Amorphous drug-polyelectrolyte nanoparticle complex with controlled release functionality, enhanced amorphous stability, and its continuous production platform

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**Amorphous Drug-polyelectrolyte Nanoparticle Complex with
Controlled Release Functionality, Enhanced Amorphous Stability,
and its Continuous Production Platform**

Dong Bingxue

SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

5 August 2019

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Date

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Kunn Hadinoto Ong

Authorship Attribution Statement

This thesis contains material from **5** paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as the first author.

Chapter 4 is published as **Bingxue Dong.** & Kun Hadinoto Ong: Amorphous nanoparticle complex of perphenazine and dextran sulfate as a new solubility enhancement strategy of antipsychotic perphenazine. Drug Development and Industrial Pharmacy 2017:1-7. Permission has been granted by the licensed publisher “Taylor & Francis” for utilization of the published content as a chapter in this thesis. [1]

The contributions of the co-authors are as follows:

- A/Prof Kunn Hadinoto Ong provided the initial project direction and edited the manuscript drafts.
- A/Prof Kunn Hadinoto Ong and I prepared the manuscript draft.
- I co-designed the study with A/Prof Kunn Hadinoto Ong and performed all the laboratory work at the School of Chemical and Biomedical Engineering, NTU. I also analyzed the data and sent the weekly reports of the experiment results, data analysis to A/Prof Kunn Hadinoto Ong.
- All microscopy, including sample preparation, was conducted by me in the Facility for Analysis, Characterization, Testing and Simulation.

Chapter 5 is published as **Bingxue Dong.** & Kun Hadinoto Ong, “Direct comparison between millifluidic and bulk-mixing platform in the synthesis of amorphous drug-polysaccharide nanoparticle complex”, International journal of Pharmaceutics 523, 42-51 (2017). Permission has been granted by the licensed publisher “Elsevier” for utilization of the published content as a chapter in this thesis.

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- All microscopy, including sample preparation, was conducted by me in the Facility for Analysis, Characterization, Testing and Simulation.
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The chapter 7 has been published as **Bingxue Dong**, Li Ming Lim, Kun Hadinoto Ong: Enhancing the physical stability and supersaturation generation of amorphous drug-polyelectrolyte nanoparticle complex via incorporation of crystallization inhibitor at the nanoparticle formation step: A case of HPMC versus PVP. *European Journal of Pharmaceutical Sciences* 2019:105035. Permission has been granted by the licensed publisher “Elsevier” for utilization of the published content as a chapter in this thesis

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- All microscopy, including sample preparation, was conducted by me in the Facility for Analysis, Characterization, Testing and Simulation.
- Lim Li Ming performed some of the basic characterizations which included the size and zeta potential, the payload and the DSC of CIP-DXT-HPMC NP, as well as for the revisions suggested by the reviewers.

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21 May 2020

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**Amorphous Drug-polyelectrolyte Nanoparticle Complex with Controlled Release
Functionality, Enhanced Amorphous Stability, and its Continuous Production
Platform**

Dong Bingxue

School of Chemical and Biomedical Engineering

A thesis submitted to the Nanyang Technological University, Singapore

in partial fulfilment of the requirement for the degree of

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PUBLICATIONS

JOURNAL ARTICLES

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2. Bingxue Dong. & K Hadinoto, “Amorphous nanoparticle complex of perphenazine and dextran sulfate as a new solubility enhancement strategy of antipsychotic perphenazine”, *Drug Development and Industrial Pharmacy*, 1-7 (2017)
3. Bingxue Dong, Cheow, W.S. & Hadinoto, K. Ternary nanoparticle complex of ciprofloxacin exhibiting sustained release at gastric pH prepared by co-complexation with polyanions and an ionic amphiphile. *Particuology* (2019).
4. Bingxue Dong, Lim LM & K Hadinoto, Enhancing the physical stability and supersaturation generation of amorphous drug-polyelectrolyte nanoparticle complex via incorporation of crystallization inhibitor at the nanoparticle formation step: A case of HPMC versus PVP. *European Journal of Pharmaceutical Sciences* (2019).
5. Bingxue Dong. & K Hadinoto, Carboxymethyl cellulose is a superior polyanion to dextran sulfate in stabilizing and enhancing the solubility of amorphous drug-polyelectrolyte nanoparticle complex. *International Journal of Biological Macromolecules* 2019.
6. MH Nguyen, H Yu, Bingxue Dong, K Hadinoto., “A supersaturating delivery system of silibinin exhibiting high payload achieved by amorphous nano-complexation with chitosan”, *European Journal of Pharmaceutical Sciences* 89, 163-171 (2016)

7. MH Nguyen, ND Pham, Bingxue Dong, THN Nguyen, CB Bui, K Hadinoto, “Radioprotective activity of curcumin-encapsulated liposomes against genotoxicity caused by gamma cobalt-60 irradiation in human blood cells”, International journal of radiation biology 93 (11), 1267-1273 (2017)
8. Yu, H, Lim, L.M, Bingxue Dong & Hadinoto, K. Proof-of-Concept Preparation and Characterization of Dual-Drug Amorphous Nanoparticle Complex as Fixed-Dose Combination of Poorly Soluble Drugs. Drug Development and Industrial Pharmacy, 1-31 (2018).

ABSTRACT

In spite of various contemporary medicinal approaches developed, there are still more than 40% of drugs in the pharmaceutical market and more than 70% of the drug candidates are insoluble in water, resulting in low oral bioavailability and low therapeutic effectivity of the drugs or drug candidates. Solubility enhancement remains a significant challenge in drug formulation technology development. Various methods have been applied to increase the solubility of poorly soluble drugs.

Rendering of drugs into their amorphous form is a promising strategy to enhance the rate and extent of the dissolution of poorly soluble drugs. Nanoization has been used as an effective method to prepare the drug formulations aimed at increasing drug solubility as well. However, most of the existing formulations of poorly soluble drugs are not able to provide fast dissolution or not able to provide the high drug loading required to decrease the pill burden. Amorphous drug-polysaccharide nanoplex with high drug loading (above 80 %) were prepared by the self-assembly electrostatic driven complexation between ionized drug molecules and oppositely charged polysaccharide. The prepared nanoplex was proven to exhibit fast drug dissolution, prolonged supersaturation. The electrostatic driven drug-polysaccharide complexation method is a simple yet effective method without thermal or mechanical stress during manufacture. To increase the drug-polysaccharide nanoplex production consistency and develop a continuous production platform, a milli-fluidic platform was designed. Millifluidic production mode and the bulk mixing production mode were directly compared for the first time on the physical characteristics, preparation efficiency, amorphous stability of the resultant nanoplex, the dissolution/supersaturation generation and the production

consistency. The millifluidic platform showed its superiority in production consistency, continuous production ability, and scalability.

The drug-polysaccharide nanoplex exhibited fast dissolution, prolonged supersaturation and suggested improved bioavailability. Nevertheless, sustained release with the enhanced extent of dissolution and high apparent solubility is still desired to meet the different requirements in different physiological environments. The bioavailability of the broad-spectrum antibiotic Ciprofloxacin (CIP) was limited because of its narrow absorption in the stomach. In Chapter 6, a ternary nanoparticle complex (nanoplex) was designed via co-complexation with polyanions and an anionic amphiphile (sodium dodecyl sulfate (SDS)) to modify the release of CIP in the stomach as well as to prolong the gastric residence time of CIP. The effect of the charge ratio of DXT to SDS on the size, zeta potential, CIP utilization rate, payload of the CIP-DXT-SDS nanoplex and the dissolution characteristics in the simulated gastrointestinal fluids were studied. The CIP-DXT-SDS nanoplex prepared with a lower charge ratio (i.e. below 80:20) were proved to exhibit sustained CIP release compared to the native CIP and the binary CIP-DXT nanoplex in SGJ.

Though the amorphous solid drug formation represents an attractive approach to enhance the solubility of poorly soluble drugs, they have a trait that the amorphous solid drug tends to become crystalline during storage due to the inherent thermodynamic instability. The poor physical stability of many amorphous drug formulations has limited their application in marketing. To address this issue, in my research, different methods were explored to increase the stability of the amorphous of the drugs. Crystallization inhibiting agents, i.e. hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) were incorporated into the nanoplex formulation to improve the amorphous state stability of CIP. With the addition of HPMC or PVP, the

nanoparticle formed (i.e. CIP-DXT-HPMC nanoplex and CIP-DXT-PVP nanoplex) has increased nanoparticle size (from 300 nm to 500 nm) and increased CIP utilization rate (increased from 65 % to 90 %, w/w) without decreasing the CIP payload. Both CIP-DXT-PVP and CIP-DXT-HPMC exhibited solubility enhancement and increased amorphous stability. In Chapter 8, another method was used to increase the amorphous stability. Carboxymethyl cellulose (CMC) was used to substitute DXT because it has the crystallization inhibiting ability. CIP-CMC nanoplex with the size at around 200 nm, zeta potential at around -49 mV, and high payload at approximately 76% were successfully prepared. The CIP-CMC nanoplex exhibited superiority over CIP-DXT nanoplex on not only the significantly improved amorphous stability but also on the higher CIP payload and production yield.

Overall, amorphous drug-polyelectrolytes nanoplex has emerged as an appealing drug formulation to address the problem of the poorly soluble drug due to its fast release, prolonged supersaturation, and simple yet highly efficient preparation. Millifluidic reactors were designed as continuous platforms to produce nanoplex with increased product consistency. The dissolutions of the nanoplexes were tunable to achieve sustained release in the stomach by designing the ternary nanoparticle complex prepared via electrostatic interaction and hydrophobic interaction. The nanoplex with increased amorphous stability was designed. Nanoplex was equipped with an adjustable release functionality and enhanced amorphous stability and continuous production. The future work I would like to recommend are 1) comparison on the co-amorphous drug formulations with the amorphous nanoplex equipped with modifiable dissolution rate functionality and improved the amorphous stability. 2) design of a one-step synthesis and recovery of the nano-complex via a flocculation and filtration process based on the

charge-driven flocculation as an intensified process for the nanoplex purification. 3)
widened application of the nanoplex on selective release functionality.

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ABBREVIATIONS

AA	Acetic acid
ACN	Acetonitrile
API	Active Pharmaceutical Ingredients
ASD	Amorphous solid dispersion
AUC	Area under the curve
C	supersaturated drug concentration
CAM	Co-amorphous drug
CE	Complexation efficiency
CIP	Ciprofloxacin
CMC	Carboxy methylcellulose
C _{ppz}	Supersaturated concentration of PPZ
C _{Sat}	Thermodynamic Saturation solubility
C _{Sat-CIP}	Thermodynamic saturation solubility of CIP
C _{Sat-PPZ}	Thermodynamic saturation solubility of PPZ
C _{Supersat}	Supersaturated concentration (mg/ml)
d	Inner diameter of the millifluidic tube (mm)
D	Molecular diffusion coefficient (cm ² /s)
DI water	deionized water
DLS	Dynamic light scattering calorimetry
DSC	Differential scanning calorimetry
DXT	Sodium dextran sulfate
F	Flow rate (ml/min)
FESEM	Field Emission Scanning Electron Microscopy

FTIR	Fourier Transform Infrared Spectroscopy
FQ	fluoroquinolone
h	Mixing length scale in the radial direction (mm)
HME	Hot melt extrusion
HPLC	High performance liquid chromatography
HPMC	Hydroxypropylmethylcellulose
HPMCAS	Hypromellose acetate succinate
L	Axial distance from the Y-junction (cm)
M _{HPMC/CIP}	Mass ratio of HPMC to CIP
M _{PVP/CIP}	Mass ratio of PVP to CIP
MW	Molecular weight
Nanoplex	Nanoparticle Complex
NP	Nanoplex
PAA	poly(acrylic acid)
PASDs	Polymeric amorphous solid dispersions
PBS	Phosphate buffered saline
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PE	Polyelectrolytes
PEG	Polyethylene glycol
PPZ	Perphenazine
PVP	Polyvinylpyrrolidone
PVP-VA	Poly(vinylpyrrolidone-co-vinylacetate)
PXRD	Powder X-ray diffraction

$R_{CIP:(DXT+SDS)}$	Charge ratio of CIP to (DXT + SDS)
$R_{CIP:DXT}$	Charge ratio of CIP to DXT
$R_{CIP:SDS}$	Charge ratio of CIP to SDS
$R_{CIP/DXT}$	Charge ratio of CIP to DXT
$R_{CMC/CIP}$	Charge ratio of CMC to CIP
$R_{DXT:SDS}$	Charge ratio of DXT to SDS at $R_{CIP} (DXT+SDS) = 1$
$R_{DXT/CIP}$	Charge ratio of DXT to CIP
$R_{DXT/PPZ}$	Charge ratio of DXT to PPZ
Re	Reynolds number
$R_{PE/drug}$	Charge ratio of polyelectrolyte to drug
SDS	Sodium dodecyl sulfate
SGJ	Simulated gastric juice
SIJ	Simulated intestinal juice
T_g	Glass transition temperature
TGA	Thermal gravimetric analysis
T_m	Melting temperature
t_{Mix}	Diffusive mixing time for diffusing half of the tube diameter (s)
t_{Res}	Residence time (s)
U	Fluid velocity (cm/s)
UV–vis	Ultraviolet visible spectroscopy
μ	Dynamic viscosity (cp) U Fluid velocity (cm/s)

CHAPTER 1 Introduction

1.1 Poor solubility of drugs restricts clinical application

With the development of high-throughput screening, combinatorial chemistry, and computational methods, a significant number of drug candidates are discovered rapidly. It is estimated that about 40% of approved drugs and 70-90% of drugs under investigation exhibit low aqueous solubility (<1 mg/ml) [2], resulting in major challenges for solubility enhancement and a bottleneck in drug formulation technology [3-7]. The Biopharmaceutics Classification System ranks active pharmaceutical ingredients (APIs) into four classes in accordance with their aqueous solubility and intestinal permeability. Drugs with low solubility and high permeability are classified as Class II drugs [4], while drugs with low solubility and low permeability are categorized into Class IV. The poor solubility of these drugs has become a serious concern to pharmaceutical scientists.

To provide the drug concentration required to attain certain therapeutic effects, high and frequent doses of poor solubility drugs are necessary. This causes a quicker consumption of drugs and reduction in patient compliance, potentially inducing toxicity. The poor solubility of drugs has become one of the major challenges facing the drug development research field, especially for oral delivery. The optimal degree of patient compliance, cost effectiveness, and ease of the administration have contributed to the wide utilization of oral delivery. The bioavailability of the orally administered drugs mainly depends on their intestinal permeability and drug solubility. Poor solubility is one of the main hurdles to achieve complete absorption in the gastrointestinal tract, resulting in poor oral bioavailability. Reliable, efficient, cost-effective and scalable formulation methods to improve drug solubility are desired.

1.2 Strategies to increase dissolution rate

The dissolution rate is always associated with the apparent solubility of the drug formulation. Solubility is defined as the saturation of the drug in the form of the drug formulation. The process of dissolution is depicted in Figure 1-1 [8]. The saturation is achieved in the water layer close to the surface of the drug formulation, where the water can be regarded as unstirred. The concentration at the saturation level is the solubility of the drug (C_s) in the drug formulation. The difference in the drug concentration in the saturated water layer and the well-stirred dissolution medium drives the dissolution and determines the dissolution rate according to the Noyes Whitney Equation (Equation 1-1) [9].

Figure 1-1: Dissolution of drug formulation in the dissolution medium

Equation 1-1 : Noyes Whitney Equation

$$\frac{dc}{dt} = \frac{D \cdot A}{h} * (C_s - C)$$

Drug dosage formulations exhibiting enhanced dissolution rate have been developed through various means to improve the bioavailability of drugs with poor aqueous solubility. Methods such as use of cyclodextrins, addition of surfactant [10], salt formulation, solid dispersions [11], polymorphism [12], micronization, nanoization,

high-pressure homogenization [13], emulsions, liposomes [7], supercritical fluid process [14-18], and cosolvency have been explored by the scientists.

Table 1-1: Limitations of some methods used to improve drug solubility

Method	Definition of the method	Limitations
using cyclodextrins	Improving the dissolution rate of drug by taking up the lipophilic drug molecule or the lipophilic moiety of the drug into the polar cyclodextrin cavity to form inclusion cyclodextrin complex [19, 20]	low aqueous solubility; poor recognition properties; and nephrotoxicity
chemical modification	Improving dissolution rate via modifying the chemical structure of the drug [21]	Using of non-biocompatible solvent which might induce toxicity
salt formulation	Increasing the dissolution rate of acidic or basic drugs by forming the salt formation of the acidic or basic drugs. Salt formation of the acidic and basic drugs generally exhibit higher solubility than the native drugs [22]	taxing process, difficulty in salt selection [23]; difficulty of synthesis, difficulty of crystallization, cost of the raw materials; precipitation;
Polymorphism drug formulation	Polymorphism drug formulations are solid drug formulations with more than one crystalline structures [24].	complicated process and supercritical conditions; low success rate
micronization	Increasing the drug dissolution rate by micronize the drug so as to increase the surface area [25].	lack of control on the morphology and surface properties of the final particle
cosolvency	Using water miscible co-solvent to increase the solubility of poorly aqueous solubility drug [26].	use of non-biocompatible solvent; poor patient compliance and commercialization
co-crystal	Co-crystals are crystalline structure materials composed of API and another or more components.[27]	supercritical conditions; low success rate

Further the Table 1-1 summarizes the limitations in using different methods to improve the dissolution rate. The cyclodextrins have a lipophilic cavity and relatively hydrophilic exterior. Although the lipophilic cavity can incorporate polar drugs or functional groups of drugs, the low aqueous solubility, poor recognition properties, and nephrotoxicity of cyclodextrins have hindered its application in the pharmaceutical field [28-30]. The drug formulation that relies on the chemical modification to enhance its solubility involves the use of a non-biocompatible solvent, which causes toxic side effects. Salt-formulations are formed by the electrostatic interaction between the acidic or basic drug and counter-ion salt which can provide the pH shifting once added into

dissolution medium and thus solubilize the drug and increase the dissolution. The taxing process of selecting an optimal salt form is also a limitation of the salt-formulation method. The salt forms are usually selected through trial and error. The difficulty of synthesis and crystallization along with the cost of the raw materials are first considered at the initial stage of the drug formulation development. If the salt was found to be nonoptimal during the subsequent process, it is usually hard to vary the salt form without delaying the formulation development program, as changing the type of salt would involve repeating the biological, toxicological, formulation and stability tests. Another specific drawback of the salt formulation is that the salt can convert into an acidic or basic form [31] as the pH changes in vivo, or precipitate out into the aqueous environment, as the salts are usually developed from organic solvents [28]. For polymorphism, co-crystal formulation involves complicated processing and supercritical conditions, resulting in a low success rate. The application of micronization is limited because of the lack of control over the morphology and surface properties of the final particle [28, 29]. Problems such as patient compliance and commercialization have limited the application of cosolvency [28, 29]. Cost-effective and simple preparation methods of drug formulations with high payload (drug loading), fast dissolution rate and high apparent solubility are desired.

1.3 A simple, economical and effective drug-polysaccharide method to prepare an amorphous nano-complex drug formulation

The self-assembly drug-polysaccharide complexation method (Figure 1-2) is quick, simple and effective, as it only involves mixing the ionic drug solution and oppositely charged polyelectrolyte solution under ambient conditions. Various amorphous drug–polyelectrolyte nanoparticulate complexations (nanoplex) exhibiting high apparent

solubility, enhanced dissolution rate, sustained supersaturation and high payload [32-36]. These merits enhance the attractiveness of the self-assembly drug-polysaccharide complexation method as a strategy for solubility enhancement.

Figure 1-2: Schematic of the self-assembly drug-polysaccharide complexation method

1.4 Existing problems with the conventional drug-polysaccharide nanoplex method

The amorphous nanoplex was conventionally prepared by bulk mixing the ionized drug and oppositely charged polysaccharide. Despite the advantages of the amorphous drug-polyelectrolyte nanoplex mentioned, the conventional bulk mixing drug-polysaccharide method has its drawbacks.

Firstly, the nanoplex prepared using the conventional bulk mixing method lacks batch-to-batch consistency [37]. The nanoplex can vary from a lot from batch to batch, with big fluctuations. This has limited its translation into the clinic [37]. Secondly, the conventional bulk mixing drug-polysaccharide nanoplex preparation is a discontinuous

production. Compared to continuous production methods, it has several disadvantages in terms of production volume, cost, and energy consumption [38, 39]. Thirdly, although the fast dissolution rate is helpful in shortening the drug onset action time, it also has various shortcomings. In different physiological or pathological environments, the desired profiles of the concentration and time for the same drug are usually different. The immutable dissolution of the drug formulations has limited the potential for drugs to be delivered to patients through different routes. For example, the pulmonary administration of the insoluble drug Ciprofloxacin (CIP) is inefficient due to its fast dissolution rate, which causes CIP to diffuse through the lung epithelium into plasma too quickly, resulting in a low CIP concentration within the lung and causing a low therapeutic effect on the pulmonary disease. On the other hand, for those drugs with small therapeutic windows, low minimum lethal concentration, or serious side effects, a fast dissolution rate might cause burst absorption and lead to grave consequences.

1.5 Stability of the amorphous drug

The amorphous drug has been considered an advancement to improve drug solubility and enhancing the bioavailability of the drug. Because of the high Gibbs' free energy and absence of the lattice energy barrier, the amorphous drug systems can generate more significant kinetic solubility compared to their crystalline counterparts.

The principal concerns associated with the amorphous drug formulation include the amorphous stability during storage and recrystallization, which causes a slow dissolution rate after a long period of time in storage [40]. The development of stable amorphous formulation thus becomes imperative for this reason.

1.6 Research Objectives

To overcome the shortcomings mentioned and expand the applications of the drug-polysaccharide nanoplex, the research objectives proposed are listed below.

1.6.1 Enhance the solubility of the poorly soluble drug

The first objective is to increase the solubility of the poorly soluble drug using the electrostatically driven drug-polysaccharide complexation method as a new solubility enhancement strategy.

1.6.2 Design millifluidic drug-polysaccharide nanoplex synthesis platforms to improve the production rate and production consistency

The second objective of my research is to develop a high-throughput continuous platform with an improved production consistency. The millifluidic synthesis platform provides a practicable procedure to evade some of the shortcomings of conventional nanoparticle syntheses, enabling a higher degree of control over reagent conditions, improved reproducibility, real-time product monitoring, and a feasible high-throughput synthetic approach. Single channel millifluidic platform were designed. A direct comparison between single-channel millifluidic and bulk-mixing methods in the synthesis of an amorphous drug-polysaccharide nanoparticle complex was conducted. The millifluidic method was superior in terms of production consistency.

1.6.3 Modify the dissolution rate of the drug-polysaccharide nano-complex with high drug loading by incorporating small molecule ions into the drug-polysaccharide nanoparticle

The fourth objective is to equip the drug-polysaccharide nanoplex with a controlled release functionality through the incorporation of ionizable small molecule ions into the

drug-polysaccharide nanoparticle. The oral bioavailability of ciprofloxacin (CIP) is limited because of its narrow absorption window in stomach. Thus, it is of particular interest to develop CIP in the form of a nanoparticle that is able to maintain a therapeutic concentration and sustained release in the stomach, to enable a longer duration of action time.

1.6.4 Enhance the amorphous stability

1.6.4.1 Incorporating crystallization inhibitor polymer into nanoparticles without decreasing the drug loading or apparent solubility

With the entry of several polymer-containing drug formulations into the market, the polymeric drug formulation plays a significant role in the drug delivery research field. The polymeric drug delivery system has shown different merits such as enhanced solubility, controlled release, prolonged circulation time, and enhanced stability. Polymers with high glass transition temperatures, such as the HPMC and PVP, have been used as crystallization inhibitors to stabilize the amorphous form of the drug.

1.6.4.2 Increasing the amorphous stability by using a polysaccharide with better crystallization inhibitor ability

The inhibition of the crystallization of polyelectrolytes in the nanocomplex has been attributed to the high glass transition temperature, impediment on the molecular mobility of the drug, and the interaction between the polymer and drug. Hence, the 3D structure (i.e., side chains) and the functional groups (i.e., hydrogen donor groups, ionic function groups) of the polyelectrolytes affect the stability of the amorphous formulation. This research study seeks to compare the amorphous stability of the nanoplex prepared using different polyelectrolytes.

CHAPTER 2 Literature review

2.1 Amorphous drugs

Drug formulations in amorphous state, so called as amorphous drugs, have been increasingly employed in the pharmaceutical field to overcome the limited solubility of the poorly soluble drugs and to increase the dissolution rate and bioavailability of drugs with poor solubility. Amorphous drugs have a higher apparent solubility than its crystalline counterpart because of the inherent higher enthalpy, entropy and free energy of the amorphous form compared to the crystalline form [40, 41]. Due to the higher free energy, the amorphous drug has the proclivity to undergo crystallization during storage, resulting in reduced solubility of the drug formulation, which has been recognized as its principle disadvantage [42].

2.1.1 Amorphous solid dispersion (ASD)

Since amorphous solid dispersion (ASD) was proposed in the 1960s [43, 44], it has received considerable attention in the pharmaceutical field. ASD refers to the amorphous solid systems prepared by dispersing active pharmaceutical ingredients (API) into polymers or excipients [45]. The inherent amorphous form of ASD leads to supersaturation and apparent solubility, which makes ASD an efficient approach to increasing the dissolution rate and oral bioavailability of poorly soluble drugs. To reduce the molecular mobility and stabilize the amorphous form of the ASD, polymers are often used as the excipients in ASD. Most of the amorphous drug formulations are ASD.

2.1.2 Co-amorphous system

Since 2009 [46, 47], “co-amorphous” has been used to refer specifically to the amorphous ASD system prepared using low molecular weight molecules to disperse the API [47, 48].

2.1.3 Nanoplex

The amorphous nanoparticle complex, nanoplex for short, is formed through self-assembly due to the drug-polysaccharide complexation driven by the electrostatic and hydrophobic interaction between the ionized drug molecules and oppositely charged polysaccharide molecules. Since it was first proposed in 2012 [33], its applicability was demonstrated for a range of drugs, such as the natural polyphenolic compound curcumin, antibiotic ciprofloxacin, and antipsychotic perphenazine [32-36, 49, 50].

2.2 Methods to prepare amorphous drugs

The development of novel drug formulations to enhance dissolution rate has become an urgent requirement in the pharmaceutical industry with the significant increase in the number of insoluble drug candidates [51]. In traditional methods, such as salt formulation, adding solubilizing excipients had its shortcomings. The salt selection is complicated and a fail-and-trial process. The large amount of solubilizing excipients used in the formulation can decrease its drug-loading and affect the therapeutic effect [52]. Among the many methods, the conversion of the crystalline drug into its amorphous form has emerged as an attractive approach to increase the apparent solubility and enhance the bioavailability of insoluble drugs.

2.2.1 Milling

Mechanical milling is a conventional and frequently used method [53-56] in the pharmaceutical industry to prepare amorphous drugs and decrease the particle size of

drugs due to its easy execution and low cost. By applying mechanical energy, milling brings about attrition and concurrently causes the accumulate disruption of the drug crystal lattice, resulting in amorphization of the drug [56, 57]. A variety of excipients, such as polyvinylpyrrolidone [58], ethyl cellulose, and polyethylene glycol, have been used during milling to prepare amorphous drugs [55, 59].

However, it is an energy-intensive and time-consuming method and lacks the fine control over the product in terms of size and surface features [60]. Milling also requires a relatively large amount of API. Moreover, milling always leads to partial amorphization, inducing quicker recrystallization [61].

2.2.2 Anti-solvent precipitation

Self-assembly drug nanoparticles can be obtained by adding the anti-solvent to the API solution to reduce the solubility of the API and trigger the supersaturation and precipitation of the API. This process is called the anti-solvent precipitation method [62]. The anti-solvent precipitation method is one of the most commonly used methods in preparing amorphous nanoparticles because of its merits, such as easy operating conditions, low cost, and scalability [63]. However, the mixing process of the anti-solvent and the API solution needs to be carefully controlled because the products are sensitive by nature and can be affected by the mixing process. Thus, to achieve the desired products, advanced mixing technologies are required.

2.2.3 Hot melt extrusion technology

Amorphous drugs can be prepared through the sudden cooling of molten material. If cooled slowly, the molecules in the molten drug can move from their current positions to form a thermodynamically stable crystalline. However, if cooled suddenly, the

molecules in the supercooled liquid will have insufficient time to form the crystal lattice and remains in the glassy state, which is the amorphous form.

Hot melt extrusion (HME) has emerged as a successful technique in the development of amorphous solid dispersion delivery systems to improve the solubility of the drugs and is aimed at improving the bioavailability and achieving the therapeutic drug concentration in the plasma. It refers to the product fabrication processes of melting and blending the drug and components at an appropriately high temperature and forcing the mixture to continuously extrude through an orifice under appropriate pressure to be cooled [64].

Since its first occurrence in 1970 [65], HME has gained popularity in both industrial applications and research, due to its many attractive merits such as its continuous production capability, robustness, industrial scalability, absence of organic solvents, controlled conditions (i.e., process temperature, shear pressure, viscosity of the molten mixture of drug and excipients, mass feed rate), use of various types of the machines (i.e., ram extrusion, screw extrusion) to suit the development objectives and prepare medicine with various morphologies, and high throughput. A myriad of the pharmaceutical dosage forms, for instance, pellets, tablets, transdermal dosage, implants, transmucosal, have been produced using HME [64]. As a continuous fabrication method, HME was expected to achieve high production efficacy and better product consistency compared to the batch-to-batch method [66]. The distributive mixing during the HME can provide a tailorable high-shear force to suit the different formulation requirements. The Food and Drug Administration's (FDA) approved drugs are prepared using HME, indicating the commercial success of this technology in the pharmaceutical field. There are also numerous ongoing research studies focused on combining HME with other

technologies, such as the use of the amphiphilic polymer to form nano or micro-sized micelles and amorphous self-micellizing solid dispersions (SmSDs) [67].

Even though, HME has various shortcomings such as unstable reproducibility, high energy requirement, the intensive process, thermal degradation of the API, and the requirement of thermally stable polymers. The thermal stability of API has posed a major challenge to the more extensive application of HME. It is possible to mix the raw drug with the excipients below the glass transition to achieve the molecular level of mixing, but in most cases, the HME is conducted above the melting temperature [68]. Excipients such as plasticizers, antioxidants, and thermal lubricants can be introduced to prevent degradation of the drugs. Nevertheless, the adding of excipients can decrease drug loading.

2.2.4 Solvent evaporation/sublimation

Converting liquid drug suspensions into solid dosages by removing water is an effective way to increase amorphous stability. The fast solvent evaporation during the spray drying and freeze-drying processes can transform the liquid suspensions into amorphous drug solid dispersions.

2.2.4.1 Spray drying

Spray drying is frequently used to prepare pharmaceutical amorphous formulations. The crystallinity, size, and morphology of the dried product can be adjusted and controlled by varying the parameters such as the feed ratio of drug and excipients, inlet temperature, concentration of the solution, flow rate, and moisture content in the chamber [60]. The properties of the dried products can also be tailored to achieve various goals by adding the excipients. For instance, surfactants can be added to increase the wettability of the spray-dried particles to increase their solubility. Spray

drying increases the compression properties as well as the plasticity and binding ability of the excipients, which makes them more suitable for the preparation of tablets with satisfactory disintegration profile, tensile strength and friability [60].

Microparticles manufactured through spray drying have been widely used in the pulmonary route. The morphology, size (especially the aerodynamic diameter) and size distribution affects the pulmonary drug delivery. Controlling the drying process and morphology of the product is still key to preparing a successful spray-dried drug formulation. Spray drying, however, has its shortcomings. Some excipients, i.e., low molecular mass sugar, are converted into a glassy state during the spray drying process, depositing onto chamber walls and forming viscous dry products. The aggregation of particles during the spray-drying process [69] and the less soluble material on the surface of the particles are other potential shortfalls of amorphous particles prepared using the method [70]. Moreover, the lack of manufacturability is another reason that limits its wide application and scalability.

2.2.4.2 Freeze drying

Freeze drying, also known as lyophilization, is a multistage processing method which has been used to dry products, such as serum, protein, peptide, vaccines and drugs. The products underwent freezing and drying process under low vacuum pressure and at low temperature. The freeze-dried formulation has improved stability during storage compared to the wet form [71].

The freeze-dried products are always porous and have a low density, which is beneficial due to the larger surface area. This advantage, together with their amorphous form, can help increase the products' dissolution and apparent solubility. The in-situ

freeze drying within capsules method is a novel method that can be used to capsule the freeze-dried products immediately without compressing the products [71].

2.3 Pharmaceutical amorphous nanoparticle

Though the success of crystalline nanosuspensions has been proven by several marketing drugs and other drugs in the clinical trial stages, the physiochemical properties of the active pharmaceutical ingredient are more susceptible in this form compared to the powder form. The pharmaceutical amorphous nanoparticle is one of the novel drug delivery systems that can be used to overcome the low bioavailability problem of the aqueous insoluble drug.

The size of the pharmaceutical nanoparticles is generally in the range of 100 to 1000 nm. The small size of the nanoparticles and the amorphous form of the drug can offer synergistic enhancement effects to improve the pharmacokinetic properties, enabling a fast drug release rate, high apparent solubility, improved onset of action time and better bioavailability. The nanoscale drug delivery system is an effective method to augment solubility. Nanoparticles have held great promise for drug delivery [72]. Several kinds of nanoparticles (i.e., Abraxane, Doxil) have been approved by the Food and Drug Administration (FDA) to treat metastatic adenocarcinoma of the pancreas and ovarian cancer. Moreover, Genexol, a polymeric micelle nanoparticle, has been approved in Korea. It has also become a promising technology to enhance the solubility and bioavailability of poorly aqueous soluble drugs [72, 73].

2.3.1 The advantages of nanoscale drug delivery systems

One of the advantages is the small size of nanoplex, which enables a large surface area to volume ratio. This can thus bring about a higher dissolution rate [74] and contribute to sustained superior saturation generation, according to the Ostwald-Freundlich

(Equation 2-1). It exhibits biological properties such as the ability to interact with the cell surfaces and enter cells, enabling its accumulation in tumor cells because of its small size. Nanoparticle drug delivery systems also have a relatively higher intracellular uptake because of the small size of the nanoparticles [75]. The realization that the emerging nanoparticles exhibit unique physical, optical, and biological properties has also sparked investigations in drug delivery disciplines [76].

Equation 2-1

$$\ln \frac{C_{s,r}}{C_{s,\infty}} = \frac{2\gamma V_m}{rRT}$$

(C_s : the solubility of drug particles with radius r (m); C_∞ : represents the solubility of drug particles with radius ∞ (m); γ : surface tension between the particles and the solution (N/m); V : the drug molecular volume (m³/mol); R : the gas constant (8.314 J/mol•K); T : the absolute temperature (K)).

In addition, many nanoparticles have demonstrated their controllability for drug release [77-80]. The modulated nanoplex constituents, material to form the nanoplex and the structure of the nanoparticle embellishes its potential ability for controlled release.

2.3.2 Methods to prepare nanoparticles

In general, most synthetic methods used to prepare nanoparticles can be classified into two main categories: “top-down” strategies and “bottom-up” strategies [81]. Top-down techniques begin with macroscopic materials. The conventional “top-down” strategies used in medical applications to reduce the size of particles are milling, grinding, and high-pressure homogenization [13]. During the process of milling or high-pressure homogenization, the physical pressure placed upon the drug might induce degradation. Top-down methods usually require high energy consumption but result in low production yield and non-uniform morphology.

Bottom-up techniques begin by synthesizing molecules into macro-scale structures [81]. Nanoencapsulation and nanoprecipitation are two common bottom-up

methods. Nanoencapsulation describes drug systems where the drug is confined to a cavity. For instance, liposome and polymeric micelles are produced using nanoencapsulation, which includes methods such as emulsion polymerization, emulsification/solvent evaporation, solvent displacement, and interfacial deposition [75]. The preparation of nanoprecipitation involves the precipitation of the drug and its counterpart. Most of the bottom-up methods consume high energy and involve complicated processes but exhibit low drug-loading.

2.3.3 Using amorphous drug formulations to increase drug solubility

The amorphous drug formulation [7, 33] has demonstrated its capability in drug solubility enhancement. The “Spring and Parachute” effect is the main mechanism of the bioavailability enhancement for amorphous drug formulations [1, 6, 51]. The intrinsic high level of free energy present in the amorphous form grants the “spring effect”, a state where the thermodynamic solubility (supersaturation) can be several times the saturated concentration of the high active pharmaceutical ingredient [6]. This high solubility driven by the high level of energy present in the amorphous form of the conventional dose can normally be maintained only for a short time, due to the precipitation. Precipitation inhibitors (such as hydroxypropylmethylcellulose, enteric derivatives of hypromellose, poly(vinylpyrrolidone), and poly(vinylpyrrolidone)-vinyl acetate copolymer) have been used to prolong the supersaturation. The main processes of drug precipitation are generally nucleation and crystal growth [82]. The interaction between precipitation inhibitors and drugs might change the properties of the solution medium and hinder the drug molecular absorption to the nuclei and prevent crystallization. The drug and polymer colloids have been proposed to be crucial in the

suppression of crystallization [83]. The bioavailability is related to the apparent solubility, which is directly related to the supersaturation level and maintenance [6].

2.3.4 Amorphous drug-electrolyte nanoparticle complex

The traditional approaches to prepare amorphous drugs involve thermal or mechanical stress during manufacturing. Among the conventional methods, milling is widely used to prepare the amorphous form of drugs is milling, where the crystalline drug is milled into small-sized amorphous forms, a shortcoming of the milling method. Recently, intraoral delivery formulations were prepared in the amorphous form to exploit the fast dissolution afforded by the metastable amorphous form in the form of either an amorphous solid dispersion [11, 84] or cyclodextrin inclusion complex [84-86]. Despite shortcomings such as complicated processes, high energy consumptions, high costs, supercritical conditions, and low success rates of the production methods mentioned above, most of the drug formulations created to improve the solubility of insoluble drugs share a common trait—their low drug payloads [87-90].

In our lab, various metastable amorphous electrostatic complexations with high payload, fast dissolution and prolonged high-level supersaturation [1, 32, 33, 35, 36] are prepared using the quick, simple, and effective self-assembly drug-polysaccharide complexation method, which only involves the mixing of the drug solution and polymer solution under ambient conditions [33, 35, 36]. Wean Sin Cheow et al. [33] prepared the ionized drug-polyelectrolyte nanoplex for the first time in 2012.

The amorphous drug-polysaccharide nanoplex represents a promising drug formulation for poorly soluble drugs due to the capability of the amorphous nanoplex to generate a highly supersaturated drug concentration [36, 91] and fast dissolution rate afforded by the large specific surface area of the nanoparticles [92-94].

2.4 Methods to enhance amorphous stability

The amorphous drug has been considered an advancement to improve drug solubility and enhance the bioavailability of the drug. Because of the high Gibbs' free energy and absence of the lattice energy barrier, the amorphous drug systems can generate more significant kinetic solubility compared to their crystalline counterparts.

However, the thermodynamic instability of amorphous drugs has been one of the major challenges in amorphous drug formulation research. The amorphous formulation tends to convert to the crystalline form, which drastically decreases its solubility. The crystallization of a drug in the amorphous form involves nucleation and crystal growth [95].

Pure amorphous drugs fail to be developed into marketing products because of their inherent instability. A broad range of excipients such as surfactants, proteins, carbohydrates, lipids, amino acids [96], sugars, organic acids [97], and urea [98] have been studied and used to stabilize the amorphous drug formulation [40].

2.4.1 Polymeric amorphous formulations: Using polymer to reduce the molecular mobility and prevent the crystalline

The polymer-drug amorphous solid has been a mainstay in the pharmaceutical field. Commercialized polymeric amorphous drug formulations, such as Novir®, Kaletra®, and Sporana®, demonstrated the success of the polymer in the stabilization of the amorphous form and increment of its apparent solubility and dissolution [99].

With the entry of several drug formulations containing polymer into the market, the polymeric drug formulation has played a significant role in the drug delivery research field. The polymeric drug delivery system has shown different merits such as

enhanced solubility, controlled release, prolonged circulation time, and enhanced stability.

The large and flexible 3-dimensional structures of the polymer and the interactions (i.e., electrostatic interaction, hydrogen bonding) between the polymer and the drug have the potential to inhibit the molecular mobility of the drug [40] and prevent the crystallization of the drug. A myriad of polymers has been used to molecularly distribute the API molecules inside the polymer matrix to prepare the amorphous drug formulation [40]. The miscibility of the polymer and the drug has been studied by different models, for instance, the Fory-Huggins (FH) lattice theory and Hildebrand regular solution theory [100-103].

Polymer has been used in amorphous drug formulation to decrease the amorphous stability of its repetitive 3D structure and the bond (i.e., hydrogen bond, electrostatic interaction bond [1, 104, 105]) between the polymer and the API molecules. This inhibits the molecular mobility of the drug [40] and impedes the crystallization, maintaining the amorphous form of the drug. The polymers can inhibit the crystallization and precipitation of the drug in the dissolution medium and maintain the high supersaturation concentration level for a significantly extended period [1, 106, 107]. The existence of the polymer excipients can dilute the free drug molecules available for nucleation and increase the viscosity, which can slow down the transport of free atoms/molecules to the surface of the nucleus and prevent the growth of the crystalline.

In summary, the chemical structure, molecule weight, melting temperature (T_m), glass transition temperature (T_g), viscosity, structure flexibility, and bond formulation between the drug and the polymer are important for the stability of the amorphous solid

dispersion system. Various methods such as the solubility parameter approach, melting enthalpy approach, Flory-Huggins theory, and molecular modeling are developed as tools to predict the rational formulation of the drug-polymer amorphous solid dispersion.

2.4.1.1 Diminution of the molecular mobility in polymer amorphous formulations

Molecular mobility is consisted of global mobility (also referred as α -relaxation or structural relaxation) and local mobility (also called β -relaxation). Molecular mobility plays a big role in the recrystallization of the amorphous drug formulations and is regarded as a predictor of amorphous state stability in the pharmaceutical field [108-111]. The nucleation, crystalline growth and diffusion progress of both phase separation and crystallization involve molecular mobility. The phase separation and crystallization can occur when sufficient molecular mobility is achieved. Global mobility has been found to be coupled with the crystallization onset of many drugs [42]. Reducing molecular mobility of amorphous drug formulations is a useful approach to improve the physical stability of amorphous drug [109, 112].

Dielectric spectroscopy analysis [42], DSC, and solid-state nuclear magnetic resonance (ss NMR) are the tools commonly used to examine molecular mobility. Correlations between molecular and amorphous state stability have been reported in many research papers [42, 110, 113-120]. In the literature, it is reported that polymers such as PVP, HPMC, and poly(acrylic acid) (PAA) have been reported to deduce the molecule mobility and increase the physical stability of amorphous pharmaceuticals [40, 108].

2.4.1.2 Drug-polymer interaction enhances amorphous form stability

The interaction between polymers and the excipients has been reported to stabilize the amorphous drug. For example, PVP and drugs (such as indomethacin, lecodeine, and

nifedipine) can interact through the pyrrolidone ring and hydrogen donor groups [121]. In literature, it is reported that polymer-drug interactions such as hydrogen bonding [122-125], van der Waals force [126], and hydrophobic and electrostatic interaction [32, 35] contribute to the stability of the amorphous drug as they restrict molecular mobility and hinder crystallization.

The strength of the interaction between the drug and the polymer plays an important role in crystallization inhibition. Kothari et al. compared the H-bonding between different polymers (i.e., PVP, hypromellose acetate succinate (HPMCAS) and poly(acrylic acid) (PAA)) and found that stronger H-bonding leads to a stronger amorphous stabilizing ability [127]. Electrostatic interaction stabilized amorphous drug formulations have enhanced stability because of the strong ionic bonding [53-55, 128-132].

2.4.1.3 High T_g and anti-plasticization of the polymer contributes to amorphous stability

The crystallization of the amorphous drug involves the nucleation of the drug and crystalline growth, both of which require energy. The anti-plasticization, which means the reduction of plasticity, leads to an increase in T_g and increases the energy required by the amorphous drug to convert to the crystalline form [35]. Khougaz et al. compared the crystallization inhibition of five polymers and concluded that the polymers with higher T_g have better stabilizing effects [126].

2.4.2 Enhance the amorphous stability using co-amorphous drugs

The concept “co-amorphous drug” (CAM) refers to the drug-small molecule system associated with weak and discrete force of interaction between the drug and the small molecule components [133, 134]. The CAM system has gained a lot of attention as an

alternative to polymeric amorphous solid dispersions (PASDs) since it was first coined in 2009 [135] for its potential superiority in enhancing amorphous stability and increasing the drug payload. The polymer was unfavored due to its large molecule weight, limited drug miscibility, and hygroscopic nature, compared to small molecules.

However, the weak interaction force between the drug and the counter-molecule might not be sufficient to ensure the amorphous stability after a long time in storage.

2.5 Dissolution of amorphous drug

2.5.1 The typical spring and parachute of amorphous drug supersaturation

The thermodynamically [119] unstable amorphous drug formulation always has a higher energy level compared to its crystalline counterpart, and tends to generate rapid dissolution .

Figure 2-1: Schematic of supersaturation generated in the “Spring and Parachute” model, spring model and generated by crystalline drug

This leads to a high drug concentration in the dissolution medium, which is followed by a decrease of drug concentration due to the recrystallization and precipitation of the drug in the solution. The rapid dissolution appears as a peak and is called “spring”, while the decrease in solubility is known as “parachute” (Figure 2-1).

Typical spring and parachute of amorphous drug supersaturation improves drug solubility and dissolution rate.

2.5.2 Fast is not enough

The ability of amorphous drug to generate a fast dissolution and high thermodynamic concentration using amorphous drugs has been confirmed in many studies [1, 32, 33, 105]. However, the fast dissolution can generate a concentration that is too high and beyond the therapeutic window, causing toxicity. This is very important for drugs with small therapeutic windows. Moreover, the high supersaturation created in the stomach will lead to precipitation because of the pH variation between the stomach and the small intestine. The fast release of the drug in the stomach or intestine should be maintained for a significant amount of time to ensure absorption of the drug. The fast in vitro dissolution of drugs does not transfer to optimal in vivo absorption because of the pH variation and fast excretion [136]. Compared to the burst supersaturation release, the moderate prolonged release which maintains the therapeutic concentration for a longer time is preferred, as the prolonged supersaturation can generate less drug precipitation and achieve higher bioavailability, reducing the frequency of drug dosage and enhancing patient compliance.

Though the amorphous drug formulation has emerged as an attractive method to address low-solubility issues and provide fast dissolution, the desired dissolution rates are different when the therapeutic aims are different. Thus, drug formulations with enhanced solubility and variable dissolution rates are highly demanded.

2.5.3 Strategies to sustain the burst dissolution of amorphous drugs

Polymers, such as HPMC and PVP, can inhibit the crystallization of drugs during dissolution and delay the supersaturation by interfering with the nucleation process

[137]. Moreover, interactions between drugs and excipients have been used to prolong the supersaturation in order to achieve sustained release in a non-sink condition.

There is growing interest in the use of a precipitation inhibitor to prolong the supersaturation to achieve sustained release in a non-sink condition. Polymers can inhibit the crystallization of drugs during dissolution and extend the supersaturation duration by interfering with the nucleation process [137, 138]. Using interactions between drugs and excipients has been applied as an approach to sustain the release as well.

Table 2-1: Several examples of polymers capable of stabilizing the amorphous drug and enhancing the bioavailability

Polymer	Mechanism that stabilizes the amorphous form	Mechanism that increases bioavailability
PVP	Polymer viscosity [139]; interactions between the pyrrolidone ring of PVP and hydrogen donor groups of drug [121]; 3D structure inhibits the mobility [40]; anti-plasticization [140]	Amorphous form; hydrophilicity
PVP-VA	Hydrogen bonding between the drug and PVP [141]	Amorphization of drug [142]; prolonged sustained time [141, 143]
Hydroxypropyl methylcellulose	Polymer viscosity; Drug-polymer interaction (i.e., H-bonding) [144]; anti-plasticization [145]	Hydrophilicity
Chitosan	Drug-polymer electrostatic interaction and hydrophobic interaction [32]	Amorphous nanoparticle drug [32, 35]

Chengyu et al. used the interaction between sorafenib, poly(vinylpyrrolidone-co-vinylacetate) (PVP-VA) and Sodium dodecyl sulfate (SDS) to adjust the “spring” and “parachute” progress to achieve a high and sustained drug supersaturation aimed at increasing the bioavailability in gastrostomies [143]. The interaction between the drug and the PVP-VA is able to sustain the “parachute”. The SDS interaction with the PVP-

VA competes with the drug to affect the parachute. The molecular interactions between the drug, PVP-VA, and SDS were successfully used to modify the supersaturation, which is the dissolution of the drug under a non-sink condition. Moreover, counter ions are used to form the nanoplex dose formulation with controlled dissolution rates. The counter-ions that exhibit charges also have the potential to form complexes with ionized drugs. For instance, sodium dodecyl sulfate (SDS) can provide the counter-ion— SO_3^- . Moreover, the SDS-ionized drug has been proven to have low solubility [146], which indicates its potential ability to slow down the drug release. The ions-drug complex has recently been used to sustain drug release.

The interaction between drugs and ions has appealed attention from pharmaceutical scientists. For example, the co-administration of CIP with cations like Mg^{2+} and Al^{3+} has been reported to be able to reduce oral availability [147, 148]. Further, the complexation's reduced diffusion through the intestine has been demonstrated [149-151]. The function group of the synthetic broad-spectrum quinolones antibiotics, such as the carboxylic acid at the 3-position, the basic piperazinyl ring at the 7-position, and the carbonyl oxygen atom at the 4-position can bind to metal ions (such as Ca^{2+} , Cu^{2+} , Ni^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , and Fe^{3+}) to form complexes of various stoichiometry [89, 151, 152]. The stability constants and apparent association constants of the cation-CIP complex have been studied [151-153]. The formed complex has a higher molecular weight, more complex molecular structure and is electrically charged. These traits are believed to be the reason for its limited diffusion through the intestinal and lung epithelium [147]. In acidic conditions, the amphiphilic quinolones molecule can be protonated, resulting in an ionized molecular structure [33, 154]. At $\text{pH} < \text{pKa}$, protonated quinolones act as cations to form complexes with negatively charged ions

[147]. Likewise, the ion-quinolones complex also exhibits the possession of electric charges and has the potential to interact with negatively charged ions.

The drug-ion complex has been proven to allow sustained release without any additional adjunct or complicated procedure. Seung Yeon et al. using the positively-charged antibiotic tetracycline and oppositely charged multivalent ions HPO_4^{2-} as models for the drug and counter-ion, respectively, have developed an ion-complexed antibiotic formulation which permits prolonged drug release [155]. The ion-complexed antibiotic was prepared through the simple mixing of an ionized drug solution and multivalent counter ionic solution. It is exciting that the formed yellow ion-antibiotic complexes showed an unceasing release for 21 days, before finally reaching 100% release. In comparison, it takes only two days for TC to be released from native TC powders [155]. Seung Yeon et al. [155] also reported their fabrication of ion-complexed antibiotics using positively-charged Doxycycline Hyclate (DX) and negatively-charged multivalent HPO_4^{2-} in another paper. The ion-DX complex showed controlled release that lasted 6 weeks, while the release of DX from the native DX powder only takes 4 hours [156].

Brillault J et al. [153] has proven *in vitro* that compared to the native CIP, the permeability through the epithelial of the ion-drug complexation decreased while the antibiotic efficacy and cell toxicity remained almost the same. Frederic Tewes et al. have developed inhalable CIP- Ca^{2+} complex microparticles with calcium hydroxide ($\text{Ca}(\text{OH})_2$) and CIP using the spray drying method [157]. Hyaluronate and formic acid (HCOOH) were also used to increase the solubility of calcium hydroxide. Compared to the native CIP, this fluoroquinolone (FQ)-ion formulation exhibited sustained CIP release and lower apparent permeability owing to the ion-CIP interaction. Furthermore,

it exhibits less dependence on pH compared to the native CIP [157]. Nazende et al. mentioned the counter-ion concept [146]. They formed the ion complex using sodium dodecyl sulfate (SDS) as the counter-ion and fluoroquinolone (Figure 2-2(A)) antibiotic ciprofloxacin as the model drug (Figure 2-2 (C)). In an acidic condition, the secondary amine of piperazinyl in the C7 position of the CIP can be protonated (Figure 2-2(B), (D)). The protonated C binds with the negatively charged counter-ion SDS through electrostatic interaction. The formed complex exhibited a suppressed pH-dependent solubility. Moreover, at a low pH (pH 1.2 to 4.5), the solubility of CIP-SDS is much lower than the native CIP, indicating that the CIP release from the CIP-SDS complex would probably be slower than that of the native CIP drug. The ion-drug complex was also successfully prepared with other drugs (aminoglycosides gentamycin and tobramycin) [146].

Figure 2-2: (A) Molecular structure of Quinolones [158] (B) Molecular structures of CIP, ciprofloxacin [159]; (C) Molecular structure of negatively-charged sodium dodecyl sulphate ions; (D) Protonated CIP

Moreover, the solubility independence from pH can expand the application of CIP. The pH of the liquid in the human body can vary. For example, the pH of gastric juice is easily affected by the food consumed, physiological metabolism and other pathological reasons [147, 160-163]. The pH of gastric juice varies from 1.5 to 3.5 [147,

164, 165]. The pH of the infected site is always lower than the regular counters [166]. The suppressed pH-dependent solubility can contribute to the constant release of the drug [157]. Furthermore, the amorphous CIP-DXT nanoplex lacks stability after long-term storage. The surfactant can be used as a component to improve the wettability and stabilization of the nanoplex [5, 6, 167-169].

The counter-ion is a promising concept to obtain modified dissolution profiles [146]. However, the formed ion-drug complex is either not nanosized [155-157], not amorphous [146], and has a low payload [146, 157]. The drug-polyelectrolyte nanoplex driven by the electrostatic interaction between the ionized drug and the charged polymer is amorphous [1, 33, 35, 36, 105]. By adjusting the ratio of drug, polysaccharide, and ion, drug-polysaccharide-ion formulations with the amorphous form was obtained.

The drug-polysaccharide nanoplex was prepared using the drug-polysaccharide complexation method based on the electrostatic and hydrophobic interaction between the polysaccharide and the ionized drug. Both polyelectrolyte DXT and sodium dodecyl sulfate (SDS) can get negatively charged in the solution because of the functional group —SO_3^- . Both SDS and DXT have the potential to form complexes together with ionized drug molecules. The SDS-ionized drug had been proven to have low solubility [146], which results in its slow dissolution rate. However, the low solubility of the SDS-drug is not desired, as the low solubility limits the bioavailability of insoluble drugs. CIP, DXT, SDS were used as the drug, polysaccharide and counter-ion model, respectively, to form a complex that exhibits a modified dissolution rate with high apparent solubility. By adjusting the ratio of the CIP, DXT and SDS in the complex, novel drug-polysaccharide-ion formulations that can perform sustained release, and with a high bioavailability is developed.

2.6 Millifluidic: A high-throughput platform with batch-to-batch reproducibility

The millifluidic synthesis platform has been significantly proven to be a high throughput [39, 170] synthesis system with controllability on the nanoplex product properties [72] (such as size and zeta potential) [107]. The millifluidic synthesis platform that operates under a laminar flow has been designed to produce nanoplexes with consistent size, surface chemistry, surface charge and controlled composition [5, 171, 172]. Merits such as easy manipulation, reproducibility as well as high production throughput make the millifluidic method an attractive synthesis platform [5].

Figure 2-3: Schematic of the millifluidic reactor setup used in the production of amorphous perphenazine (PPZ) and dextran sulfate (DXT) nanoparticle complex (nanoplex).

Despite the advantages of the nanoplex mentioned above, the limited batch-to-batch reproducibility, controllability in terms of physicochemical properties (e.g., size, size distribution, zeta potential), and the discontinuous production of the conventional bulk mixing method have decelerated the clinical development and clinical translation of the nanoplex. The physicochemical properties, such as size, payload, and zeta potential can strongly affect the physiological effect of the nanoparticles [72]. Hence, a controlled and reproducible synthesis of the nanoplex is desired and critical for clinical translation and industrial-scale production [72, 170, 173].

Unlike bulk mixing, the laminar flow and uniform mixing in the millimetric coaxial reactor has been found to be effective in forming nanoplexes with uniform size [5]. The millifluidic method can produce nanoplexes with a narrow distribution size [174, 175] because of the purely laminar hydrodynamic flow with a small Reynolds's number (smaller than 20) in the microfluidic systems [105, 176]. The laminar flow and uniform mixing in the millifluidic are not sensitive to volume, providing a uniform chemical and thermal environment in the millifluidic reactor, resulting in uniform products. The slow laminar diffusion at the liquid-liquid interface can bring about well-controlled nanoplexes. The millifluidic facilitates the size-control capability of the synthesis of nanoplexes by manipulating the flow rate and reactor length [170, 176-180]. The millifluidic synthesis platform has been proved to enable size control of the nanoplex by varying the length of the millifluidic reactor and the residence time [105]. Tran et al. used the millifluidic platform to prepare a curcumin-chitosan nanoplex with prolonged supersaturation, faster dissolution, high drug loading, and improved utilization rate [176].

At present, nanoparticles are usually prepared in the laboratory scale (small volumes), which is appropriate for investigating the fundamental properties and the principle of nanoplex formulation [173]. Though the scaling-up and high-throughput nanoplex synthesis seems like a simple concept, it faces a significant challenge because the increasing of the concentration or the volume of the reaction can significantly alter the effectiveness of reagent mixing and thermal transport, resulting in products with uniform properties [170]. The laminar flow and uniform mixing in the millifluidic channels can provide controls over the reaction, which avoids the challenges mentioned above associated with the bulk mixing method. Theoretically, increased throughput can

be achieved by operating the reaction continuously and running the reaction in parallel channels. Samuel E. Lohse, et al. designed millifluidic reactors facilitating the gram-scale synthesis of gold nanoparticle, which scaled up the gold nanoparticle for more than 200 times [170] while few gold nanoparticle synthesis are on the product scale larger than 50 mg though the synthesis of gold nanoplex have been studied for decades [181-190]. The numbering-up has been widely applied to increase the throughputs in chemical synthesis or pharmaceutical fields for the reactions involved sensitive conditions [191, 192]. A system of parallel channels was able to reach the product rate at kilograms/h [193]. A continuous millifluidic platform with a scale up ratio of 5000 compared to lab batch experiments for nanostructured CaCO_3 was successfully designed by Anne Aimable et al [194]. The reactor was designed for nanosized ZnO powders with a scale up ratio of 250 compared to lab batch experiments [191]. Adrian et al also designed multichannel millifluidic droplet reactor for high throughput synthesis of nanocrystals with a production rate upto 145g/day [195]. The continuous millifluidic technologies have the advantage of high throughput and complete manipulation over the reagent flows in the reactors, offering an option to increase the scale of synthesis of nanoparticles [196].

The millifluidic device can be assembled and disassembled easily with recyclable elements such as tubing and connections. Compared to the microfluidic process, the millifluidic is a cost-effective and less time-consuming process. The millifluidic synthesis platform provides better controllability compared to conventional bulk mixing [37, 105, 197], and holds promise for continuous high-throughput nanoplex synthesis. Furthermore, the high-throughput millifluidic synthesis platform can connect with monitoring and purification techniques for quality control [170].

CHAPTER 3 Materials and Methods

In this chapter, the materials and methodology are described in general. The detailed descriptions and justifications for the methodology are provided in the ensuing chapters.

3.1 Materials

Perphenazine (PPZ), CIP ($\geq 98\%$ purity), SDS ($\geq 99\%$ purity), low molecular weight chitosan (CHI) (50–190 kDa), sodium phosphate dodecahydrate ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), hydrochloric acid (HCl), potassium hydroxide (KOH), SDS and phosphate buffered saline (PBS, pH 7.4), Hydroxypropylmethylcellulose (HPMC) (MW = 26 kDa), PVP (MW = 40 kDa), potassium phosphate monobasic (KH_2PO_4), Sodium carboxymethyl cellulose (Average Mw $\sim 250,000$, degree of substitution 1,2), Pluronic F68, and dialysis tube were purchased from Sigma-Aldrich (USA).

Figure 3-1: Molecular structure of dextran sulfate (DXT).

Glacial acetic acid (AA), acetonitrile (HPLC grade), and ethanol absolute ($> 99.7\%$, HPLC grade) were purchased from VWR Singapore Pte Ltd (Singapore). Stainless steel test sieves (JIS Z 8801, Diameter 75 mm * Height 20 mm) with $75\ \mu\text{m}$ mesh and stainless-steel test sieves (JIS Z 8801, Diameter 75 mm * Height 20 mm) with $38\ \mu\text{m}$ mesh were purchased from Tokyo Screen Co, Ltd (Japan).

Low molecular weight DXT (MW = 5 kDa) was purchased from Wako Pure Chemical Industries (Japan).

3.2 Methods

3.2.1 Preparation of nanoplex

The amorphous nanoplex was formed via the self-assembly electrostatically driven complexation method between the ionized drug molecules and oppositely charged polymers or counter surfactant.

3.2.1.1 Preparation of PPZ-DXT nanoplex

The PPZ-DXT nanoplex was prepared using the drug-polysaccharide method. To prepare the PPZ-DXT nanoplex, PPZ was first dissolved in AA solution to produce protonated PPZ. DXT was dissolved in deionized water to produce negatively charged DXT. Protonated PPZ was mixed with oppositely charged DXT, resulting in the formulation of PPZ-DXT complex. To investigate the effect of pH, PPZ was dissolved in aqueous AA solutions of varying concentrations (i.e., AA = 0.05 to 0.5% v/v corresponding pH of 3.4 to 2.9). By varying the concentration of PPZ and DXT, the charge ratio of DXT to PPZ ($R_{DXT/PPZ}$) also varied during the investigation (i.e., $0.8 \leq R_{DXT/PPZ} \leq 1.8$). The details on how to prepare PPZ-DXT nanoplex are explained in the section 4.2 of Chapter 4 and section 5.2 of Chapter 5.

- **Bulk mixing method:** Briefly, PPZ was dissolved in aqueous AA solutions of varying concentrations (i.e., AA = 0.05 to 0.5% v/v corresponding pH of 3.4 to 2.9). DXT was dissolved in neutral deionized water. The amount of DXT dissolved in water was varied as the charge ratio $R_{DXT/PPZ}$ varies. Next, 1 ml cationic PPZ solution was added into 1 ml oppositely charged DXT solution using a pipette, followed by an

immediate vortex to obtain a well-mixed suspension. The resultant suspension was centrifuged for 15 minutes at 14,000 g for two cycles to remove the excess PPZ and DXT. The details on how to prepare PPZ-DXT nanoplex in bulk mixing method are explained in Chapter 4.

- **Millifluidic synthesis platform:** PPZ was dissolved in aqueous AA solutions of varying concentrations to investigate the effect of pH. The pH varied between 3.4 to 2.9 while the AA concentrations varied from 0.05% (v/v) to 0.5% (v/v). In this pH range, PPZ is protonated to cationic PPZ with charge density of 2.48×10^{-6} mol-charge/mg. DXT was dissolved in water. To study the charge ratio of DXT to PPZ, PPZ concentration was fixed while the DXT concentration was varied to get different $R_{DXT/PPZ}$. For each tube inner diameter (d) investigated, the PPZ-DXT nanoplex was collected at different axial distance (L) from the Y-junction (i.e., 5, 10, and 15 cm) to study the effect of residence time (t_{Res}). To study the effect of the tube diameter, t_{Res} was fixed by changing the flow rate. Two syringe pumps were operated at flow rate equals to 0.25 ml/min, 1.09 ml/min, and 2.44 ml/min for $d = 0.762$ mm, 1.588 mm, and 2.381 mm, respectively. After collection, the nanoplex suspension was centrifuged for 15 minutes at 14,000 g to remove the excess PPZ and DXT. The details on how to prepare PPZ-DXT nanoplex in millifluidic synthesis platform are explained in Chapter 5.

3.2.1.2 Preparation of ternary nanoparticle complex of drug, polyanions and ionizable small molecules

The CIP-DXT nanoplex was prepared using the bulk mixing method. CIP was electrically absorbed to the oppositely charged DXT polyelectrolyte, forming the complex. The formed complex was precipitated out to form the CIP-DXT complex due to the hydrophobic interactions and increasing aggregation concentration. In this work, the CIP concentration was fixed at 5 mg/ml. The anion DXT-SDS solution was prepared by mixing SDS and DXT aqueous solutions. There are 24 —OSO₃⁻ in each DXT molecular and 1 —OSO₃⁻ in each SDS molecule, resulting in charge densities of 4.8×10^{-6} mol-charge/mg DXT and 3.467743×10^{-6} mol-charge/mg SDS. The charge ratio of DXT to SDS in the DXT-SDS solution varies from 1:0 to 0.1:0.9, whereas the charge ratio of CIP: DXT: SDS varies from 1:1:0, 1:0.9:0.1, 1:0.8:0.2 ... to 1:0.1:0.9. 1 ml CIP solution in 0.4% (v/v) AA (pH=3.01) was added into an equal volume of anion solution in a 2 ml micro-centrifuge tube. White precipitation was observed immediately upon the addition of CIP solution into the anion solution. To remove the excess CIP, SDS, and DXT, the nanoplex suspension was washed through three cycles of centrifugation and resuspension. Thereafter, the nanoplex was sonicated for 10 seconds. for the later physical or dissolution examination. The details on how to prepare the CIP-SDS-DXT ternary nanoplex is explained in the section 6.2 of the Chapter 6.

3.2.2 Physical characterizations of the nanoplex

3.2.2.1 Size, polydispersity, zeta potential and colloidal stability

The size, polydispersity and zeta potential of the nanoplex were examined via photon correlation spectroscopy (PSC) using the Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The nanoplex suspension was diluted with deionized water prior to the PCS measurement at 25 °C.

3.2.2.2 Colloidal stability

The colloidal stability in deionized water at 25 °C and neutral pH was evaluated over an extended period by monitoring the changes in the size and zeta potential measured by PCS.

3.2.2.3 The efficiency of nanoplex preparation

The payload of the nanoplex was defined as the percentage of the mass of the active pharmaceutical ingredient (API) in the freeze-dried nanoplex (Equation 3-1). The payload of the nanoplex was determined by dissolving a known mass of freeze-dried nanoplex into a sufficient volume of 1 × PBS solution (pH 7.4) or other solvent. The concentration of API in the solution was determined by a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan) or HPLC at 24 hours, 48 hours, and 72 hours after the nanoplex powder was added into the PBS solution. The API concentration in the solution does not further increase after 48 hours. Hence, the nanoplex was deemed to have completely disintegrated and the API in the nanoplex was deemed to have been released completely.

Equation 3-1: Definition of Payload of PPZ nanoplex

$$\text{Payload (\%)} = \frac{\text{mass of active pharmaceutical ingredients in the nanoplex}}{\text{Mass of nanoplex}} \times 100 \%$$

After the API in the nanoplex was completely dissolved, we used the API concentration and volume of solution to calculate the mass of API in the nanoplex. The complexation efficiency is the mass percentage of the drug that forms the nanoplex based on the amount of drug that was initially added (see Equation 3-2). The mass of API that formed the nanoplex was calculated from the difference between the initial mass of API added and the mass of API recovered in the supernatant after the first centrifugation. The supernatant after the first centrifugation was diluted 100 times, and

afterward, the PPZ concentration was quantified by a UV-Vis spectrophotometer or HPLC.

Equation 3-2: Definition of complexation efficiency of methods to prepare PPZ nanoplex

$$\text{Complexation efficiency (\%)} = \frac{\text{mass of Active pharmaceutical ingredients that formed nanoplex}}{\text{Initial mass of active pharmaceutical ingredients added}} \times 100 \%$$

Equation 3-3: Definition of production yield of methods to prepare PPZ nanoplex

$$\text{production yield (\%)} = \frac{\text{total mass of nanoplex formed}}{\text{total ingredients added}} \times 100 \%$$

3.2.2.4 Solid-state characterizations

- Fourier Transform Infrared Spectroscopy (FTIR): The presence of the active pharmaceutical ingredient in the nanoplex was verified by the Fourier Transform Infrared Spectroscopy (Spectrum One, Perkin-Elmer, USA) performed between 400 and 4500 cm⁻¹ at 4 cm⁻¹ spectral resolution. The pellets for the FTIR were prepared by pressing a mixture of the nanoplex and KBr (mass ratio of nanoplex to KBr is around 1:50) in a tie at 10 tons for 30 seconds.
- Field Emission Scanning Electron Microscope (FESEM): The nanoplex suspension was first lyophilized (primary drying: -10 °C, 0.16 mbar; secondary drying: 5 °C and later 30 °C, 0.06 mbar) with a laboratory freeze-dryer alpha 1-4 (Martin Christ GmbH, Osterode, Germany). Then, the morphology of the freeze-dried nanoplex was coated with platinum and examined using the Field Emission Scanning Electron Microscope (FESEM) (JSM-6700F, JEOL, USA).
- PXRD: The amorphous form of the nanoplex was verified by its Power X-ray Diffraction (PXRD) patterns from 5° to 70° (2θ) with a step size of

0.02°/s using a D8 Advance X-ray diffractometer equipped with Cu K α radiation (Bruker, Germany).

- TGA/DTA: Samples were heated from 25 to 450 °C at a heating rate of 10 °C/min using the GA- Mettler Toledo TGA/DSC machine.
- DSC: Samples were heated from 25 to 300 °C at a rate of 2 °C/min within a nitrogen atmosphere (purity 99.99%, flow 40 ml/min) using the Cryo DSC- Mettler Toledo 822e.

3.2.2.5 Dissolution rate

The nanoplex disassociates in aqueous salt solutions. The presence of salt potentially neutralizes the electrostatic interaction between the drug and polyelectrolyte in the nanoplex, which leads to the de-complexation [33, 34]. The thermodynamic saturation of API in the dissolution medium (i.e., PBS, SIJ, SGJ) was first determined by incubating the excess native API in a fixed volume of dissolution medium at 37 °C for 72 hours with constant stirring. Afterward, the solution in dissolution medium was centrifuged to remove the non-dissolved API and the amount of API in the supernatant was quantified using UV-Vis spectrophotometry or HPLC to determine its saturation.

- Dissolution of PPZ-DXT nanoplex or TC complexes under sink condition using dialysis bags: The in vitro dissolution rate of the nanoplex is determined under a sink condition using the dialysis bag method. The nanoplex suspension or native API was added into the dialysis bag. The dissolution rate of the native API and nanoplex were determined in triplicates through dialysis bags immersed in dissolution medium. The PBS, simulated gastric juice (SGJ), and simulated intestinal juice (SIJ) were used as dissolution mediums. The SGJ was prepared by adjusting the pH of 0.2%

(w/v) NaCl solution to pH 1.2 using 1.0 M HCl, whereas the SIJ was prepared by dissolving 0.68 g KH_2PO_4 in 75 ml deionized water, adjusting the pH to 6.8 by adding 0.2 M KOH, and increasing the volume to 100 ml [32, 198].

- Dissolution of CIP-DXT-SDS nanoplex: The dissolution rate of freshly prepared CIP-DXT-SDS nanoplex was determined in triplicates by adding the nanoplex suspension (containing 2 mg CIP) into a dialysis bag immersed in 80 ml solution medium (A). Solution medium (A) was prepared by dissolving 1.7 g NaH_2PO_4 into 900ml of DI water, then adjusting the pH to 6.8 using KOH solution, before increasing the total volume to 1L. The ion strength in solution medium (A) is $\frac{1}{4}$ times of that in SIJ.

3.2.2.6 Supersaturation

The supersaturation of the prepared nanoplex was characterized in triplicates by adding excess nanoplex into a fixed volume of dissolution medium (i.e., PBS) in a shaking incubator at 37 °C. A fixed volume of aliquot was withdrawn at fixed time intervals over a period of time. The aliquot was filtered and diluted to prevent the precipitation of API from the supersaturated solution. The API concentration in the aliquot, which represented the supersaturated API concentration, was determined with UV-Vis or High-Performance Liquid Chromatography (HPLC) in triplicates. The supersaturation level was reported as the ratio of C to C_{eq} .

CHAPTER 4 Amorphous nanoparticle complex preparation using perphenazine and dextran sulfate as a new solubility enhancement strategy of antipsychotic perphenazine

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The objective of this study is to develop a new solubility enhancement strategy of antipsychotic drug – perphenazine (PPZ) – in the form of its amorphous nanoparticle complex (or nanoplex) with polyelectrolyte dextran sulfate (DXT). Significance: Poor bioavailability of PPZ necessitated the development of fast-dissolving PPZ formulations regardless of delivery routes. Existing fast-dissolving formulations, however, exhibited low PPZ payload. The high-payload PPZ-DXT nanoplex represents an attractive fast-dissolving formulation, as dissolution rate is known to be proportional to payload.

The nanoplex was prepared by electrostatically driven complexation between PPZ and DXT in a simple process that involved only ambient mixing of PPZ and DXT solutions. We investigated the effects of key variables in drug-polyelectrolyte complexation (i.e. pH and charge ratio $R_{DXT/PPZ}$) on the physical characteristics and preparation efficiency of the nanoplex produced. Subsequently, we characterized the colloidal and amorphous form stabilities, dissolution enhancement, and supersaturation generation of the nanoplex prepared at the optimal condition.

The physical characteristics of nanoplex were governed by $R_{DXT/PPZ}$, while the preparation efficiency was governed by the preparation pH. Nanoplex having size of $\approx 80\text{nm}$, zeta potential of $\approx (-) 60\text{ mV}$, and payload of $\approx 70\%$ (w/w) were prepared at nearly 90% PPZ utilization rate and $\approx 60\%$ yield. The nanoplex exhibited superior dissolution than native PPZ in simulated intestinal juice, resulting in high and prolonged apparent solubility with good storage stabilities.

The simple yet efficient preparation, excellent physical characteristics, fast dissolution, and high apparent solubility exhibited by the PPZ-DXT nanoplex established its potential as a new bioavailability enhancement strategy of PPZ.

4.1 Introduction

Perphenazine (PPZ) is a first-generation phenothiazine-type antipsychotic drug primarily used in the treatment of schizophrenia, depressants and severe nausea [199-201]. While the introduction of newer-generation antipsychotic drugs has diminished the role of PPZ as the mainstay therapy for psychotic disorders, PPZ remains a highly valuable antipsychotic drug attributed to its significantly lower costs, effective diffusion through the blood-brain barrier [202] and comparable therapeutic efficacies compared with the newer drugs [200, 203]. It is a Class II drug molecule according to the Biopharmaceutical Classification System [204], with a LogP value at 4.2 [205]. The conventional administration of PPZ is via the oral route, where multiple dosing is administered daily to compensate for the low oral bioavailability of PPZ, which is caused by its poor aqueous solubility ($< 0.1\text{ mg/ml}$) and first-pass hepatic metabolism [206, 207]. Poor bioavailability of PPZ necessitated the development of fast-dissolving PPZ formulations regardless of delivery routes.

To improve its oral bioavailability, PPZ dosage formulations exhibiting enhanced dissolution have been developed recently by means of salt formulation [199] and amorphization [208]. Alternatively, several studies have proposed the sublingual and buccal delivery routes, in place of the oral route, to enhance the PPZ bioavailability by avoiding the first-pass hepatic metabolism [11, 84, 86, 209, 210]. For the intraoral delivery to be effective, fast dissolution of PPZ in the aqueous environment of the oral cavity is also needed, because otherwise the drug would be vulnerable to clearance by swallowing [11]. The intraoral delivery formulations of PPZ were typically prepared in the amorphous form to exploit the fast dissolution afforded by the metastable amorphous form, in the form of either amorphous solid dispersion [11, 211] or cyclodextrin inclusion complex [11, 85, 86, 211, 212].

Regardless of whether PPZ is delivered orally or intraorally, PPZ formulations exhibiting fast dissolution are, therefore, needed. The existing fast-dissolving PPZ formulations shared a common trait in their low PPZ payloads, where the payload was 20% for the formulations based on amorphous solid dispersions and cyclodextrin inclusion complex [11, 85, 86, 89, 211, 212], and <35% for the salt form of PPZ [199]. As the dissolution velocity under a sink condition is known to be proportional to the payload [213], herein we developed a high-payload fast-dissolving PPZ formulation in the form of amorphous PPZ nanoparticle complex (or nanoplex in short).

The amorphous PPZ nanoplex was prepared by self-assembly electrostatically driven complexation between PPZ and oppositely charged dextran sodium sulfate (DXT) based on the well-established drug–polyelectrolyte complexation principle [33]. The PPZ-DXT nanoplex preparation is quick and simple involving only mixing of the PPZ and DXT solutions under ambient condition. The same technique has been

employed previously to prepare amorphous nanoplex of several poorly soluble drugs, such as antibiotics (i.e. ciprofloxacin, levofloxacin, and ofloxacin) [34], anticancer (i.e. doxorubicin) [214], and antifungal (i.e. itraconazole) [215] drugs. Importantly, besides the fast dissolution, the amorphous drug nanoplex is also capable of producing apparent drug solubility that is significantly higher than the thermodynamic solubility, which is attributed to the supersaturation generation by the amorphous form [216].

The objectives of the present work were to investigate the effects of the two key process variables in drug-polyelectrolyte complexation, i.e. pH and charge ratio of DXT to PPZ ($R_{DXT/PPZ}$), on the (1) physical characteristics of the PPZ–DXT nanoplex produced (i.e. size, zeta potential, and payload) and (2) preparation efficiency (i.e. PPZ utilization rate, overall yield). Subsequently, the PPZ–DXT nanoplex prepared at the optimal pH and $R_{DXT/PPZ}$ was characterized in terms of its (i) colloidal stability, (ii) amorphous form stability, (iii) dissolution rate in simulated gastric juice (SGJ, pH 1.2) and simulated intestinal juice (SIJ, pH 6.8) for oral delivery, and (iv) supersaturation generation capability. The results coupled with the ease of preparation confirmed the promising potential of the amorphous PPZ–DXT nanoplex as a new solubility enhancement strategy of PPZ.

4.2 Materials and methods

4.2.1 Materials

PPZ, glacial acetic acid (AA), phosphate-buffered saline (PBS, pH 7.4), hydroxypropylmethylcellulose (HPMC), sodium chloride (NaCl), hydrochloric acid (HCl), potassium phosphate monobasic (KH_2PO_4), and potassium hydroxide (KOH) were purchased from Sigma Aldrich (Singapore, Singapore). Low molecular weight

(MW = 45000Da) dextran sodium sulfate (DXT) was purchased from Wako Pure Chemical Industries (Tokyo, Japan).

Figure 4-1: (A) Chemical Structure of PPZ; (B) Protonated PPZ

4.2.2 Methods

4.2.2.1 Preparation of PPZ-DXT nanoplex

To investigate the effect of pH on the nanoplex formulation, a fixed concentration of PPZ (i.e. 5mg/ml) was dissolved in aqueous AA solution of three different concentrations (i.e. 0.1, 0.2, and 0.3% v/v) corresponding to pH of 3.3, 3.1, and 3.0, respectively. PPZ having pKa of 7.94 [199] was protonated (see Equation 4-1) in this pH range to form cationic PPZ with a charge density of 2.48×10^{-6} mol-charge/mg.

Equation 4-1: Protonation of PPZ

Equation 4-2: Henderson-Hasselbalch Equation

$$\text{pH} = \text{pK}_a + \text{Log}_{10} \frac{\text{Concentration of PPZ}}{\text{Concentration of protonated PPZ}}$$

In the pH range of 3.3 to 3.0, the difference between the pH and pKa is above 4.6, then, the $\frac{\text{Concentration of pronated PPZ}}{\text{Concentration of PPZ}}$ is larger than $10^{4.6}$, which means at this pH range, the PPZ is considered as positively charged. Aqueous solution of DXT having

$pK_a \leq 2$ [217] and a charge density of 4.80×10^{-6} [217] mol-charge/mg was prepared separately at a neutral pH.

Sample calculations for the charge densities was provided in Table 4-1. The molecular weight of the DXT's monomer is 416 g/mol, hence there are approximately 12 monomers per DXT molecule. Each monomer of DXT contains two charges as depicted from the chemical structure of DXT shown in Figure 3-1. Therefore, each DXT molecule contains 24 charges. The sample calculations for $R_{DXT/PPZ}$ was given as following. For 5 mg PPZ, the amount of charge present is equal to 12.38×10^{-6} mol-charge. The mass of DXT needed for complexation with 5 mg PPZ at $R_{DXT/PPZ} = 1.6$ is equal to: $1.6 \times 12.38 \times 10^{-6} \text{ mol-charge} / (4.80 \times 10^{-6} \text{ mol-charge/mg}) = 4.28 \text{ mg}$. When X mg of DXT is used in the complexation experiment at 5 mg/ml of PPZ, $R_{DXT/PPZ}$ is calculated as in Table 4-1 and Equation 4-3:

Table 4-1: Charge density calculation of PPZ and DXT

Chemical	Molecular weight (g/mol)	No. of charge per molecule	Charge density (mol-charge/mg)
PPZ	404	1	$1/(404 \times 10^3) = 2.48 \times 10^{-6}$
DXT	5000	24	$24/(5000 \times 10^3) = 4.80 \times 10^{-6}$

The amount of DXT dissolved was varied depending on the $R_{DXT/PPZ}$ value under investigation (i.e. $0.8 \leq R_{DXT/PPZ} \leq 1.8$). Next, the cationic PPZ solution was added to the anionic DXT solution at equal volumes, followed by 10s vortexing to obtain a well-mixed solution. The resultant PPZ-DXT nanoplex suspension then underwent two cycles of centrifugation (14,000 g for 15 min) and washing with deionized water to remove the excess (i.e. not complexed) PPZ and DXT.

Equation 4-3: Calculation of $R_{DXT/PPZ}$

$$R_{DXT/PPZ} = X * 4.80 \times 10^{-6} / (12.38 \times 10^{-6}).$$

The efficiency of the PPZ-DXT nanoplex preparation was characterized in triplicates by the PPZ utilization rate and the overall yield defined in Equation 4-4 and

Equation 4-5 respectively. For the PPZ utilization rate, the mass of PPZ that formed the nanoplex was determined from the difference between the initial mass of PPZ added and the mass of PPZ recovered in the supernatant after centrifugation of the nanoplex suspension. The amount of PPZ in the supernatant was quantified by UV–vis spectrophotometry (UV Mini 1240, Shimadzu, Tokyo, Japan) after 100 dilution at absorbance wavelength of 255 nm. For the overall yield, the mass of PPZ-DXT nanoplex produced was determined by 24h freeze drying of the washed nanoplex suspension.

Equation 4-4: Definition of Complexation efficiency

$$\text{Complexation efficiency (\%)} = \frac{\text{Mass of PPZ that formed nanoplex}}{\text{Initial mass of PPZ added}} \times 100 \%$$

Equation 4-5: Definition of Production yield

$$\text{Production yield (\%)} = \frac{\text{Total mass of nanoplex produced}}{\text{Total mass of PPZ and DXT added}} \times 100 \%$$

4.2.2.2 Physical characterizations of PPZ–DXT nanoplex

The size, polydispersity index (PDI), and zeta potential of the PPZ–DXT nanoplex were determined by photon correlation spectroscopy (PCS) using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, Holtsville, NY). The size of the PPZ-DXT nanoplex prepared at the optimal condition was verified by image analysis of its field emission scanning electron microscope (FESEM) image (JSM-6700F, JEOL, Peabody, MA) using ImageJ software (NIH, Bethesda, MD) with minimum particle counts of 1000. The FESEM was carried out using freeze-dried nanoplex powders after sputter coating them with platinum. The PPZ payload, which was defined as the amount of PPZ present per unit mass of the PPZ-DXT nanoplex, was determined by dissolving a known mass of freeze-dried nanoplex powders in PBS after which the amount of PPZ was quantified by UV–Vis spectrophotometry at 255 nm. The

size, PDI, zeta potential, and PPZ pay- load were reported from a minimum of five independent replicates.

The presence of PPZ in the nanoplex was verified by Fourier Transformed Infrared Spectroscopy (FTIR) (Spectrum One, Perkin- Elmer, Shelton, CT) performed between 450 and 4000 cm^{-1} at 1 cm^{-1} spectral resolution. The FTIR was also performed for the native PPZ, native DXT, and physical mixture of PPZ and DXT. The amorphous form stability of the PPZ-DXT nanoplex prepared at the optimal condition was examined by powder X-ray diffraction (PXRD) analysis after 6-month storage at 25 °C and 55% relative humidity. The analysis was carried out between 10° and 70° (2h) with a step size of 0.02°/s using D8 Advance X-ray Diffractometer (Bruker, Karlsruhe, Germany). The PXRD analysis was also performed for the native PPZ and physical mixture of PPZ and DXT.

4.2.2.3 Dissolution rate

The thermodynamic saturation solubility of PPZ in PBS (i.e. $C_{\text{Sat-ppz}}$) was first determined by incubating excess native PPZ in 20 ml PBS at 37 °C for 72 h under constant stirring. Afterwards, the solution in PBS was centrifuged to remove the non-dissolved PPZ and the amount of PPZ in the supernatant was quantified by UV–Vis spectrophotometry at 255nm to determine $C_{\text{Sat-ppz}}$. For the dissolution medium, the SGJ was prepared following a method described in a paper [198] by adjusting the pH of 0.2% (w/v) NaCl solution to pH 1.2 using 1.0 M HCl, whereas the SIJ was prepared by dissolving 0.68g KH_2PO_4 in 75ml deionized water after which the pH adjusted to 6.8 by adding 0.2 M KOH and the volume was topped up to 100 ml.

The dissolution rates of the PPZ-DXT nanoplex and the native PPZ under sink condition in SGJ and SIJ were determined in triplicates by adding 0.86 ml of the

nanoplex suspension (or 0.57 mg of native PPZ) to a dialysis bag immersed in 40 ml SGJ or SIJ in a shaking incubator at 37 °C. At specific time points over a 24 h period, 0.5 ml aliquot was withdrawn and replaced with 0.5ml fresh SGJ or SIJ. The amount of PPZ in the aliquot was quantified by UV–Vis spectrophotometry as previously described.

4.2.2.4 Supersaturation generation

The supersaturation generation of the PPZ-DXT nanoplex was characterized in triplicates by adding excess nanoplex at $20 \times$ of $C_{\text{Sat-PPZ}}$ to 10ml PBS in a shaking incubator at 37 °C. At specific time points over 24 h, 0.5 ml aliquot was withdrawn and immediately diluted 10-fold with fresh PBS to prevent PPZ precipitation from the supersaturated solution. Subsequently, the diluted aliquot was syringe filtered using 0.2 μm membrane pores and the amount of PPZ in the aliquot was determined by UV–Vis spectrophotometry. The effect of the inclusion of HPMC in the dissolution medium as crystallization inhibitor on the supersaturation level was investigated at 2 mg/ml HPMC.

4.2.2.5 Statistical Analysis

The statistical analysis was performed using one way ANOVA with Tukey post hoc test using OriginPro 2018b software.

4.3 Results and discussion

4.3.1 Effects of $R_{\text{DXT/PPZ}}$

The investigation on the effects of $R_{\text{DXT/PPZ}}$ was carried out at a fixed PPZ concentration (i.e. 5mg/ml) and a fixed pH (i.e. pH 4.4 obtained at AA = 0.2%). At this preparation pH, both PPZ ($\text{pK}_a = 7.94$) and DXT ($\text{pK}_a \leq 2.0$) remained charged upon mixing, which ensured that the electrostatically driven complexation between PPZ and the oppositely charged DXT took place to form the PPZ–DXT nanoplex. The effects of $R_{\text{DXT/PPZ}}$ was

investigated at $R_{DXT/PPZ} = 0.8, 1.0, 1.2, 1.4, 1.6,$ and 1.8 , where the higher $R_{DXT/PPZ}$ above unity indicated the more abundant presence of charged DXT that was available for complexation with PPZ, as well as for its role as a steric/ionic stabilizer of the colloidal PPZ–DXT complex produced.

The results showed that while a cloudy suspension was formed upon mixing of the PPZ and DXT solutions at $R_{DXT/PPZ} < 1.2$, which indicated a successful formulation of colloidal PPZ–DXT complex, the colloids produced could not sustain the two-cycles of centrifugation and washing, resulting in a nearly zero yield (data not shown). Hence, a minimum $R_{DXT/PPZ}$ above unity was needed to produce a physically robust PPZ–DXT nanoplex. In this regard, the precipitation of drug-polyelectrolyte complex to form a robust nanoparticulate complex was known to occur only when a minimum critical mass had been reached [33]. Hence, the lack of physical integrity in the nanoplex prepared at $R_{DXT/PPZ} < 1.2$ was likely caused by the lack of critical mass due to the smaller presence of DXT.

At $R_{DXT/PPZ} \geq 1.2$, the PPZ–DXT nanoplex size decreased considerably from 262 ± 4 nm at $R_{DXT/PPZ} = 1.2$ to 78 ± 13 nm at $R_{DXT/PPZ} = 1.4$ (Figure 4-2 (A)) ($P < 0.05$). The size remained at ≈ 75 – 80 nm upon increasing $R_{DXT/PPZ}$ further to 1.6 and 1.8 ($P < 0.05$). The PDI of the size distribution was also improved from 0.54 ± 0.19 at $R_{DXT/PPZ} = 1.2$ to 0.2 ± 0.03 at $R_{DXT/PPZ} > 1.2$ denoting a more uniform particle size distribution for the smaller nanoplex (Figure 4-2 (A)) ($P < 0.05$). The PPZ–DXT nanoplex possessed large negative zeta potentials at around $(-)$ 65 – 75 mV independent of $R_{DXT/PPZ}$, hence denoting the significant presence of DXT on its surface, which boded well for its colloidal stability during storage (Figure 4-2 (A)). Henceforth, the PPZ–

DXT nanoplex prepared at $R_{DXT/PPZ} = 1.2$ was not pursued further as its larger size and higher PDI likely led to a slower dissolution rate.

Figure 4-2: Effects of $R_{DXT/PPZ}$ on (A) size, zeta potential, and (B) PPZ payload, PPZ utilization rate, and overall yield.

At $R_{DXT/PPZ} \geq 1.4$, the nanoplex exhibited a higher ($P < 0.05$) PPZ payload with increasing $R_{DXT/PPZ}$ = from $55 \pm 9\%$ (w/w) at $R_{DXT/PPZ} = 1.4$ to $78 \pm 7\%$ and $82 \pm 2\%$ at $R_{DXT/PPZ} = 1.6$ and 1.8 , respectively (Figure 4-2 (B)). In terms of the preparation efficiency, the effects of $R_{DXT/PPZ}$ on the PPZ utilization rate and overall yield were found to be statistically insignificant, where the PPZ utilization rate and yield were relatively constant at approximately 75–85% ($P > 0.05$) and 50–55% ($P > 0.05$), respectively (Figure 4-2 (B)). The constant PPZ utilization rate as a function of $R_{DXT/PPZ}$ suggested that a constant amount of PPZ was present in the nanoplexes prepared at different $R_{DXT/PPZ}$. Hence, the considerably higher PPZ payloads at $R_{DXT/PPZ} > 1.4$ were attributed to the smaller presence of DXT.

Based on the above results, 1.6 was determined to be the optimal $R_{DXT/PPZ}$ attributed to its superior payload compared with $R_{DXT/PPZ} = 1.4$, while exhibited highly comparable physical characteristics and preparation efficiency with $R_{DXT/PPZ} = 1.8$,

which required a larger amount of DXT in the feed. Thus, the subsequent study on the effects of pH was carried out at a fixed $R_{DXT/PPZ}$ equal to 1.6.

4.3.2 Effects of pH

In this part, AA concentration at 0.1% (v/v) to 0.9% (v/v) were investigated while the $R_{DXT/PPZ}$ was held constant at unity. When AA concentration was higher than 0.3 % (v/v), the formed PPZ-DXT nanoplex suspension contains sediment, which was not dispersible. The sediment was not regarded as nanoplex. We focus our study at AA concentration at 0.1% (v/v) to 0.3% (v/v). The pH values of the PPZ-DXT mixtures when acetic acid concentration varies from 0.1% (v/v) to 0.3% (v/v) are presented in Table 4-2. The effect of pH was investigated based on size, zeta potential, PPZ utilization.

Table 4-2: The pH of the mixture of PPZ in different AA concentrations and DXT solution

AA concentration (% v/v)	0.1	0.2	0.3
pH of AA solution	4.8	4.4	4.1

The result in Figure 4-3 (A) showed that the size, PDI, and zeta potential of the PPZ-DXT produced were largely unaffected by the variation of pH ($P > 0.05$) in the investigated range of pH, where the variations in the size, PDI and zeta potential of the PPZ-DXT nanoplex were found to statistically insignificant ($P > 0.05$). The same trend was observed for the variations in the PPZ payload as a function of the preparation pH (Figure 4-3 (B)).

While the effects of the preparation pH on the physical characteristics of the PPZ-DXT nanoplex were minimal, the same could not be said about its impact on the preparation efficiency. The results in Figure 4-3 (B) showed that both PPZ utilization rate and yield increased with increasing pH from $73 \pm 4\%$ and $35 \pm 2\%$ at AA = 0.1% (pH 4.1) to $89 \pm 2\%$ and $56 \pm 4\%$ at AA = 0.3% (pH 4.4) for the PPZ utilization rate ($P < 0.05$) and yield ($P < 0.05$), respectively. The higher PPZ utilization rate observed at

higher pH, however, did not lead to a higher PPZ payload ($P > 0.05$), which implied that the amount of DXT present in the nanoplex had also increased. The increased amount of DXT that was transformed to the nanoplex explained for the improved yield observed at higher pH.

Figure 4-3: Effects of pH (AA concentration) on size, zeta potential.

Based on these results, the optimal preparation condition of the PPZ–DXT nanoplex was determined to be at $R_{DXT/PPZ}=1.6$ and $AA = 0.3\%$. The PPZ utilization was found to steadily increase from $78.0 \pm 3.5\%$ at 0.1% (v/v) AA to $91.2 \pm 3.4\%$ at 0.3% (v/v) AA (Figure 4-3B) ($P < 0.05$).

4.3.3 Physical characteristics of the optimal PPZ–DXT nanoplex formulation

4.3.3.1 FTIR

The presence of PPZ in the PPZ–DXT nanoplex was verified by the FTIR analysis, which showed the appearance of the characteristic bands of PPZ in the FTIR spectrum of the PPZ–DXT nanoplex at 756, 1456, and 1590 cm^{-1} . These bands corresponded to the stretching vibrations of the C–Cl (756 cm^{-1}) and C=C aromatic ring (1456 and 1590 cm^{-1}) [212] groups that were present in the chemical structure of PPZ (Figure 4-1), but not in DXT. As expected, these bands also appeared in the FTIR spectra of the native

PPZ and the physical mixture of PPZ and DXT (Figure 4-4). On this note, the broad bands at 1200–1280 cm^{-1} that appeared only in the FTIR spectra of the native DXT and PPZ–DXT nanoplex corresponded to the asymmetric stretching of the sulfate group of DXT [218] (Figure 4-4). The FTIR results hence confirmed the presence of both PPZ and DXT in the nanoplex produced.

Figure 4-4: FTIR spectra of native PPZ, native DXT, PPZ–DXT nanoplex, and physical mixture of PPZ and DXT.

4.3.3.2 Amorphous form and morphology

The amorphous form of the PPZ–DXT nanoplex and its stability after the long-term storage were verified by the PXRD analysis that showed the presence of broad amorphous halos in its PXRD patterns, which were in contrast to the sharp crystalline peaks observed in the PXRD patterns of the native PPZ and the physical mixture of PPZ and DXT (Figure 4-5). The amorphous form stability was attributed to the strong binding between PPZ and DXT that restricted the molecular mobility of PPZ resulting in its suppressed crystallization propensity.

Figure 4-5: PXRD patterns of the native PPZ, native DXT, PPZ–DXT nanoplex, and physical mixture of PPZ and DXT.

The amorphous form of the PPZ-DXT nanoplex was also evident from its FESEM image that showed a highly spherical nanoparticle (Figure 4-6 (A)) as opposed to the roughly rectangular shape of the native PPZ crystals (Figure 4-6 (B)).

Figure 4-6: FESEM images of (A) PPZ–DXT nanoplex prepared at the optimal condition and (B) native PPZ

Furthermore, the image analysis of the particle size using the FESEM image of the nanoplex revealed a mean particle size of 146 ± 50 nm, which was in the same order of magnitude (albeit slightly larger) as the mean size obtained by the PCS measurement reported at 83 ± 10 nm. The FESEM result hence confirmed the formulation of

amorphous nanoparticle complex of PPZ and DXT.

4.3.3.3 Dissolution rate and colloidal stability

In the SGJ, the native PPZ exhibited a faster dissolution rate than the PPZ–DXT nanoplex attributed to the high solubility of native PPZ in acid (Figure 4-7 (A))(P < 0.05). Specifically, $86 \pm 2\%$ of the native PPZ dissolved in SGJ after 1h and complete dissolution was achieved after 5h, compared with $19 \pm 0.4\%$ (1h) and $25 \pm 0.9\%$ (5h) dissolutions of the PPZ–DXT nanoplex over the same period. In contrast, the opposite trend was observed in the SIJ, where the PPZ–DXT nanoplex exhibited a faster dissolution rate as a result of its amorphous form and large specific surface areas(P<0.05). Specifically, $23 \pm 1\%$ of the PPZ payload was released from the nanoplex in the first 1 h of dissolution compared with $17 \pm 1\%$ dissolution for the native PPZ over the same period (Figure 4-7 (A)). After 8h, which represented the typical half-life of PPZ [200], $69 \pm 2\%$ of the payload was released from the nanoplex versus $32 \pm 3\%$ dissolution for the native PPZ. These results boded well for the bioavailability enhancement capability of the PPZ-DXT nanoplex as it would dissolve slowly in the acidic environment of the stomach, hence maximizing the amount of PPZ that would be available for systemic absorption in the intestine.

Notably, the dissolution rate of the PPZ-DXT nanoplex in the SIJ was faster than the dissolution rate of an existing nanoscale PPZ delivery system, i.e. PPZ-encapsulating polymer nanoparticles [87], where $\approx 40\text{--}50\%$ of the payload was released after 8h at pH 7.4 compared with $\approx 70\%$ for the nanoplex (P<0.05). The faster dissolution rate of PPZ from the nanoplex was not unexpected because, unlike dissolution from polymer nanoparticles, the nanoplex dissolution did not occur by molecular diffusion out of the hydrolyzed polymer matrix. Instead, the nanoplex

dissolution was driven by weakened electrostatic forces that held PPZ and DXT together in the high ionic strength environment of the SIJ, resulting in the quick release of PPZ from the nanoplex.

Figure 4-7: Dissolution rates of PPZ-DXT nanoplex and native PPZ under sink condition; (B) colloidal stability of the optimal PPZ-DXT nanoplex.

On this note, it had to be mentioned that the use of dialysis bag in our dissolution experiments resulted in slower dissolution rates in terms of the absolute values for both the nanoplex and the native PPZ. Nevertheless, the smaller absolute values did not fail to distinguish the dissolution rates between the native PPZ and the PPZ-DXT nanoplex. In addition, the PPZ-DXT nanoplex was found to exhibit high colloidal stability in its aqueous suspension form as reflected by the minimal variations in the size and zeta potential over a 24-h storage at room temperature (Figure 4-7 (B)) ($P > 0.05$). The high colloidal stability of the nanoplex was attributed to its large zeta potential as a result of the significant presence of DXT on its surface.

4.3.3.4 Supersaturation generation

The PPZ-DXT nanoplex exhibited the typical “spring and parachute” supersaturation generation profile of amorphous drug formulations (Figure 4-8 (A)), where the fast dissolution in the beginning resulted in a highly supersaturated drug concentration that

continued to increase until a supersaturation peak was reached (“spring”), followed by the gradual decrease in the supersaturation level due to crystallization of the dissolved drug from the supersaturated solution (“parachute”) [216].

Figure 4-8: Supersaturation generation profile of the optimal PPZ-DXT nanoplex (A) over a 24-h period and (B) a closed-up view of the 1.5 h.

In the absence of HPMC, the PPZ-DXT nanoplex was capable of generating a maximum achievable supersaturation level at $(17.8 \pm 0.7) \times C_{Sat-ppz}$ after 10 min, where $C_{Sat-ppz}$ was equal to 32 mg/ml. The supersaturation level was maintained at $\approx 14-18 \times C_{Sat-ppz}$ for the next 1h (Figure 4-8 (B)). The supersaturation level then started to decrease to $(9.7 \pm 1.2) \times$ and $(5.8 \pm 1.1) \times C_{Sat-ppz}$ after 2.5 h ($P < 0.05$) and 6 h ($P < 0.05$), respectively, and continued to decrease to eventually drop back to $C_{Sat-ppz}$ after 24 h ($P < 0.05$). Significantly, the impact of the HPMC inclusion on the supersaturation generation of the PPZ-DXT nanoplex was found to be minimal (Figure 4-8) ($P > 0.05$). As one of the roles of HPMC was to suppress the Ostwald-ripening crystallization of the remaining solid phase undergoing dissolution [219], the similar supersaturation profiles obtained with or without HPMC suggested that the PPZ-DXT nanoplex was not prone to crystallization upon dissolution, which was likely attributed to its

aforementioned fast dissolution velocity. The high and prolonged supersaturation level exhibited by the PPZ-DXT nanoplex translated to a high apparent solubility that signified bioavailability enhancement potential of the nanoplex.

4.4 Conclusions

Amorphous nanoscale formulation of PPZ exhibiting high payload and enhanced dissolution rate was successfully prepared by electrostatically driven complexation with DXT. On one hand, the results showed that the PPZ–DXT nanoplex formulation and its physical characteristics (i.e. size and payload) were governed by $R_{DXT/PPZ}$ in which $R_{DXT/PPZ}$ above unity was needed to produce small nanoplex with a high degree of uniformity. On the other hand, the preparation efficiency was found to be governed by the preparation pH rather than $R_{DXT/PPZ}$. The optimal $R_{DXT/PPZ}$ and pH were determined to be at 1.6 and 0.3% AA concentration (pH 4.1), respectively. At this condition, nanoplex having size of $\approx 80\text{nm}$, zeta potential of $\approx (-) 60\text{mV}$, and payload of $\approx 70\%$ (w/w) were prepared at nearly 90% PPZ utilization rate and $\approx 60\%$ overall yield.

The amorphous form and colloidal stabilities of the nanoplex after prolonged storage were successfully established attributed to the stabilizing presence of DXT. Most significantly, the nanoplex exhibited superior dissolution rate than the native PPZ in the SIJ attributed to its nanoscale size and amorphous form. The fast dissolution enabled the PPZ–DXT nanoplex to produce a high and prolonged supersaturation level at around six times of the saturation solubility at the minimum, which was maintained for 6 h without the need of a crystallization inhibitor inclusion. In summary, (1) the simple yet highly efficient preparation, (2) excellent physical characteristics, (3) fast dissolution in SIJ, and (4) high apparent solubility generation of the nanoplex demonstrated in the present work established its promising potential as a new

bioavailability enhancement strategy of PPZ.

4.5 Acknowledgements

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CHAPTER 5 Designing a continuous production platform using millifluidics and conducting a direct comparison between the millifluidics and the conventional bulk mixing method

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In this PhD work, the amorphous drug-polysaccharide nanoparticle complex was prepared using the continuous production method and batch production modes, respectively represented by millifluidics and bulk mixing methods. The physical characteristics of the resultant nanoparticle complex (size, zeta potential, payload), preparation efficiency, dissolution rate/supersaturation generation and production consistency were compared directly. The millifluidics showed its superior production consistency and scalability. The nanocomplex prepared by the millifluidics method had an advantage with its product consistency.

Amorphous drug-polysaccharide nanoparticle complex (or drug nanoplex) had emerged as an ideal supersaturating delivery system of poorly-soluble drugs attributed to its many attractive characteristics. Herein direct comparison between two nanoplex synthesis platforms, i.e. millifluidics and bulk mixing, representing continuous and batch production modes respectively presented for the first time. They were compared by the resultant nanoplex's (1) physical characteristics (size, zeta potential, and

payload), (2) preparation efficiency, (3) storage stability, (4) dissolution rate/supersaturation generation and (5) production consistency. The effects of key variables in drug-polysaccharide complexation (pH, charge ratio) were investigated in both platforms. Perphenazine and dextran sulfate were used as the drug and polysaccharide models, respectively.

The results showed that both platforms shared similar dependences on pH and charge ratio with similar optimal preparation conditions, where the pH was the governing variable through its influence on size and zeta potential, Nanoplexes having mostly similar characteristics (size $\approx 70\text{--}90$ nm, zeta potential ≈ 50 mV) were produced by both platforms except for the payload where bulk mixing resulted in lower payload (65% versus 85%). The lower payload, however, resulted in its superior supersaturation generation. Nevertheless, millifluidics was favoured attributed to its superior production consistency and scalability.

5.1 Introduction

A millifluidic synthesis platform has recently emerged as an attractive alternative to microfluidics for the continuous preparation of nanoparticles having tunable sizes, as a result of its (1) ease-of-fabrication and simple operation, (2) lower susceptibility to particle fouling, and (3) higher production throughput [170]. However, unlike microfluidics in which preparation of nanoparticles from a wide range of materials (e.g. metals, polymers, lipids) have been successfully demonstrated [175, 220, 221], the application of millifluidics has been predominantly limited to the synthesis of metal nanoparticles [170, 222, 223].

The application of millifluidics to prepare amorphous drug-polysaccharide nanoparticle complex (or drug nanoplex in short) is recently presented for the first time

[107]. The amorphous nanoplex was intended as a supersaturating delivery system of poorly soluble drugs aimed at enhancing their bioavailability. The faster dissolution, prolonged supersaturation generation, high drug payload, and simple preparation of the nanoplex make it an attractive alternative to the conventional supersaturating drug delivery system, such as amorphous micro- scale solid dispersions. The drug nanoplex was formed by self-assembly electrostatically driven complexation between drug molecules and oppositely charged polysaccharides, which took place upon mixing of the drug and polysaccharide solutions under ambient condition [33].

Using curcumin and chitosan as the anionic drug and cationic polysaccharide models, respectively, Tran et al. [107] showed that the millifluidic synthesis platform enabled size control of the drug nanoplex, which was not achievable when the nanoplex was prepared by bulk mixing of the drug and polysaccharide solutions [35]. The size control of the nanoplex in millifluidics was achieved by varying the millifluidic reactor's length, where we essentially varied the residence time of the curcumin and chitosan solutions undergoing complexation. The control over the nanoplex size upon varying the residence time was investigated at different pH, molecular weight of the polysaccharides, tube diameter, and flowrate, from which the optimal millifluidic condition in terms of the nanoplex size tunability was determined.

At the optimal condition for size tunability, Tran et al. [107] showed that the millifluidic platform maintained the high drug utilization rate and high drug payload exhibited by the bulk-mixing platform. However, as the objective of Tran et al. [107] was to demonstrate the size-control capability of the millifluidic platform, the effects of the millifluidics' process variables on the other nanoplex characteristics (i.e. zeta potential, payload) and preparation efficiency (i.e. drug utilization rate, overall yield)

were not investigated. In addition, the effects of the charge ratio of the drug to polysaccharides, which together with pH represented the two most important variables in drug-polysaccharide complexation, were not examined either in Tran et al.'s paper [107].

Furthermore, direct comparison between the millifluidic and bulk-mixing platforms in terms of the physical characteristics and preparation efficiency of the nanoplexes produced had not been carried out at the optimal condition of each platform. Thus, we had not been able to answer the research question on whether the lack of size tunability in the bulk-mixing platform, which was reported by Nguyen et al. [35], could be compensated, for example, by its better preparation efficiency.

Thus, the first objective of the present work was to investigate the effects of three key variables in the drug-polysaccharide complexation by millifluidics, namely (1) pH, (2) charge ratio of polysaccharide to drug, and (3) residence time on (A) the overall physical characteristics (not limited to only the size) and (B) the preparation efficiency of the drug nanoplex produced. In this study, the effect of the residence time was investigated by varying the millifluidic reactor length at three different tube diameters.

The second objective was to carry out a direct comparison between the nanoplexes prepared at the optimal conditions of the millifluidic and bulk-mixing platforms, in terms of their (1) physical characteristics and preparation efficiency, (2) dissolution velocity, (3) supersaturation generation, (4) colloidal and amorphous form stabilities during storage, and (5) batch-to-batch (for bulk mixing) or run-to-run (for millifluidics) production consistency. The outcomes of this study would enable us to

determine the superior synthesis platform for the amorphous drug nanoplex between millifluidics and bulk mixing.

In the present work, perphenazine (PPZ) and dextran sulfate (DXT) were used as the cationic drug and anionic polysaccharide models, respectively. PPZ is a widely used first-generation antipsychotic drug, which is however notorious for its poor oral bioavailability due to its low aqueous solubility and first-pass hepatic metabolism [201, 207]. Various fast-dissolving PPZ formulations, which ranged from amorphous microscale solid dispersions and PPZ salts to nanofibers and cyclodextrin complex, had been developed (albeit at low PPZ payloads) to address the poor bioavailability issue of PPZ [11, 211, 212, 224]. As the present results showed, amorphous PPZ nanoplex with its high payload and prolonged supersaturation generation represented an improved fast-dissolving PPZ formulation.

5.2 Materials and methods

5.2.1 Materials

5.2.1.1 Materials for the nanoplex preparation and characterizations

PPZ ($\geq 99\%$), glacial acetic acid (AA) ($\geq 99.85\%$), and phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Aldrich (Singapore). Low molecular weight DXT (MW = 5 kDa) ($\geq 95\%$) was purchased from Wako Pure Chemical Industries (Japan).

5.2.1.2 Materials for the millifluidic setup

High-density polyethylene Y-junction connector (orifice ID 0.794 mm) and Tygon® microbore tubing of three different sizes, i.e. ID 0.762 mm, 1.588 mm, and 2.381 mm were purchased from Cole-Parmer (USA).

5.2.2 Methods

5.2.2.1 Millifluidic and bulk-mixing preparation of PPZ nanoplex

PPZ was dissolved at 2.5 mg/ml in aqueous AA solutions of varying concentrations (i.e. AA = 0.05 to 0.50% v/v corresponding to pH of 3.4–2.9) to investigate the effect of pH. In this pH range, PPZ having pKa of 7.94 [199] was fully protonated (see Equation 4-1, Equation 4-2 and the explanation in section 4.2.2.1 of Chapter 4) to form cationic PPZ with a charge density of 2.48×10^6 mol-charge/mg. Separately, DXT was dissolved at different concentrations in deionized water at neutral pH depending on the charge ratio of DXT to PPZ being investigated (i.e. $1.2 \leq R_{DXT/PPZ} \leq 1.8$). At this pH, DXT having pKa < 2 [225] was deprotonated to form anionic DXT with a charge density of 4.80×10^6 mol-charge/mg (refer to Table 4-1).

Equation 5-1: The calculation of $R_{DXT/PPZ}$ at PPZ concentration = 2.5 mg/ml

$$R_{DXT/PPZ} = X * 4.80 * 10^{-6} / (6.20 * 10^{-6})$$

Sample calculations for the charge densities were provided as shown in Table 4-1. The molecular weight of the DXT's monomer is 416 g/mol, hence there are approximately 12 monomers per DXT molecule. Each monomer of DXT contains two negative charges as depicted from the chemical structure of DXT shown in Figure 3-1. Therefore, each DXT molecule contains $12 \times 2 = 24$ charges. The calculation of the charge ratio was described below. The amount of charge present per ml of PPZ solution is equal to 6.20×10^{-6} mol-charge when 2.5 mg/ml PPZ was used. The mass of DXT needed for complexation with 2.5 mg PPZ at $R_{DXT/PPZ} = 1.6$ is equal to: $1.6 \times 6.20 \times 10^{-6}$ mol-charge / (4.80×10^{-6} mol-charge/mg) = 2.07 mg of DXT. $R_{DXT/PPZ}$ is calculated as in Equation 5-1 When X mg of DXT is used in the complexation experiment at 2.5 mg/ml of PPZ.

The PPZ and DXT solutions were transferred separately to two syringe pumps (Legato 200, KD Scientific, USA) connected to the millifluidic reactor made up of the Tygon® tubing (Figure 2-3) for the millifluidic preparation. Y-junction connector was placed at the reactor inlet to promote the mixing between the PPZ and DXT solutions. As illustrated in Figure 2-3, the electrostatic interaction between PPZ and DXT led to the formulation of soluble PPZ-DXT complex, which then formed aggregates as a result of the inter-PPZ hydrophobic interactions. The aggregates grew in size due to the continuous adsorptions of PPZ and DXT ions to the PPZ-DXT complex in the aggregates. The aggregates precipitated out of the solution to form the PPZ nanoplex upon reaching a critical mass.

For each tube ID (d) investigated, the PPZ nanoplex was collected at different axial distances (L) from the Y-junction (i.e. 5, 10, and 15cm) to study the effect of residence time (t_{Res}). To maintain fixed t_{Res} upon changes in d, the two syringe pumps were operated at flowrates equal to 0.25 ml/min, 1.09 ml/min, and 2.44 ml/min for d = 0.762 mm, 1.588 mm, and 2.381 mm, respectively. After collection, the nanoplex suspension was centrifuged for 15 min at 14,000 g to remove the excess PPZ and DXT after which it was washed and re-dispersed in deionized water for characterizations. For the bulk-mixing preparation, the same PPZ and DXT solutions were mixed at equal volumes by drop wise addition of the PPZ solution, followed by 10 s vortex to promote mixing between PPZ and DXT. The PPZ nanoplex was then recovered following the same procedures described above.

5.2.2.2 Physical characterizations of PPZ nanoplex

The presence of PPZ in the nanoplex was first verified by Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum One, Perkin– Elmer, USA) performed in the range of

450 and 4000 cm⁻¹ at 1 cm⁻¹ spectral resolution. The FTIR was also performed for the native PPZ, native DXT, and physical mixture of PPZ and DXT. Afterwards, the payload defined as the amount of PPZ per unit mass of the nanoplex was determined by dissolving a known mass of freeze-dried nanoplex powders in PBS. Afterwards, the amount of PPZ was quantified by UV–vis spectrophotometry (UV Mini 1240, Shimadzu, Japan) at the maximum absorbance wavelength of PPZ determined to be at 255 nm [84].

The size, polydispersity index (PDI), and zeta potential of the PPZ nanoplex were determined by photon correlation spectroscopy (PCS) using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The morphology of the PPZ nanoplex was examined from its field emission scanning electron microscope (FESEM) image (JSM-6700F, JEOL, USA), where freeze-dried nanoplex powders were used after sputter coating them with platinum. The size, PDI, zeta potential, and payload reported here were based from a minimum of six independent replicates.

Equation 5-2: Definition of complexation efficiency (CE) of PPZ-DXT nanoplex

$$CE \text{ (wt. \%)} = \frac{\text{Mass of PPZ that formed nanoplex}}{\text{initial mass of PPZ added}} \times 100$$

Equation 5-3: Definition of Yield of PPZ-DXT nanoplex

$$Yield \text{ (wt. \%)} = \frac{\text{Mass of nanoplex produced}}{\text{initial mass of PPZ and DXT added}} \times 100$$

5.2.2.3 Preparation efficiency of PPZ nanoplex

The preparation efficiency of the PPZ nanoplex was characterized in terms of (1) the PPZ utilization rate and (2) the overall production yield. The PPZ utilization rate was determined from the complexation efficiency (CE) defined in Equation 5-2, where the mass of PPZ that formed nanoplex was determined from the difference between the

initial mass of PPZ added and the mass of PPZ recovered in the supernatant after centrifugation of the nanoplex suspension. The amount of PPZ in the supernatant was quantified by UV–vis spectrophotometry after 100 dilution at 255 nm. The yield was defined in Equation 5-3 where the mass of nanoplex produced was determined by the freeze-dried mass of a known volume of the nanoplex suspension after washing.

5.2.2.4 Dissolution rate and supersaturation generation

The dissolution rates of the PPZ nanoplex and the native PPZ under a sink condition were determined in triplicates by adding 0.86 ml of the nanoplex suspension (or 0.57 mg of native PPZ) to a dialysis bag immersed in 40 ml PBS in a shaking incubator at 37 °C. At specific time points over a 30 h period, 0.5ml aliquot was withdrawn and replaced with 0.5 ml fresh PBS. The amount of PPZ in the aliquot was quantified by UV–vis spectrophotometry as previously described.

The dissolution rate of the PPZ nanoplex under a non-sink condition, which was referred here as the supersaturation generation, was determined by adding excess nanoplex at 20 of C_{Sat} to 10ml PBS in a shaking incubator at 37 °C. Here C_{Sat} represented the thermodynamic saturation solubility of PPZ in PBS, which was determined by incubating excess native PPZ in 20 ml PBS at 37 °C for 72 h under constant stirring. Afterwards, the PPZ solution in PBS was centrifuged to remove the non-dissolved PPZ and C_{Sat} was determined from the PPZ concentration in the supernatant by UV–vis spectrophotometry.

After the addition of excess PPZ nanoplex, the supersaturation generation was quantified by withdrawing 0.5ml aliquot at specific time points over a 24 h period. The aliquot was immediately diluted tenfold with fresh PBS to prevent PPZ precipitation from the supersaturated solution after which it was syringe filtered using 0.2-mm

membrane pores. The amount of PPZ in the filtered aliquot representing C_{Supersat} was determined by UV–vis spectrophotometry. The supersaturation generation was reported as the ratio of C_{Supersat} to C_{Sat} .

5.2.2.5 Physical stability and production consistency

The colloidal stability of the PPZ nanoplex was characterized by monitoring its size and zeta potential by PCS during 24 h storage at room temperature, where minimal fluctuations in the size and zeta potential indicated high colloidal stability. The amorphous form stability of the PPZ nanoplex was examined by Powder X-ray Diffraction (PXRD) analysis after three-month storage at 25 °C and 55% relative humidity. The analysis was carried out between 10° and 70° (2 θ) with a step size of 0.02°/s using D8 Advance X-ray Diffractometer (Bruker, Germany). The PXRD analysis was also carried out for the native PPZ and physical mixture of PPZ and DXT. Lastly, the production consistency of both synthesis platforms was characterized by monitoring the variations in the size and zeta potential of the PPZ nanoplexes produced from fourteen independent replicates performed on different days.

5.2.2.5 Statistical Analysis

The statistical analysis was performed using one way ANOVA with Tukey post hoc test using OriginPro 2018b software.

5.3 Results and discussion

5.3.1 Effects of preparation condition in millifluidics

5.3.1.1 Mixing in millifluidics

The millifluidic preparation of the PPZ nanoplex started at the basic condition of $d = 0.762$ mm and flowrate of 0.25 ml/min, resulting in fluid velocity (U) of 0.914 cm/s (see Equation 5-4). The flow at this condition fell under the laminar flow regime with

Reynolds number (Re) ≈ 7 indicating that the mixing between the PPZ and DXT solutions was driven predominantly by molecular diffusion (see Equation 5-5), as opposed to the convective motion driving force in bulk mixing [226]. At this condition, the time needed for molecular diffusion across half of the tube diameter (t_{Mix}) was calculated to be equal to 145 s (see Equation 5-6), which translated to axial distance (L) of ≈ 130 cm (see Equation 5-7), hence indicating a slow mixing process between the PPZ and DXT solutions.

The slow mixing in the millifluidic reactor was verified by computational fluid dynamic (CFD) simulation using COMSOL¹ version 5.0 software (USA). The CFD simulation results showed two segregated streams flowing side by side downstream of the Y-junction (image not shown), hence suggesting that the PPZ-DXT interactions, which preceded the PPZ nanoplex formulation, predominantly occurred at the liquid–liquid interface. The slow mixing between the PPZ and DXT solutions was desirable as it prolonged the period in which both molecules were in their fully ionized states with opposite charges, hence promoting the electrostatic complexation between them.

Here is an example on how to calculate the Reynolds number (Re), t_{Mix} and t_{Res} . In the case of basic condition with $d = 0.762$ mm; $F = 0.25$ ml/min, Reynolds number (Re) is computed as Equation 5-5:

Equation 5-4: Calculation of fluid velocity (U)

$$U = \frac{F}{1/4 \pi d^2} = 0.914 \text{ cm/s}$$

Equation 5-5: Calculation of Reynolds number (Re)

$$Re = \frac{Ud}{\nu} = 6.95$$

where ν is the kinematic viscosity of the PPZ and DXT solutions estimated from the viscosity of pure water ($\approx 10^{-2}$ cm²/s) as dilute concentrations of PPZ and DXT were used.

In the case of basic condition with $d = 0.762$ mm, $L = 10$ cm, Mixing time for molecular diffusion (t_{Mix}) (Re) and Axial distance (L) travelled at t_{Mix} are computed as Equation 5-6, Equation 5-7 and Equation 5-8:

Equation 5-6: Calculation of t_{Mix}

$$t_{Mix} = \frac{h^2}{D} = 145 \text{ s}$$

Equation 5-7: Calculation of axial distance (L)

$$L = U \times t_{Mix} = 0.914 \times 145 = 132 \text{ cm}$$

Equation 5-8: Calculation of t_{Res}

$$t_{Res} = \frac{L}{U} = \frac{10}{0.914} = 11 \text{ s}$$

where D is the molecular diffusion coefficient (cm²/s), $h = d/2$ and L is the axial distance at which the PPZ nanoplex was collected (i.e. 5, 10, and 15 cm). A value of D equal to 10^{-5} cm²/s was used for diffusion of small molecules. It is evident that, Reynolds number is a function of tube diameters at fixed U based on the equations.

Table 5-1: Reynolds numbers (Re) as a function of tube ID

Tube ID (d) (mm)	Flowrate (F) (ml/min)	Velocity (U) (cm/s)	Re
0.762	0.25	0.914	7.0
1.588	1.09	0.914	14.6
2.381	2.44	0.914	21.8

5.3.1.2 Effect of pH

One of the key variables in PPZ-DXT complexation was the preparation pH as it governed the degree of ionization of both molecules. The present work investigated the effect of pH by varying the AA concentration used to dissolve PPZ between 0.05% and

0.50% (v/v), resulting in the preparation pH in the range of 5.84–3.87 after mixing with the DXT solution that had a neutral pH. The pH effect study was carried out at a fixed $R_{DXT/PPZ}$, where an arbitrary value above unity (i.e. 1.6) was chosen to ensure that there were sufficient numbers of DXT molecules available for complexation with the PPZ molecules. The requirement for $R_{DXT/PPZ}$ above unity was based on our preliminary experiments that showed unsuccessful nanoplex formulation at $R_{DXT/PPZ} = 0.8$ and 1.0 (data not shown). The study was carried out at a fixed t_{Res} by collecting the nanoplex at $L = 10$ cm corresponding to t_{Res} of 11 s (Equation 5-8).

Figure 5-1: Effects of (A) the preparation pH in terms of the acetic acid (AA) concentration, and (B) residence time (t_{Res}) on the size, zeta potential, and polydispersity index (PDI) of the PPZ nanoplex prepared by millifluidics. For tube diameter (d) = 0.762 mm, charge ratio $R_{DXT/PPZ} = 1.6$, and $t_{Res} = 11$ s.

The results in Figure 5-1 (A) showed that the nanoplex size initially decreased ($P < 0.05$) with increasing AA concentration up to AA = 0.1% before the nanoplex size began to increase at higher AA concentration. Specifically, the nanoplex size decreased ($P < 0.05$) from 129 ± 31 nm at AA = 0.05% to 59 ± 5 nm at AA = 0.1%, before it gradually increased to reach 212 ± 52 nm at AA = 0.50%. A similar trend but in the opposite direction was observed for the zeta potential that increased ($P < 0.05$) from 26 ± 2 mV at AA = 0.05% to 49 ± 4 mV at AA = 0.20% before it gradually decreased ($P < 0.05$) to 39 ± 2 mV at AA = 0.50% (Figure 5-2 (A)). The negative zeta potential indicated

the predominant presence of anionic DXT near the nanoplex surface, whose presence helped in the colloidal stabilization of the PPZ nanoplex.

Significantly, these results indicated that pH variation could be used to manipulate the nanoplex's size and zeta potential in millifluidics. Whether the same trend could be there in the bulk-mixing platform was to be investigated later. On this note, the variation in the nanoplex's zeta potential as a function of AA concentrations could be attributed to two key factors, namely variations in (a) the nanoplex size and (b) spatial orientation of the DXT chains in the nanoplex. These two factors had been known to influence the charge of self-assembled colloidal complexes of polyelectrolytes and oppositely charged small molecules [227].

The optimal AA concentration in terms of the nanoplex size was determined to be in the range of $0.08\% \leq \text{AA} \leq 0.20\%$ in which small nanoplexes were produced ($\approx 60\text{--}80\text{ nm}$) as the small size typically translated to faster dissolution velocities. The larger ($P < 0.05$) nanoplex prepared at $\text{AA} > 0.20\%$ ($\approx 170\text{--}210\text{ nm}$) was likely caused by the higher degree of ionization of PPZ as the pH dropped further below its pKa, which led to increased electrostatic complexation with DXT, resulting in the faster growth of the PPZ-DXT complex. On the other end, the slightly larger ($P < 0.05$) nanoplex prepared at $\text{AA} < 0.08\%$ ($\approx 130\text{ nm}$) was postulated to be caused by coalescences of the nanoplexes driven by the lack of stabilizing force due to the low zeta potential ($\approx -26\text{ mV}$). PPZ nanoplex prepared at $\text{AA} = 0.08\%$ having higher ($P < 0.05$) zeta potential ($-37 \pm 5\text{ mV}$) compared to the PPZ nanoplex prepared at $\text{AA} = 0.05\%$ and lower ($P < 0.05$) PDI (0.20 ± 0.01) compared to the PPZ nanoplexes prepared at $\text{AA} = 0.1\%, 0.2\%, 0.3\%, 0.4\%, 0.5\%$. The smaller PDI signified higher colloidal stability and more uniform size distribution, respectively, was produced. Therefore, the

optimal AA concentration was determined to be at 0.08% corresponding to the preparation pH of 4.96. The subsequent studies on the effects of t_{Res} and $R_{DXT/PPZ}$ were carried out at this optimal AA concentration.

5.3.1.3 Effect of residence time at different tube diameters

The effect of t_{Res} by the reactor length variation was first investigated at $d = 0.762$ mm for $L = 5, 10, 15$ cm corresponding to t_{Res} of 6, 11, 17 s, respectively. The results in Figure 5-2 (B) showed that the size and zeta potential of the PPZ nanoplex was minimally affected by t_{Res} , where nanoplexes having similar sizes (≈ 55 – 60 nm) ($P > 0.05$) and zeta potentials (≈ -37 to 42 mV) ($P > 0.05$) were produced in the range of t_{Res} investigated. These results were in contrast to the finding of Tran et al. [176], which showed the high size tunability of the curcumin nanoplex by t_{Res} variations, using the same d and flowrate (hence the same U).

Figure 5-2: Effect of t_{Res} of millifluidics on the size and zeta potential at (A) $d = 1.588$ mm and (B) $d = 2.381$ mm

To investigate whether the lack of size tunability with t_{Res} variation was dependent on d , same study was carried out in millifluidic reactors of larger d (i.e. 1.588 mm and 2.381 mm), while maintaining U at 0.914 cm/s by increasing the flowrate. The flows at the larger d also fell under the laminar flow regime ($Re \approx 15$ – 22), hence slow

mixing between the PPZ and DXT solutions remained. The results presented in Figure 5-2 showed a similar trend at larger d , where the size ($P > 0.05$) and zeta potential ($P > 0.05$) of the PPZ nanoplex were minimally affected by t_{Res} . As a result, PPZ nanoplexes having similar sizes and zeta potentials in the range of $\approx 60\text{--}70$ nm and ≈ 55 to 60 mV, respectively, were produced.

The lack of size tunability by t_{Res} variation was likely caused by insufficient time window for the PPZ-DXT complex to grow as it moved along the reactor length due to the rapid convective motion at $U = 0.914$ m/s. In this regard, Tran et al.[107] had reported the diminished size tunability at higher U , hence suggesting the adverse effect of fast convective motion on the nanoplex size tunability. Operating the millifluidics at a lower U , however, was less than desirable, as it would result in a very low production throughput. Hence, size control of the PPZ nanoplex was best achieved by other means, for example, by pH variation as reported earlier.

Figure 5-3: Millifluidic production throughput (mg/h) as a function of d

Besides the size and zeta potential, the effect of t_{Res} variation on the preparation efficiency was also found to be insignificant for both $d = 1.588$ mm (Figure 5-2(A) and

$d = 2.381$ mm (B)). The CE and yield remained constant at $\approx 90\%$ ($P > 0.05$) and $\approx 30\text{--}40\%$ ($P > 0.05$), respectively, with increasing t_{Res} . The same trend was observed for the PPZ payload, where the payload showed minimal variations as a function of t_{Res} and remained at $\approx 65\text{--}75\%$ ($P > 0.05$) and $\approx 80\text{--}85\%$ ($P > 0.05$) for $d = 1.588$ (Figure 5-2A) and $d = 2.381$ mm (Figure 5-2B), respectively. Thus, these results confirmed the general ineffectiveness of t_{Res} variation as a means to manipulate the PPZ nanoplex's characteristics and preparation efficiency.

Figure 5-4: Effects of the millifluidic residence time (t_{Res}) on the preparation efficiency and payload at tube diameters (d) equal to (A) 1.588 mm and (B) 2.381 mm. For acetic acid (AA) = 0.08% and charge ratio ($R_{\text{DXT/PPZ}}$) = 1.6.

Not less importantly, the results at $d > 0.762$ mm showed that the PPZ nanoplex's size and preparation efficiency were not adversely affected by the increase in d . Furthermore, higher zeta potential and higher PPZ payload were observed at larger d . In fact, the yield was increased from $< 20\%$ at $d = 0.762$ mm to $\approx 30\text{--}40\%$ at the larger d (Figure 5-3) ($P < 0.05$). This was not unexpected as the larger d resulted in less particle clogging, hence more nanoplex could be recovered. These results boded well for the scalability of the millifluidic synthesis platform, because the production throughput per millifluidic channel could be increased multifold from 10 ± 2 mg/h at $d = 0.762$ mm to 135 ± 3 mg/h at $d = 2.381$ mm, without incurring any adverse effect on

the physical characteristics and preparation efficiency of the PPZ nanoplex (Figure 5-2).

5.3.1.4 Effect of $R_{DXT/PPZ}$

Recognizing the minimal impacts of t_{Res} and d on the PPZ nanoplex preparation, the study on the effect of $R_{DXT/PPZ}$ was carried out at fixed d (0.762 mm) and $t_{Res} = 6$ s.

Figure 5-5: Effects of charge ratio ($R_{DXT/PPZ}$) in millifluidics on the (A) size, zeta potential, and polydispersity index (PDI) and on the (B) preparation efficiency and payload. For tube diameter (d) = 0.762 mm, acetic acid.

Changing $R_{DXT/PPZ}$ from the arbitrarily chosen value of 1.6 to a lower value above unity (i.e. 1.4) was found to have minimal impacts on the size ($P > 0.05$), PDI ($P > 0.05$), and zeta potential ($P > 0.05$) (Figure 5-5A), as well as on the CE ($P > 0.05$) and yield ($P > 0.05$) (Figure 5-5B). A considerably lower ($P < 0.05$) PPZ payload at $38 \pm 4\%$, however, was observed at $R_{DXT/PPZ} = 1.4$, where there was less excess DXT available for complexation with PPZ. In contrast, increasing $R_{DXT/PPZ}$ from 1.6 to 1.8 was found to result in a slightly larger ($P < 0.05$) nanoplex (111 ± 14 nm) with higher zeta potential ($P < 0.05$) (61 ± 7 mV), while the PDI ($P > 0.05$), CE ($P > 0.05$), yield ($P > 0.05$), and payload ($P > 0.05$) were only minimally affected (Figure 5-5). These results showed that varying $R_{DXT/PPZ}$ in millifluidics was not as effective as the pH variation in manipulating the PPZ nanoplex's physical characteristics.

5.3.1.5 Physical verification of PPZ nanoplex prepared by millifluidics

The nanoscale size of the PPZ nanoplex prepared at the optimal millifluidic condition ($d = 2.381$ mm, $t_{\text{res}} = 6$ s, $R_{\text{DXT/PPZ}} = 1.6$, and $AA = 0.08\%$) was verified by the FESEM image that showed roughly spherical particles having sizes of approximately 100 nm (Figure 5-6 (A)).

Figure 5-6: (A) Field emission scanning electron microscope (FESEM) image of the perphenazine (PPZ) nanoplex prepared at the optimal condition of millifluidics; (B) Fourier transform infrared (FTIR) spectra confirming the presence of perphenazine (PPZ) and dextran sulfate (DXT) in the nanoplex.

In addition to the PPZ payload reported earlier, the presence of PPZ in the nanoplex was verified from the FTIR spectra using the characteristic bands of PPZ at 756, 1456, and 1590 cm⁻¹, which corresponded to the stretching vibrations of the C-Cl (756 cm⁻¹) and C=C aromatic ring (1456, 1590 cm⁻¹) groups of PPZ [212]. These characteristic bands appeared in the FTIR spectra of the native PPZ, PPZ nanoplex, and the physical mixture of PPZ and DXT, but not in the FTIR spectrum of native DXT, hence the presence of PPZ in the nanoplex was confirmed (Figure 5-6 B). The presence of DXT in the nanoplex was also verified by the appearance of broad bands at 1200–1280 cm⁻¹ in the FTIR spectra of the native DXT and PPZ nanoplex, which corresponded to the asymmetric stretching of the sulfate group of DXT [218].

5.3.2 Effects of preparation condition in bulk mixing

5.3.2.1 Effect of pH

In the bulk-mixing platform, the effect of pH on the PPZ nanoplex formulation at $R_{DXT/PPZ} = 1.6$ was more profound, where the nanoplex was successfully formed only at $AA \leq 0.20\%$. While turbid suspensions indicating the formulation of particulate products were observed at $AA > 0.20\%$, these particles disappeared upon centrifugation and washing, hence suggesting their lack of physical integrity likely caused by the weak bonds of the PPZ-DXT complex. This was reflected in the recovery of almost clear solutions after centrifugation at $AA > 0.20\%$, in contrast to the turbid suspensions recovered after centrifugation at $AA 0.20\%$ (Figure 5-7). The weak bonds of the PPZ-DXT complex at $AA > 0.20\%$ were postulated due to the increased charge screening effect in the bulk environment of higher ionic strengths [228].

Figure 5-7: The PPZ nanoplex suspension after the first centrifugation showing clear solution at $AA > 0.20\%$ denoting the disappearance of the particulate products after centrifugation

At $0.05\% \leq AA \leq 0.10\%$, PPZ nanoplexes having similar sizes ($P > 0.05$) and zeta potentials ($P > 0.05$) in the range of $\approx 50\text{--}70$ nm and ≈ 30 to 50 mV, respectively, were produced. In contrast, considerably larger nanoplex with size of 228 ± 44 nm (P

< 0.05) and zeta potential of -36 ± 5 mV ($P < 0.05$) were produced at AA = 0.20% (Figure 5-8A). Significantly, these results demonstrated the tunability of the PPZ nanoplex size by pH variation in the bulk-mixing platform. Similar to millifluidics, the optimal pH in terms of the physical characteristics for bulk mixing was determined to be at AA = 0.08%. The optimal pH was selected based on the superior PDI ($P < 0.05$) and zeta potential ($P < 0.05$) of the nanoplex produced compared to those produced at AA = 0.05% and 0.10%, despite having similar sizes ($P > 0.05$).

Figure 5-8: Effects of (A) the preparation pH in terms of the acetic acid (AA) concentration; (B) charge ratio ($R_{DXT/PPZ}$) on the size, zeta potential, and polydispersity index (PDI) of the perphenazine (PPZ) nanoplex prepared by bulk mixing.

5.3.2.2 Effect of $R_{DXT/PPZ}$

The effect of varying $R_{DXT/PPZ}$ from the arbitrarily chosen value of 1.6 on the size ($P > 0.05$), zeta potential ($P > 0.05$), and PDI ($P > 0.05$) was found to be minimal as shown in Figure 5-9. Similarly, the effect of $R_{DXT/PPZ}$ on the preparation efficiency (i.e. CE $\approx$ 90% ($P > 0.05$) and yield $\approx$ 20–30% ($P > 0.05$)) and payload ($\approx$ 60–65% ($P > 0.05$)) was also found to be minimal, with the exception of the lower payload ($\approx$ 30%) ($P < 0.05$) obtained at $R_{DXT/PPZ} = 1.4$ (Figure 5-9 B). These results showed that $R_{DXT/PPZ}$ variation was not an effective means for manipulating the PPZ nanoplex's characteristics by bulk

mixing. It had to be pointed out in that the nanoplex size at $R_{DXT/PPZ} = 1.6$ in the $R_{DXT/PPZ}$ study was ≈ 130 nm, instead of ≈ 50 nm reported earlier in the AA study. As discussed in more details later, this discrepancy was due to the large fluctuations in the physical characteristics of the PPZ nanoplex produced by the bulk-mixing platform.

5.3.3 Direct comparison between millifluidics and bulk mixing

5.3.3.1 Dependence on pH and $R_{DXT/PPZ}$

Both millifluidic and bulk-mixing platforms showed similar dependences on the preparation pH, where smaller nanoplexes were generally produced at lower AA concentrations (or higher pH). A similar dependence on the pH was also observed for the zeta potential. Thus, the PPZ nanoplex's size and zeta potential could be controlled by pH variation in both platforms, where nanoplexes with similar physical characteristics were produced between the two platforms.

Figure 5-9: Effect of $R_{DXT/PPZ}$ on the size, zeta potential, and PDI on the PPZ nanoplex prepared by the bulk-mixing platform

However, the range of feasible pH was narrower for the bulk-mixing platform due to the aforementioned charge screening effect at higher AA concentrations. The

same phenomenon was not observed in millifluidics because the PPZ and DXT solutions were mixed slowly, hence reducing the impact of charge screening on the PPZ-DXT complex formulation.

Both synthesis platforms also shared a similar trend upon varying $R_{DXT/PPZ}$ at the optimal pH, where the physical characteristics and preparation efficiency of the PPZ nanoplex produced were in general minimally affected by the change in $R_{DXT/PPZ}$, provided that $R_{DXT/PPZ}$ was maintained above unity. As a result, both platforms shared the same optimal pH and $R_{DXT/PPZ}$ at AA = 0.08% and $R_{DXT/PPZ} = 1.6$, respectively. These results signified the preparation pH, not $R_{DXT/PPZ}$, as the governing variable for the PPZ nanoplex formulation, regardless of the synthesis platform pursued.

Figure 5-10: Production consistency of the millifluidic and bulk-mixing platforms as characterized by the fluctuations in the size and zeta potential of the PPZ nanoplexes produced in fourteen independent replicates.

5.3.3.2 Production consistency

Next, the production consistency of the PPZ nanoplexes prepared at the optimal condition of each platform were examined by comparing the size and zeta potential of the nanoplexes produced in fourteen independent replicates. The results presented in Figure 5-10 showed large fluctuations in the size of the nanoplex prepared by bulk mixing. The size varied from the smallest values at $\approx 30\text{nm}$ (3 runs) to the largest values

at $\approx 130\text{--}150\text{nm}$ (2 runs), resulting in the mean size of $73 \pm 40\text{nm}$ with a large standard deviation. In contrast, the nanoplex prepared by millifluidics exhibited smaller fluctuations, where the smallest and largest sizes were observed at $\approx 65\text{ nm}$ (1 run) and $\approx 110\text{ nm}$ (1 run), respectively, with the majority of the runs exhibited sizes of $\approx 80\text{--}90\text{ nm}$, resulting in the mean size of $87 \pm 11\text{ nm}$.

For the zeta potential, both platform exhibited similar fluctuations ($P > 0.05$) with mean zeta potentials of $48 \pm 11\text{ mV}$ and $49 \pm 12\text{ mV}$ for millifluidics and bulk mixing, respectively. Hence, even though both platforms exhibited similar dependences on pH and $R_{\text{DXT/PPZ}}$, the millifluidic platform was vastly superior in terms of the production consistency, which was not unexpected as continuous processes had been well established to be superior to batch processes in their production consistency [229].

5.3.3.3 Physical characteristics and preparation efficiency

Next, the physical characteristics and preparation efficiency of the PPZ nanoplex prepared by each platform were compared in Table 5-2 using the results from the fourteen independent replicates.

Table 5-2: A summary of comparison between perphenazine (PPZ) nanoplexes prepared by millifluidics and bulk mixing (CE=complexation efficiency; PDI=polydispersity index; t_{Res} = residence time; d = tube diameter).

Characteristics	Millifluidics ($d = 2.381\text{ mm}$, $t_{\text{Res}} = 6\text{s}$)	Bulk mixing
Size (nm)	87 ± 11	73 ± 40
Zeta potential (mV)	-48 ± 11	-49 ± 12
PDI	0.17 ± 0.04	0.18 ± 0.04
CE (wt.%)	89 ± 2	83 ± 1
Yield (wt.%)	32 ± 2	26 ± 4
Payload (wt.%)	85 ± 2	64 ± 4

Other than the abovementioned lack of consistency in the nanoplex size for the bulk-mixing platform, the effects of the synthesis platform on the other characteristics (i.e. zeta potential, PDI, CE, yield) were relatively insignificant. In addition to its

superior production consistency, the millifluidic platform was also superior in terms of the PPZ payload, where nanoplex having a considerably higher payload than the bulk-mixing platform was produced (i.e. $85 \pm 2\%$ versus $64 \pm 4\%$). The higher payload was likely attributed to the slow mixing of the PPZ and DXT solutions in millifluidics ($t_{\text{Res}} \ll t_{\text{Mix}}$), which resulted in the smaller presence of DXT during the PPZ-DXT complex formulation. As a result, the excess DXT had fewer opportunities to interact with the growing complex, resulting in the lower payload.

5.3.3.4 Physical stabilities

First, the results of the colloidal stability test in (Figure 5-11A) showed that the PPZ nanoplex prepared by either the millifluidic or bulk-mixing platform maintained their size ($P > 0.05$) and zeta potential ($P > 0.05$) over the 24h ambient storage. These results were not unexpected considering the large negative zeta potentials of these PPZ nanoplexes. Importantly, these results boded well for the downstream processing of the nanoplex as its immediate trans-formulation into the more stable dry powder forms, which was often required for many nanoparticulate products, was not needed.

Figure 5-11: Comparison of the (A) colloidal stability and (B) amorphous form stability of perphenazine (PPZ) nanoplexes prepared by mill-fluidics and bulk mixing.

Second, the results of the amorphous form stability test in Figure 5-11 (B)

showed that both nanoplexes maintained their amorphous forms during the three-month storage as reflected by the presence of broad amorphous halos in the PXRD patterns of the nanoplexes, in contrast to the sharp crystalline peaks observed in the PXRD patterns of the native PPZ and the PPZ-DXT physical mixture.

5.3.3.5 Dissolution and supersaturation generation

The results of the dissolution test under sink condition in Figure 5-12 (A) showed similar dissolution rates ($P > 0.05$) between the PPZ nanoplexes prepared by millifluidics and bulk mixing, which was not unexpected considering their similarities in size and amorphous form. Owing to their amorphous form and nanoscale size, the PPZ nanoplexes dissolved at significantly faster rates than the crystalline native PPZ ($P < 0.05$), where $\approx 60\%$ dissolution was achieved after 2 h (with 100% dissolution after 24 h) versus $\approx 25\%$ dissolution for the native PPZ over the same period.

Figure 5-12: Comparison of the (A) dissolution rate under sink condition and (B) supersaturation generation of perphenazine (PPZ) nanoplexes prepared by millifluidics and bulk mixing.

For the supersaturation generation, the results in Figure 5-12 (B) showed that the PPZ nanoplex prepared by bulk mixing exhibited a higher supersaturation generation compared to its counterpart prepared by millifluidics in the first 2 hours. Specifically, the former generated supersaturated solution at $(13.5 \pm 1.2) \times C_{Sat}$ after 1

h, whereas the supersaturation generation of the latter was lower at $(8.8 \pm 1.3) \times C_{\text{Sat}}$ after the same period. The supersaturation generations of the two nanoplexes, nevertheless, became comparable in magnitude after 6 h at $(5.8 \pm 1.8) \times C_{\text{Sat}}$. Importantly, both nanoplexes exhibited prolonged supersaturation generation at $\approx 4 \times C_{\text{Sat}}$ for more than 9 h.

On this note, amorphous solid dispersions had been found to exhibit a slower supersaturation generation at high drug payload [230]. The reason given was because the hydrophilic polymer acting as the drug stabilizer might dissolve sooner than the drug at high payload, hence causing the dissolution to be controlled by the highly hydrophobic drug-rich layer, resulting in slower supersaturation generation. In contrast, the drug likely dissolved together with the polymer at low payload. Therefore, the faster supersaturation generation of the PPZ nanoplex prepared by bulk mixing in the early dissolution phase was attributed to its lower payload.

Figure 5-13: Supersaturation generation of the PPZ nanoplexes prepared by the millifluidic and bulk-mixing platforms over a 24 h-period

Nevertheless, both nanoplexes exhibited the typical “spring-and-parachute” supersaturation time-profiles of amorphous drug formulations [216], where a peak in the supersaturation level (“spring”) was initially reached due to the fast dissolution of the amorphous form, followed by a gradual decrease in the supersaturation level due to recrystallization of the supersaturated solution (“parachute”) to eventually drop down to nearly CS_{Sat} after 24 h (Figure 5-13).

5.4 Conclusions

First, the study on the effects of key process variables in millifluidics on the PPZ nanoplex preparation revealed the pH as the governing process variable. The residence time, tube diameter, and charge ratio (>1), on the other hand, had relatively insignificant impacts on the nanoplex preparation. Controls over the nanoplex’s size and zeta potential were achievable by pH variation, while the payload and preparation efficiency were not greatly affected by the change in the process variables. In other words, size control of the nanoplex could be achieved without any adverse effects on the other nanoplex characteristics. The minimal impact that the residence time had on the nanoplex size tunability, however, was in contrast to the previous results obtained using different drug and polysaccharides [176]. The present result hence signified the non-universality of the effects of the millifluidic operating condition.

Second, the study on the direct comparison between milli-fluidics and bulk mixing showed that the two synthesis platforms shared similar dependences on the pH and charge ratio with similar optimal values for both. More specifically, pH variation was also found effective in controlling the PPZ nanoplex’s size and zeta potential in bulk mixing, whereas the charge ratio again had minimal impacts. This finding was in

contrast to the previous finding using different drug and polysaccharide [35], which reported the infeasibility of size control in the bulk-mixing platform. These results hence reaffirmed the earlier conclusion that the effects of key process variables in drug-polysaccharide complexation, regardless of the synthesis platform, were unique depending on the drug-polysaccharide system in question.

Third, PPZ nanoplexes prepared at the optimal condition of each platform shared similar size ($\approx 70\text{--}90$ nm), zeta potential (≈ -50 mV), preparation efficiency ($\approx 90\%$ drug utilization rate and $\approx 30\%$ yield), dissolution rate, and physical stability during storage. While the nanoplex prepared by bulk mixing exhibited a lower payload (i.e. $\approx 65\%$ versus 85%), the lower payload led to slightly superior supersaturation generation. The bulk-mixing platform, however, was vastly inferior to millifluidics in terms of the production consistency. In summary, while there were minimal differences between the PPZ nanoplexes prepared by the two platforms, the superior production consistency of millifluidics, coupled with its demonstrated scalability at larger tube diameters, led us to conclude millifluidics as the superior synthesis platform for amorphous drug nanoplex.

CHAPTER 6 Modification of the dissolution rate of the drug-polysaccharide nano-complex with high drug-loading by incorporating small molecule ions into the drug-polysaccharide nanoparticle

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Here, we used the insoluble drug CIP as a model drug to develop drug formulations with enhanced apparent solubility but modifiable dissolution rates. The well-established chemotherapeutic effects of the synthetic broad-spectrum [151] fluorinated-4-quinolones antibiotic CIP [231] and its cheap price makes it very popular. It is classified as an IV compound [232] according to the biopharmaceutics classification system (BCS) because of its low solubility and permeability. The frequent administration and high dose of CIP, which is necessary to compensate for the low bioavailability, can cause drug waste and has the potential to induce antibiotic resistance. Therefore, a formulation that exhibits a high payload, an ability to achieve 100% drug release, and a low dissolution rate is needed. Our lab has proven that the CIP-DXT nanoplex prepared through the drug-polysaccharide complexation method exhibits a fast dissolution rate, high payload and 100% drug release [1, 33, 105]. However, the ideal dissolution rate varies in different physiological environments. It is necessary to develop drug dose formulations that can modify the dissolution rate to overcome different physiological

encumbrances and avert drug toxicity caused by high local concentration while retaining a high payload and 100% drug release.

Poor bioavailability of the broad-spectrum antibiotic ciprofloxacin (CIP) is caused by its narrow absorption window in the stomach. With the aim of prolonging the gastric residence time of CIP, we prepared a ternary nanoparticle complex (nanoplex) of CIP by co-complexation with polyanions (sodium dextran sulfate (DXT)) and an anionic amphiphile (sodium dodecyl sulfate (SDS)). We investigated the effect of the charge ratio of DXT to SDS on the size, zeta potential, CIP payload, and CIP utilization rate of the CIP-DXT-SDS nanoplex and its dissolution characteristics in simulated gastrointestinal fluids. Fourier transform infrared spectroscopy, powder X-ray diffraction, and differential scanning calorimetry analyses showed that the ternary nanoplex was made up of amorphous CIP-DXT and crystalline CIP-SDS complexes. The size of the CIP-DXT-SDS nanoplex prepared at a > 90% CIP utilization rate was 110–290 nm and it had a zeta potential of –16 to –39 mV, and CIP payload of 47–62%, depending on the charge ratio. At gastric pH, the CIP-DXT-SDS nanoplex prepared with a DXT:SDS charge ratio lower than 80:20 exhibited prolonged CIP release (60% dissolution after 8 h) compared with native CIP (100% dissolution after 1 h) and a binary CIP-DXT nanoplex (80% dissolution after 5 h), which was attributed to its lower solubility. The sustained release characteristics of the CIP-DXT-SDS nanoplex were comparable to those of existing CIP gastro-retentive formulations.

6.1 Introduction

Oral administration remains the preferred drug delivery route in many clinical applications because of the ease of administration [233]. Although most orally administered drugs exhibit good absorption throughout the gastrointestinal tract, many

drugs are known to have preferential absorption sites in the gastrointestinal tract, resulting in specific absorption windows [234, 235]. The broad-spectrum fluoroquinolone antibiotic ciprofloxacin (CIP) is one such example. The specific absorption site for CIP is the proximal small intestine (i.e., the duodenum and, to a smaller extent, the jejunum), resulting in a narrow absorption window [236]. Other examples of drugs with narrow absorption windows in the proximal small intestine are acyclovir, gabapentin, levodopa, and metformin [237].

CIP absorption is further complicated by its pH-dependent solubility. CIP exhibits a U-shaped pH solubility profile with high solubility ($>10\text{mg/ml}$) at pH values below 5 and above 10 because of its zwitterionic characteristics [238]. As CIP is absorbed primarily by passive diffusion, the low CIP solubility ($\ll 1\text{ mg/ml}$) at intestinal pH ($\approx 6\text{--}7.4$) is believed to be a primary cause of its poor absorption in the distal small intestine [239]. Consequently, the optimum bioavailability of CIP is achieved when CIP is delivered to the stomach ($\text{pH} < 2$), which enables CIP absorption in the duodenum [239]. For this reason, various gastro-retentive formulations aimed at prolonging the gastric residence time of CIP to maximize its bioavailability have been developed in the form of bio-adhesive, swelling, and floating tablets [240-243]. Most CIP gastro-retentive formulations exhibit extended CIP release over 24 h and make once-daily dosing of CIP a possibility. Nevertheless, the preparation of CIP gastro-retentive formulations is intricate and requires incorporation of various polymer excipients at precise pre-determined compositions [241].

However, a gastro-retentive formulation alone is not sufficient because if the native form of CIP is used, the dissolved CIP molecules will precipitate upon entering the small intestine and encountering a sudden rise in pH [244] resulting in diminished

absorption. To address this problem, the crystalline hydrochloride salt form of CIP, which has much higher solubility at intestinal pH than native CIP, has been used in CIP gastroretentive formulations. Moreover, the lower solubility of CIP·HCl compared with native CIP at acidic pH, as a result of the common-ion effect, is useful in achieving slower, sustained release of CIP in the stomach. Other crystalline CIP salts exhibiting similar gastrointestinal solubility profiles as CIP·HCl have also been developed for the same purpose [245-247].

Another strategy to increase the CIP solubility at intestinal pH is by amorphization to a metastable amorphous form that is capable of producing highly supersaturated drug concentrations upon dissolution at intestinal pH, resulting in high apparent solubility. Amorphization of CIP has been pursued in the following forms: (1) an amorphous solid dispersion (ASD) of native CIP [248, 249], (2) an amorphous CIP salt [250] (3) an ASD of a CIP salt [237], or (4) an amorphous CIP nanoparticle complex prepared by CIP complexation with polyelectrolytes [33]. Unfortunately, the ASD of native CIP has a high crystallization tendency, which leads to poor stability during storage [248, 251]. Amorphous CIP salts are more soluble than native CIP at both intestinal and gastric pH values [250, 251], rendering them less than ideal to achieve sustained release of CIP in the stomach. However, the amorphous CIP nanoparticle complex (or nanoplex for short) exhibits superior storage stability to the ASD of CIP, and this has been attributed to the molecular mobility restriction of CIP in the nanoplex as a result of its electrostatic binding with sodium dextran sulfate (DXT), which acts as a polyelectrolyte [248].

Furthermore, following complexation with anionic DXT, the protonatable amino group of CIP in the nanoplex does not contribute to its dissolution at acidic pH

[33, 252, 253]. This results in lower solubility of the CIP-DXT nanoplex at gastric pH, which is ideal for achieving sustained release of CIP in the stomach. In short, the CIP-DXT nanoplex essentially possesses similar gastrointestinal solubility characteristics as crystalline CIP salts, which bodes well for the nanoplex's ability to optimize CIP bioavailability upon its incorporation into gastroretentive formulations. Having said that, while CIP dissolution from the CIP-DXT nanoplex at acidic pH is indeed slower than that of native CIP. CIP dissolution from the CIP-DXT nanoplex still has room for improvement, particularly in terms of its sustained release. In this regard, sustained release at the nanoplex level is desirable as it would enable us to simplify gastroretentive formulations of the nanoplex, whereby the formulations would be less dependent on the type and composition of the polymer excipients and require simpler nanofluidic processing steps [254-256].

Figure 6-1: Schematic of the formulation of the ternary nanoplex of CIP-DXT-SDS via electrostatically driven co-complexation of CIP with oppositely charged DXT and SDS.

Herein, we aimed to improve the dissolution time-profile of the CIP-DXT nanoplex at acidic pH by incorporating sodium dodecyl sulfate (SDS) into the nanoplex formulation. SDS reportedly forms a crystalline ionic complex with CIP that exhibits

suppressed pH-dependent solubility [146]. We hypothesized that the addition of SDS would lead to formulation of a ternary CIP-DXT-SDS nanoplex containing both CIP-DXT and CIP-SDS complexes with improved sustained release characteristic of CIP at acidic pH. We postulated that incorporation of the CIP-SDS complex with suppressed solubility at acidic pH in the nanoplex would prolong sustained CIP release.

We envisioned the ternary CIP-DXT-SDS nanoplex would form by electrostatically driven co-complexation of protonated CIP with oppositely charged DXT and SDS, which would lead to the formulation of soluble CIP-DXT and CIP-SDS complexes (Figure 6-1, Figure 6-2 and Figure 6-3). The soluble complexes would later form aggregates among themselves as a result of hydrophobic interactions among the bonded CIP molecules. Upon aggregation, the CIP-SDS complex, which contains small molecules, would likely embed in the interstitial spaces formed between the long chains of the CIP-DXT complex. Upon reaching a critical mass, the complex aggregates would precipitate out of the solution to form a ternary CIP-DXT-SDS nanoplex made up of both CIP-DXT and CIP-SDS complexes.

Figure 6-2: Schematic of CIP and SDS binding together via electrostatic interaction

The objectives of the present work were (1) to verify the formulation of the ternary CIP-DXT-SDS nanoplex by physical characterizations, (2) to investigate the effects of the DXT:SDS charge ratio on the physical characteristics (i.e., size, zeta potential, and CIP payload) and preparation efficiency of the ternary nanoplex produced, and (3) to examine the dissolution characteristics of the CIP-DXT-SDS nanoplex in simulated gastrointestinal fluids and compare the results with those for a CIP-DXT

nanoplex and native CIP. Finally, we evaluated the ternary CIP-DXT-SDS nanoplex as a viable alternative to crystalline CIP salts for incorporation into gastroretentive CIP formulations with the aim of achieving high CIP bioavailability.

Figure 6-3: Schematic of CIP and DXT binding together via electrostatic interaction

6.2 Materials and methods

6.2.1 Materials

CIP ($\geq 98\%$ purity), SDS ($\geq 99\%$ purity), glacial acetic acid, hydrogen chloride (HCl), potassium hydroxide (KOH), sodium chloride (NaCl), methanol, acetonitrile, and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma-Aldrich (Singapore). DXT (MW = 5 kDa) was purchased from Wako Chemical (Japan).

6.2.2 Methods

6.2.2.1 Preparation of the CIP-DXT-SDS nanoplex

Briefly, we prepared solutions of CIP (10 mg/ml) in 0.4% (w/v) aqueous acetic acid (pH 3), DXT (6.25 mg/ml) in deionized water (pH 7), and SDS (8.57 mg/ml) in deionized water (pH 7). CIP has pKa values of 6.1 and 8.6 [257] and will be fully protonated at pH 3 with a positive charge density of 3.0×10^{-6} mol charge/mg. DXT and SDS have pKa values of ≤ 2 [258, 259] and will be fully de-protonated at neutral pH with negative charge densities of 4.8×10^{-6} and 3.5×10^{-6} mol charge/mg, respectively (Table 6-1). Next, 1 ml of CIP solution was added to 1 ml of a mixture of the DXT and SDS solutions (0.5 ml each), followed by 10 s of vortexing at room

temperature. The resultant CIP-DXT-SDS nanoplex was washed to remove excess CIP, DXT, and SDS by two cycles of centrifugation at $14,000 \times g$ for 10 min (Sorvall 16R, Thermo Fisher Scientific, USA) and rinsing with deionized water.

Table 6-1: Sample calculations for charge density and charge ratio in the feed

Species	Molecular weight (g/mol)	No. of charge per molecule	Charge density (mol·charge/mg)
CIP	331	1	$1/(331 \times 10^3) = 3.0 \times 10^{-6}$
SDS	288	1	$1/(288 \times 10^3) = 3.5 \times 10^{-6}$
DXT	5000	24	$24/(5000 \times 10^3) = 4.8 \times 10^{-6}$

The CIP-DXT-SDS nanoplex was prepared at a fixed charge ratio of CIP to (DXT + SDS) ($R_{CIP:(DXT+SDS)}$) with equal amounts of DXT and SDS available for complexation with CIP to ensure high CIP utilization rates. The effect of the charge ratio of DXT to SDS ($R_{DXT:SDS}$) was investigated in the range of 90:10 to 10:90 by varying the volume of the DXT and SDS solutions used for mixing with CIP while keeping the total (DXT+SDS) volume constant at 1ml. Sample calculations for the charge density can be found in Table 6-1.

Sample calculation for $R_{CIP:(DXT+SDS)}$, and $R_{DXT:SDS}$ are provided (see Equation 6-1, Equation 6-2, Equation 6-3, respectively). Two control runs with (1) the CIP-DXT nanoplex without SDS ($R_{DXT:SDS} = 100:0$) and (2) the CIP-SDS complex without DXT ($R_{DXT:SDS} = 0:100$) were prepared for comparison of $R_{CIP:DXT} = 1$ and $R_{CIP:SDS} = 1$, respectively.

Equation 6-1: Sample calculation for $R_{CIP:(DXT+SDS)}$ for the CIP-DXT nanoplex control

$$R_{CIP:(DXT+SDS)} = (3.0 \times 10^{-6} \text{ mol·charge/mg} \times 10 \text{ mg/ml} \times 1 \text{ ml}) / (4.8 \times 10^{-6} \text{ mol·charge/mg} \times 6.25 \text{ mg/ml} \times 1 \text{ ml}) = 1.0$$

Equation 6-2: Sample calculation for $R_{CIP:(DXT+SDS)}$ for the insoluble CIP-SDS complex control

$$R_{CIP:(DXT+SDS)} = (3.0 \times 10^{-6} \text{ mol·charge/mg} \times 10 \text{ mg/ml} \times 1 \text{ ml}) / (3.5 \times 10^{-6} \text{ mol·charge/mg} \times 8.57 \text{ mg/ml} \times 1 \text{ ml}) = 1.0$$

Equation 6-3: Sample calculation for $R_{CIP:(DXT+SDS)}$ for Run D (i.e. CIP-DXT-SDS nanoplex prepared at DXT:SDS = 0.6:0.4 (v/v))

$$R_{CIP:(DXT+SDS)} = (3.0 \times 10^{-6} \text{ mol} \cdot \text{charge/mg} \times 10 \text{ mg/ml} \times 1 \text{ ml}) / (4.8 \times 10^{-6} \text{ mol} \cdot \text{charge/mg} \times 6.25 \text{ mg/ml} \times 0.6 \text{ ml} + 3.5 \times 10^{-6} \text{ mol} \cdot \text{charge/mg} \times 8.57 \text{ mg/ml} \times 0.4 \text{ ml}) = 1.0$$

Equation 6-4: Sample calculation for $R_{CIP:DXT}$ for Run D (i.e. CIP-DXT-SDS nanoplex prepared at DXT:SDS = 0.6:0.4 (v/v))

$$R_{CIP:(DXT+SDS)} = (4.8 \times 10^{-6} \text{ mol} \cdot \text{charge/mg} \times 6.25 \text{ mg/ml} \times 0.6 \text{ ml}) / (3.5 \times 10^{-6} \text{ mol} \cdot \text{charge/mg} \times 8.57 \text{ mg/ml} \times 0.4 \text{ ml}) = 18/12 = 6:4 \text{ (expressed as 60:40 in the above table to correspond to the DXT:SDS volume ratio)}$$

Table 6-2: Stock solution concentrations. CIP concentration = 10 mg/ml, DXT concentration = 6.25 mg/ml, SDS concentration = 8.57 mg/ml CIP solution and (DXT+SDS) solution were mixed at equal volume (i.e. 1.0 ml each)

Run	CIP (vol.)	DXT (vol.)	SDS (vol.)	$R_{CIP:(DXT+SDS)}$	$R_{DXT:SDS}$
CIP-DXT nanoplex (control)	1.0	1.0	0.0	1.0	NA
A		0.9	0.1		90:10
B		0.8	0.2		80:20
C		0.7	0.3		70:30
D		0.6	0.4		60:40
E		0.5	0.5		50:50
F		0.4	0.6		40:60
G		0.3	0.7		30:70
H		0.2	0.8		20:80
I		0.1	0.9		10:90
Insoluble CIP-SDS complex (control)		0.0	1.0		NA

6.2.2.2 Physical characterizations of the CIP-DXT-SDS nanoplex

The size, polydispersity index (PDI), and zeta potential of the CIP-DXT-SDS nanoplex were measured in triplicate by dynamic light scattering (DLS) after $100 \times$ dilution using a Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The nanoplex morphology was examined by field emission scanning electron microscopy (FESEM) (JSM 6700F, JEOL, Japan) using the air-dried nanoplex. The CIP payload, which we defined as the amount of CIP per unit mass of the CIP-DXT-SDS nanoplex, was determined in triplicate by dissolving a known mass of the nanoplex in a 0.4% (w/v) acetic acid solution. Afterwards, the amount of CIP in

the solution was determined by UV–vis spectrophotometry (UV Mini 1240, Shimadzu, Japan) at the optimum absorbance wavelength for CIP (324 nm).

The presence of CIP in the CIP-DXT-SDS nanoplex and its complexation with DXT and SDS were examined by Fourier transform infrared spectroscopy (FTIR) using a Spectrum One FTIR Spectrometer (Perkin-Elmer, USA). The spectral range was 500 to 4000 cm⁻¹ and the spectral resolution was 1 cm⁻¹. FTIR analyses were also performed for native CIP, the CIP-DXT nanoplex, the insoluble CIP-SDS complex, SDS, and DXT. The preparation efficiency of the CIP-DXT-SDS nanoplex was characterized by the complexation efficiency (CE) of CIP (Equation 6-5). The mass of CIP that was transformed to the CIP-DXT-SDS nanoplex in Equation 6-5 was determined in triplicate from the difference between the initial mass of CIP added and the mass of CIP recovered in the supernatant after the first centrifugation of the nanoplex suspension. The amount of CIP recovered in the supernatant was determined by UV–vis spectrophotometry at 324 nm.

Equation 6-5: Definition of CE

$$\text{CE of CIP \% w/w} = \frac{\text{Mass of CIP transformed to the CIP – DXT – SDS nanoplex}}{\text{Initial mass of CIP added}} \times 100 \%$$

6.2.2.3 Solid-state characterizations of the CIP-DXT-SDS nanoplex

The crystalline/amorphous solid state of the CIP-DXT-SDS nanoplex was examined by powder X-ray diffraction (PXRD) between 10° and 70° (2 θ) with a step size of 0.02°/s using a D8 Advance X-ray diffractometer (Bruker, Germany). The thermal behaviour of the CIP-DXT-SDS nanoplex was examined by thermo-gravimetric analysis (TGA) (Pyris Diamond, Perkin-Elmer, USA) and differential scanning calorimetry (DSC) (DSC 822E, Mettler Toledo, USA). The TGA analysis was performed between 25 and 400°C with a heating rate of 10°C/min, whereas the DSC analysis was performed

between 25 to 300°C with a heating rate of 2°C/min. PXRD and DSC analyses were also performed for native CIP, the CIP-DXT nanoplex, the insoluble CIP-SDS complex, SDS, and DXT.

6.2.2.4 Release studies

The dissolution characteristics of the CIP-DXT-SDS nanoplex under sink conditions were examined in triplicate in simulated gastric juice (SGJ) and simulated intestinal juice (SIJ). Briefly, the SGJ was prepared following the paper [260] from 0.2% (w/v) aqueous NaCl solution adjusted to pH 1.2 by addition of 1.0 M HCl. The SIJ was prepared following [198] from 0.9% (w/v) aqueous KH₂PO₄ solution adjusted to pH 6.8 by addition of 0.2 M KOH. A solution of the CIP-DXT-SDS nanoplex at a concentration of one-quarter of the saturation solubility of native CIP was placed in a dialysis bag (cellulose membrane tubing with MW cut-off of 14 kDa, Sigma Aldrich). The dialysis bag was placed in a beaker containing 100 ml of SGJ (or SIJ) in a shaking incubator at 37°C. We verified that diffusion through the dialysis membrane was not the rate limiting step using an aqueous solution of native CIP. The saturation solubilities of native CIP in the SGJ and SIJ were approximately 20 and 0.12 mg/ml, respectively.

At specific time points over a 24 h period, 1-ml aliquots were withdrawn and the same volume of fresh SGJ or SIJ was added to the beaker for replenishment. The aliquot was then filtered through a 0.22µm syringe filter and centrifuged at 14,000 × g for 3min. Subsequently, the amount of CIP in the supernatant was determined by high-performance liquid chromatography with a detection wavelength of 324 nm. The high-performance liquid chromatography was carried out on an Agilent 1100 (Agilent Technologies, Singapore) using a ZORBAX Eclipse Plus C18 column (250 × 4.6 mm, 5µm particle size). The mobile phase was a mixture of an aqueous solution of 0.1 M KH₂PO₄,

acetonitrile, and methanol (60:25:15, v/v/v) with a flow rate of 1 ml/min. The CIP retention time was approximately 5 min. Dissolution of native CIP and the CIP-DXT nanoplex were characterized following the same protocols.

6.2.2.5 Statistical Analysis

The statistical analysis was performed using one way ANOVA with Tukey post hoc test using OriginPro 2018b software.

6.3 Results and discussion

6.3.1 CIP-DXT-SDS nanoplex preparation

6.3.1.1 Effects of $R_{DXT:SDS}$ on size and zeta potential

The feasibility of forming a ternary nanoplex of CIP-DXT-SDS by electrostatically driven co-complexation of CIP with DXT and SDS was first investigated by gradually increasing the amount of SDS charged in the original CIP-DXT nanoplex formulation. This is denoted by a decrease ($P < 0.05$) in the $R_{DXT:SDS}$ value from 100:0 to 10:90. Without SDS (i.e., $R_{DXT:SDS} = 100:0$), the CIP-DXT nanoplex, which represented the control run, had a size of 111 ± 12 nm ($PDI = 0.149 \pm 0.004$) and zeta potential of -39 ± 4 mV. The negative zeta potential indicated that anionic DXT and SDS were present on the nanoplex surface (Figure 6-4(a)).

After addition of SDS, the sizes of the co-complexation products remained ($P > 0.05$) in the nanometer range (110–150 nm, $PDI = 0.155–0.350$) up to $R_{DXT:SDS} = 40:60$ (Figure 6-4(a)). A further decrease in the $R_{DXT:SDS}$ to 30:70 or 20:80 led to formulation of larger ($P < 0.05$) nanoparticles (220–290nm). The zeta potential gradually decreased ($P < 0.05$) with decreasing $R_{DXT:SDS}$ to reach -24 ± 2 mV at $R_{DXT:SDS} = 80:20$ and -16 ± 2 mV at $R_{DXT:SDS} = 20:80$ (Figure 6-4(a)). The large change in zeta potential upon the addition of SDS signified successful incorporation of SDS into the co-complexation

products, where its presence as an anionic surfactant altered the surface charge distribution of the nanoparticles, which was previously dominated by DXT. The trends in the sizes and zeta potentials as a function of $R_{DXT:SDS}$ hinted at successful formulation of the ternary CIP-DXT-SDS nanoplex by the CIP co-complexation technique, which we confirmed later by solid-state characterization. On this note, the experiment performed at $R_{DXT:SDS} = 10:90$ led to formulation of considerably larger ($P < 0.05$) particles in the micrometer range. These are not plotted in Figure 6-4(a) for clarity. A similar result ($P < 0.05$) was observed for the control run without DXT (i.e., $R_{DXT:SDS} = 0:100$), where the insoluble CIP-SDS complex size was between 1 and 6 μm (data not plotted for clarity).

Figure 6-4: Effects of $R_{DXT:SDS}$ on (a) size and zeta potential, and (b) CIP payload and complexation efficiency of the CIP-DXT-SDS nanoplex.

The large size of the insoluble CIP-SDS complex suggested that in the absence of DXT, or in the presence of insufficient DXT, the CIP-SDS complex would continue to grow after its nucleation and form large precipitates. By contrast, the presence of the CIP-DXT complex at high $R_{DXT:SDS}$ inhibited the growth of the CIP-SDS complex by virtue of the reduced CIP-SDS complex formulation because of competition with the CIP-DXT formulation. The hindered spatial interactions among the CIP-SDS

complexes in the presence of long chains of the CIP-DXT complex also resulted in diminished growth ($P < 0.05$).

6.3.1.2 Effects of $R_{DXT:SDS}$ on CIP complexation efficiency and payload

The addition of SDS up to $R_{DXT:SDS} = 30:70$ had a minimal impact ($P > 0.05$) on the CIP payload of the CIP-DXT-SDS nanoplex. Specifically, the CIP payloads were between 54% and 62% (w/w) and the variations as a function of $R_{DXT:SDS}$ were statistically insignificant ($P > 0.05$) (Figure 6-4 (b)). Further decreases in the $R_{DXT:SDS}$ to 20:80 and 10:90 led to lower CIP payloads ($P < 0.05$) of $47\% \pm 2\%$ and $42\% \pm 6\%$ (w/w), respectively. For comparison, the CIP payloads of the control runs with the CIP-DXT nanoplex ($R_{DXT:SDS} = 100:0$) and the insoluble CIP-SDS complex ($R_{DXT:SDS} = 0:100$) were $61\% \pm 1\%$ and $39\% \pm 4\%$ (w/w), respectively. These results showed that the presence of sufficient DXT was crucial both to maintaining the co-complexation product size in the nanometer range and ensuring the products exhibited high CIP payloads.

For the preparation efficiency, the inclusion of SDS resulted in a higher CE ($P < 0.05$) for the CIP, which increased from $84\% \pm 2\%$ at $R_{DXT:SDS} = 100:0$ to $93\% \pm 2\%$ at $R_{DXT:SDS} = 90:10$. This indicated the CIP utilization rate increased. The higher CE was not unexpected as the addition of SDS provided another option for CIP complexation besides DXT (Figure 6-4(b)). However, increasing the SDS presence by decreasing $R_{DXT:SDS}$ to below 90:10 had minimal impact ($P > 0.05$) on the CE of CIP. The CE values remained in the narrow range of 91% to 93% for $90:10 \leq R_{DXT:SDS} \leq 10:90$.

The constant CE as a function of $R_{DXT:SDS}$ suggested that CIP, in a binary system where both DXT and SDS were available for complexation, did not exhibit a specific

preference for complexation towards DXT or SDS. Hypothetically, if CIP had exhibited preference for complexation towards DXT, the CE of CIP would have decreased at lower $R_{DXT:SDS}$ because of the lack of DXT available for complexation. The non-preference of the CIP complexation implied that the compositions of the CIP-DXT and CIP-SDS complexes in the CIP-DXT-SDS nanoplex could be manipulated by varying the $R_{DXT:SDS}$ used in its preparation. A lower $R_{DXT:SDS}$ would result in a higher proportion of the CIP-SDS complex in the ternary nanoplex.

6.3.2 FESEM and FTIR

The nanometer size of the CIP-DXT-SDS nanoplex was verified by FESEM using the co-complexation products prepared at $R_{DXT:SDS} = 80:20$ and $60:40$ as representative samples. The FESEM images (Figure 6-5) showed the presence of distinct individual nanoparticles with roughly spherical shapes and diameters between 50 and 100 nm. This was in agreement with the particle size obtained by DLS. The morphology of the CIP-DXT-SDS nanoplex resembled that exhibited by the CIP-DXT nanoplex in a previous study by Cheow and Hadinoto [33].

Figure 6-5: FESEM images of the CIP-DXT-SDS nanoplex prepared at (a) $R_{DXT:SDS} = 80:20$, and (b) $R_{DXT:SDS} = 60:40$.

The presence of CIP in the CIP-DXT-SDS nanoplex and its co-complexation with DXT and SDS were verified by FTIR using the co-complexation product prepared

at $R_{\text{DXT:SDS}} = 60:40$ as the representative sample (Figure 6-6 (a)). First, the presence of CIP was verified by the appearance of the peak at 1628cm^{-1} in the FTIR spectrum of the CIP-DXT-SDS nanoplex, which corresponded to C=O stretching of the pyridone functional group that was unique to CIP [261-264]. The C=O stretching of the pyridone shifted slightly from 1621 cm^{-1} [262] in the native CIP spectrum because of protonation of CIP in the CIP-DXT-SDS nanoplex. As expected, the C=O stretching on the pyridone also appeared at 1628 m^{-1} in the FTIR spectra of the CIP-DXT nanoplex (Figure 6-6 (a)) and the insoluble CIP-SDS complex (Figure 6-6(b)).

Figure 6-6: FTIR spectra of the (a) native CIP, CIP-DXT-SDS nanoplex, and CIP-DXT nanoplex; and (b) insoluble CIP-SDS complex, SDS, and DXT.

Next, the complexation between CIP and DXT was verified in the FTIR spectrum of the CIP-DXT-SDS nanoplex from the disappearance of the peak at 1585 cm^{-1} that corresponded to the NH_2 bending of the piperazine group of CIP [265]. The disappearance of the amine peak, which was present in the native CIP spectrum, was caused by electrostatic bonding between the protonated NH_2^+ group of CIP and the SO_3^- group of DXT. This was characterized by the peak at 1158 cm^{-1} in the DXT

spectrum (Figure 6-6 (b)). Not unexpectedly, a similar disappearance of the amine peak at 1585 cm^{-1} was also observed in the FTIR spectrum of the CIP-DXT nanoplex.

Finally, the complexation between CIP and SDS took place via a similar mechanism in which the protonated amine group of CIP formed electrostatic bonds with the sulfonyl group of SDS, as reported earlier by Türeli et al. [146]. This was evident from a similar disappearance of the amine peak at 1585 cm^{-1} in the FTIR spectrum of the insoluble CIP-SDS complex.

The presence of SDS in the CIP-DXT-SDS nanoplex was evident from the appearance of peaks at 1095 and 1229 cm^{-1} corresponding to the symmetric and asymmetric stretching of the S=O group of SDS, respectively [266]. These peaks were reduced in intensity and shifted slightly from the 1088 and 1220 cm^{-1} positions observed in the FTIR spectra of the raw SDS and the insoluble CIP-SDS complex (Figure 6-6 (b)). These shifts were likely caused by the overlapping presence of DXT and SDS in the ternary nanoplex. As expected, these peaks were not present in the FTIR spectrum of the CIP-DXT nanoplex that did not contain SDS.

6.3.3 Solid-state characterization

6.3.3.1 PXRD

The PXRD analysis was performed for CIP-DXT-SDS nanoplexes prepared at $R_{\text{DXT:SDS}} = 80:20$, $60:40$, and $40:60$ to investigate the impact of the presence of the CIP-SDS complex in the ternary nanoplex on its solid state characteristics. The results showed that crystalline peaks were present in the PXRD patterns of the CIP-DXT-SDS nanoplex prepared at $R_{\text{DXT:SDS}} = 80:20$ and $60:40$ at $2\theta = 19.5^\circ$ and 23.8° , respectively. The crystalline peaks were accompanied by amorphous halos at approximately $2\theta = 27^\circ$ (Figure 6-7(a)). Notably, the crystalline peaks of the CIP-DXT-SDS nanoplex were

distinct from the peaks observed in the PXRD pattern of native CIP (Figure 6-7(b)), signifying that CIP complexation had indeed taken place. By contrast, the PXRD pattern of the CIP-DXT nanoplex exhibited broad halos over a large 2θ range, which reflected its amorphous form as previously reported [33] (Figure 6-7(a)).

Figure 6-7: (a) PXRD patterns of the (a) CIP-DXT nanoplex, CIP-DXT-SDS nanoplexes prepared at $R_{DXT:SDS}=80:20$, $60:40$, and $40:60$; (b) native CIP, SDS, DXT, and insoluble CIP-SDS complex.

The crystalline peak of the CIP-DXT-SDS nanoplex at $2\theta = 19.5^\circ$ also appeared in the PXRD pattern of the insoluble CIP-SDS complex (Figure 6-7(b)). This indicated that the crystallinity of the CIP-DXT-SDS nanoplex was attributed to the CIP-SDS complex, while the CIP-DXT complex remained amorphous. Significantly, the crystalline peaks of the CIP-DXT-SDS nanoplex became more pronounced at lower $R_{DXT:SDS}$, reaffirming that the crystallinity was attributed to the CIP-SDS complex, whose presence supposedly increased at lower $R_{DXT:SDS}$. In fact, the PXRD pattern of the CIP-DXT-SDS nanoplex prepared at $R_{DXT:SDS} = 40:60$ showed an additional sharp crystalline peak when 2θ was approximately 9.5° (Figure 6-7(a)). The same peak was present in the PXRD patterns of native SDS and the insoluble CIP-SDS complex (Figure 6-7(b)). Therefore, the PXRD results confirmed that the compositions of the

CIP-DXT and CIP-SDS complexes in the ternary CIP-DXT-SDS nanoplex could be controlled by varying $R_{\text{DXT:SDS}}$.

6.3.3.2 TGA/DSC

The thermal behaviour of the CIP-DXT-SDS nanoplex was examined by TGA/DSC using the nanoplex prepared at $R_{\text{DXT:SDS}} = 60:40$ as a representative sample. Notably, the TGA results showed that the CIP-DXT-SDS nanoplex, the CIP-DXT nanoplex, and the insoluble CIP-SDS complex underwent significant decomposition at 260°C (data not shown). Hence, the DSC thermograms obtained at $>260^\circ\text{C}$ were not included in our analysis. The DSC thermograms (Figure 6-8) showed that the CIP-DXT-SDS nanoplex exhibited distinct thermal behaviour from that exhibited by native CIP, the CIP-DXT nanoplex, and the insoluble CIP-SDS complex.

Figure 6-8: DSC thermograms of the native CIP, CIP-DXT-SDS nanoplex, CIP-DXT nanoplex, insoluble CIP-SDS complex, physical mixture of CIP-DXT nanoplex, and insoluble CIP-SDS complex, DXT, and SDS.

Furthermore, the DSC thermogram of the ternary nanoplex was also distinct from that exhibited by the physical mixture of CIP-DXT nanoplex and CIP-SDS complex. Specifically, the CIP-DXT-SDS nanoplex exhibited a glass transition point at around 125°C, followed by a crystallization point at around 166°C, denoting its partially amorphous form, which was in agreement with the PXRD results. The native DXT showed a broad exothermic peak at the range 140 to 180 °C, which is due to its decomposition [267]. No exothermic peak happened at around 144 °C in the DSC curve of native CIP and native SDS. The native SDS showed an endothermic peak at around 90 to 120 °C . The CIP-DXT-SDS nanoplex showed an endothermic peak at around 125 °C which is not as big as the endothermic peak at around 90 to 120 °C of SDS, which is due to the decomposition of DXT. The CIP-DXT nanoplex showed an

exothermic peak at around 144 °C due to the decomposition of DXT molecules in the CIP-DXT nanoplex [267], followed by a melting point at around 182 °C, without undergoing crystallization first.

Comparison with the insoluble CIP-SDS complex and the physical mixture showed that both of their thermograms exhibited a melting point with decomposition at around 195°C, and they were distinct from the thermogram exhibited by the CIP-DXT-SDS nanoplex. The distinct DSC thermogram of the CIP-DXT-SDS nanoplex compared with the rest showed that the ternary nanoplex was successfully produced. Notably, all three complex systems (i.e., CIP-DXT, CIP-SDS, and CIP-DXT-SDS) did not exhibit the same melting point as native CIP (270°C). This further confirms that CIP complexation took place.

6.3.4 Dissolution characteristics

The dissolution time-profile of the CIP-DXT-SDS nanoplex in the SGJ was characterized for nanoplexes prepared at $R_{DXT:SDS} = 90:10$ to $60:40$ to examine the impact of varying the presence of the CIP-SDS complex in the ternary nanoplex on its dissolution characteristics at acidic pH. In agreement with our hypothesis, the CIP-DXT-SDS nanoplex exhibited improved sustained release characteristics at acidic pH compared with its CIP-DXT nanoplex counterpart. The sustained release means slower release, which is able to prolong the release time needed to achieve 100 % release. As expected, the degree of improvement increased with increasing SDS (i.e., lower $R_{DXT:SDS}$). This is discussed in more detail below. First, we demonstrated that the CIP-DXT nanoplex (without SDS) was able to slow down the CIP release in SGJ compared with native CIP ($P < 0.05$). Specifically, CIP payloads of $34\% \pm 3\%$ and $76\% \pm 5\%$ (w/w) were released from the CIP-DXT nanoplex after 1 and 5 h, respectively. This can

be compared with 100% dissolution for native CIP after just 1 h (Figure 6-9(a)). The slower CIP dissolution ($P < 0.05$) from the CIP-DXT nanoplex was attributed to the lower ($P < 0.05$) solubility of the CIP-DXT nanoplex in SGJ, which was approximately 2.8 mg/ml compared with approximately 20 mg/ml for native CIP (Table 6-3). The lower CIP solubility of the nanoplex was caused by the lack of contribution from the protonatable amino group of CIP in the nanoplex because of its electrostatic bonding to DXT. The addition of SDS into the CIP nanoplex began to have an impact on the CIP dissolution at $R_{DXT:SDS} = 80:20$. At this point, the resultant CIP-DXT-SDS nanoplex exhibited slower dissolution with rates of $26\% \pm 4\%$ and $55\% \pm 2\%$ after 1 and 5 h, respectively (Figure 6-9(a)). Nearly 100% dissolution of the CIP-DXT-SDS nanoplex was achieved only after 21 h. Increasing the SDS presence in the nanoplex by lowering $R_{DXT:SDS}$ to 70:30 or 60:40 resulted in even slower CIP dissolution, albeit slightly, of $48\% \pm 3\%$ and $49\% \pm 3\%$ after 5 h ($P > 0.05$), respectively. Nearly 100% dissolution was achieved only after 26 and 29 h, respectively, for the CIP-DXT-SDS nanoplexes prepared at $R_{DXT:SDS} = 70:30$ and 60:40 (data not shown).

The slower CIP dissolution from the CIP-DXT-SDS nanoplex was attributed to its lower solubility ($P < 0.05$) in SGJ, which was approximately 1.7mg/ml for the CIP-DXT-SDS nanoplex prepared at $R_{DXT:SDS} = 60:40$ versus approximately 2.8 mg/ml for the CIP-DXT nanoplex (Table 6-3). Coincidentally, the dissolution time-profile of the CIP-DXT-SDS nanoplex in the SGJ was highly comparable to the dissolution rate exhibited by existing CIP gastroretentive formulations at approximately 60% dissolution after 8h [241, 243]. This similarity bodes well for the CIP-DXT-SDS nanoplex's potential as an effective bioavailability enhancement strategy of CIP.

Figure 6-9: Dissolution time-profiles of the CIP-DXT-SDS nanoplexes prepared at RDXT:SDS = 90:10 to 60:40 in (a) SGJ and (b) SIJ.

As the CIP dissolution in SGJ decreased greatly upon its trans-formulation into the CIP-DXT-SDS nanoplex, it was inevitable that a small part of the nanoplex would remain intact when entering the small intestine. For this reason, the dissolution time-profile of the CIP-DXT-SDS nanoplex was also characterized in SIJ to examine whether SDS inclusion had any impact on its dissolution characteristics at intestinal pH. The results showed that the CIP-DXT-SDS nanoplex exhibited similar dissolution characteristics in SIJ as the CIP-DXT nanoplex, where dissolution rates of nearly 50% and 90% were achieved after 1 and 5 h, respectively (Figure 6-9(b)). Similar results, which signified the minimal impact of SDS inclusion on CIP dissolution in the SIJ, were observed for all CIP-DXT-SDS nanoplexes independent of RDXT:SDS.

Table 6-3: The solubilities of native CIP, the CIP-DXT nanoplex, and the CIP-DXT-SDS nanoplex prepared at RDXT:SDS = 60:40 in SGJ (pH 1.2) and SIJ (pH 6.8).

CIP form	Solubility in SGJ (pH 1.2) (mg/ml)	Solubility in SIJ (pH 6.8) (mg/ml)
Native CIP	20 ± 1	0.12 ± 0.02
CIP-DXT nanoplex	2.80 ± 0.20	0.46 ± 0.04
CIP-DXT-SDS nanoplex (RDXT:SDS = 60:40)	1.72 ± 0.02	0.33 ± 0.04

Furthermore, both nanoplexes exhibited much faster dissolution rates ($P < 0.05$) in SIJ than native CIP, which had a dissolution rate of only $37\% \pm 4\%$ after 5h. The faster dissolution rates of the nanoplexes were attributed to their higher solubilities ($P < 0.05$) in SIJ, which were approximately 0.33–0.46 mg/ml versus approximately 0.12 mg/ml for native CIP (Table 6-3). In short, the above results demonstrated that the improved sustained release characteristic exhibited by the CIP-DXT-SDS nanoplex in the SGJ were not obtained at the expense of its slower dissolution rate in SIJ, which was desirable in the event that some of the nanoplex remained intact after entering the small intestine.

6.3 Conclusions

We successfully prepared a ternary CIP-DXT-SDS nanoplex by co-complexation of CIP with DXT and SDS. This involved simple mixing of the CIP, DXT, and SDS solutions under ambient conditions. FTIR and PXRD analysis showed that the CIP-DXT-SDS nanoplex was made up of an amorphous CIP-DXT complex and crystalline CIP-SDS complex, resulting in its partially crystalline state. Nevertheless, the DSC analysis showed the thermal behaviour of the CIP-DXT-SDS nanoplex was distinct from the behaviour of its components, and indicated successful formulation of a new physical entity. Second, the compositions of the CIP-DXT and CIP-SDS complexes in the ternary nanoplex could be controlled by varying the $R_{DXT:SDS}$ used in its preparation. A lower $R_{DXT:SDS}$ would lead to a higher content of the CIP-SDS complex in the nanoplex. The lowest workable $R_{DXT:SDS}$ was 20:80, and below this, micrometer-sized particles were produced because of the predominant formulation of the CIP-SDS complex. Third, the CIP-DXT-SDS nanoplex had a size of 110 to 290 nm, zeta potential of -16 to -39 mV, and CIP payload in the range of 47% to 62%, depending on $R_{DXT:SDS}$.

The CIP-DXT-SDS nanoplex was prepared at a utilization rate of more than 90% CIP independent of $R_{DXT:SDS}$, which showed it had a high preparation efficiency. Fourth, the CIP-DXT-SDS nanoplex exhibited superior sustained release characteristics in SGJ compared with native CIP and the CIP-DXT nanoplex, which was attributed to its lower solubility at acidic pH. Moreover, the dissolution time-profile of the CIP-DXT-SDS nanoplex in SGJ was highly comparable with that exhibited by existing CIP gastroretentive formulations at about 60% dissolution after 8 h. In conclusion, using CIP as a model drug, we successfully prepared a ternary nanoplex and its counterions as an attractive formulation strategy to enhance the bioavailability of drugs with narrow absorption windows and pH-dependent solubility. We developed a simple yet highly efficient preparation that results in sustained release characteristics.

6.4 Acknowledgements

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CHAPTER 7 Enhancing the physical stability and supersaturation generation of amorphous drug-polyelectrolyte nanoparticle complex via incorporation of crystallization inhibitor at the nanoparticle formulation step: A case of HPMC versus PVP

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Abstract

Amorphous drug-polyelectrolyte nanoparticle complex (or nanoplex in short) has emerged as a highly attractive solubility enhancement strategy of poorly-soluble drugs attributed to its simple and highly efficient preparation. The existing nanoplex formulation, however, exhibits poor amorphous form stability during long-term storage for drugs with high crystallization propensity. Using ciprofloxacin (CIP) and sodium dextran sulfate (DXT) as the model drug-polyelectrolyte nanoplex, we investigated the feasibility of incorporating crystallization inhibiting agents, i.e. hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP), at the nanoplex formulation step to improve the physical stability of the CIP nanoplex. The effects of the HPMC or PVP additions on the nanoplex’s physical characteristics (i.e. size, zeta potential, CIP payload), CIP utilization rate, dissolution rate, and supersaturation generation were also examined. The results showed that the additions of HPMC or PVP increased the CIP nanoplex size (from 300 to 500 nm) and CIP utilization rate (from 65% to 90% w/w)

with minimal impacts on the CIP payload (70-80% w/w). Their additions had opposite impacts on the nanoplex's colloidal stability due to surfactant [269, 270] nature of PVP. Significantly, unlike the CIP-DXT and CIP-DXT-PVP nanoplexes, the CIP-DXT-HPMC nanoplex remained amorphous after three-month accelerated storage, while also exhibited superior solubility enhancement (15-30% higher).

7.1 Introduction

Estimated more than forty percent of drugs currently in the market [271] and nearly seventy percent of drug candidates in the pharmaceutical pipeline [94] exhibit low aqueous solubility, leading to their low oral bioavailability and consequently diminished therapeutic efficacy. Amorphous drug-polyelectrolyte nanoparticle complex (or nanoplex in short) was recently established as an effective solubility enhancement strategy of poorly soluble weak acid/base drugs, attributed to its ability to generate a highly supersaturated drug concentration by virtue of its metastable amorphous form and nanoscale size [272]. Solubility enhancement capability of the amorphous drug nanoplex has been demonstrated for a large number of drugs ranging from antibiotics (e.g. fluoroquinolones, vancomycin) [34, 273], antifungal itraconazole [215], and anti-inflammatory ibuprofen [272], to anticancer doxorubicin [214], antiparkinson ropinirole [274], antipsychotic perphenazine [1], and medicinal herbs (e.g. curcumin, silibinin) [32, 35].

The amorphous drug nanoplex was prepared by a simple technique based on electrostatically driven complexation between ionized drug molecules and oppositely charged polyelectrolytes (PE) [33]. Briefly, soluble drug-PE complexes were formed upon mixing of the drug and PE solutions by virtue of drug-PE electrostatic interactions. The soluble drug-PE complex subsequently aggregate among themselves due to the

inter-drug hydrophobic interactions between the bound drug molecules, resulting in the formulation of drug-PE complex aggregates. Upon reaching a critical mass, the complex aggregates precipitate out of the solution to form the drug nanoplex. The restricted molecular mobility of the drug molecules due to the electrostatic binding with the PE prevent them from re-arranging to ordered crystalline structure upon their precipitation.

In comparison to the other solubility enhancement strategies (e.g. size comminution, salt formulation, cyclodextrin inclusion, amorphous solid dispersion, co-amorphous systems) [19, 40, 275-277], the amorphous drug nanoplex is superior in terms of the preparation owed to its (1) simple, fast, and cost-effective preparation that involves only the mixing of drug and PE solutions under ambient condition, and (2) high drug utilization rates (> 80%), resulting in minimal drug wastage. Moreover, the amorphous drug nanoplex was demonstrated to exhibit superior supersaturation generation to the more conventional amorphous drug formulations (e.g. solid dispersion, surfactant-stabilized nanoparticles), albeit in limited studies using curcumin and itraconazole as the model poorly-soluble drugs [215, 278].

Despite its highly desirable characteristics with regard to the preparation and solubility enhancement, the physical stability of the amorphous drug nanoplex during long-term storage has not been fully examined. Long-term physical stability is mandatory for any amorphous drug formulations to be recognized as having a commercially viable shelf life [279]. A diminished stability of the amorphous form during storage, where the drug nanoplex undergoes crystallization, would cause it to lose its solubility enhancement capability.

While the long-term amorphous form stability of the drug nanoplex has been demonstrated for a number of drugs (i.e. itraconazole, perphenazine) [1, 215], the stabilization afforded by the electrostatic binding with the PE may not be adequate for drugs with high crystallization tendency [280, 281]. One example of such drug is antibiotic ciprofloxacin (CIP), which exists in the solid state as zwitterionic molecules with cationic piperazine amino group and anionic carboxylate group [250, 251]. The strong intermolecular interaction between the two charged groups render the amorphous CIP nanoplex exhibit high crystallization tendency during storage [248].

While crystallization inhibiting agents, typically polymers with high glass transition temperature, such as hydroxypropylmethylcellulose (HPMC) and polyvinylpyrrolidone (PVP) [282], could be added at the solid dosage form preparation steps of the nanoplex (e.g. granulation, tableting), this approach would present an additional challenge to the formulation scientists as they have to take into account the solid-state miscibility of the drug nanoplex with the polymers at each preparation step. In this regard, our previous study showed that achieving long-term stability of the CIP nanoplex required it to be stored as 1:1 lyophilized mixture of CIP nanoplex and HPMC [248]. Another study using curcumin as the model drug showed that a large amount of crystallization inhibitors (> 50% w/w) was needed to stabilize the curcumin nanoplex, hence inevitably lowering the drug payload in the final formulation [283].

Herein we proposed an alternative approach in which we incorporated the crystallization inhibitors (i.e. HPMC or PVP) at the nanoplex formulation step aimed at equipping the nanoplex with inherent physical stability, as illustrated in Figure 7-1. Using CIP as the model drug with sodium dextran sulfate (DXT) as the PE pair, we evaluated the feasibility of forming CIP nanoplex stabilized by both the PE and HPMC

(or PVP) at different mass ratios of polymers to CIP. Subsequently, we assessed the impacts of the HPMC and PVP inclusions on the physical characteristics (i.e. size, zeta potential, and CIP payload), CIP utilization rate, and amorphous form of the CIP nanoplex produced. Lastly, we evaluated the effectiveness of HPMC and PVP in their abilities (i) to improve the amorphous form stability of the CIP nanoplex during long-term storage, and (ii) to enhance the supersaturation generation of the CIP nanoplex.

Figure 7-1: Schematics of the incorporation of HPMC or PVP into the CIP-DXT nanoplex to produce amorphous ternary CIP-DXT-HPMC and CIP-DXT-PVP nanoplexes.

7.2 Materials and Methods

7.2.1 Materials

CIP ($\geq 98\%$), HPMC (MW = 26 kDa), PVP (MW = 40 kDa), glacial acetic acid (AA), potassium hydroxide (KOH), methanol, acetonitrile, sodium chloride (NaCl), and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma-Aldrich (Singapore). Sodium dextran sulfate (DXT) MW = 5 kDa was purchased from Wako Pure Chemical (Japan).

7.2.2 Methods

7.2.2.1 Preparation of CIP-DXT-HPMC and CIP-DXT-PVP nanoplexes

Briefly, CIP having pKa 6.1 and 8.6 was dissolved at 5 mg/ml in 0.4% (w/v) AA solution (pH 3) to form cationic CIP molecules. DXT having pKa < 2 was added to deionized water at a fixed DXT concentration of 2.4 mg/ml together with HPMC of varying concentration. The (DXT + HPMC) solution was then vortexed for 30 min to completely dissolve the HPMC. The amount of HPMC added was varied at mass ratios of HPMC to CIP ($M_{\text{HPMC}/\text{CIP}}$) equal to 1, 2, 4, and 8. At this condition, the charge ratio of CIP to DXT was equal to 1.3, hence a sufficient amount of anionic DXT molecules were available to form electrostatic binding with the cationic CIP molecules. The sample calculations for the charge ratio of CIP to DXT was presented earlier [33].

Subsequently, the CIP and (DXT + HPMC) solutions were mixed at equal volumes under gentle stirring. The resultant CIP-DXT-HPMC nanoplex suspension underwent three cycles of centrifugation ($14,000 \times g$ for 10 min) and washing to remove excess CIP, DXT, and HPMC that did not form the nanoplex. The washed CIP-DXT-HPMC nanoplex suspension was then re-suspended in deionized water for physical characterizations. The CIP-DXT-PVP nanoplex was prepared by the same procedures at mass ratios of PVP to CIP ($M_{\text{PVP}/\text{CIP}}$) equal to 1, 2, 4, and 8. Control experiments in which HPMC and PVP solutions without DXT were added to the CIP solution were performed at the abovementioned $M_{\text{HPMC}/\text{CIP}}$ and $M_{\text{PVP}/\text{CIP}}$ values. The CIP-DXT nanoplex was prepared by the same procedures with the omission of HPMC or PVP in the DXT solution.

7.2.2.2 Physical characterizations

The size (i.e. volume-averaged diameter), zeta potential, and polydispersity index (PDI) of the CIP nanoplexes in their aqueous suspension form were characterized in triplicates after $100 \times$ dilution by dynamic light scattering (DLS) using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The morphology was examined by field emission scanning electron microscope (FESEM) (JSM 6700F, JEOL, USA) using air-dried nanoplex suspension as the sample. The existence of CIP in the nanoplex was examined by Fourier transform infrared spectroscopy (FTIR) using Spectrum One FT-IR Spectrometer (Perkin-Elmer, USA). The FTIR analysis was performed between 500 and 4000 cm^{-1} at 1 cm^{-1} spectral resolution. The FTIR analysis was also performed for the native CIP, free forms of HPMC, PVP, and DXT, as well as the physical mixtures of the CIP- DXT nanoplex and free HPMC (or free PVP) at mass ratio equal to unity.

The CIP payload defined as the amount of CIP per unit mass of the nanoplex was determined in triplicates by dissolving a known mass of the CIP nanoplex in 50% (v/v) aqueous AA solution. Afterwards, the amount of CIP in the solution was determined by high performance liquid chromatography (HPLC) at detection wavelength of 324 nm . The HPLC was carried out in Agilent 1100 (Agilent Technologies, Singapore) using ZORBAX Eclipse Plus C18 column ($250 \times 4.6\text{ mm}$ and $5\text{-}\mu\text{m}$ particle size). Aqueous solution of $0.1\text{ M KH}_2\text{PO}_4$: acetonitrile: methanol at 60: 25: 15 (% v/v) was used as the mobile phase at flow rate of 1 mL/min , resulting in the CIP retention time at approximately 5 min . The CIP utilization rate was determined in triplicates following Eq. 1, where the mass of CIP that formed the CIP nanoplex was determined from the difference between the initial mass of CIP added and the mass of

CIP recovered in the supernatant after the first centrifugation of the CIP nanoplex suspension. The amount of CIP in the supernatant was determined by HPLC as previously described.

Equation 7-1: Definition of CIP utilization rate

$$\text{CIP Utilization rate (\% w/w)} = \frac{\text{Mass of CIP that formed nanoplex}}{\text{Initial mass of CIP added}} \times 100 \%$$

The amorphous form of the CIP nanoplexes immediately after their preparation was verified by powder x-ray diffraction (PXRD) performed between 10° and 70° (2θ) with a step size of 0.02°/s using D8 Advance X-ray Diffractometer (Bruker, Germany). The glass transition point of the amorphous CIP nanoplexes was examined by differential scanning calorimetry (DSC) (DSC 822E, Mettler Toledo, USA) performed at heating rate of 2 °C/min between 25 °C to 300 °C. Thermogravimetric analysis (TGA) was performed at heating rate of 10 °C/min between 25°C and 400 °C (Pyris Diamond, Perkin-Elmer, USA). The PXRD, DSC, and TGA analysis were also performed for the native CIP, and the free forms of DXT, HPMC, and PVP.

7.2.2.3 Physical characterizations

The dry-powder forms of the CIP nanoplexes were prepared by 48 h lyophilization without cryoprotectants at -52 °C and 0.05 mbar in Alpha 1-2 LD Plus (Martin Christ, Germany). The CIP nanoplex powders were stored for three months in an open glass container placed in a desiccator conditioned at 40 °C and 75% relative humidity after which the PXRD analysis of the stored nanoplexes was performed. The 75% relative humidity in the desiccator was generated by equilibrating saturated NaCl solution at 40°C. The accelerated storage at 40°C and 75% humidity for three months was used to simulate twelve-month storage under normal condition at 25 °C and 60% humidity [284].

7.2.2.4 Dissolution characteristics under sink condition

The dissolution time-profiles of the CIP nanoplexes in their dry-powder forms were characterized in triplicates under sink condition in simulated intestinal juice (SIJ). The SIJ was prepared following [198] from 0.9% (w/v) aqueous KH_2PO_4 solution adjusted to pH 6.8 by the addition of 0.2 M KOH. Briefly, the CIP nanoplex powders were added to 40 mL SIJ held at 37°C in a shaking incubator at $1/5 \times C_{\text{Sat}}$, where C_{Sat} represented the thermodynamic saturation solubility of CIP in the SIJ determined previously to be equal to 0.12 mg/mL [253]. Subsequently, 0.5 mL aliquot was withdrawn at specific time points over a six-hour period and 0.5 mL fresh SIJ was added back to the dissolution vessel. The aliquot was then centrifuged at $14,000 \times g$ for 3 min after which the amount of CIP in the supernatant was determined by HPLC as previously described.

7.2.2.5 Supersaturation generation under non-sink condition

The CIP nanoplex powders were added in excess at $20 \times C_{\text{Sat}}$ to 10 mL SIJ held at 37°C in a shaking incubator. Afterwards, 300 μL aliquot was withdrawn at specific time points over 24 h. The aliquot was syringe filtered using 0.22- μm pore size and was immediately diluted hundredfold with fresh SIJ to prevent the precipitation of CIP from the supersaturated solution. The amount of CIP in the aliquot after dilution was then determined by HPLC as previously described. The supersaturation generation was reported from three replicates as the ratio of the supersaturated CIP concentration in the solution, i.e. C to C_{sat} .

7.2.2.6 Statistical Analysis

The statistical analysis was performed using one way ANOVA with Tukey post hoc test using OriginPro 2018b software.

7.3 Results and discussion

7.3.1 CIP-DXT-HPMC nanoplex

7.3.3.1 Physical characteristics

The successful incorporation of HPMC into the CIP nanoplex was evident from trends in the size and zeta potential of the CIP nanoplex produced as a function of $M_{\text{HPMC/CIP}}$. In the absence of HPMC ($M_{\text{HPMC/CIP}} = 0$), the CIP nanoplex exhibited size and zeta potential of 298 ± 7 nm ($\text{PDI} = 0.46 \pm 0.06$) and -32 ± 0.5 mV, respectively (Fig. 2A), where the negative zeta potential indicated the presence of anionic DXT on the nanoplex surface acting as colloidal stabilizer. Upon incorporation of HPMC at $M_{\text{HPMC/CIP}} = 2$, the size and zeta potential of the resultant nanoparticles were found to increase ($P < 0.05$) and decrease ($P < 0.05$), respectively, to 484 ± 4 nm and -25 ± 0.6 mV (Figure 7-2A). Further increasing $M_{\text{HPMC/CIP}}$ to 8 resulted in even larger particles with lower zeta potential at 586 ± 27 nm and -18 ± 0.2 mV, respectively, while the PDI was minimally impacted (Figure 7-2 A).

As HPMC is a nonionic polymer [285], one of the first indications that HPMC was successfully incorporated into the CIP nanoplex was in the form of a decrease in the zeta potential, as a result of the presence of nonionic HPMC that altered the surface charge distribution of the CIP nanoplex. On this note, the control run in which the CIP solution was mixed with the HPMC solution without DXT did not produce any precipitates, indicating that the interactions between CIP and HPMC (if any) did not lead to particle formulation in the absence of DXT.

In addition to its influence on the size and zeta potential, the inclusion of HPMC also led to an increase in the CIP utilization rate. Specifically, the CIP utilization rate, which was equal to $65 \pm 6\%$ (w/w) in the absence of HPMC, increased ($P < 0.05$) to 82

$\pm 5\%$ (w/w) at $M_{\text{HPMC}}/\text{CIP} = 1$ and increased further to $94 \pm 1\%$ ($P < 0.05$) at $M_{\text{HPMC}}/\text{CIP} = 8$ (Figure 7-2B). As the nanoplex was prepared at a fixed CIP concentration, the higher CIP utilization rate indicated an increasing amount of CIP present in the nanoplex at higher $M_{\text{HPMC}}/\text{CIP}$.

Figure 7-2: Effects of $M_{\text{HPMC}}/\text{CIP}$ on the (A) size and zeta potential, (B) CIP utilization rate and CIP payload of the CIP-DXT-HPMC nanoplex produced.

The increased amount of CIP in the nanoplex at higher $M_{\text{HPMC}}/\text{CIP}$, however, did not lead to an increase in the CIP payload, which remained constant at $\approx 70\text{--}75\%$ independent of $M_{\text{HPMC}}/\text{CIP}$ (Figure 7-2B). This signified a constant mass ratio of CIP to the polymers (i.e. DXT + HPMC) in the nanoplexes prepared at different $M_{\text{HPMC}}/\text{CIP}$. The constant CIP payload observed at higher $M_{\text{HPMC}}/\text{CIP}$ despite the higher CIP utilization rate was caused by the presence of HPMC that in turn increased the polymer contents in the nanoplex. In terms of the morphology, the FESEM analysis showed that the CIP-DXT-HPMC nanoplex exhibited a roughly spherical shape that was similar to that of the CIP-DXT nanoplex (Figure 7-6).

7.3.3.2 FTIR

The presence of CIP and HPMC in the CIP-DXT-HPMC nanoplex and CIP complexation with DXT were examined by FTIR using the nanoplex prepared at $M_{\text{HPMC}}/\text{CIP} = 2$ as the representative sample. First, the presence of CIP was evident by the appearance of the peak at 1628 cm^{-1} in the FTIR spectrum of the CIP-DXT-HPMC nanoplex (Figure 7-7) corresponding to the C=O stretching of the pyridone functional group that was unique to CIP [261]. The pyridone peak in the CIP nanoplex was shifted slightly from 1621 cm^{-1} observed in the native CIP's spectrum due to the protonation of CIP in the nanoplex. The same peak appeared in the FTIR spectrum of the CIP-DXT nanoplex, as well as in the FTIR spectrum of the physical mixture of the CIP-DXT nanoplex and HPMC. The FTIR spectra of the free DXT and free HPMC were presented in Figure 7-3.

Figure 7-3: FTIR spectra of the free DXT and free HPMC

Second, the complexation between CIP and DXT was evident in the FTIR spectrum of the CIP-DXT-HPMC nanoplex in the form of the disappearance of the peak at 1585 cm^{-1} corresponding to the NH_2 bending of the piperazine group of CIP [265].

The disappearance of the amine peak, which was present in the FTIR spectrum of the native CIP, was attributed to the electrostatic binding between the protonated NH_2^+ group of CIP and the SO_3^- group of DXT, which was represented by the peak at 1158 cm^{-1} in the FTIR spectrum of DXT. A similar disappearance of the amine peak was observed as expected in the FTIR spectra of the CIP-DXT nanoplex and the physical mixture. Third, the presence of HPMC in the CIP-DXT-HPMC nanoplex was evident from the appearance of the characteristics peaks of HPMC corresponding to the C-H stretching vibration at 2930 and 2840 cm^{-1} [286]. The same peaks appeared in the FTIR spectra of the physical mixture and the free HPMC, but not in the spectra of the native CIP and the CIP-DXT nanoplex.

Besides its electrostatic interaction with DXT, CIP theoretically could also interact with HPMC in the nanoplex via hydrogen bond interaction between the uncharged COOH group of CIP and the C-O-C group of HPMC represented by the peak at 1050 cm^{-1} in the FTIR spectrum of the free HPMC. The C=O stretching of the COOH group of CIP in the nanoplex was represented by the peak at 1732 cm^{-1} in the FTIR spectra of the CIP-DXT nanoplex and the physical mixture. Notably, this peak was not present in the FTIR spectrum of the native CIP as the COOH group was charged in the native CIP due to its zwitterionic nature [251]. The hydrogen bond interaction between CIP and HPMC, however, was minimal at best based on the FTIR spectrum of the CIP-DXT-HPMC nanoplex, where the COOH peak at 1732 cm^{-1} was not shown to shift or change in intensity compared to that observed in the CIP-DXT nanoplex, denoting the absence of hydrogen bond interaction involving the COOH group of CIP.

7.3.3.3 PXRD and DSC

The amorphous form of the CIP-DXT-HPMC nanoplex prior to the long-term storage was verified by PXRD using the nanoplexes prepared at $M_{\text{HPMC/CIP}} = 2$ and 8 as the representative samples.

Figure 7-4: TGA thermographs of the CIP-DXT, CIP-DXT-HPMC, CIP-DXT-PVP nanoplexes, native CIP, free DXT, free HPMC, and free PVP

Similar to that of the CIP-DXT nanoplex, the PXRD patterns of both the CIP-DXT-HPMC nanoplexes showed the absence of sharp crystalline peaks at $2\theta \approx 7$ to 27° that were present in the PXRD pattern of the native CIP (Figure 7-8A). Nevertheless, the absence of broad amorphous halos in the PXRD patterns of the CIP nanoplexes suggested that the nanoplexes were not fully amorphous containing minute crystalline contents. The thermal stability of the amorphous form of the CIP-DXT-HPMC nanoplex ($M_{\text{HPMC/CIP}} = 2$) was examined by the TGA/DSC analysis in comparison to that of the CIP-DXT nanoplex. On this note, the TGA data showed that both CIP nanoplexes and their constituents (i.e. DXT, HPMC, and also PVP) had undergone

significant decomposition at 240°C (Figure 7-4), thus the DSC thermographs above 240 °C was excluded from the analysis of the CIP nanoplexes.

The DSC thermographs of the CIP-DXT-HPMC and CIP-DXT nanoplexes showed the presence of broad exothermic bands at around 110-150 °C indicating the amorphous-to-crystalline trans-formulation of the nanoplexes upon heating. Upon further heating, strong endothermic peaks at approximately 200 °C and 185 °C appeared in the DSC thermographs of the CIP-DXT-HPMC and CIP-DXT nanoplexes, respectively, attributed to the melting points of the crystalline CIP (Figure 7-8 B). The melting point of the two nanoplexes was notably lower than the melting point of the crystalline native CIP observed at 270°C.

7.3.2 CIP-DXT-PVP nanoplex

7.3.2.1 Physical characteristics

The addition of PVP was found to increase the zeta potential of the CIP nanoplex produced from -32 ± 0.5 mV in the absence of PVP ($M_{PVP/CIP} = 0$) to -42 ± 0.9 and -44 ± 0.3 mV at $M_{PVP/CIP} = 1$ and 8, respectively (Figure 7-5 A). The higher zeta potential observed at higher $M_{PVP/CIP}$ was not unexpected because amphiphilic PVP had been well established as an effective nonionic dispersant for nanoparticles owed to the steric hindrance effect afforded by its hydrophobic carbon chains [270]. The addition of PVP also led to larger CIP nanoplex likely caused by the formulation of PVP coating on the nanoplex surface. Specifically, the CIP nanoplex size increased from 298 ± 7 nm ($PDI = 0.46 \pm 0.06$) in the absence of PVP to 322 ± 14 ($PDI = 0.41 \pm 0.02$) and 493 ± 29 nm ($PDI = 0.37 \pm 0.05$) at $M_{PVP/CIP} = 1$ and 8, respectively (Figure 7-5A). On this note, the control run in which the CIP solution was mixed with the PVP solution without DXT

did not produce any precipitates, indicating that the interactions between CIP and PVP (if any) did not lead to particle formulation in the absence of DXT.

Figure 7-5: Effects of $M_{PVP/CIP}$ on the (A) size and zeta potential, (B) CIP utilization rate and CIP payload of the CIP- DXT-PVP nanoplex produced

Similar to the trend observed in the CIP-DXT-HPMC nanoplex, the inclusion of PVP also led to higher CIP utilization rates, which increased from $65 \pm 6\%$ (w/w) in the absence of PVP to $81 \pm 4\%$ and $90 \pm 4\%$ at $M_{PVP/CIP} = 1$ and 8, respectively (Figure 7-5B). In this regard, the formulation of drug nanoplex was dependent on the competition between the long-range electrostatic repulsion among the drug-PE complexes and the inter-drug hydrophobic attraction of the bound drug molecules, where stronger inter-drug hydrophobic attraction than the electrostatic repulsion would lead to more drug molecules being transformed to the nanoplex [127]. The present results indicated that the physical presence of PVP and HPMC in the complexation solution reduced the long-range electrostatic repulsion, resulting in the higher CIP utilization rates. Also similar to the trend observed in the CIP-DXT-HPMC nanoplex, the CIP payload of the CIP-DXT-PVP nanoplex remained relatively constant at approximately 75-80% ($P > 0.05$) independent of $M_{PVP/CIP}$, despite the increase in the

CIP utilization rate at higher $M_{PVP/CIP}$ (Figure 7-8B). The constant CIP payload observed at higher $M_{PVP/CIP}$ was attributed to the inclusion of PVP that in turn increased the polymer content in the nanoplex.

Figure 7-6: FESEM images of (A) CIP-DXT nanoplex, (B) CIP-DXT-HPMC nanoplex, and (C) CIP-DXT-PVP nanoplex

In terms of the morphology, the FESEM image of the CIP-DXT-PVP nanoplex showed nanoparticles with a roughly spherical shape similar to that of the CIP-DXT and CIP-DXT-HPMC nanoplexes (Figure 7-6). The CIP-DXT-PVP nanoplex, nevertheless, appeared to have a more elongated shape compared to its counterparts likely caused by the presence of PVP on its surface.

7.3.2.2 FTIR

Similar to the FTIR analysis performed on the CIP-DXT-HPMC nanoplex, the presence of CIP in the CIP-DXT-PVP nanoplex was reaffirmed by the appearance of the pyridone peak unique to CIP at 1628 cm⁻¹ (Figure 7-7).

Figure 7-7: FTIR spectra of the native CIP, CIP-DXT nanoplex, CIP-DXT-HPMC nanoplex, CIP-DXT-PVP nanoplex, and the physical mixtures of CIP-DXT nanoplex with free HPMC or free PVP

Likewise, the successful CIP-DXT complexation in the presence of PVP was verified by the disappearance of the amine peak at 1585 cm⁻¹ belonging to the piperazine group of CIP. The FTIR analysis was performed using the CIP-DXT-PVP nanoplex prepared at $M_{\text{PVP/CIP}} = 2$ as the representative sample. The presence of PVP in the CIP-

DXT-PVP nanoplex was evident by the appearance of the peak at 870 cm^{-1} corresponding to the pyrrolidone ring that was unique to PVP [287]. The same peak appeared in the FTIR spectra of the physical mixture and the free PVP (Figure 7-3), but not in the FTIR spectra of the native CIP or the CIP-DXT nanoplex. While PVP possessed carbonyl functional groups represented by the peak at 1660 cm^{-1} that could act as hydrogen bond acceptors for hydrogen bonding with the COOH group of CIP, the existence of the hydrogen bond between CIP and PVP was not evident in the FTIR spectrum of the CIP-DXT-PVP nanoplex. The peak at 1732 cm^{-1} corresponding to the COOH group of CIP was not shifted or changed in intensity compared to that observed in the spectrum of the CIP-DXT nanoplex, hence suggesting there was minimal hydrogen bond interaction between CIP and PVP, similar to the observation made for the hydrogen bond interaction between CIP and HPMC.

Figure 7-8: (A) PXRD patterns of the native CIP, CIP-DXT nanoplex, CIP-DXT-HPMC nanoplex ($M_{\text{HPMC/CIP}} = 2$ and 8), and CIP-DXT-PVP nanoplex ($M_{\text{PVP/CIP}} = 2$ and 8) prior to the long-term storage; (B) DSC thermographs of the native CIP, CIP-DXT nanoplex, CIP-DXT-HPMC nanoplex.

7.3.2.3 PXRD and DSC

The PXRD analysis performed on the CIP-DXT-PVP nanoplexes prepared at $M_{\text{PVP/CIP}} = 2$ and 8 as the representative samples showed the amorphous form of the nanoplex

prior to the long-term storage (Figure 7-8 A). Nevertheless, not unlike the CIP-DXT-HPMC nanoplex, the CIP-DXT-PVP nanoplex was not fully amorphous either as evidenced by the absence of broad amorphous halos. Similar to the CIP-DXT and CIP-DXT-HPMC nanoplexes, the DSC analysis showed that the amorphous CIP-DXT-PVP nanoplex ($M_{PVP/CIP} = 2$) had undergone crystallization upon heating as indicated by the presence of broad exothermic band at around 110-150°C (Figure 7-8B). The CIP-DXT-PVP nanoplex exhibited an endothermic peak at around 170°C upon further heating attributed to the crystalline melting point.

7.3.3 Comparison of CIP-DXT-HPMC versus CIP-DXT-PVP nanoplexes

7.3.3.1 Physical characteristics

The effects of incorporating HPMC and PVP on the size of the CIP nanoplexes produced were similar in which the nanoplex size increased ($P < 0.05$) from ≈ 300 nm in the absence of HPMC or PVP to ≈ 500 nm ($P < 0.05$) at the highest $M_{HPMC/CIP}$ and $M_{PVP/CIP}$ investigated. The effects of HPMC and PVP inclusions on the zeta potential, on the other hand, were exactly opposite, where the presence of PVP increased the zeta potential owed to its surfactant-like properties, which was in contrast to the lower zeta potential observed in the presence of HPMC. The HPMC and PVP incorporations also had similar effects on (i) the CIP utilization rates, which increased at the same rates with $M_{HPMC/CIP}$ and $M_{PVP/CIP}$, and on (ii) the CIP payload, which remained constant as a function of $M_{HPMC/CIP}$ and $M_{PVP/CIP}$. The amorphous forms of the CIP-DXT-HPMC and CIP-DXT-PVP nanoplexes exhibited similar recrystallization tendency upon heating at around 110-150°C denoting their similar thermal stability.

7.3.3.2 Long-term physical stability

The PXRD analysis performed after the three-month accelerated storage showed that the five CIP nanoplex formulations investigated (i.e. CIP-DXT, CIP-DXT-HPMC prepared at $M_{\text{HPMC}/\text{CIP}} = 2$ and 8, and CIP-DXT-PVP prepared at $M_{\text{PVP}/\text{CIP}} = 2$ and 8) had undergone crystallization (Figure 7-9), with the exception of one formulation, namely the CIP-DXT-HPMC nanoplex prepared at $M_{\text{HPMC}/\text{CIP}} = 8$. The PXRD patterns of the CIP nanoplexes that had undergone crystallization during storage showed sharp crystalline peaks at $2\theta \approx 13, 22$, and 27 , which were different from the predominant peaks observed in the PXRD pattern of the native crystalline CIP at $2\theta \approx 7, 18, 23$, and 27 , hence indicating that the CIP nanoplexes had crystallized to different crystal structures after storage.

These results signified that the incorporation of HPMC was superior to that of PVP in stabilizing the amorphous form of the CIP nanoplex during prolonged storage, albeit high $M_{\text{HPMC}/\text{CIP}} = 8$ was required to achieve the desired results. It had to be pointed out that, despite the high $M_{\text{HPMC}/\text{CIP}}$ required in the feed solution, the CIP payload remained high at approximately 75% (w/w) as the CIP payload was not affected by $M_{\text{HPMC}/\text{CIP}}$ in the feed. As the hydrogen bond interactions between CIP and HPMC were found to be a non-factor, we postulated that the increased physical separation among the CIP molecules afforded by the presence of HPMC in the nanoplex was responsible for the amorphous stabilization by HPMC, which supplemented the molecular mobility restriction afforded by the CIP electrostatic binding with DXT. The inferior stabilization of PVP was believed to be caused by the aforementioned tendency of PVP to predominantly occupy the nanoplex surface owed to its surfactant properties,

resulting in its lower effectiveness in preventing crystallization of the amorphous CIP from other parts of the nanoplex.

Figure 7-9: PXRD patterns of the native CIP, CIP-DXT nanoplex, CIP-DXT-HPMC nanoplex ($M_{\text{HPMC}}/\text{CIP} = 2$ and 8), and CIP-DXT-PVP nanoplex ($M_{\text{PVP}}/\text{CIP} = 2$ and 8) after the three-month accelerated storage

7.3.3.3 Dissolution characteristics under sink condition

The presence of HPMC in the CIP-DXT-HPMC nanoplex resulted in its slower dissolution rate under sink condition compared to the CIP-DXT nanoplex, which was not unexpected as HPMC had been well established as a sustained release polymer delivery system [288]. Specifically, the CIP-DXT-HPMC nanoplexes prepared at $M_{\text{HPMC}}/\text{CIP} = 2$ and 8 reached $\approx 50\%$ (w/w) dissolution after 1.5 h and 2 h, respectively, compared to 50 min for the CIP-DXT nanoplex (Figure 7-10A). After 6 h, the CIP-DXT nanoplex reached nearly 100% dissolution, whereas the CIP-DXT-HPMC nanoplexes prepared at $M_{\text{HPMC}}/\text{CIP} = 2$ and 8 reached $\approx 80\%$ and 60% dissolution, respectively. The slower dissolution rate exhibited by the CIP-DXT-HPMC nanoplex prepared at $M_{\text{HPMC}}/\text{CIP} = 8$ was attributed to its higher HPMC content.

A similar trend in the nanoplex dissolution was observed upon the inclusion of PVP - another well-established sustained release polymer delivery system [289]. The CIP-DXT-PVP nanoplex was observed to dissolve more slowly than the CIP-DXT nanoplex and the dissolution rate was slowed further for the CIP-DXT-PVP nanoplexes prepared at higher $M_{PVP/CIP}$ (Figure 7-10). While the CIP-DXT-PVP nanoplex exhibited faster dissolution initially (< 1 h) than the CIP-DXT-HPMC nanoplex, its dissolution rate slowed down considerably after 1 h, resulting in its slower overall dissolution rates. After 6 h, the CIP-DXT-PVP nanoplexes prepared at $M_{PVP/CIP}= 2$ and 8 exhibited $\approx 65\%$ and 55% dissolution, respectively.

Figure 7-10: (A) Dissolution rates under sink condition and (B) supersaturation generation of CIP-DXT nanoplex, CIP-DXT-HPMC nanoplex ($M_{HPMC/CIP} = 2$ and $M_{HPMC/CIP} = 8$)

7.3.3.4 Supersaturation generation

In comparison to the CIP-DXT nanoplex, the CIP-DXT-HPMC nanoplex exhibited superior supersaturation generation with a higher peak supersaturation level. The supersaturation level then gradually decreased over 24 h due to crystallization of the supersaturated CIP solution. The supersaturation generation of the nanoplex thus followed the typical “spring-and-parachute” supersaturation time-profile of amorphous

drugs. Specifically, the CIP-DXT-HPMC nanoplex prepared at $M_{\text{HPMC}}/\text{CIP} = 2$ reached a peak supersaturation level of $(4.5 \pm 0.2) \times C_{\text{Sat-CIP}}$ after 1.67 h, followed by its gradual decrease to $3.5 \pm 0.1 \times C_{\text{Sat-CIP}}$ and $2.2 \pm 0.2 \times C_{\text{Sat-CIP}}$ after 8 h and 24 h, respectively (Figure 7-10B). For comparison, the CIP-DXT nanoplex reached a lower peak supersaturation level of $3.3 \pm 0.1 \times C_{\text{Sat-CIP}}$ after 2.17 h, followed by its gradual decrease to $2.9 \pm 0.2 \times C_{\text{Sat-CIP}}$ and $2.0 \pm 0.1 \times C_{\text{Sat-CIP}}$ after 7 h and 24 h, respectively.

The superior supersaturation generation of the CIP-DXT-HPMC nanoplex was attributed to the swelling of HPMC in the nanoplex upon dissolution, resulting in the formulation of swollen polymer layer that in turn limited further intake of the liquid from the dissolution medium to the nanoplex. This in turn suppressed the solution-mediated crystallization of the amorphous solid phase of the nanoplex during dissolution. Not unexpectedly, the supersaturation generation was further improved for the CIP-DXT-HPMC nanoplex prepared at $M_{\text{HPMC}}/\text{CIP} = 8$ owed to its higher HPMC content, where the peak supersaturation level was equal to $(5.9 \pm 0.2) \times C_{\text{Sat-CIP}}$ after 1.17 h, followed by its gradual decrease to $(4.1 \pm 0.1) \times C_{\text{Sat-CIP}}$ and $(2.8 \pm 0.05) \times C_{\text{Sat-CIP}}$ after 8 h and 24 h, respectively (Figure 7-10B).

As a result of the higher peak supersaturation level, the CIP-DXT-HPMC nanoplexes exhibited larger area under the curve (AUC) in their supersaturation time profiles. The supersaturation AUCs were equal to (73.4 ± 5.4) and (87.6 ± 0.08) mg/ml·h for the nanoplexes prepared at $M_{\text{HPMC}}/\text{CIP} = 2$ and 8, respectively. For comparison, the CIP-DXT nanoplex exhibited supersaturation AUC of (2 ± 2.1) mg/ml·h, hence it was approximately 15-30% lower ($P < 0.05$) than that of the CIP-DXT-HPMC nanoplexes. The larger supersaturation AUC ($P < 0.05$) of the CIP-DXT-

HPMC nanoplex signified its ability to maintain high apparent solubility of CIP over a prolonged period.

Figure 7-11: (A) Dissolution characteristics of CIP-DXT-PVP nanoplex under sink condition, (B) Supersaturation generation of CIP-DXT-PVP nanoplex, both in comparison to the CIP-DXT nanoplex

In contrast, the CIP-DXT-PVP nanoplex did not exhibit significantly superior supersaturation ($P > 0.05$) generation to the CIP-DXT nanoplex. In fact, the CIP-DXT-PVP nanoplex prepared at $M_{PVP/CIP} = 2$ exhibited comparable supersaturation generation to that of the CIP-DXT nanoplex (Figure 7-11). The supersaturation generation was slightly improved for the CIP-DXT-PVP nanoplex prepared at $M_{PVP/CIP} = 8$, where the peak supersaturation level was equal to $(4.1 \pm 0.2) \times C_{Sat-CIP}$, followed by its gradual decrease to $(3.2 \pm 0.1) \times C_{Sat-CIP}$ and $(2.4 \pm 0.1) \times C_{Sat-CIP}$ after 7 h and 24 h, respectively. We postulated that the lack of improvement in the supersaturation generation of the CIP-DXT-PVP nanoplex was caused by its aforementioned slower dissolution rates, which in turn rendered the amorphous solid phase prone to Ostwald ripening crystallization. In short, the CIP-DXT-HPMC nanoplex is superior to its CIP-DXT-PVP counterpart in terms of long-term physical stability and solubility enhancement.

7.4 Conclusions

We successfully incorporated crystallization inhibitor HPMC and PVP to the CIP nanoplex at the nanoplex formulation step, instead of at the later stages in the solid dosage formulation step, to produce ternary amorphous nanoplexes of CIP-DXT-HPMC and CIP-DXT-PVP. In comparison to the CIP-DXT nanoplex, the additions of HPMC and PVP had similar impacts on the CIP nanoplex's physical characteristics, where they both increased the nanoplex size and CIP utilization rate with minimal impacts on the CIP payload. The impacts of HPMC and PVP additions, nevertheless, were opposite for the nanoplex's zeta potential, where the presence of PVP increased the zeta potential attributed to the surfactant property of PVP, whereas lower zeta potential was observed in the presence of HPMC. As expected, the additions of HPMC and PVP slowed down CIP dissolution rates from the nanoplex. The impacts of the HPMC and PVP additions on the nanoplex's physical and dissolution characteristics were magnified with increasing $M_{\text{HPMC/CIP}}$ and $M_{\text{PVP/CIP}}$ due to the increased polymer contents in the nanoplex. Between the CIP-DXT-HPMC and CIP-DXT-PVP nanoplexes, the former was found to exhibit (i) superior amorphous form stability during long-term storage, despite their comparable thermal stability, and (ii) superior solubility enhancement. In conclusion, the present results established the feasibility of incorporating HPMC or PVP at the nanoplex formulation step and HPMC represented the more effective crystallization inhibitor for the amorphous CIP nanoplex.

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CHAPTER 8 Carboxymethyl cellulose is a superior polyanion to dextran sulfate in stabilizing and enhancing the solubility of amorphous drug-polyelectrolyte nanoparticle complex

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Abstract

Amorphous drug-polyanion nanoplex represents an effective solubility enhancement strategy of weakly-basic poorly-soluble drugs. While dextran sulfate (DXT) was chosen in most studies as the polyanion for nanoplex formulation, drug-DXT nanoplex demonstrated poor long-term physical stability for drugs with high crystallization propensity, such as ciprofloxacin (CIP). Herein we hypothesized that amorphous form stability of CIP nanoplex could be improved by substituting DXT with carboxymethyl cellulose (CMC) known for its crystallization inhibiting activity. The optimal preparation condition of the CIP-CMC nanoplex was determined by investigating the effects of CMC/CIP charge ratio and pH on the resultant nanoplex's physical characteristics and preparation efficiency. At the optimal condition, the CIP-CMC nanoplex possessed size, zeta potential, and CIP payload of approximately 200 nm, -49 mV, and 76%, respectively. Its preparation was highly efficient with CIP utilization rate and overall yield of roughly 89% and 46%, respectively. Compared to the CIP-DXT nanoplex, the CIP-CMC nanoplex was larger and with higher CIP payload attributed to

CMC's higher chain stiffness. The CIP-CMC nanoplex exhibited superior physical stability after twelve-month storage and improved solubility enhancement capability (30% higher), despite its slower dissolution. These results clearly established CMC as the superior polyanion to DXT for nanoplex formulation of weakly-basic drugs.

Keywords: cellulose; polyelectrolyte complex; nanoparticles

Highlights:

- Charge ratio and pH have large influence on CIP payload and preparation efficiency
- Optimal condition to prepare CIP-CMC nanoplex is at $R_{CMC}/CIP = 1.6$ and pH 3.94
- Chain stiffness of DXT and CMC impacts the resultant nanoplex characteristics
- CIP-CMC nanoplex remains amorphous after twelve-month storage unlike CIP-DXT nanoplex
- CIP-CMC nanoplex has higher payload and superior solubility enhancement than CIP-DXT nanoplex

8.1 Introduction

Drugs exhibiting poor aqueous solubility constitute nearly 40% of the total number of approved drugs in the market [291]. The percentage is even higher for new chemical entities that are currently under development in the pharmaceutical pipeline in which 75-90% percent of them are poorly soluble due to their increasingly complex chemistry [292]. A majority of these poorly soluble drugs are weak organic acids or base [293]. For such drugs, amorphous drug-polyelectrolyte nanoparticle complex (or nanoplex in short) represents an effective solubility enhancement strategy by virtue of its ability to rapidly generate highly supersaturated drug concentration upon its dissolution [272]. The supersaturation generation is attributed to the metastable amorphous form and

nanosize of the drug nanoplex, resulting in drug's kinetic solubility that is multi-fold higher than its thermodynamic solubility.

The amorphous drug nanoplex is formed by electrostatic complexation between the ionized form of the weak acid/base drug molecules and oppositely charged polyelectrolytes (PE) to produce soluble drug-PE complex. The drug-PE complex subsequently form aggregates among themselves as a result of the inter-drug hydrophobic interactions among the bound drug molecules in the complex [294]. In addition, the cooperative binding between the bound drug molecules in the complex and free drug molecules further contributes to the growth of the aggregate mass. Upon reaching a critical mass, whose value was dictated by the drug's hydrophobicity [295], the complex aggregates undergo a phase transition from soluble to insoluble complex, resulting in the formulation of the drug-PE nanoplex. The electrostatic binding between the drug and PE restricts the mobility of the drug molecules to arrange themselves to ordered crystalline structures upon phase transition, leading to the formulation of an amorphous form.

While simultaneous amorphization and nanonization has been well established as an effective solubility enhancement strategy of poorly soluble drugs [52, 296], the amorphous drug nanoplex possesses one key advantage over conventional amorphous nanopharmaceutical formulations in its simpler and energy-minimal preparation. The preparation techniques of conventional amorphous nanopharmaceuticals, such as high-pressure homogenization [297], wet milling [298], nano spray drying [299], controlled nanoprecipitation [300], microfluidic precipitation [301], are often energy intensive, lengthy, or highly intricate with extensive use of organic solvents.

In contrast, the preparation of amorphous drug nanoplex only requires bulk mixing of the ionized drug solution in acid/base with the aqueous PE solutions under ambient condition [283]. In addition to its simplicity, zero solvent use, and minimal energy requirement, the nanoplex formulation is also characterized by high drug utilization rates (>80% w/w) defined as the mass percentage of the initial drug mass that is successfully transformed into the nanoplex. The high drug utilization rate leads to not only minimal drug wastage, but also high drug payload. For a given drug-PE pair, previous studies showed that the nanoplex's physical characteristics were governed mainly by these three process variables, namely (1) drug concentration, (2) charge ratio of PE to drug ($R_{PE/drug}$), and (3) pH that influences the degree of ionization of the weak acid/base drugs [33, 35]. As can be seen here, the amorphous nanoplex is a highly attractive solubility enhancement strategy provided the poorly soluble drugs possess weakly acidic or weakly basic properties.

At its current state of development, a large number of poorly soluble drugs have capabilities [302] both in vitro and in vivo [1, 32-35, 167, 214, 274, 303]. For example, antibiotics (e.g. fluoroquinolones, vancomycin) [34, 273], antifungal itraconazole [167], anti-inflammatory ibuprofen [272], anticancer doxorubicin [214], bioactive curcumin [35], anti-Parkinson ropinirole [32], and antipsychotic perphenazine [1]. Even though a large number of drugs have been investigated, the effects of employing a different type of PE on the resultant nanoplex's characteristics have not been adequately examined. In fact, for weakly basic poorly soluble drugs, a large majority of the studies employed polyanionic dextran sulfate (DXT) as the PE in their nanoplex formulation. The popularity of DXT was owed to its strong polyelectrolyte nature and Generally Recognized as Safe (GRAS) status.

Besides DXT, a second polyanion that has been studied for the nanoplex formulation of weakly basic drugs was κ -carrageenan. However, the resultant nanoplex was found to be inferior to its DXT counterpart in terms of its solubility enhancement and preparation efficiency due to the lower charge density and higher chain stiffness of κ -carrageenan [252]. Investigations on the effects of PE's characteristics were limited to a study on the effect of molecular weight (MW) of DXT on the nanoplex's physical and dissolution characteristics, where the effects of MW of DXT were reported to be minimal [304].

While the solubility enhancement capability of amorphous drug-DXT nanoplexes has been well established, their amorphous form stability during long-term storage has not been adequately assessed. A reduced stability during storage in which the drug nanoplex undergoes crystallization would negate its solubility enhancement capability. In this regard, the long-term amorphous form stability of drug-DXT nanoplexes had been demonstrated in limited studies using itraconazole and perphenazine as the model drugs [1, 167].

For drugs with high crystallization propensity, however, the drug-DXT electrostatic binding might not be sufficient to restrict the drug molecular mobility to gradually rearrange to the more highly ordered crystalline state [250]. Antibiotic ciprofloxacin (CIP) is one example of weakly basic drugs exhibiting a high crystallization propensity due to its zwitterionic nature, where the strong intermolecular interaction between the cationic piperazine amino group of CIP and its anionic carboxylate group enables its solid-state molecular mobility [280]. Consequently, amorphous CIP-DXT nanoplex was shown to crystallize after twelve-month storage in the absence of crystallization inhibiting agents [248].

Herein we aimed to improve the physical stability of the amorphous CIP nanoplex by replacing DXT with a polyanion known for its crystallization inhibiting activity, namely carboxymethyl cellulose (CMC) [305]. CMC has been extensively used in pharmaceutical applications mostly as the carrier matrix for controlled-release drug delivery systems owed to its pH-dependent swelling characteristics [306-308]. The crystallization inhibiting activity of CMC, and cellulose ethers in general, was attributed to the strong hydrophobic interactions between the alkyl groups of the cellulose ether and the drug molecules, resulting in restricted drug molecular mobility. The strong drug-CMC interactions were afforded by the extended con-formation of the CMC chains, which enabled CMC to interact effectively with the drug molecules [309]. The extended con-formation of CMC was as a result of the presence of carboxylate groups in CMC that minimized intramolecular interactions of the CMC chains

First, we aimed to determine the optimal preparation condition for the amorphous CIP-CMC nanoplex by investigating the effects of charge ratio of CMC to CIP ($R_{\text{CMC/CIP}}$) and pH on (1) the physical characteristics (i.e. size, zeta potential, CIP payload, and colloidal stability) and (2) preparation efficiency (i.e. CIP utilization rate, overall production yield). Second, using the CIP-CMC nanoplex prepared at the optimal condition, we aimed to evaluate the CIP-CMC nanoplex against the CIP-DXT nanoplex developed in our previous study [304], in terms of their (1) physical characteristics, (2) dissolution characteristics in simulated gastrointestinal fluids, (3) amorphous form stability during long-term storage, and lastly (4) in vitro solubility enhancement capability.

8.2 Materials and Methods

8.2.1 Materials

Ciprofloxacin (CIP) ($\geq 98\%$ purity), sodium dextran sulfate (DXT) (MW 500 kDa), sodium carboxymethyl cellulose (CMC) (MW 250 kDa with degree of substitution of 1.2), glacial acetic acid (AA), Pluronic F68, potassium hydroxide (KOH), methanol, acetonitrile, sodium chloride (NaCl), and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma-Aldrich (Singapore).

8.2.2 Methods

8.2.2.1 Preparation of CIP-CMC and CIP-DXT nanoplexes

The schematic representation of the CIP nanoplex formulation was presented in Figure 8-1. CIP having pK_a 6.1 and 8.6 [257] was dissolved at 5 mg/ml in 1.6% (v/v) AA solution (pH 2.6) to form cationic CIP molecules with charge density of 3.0×10^{-6} mol·charge/mg, whereas CMC having pK_a 3.7 [310] was dissolved in 0.05 M KOH (pH 12.7) to form polyanionic CMC molecules with charge density of 4.6×10^{-6} mol·charge/mg. The CMC concentration was varied between 3.3 and 6.5 mg/ml to study the effects of $R_{\text{CMC/CIP}}$ in the range of $1.0 \leq R_{\text{CMC/CIP}} \leq 2.0$. The AA concentration used to dissolve CIP was varied between 0.8% to 2.4% (v/v) to study the effect of pH in the range of $3.79 \leq \text{pH} \leq 4.05$. The sample calculations for the charge density and $R_{\text{CMC/CIP}}$ were provided in Table 8-1. The molecule weight of the DXT's monomer is 416 g/mol, hence there are approximately 1200 monomers per DXT molecule. Each monomer of DXT contains two SO_3^- charges, therefore each DXT molecule contains 2400 charges. Similarly, the molecule weight of the CMC's monomer is 263 g/mol, hence there are approximately 950 monomers per CMC molecule. Each monomer of CMC contains 1.2

O=C-O- charge for degree of substitution = 1.2, therefore each CMC molecule contains 1140 charges.

Table 8-1: Charge density calculation

Chemical	Molecular weight (g/mol)	No. of charge per molecule	Charge density (mol·charge/mg)
CIP	331	1	$1/(331 \times 10^3) = 3.0 \times 10^{-6}$
CMC	250,000	1140	$1140/(250,000 \times 10^3) = 4.6 \times 10^{-6}$
DXT	500,000	2400	$2400/(500,000 \times 10^3) = 4.8 \times 10^{-6}$

Equation 8-1: Definition of Charge ratio CMC to CIP $R_{CMC/CIP}$

$$R_{CMC/CIP} = \frac{\text{Charge density of CMC} \times \text{Concentration of CMC}}{\text{Charge density of CIP} \times \text{Concentration of CIP}}$$

Equation 8-2: Sample calculations to determine the concentration of CMC in mg/ml required to produce $R_{CMC/CIP} = 1.6$

$$\begin{aligned}
 R_{CMC/CIP} &= \frac{\text{Charge density of CMC} \times \text{concentration of CMC}}{\text{Charge density of CIP} \times \text{concentration of CIP}} \\
 &= \frac{\text{Concentration of CMC} \times 4.6 \times 10^{-6}}{5 \times 3.0 \times 10^{-6}} \\
 &= 1.6
 \end{aligned}$$

Hence, the concentration of CMC required to produce $R_{CMC/CIP} = 1.6$ is 5.2 mg/ml.

Equation 8-3: Sample calculations to determine the concentration of CMC in mg/ml required to produce $R_{CMC/CIP} = 1.0$

$$\begin{aligned}
 R_{DXT/CIP} &= \frac{\text{Charge density of DXT} \times \text{concentration of DXT}}{\text{Charge density of CIP} \times \text{concentration of CIP}} \\
 &= \frac{\text{Concentration of DXT} \times 4.8 \times 10^{-6}}{5 \times 3.0 \times 10^{-6}} \\
 &= 1.0
 \end{aligned}$$

Hence, the concentration of CMC required to produce $R_{CMC/CIP} = 1.0$ is 3.2 mg/ml.

Equal volumes of the CIP and CMC solutions were then mixed under gentle stirring resulting in the formulation of the CIP-CMC nanoplex without the need for ultrasonication. The nanoplex suspension then underwent three cycles of centrifugation

($14,000 \times g$ for 10 min) and rinsing with deionized water to remove excess CIP and CMC that did not form the nanoplex. Next, the washed CIP-CMC nanoplex suspension was re-suspended in deionized water in the presence of 6.5 mg/mL Pluronic F68. The nanoplex suspension was subsequently freeze dried without cryoprotectants for 48 h at $-52\text{ }^{\circ}\text{C}$ and 0.05 mbar in Alpha 1-2 LD Plus freeze dryer (Martin Christ, Germany). Two types of control experiments, i.e. (1) 5 mg/mL CIP solution in 1.6% AA was mixed with 0.05 M KOH solution without CMC, and (2) 3.3 mg/mL CMC solution in 0.05 M KOH was mixed with 1.6% AA solution without CIP, were performed for comparison.

Figure 8-1: Schematic representation of the CIP nanoplex formulation

The CIP-DXT nanoplex was prepared at the optimal condition established in Yu and Hadinoto [304] with slight modifications. Briefly, CIP was dissolved at 5 mg/mL in 0.7% (v/v) AA (pH 2.9), while DXT having $\text{pK}_a < 2$ [259] was dissolved in deionized water at 3.2 mg/mL ($R_{\text{DXT/CIP}} = 1.0$) to form polyanionic DXT molecules with charge density of 4.8×10^{-6} mol-charge/mg. The CIP and DXT solutions were then mixed at equal volumes under gentle stirring and the resultant CIP-DXT nanoplex was centrifuged, washed with 6.5 mg/mL Pluronic F68 solution, and freeze dried for characterizations, following similar procedures described in the above.

8.2.2.2 Physical characterizations

The size, zeta potential, and polydispersity index (PDI) of the CIP nanoplex suspension were characterized in triplicates by dynamic light scattering (DLS) using Brookhaven 90 Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corporation, USA). The DLS measurement was performed after $100 \times$ dilution of the aqueous nanoplex suspension. The CIP-CMC nanoplex's morphology was examined by field emission scanning electron microscope (FESEM) (JSM 6700F, JEOL, USA) using air-dried nanoplex as the sample. The colloidal stability of the CIP-CMC nanoplex prepared at the optimal condition was evaluated by monitoring the variations in its size and zeta potential using DLS at room temperature over a 24-h period.

The CIP payload defined as the amount of CIP per unit mass of the nanoplex was determined in triplicates by dissolving a known mass of the CIP nanoplex in 50% (v/v) AA. Afterwards, the amount of CIP in the solution was determined by high performance liquid chromatography (HPLC) at detection wavelength of 324 nm. The HPLC was carried out in Agilent 1100 (Agilent Technologies, Singapore) using ZORBAX Eclipse Plus C18 column (250×4.6 mm and 5 μ m particle size). The mobile phase was 0.1 M KH_2PO_4 : acetonitrile: methanol (60: 25: 15 v/v) at flow rate of 1 mL/min, resulting in the CIP retention time of approximately 5 min.

The existence of CIP in the CIP-CMC nanoplex and its complexation with CMC was examined by Fourier transform infrared spectroscopy (FTIR) using Spectrum One FT-IR Spectrometer (Perkin-Elmer, USA). The FTIR analysis was performed between 500 and 4000 cm^{-1} at 1 cm^{-1} spectral resolution. The FTIR analysis was also performed for the native CIP, free CMC, and the physical mixture of the native CIP and free CMC.

The amorphous form of the CIP-CMC nanoplex immediately after their preparation was verified by powder X-ray diffraction (PXRD) performed between 10° and 70° (2θ) with a step size of 0.02°/s (X-ray wavelength: Cu anode, 1.542 nm; X-ray generation: 30 kV/10 mA) using D8 Advance X-ray Diffractometer (Bruker, Germany). The thermal stability of the amorphous form of the CIP-CMC nanoplex was examined by differential scanning calorimetry (DSC) (DSC 822E, Mettler Toledo, USA) performed at heating rate of 2 °C/min between 25 °C to 300°C. Thermogravimetric analysis (TGA) was performed at heating rate of 10°C/min between 25 °C and 400 °C (Pyris Diamond, Perkin-Elmer, USA). The PXRD, DSC, and TGA analysis were also performed for the native CIP, free CMC, and the CIP-DXT nanoplex.

8.2.2.3 Preparation efficiency

The CIP utilization rate was characterized in terms of the complexation efficiency (CE) defined in Equation 8-4, where the mass of CIP that formed the CIP nanoplex was determined from the difference between the initial mass of CIP added and the mass of CIP recovered in the supernatant after the first centrifugation of the CIP nanoplex suspension.

Equation 8-4: Definition of CE

$$CE (\% w/w) = \frac{\text{Mass of CIP that formed nanoplex}}{\text{Initial mass of CIP added}} \times 100\%$$

Equation 8-5: Definition of Yield

$$Yield (\% w/w) = \frac{\text{Mass of nanoplex produced}}{\text{Initial mass of CIP and DXT or CMC added}} \times 100\%$$

The amount of CIP in the supernatant was determined in triplicates by HPLC as previously described. The overall production yield was characterized in triplicates following Equation 8-5, where the mass of CIP nanoplex produced was determined from the dry mass of the CIP nanoplex suspension after 48-h freeze drying.

8.2.2.4 Dissolution characteristics under sink condition

The dissolution rates of the CIP nanoplexes were characterized in their dry-powder forms under sink conditions in simulated gastric juice (SGJ) and intestinal juice (SIJ). The SGJ was prepared from 0.2% (w/v) NaCl solution adjusted to pH 1.2 by the addition of 1.0 M HCl. The SIJ was prepared from 0.9% (w/v) KH_2PO_4 solution adjusted to pH 6.8 by the addition of 0.2 M KOH [198]. The CIP nanoplex powders were added at $1/4 \times C_{\text{Sat}}$ to a dialysis bag immersed in 40 ml of the dissolution medium held at 37°C in a shaking incubator. Herein C_{Sat} denoted the thermodynamic saturation solubility of CIP in the dissolution medium, where the C_{Sat} of CIP in the SGJ and SIJ were determined previously to be equal to 21.3 and 0.12 mg/mL, respectively [305]. Afterwards, 0.3 mL aliquot was withdrawn at specific time points over a 24-h period and 0.3 mL fresh medium was added back to the dissolution vessel. The aliquot was then centrifuged at $14,000 \times g$ for 3 min after which the amount of CIP in the supernatant was determined by HPLC as previously described.

8.2.2.5 Supersaturation generation under non-sink condition

The solubility enhancement capabilities of the CIP nanoplexes were characterized in their dry-powder forms in terms of the supersaturation level defined as the ratio of C/C_{Sat} where C denoted the supersaturated CIP concentration. Briefly, the CIP nanoplex powders were added in excess at non-sink condition of $20 \times C_{\text{Sat}}$ to 20 mL SIJ held at 37 °C in a shaking incubator. Next, 0.35 mL aliquot was withdrawn at specific time points over a 24-h period after which the aliquot was syringe filtered (0.22- μm pores) and immediately diluted twentyfold with fresh medium to prevent the precipitation of CIP from the supersaturated solution. The amount of CIP in the aliquot

was then determined by HPLC as previously described. Both dissolution and supersaturation generation experiments were performed in triplicates.

8.2.2.6 Amorphous form stability during storage

The amorphous form stability of the dry-powder forms of the CIP nanoplexes during long-term storage was assessed by the changes in their PXRD patterns before and after storage. Briefly, the CIP nanoplex powders were stored in duplicates for three months in an open glass container placed in a desiccator maintained at 40°C and 75% relative humidity. The environment of 75% relative humidity in the desiccator was generated by equilibrating saturated NaCl solution at 40 °C. The accelerated storage at 40°C and 75% humidity for three months was used to simulate twelve-month storage under normal condition at 25°C and 60% humidity [311].

8.2.2.6 Statistical Analysis

The statistical analysis was performed using one way ANOVA with Tukey post hoc test using OriginPro 2018b software.

8.3 Results and discussion

8.3.1 Optimal preparation condition of the CIP-CMC nanoplex

8.3.1.1 Effects of $R_{CMC/CIP}$

The investigation on the effects of $R_{CMC/CIP}$ was performed at $1.0 \leq R_{CMC/CIP} \leq 2.0$, while keeping the pH constant at 4.05 (AA = 1.6% v/v). The effects of $R_{CMC/CIP}$ were investigated at values larger than unity (at which there were excess CMC charges compared to CIP charges) to ensure a sufficient number of anionic CMC molecules were available for complexation with the oppositely charged CIP molecules [312]. At $R_{CMC/CIP} = 1.0$, the CIP-CMC nanoplex exhibited size and zeta potential of 321 ± 17 nm (PDI = 0.342 ± 0.027) and -47.0 ± 0.4 mV, respectively (Figure 8-2 A). On this note,

the two control runs did not yield any particulate product, hence confirming that the resultant nanoplex was the product of complexation between CIP and CMC, and was not a product of pH shift precipitation of the acidic CIP solution upon mixing with the basic CMC solution.

The negative zeta potential signified that the anionic CMC was predominantly present in the vicinity of the nanoplex surface to act as its colloidal stabilizer working in tandem with the nonionic Pluronic F68 surfactant. The nonionic surfactant Pluronic F68 is surfactant which is used to stabilize the nanoplex formed. For the most part, the size and zeta potential of the CIP-CMC nanoplex were minimally affected ($P > 0.05$) by the change in $R_{CMC/CIP}$ (Figure 8-2 A). Only upon raising $R_{CMC/CIP}$ to 2.0, the nanoplex size decreased ($P < 0.05$) to 219 ± 19 nm ($PDI = 0.335 \pm 0.023$), while the zeta potential remained relatively unchanged at -46.9 ± 0.6 mV. A similar trend was observed for the CIP payload, where the CIP payload was equal to $77.2 \pm 3.2\%$ (w/w) at $R_{CMC/CIP} = 1.0$ and remained ($P > 0.05$) at approximately that value up to $R_{CMC/CIP} = 1.6$, before the CIP payload decreased ($P < 0.05$) to $69.9 \pm 2.6\%$ and $67.5 \pm 1.8\%$ (w/w) at $R_{CMC/CIP} = 1.8$ and 2.0, respectively (Figure 8-2 B).

With regard to the preparation efficiency, increasing $R_{CMC/CIP}$ had a positive impact on the CIP utilization rate, where the CE increased from $65 \pm 1\%$ (w/w) at $R_{CMC/CIP} = 1.0$ to $\approx 78-80\%$ (w/w) at higher $R_{CMC/CIP}$. This was attributed to an increased number of CMC molecules available for complexation with CIP at higher $R_{CMC/CIP}$ (Figure 8-2 B). As the investigation was performed at a fixed CIP concentration (i.e. 5 mg/ml), the higher CIP utilization rate ($P < 0.05$) indicated that a larger amount of the CIP molecules was transformed into the nanoplex at higher $R_{CMC/CIP}$. The increased amount of CIP in the nanoplex, however, did not lead to a higher CIP payload at higher

$R_{CMC/CIP}$. In fact, as mentioned before, the CIP payload decreased ($P < 0.05$) as $R_{CMC/CIP}$ was increased to above 1.6. This suggested that besides the CIP molecules, a larger amount of the CMC molecules was also transformed into the nanoplex at higher $R_{CMC/CIP}$. The increased numbers of both CIP and CMC molecules that were transformed to the nanoplex at higher $R_{CMC/CIP}$ were also reflected in the trend in the overall production yield, which increased with increasing $R_{CMC/CIP}$ from $36 \pm 2\%$ (w/w) at $R_{CMC/CIP} = 1.0$ to $47 \pm 4\%$ (w/w) at $R_{CMC/CIP} = 1.8$ (Figure 8-2 B).

Figure 8-2: Effects of $R_{CMC/CIP}$ on the (A) size and zeta potential; and (B) complexation efficiency, production yield, payload of the CMC-CIP nanoplex produced at AA = 1.6% v/v. Data were plotted as the mean $\pm$ standard deviation ($n = 3$);

The production yield, nevertheless, dropped to $39 \pm 2\%$ (w/w) upon a further increase in $R_{CMC/CIP}$ to 2.0 likely caused by too high of excess of CMC in the feed solution. The optimal $R_{CMC/CIP}$ was thus determined to be equal to 1.6 below which the CIP utilization rate was not maximized and above which the CIP payload and the production yield became lower. Compared to the size of nanoplex prepared at $R_{CMC/CIP} = 1.8$, nanoplex size at $R_{CMC/CIP} = 2.0$ decreased. This might due to that more CIP molecules and CMC molecules failed to bind together to grow the nanoplex, which is

indicated by the decreased CIP payload, lower CIP utilization rate and lower production yield at $R_{CMC/CIP} = 2.0$.

8.3.1.2 Effects of pH

The investigation on the effects of pH was performed at the optimal $R_{CMC/CIP} = 1.6$ and AA concentrations of 0.8, 1.6, 2.0, and 2.4% (v/v), resulting in the net pH of the mixed (CIP + CMC) solutions of 4.26, 4.05, 3.94, and 3.79, respectively. In this regard, preliminary experiments at $AA < 0.8\%$ ($pH > 4.26$) produced large precipitates that were not re-dispersible after centrifugation, hence they were not investigated further. The large precipitates were likely products of the pH-shift precipitation of CIP as the net pH of the mixed solution (≈ 4.6) fell in the range in which CIP was less soluble [146].

Figure 8-3: Effects of pH (or the acetic acid concentration) on the (A) size and zeta potential; and (B) complexation efficiency, production yield, payload of the CMC-CIP nanoplex produced at $R_{CMC/CIP} = 1.6$. Data were plotted as the mean $\pm$ standard deviation ($n = 3$);

The results showed that the CIP-CMC nanoplex gradually decreased with decreasing pH from 417 ± 33 nm ($PDI = 0.332 \pm 0.012$) at pH 4.26 to 181 ± 21 nm ($PDI = 0.337 \pm 0.009$) at pH 3.79 (Figure 8-3 A). The zeta potentials, nevertheless, were relatively constant at ≈ -48 - -49 mV for pH between 4.26 and 3.94, and it only exhibited

a significant change at the lowest pH investigated (pH 3.79) at which the zeta potential decreased to -43.6 ± 1.3 mV (Figure 8-3 A).

The preparation efficiency was found to benefit from operation at lower pH to a certain extent. Specifically, the CE increased from $77 \pm 1\%$ at pH 4.26 to $89 \pm 2\%$ at pH 3.94, denoting higher CIP utilization rates attributed to the higher degree of ionization of the CIP molecules at lower pH (Figure 8-3 B). The production yield correspondingly increased ($P < 0.05$) from $35 \pm 1\%$ at pH 4.26 to $46 \pm 2\%$ (w/w) at pH 3.94 owed to the higher CIP utilization rate (Figure 8-3 B). The CE, however, decreased ($P < 0.05$) to $82 \pm 2\%$ upon a further decrease in the pH to 3.79 (Figure 8-3 B). In this regard, as the net pH became lower approaching the pKa of CMC, the net charges of CMC molecules became lower, resulting in their less effective electrostatic binding with CIP, which in turn produced the lower CE.

As a result of the higher CE, the CIP payload increased from $65.4 \pm 4.7\%$ at pH 4.26 to $76.3 \pm 1.8\%$ (w/w) at pH 3.94 (Figure 8-3 B). The trends in the nanoplex characteristics and preparation efficiency as a function of pH suggested benefits of operating at a lower pH above pH 3.79. The optimal pH was determined to be equal to pH 3.94 achieved at AA = 2.0% (v/v) by virtue of its higher CE. On this note, our investigation also revealed the narrow window of pH (i.e. $3.94 \leq \text{pH} \leq 4.26$) in which the CIP-CMC nanoplex produced would exhibit optimal CIP payload and preparation efficiency. One of the reasons for the narrow window of pH was the strong pH-dependent solubility of CIP [146] that hindered the CIP-CMC nanoplex formulation at $\text{pH} > 4.26$. The narrow window of feasible pH made pH, not $R_{\text{CMC/CIP}}$, as the process variable that dictated the CIP-CMC nanoplex preparation. In the next section, the solid-

state physical characteristics and dissolution profiles of the CIP-CMC nanoplex prepared at the optimal $R_{CMC/CIP}$ and pH were examined.

8.3.2 Physical characteristics of the optimal CIP-CMC nanoplex

8.3.2.1 FESEM, colloidal stability

The nanoscale size of the CIP-CMC nanoplex was verified by the FESEM analysis in which roughly spherical nanoparticles with individual sizes of approximately of 100 nm were observed (Figure 8-4 A). The individual nanoplex size from the FESEM was slightly smaller than that obtained by DLS (≈ 200 nm) suggesting the agglomeration tendency of the nanoplex, despite its high zeta potential. The colloidal stability of the CIP-CMC nanoplex at room temperature therefore merited an investigation. The nanoplex size was found to increase ($P < 0.05$) from 208 ± 9 nm (PDI = 0.379 ± 0.022) observed after its preparation to 399 ± 30 nm (PDI = 0.360 ± 0.025) after 2 h (Figure 8-4 B).

Figure 8-4: (A) FESEM and (B) Colloidal stability after preparation of the CIP-CMC nanoplex prepared at $AA = 2.0\%$ and $R_{CMC/CIP} = 1.6$. Data were plotted as the mean $\pm$ standard deviation ($n = 3$);

The nanoplex size increased ($P < 0.05$) to 473 ± 13 nm (PDI = 0.556 ± 0.038) after 24 h, while the zeta potentials remained relatively unchanged. The larger nanoplex size with time, and more tellingly the worsened PDI, hinted at the occurrence of

nanoplex agglomeration. For this reason, the CIP-CMC nanoplex was immediately transformed to its dry-powder forms by freeze drying prior to characterizing its dissolution.

8.3.2.2 FTIR

First, the presence of CIP in the CIP-CMC nanoplex was verified by the appearance of peaks at 1621 and 3046 cm^{-1} in the FTIR spectrum of the CIP-CMC nanoplex (Figure 8-5) corresponding to the C=O stretching of the pyridone functional group and the -C-H stretching of the aromatic ring, respectively [261]. These two peaks, which were unique to CIP, were as expected present in the FTIR spectra of the native CIP and the physical mixture of the native CIP and free CMC. The FTIR analysis of the CIP-CMC nanoplex also revealed that the carboxylic acid group of CIP in the nanoplex was uncharged as indicated by the appearance of the peak at 1735 cm^{-1} . The COOH peak was not present in the FTIR spectrum of the native CIP, or in the spectrum of the physical mixture, because the CIP molecules in the native CIP existed in their zwitterionic state with COO^- in place of COOH.

Figure 8-5: FTIR analysis confirmed the existence of CIP in the CIP–CMC nanoplex

Second, the presence of CMC in the nanoplex was evident from the appearance

of peaks at 1057 and 2940 cm^{-1} that were unique to CMC corresponding to the C-O stretching of the ether linkage and the C-H stretching vibration of the glucose ring of the CMC molecules [313]. These peaks were also present in the FTIR spectra of the free CMC and the physical mixture. Third, the complexation between CIP and CMC was evident in the FTIR spectrum of the nanoplex from the disappearance of the peak at 1585 cm^{-1} corresponding to the NH_2 bending of the piperazine group of CIP [265]. The disappearance of this amine peak, which was present in the spectra of the native CIP and the physical mixture, was caused by the electrostatic binding between the protonated NH_2^+ group of CIP and the carboxyl group of CMC represented by the 1637 cm^{-1} peak in the FTIR spectrum of the free CMC (Figure 8-5).

8.3.2.3 PXRD and DSC

The amorphous form of the CIP-CMC nanoplex prior to its long-term storage (i.e. 0 month) was evident from the PXRD patterns, which showed the absence of sharp crystalline peaks at $2\theta \approx 15, 21$, and 25 that were visible in the PXRD pattern of the crystalline native CIP (Figure 8-6 A). A similar absence of sharp crystalline peaks was observed in the PXRD patterns of the CIP-DXT nanoplex prior to the long-term storage.

The thermal stability of the amorphous form was subsequently examined by TGA and DSC, where the TGA results showed that the CIP-CMC nanoplex had experienced a significant mass loss at around 260 $^{\circ}\text{C}$ due to CMC decomposition (Figure 8-7). The CMC decomposition was also evident from the strong exothermic events beginning at around 250 $^{\circ}\text{C}$ in the DSC thermograph of the free CMC (Figure 8-6 B). The DSC thermographs above 250 $^{\circ}\text{C}$ thus were not analysed. The DSC thermograph of the CIP-CMC nanoplex displayed an exothermic event at $\approx 80\text{-}100$ $^{\circ}\text{C}$

indicating crystallization of the amorphous form of the nanoplex upon heating (Figure 8-6 B).

Figure 8-6: (A) PXRD analysis confirmed the amorphous state of the CIP–CMC nanoplex and its stability after twelve-month storage and (B) DSC of the Native CIP, CIP–CMC nanoplex, CIP–DXT nanoplex and free CMC.

Upon further heating, the DSC thermograph showed an endothermic peak at $\approx 192^{\circ}\text{C}$ attributed to the melting point of the crystalline CIP.

Figure 8-7: TGA thermographs of the native CIP, free CMC, CIP–CMC nanoplex, and CIP–DXT nanoplex

For comparison, similar exothermic crystallization event and endothermic melting point were also observed for the CIP–DXT nanoplex at $\approx 120\text{--}150^{\circ}\text{C}$ and $\approx 182^{\circ}\text{C}$

°C, respectively. Nevertheless, the melting points of the CIP nanoplexes were observed at a lower temperature compared to that observed for the native CIP at 270 °C. The crystallization point of the CIP-DXT nanoplex started at a higher temperature denoting its slightly higher thermal stability than the CIP-CMC nanoplex.

8.3.3 Evaluation of the CIP-CMC nanoplex versus CIP-DXT nanoplex

8.3.3.1 Physical characteristics

The physical characteristics of the CIP-CMC and CIP-DXT nanoplexes prepared at their respective optimal conditions were presented in Table 1. In comparison to the CIP-DXT nanoplex, the CIP-CMC nanoplex was larger ($P < 0.05$) (≈ 210 nm versus 110 nm) and possessed a considerably higher ($P < 0.05$) PDI (≈ 0.38 versus 0.15) denoting its lower monodispersity.

Table 8-2: Comparison on the physical characteristics of CIP-CMC nanoplex and CIP-DXT nanoplex

Physical characteristics	CIP-CMC nanoplex	CIP-DXT nanoplex
Size (nm)	208 ± 9	111 ± 12
PDI	0.379 ± 0.022	0.149 ± 0.004
Zeta potential (mV)	-48.9 ± 0.5	-39.5 ± 4.1
CIP payload (% w/w)	76.3 ± 1.8	60.5 ± 1.2
CE (% w/w)	89 ± 2	85 ± 2
Yield (% w/w)	46 ± 2	64 ± 6

While they exhibit similar charge densities, CMC and DXT differed greatly in their persistence lengths, which characterized chain stiffness of polymers. In this regard, the persistence length of the CMC chains was equal to 12-16 nm [314], which was significantly longer than 1.6 nm exhibited by the DXT chains [295], therefore denoting the former's higher chain stiffness. The larger size of the CIP-CMC nanoplex was believed to be caused by the higher stiffness of the CMC chains, which made them preferentially exist in their extended conformation upon complexation with CIP, resulting in the formulation of larger nanoplex. While the CIP-CMC and CIP-DXT

nanoplexes exhibited highly negative zeta potentials at ≈ -49 and -40 mV, respectively, the present investigation and our previous study on the CIP-DXT nanoplex [304] showed that both of them exhibited a tendency to agglomerate. The agglomeration, nevertheless, did not have a severe impact as the nanoplexes remained in the submicron size range after agglomeration. With regard to the preparation efficiency, they exhibited highly comparable CEs ($\geq 85\%$) with a higher production yield ($P < 0.05$) for the CIP-DXT nanoplex ($\approx 64\%$ versus 46%) attributed to its lower optimal $R_{PE/drug}$ ($R_{DXT/CIP} = 1.0$ versus $R_{CMC/CIP} = 1.6$).

Despite their highly comparable CEs, which signified that almost the same number of CIP molecules were transformed to the nanoplexes, the CIP-CMC nanoplex was superior ($P < 0.05$) in terms of the CIP payload at $\approx 76\%$ compared to $\approx 60\%$ for the CIP-DXT nanoplex. In other words, for a given CE, a smaller number of PE molecules ended up in the nanoplex when CMC was used as the polyanion in place of DXT. This result signified that CMC had a superior CIP loading capacity to DXT attributed to the former's higher chain stiffness. The extended conformation of the CMC chains enabled their optimal interactions with the CIP molecules and at the same time optimized the cooperative binding between the free and bound CIP molecules.

8.3.3.2 Dissolution characteristics under sink condition

In the SGJ, both CIP-CMC and CIP-DXT nanoplexes exhibited slower dissolution rates than the native CIP due to the lower solubility of the CIP complex at acidic pH as the electrostatic binding between the amine group of CIP and the PEs prevented the amine group from being protonated in an acidic environment. Specifically, the native CIP reached nearly 100% (w/w) dissolution in less than 2 h, whereas both CIP nanoplexes reached less than 50% (w/w) dissolution over the same period (Figure 8-8 A).

Comparing between the CIP-DXT and CIP-CMC nanoplexes, the CIP-CMC nanoplex exhibited a slower dissolution rate in the SGJ with 16 ± 1 and $38 \pm 2\%$ dissolutions after 1 h and 8.5 h, respectively, versus 33 ± 1 and $72 \pm 3\%$ after the same period for the CIP-DXT nanoplex.

Figure 8-8: Dissolution rates of the CIP-CMC and CIP-DXT nanoplexes under sink condition in (A) SGJ and (B) SIJ.

In the SIJ, both CIP-CMC and CIP-DXT nanoplexes exhibited faster dissolution rates than the native CIP attributed to their amorphous form and nanosize. Specifically, both CIP nanoplex reached $>80\%$ dissolution after 8 h, in contrast to only 45% dissolution for the native CIP (Figure 8-8 B).

Similar to the dissolution results in the SGJ, the CIP-CMC nanoplex again exhibited a slower dissolution rate ($P < 0.05$) than the CIP-DXT nanoplex. Specifically, the CIP-CMC nanoplex reached $44 \pm 2\%$ and $84 \pm 4\%$ dissolutions after 1 h and 9 h, respectively, compared to $61 \pm 2\%$ and $98 \pm 3\%$ dissolutions for the CIP-DXT nanoplex after the same period. The slower dissolution rates of the CIP-CMC nanoplex were attributed to the hydrogel characteristics of CMC that swelled upon dissolution,

resulting in the formulation of swollen polymer layer that in turn limited outward diffusion of the drug molecules [306] .

8.3.3.3 Stability of the amorphous form

After twelve months of storage, the CIP-CMC nanoplex remained in the amorphous form as reflected by the minimal change in its PXRD pattern before and after storage (Figure 8-6A). In contrast, the PXRD pattern of the CIP-DXT nanoplex after the twelve-month storage displayed high-intensity peaks at $2\theta = 12$ and 25 and several lower-intensity peaks $2\theta = 15$ and 20 , indicating the presence of crystalline contents in the nanoplex (Figure 8-8 A). The superior physical stability of the CIP-CMC nanoplex during long-term storage was not unexpected as CMC, unlike DXT, was known for its crystallization inhibiting activity. Notably, the superior storage stability of the CIP-CMC nanoplex to the CIP-DXT nanoplex was observed despite the former's aforementioned lower thermal stability.

8.3.3.4 Solubility enhancement

Both CIP-CMC and CIP-DXT nanoplexes exhibited the “spring-and-parachute” supersaturation time-profiles characteristics of amorphous drug formulations, where the supersaturation level (C/C_{Sat}) continued to increase upon dissolution until a peak supersaturation was reached (“spring”), after which it gradually decreased due to crystallization of the supersaturated solution to eventually settle back at the C_{Sat} value (“parachute”) [40]. Owing to the crystallization inhibiting activity of CMC, the CIP-CMC nanoplex exhibited superior supersaturation generation to the CIP-DXT nanoplex in which high apparent solubility of CIP over a prolonged period was observed (Figure 8-8 B). Specifically, the CIP-CMC nanoplex reached a maximum supersaturation level

of $(8.47 \pm 0.25) \times C_{\text{Sat}}$ after 70 min (1.17 h), followed by its gradual decrease to $(4.41 \pm 0.17) \times C_{\text{Sat}}$ and $(2.77 \pm 0.40) \times C_{\text{Sat}}$ after 8 h and 24 h, respectively.

Figure 8-9: Supersaturation generations of CIP-CMC and CIP-DXT nanoplexes over 48 h

In comparison, the CIP-DXT nanoplex reached a lower maximum supersaturation level of $(4.75 \pm 0.05) \times C_{\text{Sat}}$ after 100 min (1.67 h), followed by its gradual decrease to $(3.60 \pm 0.15) \times C_{\text{Sat}}$ and $(2.01 \pm 0.02) \times C_{\text{Sat}}$ after 8 h and 24 h, respectively (Figure 8-9). In addition to DXT's lack of crystallization inhibiting activity, the inferior supersaturation generation of the CIP-DXT nanoplex could also be attributed to its fast dissolution rate. The fast dissolution rate of the CIP-DXT nanoplex resulted in a high CIP supersaturation level after just a short period, which, however, was short-lived due to the high crystallization driving force of the supersaturated solution at a high degree of supersaturation. In this regard, the areas-under-the-curve (AUC) of the supersaturation time-profiles of the CIP-CMC and CIP-DXT nanoplexes were determined to be equal to $(100.9 \pm 1.7) \times C_{\text{Sat}}$ and $(78.9 \pm 0.7) \times C_{\text{Sat}}$ mg·h/ml, respectively. Thus, the CIP-CMC nanoplex produced roughly 28% higher supersaturation AUC than the CIP-DXT nanoplex. On this note, the supersaturation

level generated by the CIP-DXT nanoplex continued to decrease beyond 24 h to eventually settle at approximately the C_{Sat} value $(1.02 \pm 0.32) \times C_{\text{Sat}}$ after 48 h, while the decrease in the supersaturation level of the CIP-CMC nanoplex was not unexpectedly slower to reach $(2.03 \pm 0.23) \times C_{\text{Sat}}$ after 48 h (Figure 8-9).

8.4 Conclusions

The present work successfully established CMC as the superior polyanion to DXT as the complexation partner for CIP to prepare amorphous CIP nanoplex intended as a solubility enhancement formulation of poorly soluble CIP. The optimal preparation condition of the CIP-CMC nanoplex was determined at $R_{\text{CMC/CIP}} = 1.6$ and pH 3.94, where $R_{\text{CMC/CIP}}$ and pH were found to have greater influences on the preparation efficiency and CIP payload, while their impacts on the size and zeta potential were not as significant. At the optimal condition, the CIP-CMC nanoplex exhibited size and zeta potential of ≈ 200 nm and ≈ -49 mV, respectively, with CIP payload of $\approx 76\%$ and CIP utilization rate of $\approx 89\%$. In comparison to the CIP-DXT nanoplex, the CIP-CMC nanoplex was larger and possessed lower monodispersity, but with higher CIP payload ($\approx 76\%$ versus 60%), which we postulated to be caused by the higher chain stiffness of CMC. Their distinct physical characteristics, nevertheless, did not affect their preparation efficiency which were highly comparable. The CIP-CMC nanoplex exhibited slower dissolution rates than its DXT counterparts owed to the swelling hydrogel nature of CMC. Despite its slower dissolution rate, the CIP-CMC nanoplex exhibited superior solubility enhancement capability with supersaturation levels of $> 4.5 \times C_{\text{Sat}}$ maintained over 8 h, attributed to the crystallization inhibiting activity of CMC. Most importantly, in contrast to the CIP-DXT nanoplex, the CIP-CMC nanoplex remained amorphous after twelve-month storage denoting its high long-term physical

stability. The superior solubility enhancement and physical stability of the CIP-CMC nanoplex clearly established CMC as the better polyanion for nanoplex formulation of weakly basic poorly soluble drugs.

8.5 Acknowledgement

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CHAPTER 9 Conclusion and future work

9.1 Conclusions

In summary, my Ph.D. thesis focuses on the following topics: 1) Improving the solubility of insoluble drugs by developing amorphous drug nanoparticles with high drug loading, fast release, and prolonged supersaturation; 2) Enhancing the amorphous stability of amorphous nanoparticle formulations exhibiting high drug loading, fast release, and prolonged supersaturation; 3) Modification of the drug release of nanoparticle formulations via a range of methods; 4) Increasing the consistency of the nanoparticle products by building a millifluidic platform.

In **Chapter 4**, amorphous nano-complexes aimed at increasing the solubility of the poorly soluble drug were prepared. The antipsychotic drug perphenazine with low solubility was used as the drug model, and dextran sodium sulfate was used as the polysaccharide model. The poorly soluble drug and the ionizable polysaccharide were dissolved in a solution to get ionized. Under ambient conditions, the ionized drug and the ionized polysaccharide molecules were driven by electrostatic and hydrophobic interactions to form the complex at nanosize. The nanoparticle complex formed was demonstrated to exhibit high drug loading, fast dissolution rate, and prolonged supersaturation, all of which makes the formulation a promising alternative in resolving the low solubility problem faced by many drugs in the drug development pipeline.

In **Chapter 5**, a millifluidic platform was set up to increase the consistency of the drug-polysaccharide nanoplex. Amorphous drug-polysaccharide nanoparticle complexes were prepared by the continuous production method and batch production modes, represented by millifluidics and bulk mixing methods., respectively The physical characteristics (size, zeta potential, and payload) and preparation efficiency,

dissolution rate, supersaturation generation, and production consistency of the resultant nanoparticle complexes were compared directly. The millifluidics showed its superior production consistency and scalability. Both the drug-polysaccharide nanoplexes prepared by millifluidic and bulk mixing method exhibited fast dissolution rate, prolonged supersaturation which bodes well for the ability of the drug-polysaccharide nanoplexes in increasing the bioavailability of drug. However, fast dissolution rate is not enough. Sustained release functionality and controlled release functionality are also required in order to improve patient compliance and to decrease the potential of toxicity.

In **Chapter 6**, nanoplex with sustained release functionality was successfully developed. Sustained release was achieved by adding the counter ion (SDS) to the nanoplex. The dissolution of the nanoplexes were controlled successfully by modifying the ratio of the drug, counter ion and polysaccharide ratio.

Chapter 7 discusses how amorphous stability of nanoplex was enhanced by incorporating a second polysaccharide without decreasing the drug loading or decreasing the apparent solubility. The crystallization inhibitor (i.e. HPMC, PVP) was incorporated into the nanoplex without decreasing the drug loading. It incorporated nanoplex exhibited superior amorphous stability during long-term storage in the accelerated amorphous test study.

Chapter 8 also focuses on enhancing the amorphous stability of nanoplex by applying a polyanion with crystallization inhibiting ability, drug-polysaccharide nanoplex with high long-term amorphous stability was successfully designed.

9.2 Recommendations for Future Work

In my research work, self-assembled nanoplexes exhibiting high drug loading, sustained release functionality, and enhanced amorphous stability were prepared via

electrostatic and hydrophobic interactions. In future, I would like to recommend the work as stated below.

9.2.1 Compare the co-amorphous system and the drug-polysaccharide nano-complex systems, especially based on their amorphous stability

“Co-amorphous drug” (CAM) refers to the drug-small molecule system associated with weak and discrete forces of interaction between the drug and the small molecule components [133]. The co-amorphous system is emerging as a new formulation approach and shows potential superiority over amorphous polymeric solid dispersions due to the small molecular weight components used in preparing the co-amorphous, which occupy a smaller volume and weight ratio in the drug formulation, enabling a higher drug loading.

The co-amorphous system offers advantages such as an increased aqueous solubility due to the amorphous form and amorphous stabilization brought about by the interaction (i.e. hydrogen binding, π - π interaction) between the drug and the counter molecular in the co-amorphous system.

However, the interaction between the drug and the counter molecule in the co-amorphous system might not be sufficient to stabilize the amorphous form in longer periods of storage time, compared to the drug-polysaccharide nanoplex prepared by the electrostatic and hydrophobic interaction. A fair comparison between the two amorphous systems in terms of stability and dissolution rate has not been carried out.

The bonding (hydrogen bonding and Van der Waals forces) between the drug and the counter molecule in the co-amorphous system is believed to maintain the amorphous form. However, the bonding force [48] is weak compared to the ionized drug and polyelectrolyte in the drug-polysaccharide nanoplex formed, due to the

electrostatic interaction and hydrophobic interaction between the drug and the polysaccharide. Thus, the amorphous stability of the drug-polysaccharide nanoplex could be better than the co-amorphous system. I would recommend work on comparing the co-amorphous system and drug polysaccharide nano-complex based on their dissolution rate and amorphous stability.

9.2.2 Develop a one-step synthesis and recovery of the nano-complex via a flocculation and filtration process, using charge-driven flocculation as an intensified process for nanoplex purification and nanoplex granulation

The fabricated nanoplex was purified through several cycles of centrifugation and washing. This purification process is time-consuming and energy-intensive [1, 32, 33, 35, 105, 107]. These reasons have limited its industrial application. For future work, I would like to recommend work on developing a one-step synthesis and recovery process for the nanoplex via a flocculation and filtration process. The role played by the nanoplex in enhancing the bioavailability of the poorly soluble drug has been well recognized. The improved bioavailability results in a lower drug dose requirement, which reduces the drug wastage and energy consumption. However, the centrifugation used to separate the nanoplex in the bulk mixing and millifluidic methods is energy-intensive and time-consuming [1, 32, 33, 35, 105, 107].

To address this issue, I would like to recommend work on developing a fast and energy efficient method to purify the nanoplex and skip the centrifugation. The charge driven flocculation and the subsequent filtration show promise as an alternative to the centrifugation process.

There are several studies on the production of nanoparticle flocculation. Nanoparticles can flocculate with sodium sulfate [161, 315, 316]. The formed

flocculation is reversible and can be dispersed into the nanoplex suspension. The salt concentration can be optimized to control the flocculation [316]. Negatively charged gold nanoparticle (diameter $\approx 14\text{nm}$) sol was found to bind with the positively-charged poly(L-lysine) (PLL) to form submicrometer-sized spherical aggregates [315]. Nanoparticle adsorption to oppositely charged substrates has also been used for nanoplex granulation. Yue Yang et al. [49] used the positively-charged and negatively charged CHI microparticles as the substrate to form granules with oppositely charged ciprofloxacin and curcumin nanoplex, respectively, via electrostatic absorption. Flocculation was observed due to three reasons. First, the salt/electrolyte in the solution can provide positively-charged or negatively-charged ions in an aqueous solution. These ions bind to the surface of oppositely charged nanoparticles, which reduces the absolute apparent zeta potential of the nanoplex and weakens the repulsive force between the nanoparticles, breaking in the balanced force in the nanoparticle sol and resulting in the aggregation of nanoparticles. Second, charged nanoparticles can bind to the oppositely charged polyelectrolyte, causing the shielding of the charge in the polymer chain, which decreases the repulsive force between the polymer chain, leading to precipitation. Third, the electrostatic interactions between the oppositely charged nanoplex and substance might absorb each other and suppress the formed complex, resulting in the precipitated granule. A visibly flocculated nanoplex can be filtered rapidly. Filtration after flocculation is a simple, highly efficient and easily reproducible process used to purify the nanoplex [316]. Moreover, the dispersed nanoplex is supposed to exhibit a certain desired size, dissolution rate, and high apparent solubility. Preliminary work related to this has been done by me.

9.2.3 Increase the amorphous stability and payload by using ionizable small molecules as ion pairs to the drug

The amorphous nanoparticle drug has proven its ability to overcome poor drug solubility. However, the development of a nano-amorphous drug formulation with high drug loading and good stability in the amorphous form still remains challenging. One approach is use the small ionizable molecules to replace the polysaccharide to develop an easy yet effective strategy to prepare an amorphous nanoparticle complex exhibiting sustained release, higher drug loading, and good drug release efficiency.

9.2.4 Embedding the nanoparticles with the modifiable release functionality into micro size with suitable aerodynamic properties to treat respiratory infections

The systemic antibiotic CIP has been used clinically to treat respiratory infections such as chronic obstructive pulmonary disease and cystic fibrosis, which are mainly caused by *Pseudomonas aeruginosa* [317, 318]. Compared to the nasal and sublingual cavities, the lungs have a huge available surface area and epithelial permeability, which makes pulmonary administration an interesting approach [319]. Moreover, in pulmonary administration, CIP can deposit directly on the lung and reach the defect site directly, potentially increasing its therapeutic efficacy [157].

However, the burst absorption can cause a high local plasma concentration and increase the risk of toxicity and bacteria resistance [157]. Furthermore, the fast diffusion through the lung epithelium [320] reduces the amount of CIP remaining within the lung and causes the rapid clearance of CIP, which results in failure to maintain the therapeutic effect over an adequate period of time. The main challenge with lung administration lies with the uncontrollable release of the drug within the lung. Therefore, an alternative CIP dose formulation with sustained release or lower permeability is

desired for the purpose of pulmonary administration. Embedding nanoparticles into micro size with suitable aerodynamic properties represents a promising approach for the delivery of the drug deep into the deep lungs, as the use of the dry powder inhaler system would provide a high drug pulmonary deposition due to the suitable aerodynamic size of the microparticles. The followed release of nanoparticles with high drug loading can thus provide superior penetration of the mucus and slow down clearance from the lungs, with adequate drug concentration for therapeutic effect. CIP is an antibiotic commonly used to treat lung diseases but has a significant disadvantage. CIP's rapid systemic absorption in the lung can increase the risk of toxicity. Based on this, I would like to recommend working on embedding the nanoparticles into micro size to exhibit suitable aerodynamic properties and with the modifiable release functionality to treat respiratory infections using CIP as a drug model.

9.2.5 Selective release function and nanoplex stability

9.2.5.1 Equipping the nanoplex with selective release functionality

Currently, the nanoplex can release the drug in any liquid body with electrolyte. Conserving the API from unnecessary release improves bioavailability. Ideally, the nanoplex should remain assembled before it reaches the absorption site. For the drug to be absorbed in the intestine, it will be better for the drug nanoplex to remain "complete" in the stomach and release the drug only in the intestine, because the release of the drug in non-absorption sites will not have the desired therapeutic effect. The drug released in the stomach juice will precipitate out when it reaches the intestine due to the change of pH, resulting in the decrease of its bioavailability. Nanoplex with more selective dissolving functionality or coated with a protective layer to avoid its disintegration in the gastric juice was possible to be designed.

9.2.5.2 Improving the stability and prolonging the lifetime of the nanoplex

For the nanoplex to be administrated via intravenous injection, the nanoplex should remain stable for a long enough time, so that the drug can be released at the disease site. However, the current nanoplex tends to dissolve quickly in the PBS, which indicates its instability and short lifetime in the human body. Nanoplexes can be coated with protective layers (such as PLGA, PVP) can be designed in future to avoid this problem. Suitable protective layers should be explored.

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