were to compare the outcome produced using different mills (a Retsch mixer mill MM400, a Retsch planetary ball mill PM100, and a Fritsch vibration mill), and to perform a scale up of the process. A focus remained on determining whether the use of different mills leads to the production of reproducible or contrasting results. This is important since multiple models and makes of mills exist and are being utilized for research purposes today. Lastly, this chapter aimed to determine the mechanism of the formation of LVX:EL100 binary amorphous systems. Solid-state analysis and in-situ measurements using synchrotron radiation were also performed to develop a deeper understanding of changes to the LVXh crystalline structure and its response when exposed to mechanical conditions. Finally, the physical stability of the binary systems as a function of temperature and humidity was determined.

3.2 Experimental Section

3.2.1 Materials

Materials were purchased as described in **Section 2.2.1**. EL100 was received from Evonik Industries (Germany).

3.2.2 Methods

3.2.2.1 Retsch Mixer Mill MM400

Different weight ratios that comprised 50-90% (w/w) LVXh to form LVXh:EL100, were BMed using a Retsch mixer mill MM400 (Haan, Germany) at 25 Hz (1/s). A 25 mL ZrO₂ grinding jar was used. A sample of 0.5 g and two ZrO₂ balls with a diameter of 1.5 cm were added to the jar (**Table 3.1**). The samples were milled for a duration of 1 hour with no breaks in between. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. The samples were analyzed via PXRD and DSC (**Sections 2.2.2.3 and 2.2.2.4**). The 50, 70, 80, and 90% (w/w) LVXh samples were analyzed via FTIR (**Section 2.2.2.7**). The 50% (w/w) LVXh sample was analyzed via DVS (**Section 2.2.2.8**).

3.2.2.2 Retsch Planetary Ball Mill PM100

Different weight ratios that comprised 50-90% (w/w) LVXh to form LVXh:EL100, were BMed using a Retsch planetary ball mill PM100 (Haan, Germany) at 400 rpm (described in **Section 2.2.2.1.1 (Method 1)**, and **Table 3.1**). Ball milling was conducted at room temperature for a maximum of 30 min at a time. A 15-minute break was implemented after every 30 min of milling to prevent overheating due to the longer processing durations. The samples were milled for a total duration of 4 hours at timepoint measurements taken every 0.5, 1, 2, 3, and 4 hours. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling. Around 25 mg of the sample was removed for analysis via PXRD and DSC (**Sections 2.2.2.3 and 2.2.2.4**). Approximately 10 mg of the sample was removed for FTIR analysis (**Section 2.2.2.7**) and 15 mg for DVS analysis (**Section 2.2.2.8**) after 4 hours of milling.

Three stainless steel milling balls with a diameter of 2 cm and weight of 32 g each were used to compare results for 60% (w/w) LVXh. The sample was milled for a total duration of 4 hours (described in **Section 2.2.2.1.1**). Approximately 5 mg of the sample was removed for analysis via PXRD after 0.5, 1, 2, 3, and 4 hours of ball milling (**Sections 2.2.2.3**). The 60%

Table 3.1 Conditions of milling used in this work. ZrO₂ - zirconium oxide, S.S. - stainless steel.

(w/w) LVXh sample after ball milling for 4 hours was analyzed via FTIR (Section 2.2.2.7). Mill	Type and volume of jars (mL)	Type and number of balls, diameter (cm)	Frequency/Speed	Duration (hours)	Powder Load (g)	LVXh Content Tested (% (w/w))
Retsch Mixer Mill MM400	ZrO ₂ , 25	2 ZrO ₂ balls, 1.2	25 Hz	1	0.5	50-90
Retsch Planetary Ball Mill PM100	S.S., 50	3 S.S. balls, 1.5	400 rpm	0.5, 1, 2, 3, 4	2.0	30-90
Retsch Planetary Ball Mill PM100	S.S., 50	3 S.S. balls, 2.0	400 rpm	0.5, 1, 2, 3, 4	2.0	60
Fritsch Vibration Ball Mill	S.S., 12	3 S.S. balls, 0.4	50 Hz	5	0.14	50
Fritsch Vibration Ball Mill	S.S., 12	1 S.S. balls, 0.9	50 Hz	5	0.14	50

3.2.2.3 Fritsch Vibrational Ball Mill

LVXh:EL100 that contained 50% (w/w) LVXh was BMed using a Fritsch Vibration Ball Mill (Pulverisette 23, Fritsch, Germany) at 50 Hz. Ball milling was executed at room temperature for 30 minutes with a 15-minute breaks every 30 minutes for a total of 5 hours. A 1.2 cm diameter Perspex jar was used,²²⁴ and a total of 0.14 g of sample was added to jar. Three stainless steel milling balls with a diameter of 0.4 cm each were used for the most part of this study. One stainless steel milling ball with a diameter of 0.9 cm was used to compare to the previous parameters (**Table 3.1**).

3.2.2.4 Powder X-Ray Diffraction (PXRD)

As described in **Section 2.2.2.3**.

3.2.2.5 In-Situ Powder X-Ray Diffraction Measurements

In-Situ Powder X-Ray Diffraction Measurements were performed, and data was analyzed primarily by Dr Lucia Casali (Federal Institute for Materials Research and Testing, Berlin, Germany), with involvement from Prof. Franziska Emmerling (Federal Institute for Materials Research and Testing, Berlin, Germany and Humboldt-Universität zu Berlin, Germany).

In situ X-ray diffraction measurements were performed at 1-minute intervals at the μ Spot beamline (BESSY II, Helmholtz Centre Berlin for Materials and Energy).²²⁵ The experiments were conducted with a wavelength of 0.7314 Å using a double crystal monochromator and the resulting scattering images were integrated with the Dpdak-software.²²⁶ The data obtained were plotted with the software OriginPro 2023 to obtain a temporal evolution of the system mechanically activated. No background correction was applied to avoid artifacts and misleading information regarding the intensity variation of the peaks.

3.2.2.6 Differential Scanning Calorimetry (DSC)

As described in **Section 2.2.2.4**.

3.2.2.7 Solid-State Fourier Transform Infrared Spectroscopy (FTIR)

Powders (as described in **Sections 3.2.2.1** and **3.2.2.2**) were mixed with dried KBr using a spatula in a ratio of 1:30 and analyzed by FTIR as described in **Section 2.2.2.7**.

3.2.2.8 Dynamic Vapor Sorption (DVS)

DVS was used to investigate the BMed binary sample by use of an Advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., UK) (as described in **Section 2.2.2.8, Method 1**).

3.3 Results and Discussion

Previous studies by Mesallati *et al.* showed that ball milling CIP with an acidic polymer using the Retsch PM100 ball mill allowed fully amorphous powders to be obtained.¹⁹ In those systems, CIP demonstrated the ability to form a cation, which reacted with an acidic polymer to yield the formation of amorphous polymeric salts.¹⁹ Thus, based on the prior work, a similar approach of co-milling LVXh with an acidic polymer, EL100 using a MM400 and a PM100 was utilized.

3.3.1 Ball Milling using a MM400

Figure 3.2a shows the crystalline nature of LVXh starting material powder and PXRD of EL100, which is an amorphous polymer. Different weight ratios of LVXh:EL100 comprising 50-90% (w/w) LVXh were BMed via a MM400 in an attempt to attain complete amorphization of LVXh. It is evident that the intensity of LVXh can be reduced on co-milling with EL100, in agreement with previous studies (**Chapter 2**). LVXh:EL100 comprised of 80-90% (w/w) LVXh demonstrated a reduction in crystalline Bragg peaks and Bragg peak broadening. However, sharp Bragg peaks were still prominent in those samples, particularly for 90% (w/w) LVXh, confirming the semi-crystalline nature of the samples.²²⁷ Thus, the EL100 content was increased in an endeavor to further facilitate phase transformation and achieve complete amorphization. Complete amorphization was confirmed when $\leq 70\%$ (w/w) LVXh was present in the co-processed samples after 1 hour of milling, confirmed by the formation of a halo and the complete abolishment of crystalline Bragg peaks (**Figure 3.2a**). This was in contrast to LVXh starting material powder, which was processed using the MM400 and the PM100 in previous studies performed (**Chapter 2**). These samples depicted the remainder of crystalline Bragg peaks, with slight reductions to the intensity. The findings demonstrated the incapability of fully amorphizing LVXh (**Chapter 2**).

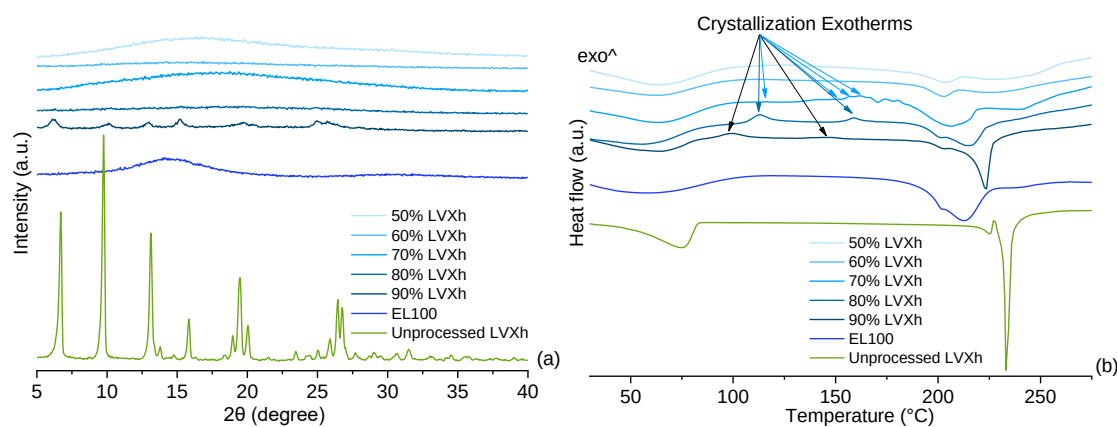


Figure 3.2 (a) PXRD analysis of unprocessed LVXh, EL100, and processed LVXh:EL100 in different weight ratios. (b) DSC thermograms of unprocessed LVXh, EL100, and processed LVXh:EL100 in different weight ratios. The Retsch mixer mill MM400 was used with conditions specified in **Table 3.1**.

Thermograms of the MM400 processed samples demonstrated alterations with respect to the dehydration and melting peaks. The DSC data for unprocessed LVXh depicts a dehydration peak at 74.8 °C, which represents $\frac{1}{2}$ H₂O that is embedded in the crystal structure of the hemihydrate LVX. In contrast, EL100 presents a dehydration peak due to its amorphous structure which is known to retain moisture and is determined at 58.9 °C. However, the dehydration peaks for LVXh:EL100 samples consisting of 50-90% (w/w) LVXh after BMing for 1 hour, shifted to lower peak temperatures (**Figure 3.2b**). Unprocessed LVXh powder did not present an exotherm after the dehydration peak, however, BMing of LVXh:EL100 samples with 70-90% (w/w) LVXh resulted in the emergence of exothermic peaks of cold crystallization. The samples comprising 70% (w/w) LVXh had multiple exotherms whilst 80 and 90% (w/w) of LVXh had two exothermic peaks. These exotherms indicate that LVXh amorphized upon processing. No exotherm was present for the 50 and 60% (w/w) LVXh samples. Since amorphization between 50-70% (w/w) LVXh was confirmed by PXRD analysis (**Figure 3.2a**), the absence of an exotherm may suggest stability of the amorphous phase.

Unprocessed LVXh exhibits melting/decomposition at onset values of 221.6 and 231.9 °C, and a T_g of 80 °C (**Section 2.3.4**). Unprocessed EL100 displays decomposition at an onset value of 193.1 °C (**Figure 3.2b**). Furthermore, the T_g of EL100 is reported at 130 °C,^{19, 228} although much ambiguity exists regarding the T_g of this polymer. The presence of EL100 resulted in a decrease in the onset temperatures for LVXh:EL100 processed samples, which may be impacted by the polymer which is known to depress the melting point of an API upon addition and/or result in dissolution.²²⁹ A distinction is observable whereby the sharp

melting/decomposition peak exhibited for unprocessed LVXh and 90% (w/w) LVXh sample undergo drastic change to a broad peak, upon decreased LVXh content.

To conclude, the PXRD studies on samples processed by the MM400 mill demonstrated that $\leq 70\%$ (w/w) LVXh resulted in the complete formation of LVXam after just one hour of milling. Moreover, DSC analysis unveiled that the powders containing 70-90% (w/w) LVXh comprise LVXam. Thus, in accordance with PXRD and DSC findings, the powders containing $\leq 70\%$ (w/w) of the drug are fully amorphous. Furthermore, a combination of these techniques led to the disclosure that the absence of an exothermic peak for 50-60% (w/w) LVXh samples may indicate the stability of the amorphous systems.

3.3.2 Ball Milling using a PM100

A scale-up reaction was performed from 0.5 g to 2 g of LVXh:EL100. As a result of a larger batch size, the samples would ultimately require additional milling time to provide the opportunity for all material to endure CLCs, and ultimately, allow the reaction to reach a steady state. CLC is a mechanical term used to describe the generation of sufficient mechanical stress that results in mechanical deformation as a preliminary step for the initiation of phase transformation.¹⁰⁴ CLCs only ensue in small volumes within the powder volume which is trapped between the ball and wall of the grinding jar, or between two balls for example. It is these powders that experience CLCs that result in product formation. Additionally, the different mechano- and tribo-chemical impact of the two mills was taken into consideration when choosing BM durations since a PM100 does not produce impact forces which result in less energy being produced, and thus, longer BMing duration times.¹⁰¹ The PM100 is equipped for long milling durations and breaks can be automatically implemented into the milling method. The implementation of breaks is a common practice when utilizing mechano- and tribo-chemical processes to prevent overheating of the mill and achieve optimal safety.^{230, 231} Thus, a change of mill from a MM400 to a PM100 was implemented to perform scale up reactions.

3.3.2.1 Powder X-Ray Diffraction (PXRD)

LVXh exists and is stable in the crystalline form. Thus, previous studies were performed using a MM400 and a PM100 in an attempt to amorphize LVXh, without the use of any excipients (Chapter 2). Nevertheless, the crystalline Bragg peaks were not successfully eliminated, with slight reductions in the intensity of the peaks (**Section 2.3.2**). Different weight ratios of LVXh:EL100 were investigated, which included samples consisting of 30-90% (w/w) LVXh. These samples were BMed for 0.5 and 4 hours, followed by PXRD analysis. The

reduction of peak intensities and peak broadening was displayed, indicating crystallite size reduction (**Figure 3.3**).²³² Almost complete amorphization was achieved when the content of LVXh was $\leq 50\%$ (w/w) and the duration of ball milling was 4 hours. This was confirmed by the noticeable decrease in the Bragg peak intensity and the development of a broad halo (**Figure 3.3b**).²²⁷ This is in contrast to the samples that underwent BMing for shorter times and resulted in partially crystalline material (**Figure 3.6**). These results are in contrast to those produced via a MM400, which demonstrated amorphization by PXRD analysis for samples comprising $\leq 70\%$ (w/w) LVXh (**Figure 3.2a**). This also highlights that a higher percentage of LVXh can be amorphized when using a MM400 as opposed to a PM100.

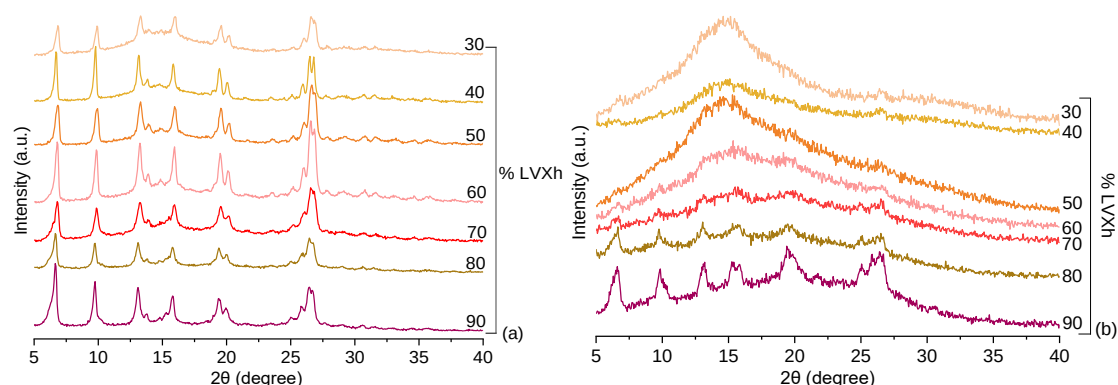


Figure 3.3 PXRD analysis of processed LVXh:EL100 in different weight ratio. The Retsch planetary ball mill PM100 and three 1.5 cm balls were used with conditions specified in **Table 3.1** after powders were subjected to ball milling for (a) 0.5 hours and (b) 4 hours.

3.3.2.2 Differential Scanning Calorimetry (DSC)

Physical mixtures (PMs) and BMed LVXh:EL100 samples were analyzed via DSC and compared to unprocessed LVXh and EL100 (**Figure 3.4**). LVXh:EL100 PMs and 0.5 hour BMed samples with weight ratios between 30-90% (w/w) LVXh displayed dehydration peak temperatures between 69.7-76.6 °C (**Figure 3.4a-b**). Nevertheless, 4 hours BMed LVXh:EL100 samples with a weight ratio of 30-70% (w/w) displayed no dehydration peak. For 80 and 90% (w/w) LVXh samples dehydration peak temperatures exhibited at 66.8 and 68.6 °C (**Figure 3.4c**). Thus, the %LVXh present and the duration of BMing have an impact on the dehydration peak, and therefore, the changes of water in the crystal lattice. This highlights that the removal of water from LVXh is possible by milling, despite failure to do so by drying the crystalline material to 80-200 °C in a vacuum oven for 24-72 hours. It is therefore likely that the reduction of the dehydration peak reflects an increase in the amorphization. This is in agreement with studies by Otsuka and Kaneniwa which demonstrate that the grinding process reduces the extent of crystallinity and decreases dehydration temperatures of the ground products, resulting in the

potential removal of lattice water, and thus, facilitating amorphization.^{233,234} Thus, it is easier to remove water from a disordered solid than from an ordered, crystalline structure.

Previous research conducted in **Chapter 2** explored LVXh BMed on its own and identified the appearance of exothermic peaks of cold crystallization between 92.4-94.7 °C when LVXh underwent milling for 0.5-4 hours (**Chapter 2**). Thus, exothermic peaks were closely examined for PMs and BMed samples. No exotherms were present for PMs. Upon BMing, the exothermic peaks of cold crystallization were observed for several LVXh:EL100 samples that were BMed for a duration of 0.5 hours with 70 and 80% (w/w) LVXh content (**Figure 3.4b**) and 40-90% (w/w) LVXh for 4 hour samples, with the peaks increasing steadily from 95.9 to 104.9 °C (**Figure 3.4c**). The appearance of these exotherms confirms the presence of an amorphous phase and the crystallization of LVX γ (Figure 3.10), in agreement with previous studies performed (**Chapter 2**). No exotherm was depicted for 30% (w/w) LVXh sample. Nevertheless, a combination of PXRD and DSC analysis suggests that the absence of an exothermic peak indicates the stability of the amorphous system. The exotherm shifts marginally toward an increased temperature upon a decrease in LVXh content. This may convey an increase in the physical stability of the amorphized material upon the addition of EL100.²²⁷ The exotherms produced in combination with PXRD data (**Figure 3.3**) verify that differences exist between the samples. Thus, the combination of both techniques reveals that the 40 and 50% (w/w) LVXh samples are fully amorphous.

No endothermic peaks were obtained for PM (**Figure 3.4a**). However, after BMing LVXh:EL100 for 0.5 hours, an endothermic peak at 203.3, 204.6, and 206 °C appeared for samples containing 70-90% (w/w) LVXh. A subtle decrease in peak temperature became apparent when LVXh content decreased. However, no endothermic peaks were detected for samples with $\leq 60\%$ (w/w) LVXh (**Figure 3.4b**). The samples that were further BMed for 4 hours showed the appearance of an endothermic peak for all samples between 40-90% (w/w) LVXh material. The endothermic peak temperature subtly increased upon the increase of LVXh content. Temperatures increased gradually from 196.8 to 203.6 °C between 40-90% (w/w) LVXh (**Figure 3.4c**).

The melting/decomposition peaks for LVXh:EL100 PMs and BMed samples are displayed in **Figure 4**. The decomposition onset temperatures consistently increased from 215.3 to 224.6 °C for the PM samples (**Figure 3.4a**), from 202.3 to 224.4 °C for the 0.5-hour processed samples (**Figure 3.4b**), and from 201.4 to 220.5 °C for the 4-hour processed samples, with LVXh

weight ratios ranging from 30% to 90% (w/w) (**Figure 3.4c**). In all cases, the onset decomposition temperatures were lower than that of unprocessed LVXh, which has an onset value of 231.9 °C. The decomposition peaks for PMs and BMed samples at 0.5 and 4 hours became sharper upon an increase of %LVXh. DSC confirms complete amorphization by the elimination of the dehydration peak, in addition to the formation of the broad decomposition peak. These results in combination indicate that samples with $\leq 70\%$ (w/w) LVXh after being subjected to milling for 4 hours are amorphous (**Figure 3.4c**). However, this is in contrast to results detected via PXRD which suggests complete amorphization for 30-50% (w/w) LVXh samples only (**Figure 3.3**).

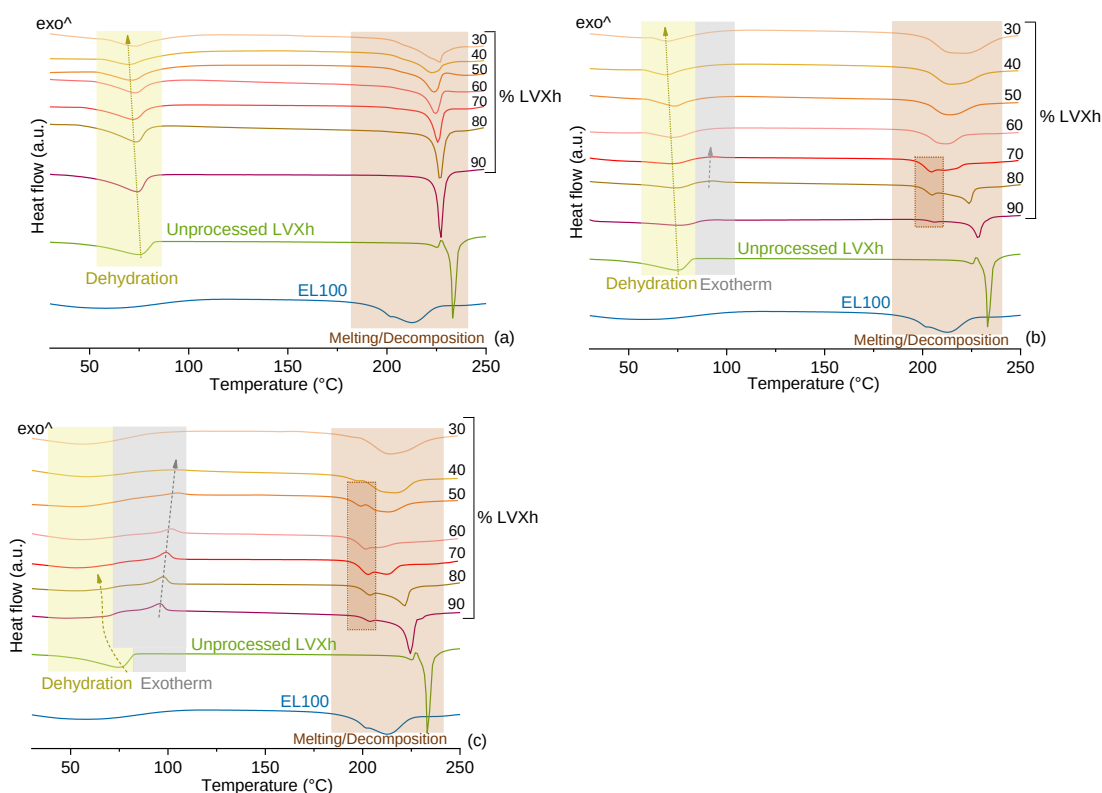


Figure 3.4 DSC thermograms of unprocessed LVXh and EL100, LVXh:EL100 physical mixtures, and processed LVXh:EL100 in different weight ratios. (a) LVXh:EL100 physical mixtures and a Retsch planetary ball mill PM100 and three 1.5 cm balls were used with conditions specified in **Table 3.1** after powders were subjected to ball milling for (b) 30 minutes and (c) 4 hours. The dehydration (yellow), exotherm of crystallization (grey), and melting/dehydration (light and dark brown) peaks of unprocessed LVXh and LVXh:EL100 samples are shown.

The onset temperatures of the dehydration peaks were investigated for processed LVXh. which showed a slight yet steady increase in temperature. In contrast, processed LVXh:EL100 samples showed more prominent fluctuations in the onset temperatures. In general, the dehydration onset temperature for 80 and 90 % LVXh samples decreased, however, the samples

with ≤ 70 % LVXh displayed increased dehydration onset values, in comparison to pure LVXh, with complete elimination of this peak after 2 and/or 3 hours of milling (**Figure 3.5a**). This may suggest that the higher the content of EL100, the greater the impact of this polymer on the ability to release the crystalline water from the LVXh structure during processing.

The onset temperatures of the exothermal peak, ascribed to the crystallization of amorphous LVX, gradually increased with the duration of BMing. The 50–90 % LVXh samples demonstrated a significant increase in onset temperature of the exotherm, compared to that of LVXh processed on its own, with 70 % LVXh achieving the highest onset temperatures (**Figure 3.5b**). Therefore, it could be assumed that the presence of the polymer increased the stability of the amorphous LVX phase formed upon BMing of the mixture, as it crystallized at a higher temperature in comparison to that of the amorphous phase formed by pure LVXh (LVXam).

The enthalpy of crystallization (ΔH_{Cryst}) was calculated solely based on the crystallization exotherm of LVXam. It is noticeable that ΔH_{Cryst} of processed LVXh gradually increased upon BMing duration. The existence of the amorphous phase in processed LVXh is consistent with previous findings (**Chapter 2**).²³⁵ The elevation in values suggests that amorphization gradually increases upon milling. 40-70% (w/w) LVXh samples displayed higher ΔH_{Cryst} values compared to processed LVXh on its own and the sample with 70 % LVXh demonstrated the greatest ΔH_{Cryst} values (**Figure 3.5c**). Collectively, the thermal analysis data suggest that BMing of LVXh and EL100 leads to significant changes in the solid-state of the active with the polymer aiding in LVXh dehydration and amorphization and that the resulting LVXam has better thermal stability than the phase produced upon BMing of LVXh alone.

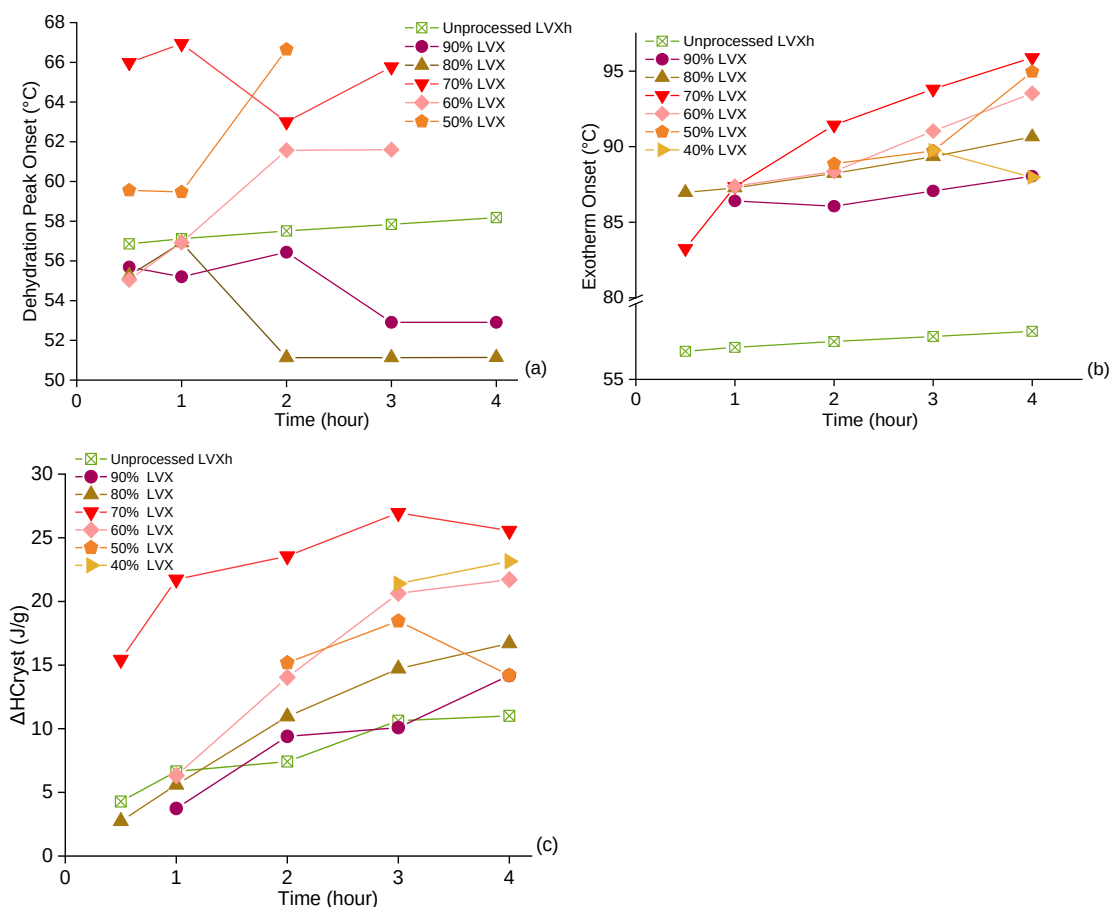


Figure 3.5 (a) DSC dehydration peak onset data analysis of processed LVXh and processed LVXh:EL100 subjected to milling using different percentages of LVXh for durations between 0.5-4 hours. (b) DSC onset data of exotherm crystallization peak analysis of processed LVXh and processed LVXh:EL100 subjected to milling using different percentages of LVXh for durations between 0.5-4 hours. (c) DSC ΔH_{Cryst} data of exotherm crystallization peak analysis of processed LVXh and processed LVXh:EL100 subjected to milling using different percentages of LVXh for durations between 0.5-4 hours.

3.3.2.3 Solid-State Changes

A summary of the crystallization and amorphization events, when analyzed via PXRD and DSC at various % (w/w) LVXh and BMing durations using a PM100, is presented in the schematic below (**Figure 3.6**). The green area highlights the crystalline and semi-crystalline LVXh samples according to PXRD analysis, with no appearance of crystallization via DSC analysis. This reveals that the material remains crystalline, despite the interference to the crystal structure shown by PXRD. The red section displays semi-crystalline LVXh via PXRD (presence of Bragg peaks) and crystallization of LVXh via DSC. This confirms the presence of the amorphous material. The blue region represents the complete amorphization of LVXh by PXRD analysis, in agreement with DSC analysis which demonstrates the crystallization of amorphous LVX. The turquoise zone presents unique results in that complete amorphization is

also confirmed via PXRD analysis, yet no crystallization of amorphous LVX occurs. This implies that the formation of a stable composite, amorphous system occurred with 30% (w/w) LVXh after 4 hours of BMing. This finding is in comparison to the MM400 which displayed amorphization via PXRD, and no crystallization of amorphous LVX at 50 and 60% (w/w) LVXh.

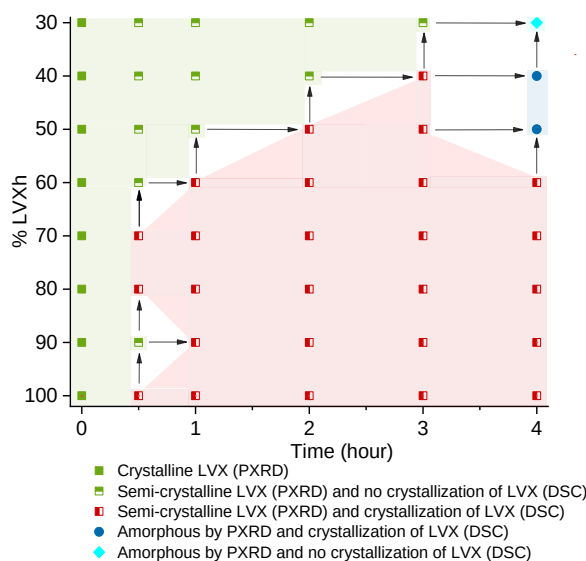


Figure 3.6 Schematic of crystallization and amorphization events via PXRD and DSC analysis for a PM100 for different % (w/w) LVXh and BMing durations. The different pathways of transformation to the independent phases are conveyed using black arrows.

3.3.3 In-Situ Measurements

The in-situ measurements of 50 % LVXh system were conducted while BMing the sample for 5 hours using three 0.4 cm stainless steel balls (**Figure 3.7**). The results demonstrated a slow decrease in the crystallinity of the system during the milling period. The slow amorphization kinetics allowed each of the crystallographic planes to be individually analyzed at specific time intervals. The peaks at approximately 4.5 and 7 nm⁻¹ displayed higher intensities compared to the other peaks detected. Nevertheless, it was discovered that each of the crystallographic planes of LVXh amorphized at the same time. This was confirmed by the presence of each of the LVXh peaks, which persisted throughout the entire measurement, with the relative intensities proportioned to the initial ones.

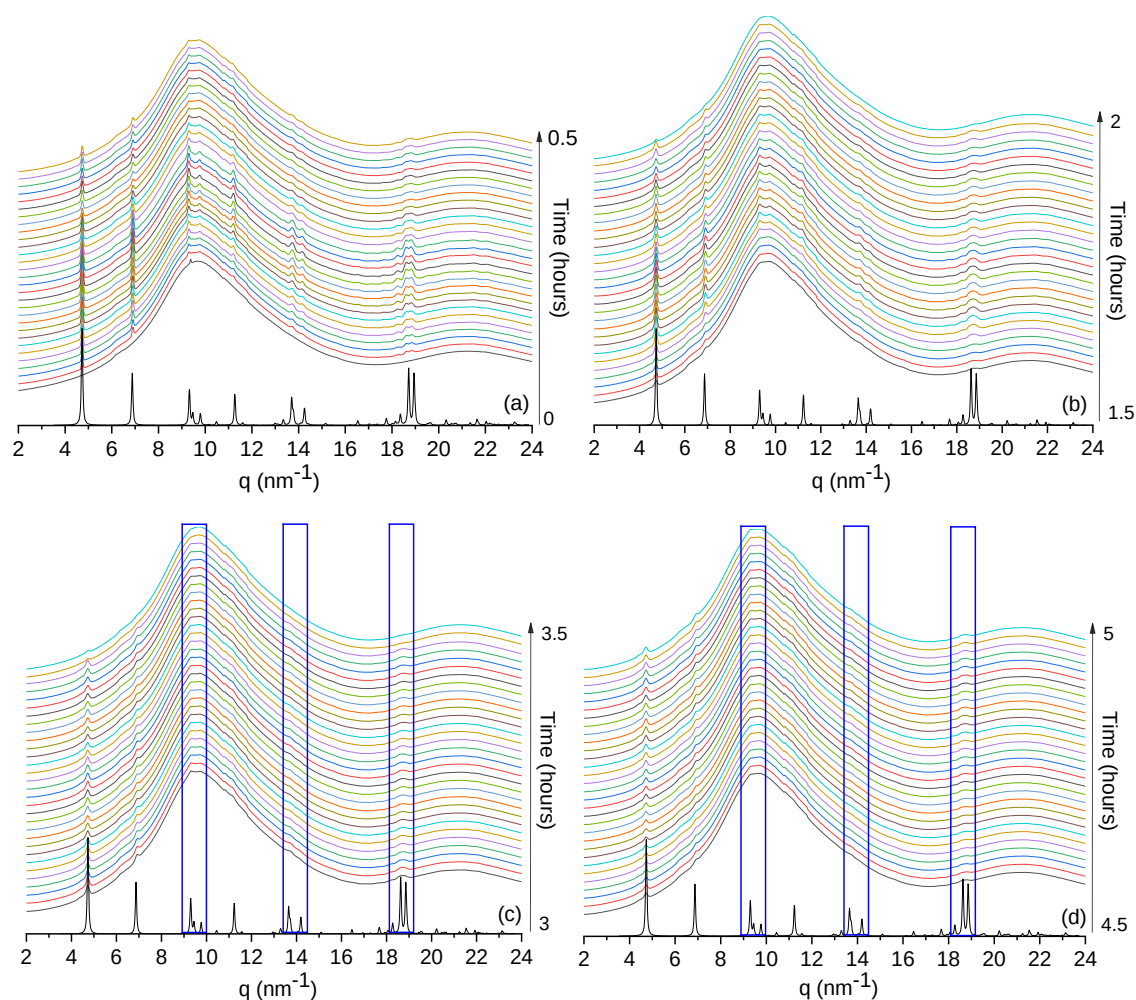


Figure 3.7 In-situ analysis of 50% (w/w) LVXh subjected to milling using Fritsch Vibration Ball Mill, 12 mL stainless steel jar at 50 Hz, using three 0.4 cm stainless steel balls for (a) 0-0.5 hours (b) 1.5-2 hours (c) 3-3.5 hours and (d) 4.5-5 hours.

Although amorphization occurred, complete amorphization was not achieved. This is in contrast to the MM400 and the PM100 % (w/w) LVX samples which displayed that $\leq 70\%$ (w/w) LVXh and $\leq 50\%$ (w/w) LVXh were completely amorphous via PXRD (**Figure 3.2a** and **3.3b**). Furthermore, this finding contrasts with PXRD and DSC as the combination of both analyses revealed that 70% (w/w) LVXh samples processed by the MM400 (**Figure 3.2**) and 40 and 50% (w/w) LVXh samples processed by a PM100 (**Figure 3.3b**, **Figure 3.4c**, and **3.6**) are fully amorphous.

Additional investigations were performed on the 50% (w/w) LVXh sample, however using a larger ball size of 0.9 cm with the aim of enhancing the mechanical impact (**Figure 3.8**).²³⁶ Visual comparisons were observed for the samples produced using different ball sizes and number of balls. The 0.9 cm stainless steel ball reaction produced a sample yellow color,

while the former sample that utilized three 0.4 cm balls generated an off-white sample color. This finding is comparable to other fluoroquinolone systems which were processed using a MM400 and a PM100 to produce a dark yellow/orange color (**Figure A.3.1**). Mesallati *et al.* also noted a change in the color of BMed CIP from “off-white” to “pale yellow”,⁵⁰ ASDs of BMed CIP and amino acids to an “intense yellow”, and semi-crystalline solid dispersions (SDs) were “pale yellow”,¹⁸ ASDs of BMed CIP and amino acids to an “intense yellow”, and semi-crystalline solid dispersions (SDs) were “pale yellow”,¹⁸ ASDs of BMed CIP and acidic polymers to a “dark yellow/orange” color,²²⁸ and ENRO from “pale yellow” to a “golden color” upon quench cooling.²⁰ This color change may be attributed to proton transfer.²³⁷

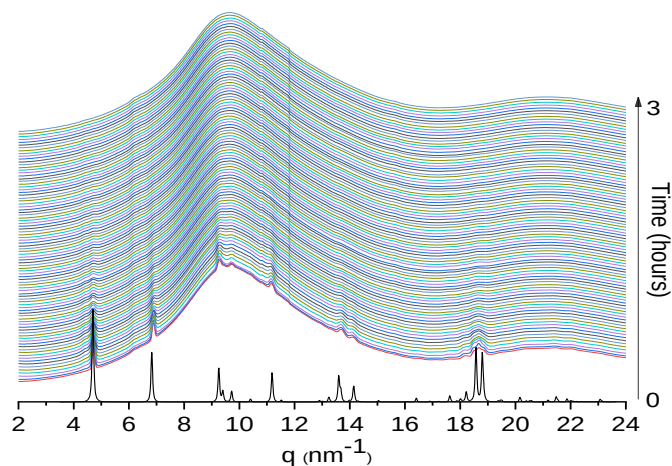


Figure 3.8 In-situ analysis of 50% (w/w) LVXh subjected to milling using Fritsch Vibration Ball Mill, 12 mL stainless steel jar at 50 Hz, using one 0.9 cm stainless steel ball for 0-3 hours.

The in-situ data showed that in harsher conditions using a larger ball, the complete amorphization of LVXh ensued at a faster rate i.e., within 3 hours (**Figure 3.8**). It was seen that the reduction of the crystalline planes occurred in the same manner as when three 0.4 cm balls were used (**Figure 3.7**). Thus, the crystallographic planes of LVXh gradually amorphized simultaneously, implying no weak crystallographic plane(s). The amorphization of LVX via mechanochemistry was successfully monitored via in-situ measurement. Overall, the findings strengthen the observation that different mills and parameters used produce different results (**Figure 3.7** and **Figure 3.8**).^{238, 239} In the case of altered parameters, Linberg *et al.* demonstrated that changes to the kinetics of transformation can occur based on the material of the jar, milling frequency, or ball size, which may impact the rate of activation.²³⁹ Likewise, Michalchuk *et al.*, highlighted the importance of the diameter and mass of the ball(s) on the reaction rate.²³⁸ Nevertheless, despite the research conducted on the impact of parameter change, two mills

utilizing different mechano- and tribo-chemical forces were compared for the first time to show that varying outcomes can be produced as a result.

The three mills that were chosen for comparative purposes i.e., the MM400, the PM100, and the vibration ball mill highlight the changes that exist in relation to the crystalline Bragg peaks as a result of varying forces and parameters. After 1 hour of milling, the MM400 displayed complete amorphization with $\leq 70\%$ LVXh (**Figure 3.2a**), and incomplete amorphization remained for samples milled using a PM100 (**Figure 3.6**) and a vibration ball mill (**Figure 3.8**). Nevertheless, complete amorphization ensued utilizing both processed upon further milling.

3.3.4 Infrared Analysis and the Mechanism of LVX:EL100 System Formation

The spectrum of EL100 presents characteristic absorption bands of the C=O stretching of the esterified carboxylic acid at 1730 cm^{-1} and the C=O stretching of the -COOH moiety at 1705 cm^{-1} . It is evident from **Figure 3.9a** that subtle changes occurred to the LVXh carbonyl group of the carboxylic acid in co-milled LVXh:EL100 formulations. Slight shifts from 1725 cm^{-1} for unprocessed LVXh²⁰⁴ to 1729 cm^{-1} presented for all ratios of LVXh:EL100, which may be as a result of H-bonding interactions between LVXh and EL100 and/or due to the presence of EL100.²⁰² Also, a shoulder at 1606 cm^{-1} was noted for the co-processed samples, most likely due to H-bond interactions in addition to some other slight changes to the band position and intensity in the fingerprint region. Previous studies by Mesallati *et al.* showed the possibility of proton transfer occurring from the carboxylic acid of the acidic polymer, EL100, to a proton acceptor group on CIP,¹⁹ a compound structurally similar to LVX. According to the literature, there have been reports that N2 and N3 nitrogen atoms on LVX have undergone protonation to form piperazinium salts in fluoroquinolone drugs.²⁴⁰ It is evident from IR that the absorption band of the acidic carbonyl group of LVX did not undergo significant changes. This suggests that the carboxylic acid of LVXh did not act as a proton donor group to facilitate proton transfer. However, the carboxylic group of EL100 is capable of ionization with the characteristic stretching vibration of the -COO⁻ group reported at 1556 and 1392 cm^{-1} .²⁴¹ Both bands were visible, especially in the 50% (w/w) LVXh co-milled sample with the former represented as a shoulder and the latter band had an increased intensity. Therefore, this indicated a potential acid-base interaction between LVX and EL100, consistent with previous studies.¹⁸⁻²⁰

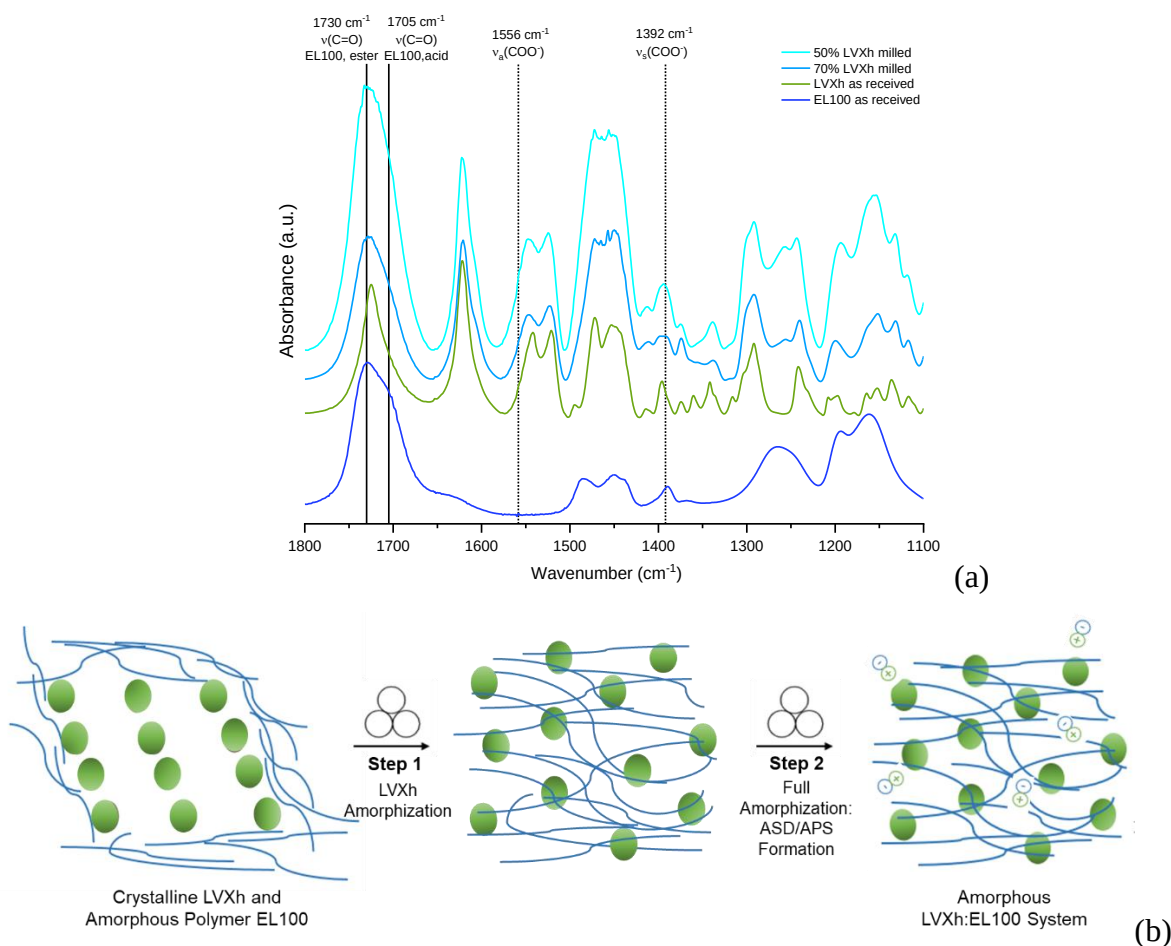


Figure 3.9 (a) FTIR analysis of unprocessed LVXh, EL100, and 50 and 70% (w/w) LVXh:EL100 subjected to milling for a duration of 1 hour using a MM400. (b) Proposed mechanism of formation of an ASD – amorphous solid dispersion; APS – amorphous polymeric salt LVXh:EL100 systems. The drug (green), polymer (blue), and charges (green and blue) are shown. The three balls denote mechano- and tribo-chemistry.

Overall, considering all co-milling efforts, it can be assumed that the formation of the amorphous LVX:EL100 systems is not instantaneous, but rather occurs as a two-step process. The first step involves drug amorphization and the second step engages in the reaction between the amorphized drug and the polymer, leading to polymer ionization (**Figure 3.9b**). The exothermic peak evident for most of the co-processed ratios, represents the crystallization of an amorphous drug. Upon the addition of increased polymer, the exothermic peak becomes broader and eventually disappears (**Figure 3.2b**). However, the proton transfer between the drug and EL100 can be confirmed by FTIR (**Figure 3.9a**). This perhaps is illustrated by the disappearance of the exotherm (**Figure 3.4c**), confirming the transformation from the amorphous drug phase to the APS form. The formation of APS is possible due to the existence and availability of LVXh proton donor and acceptor sites.²⁴⁰

3.3.5 Physical Stability of Amorphous LVX:EL100 Systems

3.3.5.1 Stability on Thermal Treatment

Samples containing 70-90% (w/w) LVXh were BMed between 0.5-4 hours and heated to the exotherm and past the endotherm peaks at 100 and 206 °C, respectively (**Figure 3.4**), and analyzed via PXRD in order to conduct a solid-state investigation of the samples. The identification of the anhydrous/dehydrate form of LVXh, known as anhydrous LVX form γ (LVX γ), appeared as discovered in previous findings upon mechanical processing of LVXh in the solid-state (**Figure 3.10**) (**Chapter 2**).²³⁵

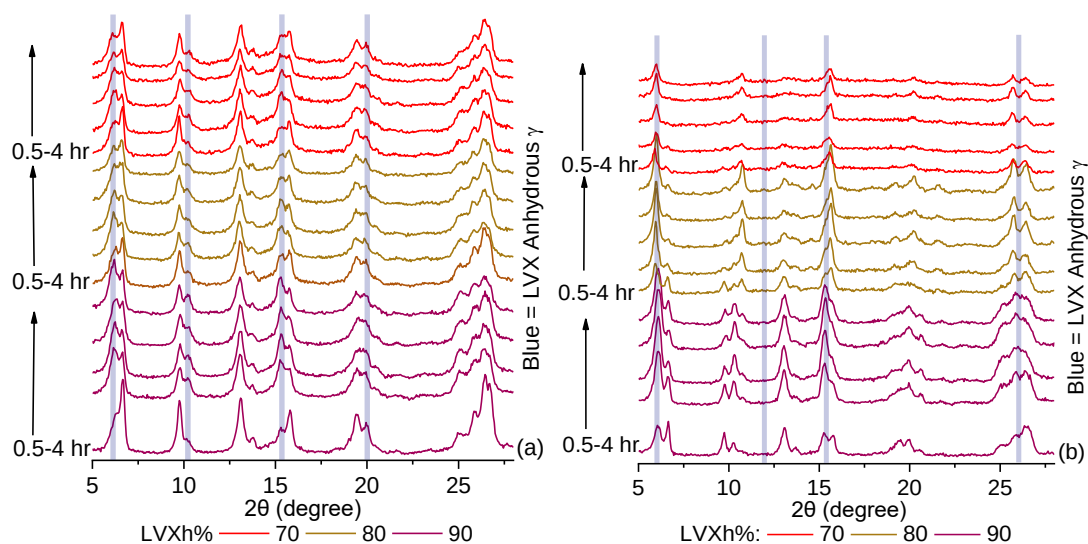


Figure 3.10 PXRD analysis after ball milling at different durations using different % (w/w) LVXh to investigate the polymorphic transformation of LVX following heating (a) to 100 °C (b) to 206 °C.

3.3.5.2 Stability upon Exposure to Humidity

DVS was used to analyze LVXh:EL100 with 40 and 50% (w/w) LVXh using different mills (**Figure 3.11**). These analyses were carried out to evaluate the effect of moisture on the solid-state stability of the BMed samples. A sample mass increase was shown for both samples when RH was increased. The powders sorbed 12 (sample post PM100 milling) and 18% (w/w) (sample post MM400 milling) of moisture at 90% RH. In contrast, the desorption plots illustrated a rapid decrease in mass (%), which highlights the quick weight loss and dehydration of the samples (**Figure 3.11a**). The gap between the sorption and desorption partial pressure ranges exhibits a typical hysteresis pattern, commonly observed for organic, non-rigid solids. The 40 and 50% (w/w) LVXh sample lost 12 and 18% (w/w) moisture between 90-0% RH (desorption) over 1,250 and 500 minutes (≈ 21 and 8 hours) (**Figure 3.11b**). The hygroscopicity demonstrated is expected for amorphous materials due to the random orientation of their

molecules facilitating water uptake due to the increase in free volume. Water sorption can offer disadvantages in terms of drug stability by potentially lowering the T_g , resulting in an increase in molecular mobility and plasticization, and thus, encouraging crystallization.²⁴²

The Young and Nelson model was used for this hysteresis to deconvolute moisture sorption into three mechanisms (**Figure 3.11d** and **Table A.3.1**).²⁴³ These include surface/monolayer adsorption, multilayer adsorption, and bulk/internal absorption. The best fit and experimental data are shown in **Figure 3.11c** and **3.11d**. Bulk absorption is the leading mechanism for 50% (w/w) LVXh. The 40% (w/w) LVXh sample has substantially lower internally absorbed moisture (0.07 g water/g material) than that of 50% (w/w) LVXh. The amount of internally absorbed moisture is comparable to that of the multilayer moisture (0.05 g water/g material) for the 40% (w/w) LVXh sample. Lastly, water was also evident on the surface/monolayer of the 40% (w/w) LVXh sample while absent for 50% (w/w) LVXh sample.

After DVS analysis, PXRD analysis was carried out on both samples and compared to the starting material. It was observed that the 40% (w/w) LVXh sample showed slight evidence of crystallization while that comprising 50% (w/w) LVXh remained amorphous (**Figure A.3.2**). The traces of crystallization for the 40% (w/w) sample is in alignment with findings from **Figure 3.6**, which suggests that the most stable sample produced by a PM100 is that of 30% (w/w) LVXh. The excellent stability of 50% (w/w) LVXh sample made using a MM400 is in agreement with **Figure 3.2b** which displays the absence of an exothermic peak, suggesting improved stability.

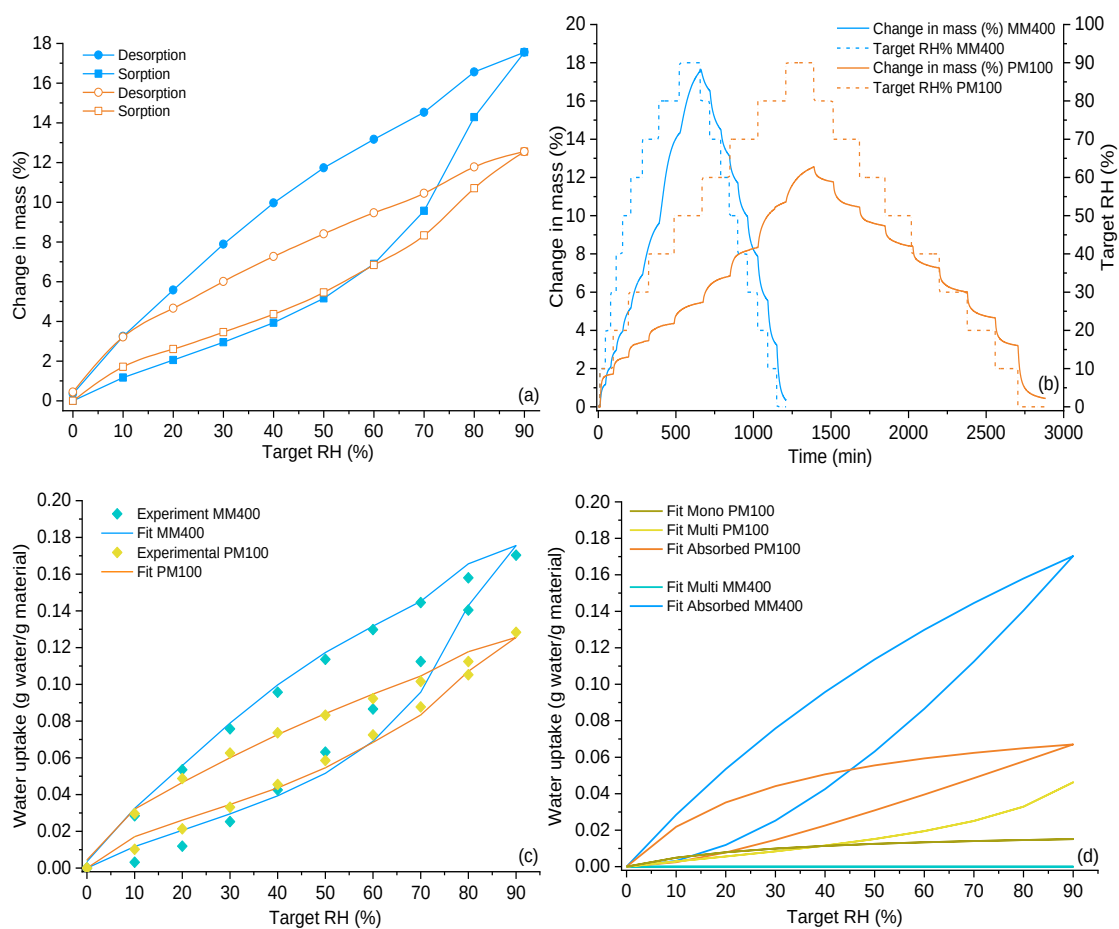


Figure 3.11 DVS analysis of 40% (w/w) LVXh after being subjected to milling for 4 hours at 400 rpm using a PM100 (orange and yellow) and 50% (w/w) LVXh after subjected to milling for 1 hour at 25 Hz using a MM400 (blue and turquoise) (a) isotherm plot (b) mass plot (c) Young-Nelson model best fit and (d) deconvoluted sorption behavior for the bulk, monolayer and multilayers.

3.4 Conclusions

An investigation into BMing LVXh with an excipient, EL100, was conducted for the first time, according to the literature. The solid-state changes were identified using PXRD, in-situ PXRD, DSC, FTIR, and DVS. Different mills such as a Retsch mixer mill MM400, a Retsch planetary ball mill PM100, and a Fritsch vibration mill were utilized to compare LVXh:EL100 samples and the impact that the same process may have on product outcome. Indeed, the findings revealed variability in the product outcome depending on the mill used, and in some cases, the parameters chosen.

PXRD analysis for the PM100 and the MM400 samples confirmed complete amorphization when ≤ 50 or $\leq 70\%$ (w/w) LVXh was present in the co-processed samples. This highlights that a higher percentage of LVXh can be incorporated into the system and successfully amorphized when using a MM400. DSC analysis for the PM100 and the MM400 samples disclosed that the powders containing 40-90 or 70-90% (w/w) of the drug comprised of LVXam. A combination of PXRD and DSC analysis on the PM100 and the MM400 samples revealed that the 40 and 50% (w/w) LVXh (PM100) or 70% (w/w) LVXh (MM400) samples are fully amorphous. Furthermore, a combination of these techniques led to the disclosure that the absence of an exothermic peak for 30% (w/w) LVXh (PM100) or 50-60% (w/w) (MM400) LVXh samples may indicate the stability of the amorphous systems.

In-situ measurements disclosed that each of the crystallographic planes of LVXh amorphized at the same time. FTIR indicated a potential acid-base interaction between LVX and EL100. This finding, in combination with PXRD and DSC, assisted in revealing the mechanism of LVX:EL100 system formation. It may be the case that the formation of the amorphous LVX:EL100 systems did not occur instantaneously, but rather as a two-step process. The first step may undergo amorphization of the drug, and the second step may consist of a reaction between the amorphized drug and the polymer, ultimately, leading to polymer ionization. Thermal treatment showed the identification of the anhydrous/dehydrate form of LVXh, known as anhydrous LVX form γ (LVX γ). This form may play a role in achieving the desired outcome of amorphous material. DVS displayed substantial moisture sorption, 12 and 18% (w/w) at 90% RH. PXRD analysis after the DVS measurements showed that 40% (w/w) LVXh sample, processed by a PM100, only slightly crystallized back to LVXh while the 50% (w/w) LVXh system, milled by a MM400, remained amorphous. This strengthens the hypothesis

that the presence of an exotherm alludes to a less stable amorphous system, whilst the disappearance of the exotherm implies improved physical stability.

In summary, these findings demonstrate that the production of amorphous systems is possible by utilizing all milling processes investigated in this work. Nevertheless, variability in product outcome was demonstrated, highlighting the importance of mill selection on process and product optimization. The mechanism by which amorphization and optimal stability of LVXh:EL100 systems was revealed. To conclude, a more sustainable approach for achieving amorphization of the fluoroquinolone drug, LVXh, was accomplished, and advancements to the fast-growing world of mechano- and tribo-chemistry were made.

Chapter 4.0

Solid-State Characterization and Stabilization of Amorphous and Semi-Crystalline Polymeric Systems of Ciprofloxacin Prepared by Mechanical Activation

Chapter 4.0 Solid-State Characterization and Stabilization of Amorphous and Semi-Crystalline Polymeric Systems of Ciprofloxacin Prepared by Mechanical Activation

4.1 Introduction

Poor solubility of BCS Class II and IV drugs remains one of the biggest challenges facing the pharmaceutical industry today. The solubility of poorly soluble drugs can be enhanced by disrupting the crystal lattice by means of amorphization and/or salt formation.²¹ The amorphous form of a drug is the most energetic form in the solid-state due to its disordered make up and absence of long-range order.²² In consequence, less energy is required to enter into solution, and thus, the amorphous form of the drug is known to be more soluble than the crystalline form.²³ However, the amorphous form of the API is known to be poorly stable and often converts to its most stable, crystalline form. Fortunately, the use of polymer(s) can generate an anti-plasticizing effect and increase the overall Tg of the system produced. Furthermore, additional hydrogen bond interactions can form between the API and the polymer. A combination of these outcomes can reduce the molecular mobility of the API and enhance the stability of the amorphous drug. This approach is known as an ASD. An ASD is a term used to describe a system where the API is in the amorphous form and is distributed within a polymer matrix in the solid phase. However, the stability of ASDs may be further developed by the inclusion of an ionic interaction, resulting in the formation of an APS.^{19, 20}

CIP, a fluoroquinolone antibiotic was chosen for the investigation of ASDs/APSs for several reasons. Firstly, CIP is an important drug as it has a broad spectrum of antimicrobial activity against Gram positive and Gram negative bacteria and is listed by the WHO as an essential medicine.¹²⁸ Furthermore, CIP is a poorly soluble BCS Class IV drug due its possession of an overall neutral charge and strong intermolecular interactions, resulting in a solubility of 0.06 and 0.08 mg/mL in water at 25 and 37 °C, respectively.¹²⁹ Lastly, CIP can form a salt owing to the presence of both an acidic and basic group and its ionizable, commercially available, zwitterionic form which exists in equilibrium (**Figure 1.10**).^{19, 50, 131} This was demonstrated by Mesallati *et al.* by the deprotonation of an acidic counterpart, such as polymers or amino acids, to facilitate ASDs/APSs formation of CIP.^{18, 19} Salts of CIP and other fluoroquinolone drugs such as ENRO have been formed and reported in the literature using other counterparts such as sweeteners,²⁴⁴ and carboxylic acids/²⁴⁵acidic counterions.^{19, 129}

Amorphization and salt formation of CIP is challenging due to its poor solubility in common solvents such as ethanol, methanol, propanol, acetone, and chloroform,¹³⁰ and its thermal degradation properties.⁵⁰ These restrictions reduce the potential processes/techniques that can be applied to convert the drug. Fortunately, the BMing process can overcome these limitations as demonstrated for fluoroquinolone drugs LVXh (**Chapter 3**), CIP, and ENRO.^{4, 5, 9, 10} This was displayed by the formation of ASD/APSs with excipients and the elimination of solvents, whilst preventing degradation.^{18, 19}

Mechanochemistry is an approach that can produce forms of the drug with improved solubility and properties while eliminating/reducing solvents for the production of APIs. Mechanochemistry plays an important role in the future of green chemistry for the pharmaceutical industry, and thus, there is a need for further contributions to this field of chemistry. Parameters utilized during the BMing process and different mills used must be further investigated in order to fully understand the impact of milling conditions. According to the literature, research has been carried out on the ball mill to produce the amorphous form of the disaccharide sugar, lactose, and the API, curcumin using different ball sizes.¹⁵ An exploration into ASDs of the API, ibuprofen, and the natural compound, resveratrol using different ball mill frequencies was also performed.¹⁵ An analysis into BMing durations for LVXh (described in **Chapter 3**), CIP, and ENRO was performed previously to form amorphous material, ASDs, and/or APSs.^{5, 6} Furthermore, different ball mills and parameters such as number of balls used, ball and jar sizes, ball and jar materials, and sample size were examined for the first time on the fluoroquinolone drug, LVXh, as described in **Chapter 3**.

CIP does not contain the same level of complexity as LVXh in terms of its solid-state, however, its poor solubility makes it a desirable drug for ASD/APS formation. Thus, an investigation into different mills and parameters including the number of balls used, ball and jar sizes, and ball and jar materials was reported for the first time in the literature for CIP ASD/APSs. Lastly, according to the literature, neutral polymers displayed the inability to fully amorphize CIP¹⁹ or ENRO,²⁰ and thus, deprotonation of the polymer was not investigated, nor reported. Therefore, up until now, salt formation of fluoroquinolone drugs with neutral polymers has not been reported in the literature.¹⁹ The stability of the amorphous phase within a semi-crystalline polymeric system and its dependence on the type of polymer was investigated. In summation, an investigation into BMing parameters to produce ASD/APSs of CIP and the salt

formation and stability of amorphous and semi-crystalline polymeric systems was investigated in this chapter.

In this study, different parameters were considered to investigate the impact on the solid-state properties of the systems CIP:EL100, CIP:HPMCP-55, CIP:HPMC, and CIP:KolVA64, concentrating on the reduction of crystallinity. Moreover, investigations were carried out to identify drug-polymer interactions between CIP and the individual polymers. CIP:EL100 and CIP:HPMCAS-L have been previously investigated,¹⁹ however, HPMCP-55, HPMC, and KolVA64 have not been explored in binary systems with CIP. These polymers were selected due to their amphiphilic and semi-rigid properties, and high glass transition temperatures (T_g) (as described in **Chapter 1**). The samples were characterized in the solid-state form by powder X-ray diffraction, thermal analysis, FTIR, SSNMR, and DVS analysis.

4.2 Experimental Section

4.2.1 Materials

Anhydrous CIP was purchased from Carbosynth Limited (UK). The listed polymers were purchased: methacrylic acid methyl methacrylate copolymer (Eudragit® L100, Evonik, Germany), copovidone, Copolyvidone, VP/VAc copolymer 60/40, copolymer of 1-vinyl-2-pyrrolidone and vinyl acetate in a ratio of 6:4 by mass (Kollidon® VA64, BASF, Germany). The following polymers were donated by Shin-Etsu Chemical Co., Ltd., Japan: Cellulose Mw 20,000 g/mol (HPMC) and cellulose, hydrogen 1,2-benzenedicarboxylate, 2-hydroxypropyl methyl ether (HPMCP-55). Deionized water (14.0 MΩ·cm) prepared using an Elix 3 connected to a Synergy UV system (Millipore, UK) was used in all experiments. Potassium bromide FTIR grade, ≥ 99% trace metals basis, was purchased from Merck (UK).

4.2.2 Methods

4.2.2.1 Milling

4.2.2.1.1 Retsch Mixer Mill MM400

CIP was BMed with polymers to design binary systems using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s). A 25 mL ZrO₂ jar was used. A sample of 1 g was added to the jar. Two ZrO₂ balls of 1.2 cm and weight of 5 g each was used. This condition will be referred to as **MM400_Cond1**. The binary acidic polymer sample CIP:EL100 and the binary neutral polymer samples CIP:KolVA64 and CIP:HPMC were milled for a total duration of 99 minutes with no breaks in between. Approximately 2 mg of the sample was removed for PXRD analysis (described in **Section 2.2.2.3**). Approximately 0.1 g of sample was removed for SSNMR analysis (described in **Section 4.2.2.7**).

CIP was BMed with polymers to design binary systems using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s). A 25 mL ZrO₂ jar was used. A sample of 1 g and ten ZrO₂ balls of 1 cm and weight of 3 g each was added to jar. This condition will be referred to as **MM400_Cond2**. Ball milling was conducted at room temperature with maximum intervals of 99 minutes. A 15-minute break was implemented after every 99 minutes of milling. The binary acidic polymer sample CIP:HPMCP-55 was milled between 3-6 hours at timepoints taken at 1 hour intervals. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if necessary. Around 2 mg of sample was

removed for PXRD analysis (described in **Section 2.2.2.3**). Around 15 mg of sample was removed for analysis via DSC (described in **Section 2.2.2.4**).

CIP was BMed with polymers to design binary systems using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s). A 50 mL stainless steel grinding jar was used. A sample of 2 g and eight stainless steel milling balls with a diameter of 1.2 cm and weight of 7 g each was added to jar. This condition will be referred to as **MM400_Cond3**. Ball milling was conducted at room temperature with maximum intervals of 99 minutes. A 15-minute break was implemented after every 99 minutes of milling. The binary acidic polymer sample CIP:HPMCP-55 was milled between 3-6 hours at timepoints taken at 1 hour intervals. The samples were taken by opening the jar and removing the sample amount required for analysis using a spatula. Once the sample was removed, the jar was closed again before the continuation of further ball milling, if necessary. Around 2 mg of sample was removed for PXRD analysis (described in **Section 2.2.2.3**). Around 15 mg of sample was removed for analysis via DSC (described in **Section 2.2.2.4**).

4.2.2.1.2 Retsch Planetary Ball Mill PM100

CIP was BMed with polymers to design binary systems using a Retsch planetary ball mill PM100 (Germany) at 400 rpm (described in **Section 2.2.2.1.1, Method 1**). The binary acidic samples CIP:HPMCP-55 and CIP:EL100 were milled between 3-6 hours at timepoints taken at 1 hour intervals. The binary neutral polymer samples CIP:KolVA64 and CIP:HPMC were milled for 4 and 6 hours. Around 2 mg of sample was removed for PXRD analysis (described in **Section 2.2.2.3**). Around 30 mg of sample was removed for DSC and MTDSC analysis (described in **Section 2.2.2.4** and **Section 2.2.2.5**). An additional 5 mg of sample was removed for analysis via FTIR (described in **Section 2.2.2.7**), and 15 mg of sample was removed for analysis via DVS (described in **Section 2.2.2.8**).

Table 4.1 Conditions of milling using the Retsch mixer mill (MM400) and the Retsch planetary mill (PM100).

Mill	Condition	Jars (mL)	Balls (cm Ø)	Frequency/Speed (rpm or Hz)	Duration (hours)	Powder Load (g)	Samples
Retsch Mixer Mill MM400	MM400_Cond1	ZrO ₂ 25	2 ZrO ₂ balls, 1.2	25 Hz	1.65	1	CIP:EL100 CIP:KolVA64 CIP:HPMC
Retsch Mixer Mill MM400	MM400_Cond2	ZrO ₂ 25	10 ZrO ₂ balls, 1.0	25 Hz	3, 4, 5, 6	1	CIP:HPMCP-55
Retsch Mixer Mill MM400	MM400_Cond3	S.S. 50	8 SS balls, 1.2	25 Hz	3, 4, 5, 6	2	CIP:HPMCP-55
Retsch Planetary Ball Mill PM100	PM100_Cond1	S.S. 50	3 SS balls, 1.5	400 rpm	A) 3, 4, 5, 6 B) 4 or 6	2	A) CIP:HPMCP-55 CIP:EL100 B) CIP:KolVA64 CIP:HPMC

4.2.2.2 Powder X-Ray Diffraction (PXRD)

As described in **Section 2.2.2.3**.

4.2.2.3 Differential Scanning Calorimetry (DSC)

As described in **Section 2.2.2.4**.

4.2.2.4 Modulated Temperature Differential Scanning Calorimetry (MTDSC)

As described in **Section 2.2.2.5**. ≈ 7 to 8 mg samples were analyzed.

4.2.2.5 Calculation of Theoretical Glass Transition (T_g) Values with Gordon–Taylor Equation

The theoretical T_g s of the binary systems were calculated using the Gordon–Taylor equation:^{246, 247}

Equation 4.1 Gordon–Taylor equation.^{239, 240}

$$T_g = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \quad \text{Eqn. (4.1)}$$

Equation 4.2 K value for the Gordon–Taylor equation.^{239, 240}

where K is approximately equal to:

$$K \approx \frac{T_{g1} \rho_1}{T_{g2} \rho_2} \quad \text{Eqn. (4.2)}$$

w_1 and w_2 are the weight fractions of the components, T_{g1} and T_{g2} are the glass transition temperatures of CIP and the polymer, and ρ_1 and ρ_2 are the true densities of the two constituents. The T_g of pure amorphous CIP was previously determined as 86.7 °C.⁵⁰ The T_g s of the polymers and binary systems were obtained as described in **Section 2.2.2.5**. However, the T_g values of the polymers (**Table 1.1**) and the average true density data were also sourced from the literature.^{19, 248} The experimental values measured by MTDSC were compared to the theoretical T_g s using the Gordon–Taylor (G-T) equation (**Equations 4.1 and 4.2**).

4.2.2.6 Solid State Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was carried out (as described in **Section 2.2.2.7**). Unprocessed CIP and BMed samples were first mixed with dried KBr using a spatula in a ratio of 1:30 for CIP and 1:20 for BMed samples.

4.2.2.7 Solid State Nuclear Magnetic Resonance (SSNMR)

SSNMR was performed and analyzed by Mr. Mateo Flores Naranjo, with involvement from Prof. Sarah Hudson (University of Limerick, Ireland). The ^{13}C cross polarization magnetic angle spinning (CPMAS) SSNMR spectra were collected on a Bruker Avance III HD NMR spectrometer operating at a 9.4 T magnetic field with ^1H and ^{13}C resonance frequencies of 400.1 and 100.6 MHz respectively. The samples were filled in 4 mm zirconia rotors at room

temperature and ambient conditions and capped (**Table 4.2**). The spectra were referenced using the high frequency ^{13}C resonance of adamantane to 38.48 ppm. The spectra were acquired at natural abundance with a double resonance triple channel 4 mm Bruker MAS probe. Proton decoupling was carried out with the SPINAL6466 decoupling sequence at 100%. For each sample, the ^1H T1 relaxation time(s) were checked using the saturation recovery pulse sequence to ensure that the recycle delay allowed for adequate relaxation between the collection of subsequent transients (at least $1.4 \times \text{T1}$ values). The data was acquired by cross-polarization pulse experiment with a magic angle spinning rotor frequency of 10 KHz. All acquisition parameters were optimized for each sample, and the relaxation delay (D1) was standardized to 16.3 seconds (**Table 4.2**).

Table 4.2 Relaxation times and acquisition parameters (contact times and number of scans) for CIP, polymers, and binary amorphous polymeric systems.

Samples	Relaxation time T1 (second)	Contact Time (μ second)	Number of scans
CIP (“as supplied”)	10.17	1500	1000
EL100	0.57	1500	1000
HPMCP-55	1.5	2500	1000
CIP:EL100 1:1 (amorphous)	1.24	1000	1000
CIP:HPMCP-55 1:1 (amorphous)	1.71	500	1000

4.2.2.8 Dynamic Vapor Sorption (DVS)

DVS was used to investigate BMed binary samples by use of an Advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., UK) (as described in **Section 2.2.2.8, Method 1**).

4.3 Results and Discussion

4.3.1 Production of Binary Amorphous Solid Dispersions

A range of binary systems of CIP using acidic and neutral polymers were produced by ball milling. The amorphous, acidic polymers chosen for investigation were HPMCP-55 and EL100, and the neutral polymers selected were KolVA64 and HPMC. Although similar studies on ASDs of CIP were previously described by Mesallati *et al.*,¹⁹ an investigation into different parameters of a MM400 and the use of HPMCP-55, KolVA64, and HPMC had not yet been investigated. It is known that increased polymer content % (w/w) and induced heat can assist in producing fully amorphous material. Thus, 50% (w/w) polymer content was chosen rather than the previously explored 40% (w/w) polymer content, in most instances.¹⁹ However, rather than inducing heat which may cause scale-up limitations in a pharmaceutical setting, different parameters, impacting the solid-state of the resulting systems, were explored.

4.3.2 Powder X-Ray Diffraction (PXRD)

Two separate milling conditions were conducted to investigate CIP:EL100 using 40 and 50% (w/w) CIP as discussed in **Section 4.2.2.1** and **Table 4.1**. The implementation of the **MM400_Cond1** led to the complete abolishment of crystalline Bragg peaks (**Figure 4.1**). This is comparable to the MM400 milled samples of LVXh:EL100 samples that demonstrated an amorphous halo after 0.5 hours of milling with a powder load of 0.5 g (**Chapter 3**). Although the conditions used are slightly different in that a scale-up of 1 g was conducted in these studies, the results show comparability despite scale-up and a change of fluoroquinolone API.

The PM100_Cond1 was utilized and a scale-up from 1 g (**MM400_Cond1**) to 2 g samples was investigated using 40 and 50% (w/w) CIP samples. A sample containing 40% (w/w) CIP content demonstrated the persistence of peak 4 at $25.5^\circ 2\theta$ after 6 hours. The sample containing 50% (w/w) CIP presented the persistence of Bragg peaks 1, 2, 3, and 4 at 14.5 , 16.7 , 20.9 , and $25.5^\circ 2\theta$ after BMing for 3 hours. Further processing after 4 and 5 hours led to the abolishment of peak 2, followed by the elimination of peaks 1 and 3 after 6 hours of milling. The remaining peak 4 at $25.5^\circ 2\theta$ after 6 hours of milling highlights the strength of this peak. However, further processing of 7 hours led to the elimination of peak 4 at $25.5^\circ 2\theta$. This demonstrates that further processing time is required to completely abolish CIP peaks using a PM100 compared to a MM400.

Similar studies using a MM400 and the same parameters (**Section 3.2.2.1** and **Table 3.1**) were performed on LVXh:EL100 samples. The results showed the complete formation of an

amorphous halo after a process duration of 4 hours using 50% (w/w) LVXh (**Chapter 3**). This demonstrates that additional energy is required to decrease the level of crystallinity of CIP compared to LVXh since further process times were necessary to further eliminate crystalline Bragg peaks of CIP. Thus, the CIP:EL100 results obtained and the comparison to LVXh:EL100 samples display that a decrease in the degree of crystallinity of the drug is API dependent, and further processing of the material may be required to achieve the same desired outcome.

Overall, the results obtained demonstrate the advantages of utilizing a MM400 over a PM100 for the production of completely amorphous material and reduced process durations. This is also in agreement with previous studies described using LVXh:EL100 systems (as described in **Chapter 3**).

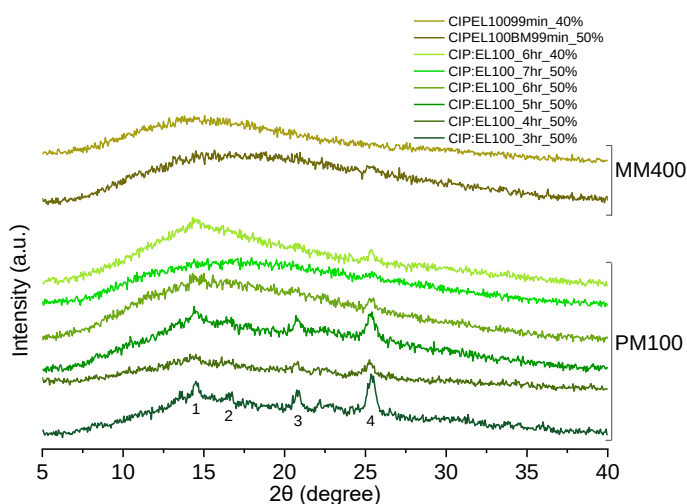


Figure 4.1 PXRD analysis of unprocessed CIP and processed CIP with EL100 at a 40 and 50% (w/w) concentration subject to milling for 99 minutes using the MM400_Cond1 and between 3-7 hours using the PM100_Cond1 to generate ASDs.

Three different milling conditions were used to investigate CIP:HPMCP-55 using 50% (w/w) CIP. The PM100_Cond1 was compared to the MM400_Cond2 and the MM400_Cond3 for CIP:HPMCP-55 and CIP:EL100 samples (**Section 4.2.2.1** and **Table 4.1**) since the decrease in the degree of crystallinity was seen using MM400 in **Chapter 3**. The MM400_Cond2 and the MM400_Cond3 methods were used to investigate the effect of using an increased number of balls to allow more powder to be affected by CLC and generate more impact forces, which may result in the production of higher energy systems.

The PM100_Cond1 displayed Bragg peaks 1, 2, 3, and 4 at 14.5, 16.7, 20.9, and 25.5° 2θ after 3-5 hours of milling with peaks 1, 3, and 4 remaining the most prominent of the four peaks. Continuous milling of CIP:HPMCP-55 for 6 hours presented peaks 1, 3, and 4 at 14.5, 20.9, and 25.5° 2θ (**Figure 4.2**). The remainder of crystalline Bragg peaks is similar to

CIP:EL100 sample which displayed Bragg peaks 1-4 after 3-5 hours of milling. However, the CIP:HPMCP-55 results produced after 6 hours of milling are in slight contrast to CIP:EL100 which showed the abolishment of peaks 1-3, while peak 4 remained unchanged (**Figure 4.1**). Thus, the polymer chosen may influence the degree of crystallinity of CIP. Again, this is in contrast to LVXh:EL100 which demonstrated the complete abolishment of crystalline Bragg peaks. This highlights the impact of both the API and polymer selection in reducing the degree of crystallinity of the drug.

The MM400_Cond2 is comparable to the PM100_Cond1 which highlights that utilizing a softer material (ZrO_2) can be comparable to stainless steel if CLCs are enhanced. Furthermore, it shows that the increase in impact forces or the use of a harder material (stainless steel) in isolation may not necessarily result in the complete abolishment of peaks (**Figure 4.2**). Thus, a combination of stainless steel and the CLC may have a significant impact on the degree of crystallinity of the API.

Milling CIP:HPMCP-55 using the MM400_Cond3 indicated that peak 4 at $25.5^\circ 2\theta$ was the strongest peak since traces existed after 3 hours of milling following the final abolishment of peaks 1, 2, and 3 (**Figure 4.2**). This finding is in agreement with reports by Mesallati *et al.* which suggested that $25.3^\circ 2\theta$ for CIP may be the cleavage plane of CIP zwitterion.⁵⁰ This is in contrast to LVXh:EL100 sample which showed that each of the crystallographic planes of LVXh amorphized at the same time (**Chapter 3**). Nevertheless, CIP:HPMCP-55 amorphized after 4 hours of milling. Thus, further BMing demonstrated that it is possible to eliminate all peaks in the CIP:HPMCP-55 50% (w/w) system by altering parameters without increasing the polymer content. The complete amorphization of CIP using HPMCP-55 and the MM400_Cond3 is in contrast to results produced using the PM100_Cond1 and the MM400_Cond2. However, the findings are in agreement with CIP:EL100 sample results which used the MM400_Cond1. Nevertheless, CIP:EL100 displayed an amorphous system without the need to alter parameters, in contrast to the CIP:HPMCP-55 sample.

The MM400_Cond3 was the only condition used that demonstrated complete amorphization of CIP when using HPMCP-55. Complete removal of crystalline Bragg peaks was achieved using this condition after 4 hours of milling.

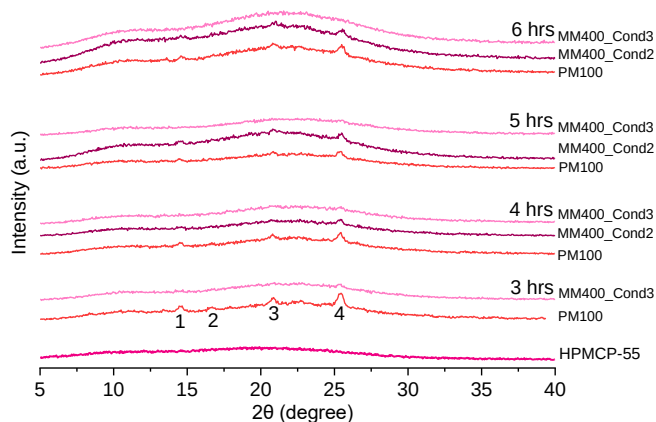


Figure 4.2 PXRD analysis of processed CIP with HPMCP-55 at a 50% (w/w) concentration subject to milling between 3-6 hours using the PM100 (red), the MM400_Cond2 (plum purple), and the MM400_Cond3 (light pink).

Two separate milling conditions, the MM400_Cond1 and the PM100_Cond1, were used to investigate CIP:KolVA64 and CIP:HPMC using 50% (w/w) CIP (**Section 4.2.2.1** and **Table 4.1**). Both conditions and mills are comparable in that crystalline Bragg peaks were reduced and broadened, suggesting crystallite size reduction and lattice microstrains.^{227, 232} Nonetheless, complete amorphization of CIP was not achieved using neutral polymers HPMC or KolVA64 (**Figure 4.3**). This is in alignment with previous studies by Mesallati *et al.* whereby the incorporation of PVP, Soluplus[®], or PEG 4000 failed to amorphize CIP.¹⁹ Despite the theoretical ability of CIP to form hydrogen bond interactions with neutral polymers, the interactions may be too weak to achieve complete amorphization,¹⁹ however, partial amorphization may be achieved through this type of bond interaction.

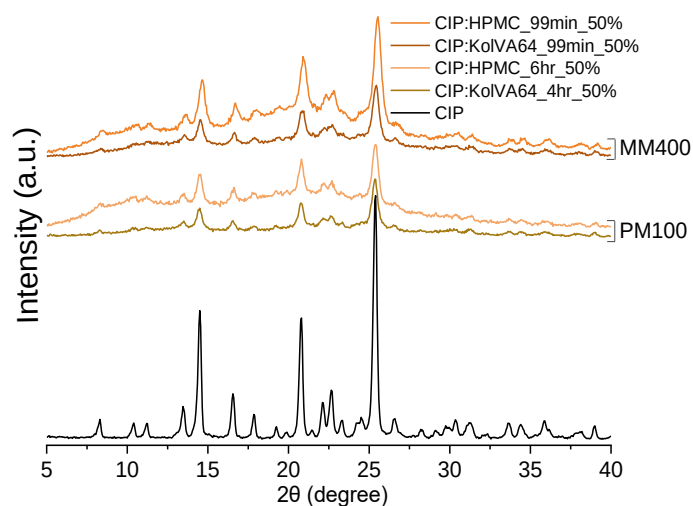


Figure 4.3 PXRD analysis of processed CIP with neutral polymers KolVA64 or HPMC at a 50% (w/w) concentration subject to milling at 4 and 6 hours using a PM100, 50 mL stainless steel jar, and three 1.5 cm balls at 400 rpm (PM100_Cond1) and 99 minutes using a MM400, 25 mL ZrO₂ jar, and two 1.2 cm ball at 25 Hz (MM400_Cond3).

4.3.3 Thermal Analysis

Thermograms of all samples were investigated for a dehydration peak (**Figure 4.4**). Unprocessed EL100 had a dehydration temperature at 62.3 °C and CIP:EL100 processed samples depicted subtle decreases in dehydration peak temperatures to 60.1-60.8 °C after 6 and 7 hours of milling. The thermogram of unprocessed HPMCP-55 showed a dehydration peak at 54.7 °C. The CIP:HPMCP-55 processed samples using the PM100_Cond1, the MM400_Cond3, and the MM400_Cond2, displayed dehydration peaks at 51.6, 63.1 and 66.6 °C, after 6 hours of milling.

Since amorphization was detected for CIP:EL100 or CIP:HPMCP-55 samples via PXRD analysis (**Figure 4.1** and **Figure 4.2**), thermograms were investigated for exothermic peaks of crystallization (**Figure 4.4**). No crystallization peaks were observed for CIP:EL100 (**Figure 4.4a**) or CIP:HPMCP-55 (**Figure 4.4b**) samples using the PM100_Cond1. This may indicate the stability of the amorphous form of the drug and its ability to withstand exposure to high temperatures. This is in contrast to LVXh:EL100 samples which displayed exotherms for 40-90% (w/w) LVXh while utilizing the PM100_Cond1. No exotherm was detected for LVXh:EL100 containing 30% (w/w) LVX (**Chapter 3**). This confirms that perhaps more CIP can be incorporated into the system and achieve stability of the amorphous drug than the API LVXh. This highlights the differences that exist between the fluoroquinolone drugs since process parameters were unchanged. This confirms that different stabilities of the amorphous

phases exist depending on the API chosen, and thus, different drug loading capabilities exist for individual APIs such as CIP and LVXh. CIP:HPMCP-55 was further examined using the MM400_Cond2 and the MM400_Cond3 (**Figure 4.4b**). Both conditions also displayed the absence of any exotherm peak of crystallization, indicating the stability of the amorphous form. Additional studies were performed for LVXh:EL100 that used a MM400 for comparative purposes. The findings revealed the ability to produce stable amorphous forms with 50-60% (w/w) LVXh (**Chapter 3**). This was indicated by the absence of an exotherm of crystallization. This highlights that improvements may be made to the stability of the amorphous phase and the drug loading % (w/w) by utilizing a MM400. The semi-crystalline samples were closely investigated for exothermic peaks of crystallization. CIP:HPMC did not display an exotherm of crystallization (**Figure 4.4c**). This is in contrast to the semi-crystalline CIP sample containing neutral polymer KolVA64. CIP:KolVA64 displayed an exothermic peak of crystallization at 199.4 °C. This confirms the presence of the amorphous form of the drug and that the sample is semi-crystalline in nature, in agreement with the reduction of Bragg peaks seen by PXRD (**Figure 4.3**). This may further confirm the stability of the amorphous phase of CIP:HPMC sample since no exotherm of crystallization was detected. This finding may indicate that the amorphous component of CIP in CIP:HPMC is more stable than the amorphous phase in the CIP:KolVA64 system.

Thermograms of unprocessed EL100 showed the melting/decomposition onset value of EL100 was 193.1 °C (**Figure 4.4a**). Upon processing, the melting/decomposition onset value of CIP:EL100 moved to 191.3 °C after 6 hours and further reduced to 187.3 °C after 7 hours of milling. The melting/decomposition of HPMCP-55 was 180.5 °C (**Figure 4.4b**). Processed CIP:HPMCP-55 samples using the PM100_Cond1, the MM400_Cond2, and the MM400_Cond3 displayed reduced melting/decomposition onset values between $\approx$ 163.4-167.4 °C. The melting/decomposition temperatures of KolVA64 and HPMC were 292.2 and 293.4 °C, respectively (**Figure 4.4c**). The CIP:HPMC and CIP:KolVA64 systems display melting/dehydration onset values at 222.4 and 241.4 °C.

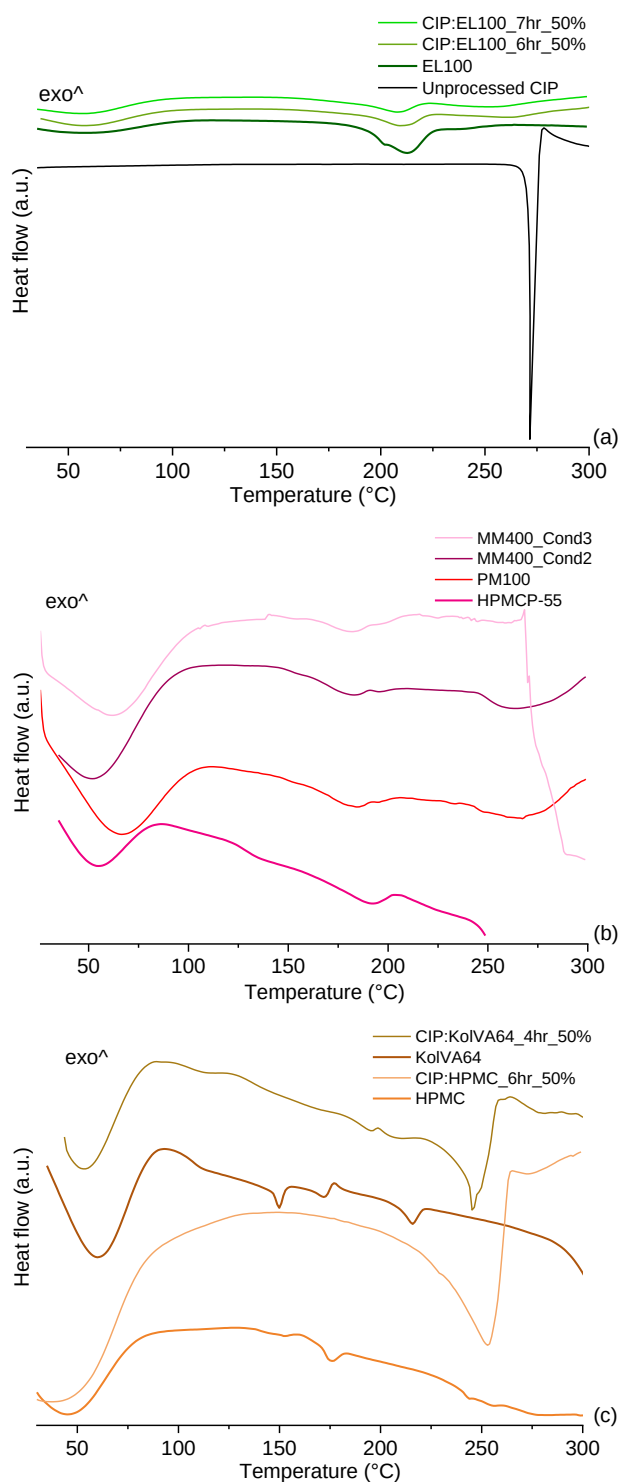


Figure 4.4 DSC analysis of (a) unprocessed CIP and EL100, and processed CIP at 50% (w/w) concentration and EL100 subject to milling for 6 and 7 hours using the PM100_Cond1, (b) HPMCP-55 subject to milling for 6 hours using the PM100_Cond1 (red), the MM400_Cond2 (plum purple), and the MM400_Cond3 (light pink), (c) KolVA64 or HPMC subject to milling at 4 and 6 hours using the PM100_Cond1.

The experimental T_g values of the binary systems were investigated via MTDSC analysis and compared to the theoretical T_gs calculated using the G-T equation (**Figure 4.5** and **Equation 4.1**). The binary systems demonstrated experimental T_g values between 102 and 129 °C, thus, exceeding the T_g value of CIP (86.7 °C) and the theoretical T_g values.⁵⁰ It is evident that the semi-crystalline samples displayed only one T_g (assuming complete amorphization), indicating compatibility and miscibility between neutral polymers and CIP. This highlights that miscibility may be possible, while the neutral polymers HPMC and KolVA64 remain incapable of disrupting the crystal lattice to achieve complete amorphization. However, the polymer may interact differently depending on the phase with which it is in contact i.e., crystalline and/or amorphous CIP. Therefore, Hansen solubility parameters were investigated to theoretically investigate the compatibility between amorphous CIP and the polymers discussed. The total Hansen solubility values of CIP and polymers were determined from the literature (**Table 4.3**). In theoretical terms, to obtain at least partial miscibility between the drug and the polymer, the solubility parameter difference ($\Delta\delta$) between the two must be < 7.0 Mpa^{0.5}.²⁴⁹ The difference between CIP and each polymer was < 7.0 Mpa. Thus, the values calculated display the theoretical miscibility of CIP and polymers in all cases (**Table 4.3**).

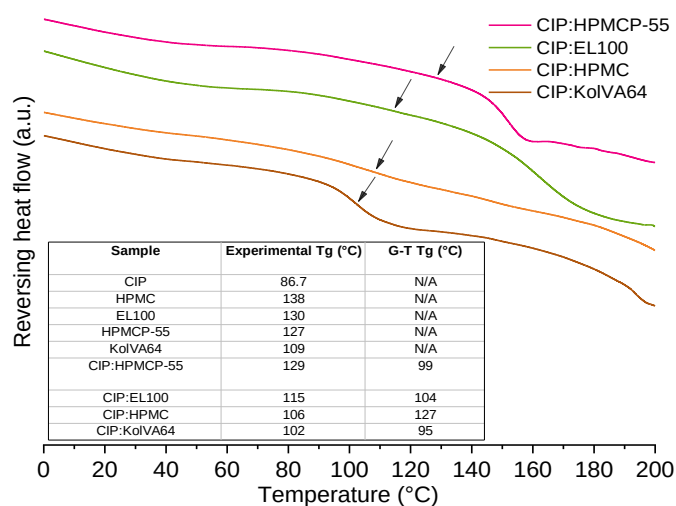


Figure 4.5 MTDSC analysis of processed CIP with acidic polymers EL100 (6 hours) or HPMCP-55 (7 hours) at a 50% (w/w) concentration subject to milling using the PM100_Cond1, and neutral polymers HPMC or KolVA64 (1.65 hours) at a 50% (w/w) concentration subject to milling using the MM400_Cond1.

Table 4.3 Hansen solubility values of drug and polymers.²⁵⁰⁻²⁵³

Drug or Polymer	δ (Mpa ^{0.5})	$\Delta\delta$ (Mpa ^{0.5})
CIP	25	N/A
EL100	21	4
HPMCP-55	22	3
HPMC	22	3
KolVA64	21	4

4.3.4 Infrared Analysis

The IR spectra of unprocessed CIP, acidic and neutral polymers, and BMed systems are displayed in **Figure 4.6**. It is known that anhydrous CIP is usually commercially available in its zwitterionic form in the solid-state. Thus, the absence of the carboxylic acid group of CIP at approximately 1720 cm⁻¹ is to be expected.

An investigation into the CIP:EL100 and CIP:HPMCP-55 samples were initially carried out (**Figure 4.6a** and **4.6b**). The presence of the carboxylic acid group was confirmed for the systems. However, this does not necessarily verify that proton transfer has taken place since acidic polymers contain a carboxylic acid group. Nevertheless, the CIP:EL100 sample displayed the absence of the carboxylate group with a trace amount existing for CIP:HPMCP-55 at 1588 cm⁻¹. This finding may indicate that proton transfer occurred for both samples. A peak between 1619 and 1629 cm⁻¹ was evident for all samples due to the carbonyl stretch of the carboxylic acid. However, CIP:HPMCP-55 sample displayed a split and more broad peak at 1612 cm⁻¹.¹⁹ This may suggest the engagement of the carbonyl group in hydrogen bonds. A peak at 1543 cm⁻¹, which could be of the amine group, can be detected for CIP and also persisted for CIP:EL100 and CIP:HPMCP-55 amorphous polymeric systems, however, the peaks shifted to 1548 and 1558 cm⁻¹, along with being less detectable for CIP:HPMCP-55. This indicates that the amine group of CIP is involved in H-bonding interactions.

The semi-crystalline systems CIP:HPMC and CIP:KolVA64 were examined (**Figure 4.6c** and **4.6d**). The detection of the carboxylic acid group was observed at 1733 and 1726 cm⁻¹ for the CIP:HPMC semi-crystalline sample which showed that proton transfer occurred.¹⁹ Proton transfer for CIP:KolVA64 sample was more difficult to detect than CIP:HPMC since vinyl acetate peak also exists at 1737 cm⁻¹.²⁵⁴ However, upon closer analysis, two peaks were detected at 1737 and 1733 cm⁻¹ confirming the presence of both groups, indicating potential proton transfer. The weaker detection of the carboxylic acid group observed at 1726 cm⁻¹ for the CIP:HPMC and CIP:KolVA64 samples may represent only residual amounts of amorphous

material, in agreement with **Figure 4.4c**. The samples also showed the presence of CIP carboxylate group at 1590 and 1375 cm^{-1} , expected due to their semi-crystalline nature. Lastly, a peak at 1543 cm^{-1} , which may be the amine group, can be detected for the semi-crystalline systems and is comparable to that of CIP, indicating the possibility of little to no H bond interactions, in contrast to the amorphous polymer systems.

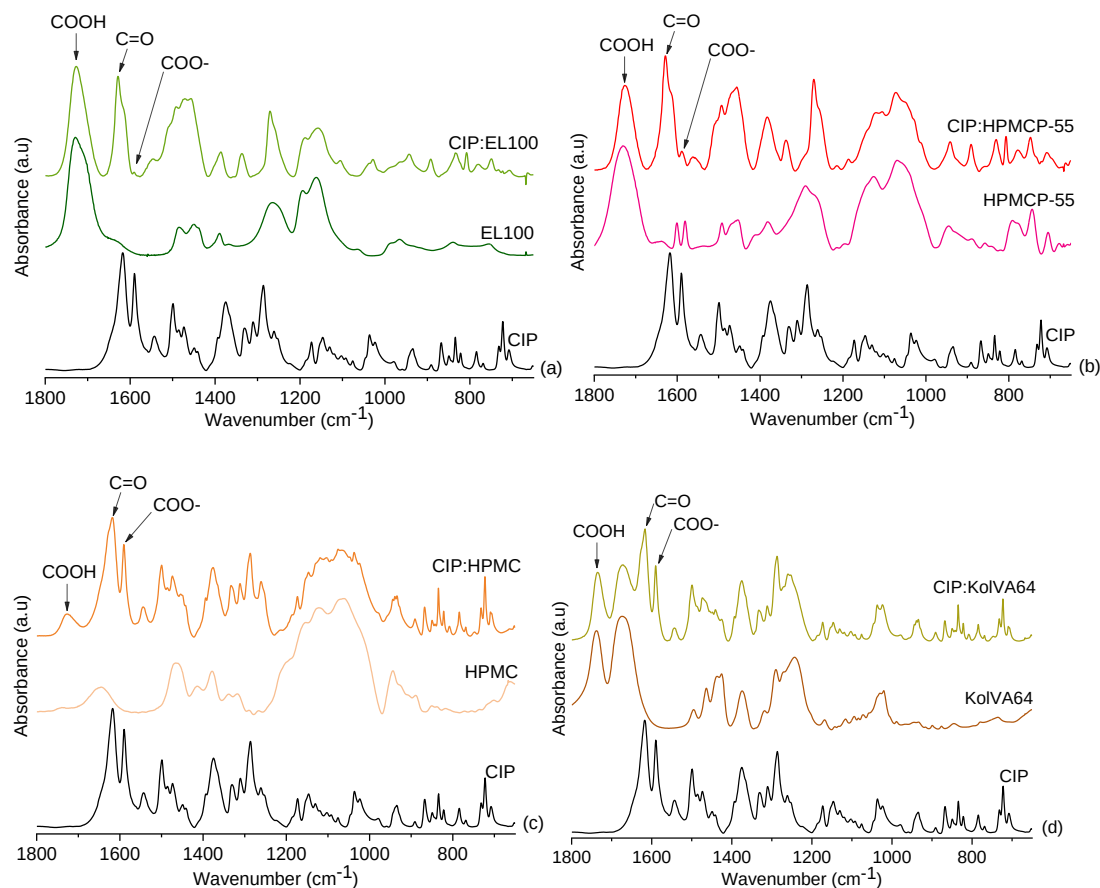


Figure 4.6 FTIR analysis of unprocessed CIP and polymer, and processed CIP:polymer with 50% (w/w) CIP using a PM100, 50 mL stainless steel jar, and three 1.5 cm balls at 400 rpm (a) CIP:EL100, (b) CIP:HPMCP-55 (c) CIP:HPMC and (d) CIP:KolVA64.

4.3.5 Solid State Nuclear Magnetic Resonance (SSNMR)

The ^{13}C CPMAS spectrum of crystalline CIP showed chemical shifts for key functional groups including the carboxylate ion, the ketone carbon, and the piperazine ring which confirms its zwitterionic state, as previously reported. When in a zwitterionic state, the carboxylate ion (C18) exhibits a chemical shift of 177.09 ppm, while the ketone carbon (C13) shows a shift of 177.77 ppm. The carbons in the piperazine ring are accurately located within the 40-50 ppm range (**Table 4.4**).

Table 4.4 Chemical shifts of carboxylate, ketone, and piperazine ring (ppm) of CIP and binary amorphous polymeric systems.

Sample	Chemical Shifts (ppm)					
	Carboxylate	Ketone	Piperazine ring			
	C18	C13	C1	C3	C4	C6
CIP (crystalline, zwitterion)	177.09	177.77	46.39	48.10	42.40	43.23
CIP:EL100 (amorphous)	166.77	177.39	46.6	48.17	42.77	43.58
CIP:HPMCP-55 (amorphous)	168.4	177.3	46.6	48.2	42.9	43.7

Broader ^{13}C peaks observed for the CIP:EL100 sample suggest that CIP is now in an amorphous state. The peaks from the carbonyl region of CIP in its zwitterionic state are overlapped due to a higher deshielding of the carbon bonded to the ionized oxygen pushing it closer to the signal of the ketone carbon. The CIP:EL100 samples depict the separation of the carboxylic acid and ketone peaks. The carboxylic acid carbon peak then appears at 166.77 ppm (**Figure 4.7a**). This upfield shift is due to the protonation of the attached oxygen that generates a shielding effect upon that carbon. In turn, the ketone carbon is pushed downfield to 177.39 ppm (**Figure 4.7a**). This shift is assumed to happen due to an intramolecular hydrogen bond with the nearby carboxylic acid. The electron density pulled away from the ketone oxygen to the carboxylic acid hydrogen during H-bonding generates a deshielding effect on the ketone carbon. Such shifts strongly suggest a proton transfer to the carboxylate group of CIP in the CIP:EL100 sample. Similarly, there is a slight downfield shift of the signals from the carbons in the piperazine ring (**Figure 4.7b**). Though the shifts are not considerable, it might be the case that N5 is deprotonated generating a deshielding effect, and/or is participating in hydrogen bonding.

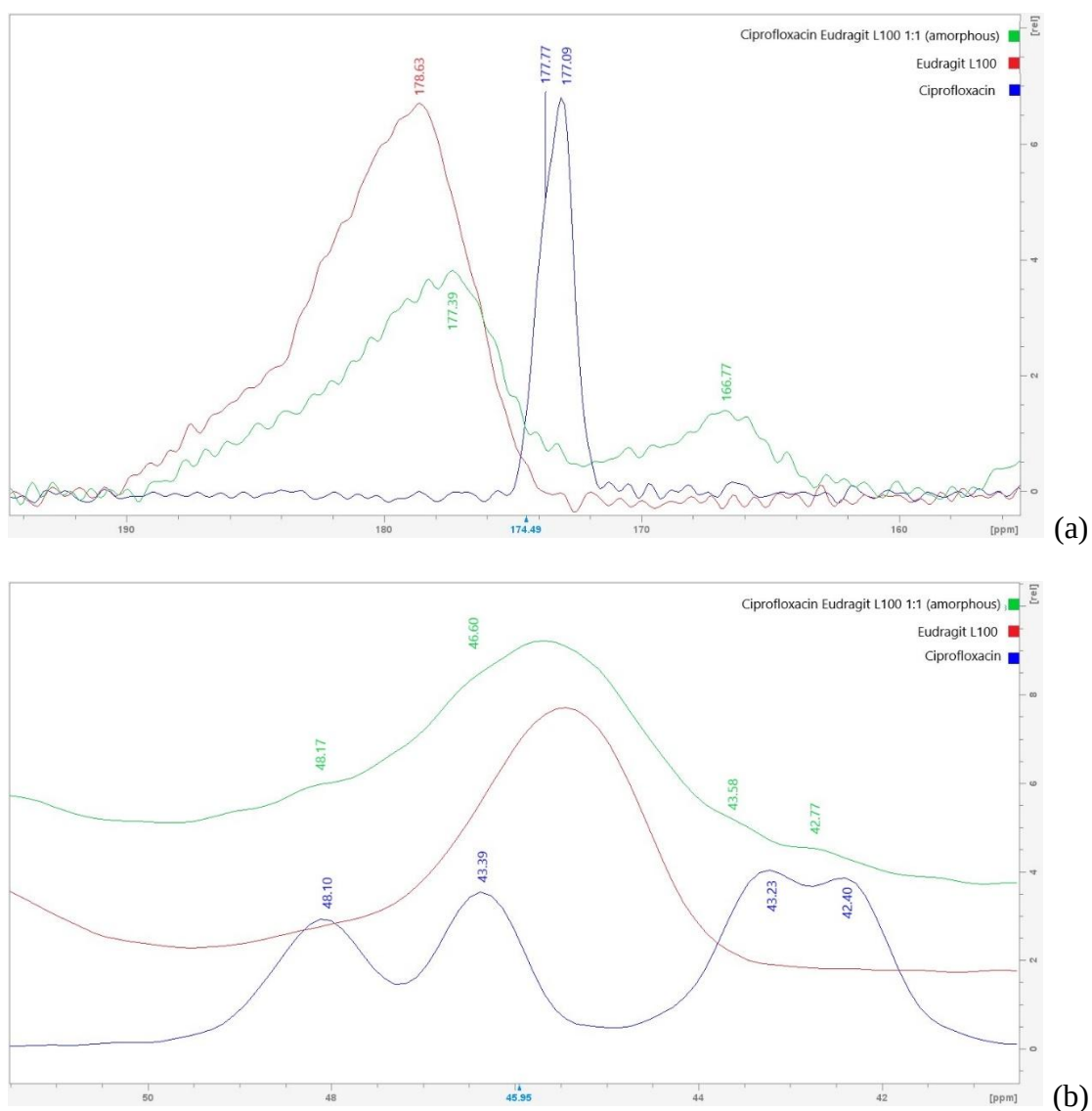


Figure 4.7 ^{13}C CPMAS spectra for CIP, EL100, and CIP:EL100 processed sample for (a) carboxylate region and (b) piperazine ring region.

The CIP:HMPCP-55 sample displayed the separation of the chemical shifts of the ketone and carboxylate carbons, similar to the CIP:EL100 sample, confirming the protonation of the carboxylate group of CIP (**Figure 4.8a**). Similarly, downfield shifts from carbons 4,6 in the piperazine ring suggest a deshielding effect from deprotonation of the nitrogen 5. Carbons 1,3 show also a downfield shift but are weaker than those of the carbons closer to the nitrogen (**Figure 4.8b**).

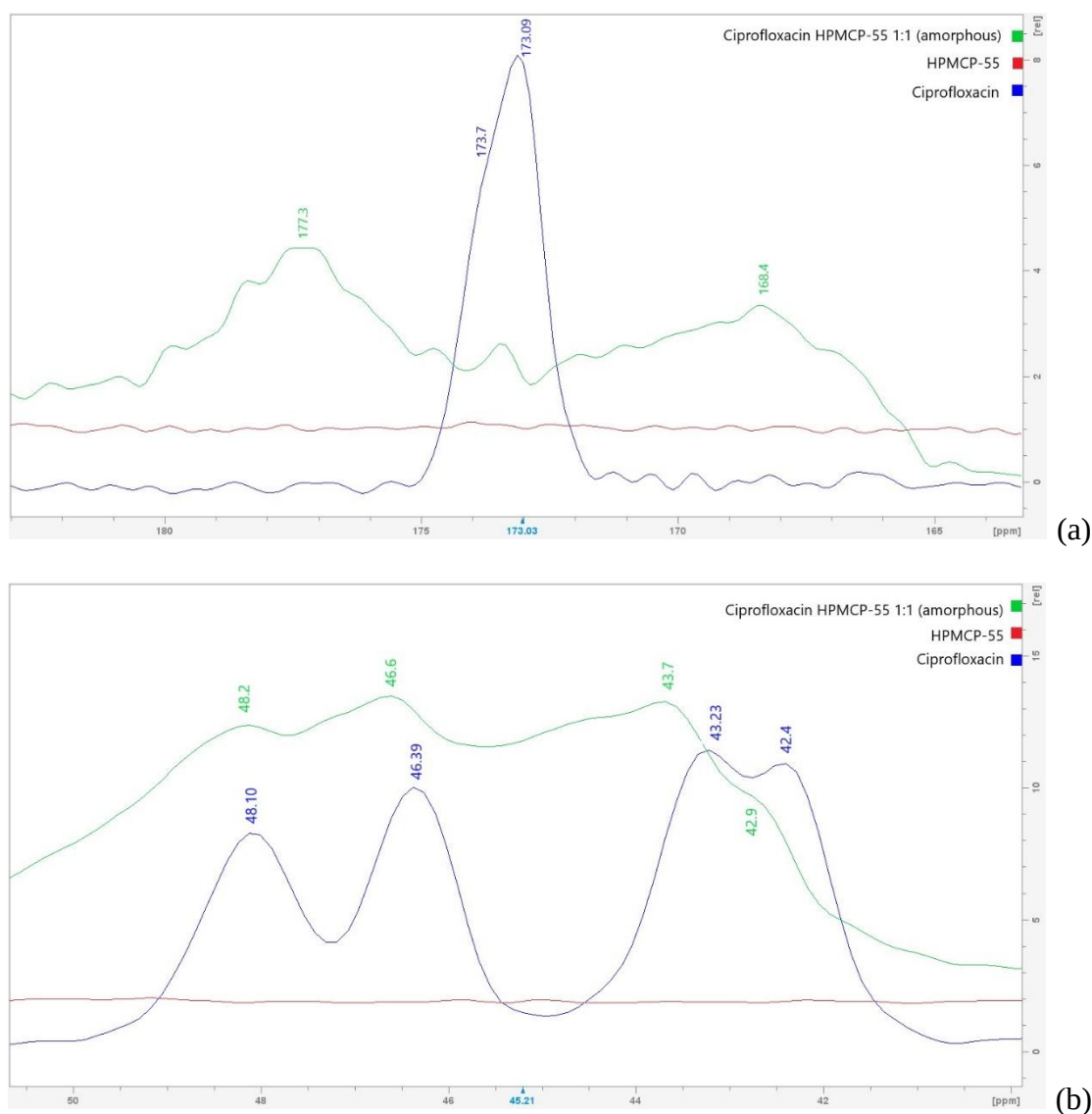


Figure 4.8 ^{13}C CPMAS spectra for CIP, HPMCP-55, and CIP:HPMCP-55 processed sample for (a) carboxylate region and (b) piperazine ring region.

The ^{13}C CPMAS analysis of the binary amorphous polymeric systems CIP:EL100 and CIP:HPMCP-55 discussed, reinforce the hypothesis of a proton transfer to the carboxyl region of CIP upon BMing. However, it is unclear if the piperazine region of the molecule participates in the proton transfer process or inter/intra molecular interactions. Furthermore, the spin-lattice relaxation times (1H T1) of the binary samples further confirm the amorphous nature of the samples, with lower relaxation times than crystalline CIP (**Table 4.2**).

4.3.6 Physical Stability of Binary Systems

4.3.6.1 Physical Stability of Binary Systems Containing an Acidic Polymer

DVS was firstly used to analyze processed samples CIP:EL100 and CIP:HPMCP-55, which contained an acidic polymer using the PM100_Cond1 (**Figure 4.9** and **4.10**). These analyses were carried out to evaluate the effect of moisture on the solid-state stability of the amorphous BMed samples. The samples illustrated a sample mass increase when relative humidity (RH) increased. There was no evidence of partial crystallization despite the powders sorbing around 17% (w/w) of moisture at 90% RH. The CIP:EL100 and CIP:HPMCP-55 samples lost approx. 17% (w/w) moisture between 90-0% RH (desorption) over 1,000 and 1,400 minutes ($\approx$ 17 and 23 hours), respectively. The hygroscopicity demonstrated is expected for amorphous materials due to the random orientation of their molecules facilitating water uptake due to the increase in free volume. Water sorption can offer disadvantages in terms of drug stability by potentially lowering the T_g , resulting in an increase in molecular mobility and plasticization, and thus, encouraging crystallization.²⁴² However, both systems investigated remained amorphous following DVS analysis, as shown by PXRD (**Figure 4.11**). The amino group of CIP and carboxylic acid of the polymers may contain electrostatic forces which may act as a stabilizer for the APSs,¹⁹ along with ionic and H bond interactions with the carboxylic acid and carbonyl group of CIP, preventing crystallization during the study. Moreover, long chain polymers can postpone crystallization via steric hindrance ultimately averting the diffusion of drug molecules, barricading sites of crystal growth, and enhancing the kinetic barrier to nucleation.^{255, 256}

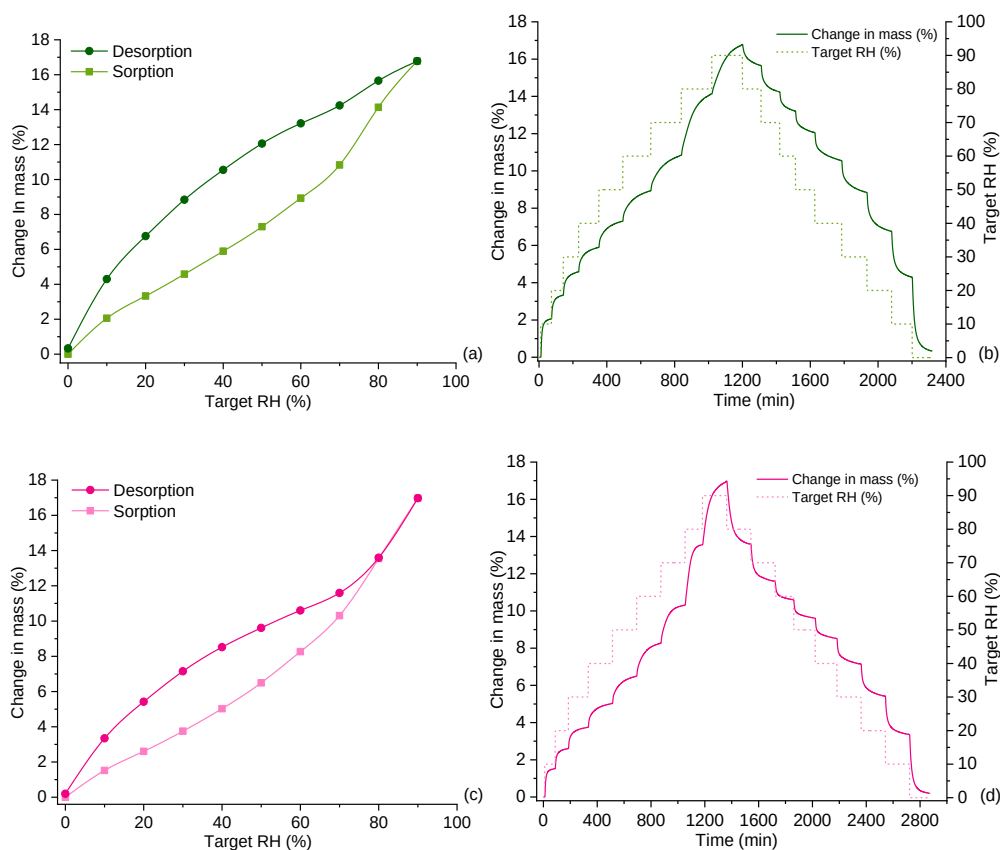


Figure 4.9 DVS plots of processed CIP with EL100 (green) and CIP with HPMCP-55 (pink) at a 50% (w/w) concentration subject to milling at 7 hours using the PM100_Cond1 (a) isotherm plot of CIP:EL100 (b) mass plot over time of CIP:EL100 (c) isotherm plot of CIP:HPMCP-55 and (d) mass plot over time of CIP:HPMCP-55.

A large sorption hysteresis was seen (**Figure 4.9a** and **4.9c**). This is known to depict the high porosity of the systems.²⁵⁷ that may result in faster diffusion of moisture into the bulk of the sample by means of sorption, and ultimately, slower diffusion of water back to the surface by desorption. In accordance with the Young-Nelson model, water uptake of a sample can occur by three distinct systems adsorbed as a monomolecular layer, adsorbed as a multilayer, or absorbed into the internal solid.²⁴³

The sample of CIP:EL100 demonstrated absorption as the leading water uptake mechanism at a value of 0.006 mol/g, followed by multilayer and monolayer adsorption at about 0.001 and 0.003 mol/g, respectively. The majority of the water uptake for CIP:HPMCP-55 sample was by the mechanism of absorption and multilayer adsorption at about 0.004 and 0.0035 mol/g, respectively, followed by the monolayer adsorption at 0.001 mol/g (**Figure 4.10** and **Table 4.5**). These results suggest that more moisture exists internally for CIP:EL100 than

on the surface of the material and inner pores, whereas CIP:HPMCP-55 distributes its water uptake somewhat evenly between the internal and surface of the inner pores. It may therefore be concluded that CIP:EL100 may be more hydrophilic, and CIP:HPMCP-55 more hydrophobic in nature. This may indicate a lesser likelihood of crystallization for CIP:HPMCP-55; this sample also has a higher T_g detected by MTDSC (**Figure 4.5**).^{20, 258}

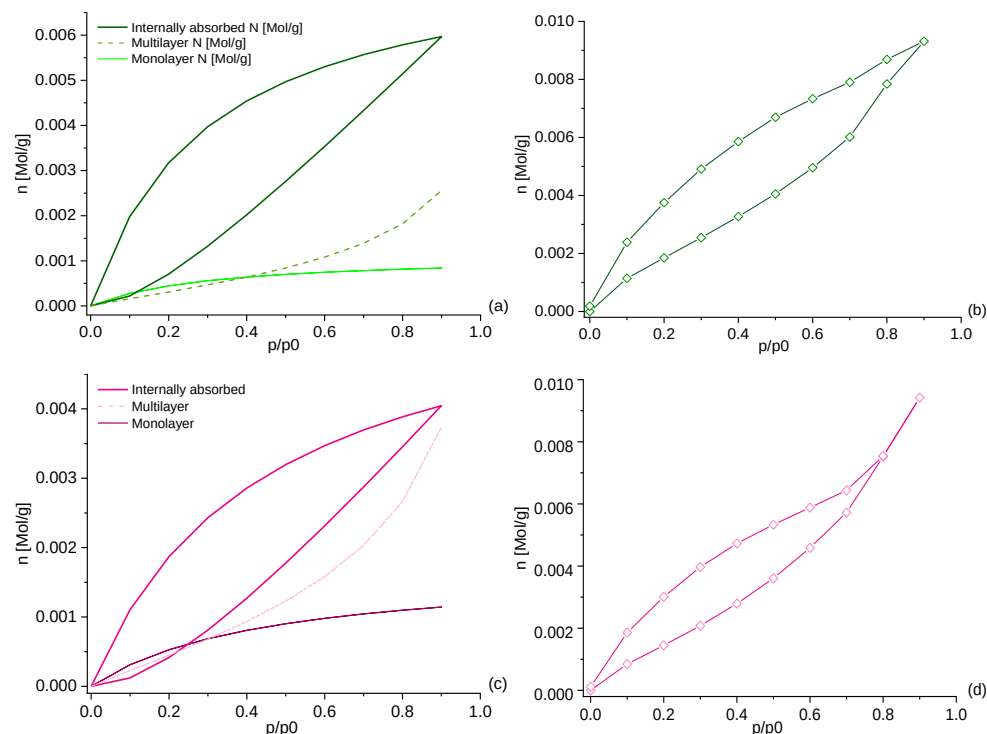


Figure 4.10 DVS plots of processed CIP with EL100 (green) and CIP with HPMCP-55 (pink) at a 50% (w/w) concentration subject to milling at 7 hours using the PM100_Cond1 of Young-Nelson model (a) deconvoluted sorption behavior for the bulk, monolayer and multilayers of CIP:EL100 (b) best fit of CIP:EL100 (c) deconvoluted sorption behavior for the bulk, monolayer and multilayers of CIP:HPMCP-55 (d) best fit of CIP:HPMCP-55.

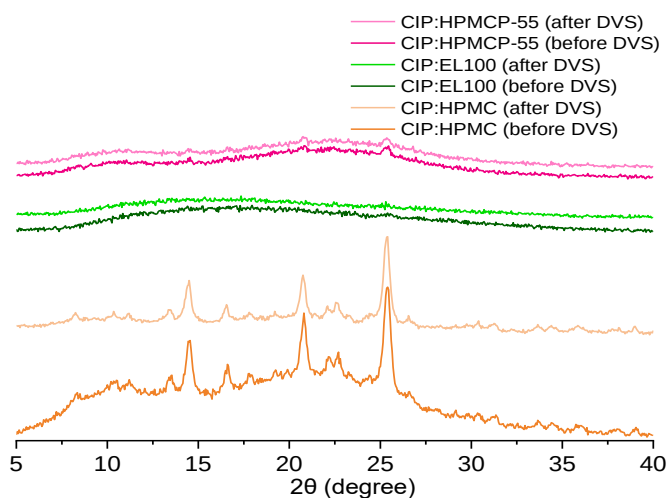


Figure 4.11 PXRD before and after DVS analysis of CIP with HPMCP-55, CIP with EL100, and CIP with HPMC at a 50% (w/w) concentration, subject to milling at 6 or 7 hours using the PM100_Con1.

Table 4.5 Young-Nelson Internally Absorbed, Multilayer, and Monolayer Parameters

Sample	Monolayer N (mol/g)	Multilayer N (mol/g)	Internally Absorbed N (mol/g)
CIP:EL100	0.0005	0.0025	0.006
CIP:HPMCP-55	0.001	0.0035	0.004
CIP:HPMC	0.0006	0.003	0.002

4.3.6.2 Physical Stability of Binary Systems Containing a Neutral Polymer

While the CIP:HMPC system is semi-crystalline, it was observed by DSC that the amorphous portion of the sample did not crystallize on heating, thus it is thermally stable (**Figure 4.4c**). The CIP:HPMC sample demonstrates a narrow, convex DVS isotherm, implying that water may be primarily adsorbed to the outer surfaces of the sample (**Figure 4.12a**), and is in alignment with **Table 4.5**. The isotherm displayed is in contrast to the fully amorphous samples CIP:EL100 and CIP:HPMCP-55, which demonstrate broader isotherms (**Figure 4.9**), again, in agreement with **Table 4.5**. The CIP:HPMC sample depicted no evidence of crystallization during the DVS cycle (**Figure 4.12**) and was confirmed by PXRD (**Figure 4.11**). This indicates that the portion of the amorphous phase within the semi-crystalline sample is stable upon exposure to moisture. The findings demonstrate that only the amorphous portion of the sample is represented by DVS analysis. This can be explained by previous studies whereby crystalline CIP was investigated for stability. The results demonstrated no significant changes

to the mass of the sample, highlighting that changes may be presented to the amorphous portion of the API rather than the crystalline phase.¹²⁹ Overall, the powder only sorbed around 10% (w/w) of moisture at 90% RH (**Figure 4.12a**). The sample lost approx. 10% (w/w) moisture between 90-0% RH (desorption) over 900 minutes (≈ 15 hours) (**Figure 4.12b**). Less moisture was sorbed compared to CIP:EL100 and CIP:HPMCP-55 (**Figure 4.9**), as expected considering the semi-crystalline nature of this system.

The Young–Nelson model for CIP:HPMC reveals that the multilayer is the predominant phase for water uptake, with a value of 0.003 mol/g, followed by internally absorbed water at 0.002 mol/g, and a monolayer value at 0.0006 mol/g (**Figure 4.12c**). This suggests that the sample may exhibit more hydrophobic characteristics, potentially repelling water from the bulk phase. The differences mentioned between the fully amorphous samples CIP:EL100 and CIP:HPMCP-55, and the semi-crystalline sample CIP:HPMC are comparable to observations made regarding enrofloxacin, whereby Mesallati *et al.* suggested that the mechanism of ASD water uptake may differ based on the polymer utilized.²⁰ In summation, CIP:EL100 is more hydrophilic, and CIP:HPMCP-55 and CIP:HPMC samples are more hydrophobic.

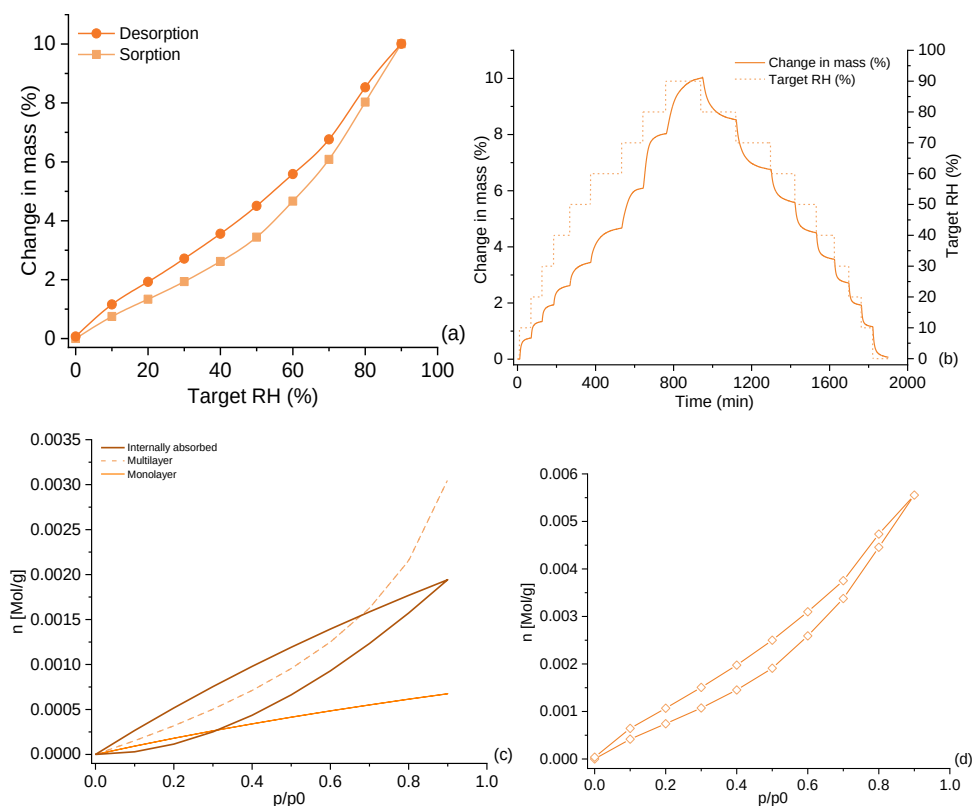


Figure 4.12 DVS plots of processed CIP with HPMC at a 50% (w/w) concentration subject to milling at 6 hours using the PM100_Cond1(a) isotherm plot and (b) mass plot over time (c) Young-Nelson Model deconvoluted sorption behavior for the bulk, monolayer, and multilayers (d) Young-Nelson Model best fit.

There was evidence of solid-state transition as the CIP:KolVA64 sample crystallized at 50 and 70% RH during the analysis and overall, the powder sorbed around 18% (w/w) of moisture at 90% RH (**Figure 4.13a**). The sample lost approx. 18% (w/w) moisture between 90-0% RH (desorption) over 1,000 minutes (≈ 17 hours) (**Figure 4.13b**). The ability to sorb large amounts of moisture as seen in **Figure 4.9** is in alignment with the characteristics of amorphous and polymeric materials.^{242, 259} The crystallization of the CIP:KolVA64 sample is in contrast to CIP:EL100, CIP:HPMCP-55, and CIP:HPMC which display no crystallization, indicating superior stability and their ability to withstand crystallization at moisture conditions of up to 90%.

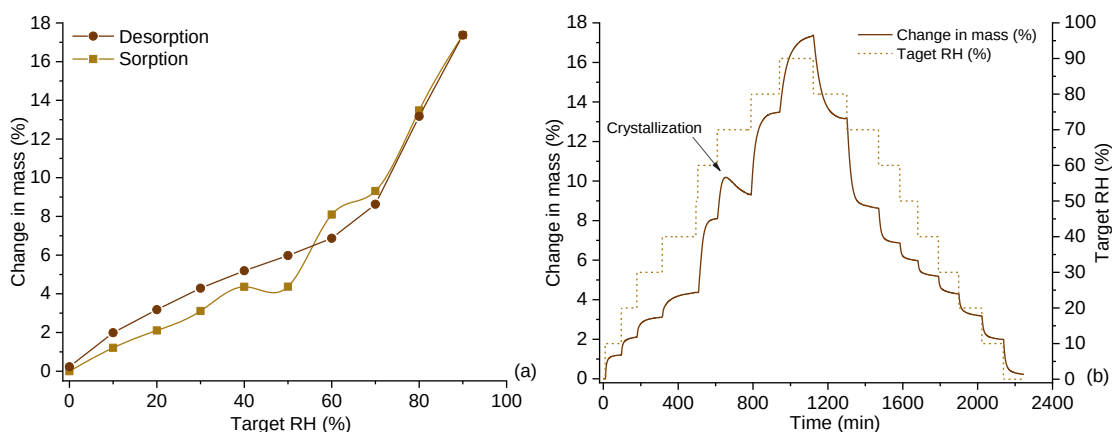


Figure 4.13 DVS plots of processed CIP with KolVA64 at a 50% (w/w) concentration subject to milling at 6 hours using the PM100_Cond1 (a) isotherm plot (b) mass plot over time.

PXRD was carried out before and after DVS analysis on samples that did not show clear signs of crystallization (**Figure 4.11**). These samples include CIP:EL100, CIP:HPMCP-55, and CIP:HPMC samples. The PXRD and DVS analysis are in agreement as no signs of crystallization were presented. CIP:KolVA64 showed clear crystallization of the sample via DVS analysis, thus, PXRD was not carried out.

4.4 Conclusions

To conclude, a range of binary systems of CIP using acidic and neutral polymers were produced using the milling process and analyzed using PXRD, DSC, IR, SSNMR, and DVS. The MM400 reduced the degree of crystallinity, and the milling duration required to achieve such results, compared to the PM100. The MM400 displayed the complete amorphization of the API by altering the parameters while maintaining the % API. The IR results demonstrated residual amounts of the carboxylic acid group in the semi-crystalline CIP:HPMC system, confirming proton transfer. This may also be the case for the CIP:KolVA64 system. Thermal analysis of CIP:HPMC showed no crystallization peaks, indicating the stability of its amorphous form of the API. In contrast, the CIP:KolVA64 sample displayed an exothermic crystallization peak, suggesting that its amorphous form is less stable compared to that of CIP:HPMC. Furthermore, the values calculated using Hansen solubility showed theoretical miscibility of CIP and polymers for all systems, in agreement with the display of one T_g for all samples. The ¹³C CPMAS analysis of the amorphous polymeric systems reinforced the hypothesis that proton transfer occurred to the carboxyl region of CIP. However, it remains unclear if the piperazine region of the molecule participates in the proton transfer process and/or hydrogen bond interactions. Lastly, the effect of moisture on the solid-state stability of the BMed samples was investigated by DVS analysis. The amorphous component in the semi-crystalline system of CIP:HPMC was stable, in contrast to that of CIP:KolVA64. The Young-Nelson model confirmed the hydrophobic nature of the CIP:HPMC system which may have contributed to the stability of the amorphous phase of the API. In summation, the solid-state characterization and stabilization of amorphous and semi-crystalline polymeric systems of CIP prepared by mechanical activation were thoroughly investigated.

Chapter 5.0 Effects of Polymer Addition to Binary Amorphous Polymeric Systems using Mechanical Energy

Chapter 5.0 Effects of Polymer Addition to Binary Amorphous Polymeric Systems using Mechanical Energy

5.1 Introduction

ASDs are commonly used to improve the bioavailability of poorly soluble APIs.^{19, 20} ASD is a term used to describe a system whereby the API is the amorphous form, usually converted from its original most stable crystalline form, and is distributed within an excipient matrix, usually a polymer of some kind, in the solid form.²⁶⁰ The amorphous API contains a higher Gibbs free energy and exhibits a greater surface area in comparison to the stable crystalline form. The stability of ASDs still remains an issue in the present day as the amorphous form often recrystallizes to its most stable crystalline form. This poses concerns for an acceptable shelf-life of the drug product that falls within FDA guidelines. Nevertheless, over the years, research has shown that a combination of two polymers to form ternary ASDs can significantly enhance the stability of the drug compared to binary systems by providing additional interactions between the drug and polymers.⁵² Moreover, salts offer further stability due to their strong ionic interactions.¹⁸⁻²⁰ Thus, ternary APSs were of interest for this study.

CIP, a fluoroquinolone antibiotic with a broad spectrum of antimicrobial activity, was chosen for this investigation.¹²⁸ CIP has an acidic and basic group, and thus, is a favorable drug for salt formation.¹³¹ This approach, along with amorphization is a promising combination for improved solubility of poorly soluble drugs. The unionized and ionized/zwitterionic form of the drug exist, with the ionized form being commercially available.⁵⁰ Since the zwitterionic form has an overall neutral charge, it is practically insoluble in aqueous media. The solubility of CIP in water has been stated as 0.06 and 0.08 mg/mL at 25 and 37 °C, respectively.¹²⁹ Changing the form of CIP to improve the properties of the drug is challenging due to its poor solubility in common solvents¹³⁰ and its thermal degradation capabilities, posing as limitations.⁵⁰ These constraints decrease the potential processes that can be employed to improve the properties of the drug. Nevertheless, the ball milling (BMing) process has been demonstrated to overcome these limitations, as it enables the production of amorphous polymeric systems of LVXh, CIP (**Chapters 3 and 4**), and ENRO without the need for solvents, while also preventing degradation.^{18, 19}

Several technological approaches have been pioneered for preparing ASD formulations for BCS Class II and IV drugs, from the first being approved in 1985 utilizing the solvent

evaporation method. Since then > 30 ASDs have been prepared using methods such as fluid bed bead layering, solvent/anti-solvent precipitation, spray drying, and melt extrusion to produce ASDs, with spray drying and melt extrusion being the most popular.^{145, 261, 262} Unfortunately, there have been no ASDs approved using mechano- and tribo-chemistry, a green approach to enhance the solubility of drugs. This highlights the need to further contribute to the area of mechano- and tribo-chemistry to encourage the pharmaceutical industry to move in the direction of sustainability whilst reducing costs. It is known in the literature that a combination of amorphous ternary systems and ionization can potentially improve the solubility and stability of the API. According to the literature, two fluoroquinolone drugs, OFX and NOR, have been studied as ternary systems. OFX was investigated in combination with a polymer, polyethylene glycol, and a surfactant, polysorbate 80,²⁶³ while NOR was combined with egg albumin and olive oil.²⁶⁴ Additionally, several ternary ASD investigations have been performed using the ball milling process where at least one excipient was a polymer; Indomethacin, KolVA64-Polyvinylpyrrolidone VA64 (PVP) copolymer, and amino acid -L-arginine,²⁶⁵ meloxicam, and moringa and PVP,²⁶⁶ candesartan cilexetil, and hydrochlorothiazide as fixed-dose combination, and HPMCAS,²⁶⁷ and itraconazole and Soluplus®-HPMCP.²⁶⁸ Lastly, co-amorphous ternary system analyses using the ball milling process were carried out using two co-formers: carbamazepine with citric acid and L-arginine,²⁶⁹ mebendazole with glutamic acid and arginine,²⁷⁰ as well as naproxen with tryptophan or arginine and proline.²⁷¹ CIP binary systems were designed containing polymers (**Chapter 4**) or amino acids.^{18, 19} To build on this investigation, ternary ASD/APSs of fluoroquinolone drugs were investigated, for the first time, using two polymers as the excipients while implementing the ball milling process.

The aim of this study was to investigate ternary amorphous polymeric systems of CIP for their solid-state characteristics and physical stability. An additional aim was to investigate the increase or decrease in the degree of crystallinity to the original binary system upon the addition of a third component and the % (w/w) of the third component and to rank the polymers accordingly. Lastly, the hydro-philic and -phobic properties of the ternary systems were investigated. To date, CIP ternary ASD/APSs have not been created using two polymers, nor have any fluoroquinolone APIs been explored in combination with two polymers, highlighting the significance of this research.

5.2 Experimental Section

5.2.1 Materials

The materials were purchased or donated as described in **Section 4.2.1**.

5.2.2 Methods

5.2.2.1 Milling

5.2.2.1.1 Retsch Planetary Ball Mill PM100

CIP was BMed with polymers to design ternary systems using a Retsch planetary ball mill PM100 (Germany) at 400 rpm (as described in **Section 2.2.2.1.1, Method 1**). The ternary acidic samples CIP:EL100:HPMCP-55, CIP:EL100:HPMCAS-H and CIP:EL100:HPMCAS-L were milled for a total of 7 hours at ratios 40:40:20. The ternary acidic and amphiphilic samples CIP:EL100:KolVA64 and CIP:EL100:HPMC were milled for a total of 7 and 8 hours at ratios 45:45:10 and 40:40:20 % (w/w). The ternary acidic and amphiphilic samples CIP:HPMCP-55:HPMC and CIP:HPMCP-55:KolVA64 were milled for a total of 7, 8, and 9 hours at ratios 30:30:40, 35:35:30 and 45:45:10 % (w/w). Some samples were milled altogether, and others were milled first as binary systems before transforming to ternary systems (**Table 5.1**). Around 2 mg of the sample was removed for PXRD analysis (described in **Section 2.2.2.3**). Around 30 mg of the sample was removed for DSC and MTDSC analysis (described in **Sections 2.2.2.4 and 2.2.2.5**). An additional 10 mg of the sample was removed for analysis via FTIR (described in **Section 2.2.2.7**), and 15 mg of the sample was removed for analysis via DVS (described in **Section 2.2.2.8**).

Table 5.1 Conditions of polymer addition using a Retsch planetary ball mill PM100.

Binary system	BMing duration of binary system (hours)	Ternary polymer or system	BMing duration of ternary system (hours)	Total BMing duration (hours)	Ratio % (w/w)
CIP:EL100	6	HPMCP-55	1	7	40:40:20
CIP:EL100	6	HPMCAS-H	1	7	40:40:20
CIP:EL100	6	HPMCAS-L	1	7	40:40:20
N/A	N/A	CIP:EL100: KolVA64	7	7	45:45:10
N/A	N/A	CIP:EL100: KolVA64	8	8	45:45:10
CIP:EL100	6	KolVA64	1	7	45:45:10
CIP:EL100	7	KolVA64	1	8	40:40:20
CIP:EL100	6	HPMC	1	7	40:40:20
N/A	N/A	CIP:HPMCP - 55:KolVA64	8	8	45:45:10
CIP:HPMCP-55	6	HPMC	1	7	35:35:30
CIP:HPMCP-55	6	HPMC	1	7	30:30:40
CIP:HPMCP-55	8	KolVA64	1	9	30:30:40

5.2.2.1.2 Retsch Mixer Mill MM400

CIP was BMed with polymers to design ternary systems using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s) (as described in **Section 2.2.2.1.2, MM400_Cond1**). The ternary acidic samples CIP:EL100:HPMCAS-H and CIP:HPMCP-55:HPMC were milled for a total of 114 minutes at ratios 30:30:40. One sample was milled altogether, and another was milled with two polymers before the addition of the API, and the remainder were milled firstly as binary systems for 99 minutes, followed by ternary systems for 15 minutes (**Table 5.2**). Around 2 mg of the sample was removed for PXRD analysis (described in **Section 2.2.2.3**). Around 15 mg of the sample was removed for DSC analysis (described in **Section 2.2.2.4**). An additional 10 mg of the sample was removed for analysis via FTIR (described in **Section 2.2.2.7**), and 15 mg of the sample was removed for analysis via DVS (described in **Section 2.2.2.8**).

***Table 5.2** Conditions of polymer addition using a Retsch mixer mill MM400.*

Binary system	BMing duration of binary system (minutes)	Ternary polymer/ component or system	BMing duration of ternary system (minutes)	Total BMing duration (minutes)	Ratio % (w/w)
CIP:EL100	99	HPMCAS-H	15	114	40:40:20
N/A	N/A	CIP:HPMCP-55:HPMC	N/A	99	30:30:40
HPMCP-55:HPMC	15	CIP	99	114	30:30:40
CIP:HPMCP-55	99	HPMC	15	114	30:30:40

5.2.2.2 Powder X-Ray Diffraction (PXRD)

As described in **Section 2.2.2.3**.

5.2.2.3 Differential Scanning Calorimetry (DSC)

As described in **Section 2.2.2.4**.

5.2.2.4 Modulated Temperature Differential Scanning Calorimetry (MTDSC)

As described in **Section 2.2.2.5**.

5.2.2.5 Calculation of Theoretical Glass Transition (T_g) Values with Gordon–Taylor Equation

The theoretical T_gs of the ternary were calculated by extension of the Gordon–Taylor equation (as described in **Section 4.2.2.5**)^{246, 247} to a three-component equation:²³⁸

Equation 5.1 Extension of the Gordon–Taylor equation.^{246, 247}

$$T_g = \frac{w_1 T_{g1} + K_1 w_2 T_{g2} + K_2 w_3 T_{g3}}{w_1 + K_1 w_2 + K_2 w_3} \quad \text{Eqn. (5.1)}$$

where K is approximately equal to:

Equation 5.2 K value for the extension of the Gordon–Taylor equation.^{246, 247}

$$K_1 \approx \frac{T_{g1} \rho_1}{T_{g2} \rho_2} \quad \text{and} \quad K_2 \approx \frac{T_{g1} \rho_1}{T_{g3} \rho_3} \quad \text{Eqn. (5.2)}$$

The T_gs of the ternary systems were obtained (as described in **Section 2.2.2.5**), and the parameters using in **Equations 5.1** and **5.2** are described in **Section 4.2.2.5**.

5.2.2.6 Solid State Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted as described in **Section 2.2.2.7**. Unprocessed CIP and BMed CIP ternary samples were first mixed with dried KBr using a spatula in a ratio of 1:30 for CIP and 1:20 for BMed CIP ternary samples.

5.2.2.7 Dynamic Vapor Sorption (DVS)

DVS was used to investigate BMed ternary samples by use of an Advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., UK) (as described in **Section 2.2.2.8, Method 1**).

5.3 Results and Discussion

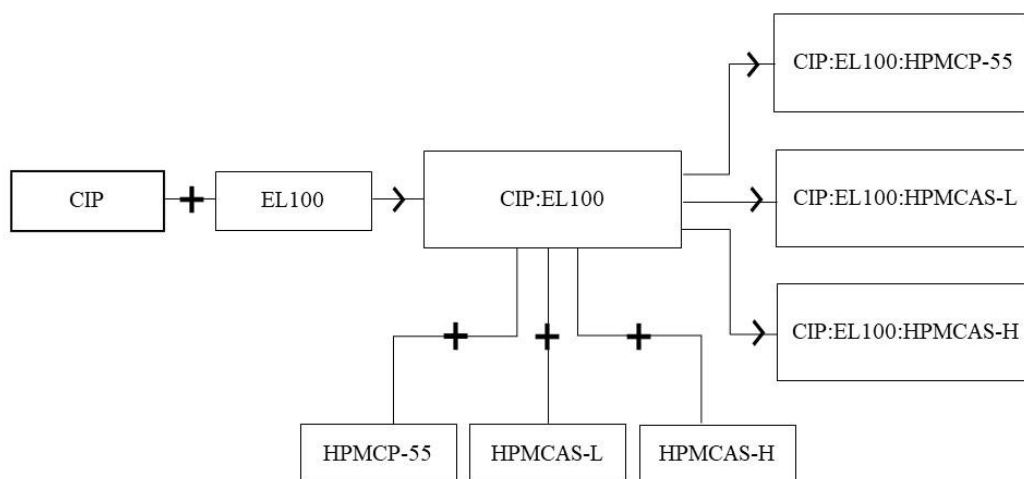
5.3.1 Ball Milling of Ciprofloxacin Ternary Systems

5.3.1.1 Powder X-Ray Diffraction

The binary systems CIP:EL100 and CIP:HPMCP-55 were explored in **Chapter 4**. Since many benefits of formulating ternary ASDs for the purpose of enhancing dissolution, solubility, and stability have been reported in the literature, a study on ternary systems was examined. An acidic polymer has proven capable of producing ASD/APSs of CIP as described in **Chapter 4**. Nevertheless, in alignment with the literature, neutral, amphiphilic polymers provide advantages due to their hydrophilic and hydrophobic properties. Thus, the addition of a second polymer, either acidic or neutral (amphiphilic), to generate ternary systems using a Retsch planetary ball mill PM100 was considered and compared to a Retsch mixer mill MM400. A study on the addition of the polymers and/or API, the % (w/w) of the polymers, the duration of milling, and a comparison of mills (PM100 and MM400) was conducted. The samples were analyzed by PXRD in the solid-state as the primary method for ranking the polymers used in this study, and/or their order of addition.

5.3.1.1.1 Addition of Another Acidic Polymer to CIP:EL100

This section focused on the generation of a ternary system using two acidic polymers. The aim of this study was to investigate whether an additional acidic polymer has the ability to increase or decrease the degree of crystallinity in the CIP:EL100 system. An exploration of the addition of HPMCP-55, HPMCAS-L, and HPMCAS-H to the CIP:EL100 amorphous polymeric system was conducted using a PM100 mill. Investigations into CIP:EL100:HPMCAS-H were also performed using a Retsch mixer mill MM400, that produce impact forces, a dominant force known to improve reaction rate and product formation. The processing of CIP ternary sample CIP:EL100:HPMCAS-H by the MM400 was implemented as a comparative process to the PM100 (**Scheme 5.1** and **Figure 5.1**).



Scheme 5.1 Addition of another acidic polymer to CIP:EL100.

All ternary samples contained ratios of 40:40:20 and a milling duration of 7 hours was used with a Retsch planetary ball mill PM100 (**Figure 5.1**). All samples produced an amorphous halo with the remainder of one or more crystalline Bragg peak(s), with reduced intensities. The CIP:EL100 sample containing HPMCP-55 displayed the presence of one minute Bragg peak at $25.5^\circ 2\theta$, comparable to the binary system. Thus, the addition of HPMCP-55 to the system did not increase nor decrease the degree of crystallinity of the CIP:EL100 system. The ternary samples consisting of HPMCAS-L/H depicted Bragg peaks at 14.5° , 20.9° , and $25.5^\circ 2\theta$. Despite both polymers resulting in an increase in the level of crystallinity of the CIP:EL100 system, the intensities of the Bragg peaks for the sample containing HPMCAS-H were slightly higher than for the sample containing HPMCAS-L. The peak at $25.5^\circ 2\theta$ remained for all ternary samples which may represent the strongest point in the crystalline lattice,⁵⁰ and remain a challenge when attempting to decrease the level of crystallinity of the CIP:EL100 system. Furthermore, the CIP:EL100:HPMCAS-H sample was prepared using the MM400 and resulted in a decrease in the degree of crystallinity of the CIP:EL100 system. This is in contrast to the sample milled using a PM100 (**Figure 5.1**), highlighting the benefits of impact forces and increased energy into the system (as described in **Chapters 2** and **3**). Furthermore, the processing time was reduced from 7 hours to 99 minutes by using the MM400.

To conclude, the addition of HPMCP-55 to CIP:EL100 did not increase or decrease the solid-state arrangement of CIP:EL100. In comparison, the addition of HPMCAS-L/H resulted in an increase in the degree of crystallinity of the CIP:EL100 system. The acidic polymers added to the CIP:EL100 system can be ranked by performance using the PM100 in the order of

HPMCP-55, HPMCAS-L, and HPMCAS-H. The level of crystallinity of the CIP:EL100 system upon the addition of HPMCAS-H was reduced using the MM400, in contrast to the sample milled using the PM100.

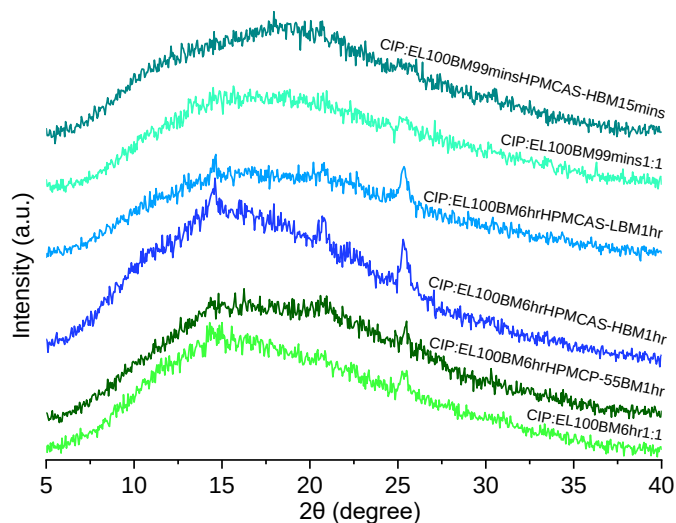
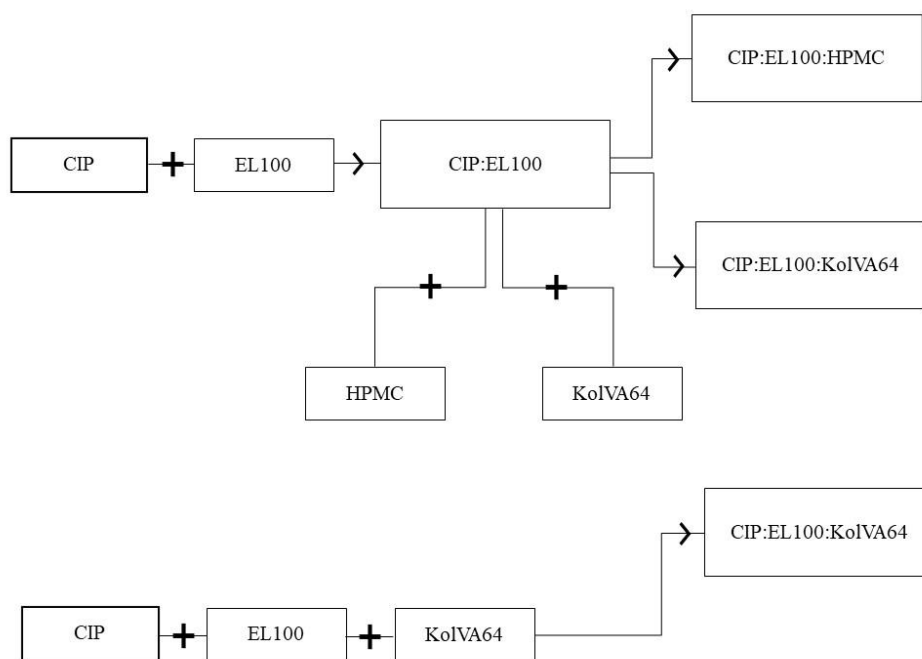


Figure 5.1 PXRD analysis of processed binary and ternary systems using the Retsch mixer mill MM400.

5.3.1.1.2 Addition of Amphiphilic Polymer to CIP:EL100

Following on from the ternary investigation in **Section 5.3.1.1.1**, CIP:EL100 was further explored. The aim of this investigation was to explore the addition of a neutral polymer with amphiphilic character, HPMC and KolVA64, on the solid-state properties of the CIP:EL100 system. Moreover, an investigation was performed on the type of amphiphilic polymer added, the % (w/w) of the amphiphilic polymer, and the milling duration (**Figure 5.2**). Lastly, the addition of all excipients together was compared to the addition of KolVA64 to the CIP:EL100 amorphous polymeric system (**Scheme 5.2**).



Scheme 5.2 Addition of amphiphilic polymer to CIP:EL100.

The CIP:EL100:KolVA64 sample components were milled altogether at a ratio of 45:45:10 and depicted Bragg peaks at 14.5, 16.6, 20.9, and 25.5° 2 θ after 7 hours of milling, and at 14.5, 20.9, and 25.5° 2 θ after 8 hours of milling (**Figure 5.2**). Thus, despite further processing of the sample, both samples had a greater degree of crystallinity compared to CIP:EL100 system. Following on, CIP:EL100 was first processed as a binary system, followed by the addition of 10% (w/w) KolVA64. The sample displayed a comparable degree of crystallinity to that of CIP:EL100 system. Nevertheless, upon increasing the KolVA64 content to 20% (w/w), the level of crystallinity decreased compared to the CIP:EL100 system, showing the abolishment of the strongest peak at 25.5° 2 θ . Therefore, it is possible for KolVA64 to assist in Bragg peak reduction of CIP:EL100 as part of a ternary system. However, the decrease in crystallinity achieved may be dependent on the % (w/w) of KolVA64 added and by firstly milling the system as a binary sample before the addition of KolVA64. The CIP:EL100:HPMC system was explored using 20% (w/w) HPMC. The sample demonstrated the presence of peaks at 14.5, 16.6, 20.9, and 25.5° 2 θ , indicating that 20% (w/w) HPMC can increase the degree of crystallinity of the CIP:EL00 system. These findings are similar to CIP:EL100:HPMCAS-L/H using the PM100 (**Figure 5.1**). The CIP:EL100:HPMC system (20% (w/w) HPMC) was further examined utilizing the MM400 as a comparative method (**Figure 5.2**). The results demonstrated

a decrease in the level of crystallinity, as seen for CIP:EL100:HPMCAS-H sample using the MM400 (**Figure 5.1**).

In conclusion, 10% (w/w) KolVA64 with all three components milled together resulted in a slight increase in crystallinity compared to CIP:EL100, 10% (w/w) KolVA64 added to the binary system of CIP:EL100 demonstrated no changes to the degree of crystallinity of the system, 20% (w/w) KolVA64 added to the binary system decreased the level of crystallinity of the CIP:EL100 system and the addition of 20% (w/w) HPMC to the binary system increased the degree of crystallinity of the CIP:EL100 system. Hence, the polymers and their respective percentages (w/w) added to the CIP:EL100 system can be ranked in order of effectiveness as follows: 20% (w/w) KolVA64, 10% (w/w) KolVA64, and 20% (w/w) HPMC, based on the results from processing using the PM100. The MM400 displayed a complete reduction in the intensity of the Bragg peaks for CIP:EL100:HPMC compared to the sample milled using the PM100.

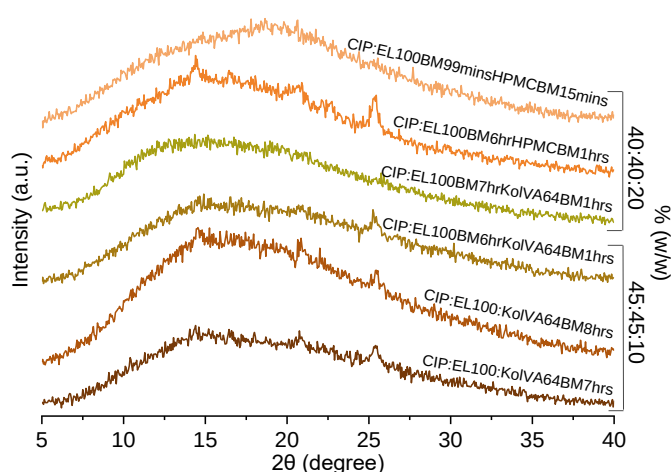
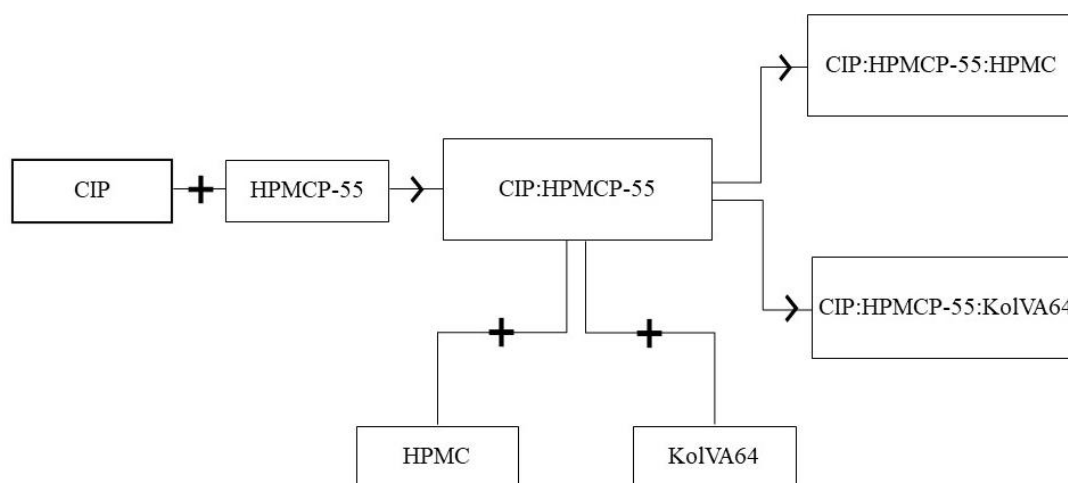


Figure 5.2 PXRD analysis of processed ternary systems using the Retsch planetary mill **PM100** and the Retsch mixer mill **MM400**.

5.3.1.1.3 Addition of Amphiphilic Polymer to CIP:HPMCP-55

The aim of this investigation was to reveal whether the addition of an amphiphilic polymer to the CIP:HPMCP-55 system has the ability to increase or decrease the level of crystallinity of the system. A study of the independent incorporation of HPMC and KolVA64 to the CIP:HPMCP-55 system and the different % (w/w) ratios was performed (**Scheme 5.3** and **Figure 5.3**).



Scheme 5.3 Addition of amphiphilic polymer to CIP:HPMCP-55.

The CIP:HPMCP-55:HPMC sample with a ratio of 35:35:30 contained Bragg peaks at 14.5, 20.9, and 25.5° 2 θ (**Figure 5.3**). The PXRD trace of this sample is comparable to CIP:HPMCP-55 binary system whereby peaks remained at 14.5, 20.9, and 25.5° 2 θ . Therefore, the addition of 30% (w/w) HPMC to the CIP:HPMCP-55 system did not increase or decrease the level of crystallinity of the system described. This is in disagreement with CIP:EL100 containing 20% (w/w) HPMC (**Figure 5.2**). The CIP:HPMCP-55:HPMC and CIP:HPMCP-55:KolVA64 samples using ratios of 30:30:40 displayed Bragg peaks at 14.5 and 25.5° 2 θ , with slight further reductions to the intensity of the Bragg peaks for the sample comprising KolVA64. Nevertheless, the addition of 40% (w/w) HPMC or KolVA64 to the CIP:HPMCP-55 system slightly decreased the level of crystallinity of the CIP:HPMCP-55 system. This is in agreement with the CIP:EL100 system consisting of 20% (w/w) KolVA64 (**Figure 5.2**). All ternary samples described are in contrast to the CIP:EL100:HPMC (20% (w/w) HPMC) sample which demonstrated an increase in the degree of crystallinity (**Figure 5.2**). In summary, the polymers and their respective concentrations (w/w) added to the CIP:HPMCP-55 system can be ranked by their ability to reduce the degree of crystallinity as follows: KolVA64 at 40% (w/w), HPMC at 40% (w/w), and HPMC at 30% (w/w) (**Figure 5.3**).

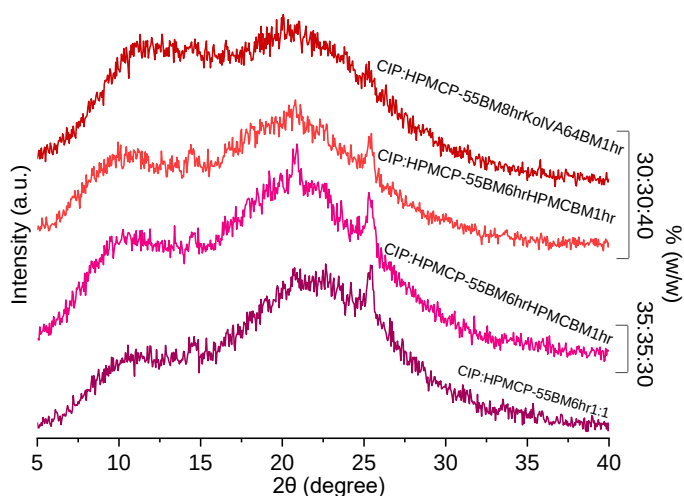
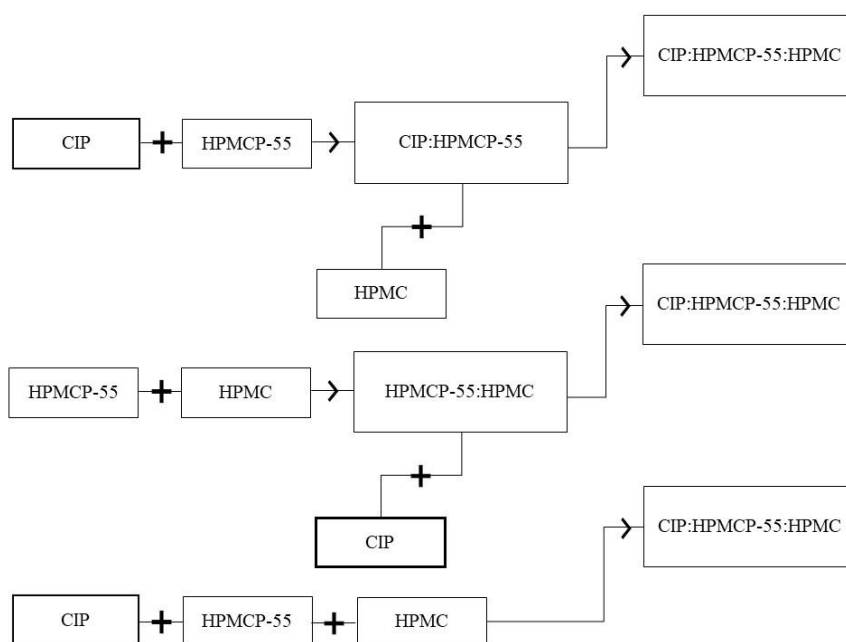


Figure 5.3 PXRD analysis of processed CIP with HPMCP-55 and HPMC or KolVA64 subject to milling for 7 and 9 hours using the Retsch planetary mill **PM100**.

5.3.1.1.4 Processing of CIP:HPMCP-55:HPMC by a MM400

An investigation into the degree of crystallinity of CIP:HPMCP-55 following the addition of HPMC was conducted using the MM400 and compared to those generated by the PM100 (**Figure 5.4**). The ratio of 30:30:40 and processing time of 99 or 114 minutes were examined for the CIP:HPMCP-55:HPMC samples. Furthermore, changes to the addition of the polymers and the API were conducted to generate a better understanding of the ternary systems produced using the MM400 (**Scheme 5.4** and **Figure 5.4**).



Scheme 5.4 Processing of CIP:HPMCP-55:HPMC using the **MM400**.

The components of the CIP:HPMCP-55:HPMC sample were milled altogether and showed the presence of crystalline Bragg peaks at 14.5° , 20.9° , and 25.5° 2θ . The diffraction peak positions were comparable to CIP:HPMCP-55 binary system, and the ternary system produced using the PM100 (**Figure 5.4**). However, the sample generated using the MM400 had a greater intensity of Bragg peaks compared to those in **Figure 5.3**. Further exploration was conducted by firstly milling the polymers together (HPMCP-55:HPMC) before the addition of CIP. This resulted in a reduction in the intensity of Bragg peaks at 20.9° and 25.5° 2θ , and the elimination of the diffraction peak at 14.5° 2θ . Thus, the level of crystallinity may have been reduced by allowing the polymers to interact before the addition of the third component, CIP. Nevertheless, further improvements were made by milling CIP:HPMCP-55 as a binary system before incorporating HPMC into the system, resulting in a decrease in the level of crystallinity of the CIP:HPMCP-55 system. This is in contrast to the CIP:HPMCP-55:HPMC systems produced using the PM100 which displayed crystalline Bragg peaks (**Figure 5.4**). These findings highlight the advantages of using the MM400 as previously discussed in **Chapters 2-4**. In addition, this study underlines the potential importance of step-by-step interactions between the polymer and the API which may lead to decreased levels of crystallinity, as was the case for CIP:HPMCP-55:HPMC.

To conclude, the use of the MM400 and the order of addition of components impacted the degree of crystallinity of the CIP:HPMCP-55 sample. The addition of the components can be ranked in terms of their effectiveness, by first the milling of the binary system CIP:HPMCP-55 prior to the addition of HPMC, followed by the milling of the two polymers (HPMCP-55:HPMC) before the incorporation of CIP, and lastly, the addition of all of the components together (CIP:HPMCP-55:HPMC). Furthermore, the complete decrease in the degree of crystallinity of CIP:HPMCP-55 can be achieved following the addition of HPMC while using the MM400, in contrast to the CIP:HPMCP-55:HPMC systems produced using the PM100.

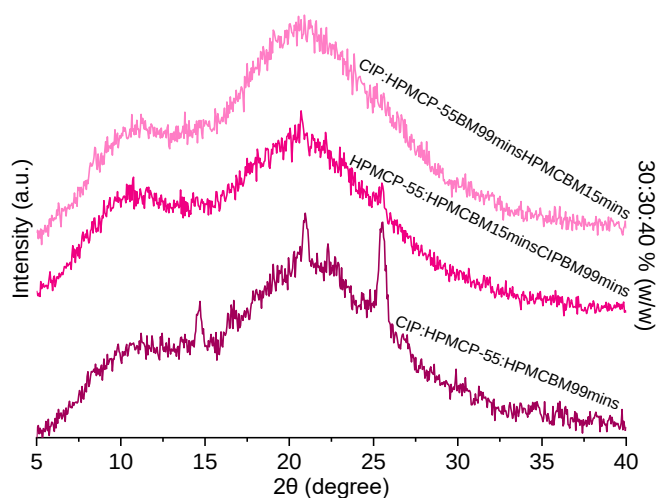


Figure 5.4 PXRD analysis of processed CIP:HPMCP-55:HPMC at 30:30:40% (w/w) subject to milling using the Retsch mixer mill **MM400**.

Although the addition of the polymers to the binary systems was successfully ranked using the PM100, the only polymers and their % (w/w) that reduced the degree of crystallinity of the original binary system, CIP:EL100, was 20% (w/w) KolVA64, and for the CIP:HPMCP-55 system was 40% (w/w) HPMC and 40% (w/w) KolVA64. In contrast, the remainder polymers and/or % (w/w) either increased or had no impact on the degree of crystallinity of the original binary systems (CIP:EL100 or CIP:HPMCP-55).

5.3.2 Thermal Analysis

CIP does not contain a dehydration peak (**Figure 4.4a**) as was the case for LVXh (**Figure 2.4**). In contrast, the polymers used tend to be hygroscopic due to their amorphous or semi-crystalline nature. Thus, it is expected that the mixtures containing these hygroscopic polymers will contain water, and since the samples produced are also primarily amorphous in nature. This was seen to be the case for thermograms of the PM100 processed samples which displayed dehydration peaks for all samples (**Figure 5.5**). This shallow dehydration peak denotes surface and/or weakly bound water evaporation. The polymers with the highest ratio used in the preparation of ternary samples were HPMCP-55 and EL100 with dehydration peak temperatures at 54.7 and 62.6 °C. The CIP:EL100 samples containing KolVA64, HPMC, HPMCP-55, HPMCAS-H, and HPMCAS-L, have dehydration peak temperature values between 57.1-61.8 °C (**Figure 5.5a**). The samples CIP:HPMCP-55:HPMC, regardless of their ratios used, have a dehydration peak at 55.6 °C (**Figure 5.5b**). The dehydration peak temperatures are consistent with the dehydration peak temperatures of the polymers utilized. Thus, no significant changes were made to the dehydration peak temperatures of these samples. Lastly, the CIP:HPMCP-

55:KolVA64 sample demonstrated a dehydration peak at 63.9 °C (**Figure 5.5b**). This value is significantly higher than that of HPMCP-55, indicating that perhaps moisture is distributed/bound differently within this sample.

Crystalline CIP undergoes melting/decomposition at 272 °C (**Figure 4.4a**). Thermograms of the PM100 processed samples displayed changes with respect to the melting/decomposition peaks. The CIP:EL100:KolVA64 sample demonstrated a melting/decomposition peak at 254 °C (**Figure 5.5a**). The CIP:EL100 samples with HPMC, HPMCP-55, HPMCAS-H, and HPMCAS-L displayed melting/decomposition temperatures between 208.8-212.4 °C. The CIP:EL100:HPMCAS-L sample displayed two additional melting/decomposition peaks at 241.7 and 262.6 °C. This is in contrast to the remaining samples CIP:EL100:HPMC, CIP:EL100:HPMCP-55, and CIP:EL100:HPMCAS-H which exhibited only one addition peak between 244.4-258.8 °C. Thus, two or three melting/decomposition peaks were detected for the ternary samples mentioned.

The melting/decomposition peaks of the CIP:HPMCP-55 ternary samples were evaluated (**Figure 5.5b**). The sample CIP:HPMCP-55:HPMC at ratios 30:30:40 and 35:35:30 % (w/w) were comparable and presented melting/decomposition peaks between 180.2-181.7 °C and 232.7-236.2 °C, and again between 267.8-270 °C. The sample CIP:HPMCP-55:KolVA64 showed melting/decomposition at 169.3 °C, and again at 213.6 and 269.1 °C. Thus, three melting/decomposition peaks were detected for the ternary samples discussed.

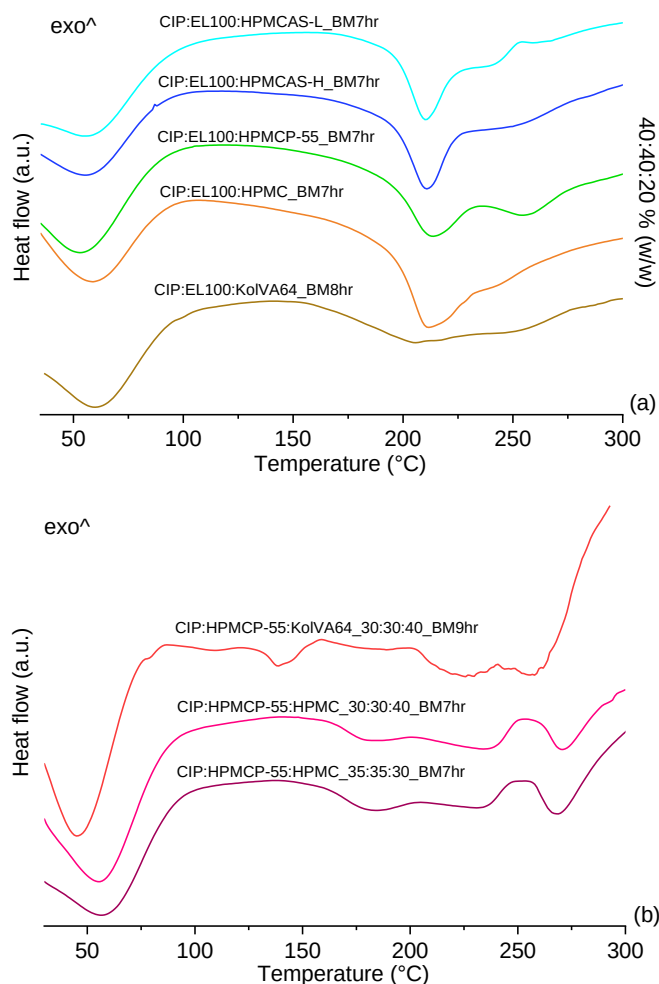


Figure 5.5 DSC analysis of processed ternary systems using the Restch planetary mill **PM100** (a) CIP:EL100 subject to milling before the addition of a ternary polymer: HPMCAS-L, HPMCAS-H, HPMCP-55, HPMC, or KolVA64 at 40:40:20% (w/w) (b) CIP:HPMCP-55 subject to milling before the addition of a ternary polymer: HPMC or KolVA64 at different % (w/w).

The Tg values of the ternary systems (**Figure 5.6**) were investigated via MTDSC analysis and compared to the Tg values of CIP, and binary systems (**Figure 4.5**). In all instances, the experimental Tg values exceeded the G-T estimated values (**Figure 5.6**). Overall, the thermograms displayed an increase in the Tg values for ternary systems compared to binary systems containing CIP:EL100, with the exception of the addition of KolVA64 (**Figures 4.5** and **5.6**). The addition of a second polymer to CIP:HPMCP-55 decreased the Tg value of CIP:HPMCP-55, particularly with the addition of KolVA64. Nevertheless, the increase in the Tg values for the CIP:EL100 ternary systems may lead to optimal stability and formulation design of the ASD/APSs. Although only one Tg was detected for all ternary samples, this does not discard the possibility that two Tgs may exist for some of, or all of the samples due to the

inability of the DSC to identify the Tgs of low polymeric concentration (i.e., ternary polymer). Thus, the possibility that minor phases separation may still be plausible.

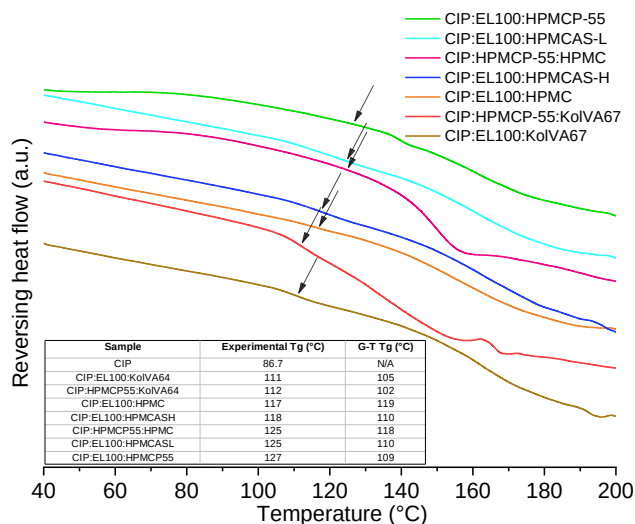


Figure 5.6 MTDSC analysis of processed ternary systems at different % (w/w) (Table 5.2) subject to milling using the Retsch mixer mill MM400.

5.3.3 Infrared Analysis

The IR spectra of unprocessed CIP, acidic and neutral polymers, and BMed ternary systems (**Figure 5.7**). The presence of the carboxylic acid group of CIP was identified for all the amorphous polymeric systems between 1723 and 1734 cm^{-1} , which is otherwise absent in unprocessed zwitterion CIP.¹⁹ The appearance of this group would normally suggest proton transfer to CIP. However, systems containing an acidic polymer, and thus, a carboxylic acid group make proton transfer difficult to detect. This is confirmed by the existence of the carboxylic acid group between 1723 and 1734 cm^{-1} for all acidic polymers. Furthermore, the vinyl acetate group in KolVA64 exists at 1737 cm^{-1} , making it difficult to detect proton transfer for any systems containing KolVA64.²⁵⁴ Since all ternary systems consist of at least one acidic polymer, proton transfer cannot be confirmed due to signals from the acidic polymers, and KolVA64 polymer where applicable.

The IR spectrum of crystalline CIP contains peaks at 1590 and 1375 cm^{-1} , corresponding to the carboxylate ion.²⁰⁴ Residual amounts of the carboxylate ion peak are seen at 1590 cm^{-1} for CIP:EL100:HPMCAS-H, indicating residual amounts of zwitterionic CIP. Interestingly, CIP:EL100 does not contain this peak, signifying that HPMCAS-H may interfere with interactions involving the carboxylate ion (**Figure 5.7a**). Furthermore, the peak at 1590 cm^{-1} is also absent for CIP:EL100:KolVA64, with no residual amounts remaining, indicating the

absence of zwitterionic CIP. The CIP:KolVA64 sample has a peak at 1590 cm^{-1} (**Figure 5.7b**), in agreement with its semi-crystalline nature as discussed in **Chapter 4**. The disappearance of this peak in the ternary sample suggests that KolVA64 does not prevent interactions with the carboxylate ion of CIP. For CIP:HPMCP-55:HPMC, residual amounts of the carboxylate ion can be seen at 1590 cm^{-1} . However, this may not be due to residual amounts of zwitterionic CIP since HPMCP-55 contains a small peak at 1590 cm^{-1} . Thus, it is difficult to detect if the identification of this peak is due to residual amounts of zwitterionic CIP, or simply the presence of the HPMCP-55 polymer (**Figure 5.7c**). The absence or residual amounts of the peak at 1590 cm^{-1} as seen for the ternary systems indicate the formation of ASDs,¹⁹ and perhaps, proton transfer.

The ketone carbonyl stretch of CIP is at approximately 1618 cm^{-1} .²⁰⁴ This peak is also present for HPMCAS-H, KolVA64, and HPMC. Each of these polymers is included in the ternary systems, thus, making specific interactions regarding the ketone carbonyl difficult to detect due to an overlap of signals from the individual components.

To conclude, the peak at 1720 cm^{-1} could not confirm the protonated carboxylic acid group of CIP due to the overlap from the carbonyl stretch originating from acidic polymers. Nevertheless, the absence or almost complete reduction of the peak at 1590 cm^{-1} from the carboxylate ion in the ternary systems indicates ASDs/proton transfer. The ketone carbonyl peak at 1618 cm^{-1} also contained overlapping signals from the polymer in the ternary systems, making it difficult to detect specific interactions with this group. The disruption of interactions to the carboxylate ion in the CIP:EL100 systems was confirmed by the addition of HPMCAS-H and was represented by the appearance of a residual peak at 1590 cm^{-1} , which was absent for the CIP:EL100 system. The CIP:KolVA64 system displayed a peak at 1590 cm^{-1} which was absent in the ternary system, demonstrating no interference to the carboxylate ion in the CIP:EL100 system with the addition of KolVA64. Thus, the addition of a second polymer has the ability to interact with the already existing binary system, and ASDs were confirmed for the ternary systems.

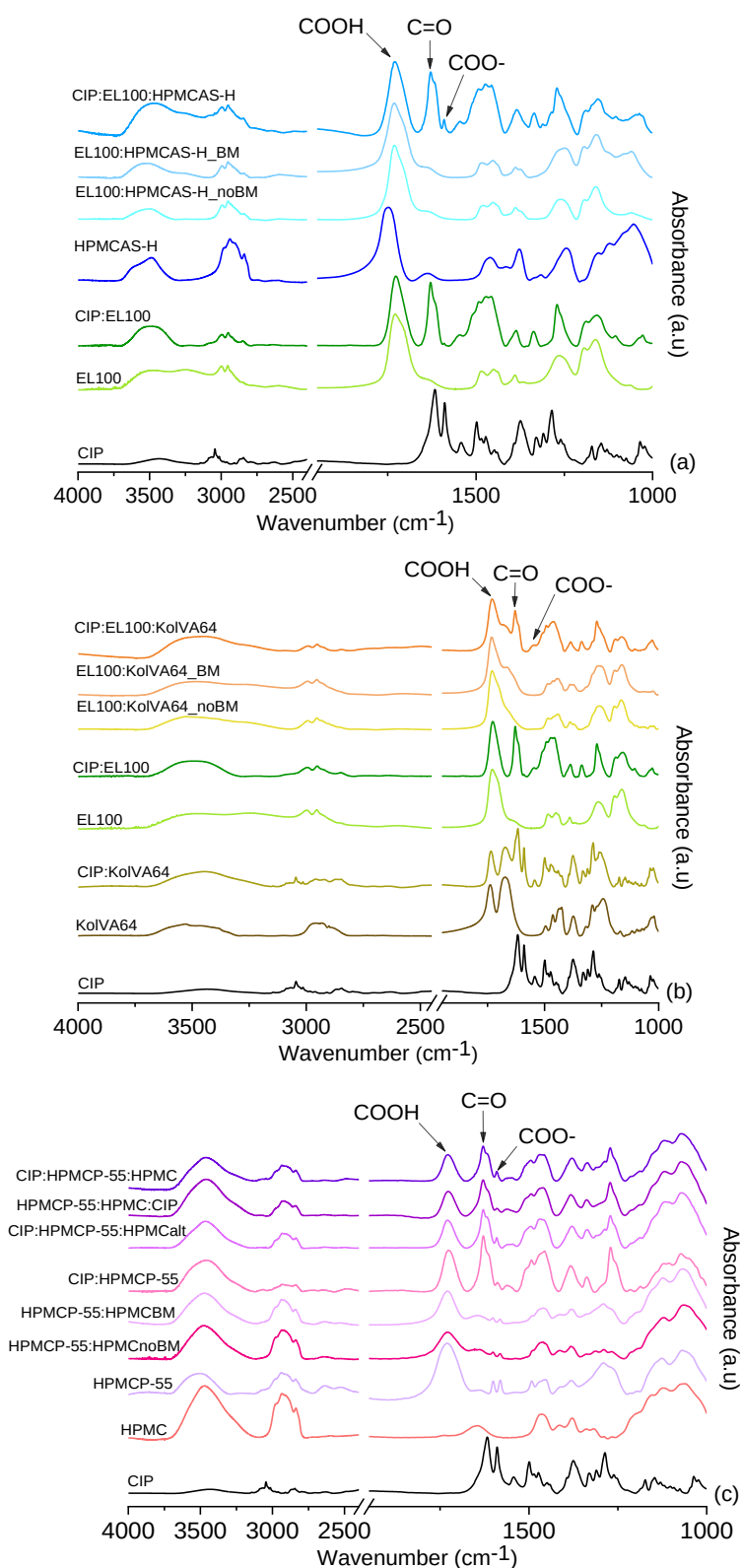


Figure 5.7 FTIR of unprocessed CIP and polymers, and processed semi-crystalline and/or amorphous polymeric systems subjected to milling to achieve ternary systems of (a) CIP:EL100:HPMCAS-H (b) CIP:EL100:KolVA64 and (c) CIP:HPMCP-55:HPMC.

5.3.4 Physical Stability of Ternary Systems

DVS analysis was carried out to evaluate the effect of moisture on the solid-state stability of the ternary BMed samples (**Figures 5.8-5.10**). A large absorption hysteresis can be seen for all samples allowing the Young-Nelson model to be employed (**Table 5.3**). A sample mass increase was depicted for all samples when RH increased. Additionally, there was no evidence of crystallization despite the powder sorbing between 16-20% (w/w) of moisture at 90% RH. The CIP:EL100 ternary samples lost approximately 16-20% (w/w) moisture between 90-0% RH (desorption) over 1,600 minutes (≈ 27 hours) (**Figure 5.8** and **5.9**). The CIP:EL100 ternary system has a longer drying time than the binary system (**Figure 4.9**), suggesting that it is more hydrophilic. Nevertheless, both the binary and ternary systems demonstrated internal absorption as the leading mechanism for water uptake. The CIP:HPMCP-55 ternary systems lost approximately 17% (w/w) moisture between 90-0% RH (desorption) between a period of 1,000-1,200 minutes (≈ 17 -20 hours) (**Figure 5.10**). The drying time for the CIP:HPMCP-55 ternary systems is shorter compared to the binary system (**Figure 4.9**), indicating that the ternary systems are more hydrophobic. This is consistent with the Young-Nelson model, which indicates that the multilayer mechanism is the primary mode of absorption (**Table 5.3**). These findings are in contrast to the samples containing CIP:EL100.

Overall, the ternary samples containing CIP:EL100 took longer to dry than the ternary samples consisting of CIP:HPMCP-55. This may be due to the binary and ternary sample(s) of CIP:EL100 having the majority of their moisture uptake internally absorbed, in all cases. In comparison, the ternary samples of CIP:HPMCP-55 primarily exhibit a multilayer water uptake mechanism (**Table 5.3**), whereas the binary system is dominated by internal absorption (**Table 4.5**). Additionally, the incorporation of a second polymer can shift the primary moisture uptake mechanism from internal absorption to the multilayer process.

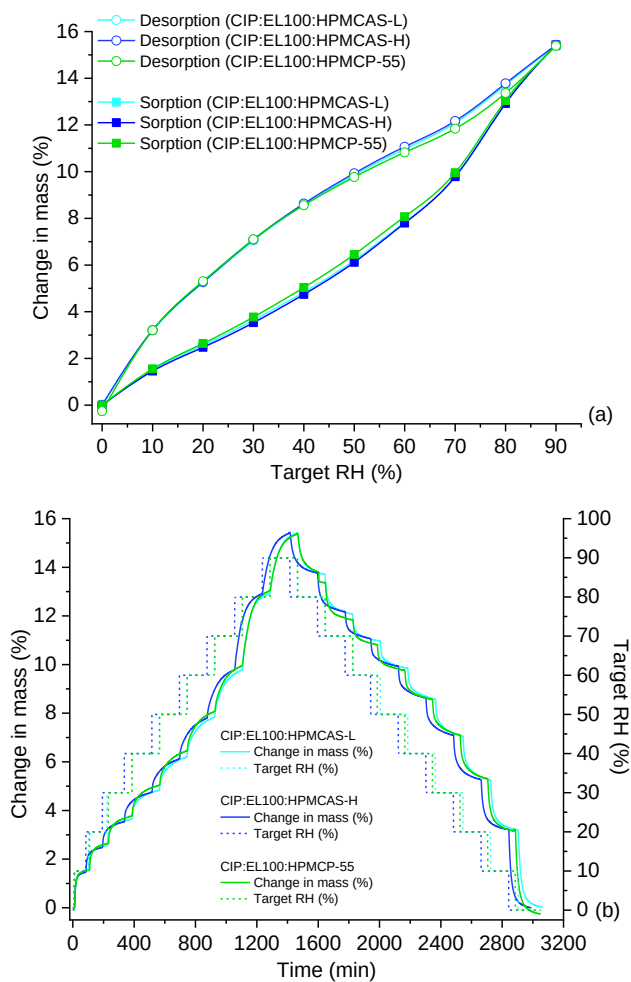


Figure 5.8 DVS plots of CIP:EL100:HPMCP-55, CIP:EL100:HPMCAS-H, and CIP:EL100:HPMCAS-L BMed for 7 hours using a PM100, 50 mL stainless steel jar, and three 1.5 cm balls at 400 rpm (a) isotherm plots (b) mass plot over time.

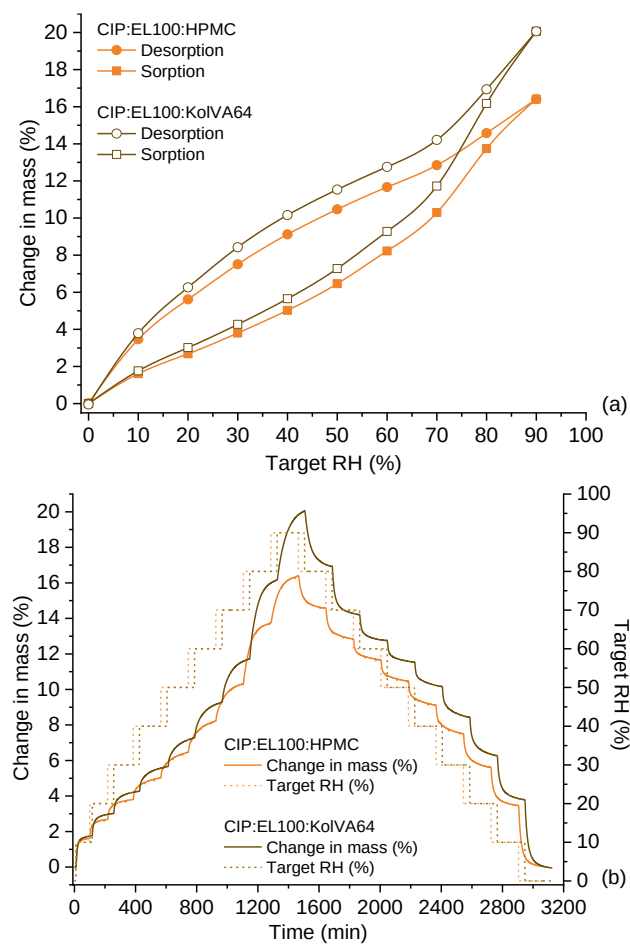


Figure 5.9 DVS plots of CIP:EL100:HPMC and CIP:EL100:KolVA64 BMed for 7 or 8 hours using a PM100, 50 mL stainless steel jar, and three 1.5 cm balls at 400 rpm (a) isotherm plots (b) mass plot over time.

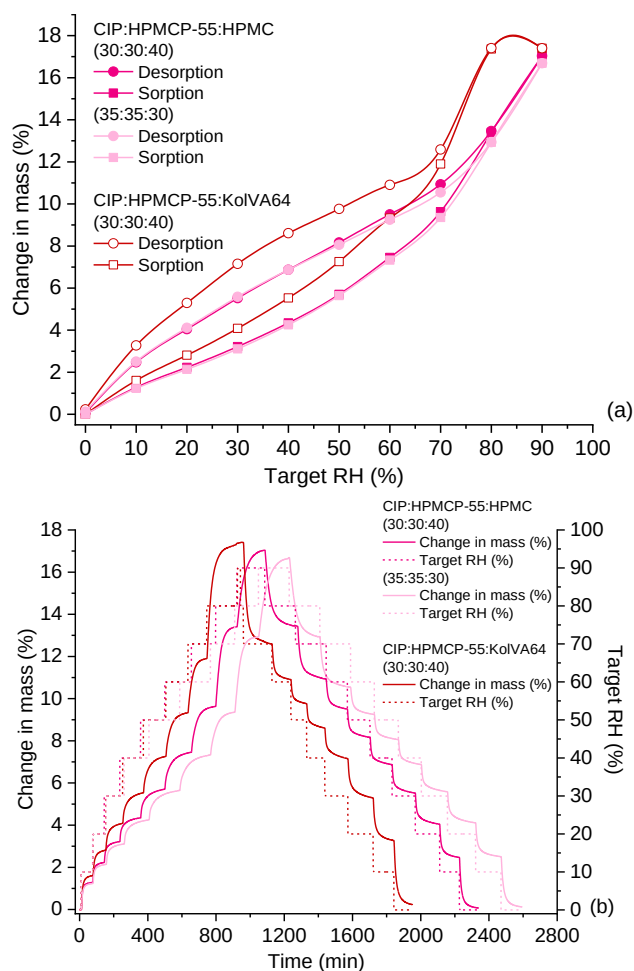


Figure 5.10 DVS plots of CIP:HPMCP-55:HPMC at different ratios, and CIP: HPMCP-55:KolVA64 BMed for 7 or 9 hours using a PM100, 50 mL stainless steel jar, and three 1.5 cm balls at 400 rpm (a) isotherm plots (b) mass plot over time.

Table 5.3 Young-Nelson Internally Absorbed, Multilayer, and Monolayer Parameters of Ternary Samples.

Sample	Monolayer N ($\approx$ mol/g)	Multilayer N ($\approx$ mol/g)	Internally Absorbed N ($\approx$ mol/g)
CIP:EL100:HPMCAS-L	0.0005	0.0025	0.005
CIP:EL100:HPMCAS-H	0.0005	0.0025	0.0055
CIP:EL100:HPMCP-55	0.001	0.003	0.0045
CIP:EL100:KolVA64	0.00075	0.0035	0.006
CIP:EL100:HPMC	0.0005	0.0025	0.0055
CIP:HPMCP-55:KolVA64	0.001	0.005	0.0045
CIP:HPMCP-55:HPMC	0.00075	0.0045	0.004

5.4 Conclusions

To conclude, the effect of the addition of various polymers including HPMCP-55, HPMCAS-L, HPMCAS-H, HPMC, and KolVA64, on the crystallinity of CIP binary and ternary systems was explored. The systems were analyzed using PXRD, DSC, MTDSC, FTIR, and DVS. The results using the PM100 demonstrated, in some cases, that the addition of certain polymers can reduce the degree of crystallinity of the original binary system. Specifically, 20% (w/w) KolVA64 reduced the crystallinity in the CIP:EL100 system, while 40% (w/w) HPMC and KolVA64 had the same effect in the CIP:HPMCP-55 system. In contrast, other polymers either had no impact or increased the degree of crystallinity of the original binary systems. The MM400 was found to reduce the degree of crystallinity in all cases where the binary system was milled before adding the second polymer. DSC analysis showed no significant changes to the dehydration peak temperatures of the ternary samples, except for CIP:HPMCP-55:KolVA64, which suggested additional water content in the sample. For the most part, the MTDSC results confirmed higher experimental T_g values than G-T estimated values. IR analysis suggested proton transfer in the systems, confirmed by the absence or almost complete reduction of the carboxylate ion peak. DVS revealed that the incorporation of a second polymer increased the hydrophilicity of the CIP:EL100 system, while the CIP:HPMCP-55 systems exhibited more hydrophobic characteristics. The moisture uptake analysis further highlighted differences in water absorption mechanisms, with CIP:EL100 ternary samples absorbing moisture internally, while CIP:HPMCP-55 systems primarily absorbed moisture via multilayer adsorption. In summation, the impact of polymer addition on the crystallinity behavior of binary amorphous polymeric systems was thoroughly investigated.

**Chapter 6.0 Mechanically Milled Binary and Ternary Amorphous
Polymeric Systems of Ciprofloxacin to Enhance Solubility and
Antibacterial Activity**

Chapter 6.0 Mechanically Milled Binary and Ternary Amorphous Polymeric Systems of Ciprofloxacin to Enhance Solubility and Antibacterial Activity

6.1 Introduction

Poor solubility of newly developed APIs is a major challenge for the pharmaceutical industry. Approximately 40% of newly developed drugs are poorly water-soluble or lipophilic in nature, and around 70–90% of all NCEs/drug molecules comprise poor aqueous solubility.¹⁻³ Therefore, it is essential to enhance the solubility of poorly soluble APIs, to allow more drug to be absorbed into the system, and ultimately, improve the bioavailability of the drug. In addition, bacterial antimicrobial resistance (AMR) is recognized by the WHO as a global public health concern.²⁷² The emergence and development of drug resistant pathogens has shown reduced susceptibility to standard antibiotics, including fluoroquinolone drugs, making it difficult to treat common infections.²⁷² Thus, CIP was chosen as a model drug for the enhancement of solubility and antibacterial activity. It is a poorly soluble BCS Class IV drug,²⁷³ with a solubility in water as low as 0.08 mg/mL at 37 °C respectively,¹²⁹ as opposed to the soluble, freely soluble, and very soluble range of 33-100, 100-1000 and ≥ 1000 mg/L.²⁷⁴ CIP is on the WHO list of essential medications, making it an even more suitable candidate for this study.¹²⁰ Improved solubility of drugs can be achieved by generating ASD/APSs that are in a high energy state as less energy is required to break the bonds due to its disordered manner compared to its ordered, crystalline counterpart. Thus, these high energy forms can generate a thermodynamically unstable, supersaturated solution of the drug,³⁵ known as the “spring”. Supersaturation is when the drug concentrations exceed the equilibrium solubility in the same medium.³⁶ If supersaturation is achieved, the free drug concentration and the thermodynamic activity of the drug will be increased beyond its solubility limit at the absorptive site, ultimately, leading to an increase in the driving force for transportation across and into the biological membrane.^{37, 38} In order to reestablish the thermodynamic equilibrium^{35, 39} of supersaturating drug delivery systems (SDDS), precipitation may occur and reduce the free drug concentration, and thus, the absorption ability. Therefore, enhancing drug solubility is heavily dependent on the generation and maintenance of the metastable supersaturated state³⁹ which can be improved by specific drug-polymer interactions,²⁹⁻³¹ and the precipitation inhibition by the use of excipients/polymers known as the “parachute” to inhibit the nucleation and crystallization of

the drug.³⁰ Thus, the supersaturation method described to improve the absorption of poorly water-soluble drugs can be summarized by the “spring and parachute” model by Guzman *et al.*^{275, 276}

Amorphous materials are thermodynamically unstable which may lead to the relaxation, nucleation, and crystal growth of the API.^{24, 25} The nucleus describes the minimum amount of a new crystalline phase that is capable of independent existence and nucleation is the formation of small nuclei. Nucleation is frequently followed by nuclei crystal growth i.e., crystallization.^{277, 278} The driving force determines the crystallization of an amorphous drug and is governed by the free energy difference between the amorphous and crystalline states, and the molecular interactions. The molecularly dispersed polymer in an ASD/APS can protect the drug against crystallization by reducing the thermodynamic driving force for crystallization.²⁶⁻²⁸ Furthermore, the polymer(s) can modify the kinetics of the system by raising the T_g which increases the kinetic barrier to crystallization.^{279, 48} However, although polymers may be able to inhibit the crystallization growth of a drug in the solid-state, this prevention is not always the case upon contact with aqueous media.²⁸⁰ A proposed mechanism suggests that surface crystallization could occur upon exposure to aqueous media, potentially leading to rapid crystal growth. This process may result in the formation of a crystalline drug layer on the surface of the otherwise amorphous polymeric system.^{280, 281} Crystallization of the amorphous drug results in a decrease in the apparent solubility. Apparent solubility is used to define the metastable or dynamic solubility of a drug, whereas solubility represents the thermodynamically stable state of a drug. The apparent solubility decreases to the thermodynamically stable solubility after a period at equilibrium.²⁸²

To date, fluoroquinolone drugs OFX²⁶³ and NOR²⁶⁴ have been investigated for enhanced dissolution profiles using ternary systems. According to the literature, ternary systems using fluoroquinolone drugs have never been investigated for enhanced solubility before. However, Mesallati *et al.* performed solubility and antibacterial studies for CIP and ENRO binary ASDs containing EL100 and HPMCAS-L/MG polymers.^{19, 20} The CIP and ENRO binary ASDs displayed superior solubility in FaSSIF solution and in water for CIP ASDs, and 2-4-fold improvements against some strains of bacteria.^{19, 20} The binary amorphous polymeric systems of CIP that were produced by milling and described in **Chapter 4** included EL100, HPMCP-55, HPMC, and KolVA64 polymers. The ternary amorphous polymeric systems of CIP that were produced by milling and described in **Chapter 5** comprised the addition of HPMCP-55,

HPMCAS-L, HPMCAS-H, HPMC, and KolVA64 to the CIP:EL100 and CIP:HPMCP-55 systems. The binary and ternary systems chosen from these chapters for solubility and antibacterial investigation are described in **Table 6.1**.

The primary aim of this study was to prepare several binary and ternary amorphous polymeric systems of CIP by BMing for solubility and antibacterial studies. For the solubility studies, the systems were exposed to aqueous, fasted, and fed state simulated intestinal fluid (FaSSIF and FeSSIF) buffers to investigate the solubility at different pH ranges and to simulate the upper part of the gastrointestinal tract in the fasted and fed states. Moreover, the samples post solubility studies were examined by PXRD analysis to investigate the stabilizing capabilities of the various polymeric systems upon exposure to different aqueous and buffer solutions. For the antibacterial studies, six strains of bacteria were investigated based on their relevance to CIP, of which three strains were ESKAPE pathogens. ESKAPE pathogens are classified as the leading pathogens for death associated with resistance,²⁸³ prioritized by the WHO^{272, 284} and listed by the Global Action Plan on AMR, the UN Interagency Coordination Group, and the One Health Global Leaders Group.²⁸⁵ This highlights the importance of including these pathogens in the study. Furthermore, three of the bacterial strains were made resistant to CIP to investigate whether the formation of the binary and/or ternary systems had the ability to change the mechanism of CIP to overcome antibiotic resistance in the strains chosen. Thus, a comparative investigation of unprocessed CIP, and CIP binary and ternary amorphous polymeric systems on solubility and stability of systems upon exposure to aqueous and FaSSIF and FeSSIF buffer solutions, and minimum inhibitory concentrations (MICs) against bacterial strains, including ESKAPE pathogens, and strains resistant to CIP were performed.

6.2 Experimental Section

6.2.1 Materials

Materials were purchased or donated as described in **Section 4.2.1**. SIF Powder Original for fasted and fed state simulated intestinal fluid (FaSSIF and FeSSIF) was purchased from biorelevant.com (UK). Sodium chloride (NaCl), sodium acetate (CH₃COONa), sodium hydroxide (NaOH), acetic acid (CH₃COOH), hydrochloric acid (HCl), monopotassium phosphate (KH₂PO₄), phosphate buffer saline (PBS), and sodium phosphate monobasic monohydrate (NaH₂PO₄·H₂O), and sodium phosphate monobasic dihydrate (NaH₂PO₄·2H₂O) were purchased from Merck (Germany). 0.2 µm, 25 mm polytetra-fluorethylene (PTFE) membrane filters (VWR, USA), and 1.5 mL reaction tubes (Greiner bio-one, Austria) were purchased. Ammonium dihydrogen phosphate (NH₄H₂PO₄) and acetonitrile were obtained from Merck (Germany). PBS tablets were obtained from Merck, Ireland, Mueller Hinton Broth (MHB) from Thermo Scientific™ Oxoid™ (UK), 96-well plates from Sarstedt (Germany), Nalgene Syringe Filter SterilePES membrane 0.45 µm 25mm from Scientific Laboratory Supplies Ltd. (Ireland), and 5 mL disposable sterile syringes from Cruinn Diagnostics Ltd. (Ireland).

6.2.2 Methods

6.2.2.1 Milling

6.2.2.1.1 Retsch Mixer Mill MM400

CIP binary and ternary systems were BMed using a Retsch mixer mill MM400 (Germany) at 25 Hz (1/s) as described in **Section 4.2.2.1.1, MM400_Cond1**. The samples were examined by/for PXRD (**Section 6.2.2.2**), solubility studies (**Section 6.2.2.3**), and bacterial studies (**Section 6.2.2.5**). A summary of the systems is displayed in **Table 6.1**.

Table 6.1 Binary and ternary systems.

	System (Binary or Ternary)	% (w/w) API:Polymer(s)	Ratio	Binary Acidic Polymer	Binary Neutral Polymer	Ternary Acidic:Acidic Polymer(s)	Ternary Acidic:Neutral Polymer(s)
1	CIP:EL100	40:60	2:3	X			
2	CIP:EL100	50:50	1:1	X			
3	CIP:HPMCP-55	50:50	1:1	X			
4	CIP:HPMC	50:50	1:1		X		
5	CIP:KolVA64	50:50	1:1		X		
6	CIP:EL100:HPMCAS-H	40:40:20	N/A			X	
7	CIP:EL100:HPMC	40:40:20	N/A				X
8	CIP:HPMCP-55:HPMC	30:30:40	N/A				X

6.2.2.2 Powder X-Ray Diffraction (PXRD)

PXRD analysis of binary and ternary samples before solubility studies as described in **Section 2.2.2.3**. PXRD analysis using the method described was performed by Dr. Michal Šimek (Zentiva, Prague, Czechia). Alternatively, PXRD patterns were obtained for binary and ternary samples before and after solubility studies with a laboratory X'PERT PRO MPD (PANalytical, Almelo, Netherlands) diffractometer with CuK α (λ = 1.542 Å) radiation. The generator was operated at an excitation voltage of 45 kV and anodic current of 40 mA. The following scan parameters were utilized: scan type-gonio, measurement range of 2-40° 2 θ , step size of 0.02° 2 θ and the time per step was 200 seconds. The samples were placed on a zero-background silica sample holder.

6.2.2.3 Dynamic Solubility Studies

6.2.2.3.1 Preparation of Aqueous and Fasted/Fed State Simulated Intestinal Fluid Solutions

Aqueous solutions and FaSSIF and FeSSIF buffers were prepared as described in **Tables 6.2** and **6.3**. Aqueous solutions were prepared to the target pH of solution (**Table 6.2**), however, the inclusion of CIP resulted in a shift in pH. Thus, the measured pH of the aqueous solutions with CIP, binary, and ternary samples is displayed in **Table 6.2**. CIP, binary, and ternary samples did not result in a pH shift in FaSSIF and FeSSIF buffers (**Table 6.3**). Blank FaSSIF and FeSSIF refers to the buffers prepared without the inclusion of fasted and fed SIF powders (**Table 6.3**).

Table 6.2 Composition of aqueous solutions with pH ranging from 1.2-6.8 used for solubility studies.

	Target pH of solution	Measured pH of CIP solution	Measured pH of binary and ternary solution	Amount	Unit
artificial gastric juice (AGJ) solution	1.2	4.6	1.5-1.6		
Volume to be prepared				0.5	L
NaCl				0.965	g
1M HCl				33.5	mL
10mM HCl solution	2.0	4.8	3.2-5.2		
Volume to be prepared				0.5	L
1M HCl				5	mL
10mM HCl + 90mM NaCl solution	2.0	5.8	5.2-6.0		
Volume to be prepared				0.5	L
NaCl				2.630	g
1M HCl				5	mL
1mM HCl + 99mM NaCl solution	3.0	4.9	4.7-5.1		
Volume to be prepared				0.5	L
NaCl				2.892	g
1M HCl				5	mL
Acetate buffer	4.5	4.8	4.1-5.3		
Volume to be prepared				0.5	L
CH ₃ COONa				0.9	g
CH ₃ COOH				0.8	mL
Phosphate buffer	6.8	6.9	6.4-7.1		
Volume to be prepared				0.5	L
NaOH				0.45	g
KH ₂ PO ₄				3.405	mL

Table 6.3 Fasted and fed state simulated intestinal fluid buffers.

	Amount	Unit
Blank FeSSIF, pH 5.0		
Volume to be prepared	0.1	L
NaCl	1.1784	g
CH ₃ COOH	0.825	mL
NaOH	0.404	g
Blank FaSSIF, pH 6.5		
Volume to be prepared	0.1	L
NaCl	0.6186	g
NaH ₂ PO ₄ .2H ₂ O	0.447	g
NaOH	0.042	g
FeSSIF, pH 5.0 v1		
Volume to be prepared	0.1	L
NaCl	1.1784	g
CH ₃ COOH	0.825	mL
NaOH	0.404	g
SIF powder	1.12	g
FaSSIF, pH 6.5 v1		
Volume to be prepared	0.1	L
NaCl	0.6186	g
NaH ₂ PO ₄ .2H ₂ O	0.447	g
NaOH	0.042	g
SIF powder	0.224	g

6.2.2.3.2 Solubility Studies of Binary and Ternary systems

About 1.5 mL of aqueous buffer, FaSSIF, or FeSSIF was added to 1.5 mL reaction tubes. Approximately 225 mg of unprocessed CIP, and processed binary and ternary systems, in excess of the expected saturated solubility, were added to the stirred vials. The resulting suspension were shaken at 37 °C for 24 hours using a grant-bio thermoshaker (Grant Instruments Ltd, UK). Subsequently, the vials were centrifuged at 10,000 rpm for 10 min using Eppendorf centrifuge 5427R (Merck, Germany). The aliquots were filtered, and the supernatant was diluted appropriately. The concentration of CIP in each of the diluted samples was determined by high-performance liquid chromatography (HPLC) (described in **Section 6.2.2.4**). The solubility studies were repeated at least in triplicate with each medium. The pH of the solutions was measured before the addition of the samples and at the end of the 24 hour study using a Thermo Orion 420A+ pH meter (Thermo Scientific, UK). The solid material left in the vials at the end of the studies was analyzed by PXRD (described in **Section 6.2.2.2**). All materials described are mentioned in **Section 6.2.1**.

6.2.2.4 High Performance Liquid Chromatography (HPLC)

The HPLC was set up and data analyzed by Dr. Tereza Krejci, Dr. Markéta Pislova, or Mr. Marek Stika (Zentiva, Prague, Czechia). The content of CIP was measured with a Waters® HPLC system (Waters Corporation, USA). 2998 PDA detector and Waters® e2695 separations modules was used for UV spectra detection for CIP at 278 nm (Waters Corporation, USA). A Gemini C6-Phenyl, 50 x 4.6mm; 3.0 mm column was used with a column temperature of 40 °C and sample temperature of 37 °C. The mobile phase consisted of 10mM ammonium dihydrogen phosphate (NH₄H₂PO₄) at pH 3.0 (solvent A) and acetonitrile (solvent B) mixed with gradients shown in **Table 6.4**. An injection volume of 10 µL and flow rate of 1.2 mL/min for 4 min was used.

Table 6.4 gradients of %A and %B at different time intervals (min).

Time (min)	%A	%B
1.0	90	10
1.5	90	10
2.5	20	80
3.0	20	80
4.0	90	10

6.2.2.5 In vitro Bacterial Studies

All bacterial studies were performed and analyzed by Mr. Stephen Ryder, with involvement from Prof. Marta Martins (Department of Microbiology, Trinity College Dublin, Ireland) (**Sections 6.2.2.5.1-6.2.2.5.3**). Six bacterial strains, namely, (1) *Staphylococcus aureus*, (2) *Pseudomonas aeruginosa*, (3) *Klebsiella pneumoniae*, (4) *Acinetobacter baumannii*, (5) *Escherichia coli*, and (6) *Salmonella enterica* serovar Typhimurium, and bacterial strains that were previously made resistant to 32 mg/L of CIP, namely *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium were examined.

6.2.2.5.1 Preparation of Ciprofloxacin Samples for Bacterial Studies

PBS solution with pH 7.5 was used to dissolve CIP, the binary and ternary systems. The maximum solubility at similar pH value i.e., approximately pH 7.0, of each binary and ternary sample was approximately halved to prevent precipitation. In the case of CIP and CIP:HPMC, the maximum solubility values were significantly lower, thus, the quantity of sample was

increased ($\frac{3}{4}$) in order for the sample to be within the microbalance limits while remaining below the solubility value. Approximately 10 mL of stock solution was prepared for the bacterial studies (as described in **Sections 6.2.2.5.2** and **6.2.2.5.3**).

6.2.2.5.2 Induction of Ciprofloxacin Resistance – Serial Passaging

Isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium were induced to 32 mg/L CIP resistance through serial passaging in increasing concentrations of the antibiotic. The induction of resistance was performed at half the minimum inhibitory concentration (MIC) of the antibiotic for each respective strain. The concentrations used were 0.007812 mg/L, 1 mg/L, and 0.03125 mg/L for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium, respectively. 2-3 colonies of each strain were used to inoculate separate tubes of 5 mL MHB and incubated at 37 °C (G24 Environmental Incubator Shaker, New Brunswick Scientific) for 18 hours and shaken at 220 ppm. 100 uL of overnight culture was used to inoculate 4.9 mL of MHB to the desired final concentrations of CIP.

To speed up the process, the 100 uL from the $\frac{1}{2}$ MIC-induced strains were used to simultaneously inoculate tubes with x1, x2, and x4 the starting MIC. The tube with the highest concentration that had turbid growth was used to concurrently inoculate three more tubes, one with the same concentration and two others with the next two multiples of the initial MIC. This process was repeated until the concentration of 4 mg/L was reached, after which doubling concentrations were used until a concentration of 32 mg/L CIP was reached. Passage controls were produced. This involved serial passage of the strains without CIP the same number of times as was required to reach the highest level of induction in each strain.

6.2.2.5.3 Assessment of Antibiotic Activity – Broth Microdilution Method

Seven compounds were assessed for antibacterial activity (**Table 6.1**). MICs for CIP and CIP binary and ternary systems were determined via the broth microdilution method. MIC testing was carried out in duplicate over three technical replicates against the six parental strains as mentioned in **Section 6.2.2.5**. MIC testing of the compounds was also carried out against bacterial strains made resistant as mentioned in **Section 6.2.2.5**. For quality control purposes, the same MICs were determined against the parental strains that were passaged in media without CIP the same number of times. MICs were determined as per the European Committee on Antibiotic Susceptibility Testing (EUCAST) guidelines.²⁸⁶ Briefly, using a multichannel P200 pipette, 100 uL of MHB was added to columns 1-11 in 96-well plates. Antibiotics were diluted

in MHB to starting concentrations of 8 mg/L for *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, 4 mg/L for *Pseudomonas aeruginosa*, and 1 mg/L for *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Using a micropipette, 200 uL of each working solution was added to separate wells of column 12 in 96-well plates. Using a multichannel pipette, each sample was diluted by transferring 100 uL from row to row in descending order while mixing before each time a transfer occurred. This was continued until column 3 was reached at which the excess 100 uL of media was discarded. To act as a positive control, column 2 received no antibiotic. Each row was then inoculated using 15 uL of overnight bacterial culture grown in MHB and standardized to 0.5 McFarland with PBS solution, leaving the first column as a sterile control (media only). Inoculated 96-well plates were incubated aerobically at 37 °C (REVCO ULTIMA Incubator Oven INB200) for 18 hours without shaking. After incubation, the 96-well plates were read at 600 nm in a plate reader (Biotek; Epoch 2 Microplate Reader) and the first well with an optical density of less than 0.1 was taken as the MIC. Where there were discrepancies between technical replicates, the most concordant value was taken as the MIC.

6.3 Results and Discussion

6.3.1 Solubility Studies

As previously stated, CIP is a zwitterionic compound consisting of a basic and an acidic group capable of ionizing under different pH environments. Therefore, it is important to study the solubility of CIP at multiple pH conditions in order to gain an insight into the behavior in the gastrointestinal tract.²⁸⁷ Solubility studies for CIP, binary and ternary systems were carried out in aqueous media, and FaSSIF and FeSSIF solutions (See **Tables 6.2** and **6.3**). The Henderson–Hasselbalch equation was used to construct the theoretical pH–solubility profile of pure CIP and the commercial hydrochloride salt, CIP HCl.¹⁹

Equation 6.1 *The theoretical pH–solubility profile of pure CIP and the commercial HCl salt using the Henderson–Hasselbalch equation.*

$$S_T = [B](1 + 10^{pK_{a1}-pH} + 10^{pH-pK_{a2}}) \quad \text{Eqn. (6.1)}$$

S_T is the total solubility of the drug in mol/L, and $[B]$ is the concentration of the free base (approximately 0.000266 M for CIP and 0.0008 M for CIP HCl). CIP has two potentially ionizable functional groups, carboxylic acid (pK_{a1}) and piperazine amine (pK_{a2}). These functional groups have pK_a values of 6.16 and 8.62, respectively.¹³² The CIP ASDs may be deemed as basic salts due to the interaction between the positively charged amino group of the drug and the negatively charged carboxylate group of the polymers.¹⁹ Following Kramer and Flynn, the pH–solubility profile of a basic salt can be characterized by two independent solubility profiles: (1) the free base as the saturation species, or (2) the salt as the saturation species.²⁸⁸ The intersection of the two curves is known as the pH of maximum solubility (pH_{max}). The dissolved solute is in equilibrium with the free base when the saturated solution is above pH_{max} . When the pH value is below pH_{max} , the dissolved solute is in equilibrium with the salt.¹³¹ **Equation 6.2** was used to predict the pH–solubility profile of the ASDs.¹³¹

Equation 6.2 *The theoretical pH–solubility profile of the ASDs in accordance with Kramer and Flynn.*

$$S_T(pH < pH_{max}) = [BH^+]_S(1 + 10^{pH-pK_a}) \quad \text{Eqn. (6.2)}$$

Where $[BH^+]_S$ is the concentration of the protonated amorphous salt. The subscript S represents the salt as the saturation species at a pH below pH_{max} .¹³¹ Unprocessed CIP, and processed binary and ternary systems, were investigated in aqueous solutions (**Figure 6.1** and **Table 6.5**). pH buffer solutions ranging between 1.2–6.8 were prepared. However, the presence of CIP caused

a pH shift to occur (**Table 6.2**). In the case of CIP binary and ternary systems, irregularity arose in the presence of 10mM HCl + 90mM NaCl and/or phosphate buffer solutions (**Figure 6.1**).

The binary samples consisting of an acidic polymer were compared to unprocessed CIP at different pH conditions (**Figure 6.1a**). The CIP:HPMCP-55 sample at pH 4.7, 5.3, and 6.4 showed an increase in solubility by 2, 28, and 78-fold, respectively. The CIP:EL100 with 40:60% (w/w) at pH 4.9, 5.9, and 6.6 demonstrated an improvement in solubility by 3, 9, and 32-fold, respectively. The CIP:EL100 50:50% (w/w) at pH 4.9 did not show improvements compared to unprocessed CIP. Nevertheless, at pH 5.9 and 6.6, the sample displayed an enhancement in solubility of 3 and 18-fold, respectively. It is evident that all samples demonstrate an improvement in solubility at increased pH. This may demonstrate the positive effects of the “spring and parachute” model and the benefits of excipients such as polymers. Furthermore, this highlights the improvements to CIP since it is a pH dependent drug with a maximum pH solubility < 5 and a minimum pH solubility near the isoelectric point at pH 6.8.²⁸⁹⁻²⁹¹ This is the point at which the neutral and zwitterionic form exists.²⁹² Additionally, reducing the API content, known as compromised dosing, for CIP:EL100 sample resulted in enhanced solubility, displaying the importance of API to polymer ratio. However, it is evident that CIP:HPMCP-55 is the optimal system for solubility enhancement. The binary samples consisting of a neutral polymer were compared to unprocessed CIP at different pH conditions (**Figure 6.1b**). The CIP:HPMC sample at pH 5.1, 6, and 7.1 showed an increase in solubility by 1, 17, and, 3-fold, respectively. The CIP:KolVA64 sample at pH 5.1, 6, and 7.1 displayed an increase in solubility by 1, 17, and 2-fold, respectively. The ternary samples consisting of acidic-acidic and acidic-neutral polymers were compared to unprocessed CIP at different pH conditions (**Figure 6.1c**). The CIP:EL100:HPMCAS-H and CIP:HPMCP-55:HPMC samples at pH of approximately 4.1-4.9 were comparable to CIP. The CIP:EL100:HPMCAS-H sample at pH 5.7 and 6.5 showed an increase in solubility by 4 and 26-fold, respectively. The CIP:HPMCP-55:HPMC sample at pH 5.3 and 6.4 displayed enhanced solubility by 8 and 31-fold, respectively.

To summarize, the binary systems containing a neutral polymer showed enhancements to the solubility of CIP at approximately pH 6. Nonetheless, further solubility enhancements were displayed for systems containing an acidic polymer at a similar pH. While both CIP:EL100 samples containing various API content displayed enhancements to the solubility of CIP, the reduction of the API content (40% (w/w)) in the CIP:EL100 sample resulted in superior

solubility compared to the sample containing 50% (w/w) API. Nevertheless, the CIP:HPMCP-55 sample displayed optimal solubility compared to the binary systems discussed (**Figure 6.1**). Furthermore, the ternary systems displayed significant solubility enhancements at approximately pH 6.5. However, the solubility improvements obtained were similar to the enhancements made by the binary systems. This highlights that overall, the addition of a second polymer to the binary systems did not further enhance the solubility of the binary systems in the aqueous media. In summation, the binary and ternary systems improved the solubility of CIP at $\text{pH} > 5$, whereas the API typically exhibits a maximum solubility at $\text{pH} < 5$ (**Table 6.5**).^{289, 290}

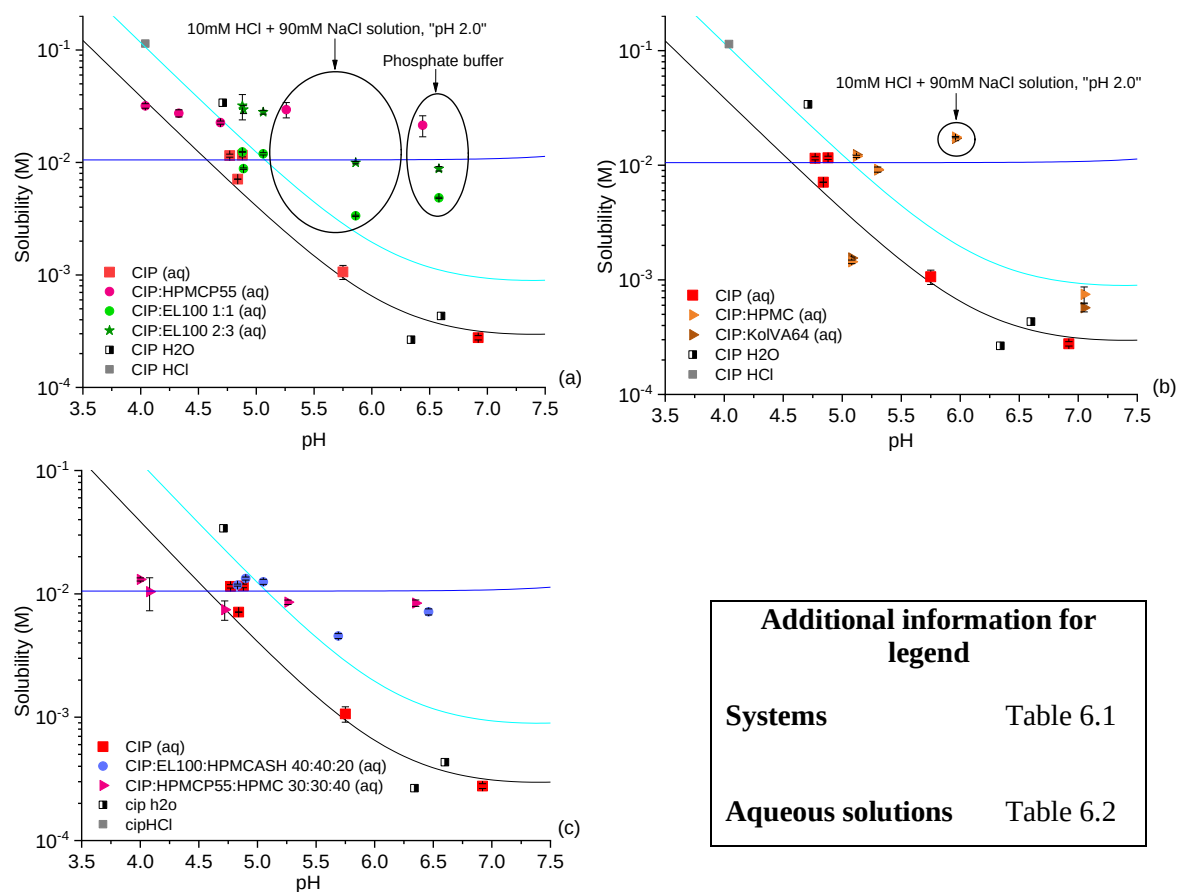


Figure 6.1 Solubility of systems in aqueous solutions (a) Binary (acidic polymer) (b) Binary (neutral polymer) (c) Ternary (acidic-acidic polymers) and (acidic-neutral polymers). Theoretical pH-solubility profile of CIP (black), CIP HCl (turquoise), and CIP ASDs (royal blue).

Table 6.5 Solubility fold increase of binary and ternary systems in aqueous solution at pH of approximately 6.8, the isoelectric point of CIP.

System (Binary or Ternary)	% (w/w) API:Polymer(s)	Solubility fold increase at pH of ≈ 6.8 (isoelectric point)
CIP:HPMCP-55	50:50	78
CIP:EL100	40:60	32
CIP:HPMCP-55:HPMC	30:30:40	31
CIP:EL100:HPMCAS-H	40:40:20	26
CIP:EL100	50:50	18
CIP:HPMC	50:50	17
CIP:KolVA64	50:50	17

The binary and ternary samples were compared to unprocessed CIP at different pH conditions using FaSSIF and FeSSIF buffer solutions (**Figure 6.2 and Table 6.6**). The buffer solutions attempt to replicate the luminal gastrointestinal conditions in fasted and fed states by capturing the buffer capacity and osmolarity of human intestinal fluid (HIF) to better predict *in vivo* drug solubility.²⁹³ The fasted and fed state buffers used in this study have a bile salt concentrations at 3 and 15 mM,^{294, 295} similar to concentrations in human volunteers which were reported at 4.61 and 12.65 mM (in the fasted and fed state).²⁹⁶ The fasted and fed state buffers also contain a phospholipid soy lecithin at 0.75 and 3.75 mM.^{294, 295} The two states display colloidal differences. The fasted and fed state has a colloidal fraction containing both the salt and phospholipid (38 and 69 nm in diameter), however, the fed state also contains an additional colloidal fraction of phospholipid vesicles (208 nm in diameter). Furthermore, the diameter of these colloids differs in the fasted and fed state.²⁹⁷ The binary samples containing an acidic polymer were examined (**Figure 6.2a**). The CIP:HPMCP-55 sample at pH 5.1 (fed) and 5.9 (fasted) showed an increase in solubility by 9 and 44-fold, respectively. The CIP:EL100 40:60% (w/w) sample at pH 5.3 (fed) and 6.1 (fasted) showed an increase in solubility by 4 and 10-fold, respectively. The CIP:EL100 50:50% (w/w) sample at pH 5.3 (fed) and 6.1 (fasted) confirmed an increase in solubility by 6 and 14-fold, respectively. The binary samples containing a neutral polymer were assessed (**Figure 6.2b**). The CIP:HPMC sample at pH 5.1 (fed) and 5.9 (fasted) showed an increase in solubility by 3 and 2-fold, respectively. The CIP:KolVA64 sample at pH

5.0 (fed) and 5.8 (fasted) showed an increase in solubility by 4 and 3-fold, respectively. The ternary samples consisting of acidic-acidic and acidic-neutral polymers were investigated (**Figure 6.2c**). The CIP:EL100:HPMCAS-H sample at pH 5.2 (fed) and 6.0 (fasted) showed an increase in solubility by 10 and 21-fold, respectively. The CIP:HPMCP-55:HPMC sample at pH 5.3 (fed) and 6.4 (fasted) displayed enhanced solubility by 9 and 30-fold, respectively. All samples in the buffer solutions were compared to blank samples which contained no fasted or fed SIF powder in the solution. The results demonstrated that in almost all cases, the addition of micelles in fasted and fed conditions increased the solubility further. However, the results varied between enhancements of 1 to 28-fold, respectively.

In summation, the most optimal samples were displayed for the CIP:EL100:HPMCAS-H, CIP:HPMCP-55:HPMC, and CIP:HPMCP-55 samples at fasted conditions with increases in solubility of CIP by 21, 30, and 44-fold, respectively. Again, CIP:HPMCP-55 displayed superior results in fasted conditions compared to all other samples. Although the fed state is reported to enhance the solubilization of poorly water-soluble drugs through additional micelle formation compared to the fasted state,²⁹⁸ It is also possible that micelles can lead to the entrapment and reduction of the free drug concentration.^{299, 300} This may explain why higher solubility values were obtained in the fasted state compared to the fed state. The solubility of CIP was improved following the investigation of the binary and ternary systems in aqueous, and FaSSIF and FeSSIF buffers. It may be hypothesized, based on the literature, that the amorphous polymeric systems designed may increase the solubility of a drug via supersaturation while maintaining permeability. This was the case for CIP binary ASDs according to previous studies performed by Mesallati *et al.*,¹⁹ in contrast to other systems designed using cyclodextrins, surfactants, and cosolvents which have been shown to negatively affect permeability while improving the solubility of the drug.^{301, 302}

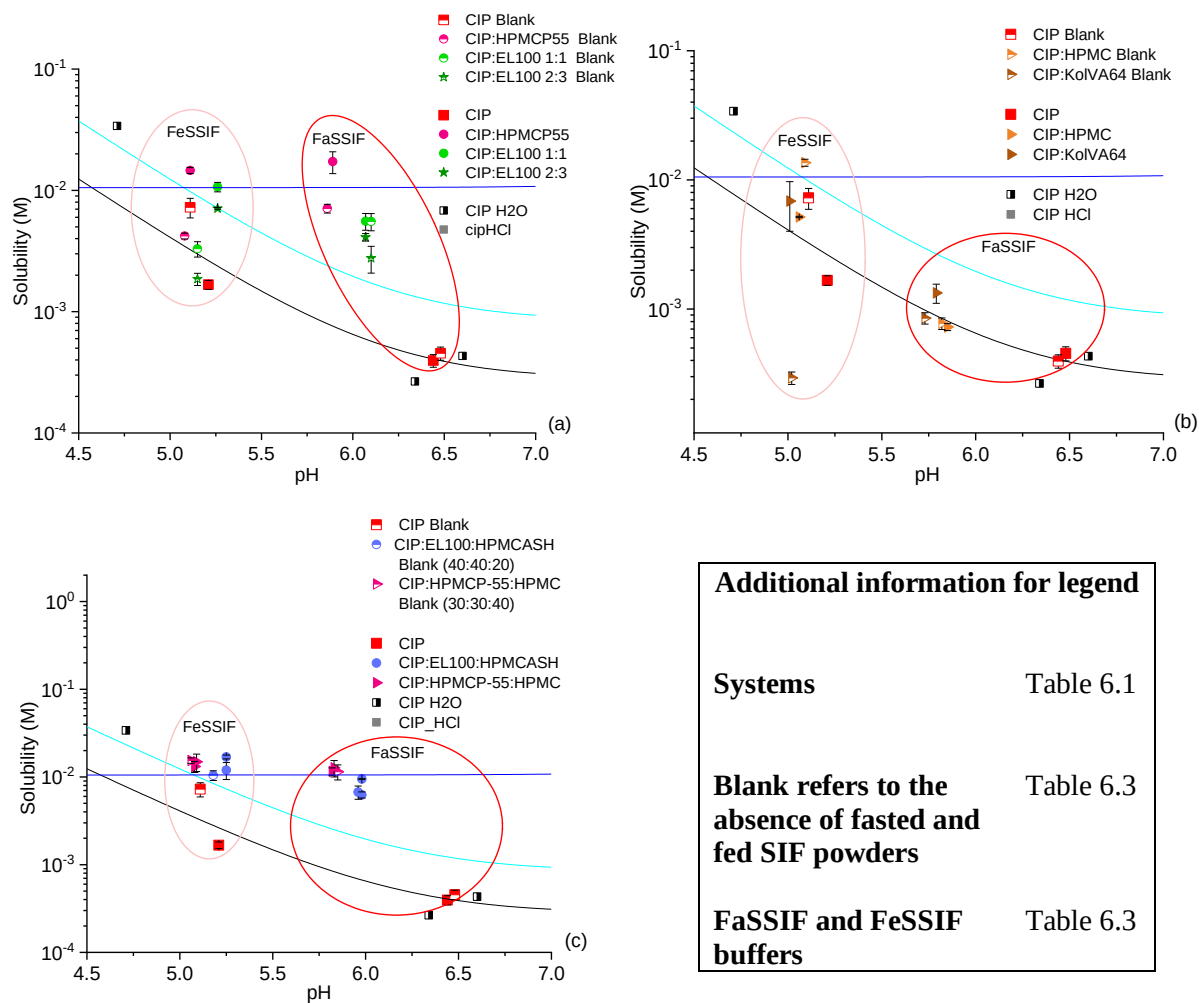


Figure 6.2 Solubility of systems in FaSSIF and FeSSIF buffers (a) Binary (acidic polymer) (b) Binary (neutral polymer) (c) Ternary (acidic-acidic and acidic-neutral polymers). Theoretical pH-solubility profile of CIP (black), CIP HCl (turquoise), and CIP ASDs (royal blue).

Table 6.6 Solubility fold increase of binary and ternary systems in FaSSIF and FeSSIF buffers at pH > 5.

System (Binary or Ternary)	% (w/w) API:Polymer(s)	Solubility fold increase in FaSSIF buffer	Solubility fold increase in FeSSIF buffer
CIP:HPMCP-55	50:50	44	9
CIP:HPMCP-55:HPMC	30:30:40	30	9
CIP:EL100:HPMCAS-H	40:40:20	21	10
CIP:EL100	50:50	14	6
CIP:EL100	40:60	10	4
CIP:KolVA64	50:50	3	4
CIP:HPMC	50:50	2	3

6.3.2 PXRD Analysis after Solubility Studies

All samples were examined before and after solubility studies in aqueous, FaSSIF, and FeSSIF media via PXRD analysis (**Figure A.6.1**). An investigation was performed to disclose the ability of the samples to withstand crystallization upon exposure to aqueous and buffer solutions as part of the solubility studies. After the solubility studies in aqueous media, the CIP:EL100 (at 40:60 and 50:50% (w/w)), CIP:HPMCP-55 and CIP:HPMCP55:HPMC samples displayed some crystallization peaks, while remaining mostly amorphous in nature. However, the samples exposed to phosphate buffer displayed more crystalline peaks than any other aqueous phase. The CIP:HPMC and CIP:KolVA64 samples became more crystalline upon exposure to the aqueous and FaSSIF and FeSSIF buffer solutions. The ternary CIP:EL100:HPMCASH samples remained fully amorphous in the aqueous solutions, displaying a superior ability to withstand crystallization. Furthermore, the sample displayed improvements in the ability to withstand crystallization in the phosphate buffer compared to other samples. After solubility studies in FaSSIF and FeSSIF buffers and blank solutions in the absence of fasted or fed SIF powders, the CIP:EL100 samples at 40:60 and 50:50% (w/w) were fully amorphous. This implies that the micelles did not facilitate the samples' ability to withstand crystallization and may be due to the solution preparation and the prevention of pH shift. The CIP:HPMCP-55 samples displayed crystallization peaks, highlighting the inability to withstand crystallization, comparable to the sample's exposure to aqueous solutions. The ternary system

CIP:EL100:HPMCASH and CIP:HPMCP55:HPMC were not examined. Lastly, some of the crystallization peaks were identified as ionized/unionized CIP, and hydrates published in the literature. However, most peaks were difficult to assign, and thus, may be due to the formation of new hydrates produced as a result of interactions with different buffer solutions and/or salts from the aqueous/buffer solutions.

6.3.3 Bacterial Studies

6.3.3.1 MICs of Binary and Ternary Systems

The minimum inhibitory concentrations (MICs) of unprocessed CIP, binary, and ternary systems were examined (**Table 6.1**). Results from the broth microdilution assays involving the bacterial strains (1) *Staphylococcus aureus*, (2) *Pseudomonas aeruginosa*, (3) *Klebsiella pneumoniae*, (4) *Acinetobacter baumannii*, (5) *Escherichia coli*, and (6) *Salmonella enterica* serovar Typhimurium are presented in **Table 6.7**. The first three bacteria strains are ESKAPE pathogens, the primary cause of global nosocomial infections with a high level of multidrug resistance.³⁰³ Overall, the bacteria strains are prevalent in common hospital and community acquired bacterial infections and CIP is amongst the leading antibiotics to inhibit their growth. The fold difference relative to the MIC of CIP is shown adjacent to the relevant MIC value. Systems 1, 2, 3, 4, and 6 (**Table 6.1**) had either no difference or a 1-fold increase in MIC relative to CIP against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Salmonella enterica* serovar Typhimurium. In *Escherichia coli* these systems had MICs 4-fold higher than that of CIP, however, it should be noted that this strain was overall more susceptible to all the systems and CIP compared to the other bacteria tested. System 8 was inferior in activity when compared to CIP in all species, with MICs 1-fold higher than that of CIP in *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, and 4- and 8-fold higher in *Salmonella enterica* serovar Typhimurium, and *Escherichia coli*, respectively. System 7 had increased efficacy relative to CIP in all strains. A 1-fold decrease in MIC was found against *E. coli*, 4-fold in *S. enterica*, *K. pneumoniae*, and *P. aeruginosa*, and an 8-fold decrease in *Staphylococcus aureus* and *Acinetobacter baumannii*, respectively. These results demonstrate improvements to MICs compared to the binary and ternary samples produced in these studies and also, binary samples reported by Mesallati *et al.*¹⁹ Mesallati *et al.* investigated MICs on CIP:EL100 and CIP:HPMCAS-L/M samples against four of the six chosen bacterial strains chosen for these studies (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and

Escherichia coli). The MIC results for the CIP:EL100 sample were comparable to CIP, whereas the CIP:HPMCAS-L/M samples displayed a 2-4-fold decrease, respectively, against the bacterial strains mentioned. MIC results are color coded for simplicity: from green as most susceptible to red as least susceptible. The related fold difference in MIC relative to ciprofloxacin for each strain is shown.

Table 6.7 Minimum inhibitory concentration values and fold change for unprocessed CIP and processed CIP binary and ternary systems against bacterial strains.

Minimum Inhibitory Concentration Values (MIC) (µg/mL)						
System	<i>Staphylococcus aureus</i> ATCC 25923		<i>Escherichia coli</i> ATCC 25922		<i>Salmonella enterica</i> serovar Typhimurium 14028S	
	MIC	CIP Comparison	MIC	CIP Comparison	MIC	CIP Comparison
CIP	1	N/A	0.007813	N/A	0.03125	N/A
1	1	No difference	0.03125	4-fold increase	0.0625	1-fold increase
2	1	No difference	0.03125	4-fold increase	0.0625	1-fold increase
3	1	No difference	0.03125	4-fold increase	0.0625	1-fold increase
4	1	No difference	0.03125	4-fold increase	0.0625	1-fold increase
6	1	No difference	0.03125	4-fold increase	0.0625	1-fold increase
7	0.125	8-fold decrease	0.003906	1-fold decrease	0.0078125	4-fold decrease
8	2	1-fold increase	0.0625	8-fold increase	0.125	4-fold increase
System	<i>Acinetobacter baumannii</i> ATCC 19606		<i>Pseudomonas aeruginosa</i> PAO1		<i>Klebsiella pneumoniae</i> ATCC 700603	
	MIC	CIP Comparison	MIC	CIP Comparison	MIC	CIP Comparison
CIP	2	N/A	0.25	N/A	1	N/A
1	2	No difference	0.25	No difference	1	No difference
2	2	No difference	0.5	No difference	2	1-fold increase
3	2	No difference	0.25	No difference	1	No difference
4	2	No difference	0.25	No difference	1	No difference
6	2	No difference	0.5	1-fold increase	2	1-fold increase
7	0.25	8-fold decrease	0.0625	4-fold decrease	0.25	4-fold decrease
8	4	1-fold increase	0.5	1-fold increase	2	1-fold increase

6.3.3.2 MICs of Binary and Ternary Systems against Strains Resistant to CIP

Unprocessed CIP and processed binary and ternary systems (**Table 6.1**), were tested against strains of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* serovar Typhimurium that were made resistant to CIP (**Table 6.8**). The results displayed in **Table 6.8** are color coded from green as the most susceptible to red as most resistant to CIP. The CIP-induced *Staphylococcus aureus* strain had an MIC of 32 ($\mu\text{g/mL}$), while CIP-induced *Escherichia coli* and *Salmonella enterica* serovar Typhimurium strains had an MIC of 64 ($\mu\text{g/mL}$). No changes occurred to the MIC values for the systems, with the exception of system 7 (CIP:EL100:HPMC), whereby the MIC decreased by 1-fold compared to unprocessed CIP in *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium strains. Nevertheless, the results indicate that the binary and ternary systems do not avoid the mechanisms of antibiotic resistance in the CIP-resistant strains examined.

Table 6.8 Minimum inhibitory concentration values of unprocessed CIP and CIP binary and ternary systems against bacterial strains induced to 32 ($\mu\text{g/mL}$) of CIP.

Minimum Inhibitory Concentrations ($\mu\text{g/mL}$) against CIP-Induced Strains			
Formulation	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Salmonella enterica</i> serovar Typhimurium ATCC 14028S
Ciprofloxacin	32	64	64
1	64	64	64
2	64	64	64
3	64	64	64
4	64	64	64
6	64	64	64
7	16	64	32
8	64	64	64

Note: Results are color coded from green as the most susceptible to red as the most resistant to CIP.

6.4 Conclusions

To conclude, the binary and ternary systems were thoroughly evaluated for their ability to enhance the solubility and antibacterial activity of CIP. In all cases, the binary and ternary systems enhanced the solubility of CIP in aqueous solutions, and FaSSIF and FeSSIF buffers. Notable solubility enhancements were observed at a pH of approximately 6.8, near the isoelectric point of CIP, in the aqueous solutions, and at pH values > 5 in FaSSIF buffers. The CIP:HPMCP-55 system exhibited a 78-fold enhancement in solubility in the aqueous solutions while containing a 50% drug load. The remaining binary and ternary systems showed solubility enhancements in aqueous solutions ranging from 32-fold and 17-fold increases relative to CIP. The CIP:HPMCP-55 system showed a 44-fold increase in the solubility of CIP in FaSSIF buffer, while the CIP:HPMCP-55:HPMC system showed a 30-fold increase. The remaining binary and ternary systems exhibited solubility increases ranging from 14-fold and 2-fold increases in the FaSSIF and FeSSIF buffers. In terms of antibacterial activity, the CIP:EL100:HPMC system demonstrated enhanced efficacy relative to CIP, showing an 8-fold decrease in MIC against *Staphylococcus aureus* and *Acinetobacter baumannii*, and a 4-fold decrease in MIC against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enterica* serovar Typhimurium, respectively. In summation, the mechanically milled binary and ternary ASD/APS systems improved the solubility and the antibacterial activity of CIP, without the use of any harmful solvents, and by employing green chemistry.

Chapter 7.0 General Discussion and Conclusions

Chapter 7.0 General Discussion and Conclusions

7.1 General Discussion

This project investigates fluoroquinolone drugs as many are included in the WHO essential medicines list. As previously discussed, this project focuses on developing new insights into the kinetic, mechanistic, and solid-state understanding of BMed fluoroquinolone drug(s) (LVXh and/or CIP) in the absence or presence of polymer(s), using mechano- and/or tribochemistry. Furthermore, poor solubility of drugs remains a huge challenge and amorphization is a known solution to this problem, despite containing its own challenges such as poor stability. Therefore, binary and ternary amorphous polymeric systems were generated to improve the solubility of CIP and the poor stability of the amorphous form. The enhancement of the solubility of CIP, the stability of the systems after solubility studies in the solid-state, and the antibacterial activity of the systems were investigated. The research chapters were successful in that the outcomes discussed were addressed, and new findings and conclusions were made. This project may be of interest to other researchers utilizing mechano- and tribochemistry since different mills were compared (Retsch mixer mill MM400 and Retsch planetary mill PM100) and parameters investigated. Furthermore, it may be of interest to other researchers who wish to improve the properties of fluoroquinolone drugs using mechanical energy while adhering to green chemistry principles. Mechanochemistry is rapidly gaining traction in the research community. However, to fully integrate this greener approach into the pharmaceutical industry, several research gaps need to be addressed as previously highlighted. This integration is crucial for mechanochemistry to replace more environmentally harmful methods of drug synthesis and enhancement. By adopting mechanochemistry, the use of hazardous and costly solvents can be significantly reduced or even eliminated, leading to improvements in human health, environmental protection, and cost efficiency. These benefits will ultimately have a global impact on human health and patient care. Therefore, this project is vital in contributing to the creation of a more sustainable world.

Chapter 1 focuses on discussing the important terms in alignment with the literature that are necessary for conducting the research investigations discussed. **Chapter 2** involves the BMing of fluoroquinolone drug LVXh, a drug listed as part of the essential antituberculosis medicines according to the WHO, while **Chapter 3** further investigates LVXh with the addition of EL100. The solid-state complexity of LVXh made it an interesting API to investigate with the promise of many learnings and transferable outcomes to be achieved from its complex

nature. **Chapter 4-6** investigated CIP, a poorly soluble drug, that is also a listed watch group antibiotic due to its high probability of developing bacterial resistance, according to the WHO.¹²⁰ Research shows that multiple fluoroquinolones have been BMed to improve the properties of the drugs such as solubility, dissolution rates, reduced particle size, enhanced crystal engineering,¹⁸⁰ and detect new polymorphic forms.¹³⁷ Thus, investigations were performed to contribute new findings to this field of research.

Chapter 2 discusses the solid-state transformations of LVXh, and the mechanism of such polymorphic transformations induced by the milling process. According to the literature, polymorphic transformations of pharmaceutical materials by mechanical treatment have been reported for sulfathiazole,^{173,174} chloramphenicol palmitate,¹⁷⁵ mannitol,¹⁷⁶ and sorbitol.¹⁷⁷ These studies contribute to the knowledge of polymorphic transformation kinetics and mechanisms produced by the mechanical process.^{176, 177} However, gaps remain in the literature regarding polymorphic transformations of materials/APIs produced by mechanical energy at a microscopic level. Therefore, **Chapter 2** focused on further contributing to this area of research.

LVX was chosen for the research conducted in **Chapter 2** as it is a complex fluoroquinolone drug. It can exist in different crystal forms in the solid-state such as,¹⁸⁶⁻¹⁹⁰ two hydrate forms i.e., hemihydrate and monohydrate,¹⁸⁹ and five water-free forms identified as amorphous, γ , δ , β , and α .¹⁸⁶⁻¹⁹⁰ Different mills and parameters were investigated to amorphize LVXh and develop a mechanistic understanding of the transformation. It was confirmed that LVXh first transforms to the anhydrous/dehydrate LVX γ as an intermediate phase, before converting to LVXam. It was also established that the reaction is reversible. The Rietveld method demonstrated that various mills and methods can affect the transformation rates of LVX γ and the quantities of LVX γ and LVXam formed. This study is important to generate a mechanistic understanding of BMed LVXh so that the learnings obtained can be transferred to other fluoroquinolone drugs with similar structures. Additionally, this study is important as it focuses on filling some of the existing gaps in mechano- and tribo-chemistry. Providing more information regarding the mechanically induced solid-state transformations of LVXh can help further advance this field of chemistry.

Chapter 3 continued investigating LVXh, however, an acidic polymer (EL100) was incorporated to fully amorphize LVXh using the mechanical process. LVXh was previously investigated for the formation of LVXam/LVX co-amorphous systems using heated mixing,²¹⁴ SDing,^{198, 215-217} freeze drying,^{217, 218} solvent evaporation/casting,^{202, 219, 220} electrospinning,^{221,}

²²² and precipitation.²²³ Although in some cases, the methods mentioned above used an acidic polymer, the mechanical processing of LVX was not applied as a method of producing LVXam. Thus, this investigation on LVXh was performed for the first time. However, the milling of fluoroquinolone drugs, CIP and ENDO, with an acidic polymer was conducted by Mesallati *et al.* The studies performed found that acidic polymers can facilitate the phase transformation of these fluoroquinolone drugs to produce the completely amorphous form using the milling process, in contrast to neutral polymers which could not completely amorphize CIP.^{19, 20} Other drugs/compounds were chosen for ASD investigations using acidic polymers and the BMing process. These included the weakly basic drug lidocaine,³⁰⁴ and a natural compound kaempferol.³⁰⁵ It is evident that ASDs using BMing are being investigated as an important strategy to enhance the pharmaceutical properties of poorly water soluble drugs. Thus, **Chapter 3** investigates the feasibility of amorphization formation of LVXh upon the addition of an acidic polymer. According to the literature, the mechanical process was not previously applied as a method of forming LVXam. This highlights the gaps and limited knowledge which exist regarding the transformation of LVX to LVXam using the milling process. Thus, since LVX had never been milled using an excipient, EL100 was added with the aim of producing amorphous polymeric systems of LVXh.

The results in **Chapter 3** displayed that the LVXh:EL100 sample was fully amorphous and the mechanism was revealed. It was uncovered that the first step involved the amorphization of the drug, and the second step may consist of a reaction between the amorphized drug and the polymer, ultimately, leading to polymer ionization. Furthermore, it was shown that a higher percentage of LVXh can be incorporated into the system and successfully amorphize depending on the mill utilized (MM400). The investigation of multiple mill types was executed and the existence of variability in the product outcome of LVXh:EL100 system depending on the mill used was presented. Lastly, stability differences between the LVXam samples produced from both mills were presented and a hypothesis was made that the presence of an exotherm alludes to a less stable amorphous system, whilst the disappearance of the exotherm implies improved physical stability. This chapter demonstrates a more sustainable approach for achieving amorphization of LVXh.

Chapter 4 produced and investigated amorphous polymeric systems of CIP using acidic or neutral polymers. The amorphous form is known to be less stable than its crystalline form and often crystallizes back to its most stable form. The use of polymer(s) to produce ASDs can

improve the stability of the amorphous form. However, the stability of ASDs may be further developed by the inclusion of an ionic interaction, resulting in the formation of an APS.^{19, 20} CIP's poor solubility makes it a desirable drug for investigation. Thus, this research chapter is important for generating systems that can improve the solubility of CIP. Previous findings were made by Mesallati *et al.* whereby CIP and ENRO were BMed with amino acids⁹ and acidic polymers to produce fully amorphous systems.¹⁸⁻²⁰ Neural polymers could not completely amorphize CIP.¹⁹ However, neural polymers had the ability to amorphize other drugs such as sulfonamide,³⁰⁶ ritonavir,³⁰⁷ nifedipine,³⁰⁸ bicalutamide,³⁰⁹ bosentan,³¹⁰ and nimodipine³¹¹ to produce ASDs using the BMing process.

Chapter 4 investigated the use of the Retsch planetary mill PM100 and the Retsch mixer mill MM400, and the milling parameters in the pursuit of generating amorphous polymeric systems of CIP with reduced processing times. According to the literature, research has been carried out on the ball mill to produce the amorphous form of the disaccharide sugar, lactose, and API, curcumin using different ball sizes.³¹² An exploration into ASDs of API ibuprofen and the natural compound, resveratrol, using different ball mill frequencies was performed.³¹² However, an investigation into different mills and parameters including the number of balls used, ball and jar sizes, and ball and jar materials has not been reported in the literature for CIP. Therefore, a gap was filled regarding the milling of CIP using acidic and neutral polymers that contribute to the further understanding of mechano- and tribo-chemistry in relation to these systems.

The Retsch mixer mill was more successful in reducing the crystallinity and the processing time compared to the Retsch planetary mill for the amorphous polymeric systems. The processing of CIP with neutral polymers (HPMC and KolVA64) did not lead to full amorphization, however, partial amorphization was confirmed. The amorphous polymeric systems produced using acidic polymers (EL100 and HPMCP-55) and the neutral polymer (HPMC) were stable at high temperatures as no crystallization was detected. This was in contrast with the amorphous portion of the semi-crystalline sample containing KolVA64 which was less stable. The results obtained are valuable as they highlight the stability differences that exist for the amorphous phase depending on the polymer selected. This demonstrates the importance of polymer selection for enhanced stability of the amorphous phase. A residual amount of the carboxylic acid group was displayed for the semi-crystalline sample with HPMC which confirms the occurrence of proton transfer and may also be the case for the sample containing

KolVA64. Proton transfer to the carboxyl region of CIP in the samples CIP:EL100 and CIP:HPMCP-55 was confirmed by ^{13}C CPMAS analysis. However, it remains unclear if the piperazine region of the molecule participates in the proton transfer process and/or hydrogen bond interactions. Lastly, the CIP binary samples including the polymers HPMCP-55 and HPMC were identified as being hydrophobic and EL100 as hydrophilic in nature. This finding contributed to the expansion of the research in **Chapter 5**.

Chapter 5 investigated amorphous polymeric systems by the generation of ternary systems containing either two acidic polymers or an acidic and neutral polymer, using the milling process. This investigation is important as ternary systems may further improve the stability of binary systems, by providing additional interactions between the drug and polymers.^{18-20, 52} According to the literature, several ternary ASD investigations have been performed using the ball milling process where at least one excipient was a polymer. The drugs investigated were indomethacin²⁶⁵ and meloxicam,²⁶⁶ and fluoroquinolone drugs OFX²⁶³ and NOR.²⁶⁴ Other studies were performed on amorphous polymeric ternary systems produced by HME, solvent evaporation, or thermal techniques to primarily investigate miscibility, stability,³¹³ dissolution,³¹³⁻³¹⁵ matrix crystallization,²⁸¹ and micro-injection moulding.³¹⁵ These studies were performed on APIs such as nimodipine,³¹³ bicalutamide,²⁸¹ and indomethacin.^{315, 314} Although amorphous polymeric ternary system investigations are being reported in the literature, there is still a need for further research on the topic of solid-state, particularly using the BMing process. Thus, the solid-state characteristics and physical stability of CIP ternary amorphous polymeric systems were investigated using the ball milling process for the first time. The increase or decrease in the degree of crystallinity to the original binary system upon the addition of a third component and the % (w/w) of the third component was investigated, and the polymers were ranked accordingly.

Chapter 5 showed that the addition of the second polymer to the binary system could increase, reduce, or have no impact on the degree of crystallinity of the original binary system. Thus, the results were dependent on the second polymer added and/or on the % (w/w) of the second polymer added. An investigation into the addition of the polymers and/or API using the MM400 was conducted and the findings were ranked by their effectiveness for decreased levels of crystallinity, by first the milling of the binary system prior to the addition of the polymer, followed by the milling of the two polymers before the incorporation of the API, and lastly, the addition of all of the components together for the CIP:HPMCP-55:HPMC system. The

CIP:EL100 ternary systems displayed a higher average T_g than the binary systems, except for the ternary system composed of KolVA64. This may indicate the potential to improve the stability of the amorphous systems by ternary formation. The absence or almost complete reduction of the carboxylate ion peak indicated proton transfer for all systems. The addition of an acidic polymer to the binary amorphous polymeric system may have the ability to interfere with interactions involving the carboxylate group of CIP. This was in contrast to the addition of a neutral polymer to the binary amorphous polymeric system which did not demonstrate interference with interactions involving the carboxylate ion of CIP. The generation of a ternary sample has the potential to make the system more hydrophilic or hydrophobic compared to the binary systems.

Chapter 6 investigated several of the binary and ternary systems (discussed in **Chapters 4 and 5**) for solubility enhancements compared to CIP and the binary systems. The systems were exposed to aqueous, and FaSSIF and FeSSIF buffers and examined post solubility studies to investigate their solubility and stability capabilities. This research is important as CIP is a poorly soluble drug listed by the WHO as an essential medicine.¹²⁰ MIC studies were performed using the binary and ternary systems produced to investigate the antibacterial activity of the systems compared to CIP for multiple strains of bacteria, including ESKAPE pathogens. This study is important as bacterial AMR is recognized by the WHO as a global public health concern.²⁷² To date, fluoroquinolone drugs OFX²⁶³ and NOR²⁶⁴ have been investigated for enhanced dissolution profiles using ternary systems. However, Mesallati *et al.* performed solubility and antibacterial studies for CIP and ENRO binary ASDs containing EL100 and HPMCAS-L/MG polymers.^{19, 20} The CIP and ENRO binary ASDs displayed superior solubility in FaSSIF solution and in water for CIP ASDs, and 2-4-fold improvements to some strains of bacteria.^{19, 20} Many solubility studies have been performed on amorphous polymeric ternary systems involving APIs/compounds such as ritonavir,³¹⁶ atorvastatin,³¹⁷ chrysin,³¹⁸ curcumin,³¹⁹ and khellin,³²⁰ indicating the importance of improving amorphous binary systems by ternary formation. However, according to the literature, there have been no investigations performed on amorphous polymeric ternary systems for enhanced solubility using mechanical energy. MIC studies on binary amorphous polymeric systems have been reported in the literature for APIs/compounds terconazole,³²¹ clotrimazole,³²² nystatin,³²² cefpodoxime proxetil,³²³ amoxicillin,³²⁴ amphotericin B,^{325, 326} azithromycin,³²⁷ and curcumin.³²⁸ Nonetheless, investigations for

enhanced antibacterial activity by MIC studies involving amorphous polymeric ternary systems have never been explored, according to the literature.

Chapter 6 presented that the solubility of CIP in aqueous solution, and FaSSIF, and FeSSIF buffers can be effectively improved by generating binary and ternary semi-crystalline and amorphous polymeric systems. An increase in the solubility of CIP was shown in the aqueous solution at pH of approximately 6.8 (isoelectric point of CIP) for all binary and ternary systems. A particular increase in the solubility of CIP was seen for CIP:HPMCP-55 (78 fold), CIP:EL100 (32 fold), and CIP:HPMCP-55:HPMC (31 fold). Considerable enhancements were shown in the fasted state for all amorphous binary and ternary samples compared to the fed state. The highest increase in solubility in FaSSIF buffers was seen for the CIP:HPMCP-55 (44 fold) and CIP:HPMCP-55:HPMC (30 fold) samples. Overall, the binary and ternary systems improved the solubility of CIP at pH > 5 in aqueous solution, and FaSSIF and FeSSIF buffers, with particular enhancements to the solubility of samples in aqueous solution and FaSSIF buffer. Binary and ternary samples were investigated for MIC studies against multiple bacterial strains. In general, the systems displayed no significant improvements to the antibacterial activity compared to CIP for the strains investigated. However, one system (CIP:EL100:HPMC) displayed a decrease in the MIC values for all bacterial strains. A 1-fold decrease in MIC was found against *Escherichia coli*, 4-fold in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enterica*, and an 8-fold decrease in *Staphylococcus aureus* and *Acinetobacter baumannii*, respectively. This research contributes to the enhanced solubility and antibacterial activity of ternary amorphous polymeric systems.

7.1.1 Conclusions

The study in **Chapter 2** is novel as LVX has never been BMed before, nor has it been SDed without the use of an excipient to successfully achieve amorphization. This chapter makes new discoveries regarding the interconversion pathways of LVXh, LVX γ , and LVXam as a result of milling LVXh and uses a new method for discovering solid-state forms of LVXh. The comparison of the two methods (BMing and SDing) showed that the success of amorphization depends on both the mechanism of formation and the chosen process. **Chapter 3** successfully prepared LVX:EL100 amorphous binary systems using the BMing process and the mechanism of formation was revealed. A comparison of the outcome produced using different mills (a Retsch mixer mill, a Retsch planetary ball mill, and a Fritsch vibration mill), and a scale up of the process was achieved. **Chapter 4** investigated the use of the planetary and mixer mills and

examined the milling parameters in the pursuit of generating amorphous polymeric systems with reduced processing times. The solid-state characteristics of the binary systems and the effect of moisture on the solid-state stability of the amorphous phase were studied for all amorphous and semi-crystalline polymeric systems, and proton transfer to the carboxyl group of CIP was confirmed. **Chapter 5** conducted research on ternary ASDs using the BMing process that has never been evaluated before, according to the literature. This involved the examination of the increase or decrease in the degree of crystallinity to the original binary system upon the addition of a third component and/or the % (w/w) of the third component and ranked the polymers accordingly. This chapter also explored the solid-state characteristics and physical stability of CIP ternary amorphous polymeric systems containing two acidic polymers or an acidic polymer and a neutral, amphiphilic polymer. This chapter contributed to the solid-state understanding of ternary ASDs generated by the BMing process, with CIP as the chosen API. **Chapter 6** displayed improvements to the solubility of CIP for the binary and ternary amorphous polymeric systems discussed. In addition, the CIP:EL100:HPMC sample demonstrated superior results against bacterial strains compared to the other systems tested and CIP, while for the most part, the other systems displayed MICs unchanged from that of CIP.

In summation, it is possible to amorphize the fluoroquinolone APIs investigated using the BMing process in the absence of harmful solvents, while achieving complete amorphization upon the addition of an acidic polymer. The amorphous systems designed can improve the solubility and antibacterial activity of a poorly soluble fluoroquinolone API while remaining stable in the solid-state and in solution. The discoveries made from each of the chapters discussed are valuable as the new findings obtained may be transferred and/or applied to current and future fluoroquinolone APIs with similar molecular structures. These discoveries enhance the understanding of mechano- and tribo-chemistry, addressing gaps in solid-state mechanochemistry and supporting the shift towards greener pharmaceutical practices. The improvements in solubility and antibacterial activity coupled with the implementation of green chemistry principles, can improve APIs while reducing manufacturing costs by eliminating costly solvents. Moreover, the reduction or removal of solvents will ultimately benefit human health and the environment. This thesis addresses several key challenges that face the pharmaceutical industry. If the proposed API and process enhancements are applied to future medicines, they have the potential to significantly improve patient outcomes worldwide.

7.2 Main Findings

7.2.1 Chapter 2

- Different mechanical actions (mechano- and tribo-chemistry) and parameters demonstrated crystallinity reduction of the diffractograms of LVXh upon processing using a PM100 and a MM400.
- Thermograms of a PM100 and MM400 Cond_2 suggested disruption of the crystal lattice, facilitating the liberation of the crystalline water of LVXh.
 - The thermograms of the LVXh samples subjected to milling revealed an exothermic peak of crystallization, indicating the existence of the amorphous phase.
- The presence of an anhydrous/dehydrated form of LVX and the event of crystallization were related to LVX γ and LVXam, respectively, confirmed by the Rietveld method (*collaboration*).
 - The mechanism discovered established that LVXh first transforms to the anhydrous/dehydrated LVX form γ (LVX γ) as an intermediate phase, before transpiring to LVXam.
 - A new mechanism of LVXam formation was discovered since LVXam was previously reported to form directly from LVXh upon dehydration.
 - A new method of forming polymorphs by the process of milling was developed, and the mechanism by which LVX γ plays an essential role in achieving amorphization was revealed.
- Incomplete amorphization for LVXh occurred upon utilization of the milling process, however, complete amorphization of LVX was achieved by spray drying from water.
- The pure phase of LVXam was reported for the first time in the literature as having a glass transition of around 80 °C.
- DVS depicted that both amorphous phases generated by BMing and SDing crystallize at 70% RH, revealing their similar capabilities to withstand crystallization under moisture conditions.

7.2.2 Chapter 3

- Variability occurred in the product outcome depending on the mill used (MM400 or PM100), and in some cases, the parameters chosen.

- PXRD analysis for the PM100 and the MM400 samples confirmed complete amorphization when ≤ 50 or $\leq 70\%$ (w/w) LVXh was present in the co-processed samples.
- DSC analysis for the PM100 and the MM400 samples disclosed that the powders containing 40-90 or 70-90% (w/w) of the drug comprised of LVXam.
- A combination of PXRD and DSC analysis on the PM100 and the MM400 samples revealed that the 40 and 50% (w/w) LVXh (PM100) or 70% (w/w) LVXh (MM400) samples were fully amorphous.
- A combination of these techniques (PXRD and DSC) led to the disclosure that the absence of an exothermic peak for 30% (w/w) LVXh (PM100) or 50-60% (w/w) (MM400) LVXh samples may indicate the stability of the amorphous systems.
- In-situ measurements disclosed that each of the crystallographic planes of LVXh amorphized at the same time (*collaboration*).
- FTIR indicated a potential acid-base interaction between LVXh and EL100.
 - This finding, in combination with PXRD and DSC assisted in revealing the mechanism of LVX:EL100 system formation.
- The formation of the amorphous LVX:EL100 system occurred as a two-step process.
- Thermal treatment showed the identification of the anhydrous/dehydrate form of LVXh, known as anhydrous LVX form γ (LVX γ).

7.2.3 Chapter 4

- The studies showed that the MM400 was more successful in reducing the crystallinity of the CIP:EL100 system than the PM100, and the process duration was significantly reduced.
- Thermal analysis displayed no exotherm of crystallization peaks for CIP:EL100, CIP:HPMCP-55, and CIP:HPMC samples, and may indicate the stability of the amorphous form of the drug and its ability to withstand exposure to high temperatures.
- In contrast, the CIP:KolVA64 sample displayed an exothermic peak of crystallization, confirming the presence of the amorphous form of the drug.
- The display of one Tg for all samples via MTDSC analysis was in agreement with the calculated Hansen solubility values that showed theoretical miscibility of CIP and polymers for all systems.

- Residual amount of the carboxylic acid group was displayed in the CIP:HPMC sample demonstrating the occurrence of partial proton transfer.
- ^{13}C CPMAS and IR analysis on the CIP:EL100 and CIP:HPMCP-55 samples showed that proton transfer occurred to the carboxyl region of CIP (*collaboration*).
- There was no evidence of partial crystallization for CIP:EL100, CIP:HPMCP-55, or CIP:HPMC samples via DVS analysis.
 - However, partial crystallization of CIP:KolVA64 was depicted.
- The amorphous polymeric systems and the amorphous component in the semi-crystalline system of CIP:HPMC were stable.
- Based on the Young-Nelson model, the CIP:EL100 sample was more hydrophilic, and the CIP:HPMCP-55 and CIP:HPMC samples were more hydrophobic in nature.

7.2.4 Chapter 5

- The addition of the polymers and/or API using the MM400 was ranked by their effectiveness for decreased levels of crystallinity, by first the milling of the binary system prior to the addition of the polymer, followed by the milling of the two polymers before the incorporation of the API, and lastly, the addition of all of the components together for the CIP:HPMCP-55:HPMC system.
- The addition of HPMCAS-H to the CIP:EL100 system may have the ability to interfere with interactions involving the carboxylate group of CIP.
 - This was in contrast to the addition of KolVA64 to the CIP:EL100 system which did not demonstrate interference with interactions involving the carboxylate ion of CIP.
 - The addition of an acidic polymer may have the ability to interact with the already existing binary system.
- Upon DVS analysis, there was no evidence of crystallization for the ternary samples.
- The incorporation of a second polymer to CIP:EL100 increased the drying time, making the system more hydrophilic.
 - This was in contrast to the ternary samples composed of CIP:HPMCP-55 which confirmed faster drying durations, making the system more hydrophobic.
- The Young-Nelson model disclosed that the majority of their moisture uptake for the ternary samples of CIP:EL100 was internally absorbed.

Conversely, the leading water uptake mechanism was the multilayer for the ternary samples containing CIP:HPMCP-55.

7.2.5 Chapter 6

- The solubility enhancement of the binary and ternary systems is in the following order:
 - The CIP:HPMC, CIP:KolVA64, CIP:EL100 (50:50% (w/w)), CIP:EL100:HPMCAS-H, CIP:HPMCP-55:HPMC, CIP:EL100 (40:60% (w/w)), and CIP:HPMCP-55 samples displayed an increase in solubility of 17, 17, 18, 26, 31, 32, and 78-fold compared to unprocessed CIP.
- The solubility enhancement of the binary and ternary amorphous polymeric systems is in the following order:
 - The CIP:EL100 (40:60% (w/w)), CIP:EL100 (50:50% (w/w)), CIP:EL100:HPMCAS-H, CIP:HPMCP-55:HPMC, and CIP:HPMCP-55 samples displayed increased solubility values of 10, 14, 21, 30, 44-fold in the fasted state, compared to unprocessed CIP.
- CIP:HPMCP-55 presented optimal solubility results in both the aqueous solution and FaSSIF buffers compared to the other binary and ternary samples, with significant enhancements in the aqueous solution.
- The CIP:EL100:HPMC sample displayed an increased efficacy relative to CIP in all bacteria strains investigated (*collaboration*).
 - A 1-fold decrease in MIC was found against *Escherichia coli*, 4-fold in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enterica*, and an 8-fold decrease in *Staphylococcus aureus* and *Acinetobacter baumannii*, respectively.
- The CIP:HPMCP-55:HPMC sample demonstrated inferior activity when compared to CIP in all species.

7.3 Future Direction

The proposed future research in optimizing the solubility and antibacterial efficacy of fluoroquinolone APIs focuses on both enhancing the molecular characteristics of formulations and addressing the persistent challenge of bacterial resistance. A key aspect of future research could involve performing kinetic studies on a broader range of BMed fluoroquinolone APIs. This would provide further insight into the transformation processes of the chosen APIs and contribute to the understanding of phase transformation for enhanced solubility and bioavailability. Specifically, kinetic investigations on the semi-crystalline CIP:HPMC system would be of interest since CIP:EL100:HPMC has shown promise in improving the MIC of CIP. This system is an ideal candidate since the system must be fully or partially crystalline in order to perform Rietveld refinement and establish a kinetic understanding of the API transformation. These studies could help to develop an understanding of the crystallization behavior and stability of the API in the system.

The most optimal samples from the solubility and MIC studies could be evaluated for *in vivo* performance to assess the safety and efficacy of the systems. Experiments could be performed to better understand the CIP:EL100:HPMC sample that led to optimal MIC values compared to CIP and the other binary and ternary systems and to ultimately improve the antibacterial efficacy of CIP. Future work could focus on investigating other hydrophilic excipients with similar properties to HPMC, such as different grades of HPMC with varying viscosities (low, medium, or high viscosity) or other hydrocolloids such as pectin, alginates, and chitosan. These excipients may enhance the swelling and gelling properties of CIP. Lastly, the use of polysaccharides such as sodium alginate (derived from brown seaweeds and soil bacteria) and pectinate (from citrus peels and apple pomace) could provide a natural, biodegradable option for stabilizing amorphous systems.

The solubility of poorly soluble fluoroquinolone APIs could potentially be improved by BMING these compounds with naturally derived fatty acids, such as omega-3, -6, or -9 fatty acids, which contain carboxylic acid groups. These fatty acids, derived from sources such as olive oil, flaxseed oil, or canola oil, could improve the solubility of APIs by interacting with the APIs molecular structure. The fatty acid could be mixed with a polymer to create ternary systems. The use of a polymer may prevent crystallization and maintain the API in its amorphous, highly solubility form.

An additional method that could be used to enhance the performance of fluoroquinolone APIs involves BM with acidic cyclodextrins to encapsulate the API. The acidic nature of certain cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin or sulfobutylether- β -cyclodextrin, may help protonate the fluoroquinolone API to increase its solubility and stability. Another study could be performed by BMing the API with an amino acid and/or acidic polymer. The amino acid and/or acidic polymer could first interact with the fluoroquinolone API to form a protonated complex, increasing the APIs solubility by altering its ionization state. Once protonated, the fluoroquinolone could be encapsulated within a neutral cyclodextrin complex.

Despite improvements in the MIC values using the CIP:EL100:HPMC system, one challenge that remains is the development of bacterial resistance. The mechanism of action of CIP is well-understood. However, bacterial resistance continues to evolve, limiting the APIs effectiveness. As a result, future research should focus on collaborations with microbiologists to design formulations that address this issue. Future studies could investigate the co-formulation of CIP with efflux pump inhibitors. For example, excipients like curcumin or verapamil could enhance the activity of antibiotics by inhibiting bacterial efflux pumps, which are often responsible for resistance. Moreover, studying how different excipients might alter the interaction of CIP with its target proteins or modify bacterial permeability could yield insights into how to overcome resistance more effectively. By investigating the molecular mechanisms of action and the associated bacterial resistance pathways, researchers could develop formulations that not only improve solubility and bioavailability but also enhance the APIs effectiveness in overcoming resistance.

In conclusion, future research could focus on optimizing the amorphous systems of fluoroquinolone APIs through kinetic studies and exploring more excipients to enhance solubility and bioavailability. Studies performed using hydrocolloids, fatty acids, and cyclodextrin encapsulation could further improve solubility and stability. However, addressing bacterial resistance remains a key challenge. Thus, establishing a collaboration with microbiologists to develop efflux pump inhibitors and molecular mechanisms of action could ultimately enhance the effectiveness of fluoroquinolones in combating bacterial resistance.

Appendices

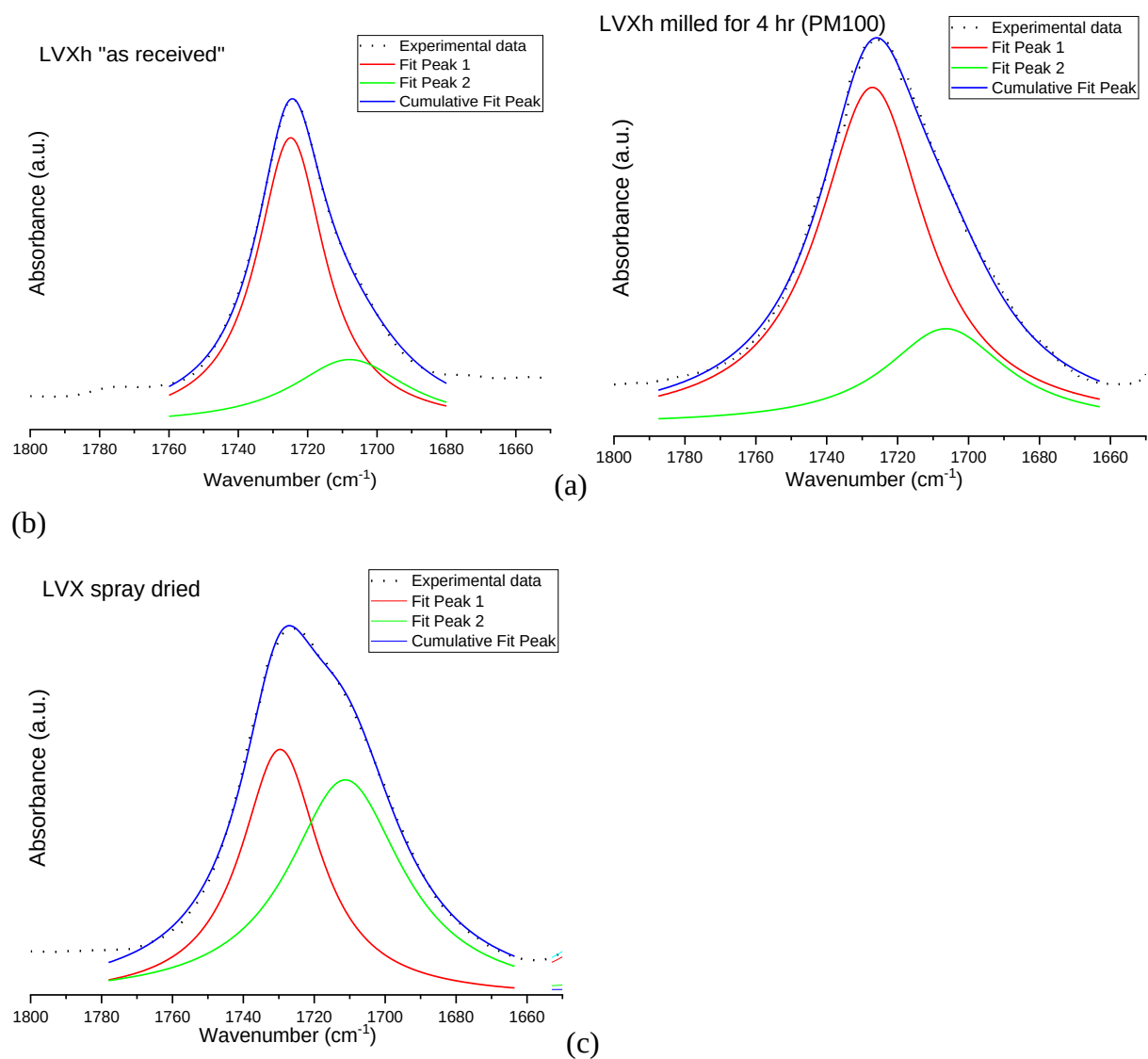


Figure A.2.1 FTIR peak deconvolution for (a) LVXh as received, (b) BMed LVXh, and (c) SDed LVX.

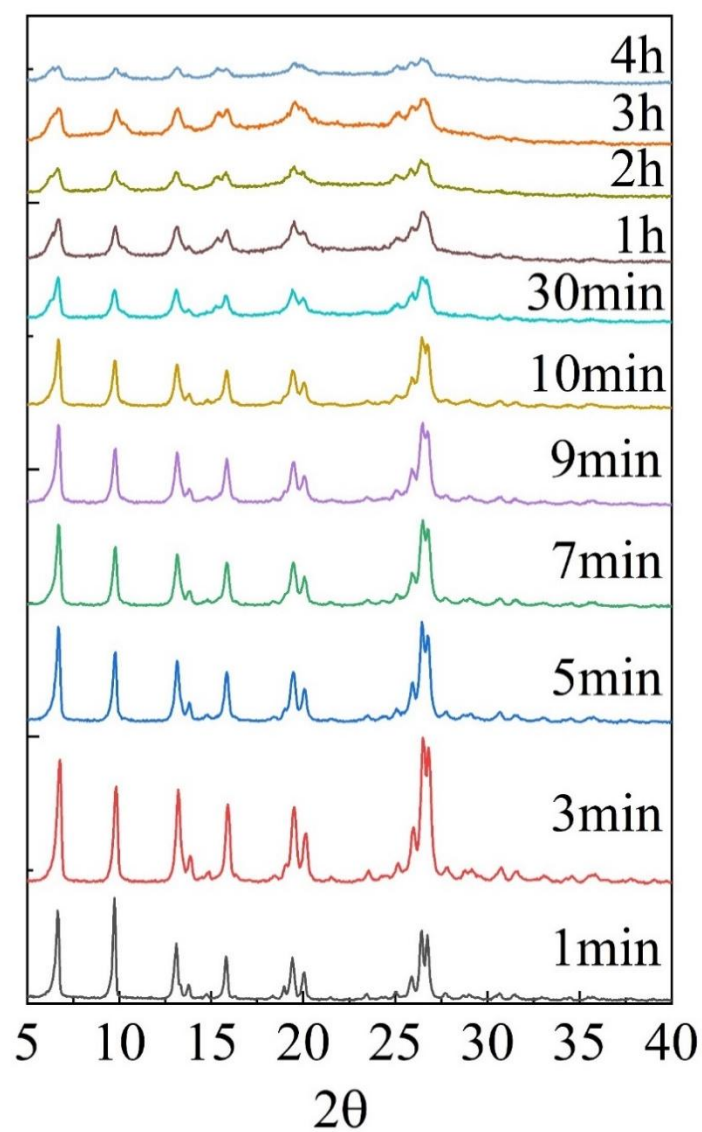


Figure A.2.2 Experimental PXRD patterns of LVXh subject to BMing using the Retsch planetary ball mill PM100 and 50 mL stainless steel jar. Data refer to different processing times, ranging from 1 min of BMing to 4 hours.

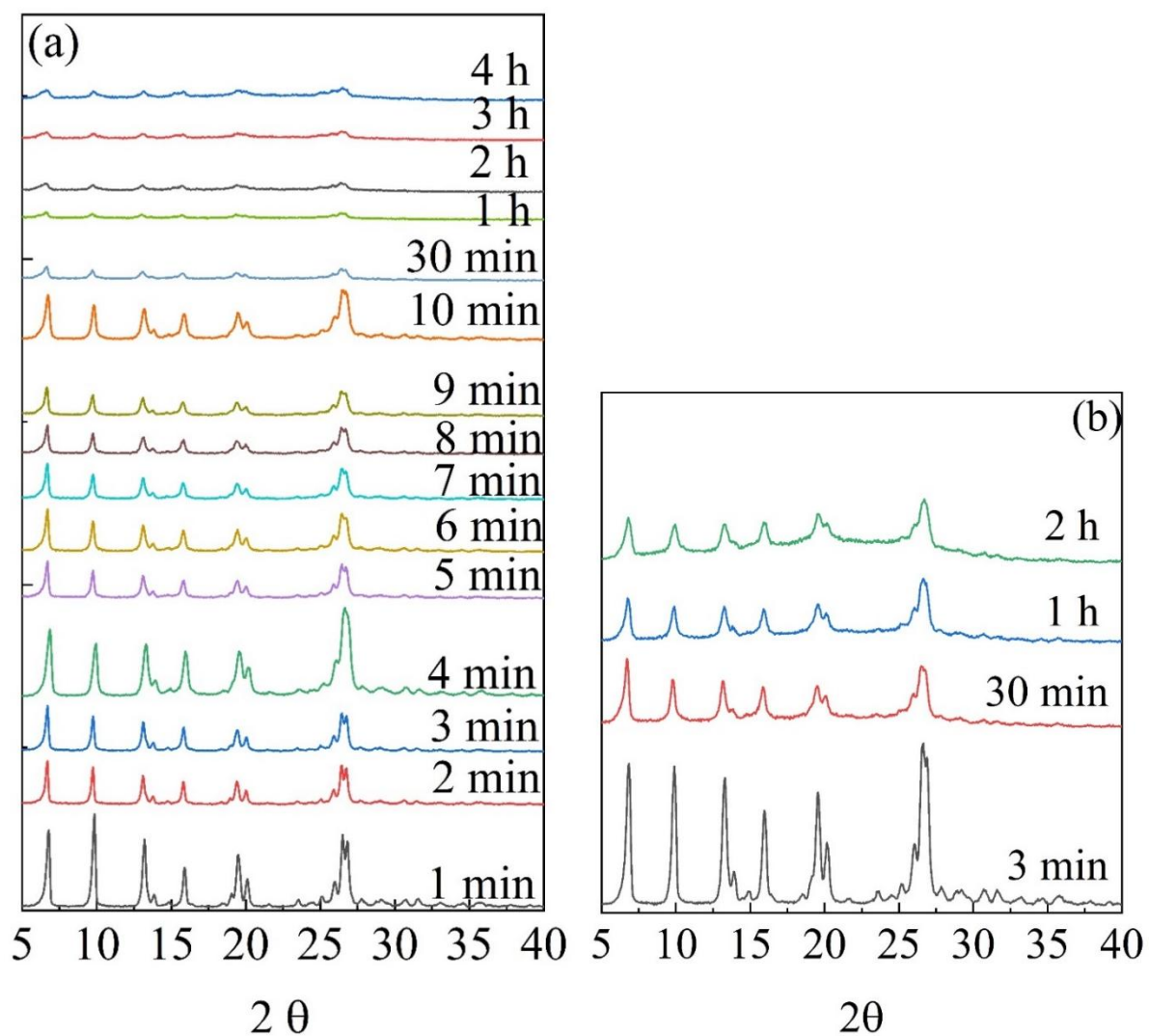


Figure A.2.3 Experimental PXRD patterns of LVXh subject to BMing using the Retsch mixer mill MM400 working at 25 Hz. The mill was equipped with a 50 mL stainless steel jar and (a) 3 stainless steel balls, (b) 5 stainless steel balls. Data refer to different processing times, ranging from 1 minute of BMing to 4 hours.

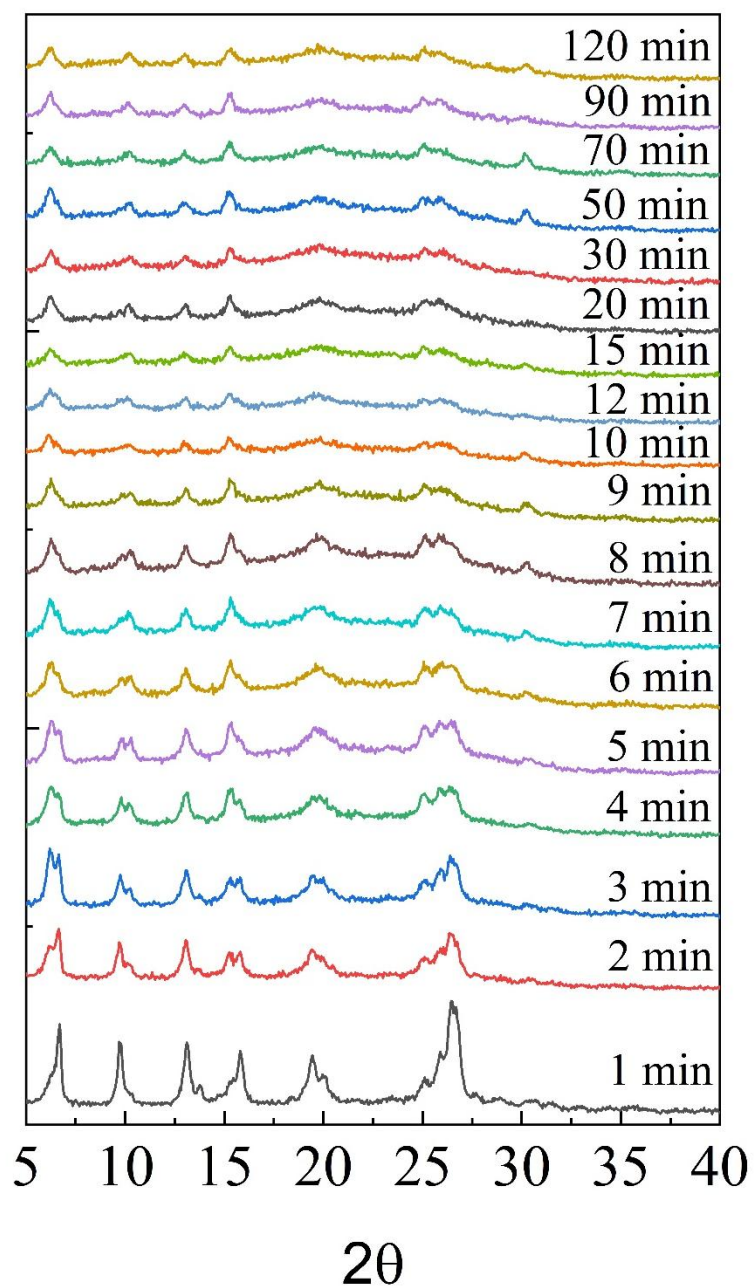


Figure A.2.4 Experimental PXRD patterns of LVXh subjected to BMing using the Retsch mixer mill MM400 at 25 Hz. The mill was equipped with a 25 mL ZrO₂ jar and one ZrO₂ ball. Data refer to different processing times, ranging from 1 minute of BMing to 2 hours.



(a)



(b)



(c)



(d)

Figure A.3.1 Photographs of (a) unprocessed and unmixed LVXh and EL100. Powders before and after milling using parameters from **Table 3.1** after (b) 1 hour of BMing using two 1.2 cm ZrO_2 balls (MM400) (c) 4 hours of milling using three 1.5 cm stainless steel balls (PM100) (d) 3 and 5 hours of BMing using one 0.9 cm stainless steel ball (left) vs three 0.4 cm stainless steel balls (right) (Fritsch Vibration Ball Mill), respectively.

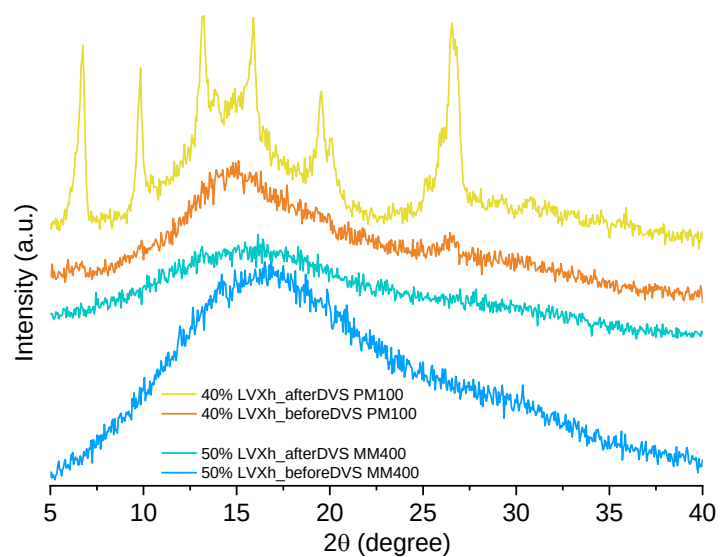


Figure A.3.2 PXRD of ball milled samples 40% (w/w) LVXh after subjected to BMing for 4 hours at 400 rpm using a PM100 (orange and yellow) and 50% (w/w) LVXh after subjected to BMing for 1 hour at 25 Hz using a MM400 (blue and turquoise) before and after DVS analysis.

Table A.3.1 Young-Nelson parameters for moisture internally absorbed, multilayer, and monolayer.

Sample	Monolayer (g water/g material)	Multilayer (g water/g material)	Internally Absorbed (g water/g material)
LVXh:EL100 40% (w/w) PM100	0.01	0.05	0.07
LVXh:EL100 50% (w/w) MM400	N/A	0.0001	0.17

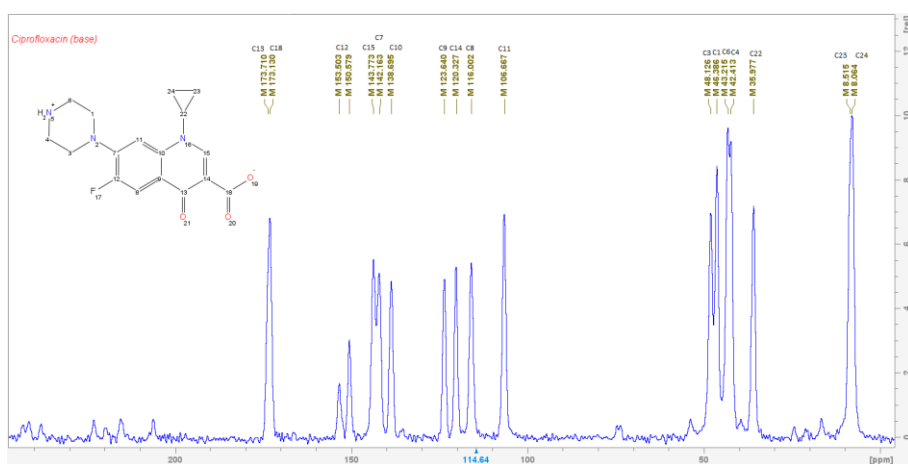


Figure A.4.1 ^{13}C CPMAS spectra for CIP.

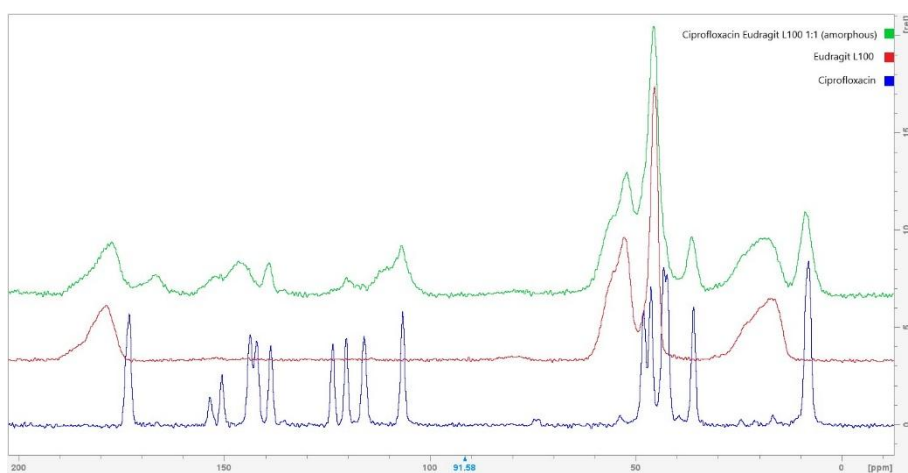


Figure A.4.2 ^{13}C CPMAS spectra for CIP, EL100, and CIP:EL100.

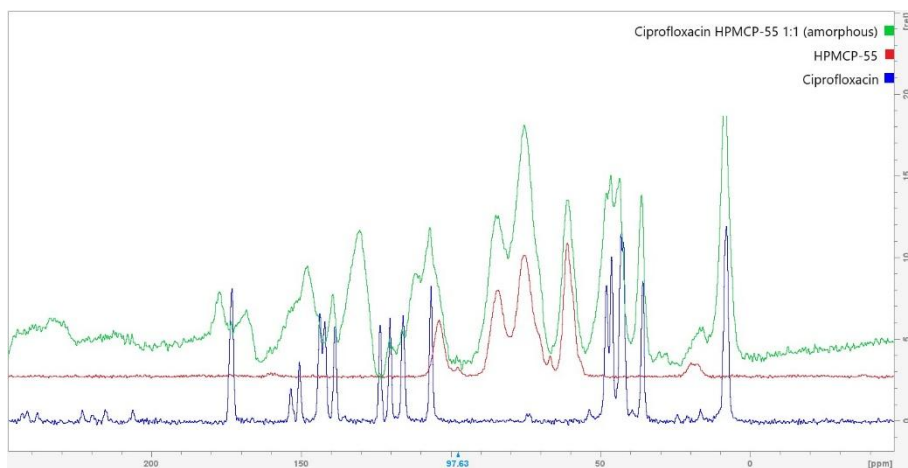


Figure A.4.3 ^{13}C CPMAS spectra for CIP, HPMCP-55, and CIP:HPMCP-55.

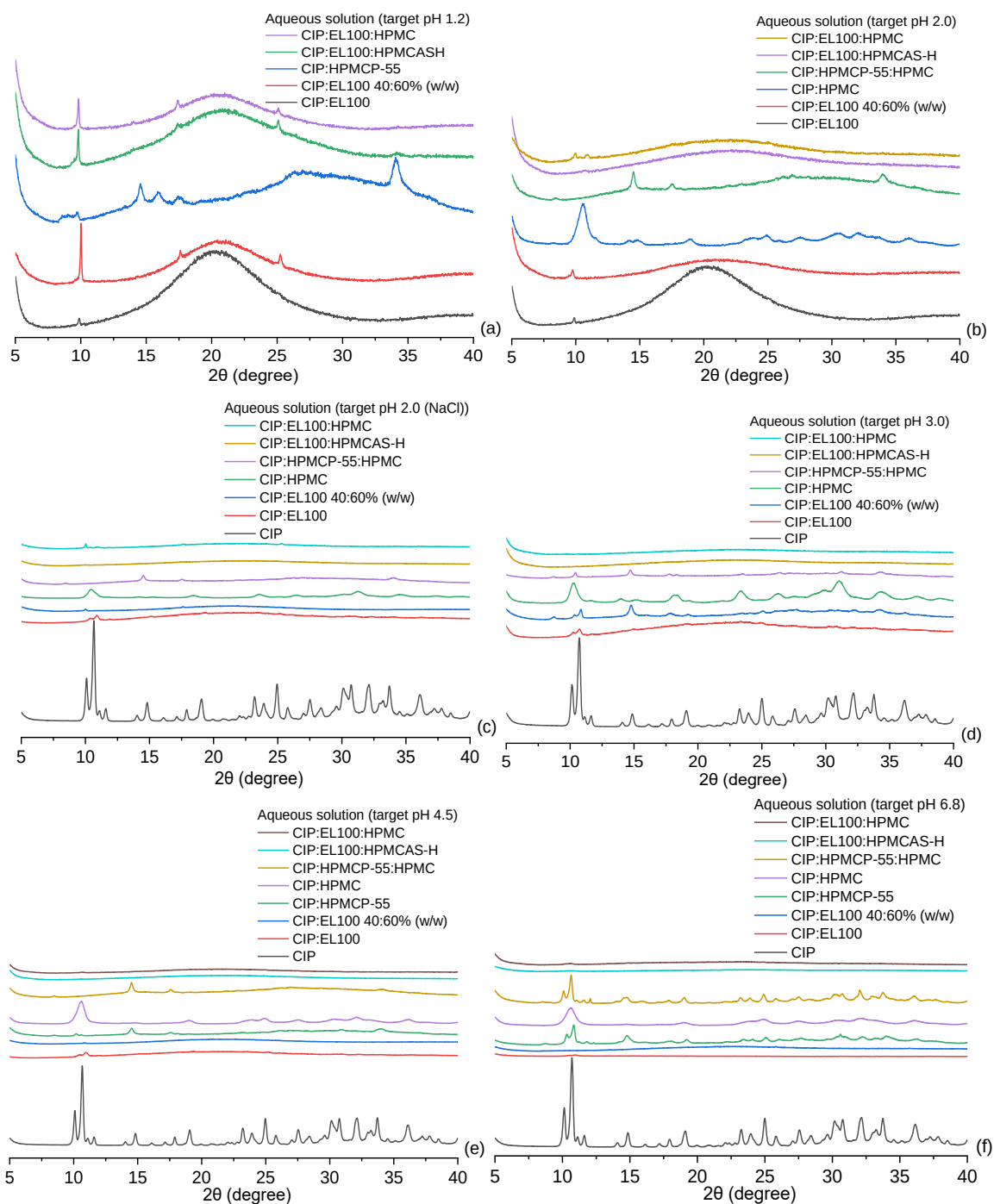


Figure A.6.1 PXR D of binary and ternary samples after solubility studies in aqueous solutions at various pH values.

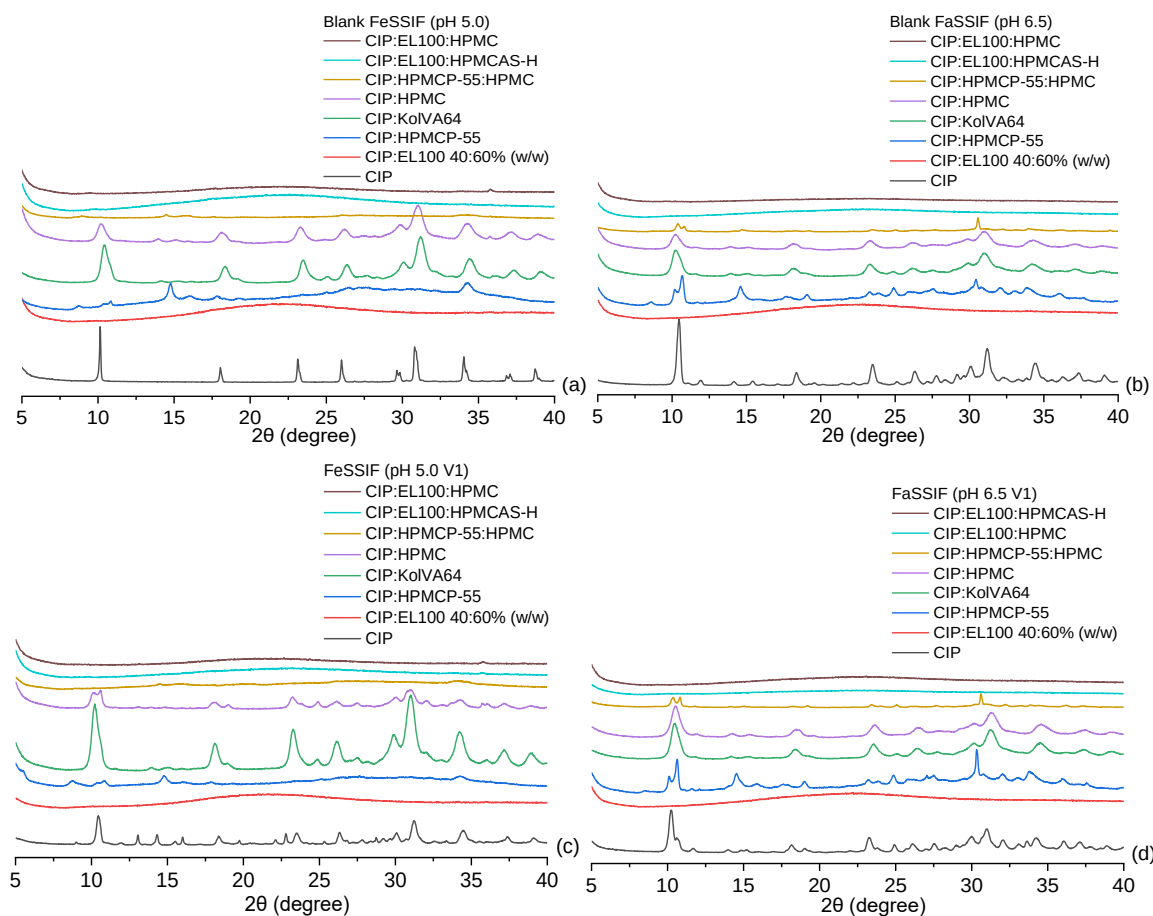


Figure A.6.2 PXRD of binary and ternary samples after solubility studies in blank and Fa/FeSSIF buffers at pH 5.0 and 6.5.

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