Summer 8-31-2014

Preparation and characterization of fast dissolving pullulan films containing griseofulvin nanoparticles for bioavailability enhancement

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ABSTRACT

PREPARATION AND CHARACTERIZATION OF FAST DISSOLVING PULLULAN FILMS CONTAINING GRISEOFULVIN NANOPARTICLES FOR BIOAVAILABILITY ENHANCEMENT

by
Zhelun Ma

The aim of this study is to enhance the bioavailability of griseofulvin, a model poorly water-soluble drug, via increasing drug dissolution rate through preparation of drug nanoparticle-laden, pullulan-based strip films. The work entails (i) wet-milling griseofulvin in a stirred media mill using pullulan (polymer) along with sodium dodecyl sulfate (surfactant) as stabilizers, (ii) preparing strip films by casting–drying a precursor suspension consisting of the mixture of the milled drug suspension and a film-forming pullulan–xanthan gum–glycerin solution, (iii) characterizing the suspensions and the films, and (iv) exploring the effects of film thickness, drug and xanthan gum loadings, and drug particle size on the drug content uniformity, mechanical properties, and in vitro drug release from the films. Results show that thin strip films exhibited excellent content uniformity, fast drug release without having excessive amount of toxic surfactants, and easy modulation of the properties, which demonstrates their effectiveness and versatility.

PREPARATION AND CHARACTERIZATION OF FAST DISSOLVING PULLULAN FILMS CONTAINING GRISEOFULVIN NANOPARTICLES FOR BIOAVAILABILITY ENHANCEMENT

by
Zhelun Ma

A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Chemical Engineering

Otto H. York Department of
Chemical, Biological and Pharmaceutical Engineering

August 2014

APPROVAL PAGE

PREPARATION AND CHARACTERIZATION OF FAST DISSOLVING PULLULAN FILMS CONTAINING GRISEOFULVIN NANOPARTICLES FOR BIOAVAILABILITY ENHANCEMENT

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谨以此文献给一直支持我的父母，祖辈，爱我和我爱的人。

The thesis work was dedicated to my beloved family.

ACKNOWLEDGMENT

I would like to express my deepest appreciation to my thesis advisor, Dr. Ecevit Bilgili, whose hardworking and relentless pursuit of science inspired me to keep going on the road of chemical engineering, and to my thesis co-advisor, Dr. Rajesh Davé, whose success set me a goal to chase. My special thanks to Dr. Robert Barat for his invaluable advice and active participation in my committee.

I also want to thank Dr. Ravikumar Chettiannan who was my mentor at the beginning of my thesis work helped me learn a lot from scratch. I am extremely grateful to my friend, Wannan Xiong, who always accompanied me and helped me finish my experiments till late at night and encouraged me a lot. I am also thankful for the advice of my fellow students, Meng Li, Jun Zhang, Scott Krull and Mohammad Azad, which helped shape my thoughts along the way.

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CHAPTER 1

INTRODUCTION

1.1 Objectives

The goal of this study is to enhance the dissolution rate and bioavailability of poorly water-soluble drugs by preparing drug nanoparticle-laden, pullulan-based oral strip films without excessive use of toxic surfactants in the formulation. To achieve this goal, the study is designed to implement the following objectives: (i) to prepare griseofulvin, a model poorly water-soluble drug, nanoparticles via wet stirred media milling (WSMM) process using pullulan (a polymer) as a steric stabilizer along with sodium dodecyl sulfate (SDS, an anionic surfactant) in small concentration, (ii) to prepare nanoparticle-laden, pullulan-based oral strip films by casting–drying a precursor suspension consisting of the mixture of the milled drug suspension and a film-forming pullulan–xanthan gum (a viscosity enhancing agent)–glycerin (a plasticizer) solution, (iii) to characterize the suspensions and the films, and (iv) to explore the effects of film thickness, drug and xanthan gum loadings, and drug particle size on the drug content uniformity, mechanical properties, and *in vitro* drug release behavior of the strip films.

1.2 Background Information

About 90% of the compounds in pharmaceutical delivery pipelines today exhibit poor water solubility (Page, 2008). Similarly, 60% of drug candidates currently under investigation can be classified as Biopharmaceutical Classification System (BCS) Class II, for which the bioavailability is limited by the dissolution rate. Recent advances in combinatorial chemistry aided the discovery of new drug molecules with such low

solubility characteristics (Müller and Peters 1998, Niwa et al. 2011). Low aqueous solubility poses a great difficulty in administering these drugs since approximately 60% of human body is made up of water. An active pharmaceutical ingredient (API), or drug, cannot reach its molecular target in the body if it remains undissolved in the gastrointestinal (GI) tract and is ultimately excreted. Simply put, drugs that do not dissolve cannot heal.

There are a number of methods that have been developed to improve drug solubility and/or dissolution rate. These include: (a) reduction of drug particle size to increase available surface area for dissolution (Bhakay et al. 2011, Merisko-Liversidge et al. 2003), as per the Noyes-Whitney equation (Noyes and Whitney 1897); (b) manipulation of solid state of drug substance to improve drug dissolution i.e., by decreasing crystallinity of drug substance through formation of solid solutions/amorphous solids (Kim et al. 2008, Shen et al. 2010, Zhang et al. 2006); (c) solubilization in surfactant systems (Carvalho et al. 2010); (d) formation of water-soluble complexes (Jansook et al. 2010); and (e) use of pro-drug and drug derivatives such as strong electrolyte salt forms that usually have higher rate of dissolution (Liu et al. 2006).

1.2.1 Wet Stirred Media Milling and Formation of Drug Nanosuspensions

A popular approach for the bioavailability enhancement of poorly water-soluble drugs is particle size reduction. Reduction in particle size leads to an increase in the specific surface area of the drug particles, which in turn improves the dissolution rate (Noyes and Whitney 1897). Ultrafine particles can be produced either by the so-called top-down approach, which includes jet milling (Chan et al. 2002), high pressure homogenization (Kamiya et al. 2009), and stirred media milling (Bilgili et al. 2006); or by the bottom-up approach (Anais

et al. 2009, Chiou et al. 2008) which involves building up particles by precipitation of dissolved molecules.

Among the methods used for particle size reduction, the use of wet stirred media milling (WSMM) has received much attention because of the effectiveness in producing microparticles and nanoparticles (Bhakay et al. 2011, Bilgili et al. 2006, Monteiro et al. 2013). During WSMM, an aqueous suspension containing drug particles and dissolved stabilizers (polymer and/or surfactant) as well as hard beads (media) is stirred at high speed by a rotor. Repeated stressing of the micron-sized drug particles that are captured between the colliding beads causes breakage and eventually production of nanoparticles provided that milling is continued for sufficient time. The milled particles in nanosuspensions (suspensions with median size typically less than a micron) interact significantly with the neighboring particles through van der Waals (interparticle) forces, hydrophobic forces, etc. due to their large surface area (Israelachvili 1992). The attractive interparticle forces can ultimately result in particle aggregation if the suspensions are not properly stabilized. In order to take advantage of potential bioavailability enhancement upon use of drug nanoparticles, it is necessary for the particle size to be preserved after milling. To preserve particle size, polymers and surfactants are used as stabilizers during wet media milling. Stabilizers adsorb on the surfaces of drug particles and provide an ionic or steric barrier (Merisko-Liversidge et al. 2003, Ploehn and Russel 1990). Bilgili and co-workers studied the impact of formulation (stabilizer type and concentration) and process parameters on the particle size, physical stability of the suspensions, and breakage dynamics during wet media milling of griseofulvin via milling experiments, polymer adsorption studies, and microhydrodynamic modeling (Afolabi et al. 2014, Bhakay et al. 2011, Bilgili and Afolabi

2012, Bilgili et al. 2006, Monteiro et al. 2013). The knowledge gained and expertise developed form the basis of the WSMM process used in this work.

Drug nanosuspensions can be used orally or can be used in ocular delivery, intravenous administration, and dermal applications. On the other hand, from a physical stability perspective for the milled suspensions as well as patient preference/compliance perspective, the general practice in pharmaceutical industry is to dry and convert these suspensions into nanocomposite microparticles (NCMPs), in the form of dry powders, via spray drying, spray freeze drying, freeze drying, and granulation with or coating onto inert excipient particles (Chaubal and Popescu 2008, Dalvi and Dave 2010, Hu et al. 2004, Lee 2003, Van Eerdenbrugh et al. 2008). NCMPs produced in such manner are generally incorporated into the final solid dosage forms such as tablets, capsules, etc. using standard pharmaceutical unit operations.

Unfortunately, during the wet-milling and drying processes, drug nanoparticles can form aggregates of several microns in size or larger (Chaubal and Popescu 2008, Lee 2003). If the drug nanoparticles are not completely recovered from these aggregates during dissolution, then the latent large surface area of the nanoparticles will not be realized. As a result, the dissolution rate and bioavailability of the drug will be smaller than that anticipated from drug nanoparticles (Lee 2003, Van Eerdenbrugh et al. 2008). Apart from dissolution testing, several studies examined the nanoparticle recovery via a redispersion test, where the NCMPs were dispersed in water (or other biorelevant fluids) and the particle size of the resulting suspension was measured and compared with the original nanosuspension (Chaubal and Popescu 2008, Lee 2003). Several studies (Chaubal and Popescu 2008, Lee 2003, Tewa-Tagne et al. 2007, Van Eerdenbrugh et al. 2008) reported

slow and incomplete recovery of nanoparticles from the NCMPs if the NCMP formulations contained either a steric stabilizer or an electrostatic stabilizer such as an ionic surfactant alone. The poor nanoparticle recovery could have been partly due to the formation of hard drug nanoparticle aggregates or drug–polymer bridging (Chaubal and Popescu 2008, Dalvi and Dave 2010, Hu et al. 2011) in the form of agglomerates. In contrast, drug nanoparticles were completely recovered, when a combination of a steric stabilizer and an ionic surfactant was used in the NCMP formulations (Hu et al. 2004, Kho et al. 2010, Lee 2003), suggesting the criticality of the presence of surfactants.

Despite the fact that surfactants are regarded to be desirable for fast nanoparticle recovery from the NCMPs, there are several issues associated with the use of surfactants such as physical instability of the drug suspensions (Vogt et al. 2008), significant particle growth in suspensions via Ostwald ripening during storage especially if used above the critical micelle concentration (CMC) (Biradar et al. 2006, Li et al. 2011), and irritation to the pulmonary epithelium in inhalation applications (Abdelwahed et al. 2006, Cheow et al. 2011, Konan et al. 2002). Thus, the surfactant concentration should be minimized and controlled during formulation development. In this study, we want to examine the feasibility of drug nanoparticle-laden, pullulan strip films as a dosage form in enhancing the drug dissolution rate without excessive use of toxic surfactants.

1.2.2 Strip Film: a Promising Dosage Form

Although solid dosage forms such as tablets and capsules are widely accepted by elders and adolescents, more and more patients tend to prefer liquid formulations that are easier to swallow (Dixit and Puthli 2009). Keeping the ease of administration and swallowing in mind, pharmaceutical research has led to the development of Orally Disintegrating Tablets

(ODTs). ODTs have been defined as "A solid dosage form containing medicinal substances which disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue". United States Food and Drug Administration (FDA) further defines ODTs as solid oral preparations that disintegrate rapidly in the oral cavity, with an *in vitro* disintegration time of approximately 30s or less, when based on the United States Pharmacopeia (USP) disintegration test method or alternative (Dixit and Puthli 2009). Research and development in the oral drug delivery segment has led to transition of dosage forms from simple conventional tablets/capsules to modified release tablets/capsules to ODTs to the recent development of oral strip films. Basically strip films can be considered as an ultra-thin strip of postage stamp size with an active agent or active pharmaceutical ingredient and other excipients. Convenience of dosing and portability can allow for wide acceptability of this dosage form among pediatric and geriatric populations. Overall, the strip films enjoy some distinct advantages over other oral dosage forms:

1. Availability of larger surface area leads to rapid disintegration and dissolution in the oral cavity.

2. The films are flexible and they are not as fragile as most of the ODTs. Therefore, the transportation of films is easier, and it is also more convenient for storage by consumers. In addition, most ODTs are brittle, which require special packages for protection during storage and transportation.

3. Administration of drug dose is much more precise with the use of films as compared with that of drops or syrup dosage. In addition, accurate dosing can be predicted per area of film due to low variability of drug content in the films.

4. The film is a convenient dosage form for patients to take. No usage of water or swallowing is required. Such dosage is pretty friendly to dysphagic patients who encounter difficulty in swallowing tablets or capsules. Also, the dosage form can be consumed at any place and any time as per convenience of the patient.

5. The film is easy to stick to buccal, which consists of substantial blood capillary for drug absorption. Hence, drugs can be absorbed directly and enter the systemic circulation without degradation from the GI tract. This advantage can be enhanced

in preparing products with improved oral bioavailability of molecules (Dixit and Puthli 2009).

6. Strip films also offer manufacturing advantages including continuous manufacturing capabilities that enable full product characterization when incorporated with in-line monitoring using Process Analytical Technologies (Dixit and Puthli 2009).

1.2.3 Integrating Drug Nanosuspensions into Oral Strip Films

Polymer strip films have great potential for the delivery of poorly soluble drugs since they provide large surface area, which leads to rapid disintegration and dissolution in the oral cavity and increased bioavailability (Dixit and Puthli 2009). Even though the interest in using strip films for drug delivery has increased based on publications and patents (Dixit and Puthli 2009, El-Setouhy and El-Malak 2010, Garsuch and Breitkreutz 2010, Li and Gu 2007, Matthews et al. 2008, Mendoza-Romero et al. 2009, Perumal et al. 2008), more research is needed especially for applications involving bioavailability enhancement of poorly water-soluble drugs.

Research and development in this field has focused on the incorporation of water-soluble drugs into strip films (Jug et al. 2009, Nishimura et al. 2009, Schmidt and Bodmeier 1999, Shimoda et al. 2009). While water soluble drugs exist in the dissolved state or as solid solution, the water insoluble drugs have to be homogenously distributed in a film in order to have an acceptable drug content uniformity. In recent patents (Yang et al. 2010), the process for making films with uniform distribution of components is described. The incorporation of poorly soluble particles into films or the issues involved when using microparticles or nanoparticles were not specifically addressed in these patents. Interactions between the drug molecule and polymer could also produce differences in the film properties (Nair et al. 2001).

Since polymer strip film has been demonstrated as a promising dosage form for drug delivery and nanosuspensions allow for dissolution and bioavailability enhancement for poorly water-soluble drugs, it is of great interest to explore the feasibility of incorporating poorly water-soluble drug nanosuspensions into strip films. To this end, (Sievens-Figueroa et al. 2012) prepared and characterized hydroxypropyl methyl cellulose films containing three different BCS Class II drugs, i.e., naproxen, fenofibrate and griseofulvin. The strip film has been characterized by SEM, XRD, Raman spectroscopy and NIR chemical imaging analysis. The SEM images showed that particles of all three model drugs were well-dispersed in the films that had good drug content uniformity detected by NIR chemical imaging. XRD and Raman spectroscopy were combined to investigate the crystal structure of the drug in the film, and results demonstrated that the drug crystallinity was preserved during the strip film preparation. These results demonstrated the feasibility and set the groundwork for the integration of WSMM with film formation process. The work also proved the advantages of films over other solid dosage forms: e.g., the films with drug nanoparticles exhibited fast and immediate drug release. Interestingly, Sievens-Figueroa et al. (2012) did not investigate the impact of some formulation and process parameters, unlike the work on traditional solvent-casting of drug solutions. Some parameters of polymer films like film thickness and excipient type/concentration are widely investigated to explore their impact on the dissolution behavior (Shimoda et al. 2009, Singh et al. 2005). For a drug-laden dosage form like films or tablets, drug loading and drug particle size are the most common dominant factors (Nair et al. 2001, Schmidt and Bodmeier 1999).

1.3 Problem Definition and Formulation Considerations for Fast Dissolving Polymer Films

This study focuses on the dissolution rate enhancement of griseofulvin, a model poorly water-soluble drug, by incorporating griseofulvin nanoparticles prepared via wet stirred media milling into pullulan-based strip films. *As a major novelty, unlike previous work (Sievens-Figueroa et al. 2012) that used HPMC, pullulan was used as both the steric stabilizer in wet milling and film-former during casting–strip film preparation.* In general, a variety of polymers (Dixit and Puthli 2009), e.g., hypromellose, hydroxy propyl cellulose, pullulan, pectin, gelatin, are available for preparation of oral strip films, and the polymers may be used alone or in combination to obtain the desired film properties. Let us consider some of the desirable film properties here. The films must be tough enough to avoid any damage during handling or during transportation. The robustness of the strip film depends on the type of polymer and its concentration in the formulation. Moreover, strip films must disintegrate fast when placed in mouth and deliver the drug to the oral cavity instantaneously. The film-forming polymer is the most essential and major component of the oral strip film; at least 45%w/w of polymer should generally be present based on the total weight of dry oral strip film (Dixit and Puthli 2009). Pullulan, which is the most commonly used polymer for preparation of oral strip films, is a natural polymer obtained from non-animal origin that does not require chemical modification (Dixit and Puthli 2009). As an edible, mostly tasteless polymer, pullulan also shows good solubility in water compared with other polysaccharides due to its linear structure. This polymer provides highly clear and homogenous films. It has low oxygen permeability and low water content, which makes it most suitable for production of polymer film. While offering

many advantages, pullulan has not been used to form oral strip films that carry nanoparticles of poorly water-soluble drugs before.

The other ingredients used in the film formulations of this study are glycerin and xanthan gum. Glycerin is used as a plasticizer because of its high solubility in water and body structure. It has been approved by FDA for oral route usage. Gum was used as a thickening agent. In its absence, viscosity of pullulan solutions was too low, which caused several issues such as excessive bubble formation in the polymer solution during shear mixing and shrinkage at the edge of the wet film during drying process. To mitigate these issues, xanthan gum was incorporated with the concentrations of 0.58%, 1.15%, 2.27% and 4.45% into the films. Other benefits of xanthan gum are as follows: it has high solubility in water and it is also approved by FDA for oral formulations.

Besides formulation, process attributes also impact the quality of strip films as well as drug release. For example, thickness determines the drug amount per unit area which may impact the dissolution rate, and it also determines the strip film texture and mechanical properties like tensile strength. Different drug loading in dry films will impact the drug content and its uniformity as well as the mechanical properties because of different polymer–drug ratio. Particles size of the drug determines the surface area from which dissolution occurs, which in turn can affect the dissolution rate directly.

In this work, a systematic study has been performed to delineate the effects of several parameters of oral strip film such as film thickness, drug loading and drug particle size, and xanthan gum loading on the dissolution rate. Through the study of these parameters, it is possible to optimize the dissolution rate and bioavailability of poorly

water-soluble drugs in oral strip films. The study also provides proof-of-concept for preparing film formulations with minimal concentration of toxic surfactants.

1.4 Scope and Organization of Thesis

The following chapters outline a study of the effects of film thickness, excipients concentration, drug loading in dry films and drug particle size on the content uniformity, mechanical properties and *in vitro* drug release behavior of dry pullulan film. Chapter 2 describes the experimental details of the study, including methods and materials for suspension and film production, as well as methods for product characterization. Results of the experiments and discussion of these results are presented in Chapter 3. Chapter 4 is summative assessment of the impact of film thickness, xanthan gum and drug loadings in dry films, and drug particle size on pullulan film.

CHAPTER 2

EXPERIMENTAL

The methods for preparing and characterizing griseofulvin nanosuspensions and nanoparticle-laden, pullulan-based oral strip films are given. Griseofulvin nanosuspensions were prepared via wet stirred media milling using pullulan as a steric stabilizer. The milled drug nanosuspensions were then incorporated into pullulan precursor solutions consisting of pullulan, glycerin, xanthan gum and deionized water. The precursor film suspensions were casted–dried. The suspensions and the films were characterized.

2.1 Preparation of Griseofulvin Nanosuspensions

2.1.1 Materials

Wet stirred media milling experiments were carried out with the poorly water-soluble drugs griseofulvin (GF) (LETCO MEDICAL). To stabilize the drug particles after milling, sodium dodecyl sulfate (SDS) (Sigma-Aldrich, Saint Louis, MO) was used as a surfactant and pullulan (DKSH, Mt. Arlington, NJ) was used as a steric stabilizer in the suspensions. The physicochemical properties of three components are presented in Table 2.1. The chemical structures of the components used for milling are shown in Figure 2.1.

Table 2.1 Properties of Components Used in Wet Stirred Media Milling

Component	Solubility at 25 ^{o}C (mg/L)	Media Molecular Weight (Da)	Melting Point (^{o}C)
Pullulan	$> 5*10^{5}$	200,000	250
SDS	150	288.38	206
GF	8.64	352.8	220

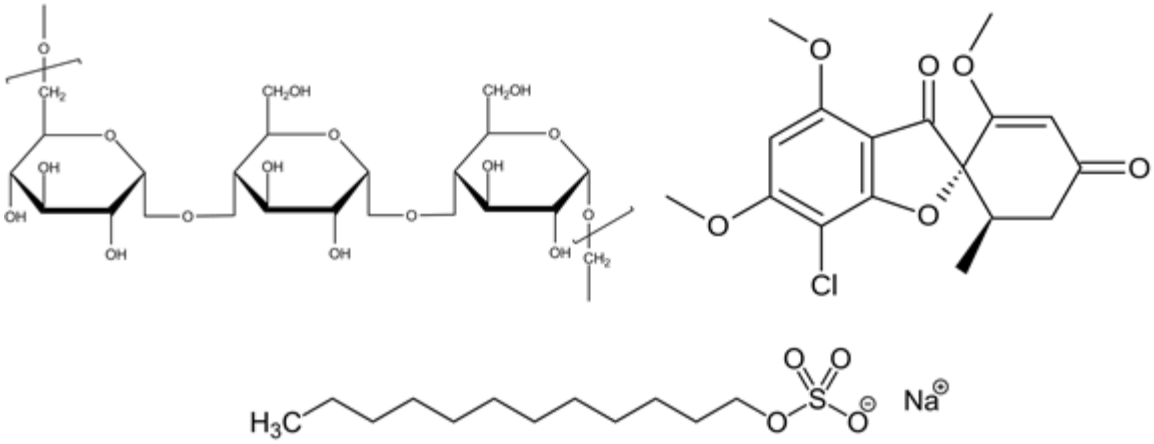

Figure 2.1 Chemical structures of pullulan (upper left) and griseofulvin (upper right) and sodium dodecyl sulfate (bottom).

Source: Wikipedia, the free encyclopedia.

2.1.2 Preparation Methods

Table 2.2 presents the suspension formulations used in this study. Feed GF suspensions were prepared using a shear mixer (Cat#. 14-503, Fisher Scientific, Pittsburgh, PA, USA) running at a fixed speed of 300 rpm. First, 0.4 g SDS was dissolved in 200 g deionized water for 15 min to make a 0.2% w/w solution. Throughout the preparation of nanosuspensions, all percentages (%) refer to w/w with respect to deionized water. 5 g pullulan was then dissolved in the SDS solution to make a 2.5% solution. 20 g griseofulvin was subsequently dispersed into the stabilizer solution with the shear mixer running for 30 min except in Runs 2–4. In Runs 2–4, different amount of GF was dissolved in stabilizer solution to make a solution of 5%, 20% and 25%, respectively. These runs were performed as part of drug loading study and it was designed according to the baseline process which refers to Run 1. Compared with the baseline process, there was one run with a lower drug loading, which had 5% drug loading and two higher drug loadings which had 20% and 25%. It is estimated that if the drug loading were lower than 5%, e.g., 1%, 3%, the drug loading in the corresponding dry film would be less than 1%. This drug loading would be

unacceptable for a variety of practical and theoretical reasons such as too low dose, difficulty to measure such low assays, etc. When the drug loading was higher than 25%, e.g., 30%, the corresponding dry film was very crisp and hard to handle.

Table 2.2 Components Percentage and Milling Time for Each Run in WSMM

Run no.	Drug loading (% w/w)	Pullulan concentration (% w/w)	SDS concentration (% w/w)	Milling time (min)
1	10	2.5	0.2	90
2	5	2.5	0.2	80
3	20	2.5	0.2	100
4	25	2.5	0.2	120
5	10	2.5	0.2	90
6	10	2.5	0.2	90
7	10	2.5	0.2	90
8	10	2.5	0.2	90
9	10	2.5	0.2	90
10	10	2.5	0.2	90
11	10	2.5	0.2	0
12	10	2.5	0.2	2
13	10	2.5	0.2	8

Separate milling experiments were conducted for different durations in Runs 1–4 in an attempt to produce similarly sized particles in suspensions with different drug loadings. In order to study the effect of drug particle size, as-received, 2 min milled, 8 min milled and 90 min milled griseofulvin suspensions were prepared (Runs 11–13 and 1, respectively). In these cases, each run corresponds to a separate milling experiment. Runs 1, and 5–7 suspensions were taken from the same milling experiment; hence, identical particle size statistics will be reported. Runs 5–7 were used to explore the impact of film thickness with the same drug nanosuspension formulation (see Section 2.2.2.1). Precursor suspensions

with the same formulation (Run 1, 5–7) were casted-dried with different wet film thickness ranging from 250 µm to 1000 µm, by adjusting the opening size of Doctor Blade (Elcometer, USA). A new batch of milled suspension was prepared for all of Runs 8–10 under identical processing conditions with those in Runs 1, and 5–7. Since the WSMM process is known to be reproducible (Bilgili and Afolabi, 2012; Afolabi et al., 2014), it is assumed that Runs 8–10 suspensions had the same particle size statistic as those of Runs 1, and 5–7. Runs 8–10 together with Run 1 were performed as part of the xanthan gum loading study, where four different xanthan gum concentrations (0.12%, 0.24%, 0.48% and 0.95%) were introduced in precursor solutions. Runs 11–13 were performed so as to study the impact of particle size. In Runs 11–13, different milling times were used to attain different drug particle sizes. The suspensions were milled for 90 min in Runs 1, 5–10. In an attempt to get similar particle sizes in the case of widely different drug loadings, Runs 1, 2, 3 and 4 were milled for 90 min, 80 min, 100 min and 120 min, respectively.

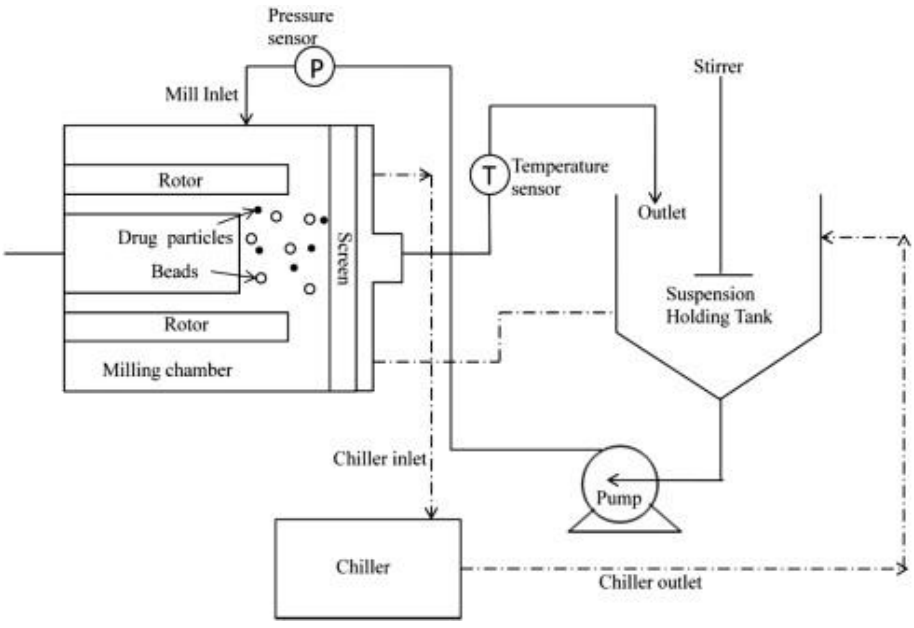

Figure 2.2 Schematic of the Netzsch stirred media mill (Model: Microcer) operating in the recirculation mode. P and T stand for pressure gauge and thermocouple, respectively. (Bhakay et al. 2013)

Drug suspensions prepared via mixing, as mentioned above, were subsequently milled in a Netzsch wet media mill (Microcer, Fine Particle Technology LLC, Exton, PA, USA). The wet stirred media milling process is depicted in Figure 2.2. In this so-called recirculation mode of operation, the suspension circulates from the holding tank through the milling chamber and back to the holding tank. Milling media (beads) are inside the milling chamber and set into motion by the rotation of the rotor. High rotor speed induces turbulent motion in the suspension, and turbulent energy dissipates during frequent bead–bead collisions (Eskin et al. 2005), which cause extensive breakage of drug particles captured between the beads (Bhakay et al. 2011, Bilgili et al. 2006). Yttria-stabilized zirconia beads with a nominal size of 400 μm were used as the milling media and a 200 μm screen was used to retain the beads in the milling chamber. The beads having 50 ml bulk volume were loaded to the milling chamber. The suspensions were fed to the milling chamber at the rate of 126 ml/min using a peristaltic pump, and the rotor speed was 3200 rpm rotor speed (tip speed of about 11.8 m/s). The temperature inside the mill was maintained below 35 ℃ with the help of a chiller (Advantage Engineering, Inc., Greenwood, IN, USA).

Samples at several milling time were taken at the outlet of the milling chamber for particle sizing. The final suspensions (after milling) were tested for shear viscosity, and they were refrigerated at 4 ℃ for a period of 7 days. Particle sizes right after milling and after 7 days of storage were compared to assess the physical stability of the suspensions.

2.2 Polymer Film Production

A schematic of the polymer films production is shown in Figure 2.3. The drug nanosuspensions prepared via wet stirred media milling and film-forming (precursor) polymer solution are mixed using a shear mixer and a Thinky mixer. The resulting precursor suspensions are then wet-casted and dried to form strip films.

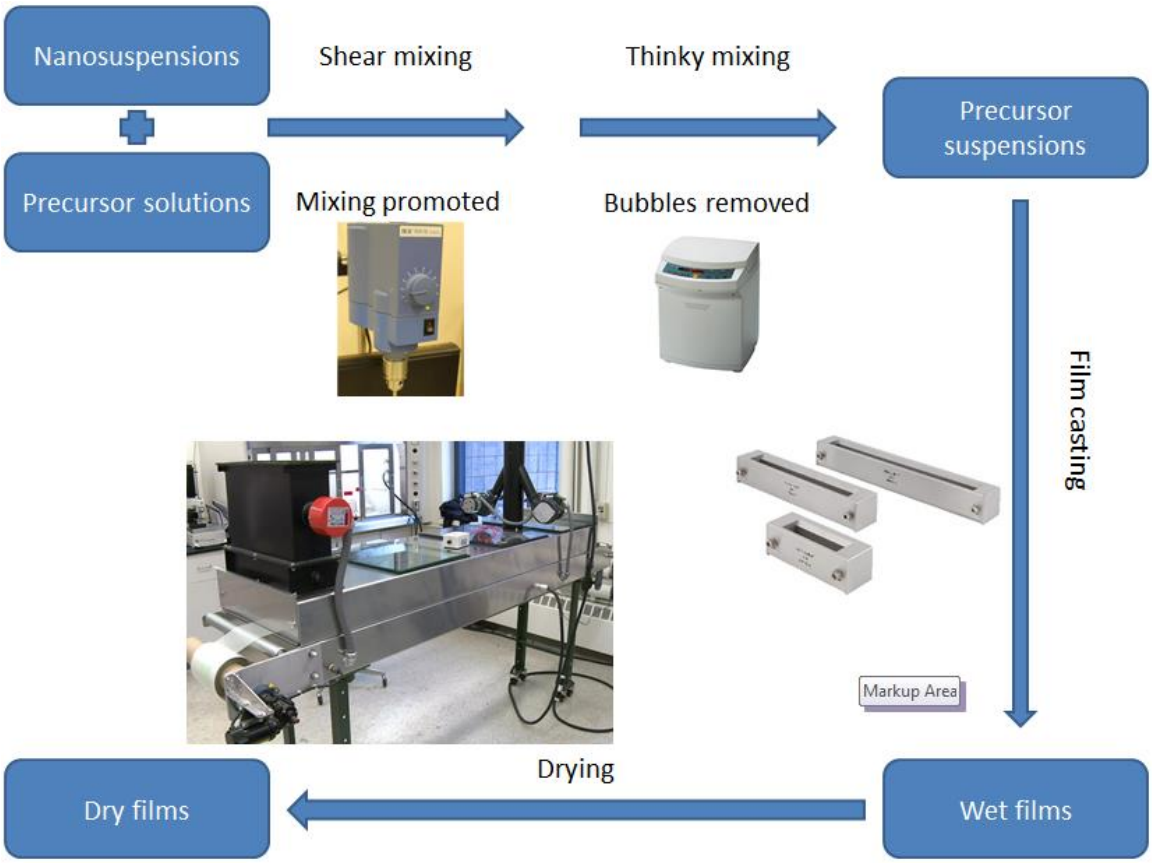

Figure 2.3 Experimental setups for the film formation process.

2.2.1 Preparation of Precursor Suspensions

2.2.1.1 Materials. In the preparation of the precursor solution, pullulan (DKSH, Mt. Arlington, NJ) was used as a film-forming polymer to be dissolved in water. Glycerin (Sigma-Aldrich, Saint Louis, MO) was used as a plasticizer while xanthan gum

(Sigma-Aldrich, Saint Louis, MO) was used as a thickening agent. The chemical structures of glycerin and xanthan gum are shown in Figure 2.4.

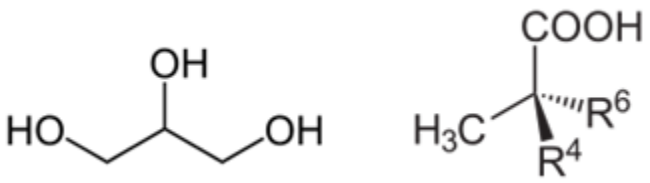

Figure 2.4 Chemical structures of glycerin (left) and xanthan gum (right).
Source: Wikipedia, the free encyclopedia.

The physicochemical properties of glycerin and xanthan gum are summarized with their median molecular weight in Table 2.3.

Table 2.3 Properties of Components Used in Precursor Solution

Component	Solubility at 25 ^{o}C (mg/L)	Media Molecular Weight (Da)	Melting Point (^{o}C)
Pullulan	> $5*10^5$	200,000	250
Glycerin	> $5*10^5$	92.09	18
X. Gum	> $5*10^5$	$2*10^6$ - $20*10^6$	270

2.2.1.2 Methods. Film precursor solutions were prepared using a dual-propeller mixer (McMaster, Catalog no. 3471K5, Los Angeles, CA, USA) attached to a motor (VWR OVERHEAD STIR VOS 16V120). The shear mixer was fixed at a speed of 40 rpm throughout the shear mixing process. A solution containing 19.88% pullulan (13 g) and 3.31% glycerin (2.16 g) was prepared by first adding the glycerin into de-ionized water (50 g) and heating to 80 ℃. This composition was chosen based on preliminary work, taking into account the final suspension viscosity and visual film quality. Pullulan was then added until well dispersed and the heat source was shut off. The temperature was decreased to room temperature to dissolve the polymer completely. Then, the required amount of

xanthan gum was dissolved in the solution for 2 h. The nanosuspensions produced from WSMM (18 g) were then added to the polymer solution (65.36 g), and then shear mixed for another 2 h. While the mixing via the dual-propeller mixer enabled intermingling of different film ingredients, unfortunately it caused incorporation of air into the suspensions in the form of bubbles, which could negatively affect the drug content uniformity, visual appearance, and mechanical properties. To mitigate these potential issues, the precursor suspensions were then transferred into specific containers, which were then used in a planetary centrifugal mixer ("THINKY MIXER" ARE-310, THINKY USA Inc.). The THINKY Mixer is a no-blade planetary centrifugal mixer that mixes materials employing "rotation" and "revolution". In much the same way the Earth revolves around the Sun, the mixing container simultaneously "revolves" around the center of the mixer while "rotating". These two forces simultaneously and thoroughly mix, disperse and deaerate materials in the container. The high centrifugal force enables simultaneous processing of mixing high-viscosity materials, which is particularly useful in this study because of the high viscosity of the precursor solution with xanthan gum. The deaeration mode allows for removal of the bubbles generated during the shear mixing process effectively, which could help obtain homogenous precursor suspensions. Each sample was mixed for 5 min and defoamed for 25 min. The revolution speed of mixing was kept at 2000 rpm while the revolution speed of defoaming was kept at 2200 rpm.

2.2.2 Dry Films Production

2.2.2.1 Methods. The final precursor suspensions were casted onto a fluoropolymer coated polyester film (3M™ Scotchpak™ 9744 Release Liner) using a Doctor Blade (Elcometer, USA). The fluoropolymer coated polyester film was installed on one of the

two rollers of a tape caster (HED® International, Inc., Ringoes, NJ, USA). The schematic of the tape caster is shown in Figure 2.5. The other roller of the tape caster was attached to a motor and the rotational speed could be controlled.

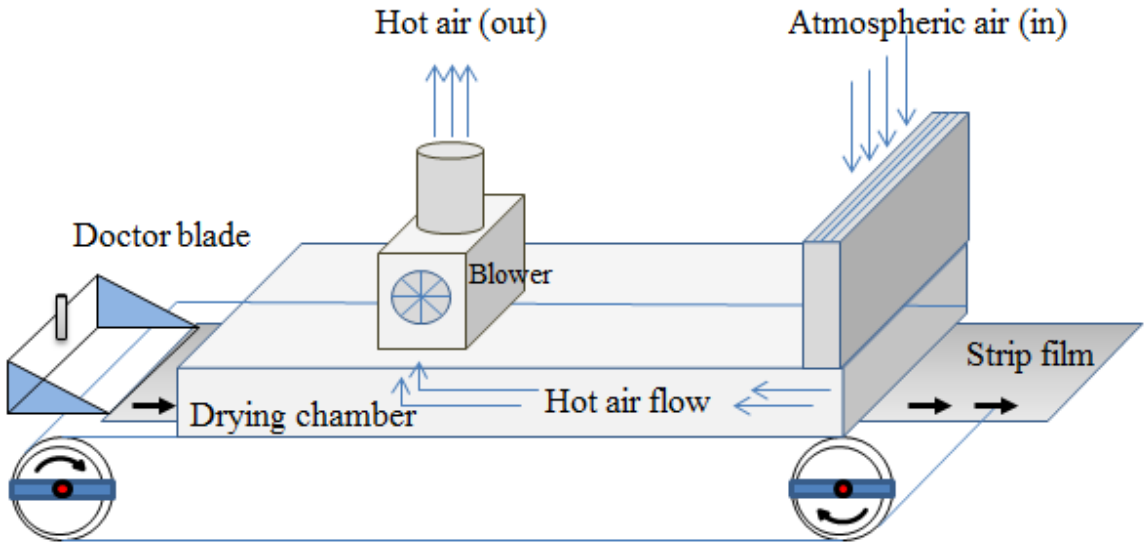

Figure 2.5 Schematic of the tape caster (HED® International, Inc., Ringoes, NJ, USA). Figure is not drawn to scale.
Source: Jun Zhang " TB2-Continuous Manufacture of Strip Films" New Jersey Institute of Technology.

The system which consists of two rollers and a polyester film is similar to a conveyor belt. Between two rollers is the drying chamber located which involves conduction heat from bottom and convection heat from the hot air that keep passing through. The flow of the hot air is opposite to the moving direction of the polyester film helps remove the moisture generated above the wet film during drying. In this study, the moving speed of the polyester film was controlled at ~0.1 m/min; the temperatures in all zones and hot air temperature were set at 50 ^{o}C. This temperature was determined according to our previous work (Susarla et al. 2013). There are two slits at the bottom of the front and rear sides of the chamber which allow the polyester film go through. Before drying, the Doctor Blade filled with precursor solution was placed next to the front sides of

the chamber. The wet film thickness was varied from 250 μm to 1000 μm by changing the opening size of Doctor Blade aperture to study the impact of thickness on drug composition (Run 1 and Runs 5–7). After the polyester film began moving, the wet films were casted by a combined force of the gravity of precursor suspension and the surface tensile strength between the precursor suspension and the polyester film. After the required length of wet film obtained, the motor was then shut down manually to let wet film stay in the middle of the drying chamber. The wet film was dried for 40 min subsequently and then peeled off from the polyester film easily. The dried films were kept in polyester bags at room temperature for further analysis.

2.3 Characterization

2.3.1 Drug Nanosuspensions

2.3.1.1 Particle Size Distribution. Particle size analysis of the suspensions was performed by laser diffraction using a Beckmann Coulter LS230. A polarized intensity differential scattering (PIDS) obscuration water optical model was employed. The PIDS was maintained between 40% and 50% while the obscuration was maintained 8% for all particle size measurements. Particle size distribution was computed by the software using the Mie scattering theory. A refractive index (RI) value of 1.65 for the GF particles (Watanabe 2002) and 1.33 for the measurement medium (DI) water were used. Prior to the size measurement, milled suspension samples (~2 ml) were diluted with 10 ml of stabilizer solution which included SDS and pullulan. The refrigerated suspension samples after 7-day storage were mixed via a digital vortex mixer (Thermo Fisher Scientific Inc., USA)

at 1500 rpm for 2 min. Then, ~2 ml samples were taken and diluted for particle size measurement using the same stabilizer solution as mentioned before.

2.3.1.2 Apparent Shear Viscosity. The apparent shear viscosity of the milled suspensions was measured using an R/S plus rheometer (Brookfield Engineering, Middleboro, MA, USA) with a water jacket assembly Lauda Eco (Lauda-Brinkmann LP, Delran, NJ, USA). A coaxial cylinder (CC 40) was used to impart controlled shear rate on the samples from 0 to 1000 1/s in 60 s. The temperature of the jacket was kept constant at 25 ± 0.2 °C. The raw data were analyzed using the Rheo 3000 software (Brook-field Engineering, Middleboro, MA, USA) of the R/S plus rheometer to obtain the apparent shear viscosity.

2.3.2 Drug Precursor Suspensions

2.3.2.1 Particle Size Distribution. The particle size distribution of GF in the precursor suspensions was also measured by laser diffraction in Coulter LS13320 (Beckman Coulter, Miami, FL, USA). ~4ml precursor suspensions samples were taken and diluted with the stabilizer solution. The diluted precursor solutions were then vibrated via vortex mixer at 1500 rpm for 4 min. The PIDS was maintained between 40% and 50%.

2.3.2.2 Apparent Shear Viscosity. The viscosity of precursor suspensions was measured using an R/S plus Rheometer (Brookfield Engineering, USA) at a shear rate of 2.2. s^{-1}, having a water jacket assembly kept constant at 25 ± 0.2 °C. The shear viscosities of each sample were measured for six times.

2.3.3 Pullulan Strip Films

2.3.3.1 Film Thickness. The thickness of the films was measured using a digital micrometer with an accuracy of 0.001 mm. Thickness of samples which were used in drug content assay, texture analysis and dissolution test were measured and used for calculating the average and relative standard deviation. Those samples were randomly chosen at different locations across the film.

2.3.3.2 Determination of Drug Content. Samples with a diameter of 3/8 inch were punched from dry films at random locations. 10 samples were punched from each film. Each sample was dissolved in 200 ml SDS (5.4 mg/ml) solution for 3 h via magnetic stirring. It is estimated that the drug amount in each sample was no more than 1.5 mg even in the thickest films and the highest drug loaded films. This amount of drug can fully dissolved in 200 ml SDS (5.4 mg/ml) solution (de Smidt et al. 1987, Rao et al. 1997).

2.3.3.3 Thermo-gravimetric Analysis. Thermo-gravimetric analysis (TGA) of films with GF nanoparticles was performed with a TGA/DSC1/SF Stare system (Mettler Toledo, Inc., Columbus, OH, USA). A small sample of a film (~2.0 mg) was placed in a ceramic crucible, heated from 25 ℃ to 150 ℃ in nitrogen atmosphere at a constant heating rate of 5 ℃/min. Finally, the sample was brought back to room temperature (25 ℃) at a cooling rate of 10 ℃/min.

2.3.3.4 SEM. A field emission scanning electron microscope (FESEM) LEO1530VP GEMINI (Carl Zeiss, Inc., Peabody, MA, USA) was used to observe the distribution of particles in the films. For the analysis of the distribution of particles in the films, a 5 mm × 5 mm piece of the film was vertically stuck to the carbon tape for the analysis of the films.

The lens was located at the edge of the samples. Samples were carbon coated using a sputter coater (Bal-Tec MED 020 HR) before analysis.

2.3.3.5 Mechanical Properties. Mechanical testing was conducted using a TA-XT Plus Texture Analyzer (Stable Microsystems, UK) to measure mechanical properties such as yield strength and Young's modulus. Six rectangular strips having dimensions of 50 mm × 15 mm were cut from a single film sheet and tested. Film thickness was measured at 4 different locations of the films and the average was used. The sample was attached to the tensile grips and the test was finished once the sample broke.

2.3.3.6 Redispersion of the Strip Films. About 10 mg of the dry film was weighed and dispersed in 20 ml deionized water for 2 min. The maximum amount of drug that can dissolve in water during the redispersion test is very small (e.g. about 0.02% of GF particles for Run 1). The samples were subsequently agitated via a digital vortex mixer (Thermo Fisher Scientific Inc., USA) at 1500 rpm for 2 min to help particles to establish good contact with water and prevent sedimentation of the particles. The particle sizes obtained from Coulter were mainly the sizes of GF particles and their clusters.

2.3.3.7 Dissolution Testing and Drug Release Kinetics. A fully automated flow-through cell dissolution apparatus (USP 4, Sotax, Switzerland) in a closed loop configuration and with cells of an internal diameter of 22.6 mm (Heng et al. 2008, Kakhi 2009) was used. A Thermo Evolution UV spectrophotometer was used to automatically measure the griseofulvin concentration using a previously generated calibration curve. A wavelength of 291 nm was used to quantify the GF concentration during the dissolution process. A 0.2-μm filter (Pall HT Tuffryn Membrane Disc Filters) was used for the study. The main purpose of the filter was to separate large aggregates of drug particles (>200 nm)

and clusters of disintegrated film from the dissolution sample with the assumptions that GF nanoparticles < 200 nm dissolve relatively fast. The temperature was maintained at 37 ± 0.5 °C during testing. The flow rates of the dissolution medium through the cells were 16 ml/min. The dissolution medium (deionized water, 250 ml) was circulated by pumping it through each cell. The solubility of GF in deionized water was 8.64 mg/L. The drug amount in the thickest films and the highest drug loaded film was only ~60% of theoretical solubility limit, i.e., 2.16 mg GF per 250 ml deionized water. Six samples were used and the average drug release and standard deviation were plotted as a function of time. The drug release profiles of GF strip-films were constructed by plotting the percent of dissolved drug as a function of time.

The equipment cell and dissolution process are described in Figure 2.6 and have been previously described by (Bhattachar et al. 2002). The cell consists of a lower cone, a cylindrical portion, and a filter head (Bhattachar et al. 2002). Dissolution medium enters at the bottom of the cone and exits through the filter head. The filter head on top holds the 0.2-µm filter. The lower cone holds a glass bead 6 mm in diameter, which serves as a check valve, preventing material to descend into the inlet tubing. Strip-film positioned at the cone section; sandwiched between 3.0 g of 1 mm round glass beads at the bottom and 2.0 g of 1 mm round beads on top, leading to a more homogeneous flow and securing the film.

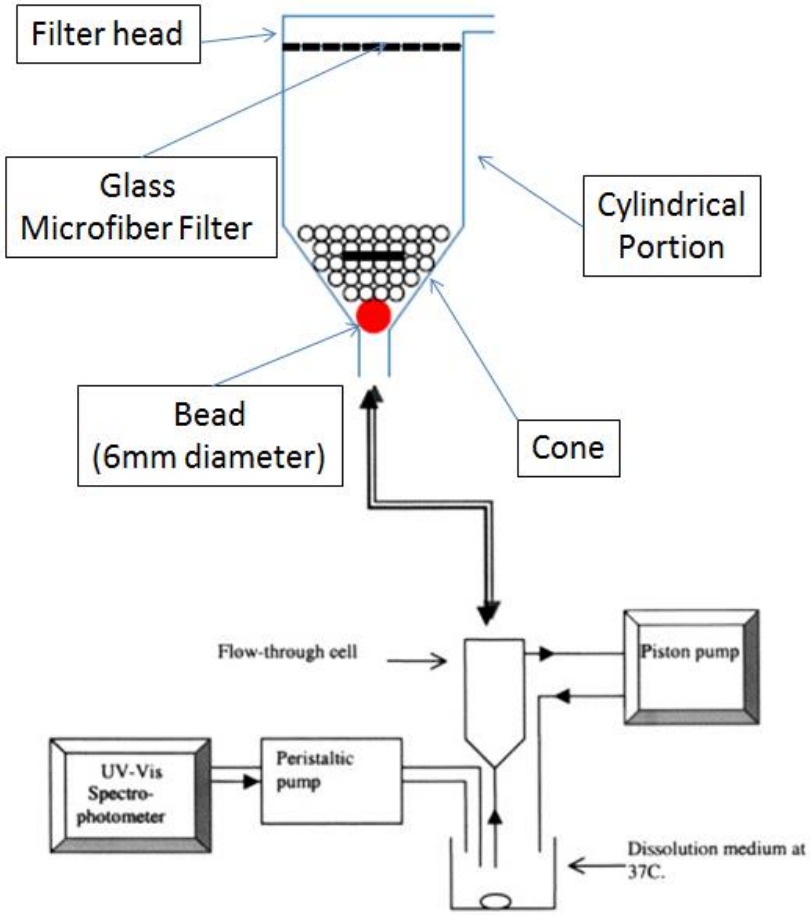

Figure 2.6 Schematic diagram of the flow-through dissolution apparatus.
(Bhattachar et al. 2002)

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Properties of Milled Suspensions

As-received microparticles of griseofulvin (GF), a model BCS class II, was wet-milled in a stirred media mill to increase its surface area, with the ultimate objective of increasing its dissolution rate, thus its bioavailability when taken by patients. The suspensions were produced by wet stirred media milling (WSMM) using pullulan (a polymer) and sodium dodecyl sulfate (SDS, an anionic surfactant) as stabilizers. Bilgili and Afolabi (2012) showed a synergistic stabilizing action of 2.5% HPC (hydroxypropyl cellulose, another polymer)–0.5% SDS in stabilizing wet media milled griseofulvin suspensions. Hence, a 2.5% pullulan–0.2% SDS stabilizer combination was selected here with the objective of minimizing the use of SDS.

Short-term physical stability of the milled suspensions for Runs 1–13 was assessed by comparing the particle size after milling and after 7-day storage (see Table 3.1). Long term stability of the suspensions and optimization of the stabilizers' concentrations were not considered in this work because the drug suspensions were used in precursor suspension and then film preparation shortly after wet media milling without long-term storage. Note that run labels are designated based on respective films. Runs 1, and 5–7 belong to the same milling batch. Since Runs 8–10 belong to a separate milling batch prepared under identical conditions with that of Runs 1, and 5–7, all of these Runs had identical particle size statistics.

Table 3.1 shows that drug loading had little impact on the median size (d_{50}) provided that the higher drug loaded suspensions were milled longer (refer to Afolabi et al.,

2014). The median sizes of Runs 1–4 suspensions did not increase dramatically over 7-day storage, signifying good physical stability, whereas the 90% passing size (d_{90}), which is too sensitive to aggregates in a particle population, increased due to limited aggregation of the milled particles via Brownian collisions. The majority of the particles in Runs 1–4 suspensions had particles smaller than 500 nm. This suggests presence of discrete nanoparticles and their relatively small aggregates in view of similar sizes observed for a fully stabilized griseofulvin suspension with 2.5% HPC–0.5% (Bilgili and Afolabi, 2012). All of these results suggest that pullulan–SDS combination was successful for stabilizing the milled griseofulvin suspensions probably due to synergistic electro-steric action, which was observed for other polymer–anionic surfactant combinations (Bilgili and Afolabi, 2012).

The particle size statistics for Runs 1, and 11–13 suspensions in Table 3.1 indicate that smaller particles were obtained by prolonging milling, while no significant size reduction occurred after 90 min at 10% drug loading (results not shown for brevity); hence, a grind limit was practically attained. The preparation of griseofulvin with widely different particle sizes will allow us to assess the impact on dissolution rate from the films. Overall, WSMM is very effective in preparing ultrafine and nanoparticles of griseofulvin in a stable suspension when 2.5% pullulan–0.5% SDS were used as stabilizers.

Figures 3.1 and 3.2 show that the apparent shear viscosity of the milled drug suspensions was not affected by drug loading and drug particle size, and all milled suspensions had a relatively low viscosity of about 6 cP. Since proper film formation entails a high viscosity on the order several thousand cP, the milled suspensions were mixed with a film-forming precursor solution of pullulan–glycerin–xanthan gum.

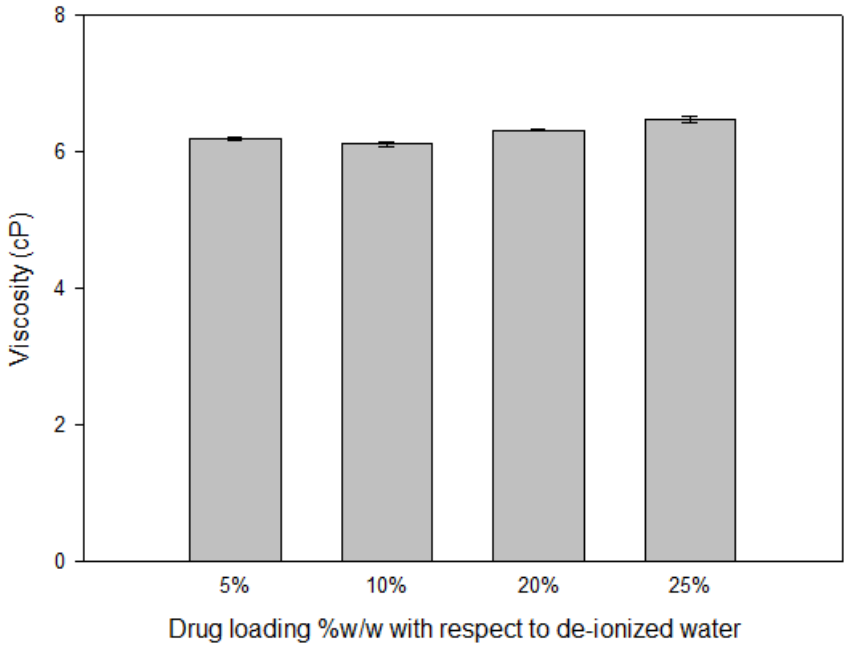

Figure 3.1 Viscosity of milled drug suspensions with different drug loading (milled suspensions from Runs 1–4).

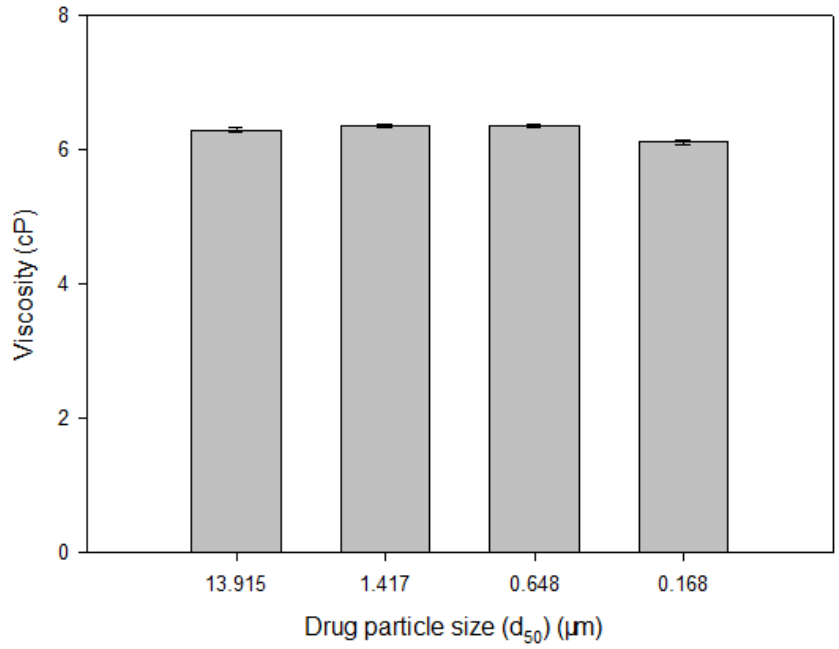

Figure 3.2 Viscosity of milled drug suspensions with different drug particle size (milled suspensions from Runs 1 and 11–13).

Table 3.1 Median Size (d_{50}) and 90% Passing Size (d_{90}) of Griseofulvin Suspensions (Freshly Milled and After 7-day Storage)

Run no.	Drug loading (% w/w)[a]	Milling time (min)	Milled d_{50} ± SD (μm)	Milled d_{90} ± SD (μm)	7-Day storage d_{50} ± SD (μm)	7-Day storage d_{90} ± SD (μm)
1	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
2	5	80	0.166 ± 0.011	0.429 ± 0.083	0.180 ± 0.009	0.466 ± 0.059
3	20	100	0.163 ± 0.007	0.274 ± 0.032	0.172 ± 0.008	0.358 ± 0.032
4	25	120	0.168 ± 0.005	0.276 ± 0.023	0.213 ± 0.016	0.435 ± 0.019
5	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
6	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
7	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
8	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
9	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
10	10	90	0.168 ± 0.007	0.329 ± 0.061	0.182 ± 0.009	0.436 ± 0.022
11	10	0[b]	13.915 ± 0.024	30.338 ± 0.762	11.293 ± 0.269	31.837 ± 0.595
12	10	2	1.417 ± 0.032	2.676 ± 0.190	1.649 ± 0.023	3.979 ± 0.319
13	10	8	0.648 ± 0.073	1.841 ± 0.153	0.563 ± 0.029	1.790 ± 0.066

[a]All milled suspensions had 2.5% (w/w) pullulan and 0.2% (w/w) SDS, with respect to de-ionized water.

[b]As-received GF particles (unmilled) considered for comparative analysis.

3.2 Properties of Film Precursor Suspensions

The milled drug suspensions were mixed with film-forming precursor solution of pullulan–glycerin–xanthan gum to prepare film precursor suspensions, which will be wet casted–dried to prepare films. The characterizations were conducted immediately after formation of the film precursor suspensions. The apparent shear viscosities of various film precursor suspensions were measured by Rheometer using the 25 cc vessel and presented in Figures 3.3, 3.4, and 3.5. Figure 3.3 shows that the suspensions with smaller griseofulvin particles, especially nanoparticles, had slightly higher viscosity. Figures 3.4 and 3.5 illustrate that the precursor suspensions with higher drug loading or xanthan gum loading had higher viscosity. Among these three factors, the xanthan gum concentration was the dominant factor. This finding is not very surprising as xanthan gum is commonly used as a viscosity enhancing agent. Based on prior experience, it is expected these film precursor suspensions with viscosities greater than >5000 cP should be amenable to proper film formation. In view of Figures 3.1 and 3.2, Figures 3.3, 3.4, and 3.5 overall suggest that the mixing of milled suspension with the film-forming solution, which has high viscosity due to the presence of high concentrations pullulan–glycerin–xanthan gum (not measured), led to ~1000 times increase in viscosity of the milled drug suspension. Hence, the addition–mixing of film-forming pullulan–glycerin–xanthan gum solution to the milled drug suspensions is expected to be a critical step for proper film formation.

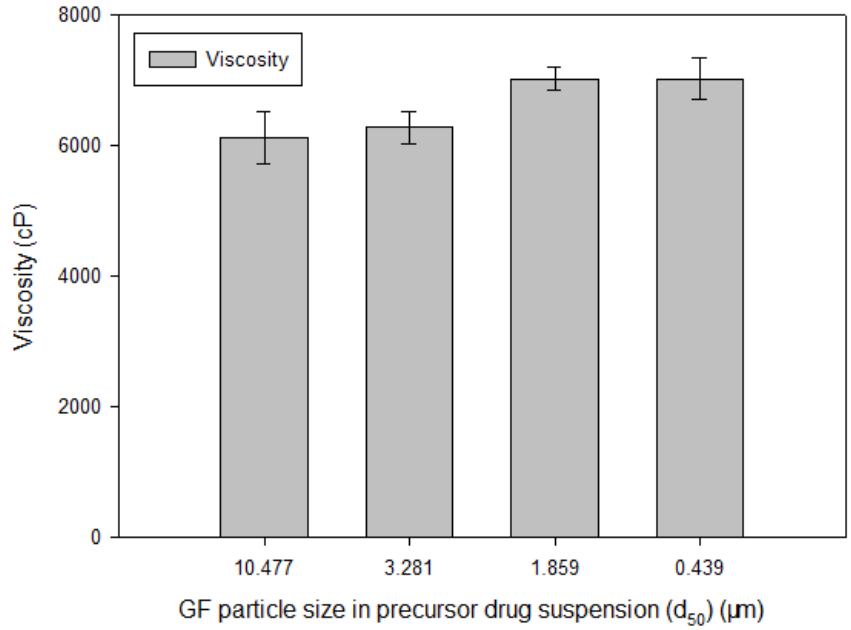

Figure 3.3 Viscosity of film precursor suspensions with various drug particle size (precursor suspensions from Runs 1, and 11–13).

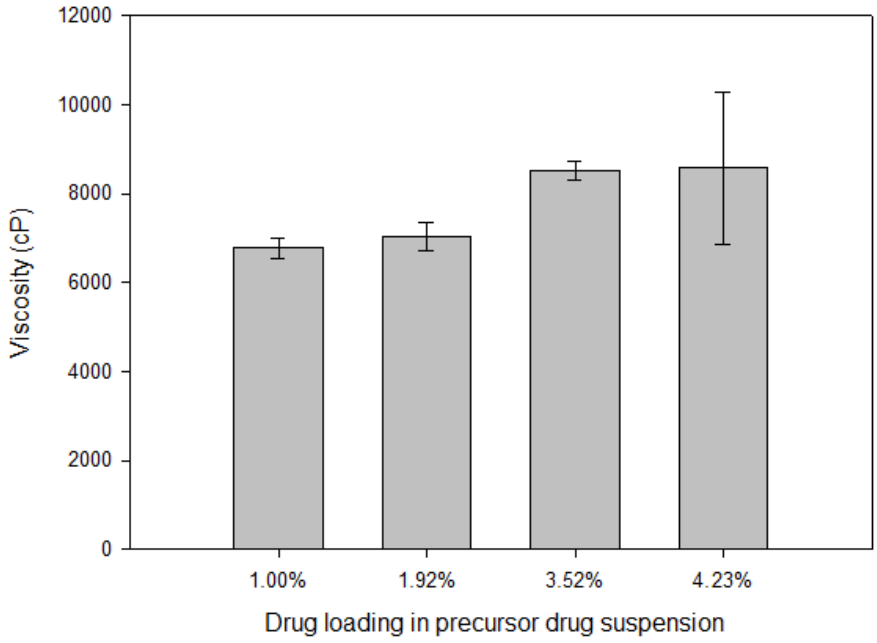

Figure 3.4 Viscosity of film precursor suspensions with various drug loading (precursor suspensions from Runs 1–4).

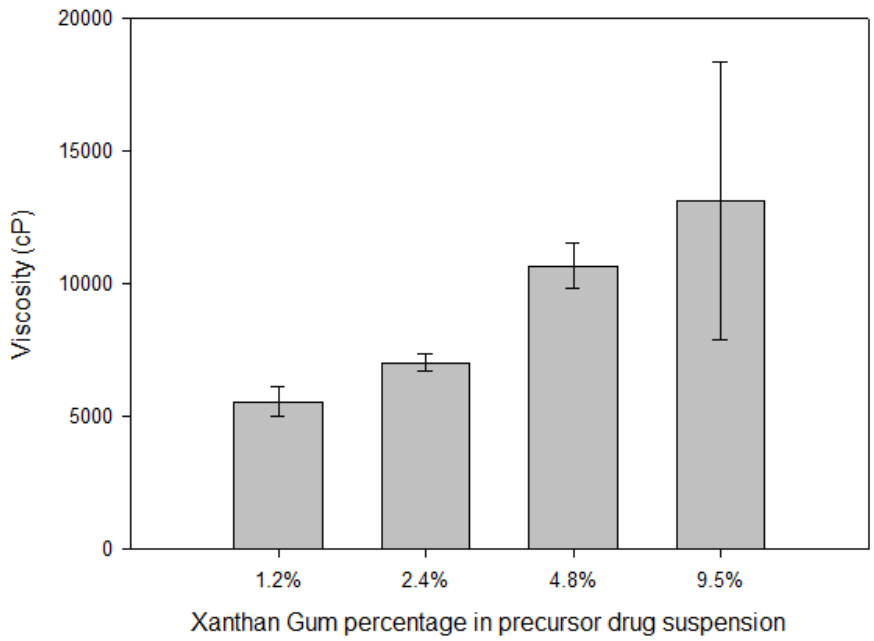

Figure 3.5 Viscosity of film precursor suspensions with various loading of xanthan gum (film precursor suspensions from Runs 1 and 8–10).

Table 3.2 present the particle size statistics of the milled drug suspensions and the film precursor suspensions, which were prepared upon addition–mixing of the film-forming solution of pullulan–glycerin–xanthan gum. It is interesting to note that d_{50} and d_{90} values were higher in the film precursor suspensions than in the milled suspensions. Run 11 was the exception, which can be related to the use of as-received (unmilled) griseofulvin (GF) particles as opposed to other runs where griseofulvin was milled via WSMM. Considerable aggregation of the GF particles took place, which could have originated from potential antagonistic interactions of pullulan–glycerin–xanthan gum–SDS as well as dilution of SDS during the mixing with the film-forming solution. Nonetheless, all milled drug suspensions that had median sizes less than 500 nm still had median sizes below 500 nm after mixing with the film-forming solution, while d_{90} increased more significantly as it is more sensitive to the presence of larger aggregates.

Table 3.2 A Comparison of the Median Size (d_{50}) and 90% Passing Size (d_{90}) of the Griseofulvin in Freshly Milled Suspensions and Film Precursor Suspensions

Run no.	Xanthan Gum amount[a] (g)	Drug loading in suspension (% w/w)[b,c]	Suspension $d_{50} \pm$ SD (μm)	Suspension $d_{90} \pm$ SD (μm)	Film precursor suspension $d_{50} \pm$ SD (μm)	Film precursor suspension $d_{90} \pm$ SD (μm)
1	0.4	10	0.168 ± 0.007	0.329 ± 0.061	0.439 ± 0.014	1.819 ± 0.032
2	0.4	5	0.166 ± 0.011	0.429 ± 0.083	0.494 ± 0.017	1.825 ± 0.025
3	0.4	20	0.163 ± 0.007	0.274 ± 0.032	0.446 ± 0.037	1.901 ± 0.146
4	0.4	25	0.168 ± 0.005	0.276 ± 0.023	0.430 ± 0.011	1.918 ± 0.032
5	0.4	10	0.168 ± 0.007	0.329 ± 0.061	0.439 ± 0.014	1.819 ± 0.032
6	0.4	10	0.168 ± 0.007	0.329 ± 0.061	0.439 ± 0.014	1.819 ± 0.032
7	0.4	10	0.168 ± 0.007	0.329 ± 0.061	0.439 ± 0.014	1.819 ± 0.032
8	0.2	10	0.168 ± 0.007	0.329 ± 0.061	0.434 ± 0.029	1.593 ± 0.125
9	0.8	10	0.168 ± 0.007	0.329 ± 0.061	0.437 ± 0.023	1.521 ± 0.085
10	1.6	10	0.168 ± 0.007	0.329 ± 0.061	0.382 ± 0.039	1.411 ± 0.055
11	0.4	10	13.915 ± 0.024	30.338 ± 0.762	10.477 ± 0.749	34.692 ± 5.659
12	0.4	10	1.417 ± 0.032	2.676 ± 0.190	3.281 ± 0.108	12.350 ± 0.762
13	0.4	10	0.648 ± 0.073	1.841 ± 0.153	1.859 ± 0.040	4.505 ± 0.261

[a]The precursor solutions had 26 g pullulan, 4.33 g glycerin and shown amount of xanthan gum in 100 g water.

[b]36 g drug suspension was mixed with the precursor solution of pullulan–glycerin– xanthan gum.

[c]All milled suspensions had 2.5% (w/w) pullulan and 0.2% (w/w) SDS, with respect to de-ionized water.

Film precursor suspensions of Runs 1–4 with significantly different drug loading had slightly different particle sizes. An increase in xanthan gum loading led to slightly smaller particles (Runs 8–10). It appears that drug and xanthan gum loadings have small impact on the particle size in the film precursor suspensions. Of course, the precursor suspensions of the as-received and coarsely milled particles (Runs 11–13) had bigger particles than those precursor suspensions with nanoparticles. It is concluded that the milling time had the most important factor that determines the particle size in the film precursor suspensions provided that WSMM produces sufficiently stable drug suspensions, as is the case here.

3.3 Properties of Strip Films

Nanoparticle-laden, pullulan-based oral strip films were produced by casting–drying the precursor suspensions. The dry films were then characterized by UV spectroscopy, SEM and laser diffraction for drug morphology–size, Texture Analyzer for mechanical properties and USP IV dissolution test for drug release.

3.3.1 Drug Content Uniformity and Drug Distribution

Table 3.3 shows the thickness, the mass, the average content of drug (dose) and the weight fraction of the drug in the films as well as the relative standard deviation (RSD) of these quantities. The casting–drying process led to films with low RSD in thickness and mass. The wet film thickness was varied from 250 μm to 1000 μm by changing the opening size of Doctor Blade aperture (Runs 1 and 5–7). An increase in aperture size led to thicker dried films with higher drug dose, as intuitively expected from an increase in the thickness of the wet film prior to drying. Film thickness and mass were not significantly affected due to

variation of drug loading in the milled suspensions, xanthan gum loading, and drug particle size, whereas drug dose increased with the use of higher drug loaded, milled suspensions besides greater film thickness as mentioned before. In view of these findings, aperture size and resulting dry film thickness as well as drug content in the milled suspension could be used to modulate drug dose in the film dosage form.

The RSD for the griseofulvin dose in all pullulan films were low, with the highest measured value being 4.30% and the lowest being only 1.08%, which suggests excellent drug content uniformity (Table 3.3) considering the very low drug doses in the films (0.3–1.3 mg). The RSD of drug dose decreased with an in increase in the drug dose, which resulted from the use of milled suspensions with higher drug loading (Runs 1–4). Similarly, thicker films had a lower RSD of drug dose than the thinner films (Runs 1 and 5–7) as the former contained a higher drug dose. An increase in the xanthan gum concentration led to more viscous precursor suspensions but the impact on drug dose RSD did not exhibit a clear trend, while the RSDs were all low. A decrease in the dose RSD resulted from the use of smaller drug particles as opposed to the as-received drug particles, which may explained by the poorer dispersion of coarser drug particles in the films. In general, drug loading and film thickness play dominant roles in determining the drug content uniformity of griseofulvin in the films. It should be noted that the normalize drug doses, i.e., weigh fraction or percentage in the films exhibit much smaller RSD than the drug dose, signifying that part of the drug dose variation results from variation in film thickness/weight. RSD values of drug percentage ranged from 0.4%–2.4% in films with milled drug. This study establishes that excellent content uniformity can be achieved even for 0.3–1.3 mg drug doses in thin strip films.

In order to assess the morphology of the griseofulvin particles distributed in the films, SEM images for the cross section of Run 1 and Run 4 films, with about 9% and 20% drug content respectively, are presented in Figure 3.6. A cautionary note is that this assessment based on these images can only be qualitative because majority of the drug particles and aggregates are covered by or encapsulated by pullulan, which is the major ingredient, matrix and film former in the film. In addition, during the preparation of the cut surface, the polymer may deform and aggregates may be broken, leading to "distorted" picture of the drug distribution. On the other hand, the following general observations can be made: the films appear to have a random distribution of drug particles, with no sign of complete phase separation, thus indicating a nanocomposite structure in the films. Discrete drug nanoparticles can be seen as well aggregates, which are more prevalent in the film with higher drug loading (Run 4).

In addition to the distribution of the drug particles in the films, the water content also plays a key role on the quality of the films. The concentration of xanthan gum, the drug loading and its particle size did not appear to significantly affect the general patterns of TGA (Thermal Gravimetric Analysis) traces (results not shown for brevity, but summarized here). Weight losses were about 5 wt% for heating the film samples from room temperature up to 100 ^{o}C, representing mostly the loss of water. The exposure of samples up to 150 ^{o}C for 15 min resulted in a weight loss of about 10–15 wt%. The significant weight loss at 150 ^{o}C can be attributed to the loss of glycerin from the films (Delgado et al. 2012). Overall the drying process is effective in keeping the free moisture content at low values of ~5 wt%, which is expected to aid in long-term stability of the films (Airaksinen et al. 2005).

Table 3.3 Thickness, Mass, Drug Dose and Drug Fraction in the Strip Films Containing Drug (Griseofulvin) Particles

Run no.	Difference from Baseline	Thickness (μm)	RSD	Mass (mg)	RSD	Drug Dose (mg)	RSD	Drug wt %	RSD
1	None[a]	56.0 ±0.8	1.46%	6.18 ±0.18	2.84%	0.545 ±0.017	3.17%	8.82 ±0.13	1.48%
2	5% drug loading	56.7 ±0.8	1.45%	6.51 ±0.22	3.33%	0.286 ±0.012	4.30%	4.40 ±0.08	1.83%
3	20% drug loading	56.2 ±0.8	1.40%	6.30 ±0.14	2.28%	1.017 ±0.022	2.12%	16.13 ±0.17	1.07%
4	25% drug loading	55.0 ±0.7	1.21%	6.01 ±0.05	0.80%	1.182 ±0.014	1.20%	19.66 ±0.25	1.28%
5	250 μm opening size	26.2 ±0.8	3.01%	2.84 ±0.07	2.41%	0.253 ±0.010	3.88%	8.91 ±0.20	2.26%
6	750 μm opening size	83.4 ±0.8	1.01%	9.24 ±0.10	1.06%	0.869 ±0.022	2.56%	9.40 ±0.23	2.40%
7	1000 μm opening size	123.2 ±2.3	1.87%	13.48 ±0.25	1.83%	1.297 ±0.024	1.84%	9.62 ±0.04	0.38%
8	0.2 g gum	56.2 ±0.9	1.64%	6.35 ±0.12	1.91%	0.578 ±0.014	2.47%	9.11 ±0.08	0.89%
9	0.8 g gum	56.7 ±0.5	0.85%	6.40 ±0.10	1.54%	0.592 ±0.006	1.08%	9.25 ±0.09	0.99%
10	1.6 g gum	57.8 ±1.0	1.79%	6.30 ±0.12	1.97%	0.552 ±0.012	2.20%	8.75 ±0.05	0.54%
11	13.915 μm median size	57.7 ±0.5	0.84%	6.16 ±0.07	1.18%	0.440 ±0.015	3.32%	7.16 ±0.30	4.19%
12	1.417 μm median size	56.1 ±0.9	1.56%	6.12 ±0.08	1.34%	0.622 ±0.012	1.96%	10.15 ±0.19	1.88%
13	0.648 μm median size	56.2 ±0.8	1.40%	6.30 ±0.09	1.40%	0.728 ±0.012	1.72%	11.56 ±0.13	1.10%

[a]Baseline: 10% drug loading, 500 μm opening size of the aperture, 0.4 g gum, and 0.168 μm median size in the milled suspension.

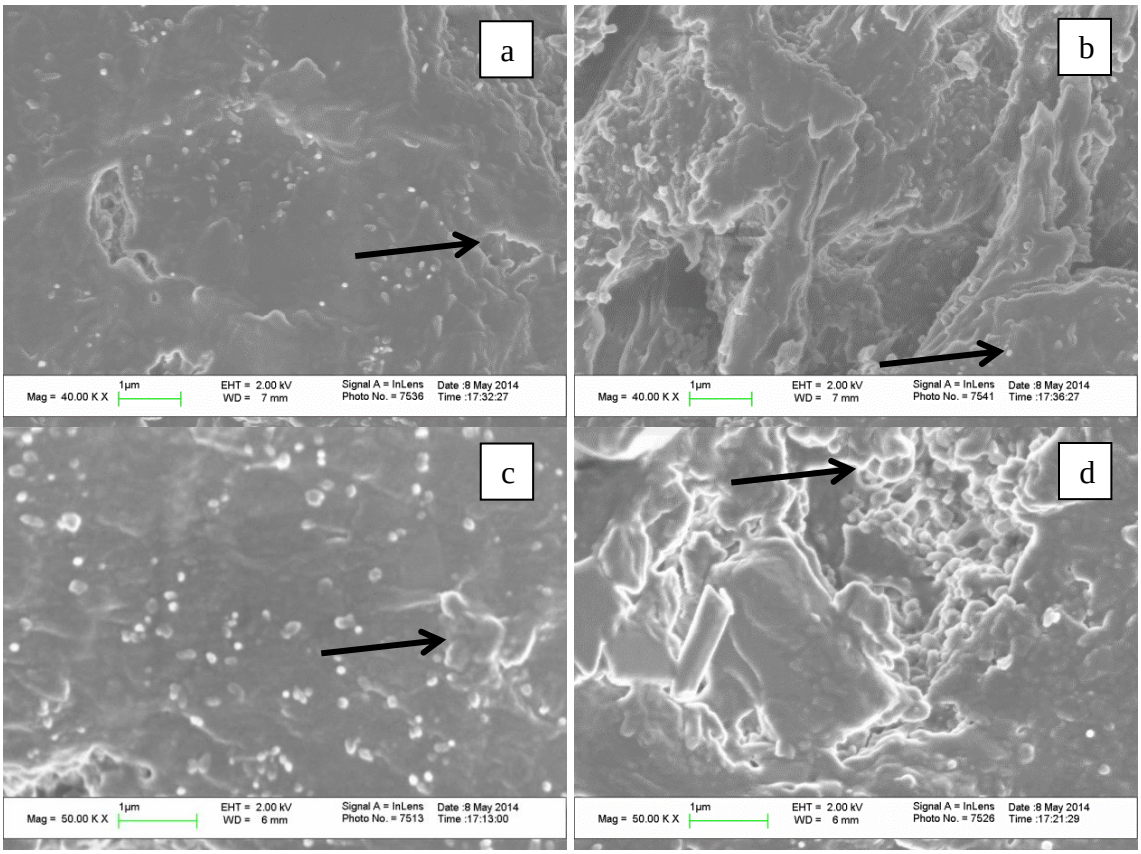

Figure 3.6 Cross-sectional SEM images of the pullulan films containing dispersed drug nanoparticles. Shown are (a) Run 1, location 1, (b) Run 1, location 2, (c) Run 4, location 1, (d) Run 4, location 2 at a high magnification. Arrows show drug aggregates in the films.

3.3.2 Mechanical Properties of the Films

It is known that the mechanical properties of polymer films are dependent on the polymer used and type and amount of plasticizers and surfactants used (Cilurzo et al. 2008) as well as the nature of the secondary phase, e.g., type–loading–size of drug particles. Films have to be strong enough and ductile to prevent rupture of the dosage form during the cutting and packaging processes (high tensile strength (TS) and high yield strength (YS)). A film that is too rigid (high Young's Modulus (YM)) could have an unpleasant sensation in the buccal cavity.

Table 3.4 shows mechanical properties of various strip films. YM values of the films ranged from 4.3–4.9 GPa except for Runs 5 and 7. The thinnest films prepared from Run 5 had the highest YM (5.79 GPa), while the thickest films from Run 7 had the lowest YM (3.93 GPa). Not only did thinner films have higher YM, but they also had lower elongation at break (EB), suggesting that thinner films were more brittle and rigid. Moreover, they had higher tensile strength (TS). In general, the films prepared in this study were more rigid and brittle than those prepared in Sievens-Figueroa et al. (2012), which made use of a much higher ratio of the plasticizer (glycerin) to the film-forming polymer. Another obvious trend is that an increase in the drug loading led to a decrease in all mechanical properties (Table 3.4). Previous work (Boateng et al. 2013, Jiang et al. 2013) showed that it is hard to obtain a high drug loaded polymer strip film with good mechanical properties and Sievens-Figueroa et al. (2012) also found that griseofulvin had a poorer interaction with the film matrix material (HPMC) than other drugs, e.g., naproxen and fenofibrate. The films with highest xanthan gum concentration (Run 10) had a higher TS and EB than the films with lower xanthan gum concentration. Films containing coarser drug particles had a slightly higher TS and EB than films with finer drug particles. These results overall suggest that strip films must be optimized to achieve the required drug dose/dissolution target from a therapeutic perspective and good/acceptable mechanical properties for proper handling–cutting–packaging of the films and patient acceptance/compliance.

Table 3.4 Mechanical Properties of the Griseofulvin-loaded Pullulan-based Strip Films

Run no.	Drug loading (% w/w)[a]	Film thickness Average ± SD (μm)	Xanthan Gum concentration (% w/w)[b]	Precursor drug suspension d_{50} ± SD (μm)	Young's Modulus (GPa)		Yield Stress (MPa)		Tensile Strength (MPa)		Elongation at break point (%)	
					Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD
1	10	56.0 ± 0.8	1.15%	0.439 ± 0.014	4.65	0.08	31.09	1.59	45.68	7.43	1.24	0.12
2	5	56.7 ± 0.8	1.15%	0.494 ± 0.017	4.68	0.83	41.96	4.37	60.34	3.95	1.82	0.27
3	20	56.2 ± 0.8	1.15%	0.446 ± 0.037	4.54	1.37	18.32	9.69	18.41	9.79	0.50	0.14
4	25	55.0 ± 0.7	1.15%	0.430 ± 0.011	4.30	0.53	15.55	9.51	20.89	6.01	0.60	0.19
5	10	26.2 ± 0.8	1.15%	0.439 ± 0.014	5.79	0.47	30.61	8.09	36.28	12.23	0.73	0.24
6	10	83.4 ± 0.8	1.15%	0.439 ± 0.014	4.34	0.43	28.40	3.40	50.57	9.01	1.61	0.36
7	10	123.2 ± 2.3	1.15%	0.439 ± 0.014	3.93	0.33	34.37	7.22	62.69	9.81	2.46	0.18
8	10	56.2 ± 0.9	0.58%	0.434 ± 0.029	4.76	0.25	34.78	3.46	52.71	5.73	1.41	0.14
9	10	56.7 ± 0.5	2.27%	0.437 ± 0.023	4.94	0.41	32.46	1.97	49.55	7.52	1.34	0.27
10	10	57.8 ± 1.0	4.45%	0.382 ± 0.039	4.84	0.47	37.22	3.46	64.27	6.87	1.86	0.35
11	10	57.7 ± 0.5	1.15%	10.477 ± 0.749	4.70	0.35	35.58	2.70	56.19	4.74	1.75	0.21
12	10	56.1 ± 0.9	1.15%	3.281 ± 0.108	4.40	0.21	36.06	3.63	53.79	4.82	1.61	0.26
13	10	56.2 ± 0.8	1.15%	1.859 ± 0.040	4.55	0.59	32.71	3.40	44.85	5.81	1.25	0.15

[a] %w/w with respect to de-ionized water in milled drug suspension.

[b] %w/w with respect to total mass in film precursor suspension.

3.3.3 Redispersion and Dissolution Response

Table 3.5 presents the d_{50} and d_{90} drug particle size statistics for the film precursor suspensions, from which the films were prepared, and the suspensions that formed upon redispersion of the strip films in water. In general, the particle size of griseofulvin after redispersion was smaller than the particle size of griseofulvin in precursor suspensions, which prove that the film format allows for acceptable dispersion of the drug aggregates during redispersion. Most of the redispersion samples had drug median sizes below 500 nm, and d_{90} values were less than 1.7 μm. On the other hand, the redispersed particles were bigger than the griseofulvin particles in the milled suspensions (refer to Table 3.2). All these results suggest that the milled drug particles aggregated mainly during the step of film precursor suspension preparation via mixing, not during the drying of the films. However, some of these aggregates dispersed back to smaller aggregates and primary nanoparticles during the redispersion. Similar trends were also observed for Runs 11–13 where coarsely milled drug particles used in the films; however, the particle sizes after redispersion were larger, which is in accordance with the use of coarser milled particles.

The dissolution behavior of the strip films containing griseofulvin particles are shown in Figures 3.8, 3.9, 3.10 and 3.11. In a previous work, Sievens-Figueroa et al. (2012) chose SDS solution (5.4 mg/ml) as the dissolution test medium in USP IV apparatus for HPMC-based drug-laden films. However, pullulan is acknowledged as a most suitable material for fast dissolving polymer strip films and shows a faster release profile than HPMC films (Dixit and Puthli 2009). Since the solubility of griseofulvin is lower in de-ionized water than in SDS solution, the use of de-ionized water for dissolution medium leads to a non-sink condition, which is more powerful for discriminating BCS Class II

Table 3.5 Median Size (*d50*) and 90% Passing Size (d_{90}) of the Griseofulvin Particle after Redispersion

Run no.	Drug loading (% w/w)[a]	Film thickness Average ± SD (μm)	Xanthan gum concentration (% w/w)[b]	Milling time (min)	Precursor drug suspension d_{50} ± SD (μm)	Precursor drug suspension d_{90} ± SD (μm)	Drug particle redispersion d_{50} ± SD (μm)	Drug particle redispersion d_{90} ± SD (μm)
1	10	56.0 ± 0.8	1.15%	90	0.439 ± 0.014	1.819 ± 0.032	0.396 ± 0.006	1.517 ± 0.032
2	5	56.7 ± 0.8	1.15%	80	0.494 ± 0.017	1.825 ± 0.025	0.429 ± 0.005	1.438 ± 0.045
3	20	56.2 ± 0.8	1.15%	100	0.446 ± 0.037	1.901 ± 0.146	0.446 ± 0.015	1.654 ± 0.026
4	25	55.0 ± 0.7	1.15%	120	0.430 ± 0.011	1.918 ± 0.032	0.425 ± 0.007	1.692 ± 0.030
5	10	26.2 ± 0.8	1.15%	90	0.439 ± 0.014	1.819 ± 0.032	0.400 ± 0.017	1.485 ± 0.053
6	10	83.4 ± 0.8	1.15%	90	0.439 ± 0.014	1.819 ± 0.032	0.421 ± 0.018	1.388 ± 0.072
7	10	123.2 ± 2.3	1.15%	90	0.439 ± 0.014	1.819 ± 0.032	0.452 ± 0.012	1.461 ± 0.041
8	10	56.2 ± 0.9	0.58%	90	0.434 ± 0.029	1.593 ± 0.125	0.424 ± 0.011	1.536 ± 0.072
9	10	56.7 ± 0.5	2.27%	90	0.437 ± 0.023	1.521 ± 0.085	0.415 ± 0.013	1.488 ± 0.079
10	10	57.8 ± 1.0	4.45%	90	0.382 ± 0.039	1.411 ± 0.055	0.379 ± 0.012	1.355 ± 0.083
11	10	57.7 ± 0.5	1.15%	0	10.477 ± 0.749	34.692 ± 5.659	8.857 ± 0.169	28.012 ± 1.662
12	10	56.1 ± 0.9	1.15%	2	3.281 ± 0.108	12.350 ± 0.762	2.283 ± 0.096	9.146 ± 0.593
13	10	56.2 ± 0.8	1.15%	8	1.859 ± 0.040	4.505 ± 0.261	1.515 ± 0.018	3.654 ± 0.158

[a] %w/w with respect to de-ionized water in milled drug suspension.

[b] %w/w with respect to total mass in pre-cast drug suspension.

drug-laden films with different thickness, drug particle size, and drug and xanthan gum loadings. Hence, de-ionized water was chosen as the dissolution medium.

Figure 3.7 shows the effect of dry film thickness on the dissolution rate of griseofulvin from the strip films. In general, the dissolution curves appeared to follow a linear trend before ~80% of drug was dissolved. The films exhibited fast/immediate release (>80% dissolved in 30 min) even for a poorly water-soluble drug like griseofulvin, with the only exception for the ~120 μm thick films. As the film thickness increased, the drug dissolution slow-down. The initial linear profiles coupled with the significant thickness dependent dissolution suggest that the dissolution was mainly limited by the slow erosion of the pullulan-based film. The film hydrates and swells while releasing drug particles from the surface as the surface of the film erodes. Figure 3.8 illustrates that an increase in xanthan gum loading in the film slows down the dissolution, while the dissolution profiles were still linear during the dissolution of most of the drug, depicting immediate release. The four profiles were almost straight lines before dissolution percentage reached 80%. Less than 18 min were needed to release 80% drug in the films except for the one which contained 4.45% xanthan gum in it (Run 10). The highest viscosity imparted by 4.45% xanthan gum made Run 10 precursor suspension very difficult to pour and wet-cast. Hence, such a high concentration of xanthan gum is not suitable for easy film forming during the production. Xanthan gum was added as the thickening agent to raise viscosity in the film precursor suspensions and at least ~0.5% was essential for film forming during the drying process. Although xanthan gum helps increase the viscosity of the film precursor suspensions, it slows down the dissolution rate. To this end, an optimal xanthan gum concentration may be selected: e.g., 1.15% used in the baseline films (Run 1).

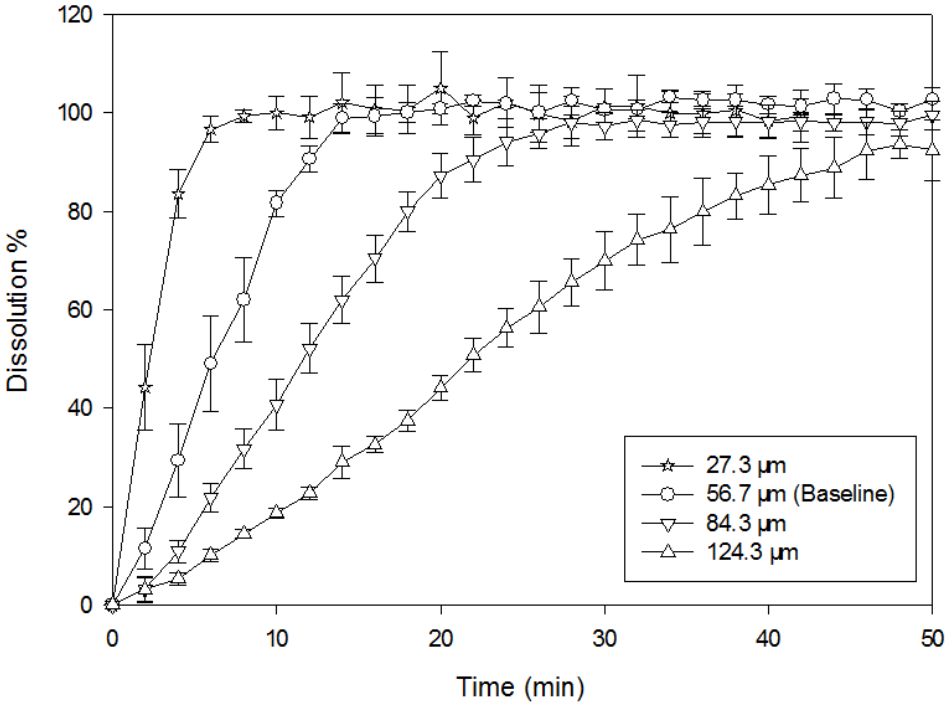

Figure 3.7 Effect of film thickness on the dissolution (mean ± standard deviation) of griseofulvin from the strip films (films prepared from Runs 1 and 5–7 precursor suspensions).

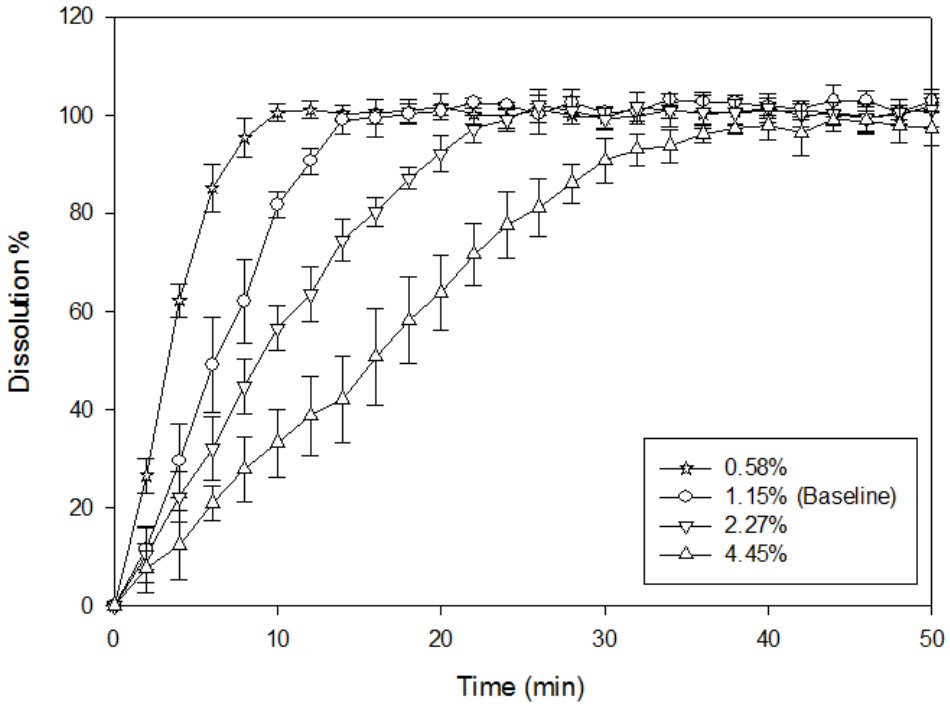

Figure 3.8 Effect of xanthan gum concentration on the dissolution (mean ± standard deviation) of griseofulvin from the strip films (films prepared from Runs 1 and 8–10 precursor suspensions).

Figure 3.9 illustrates the impact of drug loading on the dissolution profiles. The dissolution profiles were all similar and suggest immediate drug release. In the range studied, drug loading had little to no effect on the dissolution profiles. The lower steady-state drug released in the case of 19.66% drug containing films (Run 4) most probably resulted from the inability of the dissolution medium to dissolve all drug as the solubility limit of the drug was approached.

Films incorporating wet-milled drug particles led to faster drug dissolution than as-received drug particles (Figure 3.10), which can be explained by the larger surface area of the smaller particles. However, this effect appeared to get weaker when particles smaller than few microns were used. All of the films released 80% drug within 20 min.

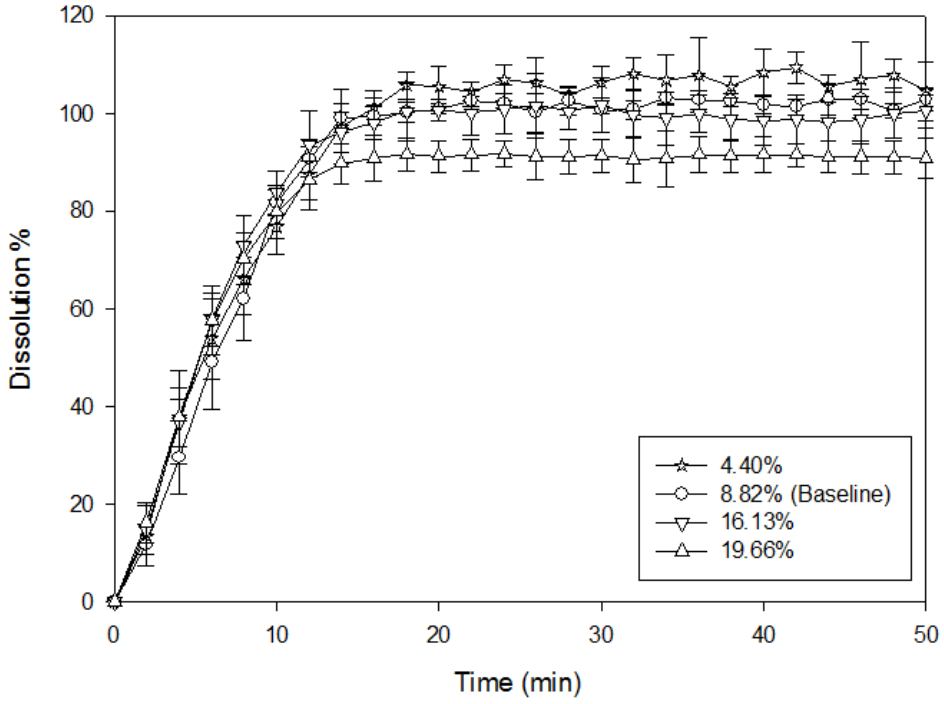

Figure 3.9 Effect of drug loading on the dissolution (mean ± standard deviation) of griseofulvin from the strip films (films prepared from Runs 1–4 precursor suspensions).

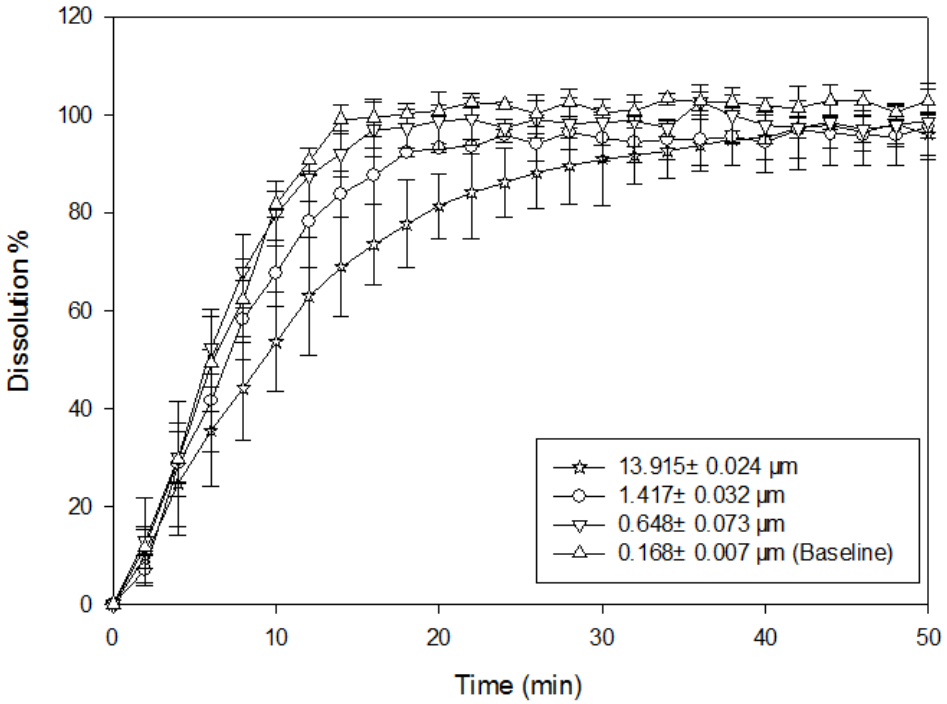

Figure 3.10 Effect of particle size on the dissolution (mean ± standard deviation) of griseofulvin from the strip films (films prepared from Runs 1 and 11–13 precursor suspensions).

CHAPTER 4

SUMMARY AND CONCLUSIONS

The aim of this study is to enhance the dissolution rate and bioavailability of griseofulvin, a model poorly water-soluble drug, by preparing drug nanoparticle-laden, pullulan-based oral strip films without excessive use of toxic surfactants in the formulation. To achieve this goal, griseofulvin suspensions were prepared via wet media milling with different drug particle size and loading, and they were incorporated into pullulan-based strip films with various drug–xanthan gum loadings after mixing with a film-forming pullulan–glycerin–xanthan gum solution. Both the suspensions and the films were thoroughly characterized.

Wet stirred media milling led to fast preparation of stable griseofulvin suspensions. The use of pullulan along with minimal amount of SDS imparted sufficient physical stability to the prepared nanosuspensions prior to casting–drying. Interestingly, the mixing of the milled drug suspensions with film-forming solution of pullulan–xanthan gum–glycerin led to some extent of aggregation in the film precursor suspension. Yet, most precursor suspensions had median sizes below 500 nm, and majority of the aggregates were still below 1000 nm. The casting–drying of the precursor milled suspensions led to the preparation of films with generally desirable properties. All films prepared under different conditions exhibited acceptable drug content uniformity, which is indicated by a relative standard deviation (RSD) of less than 6%, while most of them had excellent content uniformity (RSD < 3.5%) in view of the low dose (0.3–1.3 mg) of drug in the films. SEM images of the cross-section of the films showed presence of discrete nanoparticles as well as some aggregates in the films. The mechanical tests suggested that the films were

brittle with relatively high elastic modulus, low elongation at break, and high strength, signifying the need to increase the concentration of glycerin in the films. The redispersion of films in water led to recovery of discrete drug nanoparticles as well as aggregates. In general, the drug particle size of the redispersion was slightly smaller than that of the film precursor suspension, but much larger than that of the respective milled suspension. This finding points to the importance of characterizing the particle size of the film precursor suspension and elucidating the impact of mixing. USP IV dissolution tests overall showed that films exhibited fast/immediate release (>80% dissolved in 30 min) even for griseofulvin, a poorly water-soluble drug, in de-ionized water with the only exception for the ~120 μm thick films. An increase in film thickness and xanthan gum both led to a slow-down of the dissolution. While the drug dissolution was not very sensitive to drug loading in the film within the range of ~4–20% studied, its rate increased when milled particles as opposed to as-received particles were incorporated into the films. Considering that the SDS content of the films is estimated to be ~0.2%, it is concluded that thin pullulan-based oral strip films containing xanthan gum and glycerin can be successfully used as carrier of drug nanoparticles, which can enhance the dissolution rate of poorly water-soluble drugs, and in turn their bioavailability, without the need for excessive amount of toxic surfactants in the formulations. Film properties can be modulated, as desired for specific pharmaceutical applications, by controlling the thickness, xanthan gum and drug loadings, thus proving the versatility of the pullulan strip films for dissolution enhancement of poorly water-soluble drugs.

CHAPTER 5

FUTURE WORK

The current study has identified few challenges in the development of strip films for bioavailability enhancement of poorly water-soluble drugs based on pullulan. The standard suspension formulation used in wet media milling, i.e., 10% drug (griseofulvin)–2.5% pullulan (polymer)–0.2% SDS (surfactant) led to limited aggregate formation during milling and storage. It is recommended that an optimal loading of pullulan is to be found by studying the pullulan adsorption on griseofulvin and milling dynamics at various concentration of pullulan in the presence/absence of SDS. This will help to further suppress aggregation tendency in the milled suspensions, which are used to prepare film precursor preparation.

Another major issue observed was that griseofulvin nanoparticles severely aggregated during the mixing of the milled suspensions with the film-forming pullulan–glycerin–xanthan gum solution with the objective of preparing the film precursor suspensions. It is not clear as to which type(s) of interactions are responsible for severe aggregation. Hence, it is recommended that particle changes during the mixing should be studied in the presence/absence of one of the ingredients. If either glycerin or xanthan gum is responsible for the aggregation, then other plasticizers like PEG and viscosity enhancing agents like superdisintegrants may be used in the film forming solution.

Achieving excellent drug content uniformity with low drug doses (0.3–1.3 mg) is a major accomplishment, which paves the way to incorporating highly potent, poorly water-soluble drugs into the films. On the other hand, incorporating larger doses pose

several challenges. This study showed that higher drug loading in the films can deteriorate mechanical properties, elegance, and easy of handling-packaging of the films. Hence, drug loading may not exceed 20%, which severely restricts the drug dose. More importantly, an increase in film thickness/weight leads to serious dissolution slow-down, albeit allowing for higher drug doses. Mechanical properties can be modulated by adjusting the type/loading of the plasticizers. To maintain fast release from thicker films, the film formulations may incorporate additional functional additives such as sugars, sugar alcohols, and superdisintegrants, which may alter the erosion-based drug release mechanism. In an alternate route, brittle films with little or without plasticizers may be pulverized into a powder easily, which can be incorporated into other final dosage forms such as tablets, capsules, sachets, etc. via standard pharmaceutical unit operations.

This study has not performed a formal, comparative assessment of the capabilities of film-forming, water-soluble polymers like pullulan versus hydroxypropyl methyl cellulose (HPMC) or hydroxypropyl cellulose (HPC), which is recommended for future work. It would also be interesting to assess mixtures of different water-soluble polymers, e.g. pullulan–HPMC or pullulan–HPC mixtures. Finally, other poorly water-soluble drugs belonging to BCS Class II category should be studied to establish the generality of the approach presented in the present study.

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