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Spray Dried Nano-Crystalline Powders and In Vitro Dissolution Performance

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Spray Dried Nano-Crystalline Powders and *In Vitro* Dissolution Performance

Sumit Kumar, Ph.D.

University of Connecticut, 2014

Quality by Design principles has been applied to understand formulation and optimization of nano-crystalline spray-dried powders of poorly soluble drugs. The objectives of this research were to: 1) investigate the effect of wet media milling on nano-crystalline suspensions; 2) investigate the effect of spray-drying process and formulation parameters on nano-crystalline suspensions; 3) understand role of bulking agents during spray or freeze-drying of nano-crystalline suspensions; and 4) investigate the effect of different sized spray-dried nano-crystals on *in vitro* dissolution performance.

Poorly soluble drugs were utilized to investigate the aggregation of nano-crystals during the spray drying process. It was determined that temperature and excipient utilized in the formulations plays an important role in nano-crystal aggregation. Low inlet temperature (preferably less than drug melting temperature) for spray drying processing and the addition of stabilizers/excipients with favorable or strong interaction (such as ionic or hydrogen bonding *etc.*.) with the drug will provide better stability of the nano-crystals and thus no or minimal nano-crystal aggregation. The percent yield of spray-dried powders is dependent on the glass transition temperature of the formulations and/or bulking agents utilized. Small molecular weight bulking agents (or matrix formers) prevented nano-crystal aggregation due to favorable interactions with the stabilizers.
Stabilizers with favorable or strong interaction with the drug are preferred for the stability of nano-crystalline suspensions but this may cause physical and chemical instability (such as solid-state transformation, drug degradation *etc.*) during high intensity wet milling processing.

USP apparatus II was modified to hold dialysis sacs and this method was utilized to test and distinguish nano-crystalline formulations based on their size. The developed *in vitro* release testing method (or dissolution method) was able to distinguish nano-crystalline formulations based on size. This method can be utilized for routine quality control assays performed in the pharmaceutical industry and potentially for the development of IVIVC for these formulations.

In conclusion, QbD (or specifically DoE) studies performed in this research are examples of how this approach can be utilized to understand formation and stabilization of nano-crystalline suspensions during milling and/or drying to achieve non-aggregating stable nano-crystalline powders for desired *in vitro* and/or *in vivo* performance.

*Sumit Kumar* - *University of Connecticut, 2014*
Spray Dried Nano-Crystalline Powders and \textit{In Vitro} Dissolution Performance

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A Dissertation

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Doctor of Philosophy Dissertation

Spray Dried Nano-Crystalline Powders and In Vitro Dissolution Performance

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2014
Dedication

To my wife, parents, sister and brother-in-law
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Chapter 1

Introduction, Objectives, and Specific Aims
1.1. Introduction

In recent years, the number of poorly soluble (Biopharmaceutics Classification System class II/IV) [1] drug entities coming out of drug discovery has increased tremendously [2-5]. These brick-like molecules demand utilization of different approaches to increase their solubility and hence bioavailability before reaching the market [2-5]. Poorly soluble drugs if not formulated appropriately may pose certain challenges such as lower than expected dissolution rates and solubility, as well as high local drug concentrations due to drug aggregate deposition. Such high local drug concentrations can cause toxic effects and thus decreased systemic bioavailability [9]. There are several different methods to formulate poorly soluble drugs (BCS class II) and improve their bioavailability such as: preparation of salts, and formulation in nanosuspensions, liposomes and solid dispersions. However, all these methods have their own disadvantages. For example, loading efficiency of poorly soluble drugs in liposomes is relatively low. The formation of solid dispersions is another approach utilized to increase drug solubility but drug loading is relatively low (up to 20-30% drug). Crystalline nanosuspensions is another method to improve oral bioavailability, however although bioavailability is improved over that of coarse (micron size and above) particles, the crystalline nature still limits solubility.

Crystalline drug nanosuspensions can be defined as sub-micron colloidal dispersions of discrete drug particles, ranging from 100-1000 nm, that have been stabilized with polymers, surfactants or a mixture of both polymers and surfactant. Nanoparticles can be obtained using either a “top-down approach” (i.e. milling/grinding of the particles to achieve the required size) or a “bottom-up approach” (i.e. precipitation of drug from a solvent to an anti-solvent system) [10]. The top-down approach is very time consuming
and often leads to crystalline particles whereas the bottom-up approach is less time consuming and often leads to amorphous particles due to fast evaporation of the solvent and thus precipitation of the API as amorphous particles. The advantage of amorphous versus crystalline nanoparticles is the considerably higher solubility, which can be as much as 10 to 1600 fold. However, the stability of amorphous drugs is an important issue in the pharmaceutical field. Amorphous systems have higher free volume or enthalpy as well as high Gibbs-free energy. Accordingly, these systems are unstable and tend to crystallize to a stable polymorph of the drug, which typically would have lower solubility. The crystallization time of an amorphous drug is a kinetically controlled process (which can vary from seconds to years) and depends on several factors such as, storage temperature and moisture content. Various approaches have been used to stabilize the amorphous form of drugs. For example, crystallization inhibitors (high Tg polymers) can be added and/or the formulation may be stored at low temperature (50°C below the drug Tg) and low moisture/humidity conditions.

Nanosuspensions (nano-sized formulations) are known to improve the dissolution rate and bioavailability of poorly water-soluble active pharmaceutical ingredients (APIs) due to their increased surface area to volume ratio as described by the Noyes-Whitney equation [11]. Increase in surface-to-volume ratio and thus dissolution rate of nanoparticles improves their pharmacokinetic properties in terms of: increased rate and extent of release and absorption; rapid onset of action; reduced side effects and improved clinical performance [9, 11-13]. For example, a nanosuspension of budesonide (75 – 300 nm) has been reported to have double the C_{max} and half the T_{max} compared to larger (average 4.4 µm) suspension particles [14]. The importance of nanosuspensions to the pharmaceutical
industry can be judged by the fact that six formulations are already on the market and approximately 10 - 15 are in different stages of clinical trials [15]. However, one of the major concerns with nanosuspension formulations is the preservation of their physical and chemical stability in aqueous medium [16, 17]. Being a liquid dosage form, nanosuspensions are more susceptible to both physical instability (due to crystal growth and agglomeration) and to chemical instability (due to degradation of the active pharmaceutical ingredient(s)), when compared to solid dosage forms. In fact, of all the marketed formulations, only Megace ES is in the suspension form (nano-particulate suspension of megesterol acetate). All others are prepared as nanosuspension based solid dosage forms, as a way to overcome instability problems.

In order to convert nanosuspensions into solid dosage forms, the nanosuspensions must be dried to obtain a powder of the nanosized drug particles and this powder may then be processed into tablets or capsules. Spray and freeze-drying are the most common methods of removing water from aqueous systems. In this proposed project, spray drying will be used as a means to obtain a dry powder because of its simplicity, versatility and low cost of operation. However, any material undergoing a drying process experiences a lot of stress. In the case of spray drying the material experiences thermal stress, and in the case of freeze-drying the material experiences freezing stress, both of which may affect product performance [17, 18]. In addition, the drying process brings about concentration of the originally dispersed and dissolved materials and this may adversely affect both the physical and chemical stability of the formulation. For example, reduction in the solvent volume can lead to a decrease in the solubility of the surfactant or stabilizer, resulting in precipitation and rendering these unavailable for protection of the nanoparticles against
aggregation. In the case of drying of nanocrystals the problem of aggregation and agglomeration is compounded due to the inherent thermodynamic instability of these systems resulting from their large surface area and high interfacial tension. Strong aggregation/agglomeration of the particles will reverse the advantage of rapid dissolution, which is central to nanosuspension formulations. There are few reports [16-21], which deal with drying of nanosuspensions, but no exhaustive study has been undertaken to understand the process of drying of nanosuspensions.

1.2. Objectives

The objectives of this research are to: 1) investigate the effect of wet media milling process and formulation parameters on the size of nano-crystalline suspensions; 2) investigate the effect of spray drying process and formulation parameters on the aggregation behavior of nanosuspensions; 3) understand the mechanism of bulking agents such as, sugars in their ability to prevent aggregation of nano-crystals during spray drying; 4) investigate the effect of different sized spray-dried nano-crystals on in vitro dissolution. Such information will help in elucidating factors that contribute to improved shelf-life stability of nano-crystalline formulations.

1.3. Specific Aims

The following specific aims were developed to achieve the objectives mentioned above:

**Specific Aim I:** (Chapter 2)
Quality by Design Approach to Spray Drying Processing of Crystalline Nanosuspensions

**Specific Aim II: (Chapter 3)**
Formulation Parameters of Crystalline Nanosuspensions on Spray Drying Processing: A DoE Approach

**Specific Aim III: (Chapter 4)**
Bulking Agents to Prevent Nano-crystal Aggregation During Spray or Freeze Drying

**Specific Aim IV: (Chapter 5)**
Wet Milling Induced Physical and Chemical Instabilities of Naproxen Nano-Crystalline Suspensions

**Specific Aim V: (Chapter 6)**
Optimization and Dissolution Performance of Spray-Dried Naproxen Nano-Crystals
1.4. References:


Chapter 2

Quality by Design Approach to Spray Drying Processing of Crystalline Nanosuspensions

Abstract

Quality by Design (QbD) principles were explored to understand spray drying process for the conversion of liquid nanosuspensions into solid nano-crystalline dry powders using indomethacin as a model drug. The effects of critical process variables: inlet temperature, flow and aspiration rates on critical quality attributes (CQAs): particle size, moisture content, percent yield and crystallinity were investigated employing a full factorial design. A central cubic design was employed to generate the response surface for particle size and percent yield. Multiple linear regression analysis and ANOVA were employed to identify and estimate the effect of critical parameters, establish their relationship with CQAs, create design space and model the spray drying process. Inlet temperature was identified as the only significant factor (p value <0.05) to affect dry powder particle size. Higher inlet temperatures caused drug surface melting and hence aggregation of the dried nano-crystalline powders. Aspiration and flow rates were identified as significant factors affecting yield (p value <0.05). Higher yields were obtained at higher aspiration and lower flow rates. All formulations had less that 3% w/w moisture content. Formulations dried at higher inlet temperatures had lower moisture compared to those dried at lower inlet
2.1. Introduction

In recent years, the number of poorly soluble drug entities (Biopharmaceutics Classification System, BCS class II/IV) [1] coming out of drug discovery has increased significantly [2-5]. These brick-like molecules demand utilization of different approaches to increase their solubility and/or dissolution rate and thus oral bioavailability [6-8]. Poorly soluble drugs, if not formulated appropriately, may pose certain challenges such as high local drug concentrations due to drug aggregate deposition. Such high local drug concentrations can lead to toxicity [9]. Several different approaches can be utilized to formulate poorly soluble drugs (BCS class II/IV) and improve their bioavailability such as preparation of salts, liposomes and solid dispersions etc. However, these methods have their own shortcomings. For example, it is not possible to make stable crystalline soluble salts of all the poorly soluble drugs. The loading efficiency of poorly soluble drugs in liposomes is relatively low. Moreover, the drug loading in solid dispersions is relatively low and the physical stability can be an issue.

Crystalline nanosuspensions can be an attractive alternative to improve solubility, dissolution rate, and oral bioavailability of poorly soluble drugs. Crystalline nanosuspensions can be defined as sub-micron colloidal drug dispersions of discrete drug crystals, ranging from 100 - 1000 nm. The nanosuspensions are stabilized with polymers, surfactants or a mixture of both. Nano-crystals can be obtained using either a “top-down approach” (i.e. milling/grinding of the drug crystals to achieve the required size) or a “bottom-up approach” (i.e. precipitation of drug from a good solvent to an anti-solvent system) [10]. Crystalline nanosuspensions are known to improve the dissolution rate and
bioavailability of poorly water-soluble active pharmaceutical ingredients (APIs) due to their increased surface area-to-volume ratio [11]. Increase in surface-to-volume ratio and thus dissolution rate of nano-crystals improves their biopharmaceutical properties in terms of: increased rate and extent of release and absorption; rapid onset of action; reduced side effects and improved clinical performance [9, 11-13]. For example, a nanosuspension of budesonide (75 – 300 nm) has been reported to have double the $C_{\text{max}}$ and half the $T_{\text{max}}$ compared to larger (average 4.4 µm) suspension particles [14]. The importance of nanosuspensions in pharmaceutical dosage forms is evident from the presence of several commercially available drug products, and yet several more in clinical trials. [15]. One of the major challenges with formulation of nanosuspensions is the preservation of their physical and chemical stability in aqueous medium [16, 17]. Nanosuspensions are susceptible to both physical instability (crystal growth and agglomeration) and chemical instability (degradation) compared to solids.

In order to convert crystalline nanosuspensions into solid dosage forms, the nanosuspensions must be dried to solids. These nano-crystalline solids may then be processed into capsules. Spray and freeze-drying are the most commonly utilized to dry nanosuspensions. However, any material undergoing a drying process experiences significant stress. In the case of spray drying, the material experiences thermal stress, and in the case of freeze-drying the material experiences freezing stress, both of which may affect product quality attributes [17, 18]. For example, reduction in the solvent volume can lead to a decrease in the solubility of the surfactant or stabilizer, resulting in precipitation and rendering these unavailable for protection of the nanoparticles against aggregation. A few published reports [16-21] deal with nanosuspensions drying, but no exhaustive study
has been conducted to understand the process. The current study focuses on, spray drying as a means of obtaining dry nano-crystalline powders. The spray drying process is most practical in terms of its simplicity, versatility, low production cost and industrial applicability [22].

Quality by Design (or specifically Design of Experiment, DoE) is a versatile approach to understand the effect of critical process parameters and to optimize process conditions [23, 24]. DoE helps in identification of critical versus non-critical parameters affecting product quality attributes. Furthermore, the results of DoE afford to quantify any interactions between critical parameters on the responses (quality attributes). DoE can also be utilized for the prediction of the desired quality attributes within the design space.

A full factorial design was employed to understand the process as well as any interactions between the critical factors involved in the spray drying process. The critical process conditions investigated were: solvent flow rate (or feed rate), inlet temperature and aspiration rate. The particle size (as Z-average), moisture content, percent yield and crystallinity were investigated as responses. In addition, a response surface study (RSM) was conducted to obtain a predictive model for the yield and nano-crystals aggregation during the spray drying process. Data analysis was performed using ANOVA and multifactor analysis to: 1) elucidate interactions between critical variables; 2) establish the rank order of the critical variables; and 3) provide a predictive model for nano-crystalline suspension spray drying. To the best of our knowledge, there has been no previous quality by design (or specifically DoE) study that has addressed the process of spray drying of crystalline nanosuspensions.
2.2. Materials

Indomethacin USP (γ polymorph) was purchased from PCCA (Houston, TX). Dowfax 2A1 (alkyldiphenyloxide disulfonate) was generously gifted by Dow Chemical Company (Midland, MI). HPLC grade acetonitrile (ACROS chemicals) was purchased form Fisher Scientific (Pittsburgh, PA). Hermetic pans and lids were purchased from TA instruments.

2.3. Methods

2.3.1. Preparation of crystalline nanosuspensions

The required amount (1% w/w) of indomethacin was dispersed in 100 ml of 0.5% w/v aqueous stabilizer solution (Dowfax 2A1) using a stirrer to form a macro-suspension of the drug. Particle size reduction was carried out by processing 100 ml of macro-suspensions through a Microfluidizer model 110Y (Microfluidics, Newton, MA) at 19,000 psi for 90 min. The bulk temperature of the suspensions was maintained below 25°C during processing.

2.3.2. Spray drying of nanosuspensions

Indomethacin nanosuspension formulations were spray dried using a lab scale Buchi spray dryer B-190. Briefly, the spray dryer was pre-conditioned at the required setting of spray gas flow or airflow, aspiration rate, flow rate (or feed rate), and inlet temperature using distilled water. Once the spray dryer was equilibrated, 100 ml of the prepared nano-crystalline suspension (1% w/v) formulation was pumped into the drying chamber using a peristaltic pump (through 0.5 mm nozzle). Nano-crystalline suspension formulations were spray dried at different feed rates, temperatures and aspirator settings. Spray gas
(atomizing air) was maintained at 40 mm Hg (the air flow was approximately 600 l/hour) for all the formulations. The dried samples were removed from the collection chamber using a plastic scraper and evaluated for percent yield, moisture content, particle size and polymorphic changes, if any.

2.3.3. Powder X-ray diffraction (PXRD)

PXRD (powder X-ray diffraction) was used to study the crystallinity of the dried samples. X-ray diffraction patterns were obtained using an X-ray diffractometer (Model D5005, Bruker AXS Inc., Madison, WI) using Cu-kα radiation, a voltage of 40 kV, and a current of 40 mA. All the scans were performed at a scan rate of 2°/minute with steps of 0.02° from 5° to 50° 2θ angle.

2.3.4. Differential scanning calorimetry (DSC)

DSC was performed using a TA Q1000 calorimeter (TA instruments, New Castle, DE, USA) equipped with a refrigerated cooling accessory. The instrument was calibrated for enthalpy and heat capacity using indium and sapphire, respectively. Approximately 5-10 mg spray dried samples were sealed in hermetic pans and analyzed. The heating rate was maintained at 5°C/min from room temperature to 180°C. Nitrogen gas was used for purging at a flow rate of 50 ml/min. Data were analyzed using TA universal analysis software.

2.3.5. Determination of percent yield

For calculation of percent yield, the drug amount in liquid and spray-dried nanosuspension formulations were determined using an HPLC-UV method (as described below). Briefly,
the nano-crystalline suspension formulations were dissolved in the mobile phase and this solution was used to quantify the drug amount. The formula used for calculation of % yield was:

\[
\text{% Yield} = \frac{\text{Drug Mass Out}}{\text{Drug Mass In}} \times 100\% 
\]

Equation 2.1

Note: Drug mass in and out represents, the total amount of drug (in nanosuspensions) that was spray dried and that was recovered (dried powder), respectively.

2.3.6. HPLC-UV method

The standard curve of indomethacin was generated using a Perkin-Elmer HPLC system (series 200) connected to a UV-Vis detector (Perkin-Elmer 785). The amount of indomethacin was quantified using a C-18 Zorbax® column (Waters Corporation, USA) and the mobile phase was a mixture of two phases (A/B) at a 50:50 v/v ratio. Phase A was a mixture of water and phosphoric acid (0.2% v/v) and phase B was acetonitrile. The flow rate was maintained at 1.3 ml/min and the UV absorbance was measured at 237 nm. Various dilutions were made in the mobile phase to prepare a standard curve. The concentration range for linearity was 0.005 mg/ml to 0.2 mg/ml with a R² value of 0.9999.

2.3.7. Moisture Determination

The spray-dried powder (approximately 10 mg) was dissolved in sieve-dried methanol (1 ml). 500 µl of this drug-methanol solution was used for Karl Fisher analysis. Samples were injected into the titrimeter using a 1 ml syringe attached to a 22-gauge needle. The
moisture content was reported as weight percent.

2.3.8. Particle size measurement

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments) to determine the Z-average (at 90° scattering angle) and PDI of the nanosuspensions before and after spray drying. Briefly, the spray-dried powders were suspended in saturated and filtered (0.2 µm membrane filter) solutions of indomethacin in 30% glycerin solution to avoid any discrepancy from dissolution of nano-particles during measurement. The viscosity of this dispersant solution was measured using a Brookfield viscometer (Model DV-III) and was used to calculate the particle size of the nanosuspensions. Each sample was analyzed in triplicate and the results were reported as the mean value of these runs.

2.3.9. Design of Experiment

Based on our preliminary experiments (data not shown), it was determined that the aspirator, inlet temperature and flow (or feed) rate were important spray drying process variables. All indomethacin nanosuspension formulations (100 ml) were prepared using a microfluidizer (Microfluidics®) according to our previous published method [25]. The bulk temperature of the formulations during particle size reduction was maintained below 25°C to avoid any instability issues. A full factorial design $2^3$ (3 factors at 2 levels) was utilized. The three independent variables used at two levels in this investigation were: inlet temperature (131°C to 196°C); aspiration rate (-21 mbar to -42 mbar); and flow rate (7.2 ml/min to 11.4 ml/min). Four center points were added to the design space to identify any non-linearity in the responses. It was confirmed that all the operating conditions are
achievable. Minitab® 16 and Design-Expert 8 (Stat-ease®) software were utilized for the experimental design and analysis. To reduce systematic errors, all the experiments were completely randomized. 100 ml of indomethacin (1% w/v with 0.5% w/v Dowfax 2A1) nanosuspension (Z-average 350 nm) formulations were spray-dried at different processing conditions (according to design space) and dried powders were collected from the glass collection chamber. Multi-linear regression and ANOVA were performed to analyze the relationship between critical variables and responses.

2.3.10. Cubic central design

Based on the full factorial design results, response surface methodology was adopted for the spray drying process optimization using a cubic central design. In this study, eight additional experiments were added to the full factorial design to generate the response surface for the percent yield and particle size. Six experiments were performed at the face of each cube (full factorial design space) and two additional experiments were added to the center points. Minitab16 software was utilized for the design and analysis of the results.

2.4. Results and Discussion

2.4.1. Influence of spray drying process variables on percent yield

As shown in Table 2.1, percent yield varied from 19.58 % w/w to 51.76 % w/w. Two main factors were significant: aspiration and flow rates (as shown in Table 2.2). It was noted that increase in aspiration rate increased the percent yield from 38% w/w to 48% w/w and increase in the flow rate decreased the yield of the spray-dried powder (as shown in Figure
Both these factors affect the percent yield by acting against one another. The decreased yield with increase in flow rate could be attributed to insufficient drying resulting in sticking of liquid or solid mass inside the drying chamber or the cyclone separator, or resulting in loss at the bottom of the drying chamber. By increasing the aspirator setting from -21 mbar to -42 mbar, yield was increased due to complete drying (higher outlet temperature with higher aspirator) and consequent reduction in loss of powder to the walls of the drying chamber. The highest yield was obtained at the lowest flow rate and the highest aspirator setting as shown in the contour plot (Figure 2.2). Further analysis using ANOVA indicated a significant effect ($p < 0.05$) of the critical variables on the response (percent yield) and no significant curvature was observed ($p > 0.05$). The flow rate had the highest impact on the percent yield followed by the aspiration rate. In addition, two-way interactions between critical variables (temperature*flow rate and temperature*aspiration) were observed to be significant. The significance of these two-way interactions is considered to be a result of the interdependency of the spray drying processing variables. For example, an increase in flow rate will result in a decrease in the outlet temperature. Similarly, a decrease in aspiration rate will result in a decrease in the outlet temperature.

2.4.2. Influence of spray drying process variables on aggregation/particle size

As shown in Table 2.1, the particle size of the dried powder (nano-crystals) varied from 397 nm to 1208 nm with the larger sized particles also having larger PDIs (data not shown). The particle sizes of the liquid indomethacin nano-crystalline suspension formulations were approximately 350 nm (PDI less than 0.2) before spray drying. The
important spray-drying variable affecting the particle size/aggregation was the inlet temperature (p value less than 0.05) as shown in Table 2.3 and no other variable and/or interaction term was significant. It was observed that an increase in inlet temperature causes further aggregation as shown by particle size increase (Figure 2.3). The reason for the increase in particle size with higher inlet temperature could be due to generation of surface defects/amorphization at near or above the melting temperature of the drug (indomethacin melting temperature, 158°C). Since the nanoparticles experience this high temperature for a short time it is likely that only surface drug undergoes melting and amorphization resulting in particle aggregation. To confirm this phenomenon, DSC was performed on all the spray-dried samples and the results are shown under spray-dried powder characterization section. In addition, the change in particle size with temperature appears to be non-linear (Figure 2.3).

2.4.3. Influence of spray drying variables on moisture content

None of the spray drying variables had a significant effect (p value more than 0.05) on the moisture content of the dried samples (Figure 2.4). All the formulations had less than 3% w/w moisture as determined by Karl Fisher titrimetry (Table 2.1). The formulations dried at higher inlet temperature conditions appeared to have less moisture content compared to other formulations (shown in Figure 2.4). However, this was not statistically significant (ANOVA). A Pareto plot of the normalized effect of process variables is shown in Figure 2.5.

2.4.4. Influence of spray drying on the crystallinity of indomethacin

2.4.4.1. Powder X-ray diffraction
Powder X-ray diffraction was performed on all the DoE independent formulations (twelve conditions). No differences were observed compared to control indomethacin and Dowfax 2A1 sample mixtures as shown in Figure 2.6. All formulations contained crystalline indomethacin (γ polymorph), both before and after spray drying.

2.4.4.2. Differential scanning calorimetry

In order to determine, whether amorphization had occurred at particle surfaces, DSC analysis was performed on the spray dried nanosuspension formulations (as shown in Figure 2.7). It was observed (Figure 2.7C) that all the DoE formulations spray dried at higher temperature conditions (196°C) showed a glass transition temperature around 42° - 46°C (which corresponds to the glass transition of amorphous indomethacin) as well as a melting temperature of 158°C (crystalline indomethacin) but other samples were crystalline (Figure 2.7A and 2.7B). DSC analysis for all the spray-dried formulations is provided in Table 4. In addition, these formulations were slightly yellow in color (Figure 2.8), confirming the presence of amorphous indomethacin [26]. These results suggest that drying at higher temperatures i.e. near or above the melting temperature may pose problems (aggregation and crystal defects) in spray-dried formulations.

2.4.5. Central Cubic Design to obtain the response surface for particle size and percent yield

The Central Cubic Design (CCD) space and experiment results are shown in Table 2.5. As shown in Table 2.6A, particle size/aggregation was only dependent on the inlet temperature (p value less than 0.05). Log transformation was used to model the particle size. A few outliers were observed (probably due of aggregation of nano-crystals when
drying above the melting temperature) which resulted in non-linearity. The final equation for particle size with estimated coefficients of the critical variables is shown in Table 2.6B. The surface response for the effect of inlet temperature on particle size is shown in Figure 2.9. A model was generated for the percent yield after spray drying of indomethacin nanosuspension formulations. The model is significant (p value less than 0.05) as shown in Table 7. The surface response for percent yield shows the dependence of percent yield on the aspiration and flow rates (Figure 2.10). Other interaction terms were also observed.

2.5. CONCLUSIONS

This study demonstrates the usefulness of the quality by design approach such as, DoE and response surface methodology to gain insight and understanding of spray drying processing of nanosuspensions. All the spray-dried formulations had less than 3% w/w moisture content. Formulations dried at higher temperatures had less moisture content compared to formulations dried at lower temperatures. The nanosuspension percent yield was mainly dependent on the flow and aspiration rates (p values <0.05). The inlet temperature was identified as the only critical parameter (p value <0.05) affecting particle aggregation during nanosuspension spray drying due to crystal melting or amorphization at or above melting temperature (indomethacin melting temperature 158°C). The amorphous content was observed in the case of formulations dried above the melting temperature. Particle aggregation increased with increasing temperature due to surface amorphization/crystal defects that lead to unstable formulations. It can be concluded that spray drying of nanosuspensions should be performed below the melting temperature with
high flow and aspiration rates for maximum recovery with minimal nano-crystal aggregation.
2.5. Tables:

Table 2.1. Full factorial design space and responses for the process of spray drying of indomethacin nanosuspensions.

<table>
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<th>Sample number</th>
<th>Temperature (°C)</th>
<th>Flow Rate (ml/min)</th>
<th>Aspiration (mbar)</th>
<th>Outlet temperature (°C)</th>
<th>Moisture content (% w/w)</th>
<th>Particle size (nm)</th>
<th>% Yield</th>
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<td>2</td>
<td>760</td>
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Table 2.2. Estimated effect of spray drying variables on percent yield.

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<th>Adj MS</th>
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Table 2.3. Estimated effect of spray drying variables on particle size.

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<tr>
<td><strong>Curvature</strong></td>
<td>1</td>
<td>64470</td>
<td>64470</td>
<td>64470</td>
<td>6.35</td>
<td>0.086</td>
</tr>
<tr>
<td><strong>Residual Error</strong></td>
<td>3</td>
<td>30477</td>
<td>30477</td>
<td>10159</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pure Error</strong></td>
<td>3</td>
<td>30477</td>
<td>30477</td>
<td>10159</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11</td>
<td>1080062</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.4. DSC results showing melting, glass transition, and crystallization temperatures of all DoE formulations.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Processing inlet temperature (°C)</th>
<th>Melting temp °C (Tm)</th>
<th>Glass transition temp °C (Tg)</th>
<th>Melting enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>196</td>
<td>158.32</td>
<td>46.99</td>
<td>64.7</td>
</tr>
<tr>
<td>2</td>
<td>164</td>
<td>156.52</td>
<td>n/a</td>
<td>65.1</td>
</tr>
<tr>
<td>3</td>
<td>196</td>
<td>156.76</td>
<td>48.14</td>
<td>64.05</td>
</tr>
<tr>
<td>4</td>
<td>131</td>
<td>156.1</td>
<td>n/a</td>
<td>65.27</td>
</tr>
<tr>
<td>5</td>
<td>196</td>
<td>157.15</td>
<td>46.21</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td>164</td>
<td>156.78</td>
<td>n/a</td>
<td>66.14</td>
</tr>
<tr>
<td>7</td>
<td>196</td>
<td>158.05</td>
<td>46.63</td>
<td>62.56</td>
</tr>
<tr>
<td>8</td>
<td>131</td>
<td>156.79</td>
<td>n/a</td>
<td>60.16</td>
</tr>
<tr>
<td>9</td>
<td>164</td>
<td>159.17</td>
<td>n/a</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>131</td>
<td>155.84</td>
<td>n/a</td>
<td>64.7</td>
</tr>
<tr>
<td>11</td>
<td>131</td>
<td>156.23</td>
<td>n/a</td>
<td>64.03</td>
</tr>
<tr>
<td>12</td>
<td>164</td>
<td>155.39</td>
<td>n/a</td>
<td>65.25</td>
</tr>
</tbody>
</table>
**Table 2.5.** Cubic center design with yield and particle size of spray dried indomethacin nanosuspension formulations.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Experimental conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Aspiration (mbar)</td>
</tr>
<tr>
<td>1</td>
<td>164</td>
<td>-31</td>
</tr>
<tr>
<td>2</td>
<td>196</td>
<td>-21</td>
</tr>
<tr>
<td>3</td>
<td>131</td>
<td>-41</td>
</tr>
<tr>
<td>4</td>
<td>196</td>
<td>-41</td>
</tr>
<tr>
<td>5</td>
<td>131</td>
<td>-21</td>
</tr>
<tr>
<td>6</td>
<td>164</td>
<td>-31</td>
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<tr>
<td>7</td>
<td>131</td>
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<tr>
<td>8</td>
<td>196</td>
<td>-41</td>
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<tr>
<td>9</td>
<td>164</td>
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<td>10</td>
<td>131</td>
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<td>12</td>
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<td>-31</td>
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<tr>
<td>13</td>
<td>164</td>
<td>-41</td>
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<tr>
<td>14</td>
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<td>-31</td>
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<tr>
<td>15</td>
<td>164</td>
<td>-31</td>
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<tr>
<td>16</td>
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<td>17</td>
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<td>18</td>
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<td>-31</td>
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<td>19</td>
<td>131</td>
<td>-31</td>
</tr>
<tr>
<td>20</td>
<td>164</td>
<td>-31</td>
</tr>
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</table>
Table 2.6. Estimated effects (A) and coefficients (B) of spray drying critical variables on particle aggregation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2.46</td>
<td>3</td>
<td>0.82</td>
<td>23.23</td>
<td>&lt; 0.0001</td>
<td>significant</td>
</tr>
<tr>
<td>A-Inlet temperature</td>
<td>2.39</td>
<td>1</td>
<td>2.39</td>
<td>67.71</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>B-Aspirator</td>
<td>0.04</td>
<td>1</td>
<td>0.04</td>
<td>1.13</td>
<td>0.3033</td>
<td></td>
</tr>
<tr>
<td>C-Flow rate</td>
<td>0.03</td>
<td>1</td>
<td>0.03</td>
<td>0.84</td>
<td>0.3724</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.57</td>
<td>16</td>
<td>0.035</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.43</td>
<td>11</td>
<td>0.039</td>
<td>1.45</td>
<td>0.357</td>
<td>not significant</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.13</td>
<td>5</td>
<td>0.027</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>3.03</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\text{Ln(Particle size)} = 4.97392 + 0.16311 \times \text{Inlet temperature} + 0.012651 \times \text{Aspirator} - 0.054572 \times \text{Flow rate}
\]
Table 2.7. Estimated effect (A) and coefficients (B) of spray drying critical variables on percent yield.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>744.91</td>
<td>5</td>
<td>148.98</td>
<td>8.73</td>
<td>0.0006</td>
<td>significant</td>
</tr>
<tr>
<td>B-Aspirator</td>
<td>171.06</td>
<td>1</td>
<td>171.06</td>
<td>10.02</td>
<td>0.0069</td>
<td></td>
</tr>
<tr>
<td>C-Flow rate</td>
<td>125.03</td>
<td>1</td>
<td>125.03</td>
<td>7.32</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>147.58</td>
<td>1</td>
<td>147.58</td>
<td>8.64</td>
<td>0.0108</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>51.92</td>
<td>1</td>
<td>51.92</td>
<td>3.04</td>
<td>0.1031</td>
<td></td>
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<tr>
<td>ABC</td>
<td>249.31</td>
<td>1</td>
<td>249.31</td>
<td>14.6</td>
<td>0.0019</td>
<td></td>
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<tr>
<td>Residual</td>
<td>238.99</td>
<td>14</td>
<td>17.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>174.97</td>
<td>9</td>
<td>19.44</td>
<td>1.52</td>
<td>0.3363</td>
<td>not significant</td>
</tr>
<tr>
<td>Pure Error</td>
<td>64.02</td>
<td>5</td>
<td>12.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>983.9</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\% \text{ Yield} = 40.16556 + 3.70086 \times \text{Aspirator} - 11.95022 \times \text{Flow rate} - 0.24549 \times \text{Inlet temperature} \times \text{Aspirator} + 0.98102 \times \text{Inlet temperature} \times \text{Flow rate} - 0.01396 \times \text{Inlet temperature} \times \text{Aspirator} \times \text{Flow rate}
\]
2.6. Figures:

**Figure 2.1.** Plot showing the mean effect of aspiration and flow rates on percent yield.

**Figure 2.2.** Contour plot showing the effect of aspiration (y-axis) and flow rates (x-axis) on the percent yield.
Figure 2.3. Plot showing the effect of inlet temperatures on nanoparticle size.

Figure 2.4. Plot showing the effect of inlet temperature on the moisture content of spray dried powders.
Figure 2.5. Pareto plot showing the standardized effect of spray drying processing variables on moisture content.

Figure 2.6. PXRD of all DoE formulations (twelve samples). Refer to Table 1 for experimental conditions 1 through 12.
Figure 2.7A. DSC plot showing all formulations spray dried at the lowest inlet temperature condition (131°C).

Figure 2.7B. DSC plot showing all formulations spray dried at the center point temperature condition (164°C).
**Figure 2.7C.** DSC plot showing all formulations spray dried at the highest temperature condition (196°C).

**Figure 2.8.** Photograph showing spray-dried formulations (Note: Samples 1, 3, 5 and 7 are relatively yellow in color compared to the other samples).
Figure 2.9. Response surface to predict particle size after spray drying of indomethacin nanosuspensions.

Figure 2.10. Response surface to predict percent yield after spray drying of indomethacin nanosuspensions.
2.7. References


Abstract

Nano-crystalline suspensions offer a promising approach to improve dissolution of BCS class II/IV compounds. Spray drying was utilized as a downstream process to improve the physical and chemical stability of dried nano-crystals. The effect of nano-crystalline suspension formulation variables on spray-drying processing was investigated. Naproxen and indomethacin nano-crystalline formulations were formulated with either Dowfax 2A1 (small molecule) or HPMC E15 (high molecular weight polymer) and spray drying was performed. A DoE approach was utilized to understand the effect of critical formulation variables i.e. type of stabilizer, type of drug, ratio of drug-to-stabilizer and drug concentration. The powders were analyzed for particle size, moisture content, powder X-ray diffraction and dissolution. A dialysis sac adapter for USP apparatus II was developed which provided good discrimination between aggregated and non-aggregated formulations. Nano-crystal aggregation was dependent on the drug-to-stabilizer ratio. The glass transition temperature and the charge effect played a dominant role on spray-dried powder yield. Those formulations with low drug-to-excipient ratios were less aggregating and showed faster dissolution compared to those formulations with high drug-to-excipient ratios. All stable (less aggregated) formulations were subjected to accelerated storage stability testing. The Flory-Huggins interaction parameter (between drug and excipients)
correlated with the spray-dried nano-crystal formulations stability.

3.1. Introduction

The popularity of nanosuspensions has increased over the past decade due to the escalation (approximately 40%) in the number of poorly soluble drug candidates coming out of drug discovery [1-5]. The aqueous solubility of these drug molecules is in the order of a few nanograms to hundreds of nanograms per milliliter. These poorly soluble drug candidates must be formulated appropriately to enhance their dissolution rate and/or solubility and subsequently oral bioavailability. Apart from poor oral bioavailability, these drugs have several other disadvantages such as, fed versus fasted variation in bioavailability and the requirement of high doses to achieve therapeutic responses [6]. Several approaches have been utilized to formulate poorly soluble compounds such as solid dispersions, salt formation, prodrugs, screening for more soluble crystalline forms and preparation of nanosuspensions. The high exposed surface area of the nano-crystals increases the dissolution rate and hence oral bioavailability [6-8]. Apart from a bioavailability advantage, nano-suspension formulations have been shown to increase the chemical stability in some cases. For example, a paclitaxel aqueous nano-suspension formulation was stable up to 4 years (when stored at 4°C) [9] compared to un-milled paclitaxel (80% degradation within 25 minutes) in water [10]. The reason of nano-suspension stabilization was due to shielding or protection of the nano-sized crystalline paclitaxel by surface stabilizers.

There are several commercially available nano-particulate formulations and others are currently being evaluated in clinical trials [11]. Nanosuspensions can be defined as
suspensions of nano-sized drug particles suspended in stabilizer/s solutions. These solutions can be aqueous or non-aqueous and the typical size range for pharmaceutical nanosuspensions is 100 to 1000 nm. Nanosuspensions fall under the category of colloidal dispersions and can be prepared either via “top-down” or “bottom-up” approaches. Top-down approaches are based on milling or grinding of the drug particles in aqueous or mixtures of aqueous and non-aqueous solvents to achieve the required particle size. Bottom-up approaches are based on precipitation of the drug from solution (drug dissolved in organic solvent) using a solvent such as distilled water where the drug is insoluble. The size and stability of the nano-suspension is dependent on the HLB of stabilizer and solubility of drug in stabilizer solutions, in bottom-up and top-down approaches respectively [12]. In addition, particles generated using these approaches may yield amorphous or partially crystalline material and their physical stability can be a critical issue [13].

Crystalline nanosuspensions have high surface area to volume ratios and thus (according to the Noyes-Whitney equation, Equation 3.1) [14] have faster dissolution rates and increased oral bioavailability.

\[
\frac{dC}{dt} = \frac{DS}{Vh} (C_s - C)
\]

Equation 3.1

Where \(\frac{dC}{dt}\) is change in concentration, \(D\) is the diffusion coefficient, \(S\) is the surface area of the drug particle, \(h\) is the thickness of the diffusion layer, \(C_s\) is the saturation solubility of the drug particle, \(C\) is the concentration of the drug in solution, and \(V\) as the total volume of the solution.
In addition, according to the Kelvin equation (Equation 3.2), saturation solubility (in terms of vapor pressure) of the drug is dependent on the drug particle size (which translates to curvature effects) [15]. Theoretically, reduction in particle size will cause an increase in drug solubility. However, the predicted increase in saturation solubility for pharmaceutical nanosuspensions (approximately 300 nm) is marginal compared to un-milled particles.

\[
\frac{S}{S_0} = \exp \left(\frac{4\gamma V}{dRT}\right)
\]

Equation 3.2

Where \(S=\text{solubility, } S_0=\text{solubility of bulk material, } R=\text{gas constant, } V=\text{molar volume, } T=\text{temperature, } d=\text{diameter of particle and } \gamma=\text{surface free energy.}\)

Nanosuspensions being a liquid dosage form are physically and chemically unstable. Drug particles in aqueous environment can undergo chemical degradation such as hydrolysis. Nano-suspension formulations are known to undergo Ostwald’s ripening as well as phase separation or precipitation. To prevent chemical as well as physical instability, drying of nanosuspensions is employed. Freeze and spray-drying processes are typically used to dry nanosuspensions [16, 17]. The advantages of dried formulations are improvement in chemical and physical stability and processibility into tablets or capsules. The drying process itself can lead to issues such as, aggregation on re-dispersion of dried powders, drug degradation, delayed and dissolution rates. There are several reports which deal with spray and/or freeze-drying of nanosuspensions [16-21] but none of these explain the importance of the process and formulations parameters during nano-suspension drying.

Our previous research has focused on the effect of spray drying process parameters on the critical quality attributes (CQA) of nanosuspensions. This current study highlights the
importance of critical nano-suspension formulation variables involved during spray drying processing.

We have utilized a Design of Experiment (DoE) approach to classify and quantify all the critical formulation variables involved during spray drying of nanosuspensions. We have chosen two poorly soluble biopharmaceutical classification system (BCS) class II model drugs (i.e. indomethacin and naproxen). Dowfax 2A1 (small molecule ionic surfactant) and HPMC E15 (non-ionic polymer) were used for the stabilization of liquid nano-suspension formulations. Based on our preliminary study, we have selected four critical parameters i.e. type of drug, type of excipient, concentration of drug and ratio of drug-to-stabilizer for this study. A full factorial design was utilized with two qualitative and two quantitative factors. The CQAs were particle size, yield, moisture content, polymorphic form, dissolution and stability of the spray-dried nano-crystal powders. ANOVA was utilized to analyze the effect of critical formulation parameters on the quality attributes of spray-dried powders. To the best of our knowledge, no exhaustive DoE study has been conducted that describes the effect of nano-suspension formulation variables on the quality of the spray-dried powder.

At present, United States Pharmacopeia (USP) does not have any official or standard method(s) for in vitro release testing of colloidal/disperse dosage forms. Currently used techniques can be broadly divided into two categories: 1) sample and separation methods; and 2) membrane diffusion methods (such as dialysis sac, reverse dialysis sac, micro-dialysis, and Franz cells). These techniques are required to isolate the dosage form from the release media for analytical purposes. However, these existing methods do not use
official USP dissolution/release apparatus. Accordingly, the methodology changes with operator and laboratory, introducing undesirable variations. Therefore, results from different sources are usually not comparable. The present study aims to address this problem by designing and developing a dialysis adapter to be used in conjunction with compendial USP dissolution apparatus II.

3.2. Materials

Indomethacin USP, γ polymorph, was purchased from PCCA (Houston, TX). Naproxen was purchased from Gallipot. Dowfax 2A1 (alkyldiphenyloxide disulfonate) and HPMC E15 (premium LV) were generously gifted by Dow Chemical Company (Midland, MI). HPLC grade acetonitrile (ACROS chemicals) was purchased from Fisher scientific (Waltham, MA). Hermetic pans and lids were purchased from TA instruments (New Castle, DE) and Float-A-Lyzer dialysis sacs (MWCO 1000 kDa) were purchased from Spectrum labs (Spectrum Laboratories Inc., California).

3.3. Methods

3.3.1. Preparation of crystalline nanosuspensions

All the nano-suspension formulations were prepared using a wet media milling (top-down) approach. Briefly, the stabilizer was dissolved in distilled water and the drug was suspended in the stabilizer solution using a magnetic stirrer. The macro-suspensions were stirred to ensure complete wetting of the drug by the stabilizer. The stirred macro-suspension formulations (120 mL) were processed via a Netzsch® media mill. All nano-
suspension formulations were milled for 2 - 3 hours under controlled temperature conditions *i.e.* below 25°C. The particle size of all nano-suspension formulations was approximately 250 nm with a PDI (polydispersity index) of less than 0.2 (*i.e.* monodisperse systems).

3.3.2. Spray drying of crystalline nanosuspensions

Indomethacin and naproxen nano-suspension formulations were spray dried using a lab scale Buchi spray dryer B-290 (Buchi labortechnik AG, Flawil, Switzerland). Briefly, the spray dryer was pre-conditioned using distilled water. Once equilibrated, the required amount (either 20 ml for 5% w/v or 100 ml for 1% w/v nano-suspension formulations) of the nano-suspension formulation was spray dried at a pre-optimized condition (*i.e.* feed rate 5 mL/min, inlet temperature 150°C and aspirator setting -31 mbar). Atomizing air was maintained constant at 40 mm Hg (air flow approximately 600 L/hour) and a 0.5 mm spray nozzle was used. The dried samples were collected and evaluated for yield, particle size and drug polymorphic form.

3.3.3. Powder X-ray diffraction (PXRD)

PXRD (powder X-ray diffraction) was used to study the crystallinity of the dried samples. Diffraction patterns were obtained using an X-ray diffractometer (Bruker AXS Model D2 Phase diffractometer, Germany) using Cu-κα radiation (30 kV voltage and 10 mA current). All the scans were performed at 1°/min from 5° to 40° 2θ angle aided by goniometer.

3.3.4. Differential scanning calorimetry

Modulated temperature DSC (mtDSC) was performed using a TA Q1000 calorimeter (TA
instrument, New Castle, DE). The instrument was calibrated for enthalpy and heat capacity using indium and sapphire, respectively. About 5 - 10 mg of samples were hermetically sealed and analyzed. Nitrogen was used as a purging gas; at a flow rate of 50 mL/min. Samples were heated at 2°C/min at amplitude of +/- 0.82°C with a period of 80 seconds. Data were analyzed using TA universal analysis software.

3.3.5. Percent yield

For calculation of percent yield, the drug amounts in the liquid and dried nano-suspension formulations were determined using an HPLC-UV method (as described below). Briefly, the nano-suspension formulations were dissolved in the mobile phase and this solution was used to quantify the drug amount. The formula used for calculation of the % yield was:

\[
% \text{ Yield} = \frac{\text{Drug Mass Out}}{\text{Drug Mass In}} \times 100\%
\]

Equation 3.3

Note: Drug mass in and out represents, the total amount of drug (in nanosuspensions) that was spray dried and that was recovered (dried powder), respectively.

3.3.6. HPLC-UV method

The standard curves of indomethacin and naproxen were generated using a Perkin-Elmer (Waltham, MA) HPLC system (200 series) connected to a UV system.

3.3.6.1. Indomethacin HPLC-UV method

The amount of indomethacin was quantified using C-18 Zorbax® column (4.6 mm×150 mm, 5µm) and the mobile phase was a mixture of two phases (A/B) at 50:50 v/v ratio. Phase A was a mixture of water and phosphoric acid (0.2% v/v) and phase B was HPLC
grade acetonitrile. The flow rate was maintained constant at 1.3 ml/min and the UV absorbance was measured at 237 nm. The temperature of the column was maintained at 30°C. Various dilutions were made in the mobile phase to prepare a standard curve. The chromatographs were analyzed using a PeakSimple™ Chromatography System. The concentration range for linearity was 0.005 mg/ml to 0.2 mg/ml with an $R^2$ value 0.99.

3.3.6.2. Naproxen HPLC-UV method

Naproxen amount was quantified using C-18 Zorbax® column (4.6 mm×150 mm, 5µm) and the mobile phase was a mixture of two phases (A/B) at 50:50 v/v ratio. Phase A was a mixture of water and acetate buffer (20 mM) and phase B was HPLC grade acetonitrile. The pH of the mixture was adjusted to pH 4.4 using acetic acid. The flow rate was kept constant at 1.0 ml/min and the UV absorbance was measured at 270 nm. The temperature of the column was maintained at 30°C. Various dilutions of the mobile phase were made to prepare a standard curve. The chromatographs were analyzed using a PeakSimple™ Chromatography System. The concentration range for linearity was 0.001 mg/ml to 0.1 mg/ml with an $R^2$ value 0.9978.

3.3.7. Particle size measurement

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) to determine the Z-average and PDI of the nanosuspensions before and after spray drying processing. Briefly, the spray-dried powders were suspended in saturated and filtered (0.2 µm membrane filter) solution of the drug (either indomethacin or naproxen) in 30% glycerin solution to avoid any discrepancy from dissolution of nano-particles during the measurement. The viscosity of the dispersant solution (measured using a Brookfield viscometer model DV-III, Middleboro, MA) was
used to calculate the particle size of the formulations. Each measurement was performed in triplicate and the mean values were reported.

3.3.8. Dissolution experiment

Dissolution experiments were conducted using a Sotax® USP apparatus II. Float-A-Lyzer dialysis sacs were secured to the Sotax USP apparatus II using an in-house developed adapter (the Float-A-Lyzer cap was glued to a plastic rod which was then fixed to the cover of the Sotax USP II apparatus). The immersion depth of the dialysis sac was controlled by the length of the adapter (l) and was carefully adjusted so that the dialysis portion was fully immersed while the cap was always above the dissolution media (as shown in Figures 3.1, 3.2 and 3.3). The spray-dried formulations were re-suspended in 1 mL of distilled water and placed into the dialysis sacs (cutoff 1000 KDa). The dialysis sacs were screwed to the fixed cap (as shown in Figure 3.1 and 3.3) and the dissolution experiments were performed. All the dissolution experiments were conducted at 37°C in 900 ml (sink condition) of phosphate buffer (50 mM, pH 6.8) with 50 rpm paddle speed. At each time point, 1 ml of sample was withdrawn from the dissolution chamber (outside the dialysis sac) and replaced with fresh phosphate buffer. All the samples were analyzed using the HPLC method as described.

3.3.9. Stability study

All spray-dried formulations were stored at 40°C and 75% relative humidity (RH) for three months in closed vials. After three months, the samples were analyzed for particle size and polymorphic changes, if any.

3.3.10. Design of Experiment (DoE)
Based on our preliminary experiments, drug type, concentration of nanosuspension, type of stabilizer and drug to stabilizer ratio were identified as important formulation variables that may significantly affect the particle aggregation and yield during spray drying processing. To understand the effects of the formulation variables a design of experiment (DoE) approach was adopted and a full factorial design was utilized. For this study, two model APIs (*i.e.* naproxen and indomethacin) and stabilizers *i.e.* Dowfax 2A1 (small molecule) and HPMC E15 (large molecular weight) were tested. The experimental design space consisted of $2^4$ experiments (4 factors at 2 levels). All four independent variables at two levels were: type of drug (naproxen and indomethacin); concentration of drug nanosuspensions for spray drying (1% w/v to 5% w/v); type of excipient (Dowfax 2A1 and HPMC E15) and drug to stabilizer ratio in nanosuspensions (10 parts to 1 part and 10 parts to 5 parts). It was confirmed that all the levels of the design space can be achieved and further can be applied to other drug formulations. 20 ml, in case of 5% w/v and 100 ml, in case of 1% w/v nanosuspensions (equal amount of drug in each case) were spray-dried at an optimized condition. The dried powder was collected from the collection chamber for further analysis. Minitab® 16 (Minitab Inc. State college, PA) and Design expert 8 (Stat- ease Inc., Minneapolis, MN) were utilized to design and analysis DoE results. To reduce any effect of systematic errors all the experiments were randomized.

3.4. RESULTS AND DISCUSSIONS

3.4.1. Effect of formulation variables on nano-crystal aggregation during spray drying
As shown in Table 3.1, all spray-dried formulations showed aggregation. One critical variable was identified as a significant parameter for nano-crystals aggregation i.e. drug-to-stabilizer ratio. All other variables were not significant as shown in Table 3.2. It was noted that formulations containing high concentrations of stabilizers (i.e. either Dowfax 2A1 or HPMC E15) were less aggregated compared to formulations with lower stabilizer concentrations (Figure 3.4). Both small molecule (Dowfax 2A1) and large molecular weight stabilizers (HPMC E15) had similar effects on aggregation after spray drying processing. HPMC E15 containing formulations appeared slightly better (i.e. less aggregated) than Dowfax 2A1 containing formulations but differences were statistically insignificant. Nano-crystal aggregation was independent of the drug concentration and the drug itself (i.e. naproxen and indomethacin showed similar aggregation). Average particle sizes (z-average) of all the formulations are given in Table 3.1. A plot of the interactions between formulation variables is shown in Figure 3.5.

3.4.2. Effect of formulation variables on powder yield during spray drying

As shown in Table 1, percent yield during spray drying varied from 25% (sample 14) to 72% (sample 7). It was interesting to observe that all the formulation variables were critical to the nanosuspension yield following spray drying (as shown in Table 3.3). Naproxen containing formulations had lower yield compared to indomethacin formulations. This may be due to the lower melting temperature of naproxen compared to indomethacin (Table 3.4), which resulted in increased adhesion to the glass chambers walls. Both formulations were spray dried at an inlet temperature of 150°C. Naproxen containing formulations have a lower melting temperature i.e. 152°C (close to the inlet
temperature) and thus may form amorphous regions at the particle surfaces causing sticking to the chamber walls and lower yields. Drug concentration in the nanosuspension formulations (1% w/v and 5% w/v) also affected the yield during spray drying. Each spray drying experiment was performed with a constant amount of drug i.e. 20 ml of 5% w/v or 100 ml of 1% w/v nanosuspension. The reason for lower yields of the 1% w/v formulations may be due to the higher probability of drug particle adherence to the chambers since this formulation has a five times larger volume (i.e. 100 ml versus 20 ml) and therefore has to be spray dried for a five times longer period of time. The nanocrystal yield also showed a dependency on the type of excipient (i.e. Dowfax 2A1 and HPMC E15). All Dowfax-2A1 containing formulations had superior yields compared to the HPMC E15 containing formulations (as shown Figures 3.6 and 3.7). This maybe due to the charge effect, as Dowfax 2A1 is a negatively charged surfactant compared to HPMC E15 (non-ionic stabilizer). The Buchi spray dryer is coated with a special anti-static coating to repel charges (i.e. charged powder) away from the glass surface and thus improve the recovery. The nanosuspension formulations containing Dowfax-2A1 were highly negatively charged (high negative zeta potential) compared to the HPMC E15 containing formulations (as shown in Table 3.5). This charge effect of Dowfax-2A1 formulations could be the reason for the higher yields obtained with these formulations. The effect of drug-to-excipient ratio on the percent yield is complex. It was observed that by decreasing the drug-to-excipient ratio from 10:1 to 2:1 resulted in an increase in the yield during spray drying (Figure 3.7). In the case of Dowfax-2A1 containing formulations, an increase in Dowfax-2A1 concentration increases the negative charge and thus maybe responsible for the higher yields (as shown in Table 3.5A). In the case of HPMC E15 (non-ionic) containing
formulations, it is speculated that an increase in the HPMC E15 concentration will increase the Tg of the dried powder (greater number of HPMC E15 molecules per spray droplet) and this will cause less sticking inside the chamber. Higher glass transition temperature (Tg) formulations generally have higher yields compared to lower Tg formulations (data not shown).

3.4.3. Characterization of spray-dried powder:

3.4.3.1 Powder X-ray diffraction

As shown in powder x-ray diffraction, no changes were observed in any of the formulations (Figure 3.8 and 3.9). All the formulations were crystalline in nature.

3.4.3.2 Moisture content

All the formulations had less than 3% w/w moisture content (data not shown).

3.4.4. Dissolution performance of spray-dried product

The dissolution experiment was conducted using a newly developed dialysis sac adapter for the USP apparatus II, as described in the methods section. All dissolution experiments were performed in phosphate buffer (50 mM) at pH 6.8. Approximately 20 mg of drug as re-suspended formulations was added to the dialysis sacs and the dissolution experiments were performed in triplicate. As shown in Figures 3.10 and 3.11, aggregated formulations resulted in slower drug release or dissolution compared to less aggregated formulations. All highly aggregated formulations had lower drug-to-stabilizer ratios (as shown in Table 3.1). The initial (0-60 minutes) drug release rates of these formulations were similar, this is probably due to non-aggregated nanoparticulates in these formulations. Diffusion of
naproxen and indomethacin in solution are shown for comparison (Figures 3.12 and 3.13).

3.4.5. Stability studies:

3.4.5.1. Particle size

All less aggregated formulations (i.e. 2:1 drug-to-excipient ratio containing formulations) were stored at 40°C at 75% RH for three months and particle analysis was performed. It was observed that for the naproxen-HPMC E15 formulations (samples #15 and #16), the spray-dried nanocrystal powders were stable (i.e. less aggregation) after spray drying as well as during accelerated storage conditions compared to indomethacin-HPMC E15 formulations (sample #7 and #8), as shown in Table 3.6. This is considered due to the favorable interactions between HPMC E15 and naproxen. In the case of Dowfax-2A1 containing formulations, both naproxen-Dowfax 2A1 (samples #11 and #12) and indomethacin-Dowfax 2A1 (samples #3 and #4) spray-dried nanocrystal powders showed aggregation after spray drying as well as during storage conditions.

To further understand the mechanism of nanocrystal aggregation following spray drying and during storage stability, the Flory-Huggins interaction parameter ($\chi$ chi) was calculated from the melting point depression approach [22, 23]. This approach is used by scientists to calculate the interaction parameters between different APIs and polymer systems in order to predict the stability of solid dispersions [24, 25]. However, this method has not been applied or correlated to the stability of crystalline nanosuspension systems. The limitation of this approach (i.e. melting point depression to calculate interaction value), is that it can only be applied to systems where melting point depression is observed [22, 23, 24]. Physical mixtures of naproxen and indomethacin with HPMC E15 or Dowfax-2A1 were
prepared at different concentrations. Modulated DSC of all the physical mixtures was performed to check for depression in the drug melting point. As shown in Figure 3.13, the melting point of naproxen-HPMC E15 was greatly depressed compared to indomethacin-HPMC E15 suggesting a stronger and more favorable interaction between naproxen and HPMC E15. As shown in Figure 3.12, Dowfax-2A1 does not significantly affect the melting point of the indomethacin or naproxen, suggesting no or weak interaction. Dowfax 2A1 is a negatively charged small molecule surfactant and therefore unlikely to affect the Flory-Huggins interaction parameter, compared to HPMC E15 (un-charged polymer). The negative charge on Dowfax 2A1 is likely contributing to the nano-crystal powder stability. Dowfax 2A1-naproxen or Dowfax 2A1-indomethacin nanosuspension formulation data cannot be described by the interaction parameter determined via the melting depression approach. As little or no melting point depression was observed with Dowfax 2A1 via modulated DSC (Figure 3.12 A and B), no further chi or interaction value calculations were performed for indomethacin or naproxen with Dowfax 2A1. The interaction parameter (χ) value was calculated for both naproxen and indomethacin with HPMC-E15. The relationship of melting point depression and the interaction parameter is shown below:

\[
\left( \frac{1}{T_{M}^{mix}} - \frac{1}{T_{M}^{pure}} \right) = - \frac{R}{\Delta H_{fus}} \left[ \ln \Phi_{\text{drug}} + \left( 1 - \frac{1}{m} \right) \Phi_{\text{polymer}} + \chi \Phi_{\text{polymer}}^2 \right]
\]

Equation 3.4

Where \( T_{M}^{mix} \) is the melting temperature of the drug in the presence of the polymer, \( T_{M}^{pure} \) is the melting temperature of the drug in the absence of the polymer, \( \Delta H_{fus} \) is the heat of fusion of the pure drug, \( \Phi_{\text{polymer}} \) is the volume fraction of the polymer, \( \Phi_{\text{drug}} \) is the volume fraction of the drug and \( m \) is the ratio of the molecular volume of the polymer to
that of the lattice site (or volume of the drug). A plot of \( \left( \frac{1}{T_{M}^{\text{mix}}} - \frac{1}{T_{M}^{\text{pure}}} \right) \ast (\Delta H_{fus}/R) - (\ln \Phi_{\text{drug}}) - \left(1 - \frac{1}{m}\right) \Phi_{\text{polymer}} \) versus \( \chi \Phi_{\text{polymer}}^{2} \) will yield a linear relationship with a slope equal to \( \chi \). A negative, zero or positive value of \( \chi \) will represent favorable, ideal and no interaction between systems, respectively. The physical properties used in the calculation of \( \chi \) are shown in Table 3.7. The calculated \( \chi \) values (slope values) of indomethacin-HPMC E15 and naproxen-HPMC E15 were -0.3 and -3.4, respectively (Figure 3.14). A -3.4 value corresponds to strong interaction between naproxen and HPMC E15, which might be the reason for less nanocrystal aggregation compared with the indomethacin-HPMC E15 formulations (interaction parameter value -0.3).

**3.4.5.2. Polymorphic form**

Powder XRD (Figure 3.15) was performed for all the formulations after 3 months of storage stability. No polymorphic changes were observed during storage conditions.

**3.5. CONCLUSIONS**

This is the first report, which deals with the effect of formulation variables on the critical quality attributes of spray-dried nano-crystal powder. The aggregation tendency of spray-dried nano-crystal powders showed a dependency on the drug-to-stabilizer ratio. The formulations containing higher concentrations of excipients showed less aggregation compared to formulations containing lower concentrations of excipients. We have developed a dialysis sac adapter for USP apparatus II. This adapter can be utilized with any USP dissolution apparatus II with minor modifications. A dissolution method utilizing
dialysis sacs with USP apparatus II was able to distinguish aggregated versus non-aggregated nano-sized formulations. This dialysis sac adapter can also be utilized for other liquid formulations such as, liposome, microsphere etc. This method can be utilized for the routine quality control assays performed in the pharma industry and potentially for the development IVIVC. The nano-crystal yield during spray drying showed dependency on all factors studied \textit{i.e.} drug, excipient, drug to stabilizer ratio and concentration of the drug. The Tg and charge of the formulation played a dominant role during the spray drying process. It is observed that formulations with higher concentrations of HPMC E15 resulted in higher yields compared to formulations with lower concentrations of HPMC E15. This may be due to a difference in Tg (as higher Tg is expected with formulations containing higher HPMC-E15 concentration which will result in less sticking to the drying chamber and higher yields). On the other hand, using a charged excipient (such as negatively charged surfactants) for nano-crystal stabilization and spray drying improved the yield. In addition, we have applied chi ($\chi$) interaction parameter to the stability of polymer-stabilized nano-sized systems \textit{i.e.} nano-crystalline formulations. The stability of the spray-dried powder correlated with the interaction parameter (calculated using the melting point depression approach) between the drugs \textit{i.e.} naproxen and indomethacin and HPMC E15 (high molecular weight polymer). The melting point depression approach may also be utilized to select the best excipient/s for other BCS class II/IV APIs nano-crystalline formulations. However, different drugs and stabilizers must be tested to validate the application of this approach to nano-crystal stabilization.
3.6. Tables:

Table 3.1. Full factorial design of formulation variables involved during spray drying processing of crystalline nanosuspensions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug</th>
<th>Excipient</th>
<th>Ratio of drug-to-excipient</th>
<th>Conc. of Drug (w/w)</th>
<th>Particle Size (% Increase)</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>5</td>
<td>1021.41</td>
<td>52.54</td>
</tr>
<tr>
<td>2</td>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>1</td>
<td>857.91</td>
<td>48.76</td>
</tr>
<tr>
<td>3</td>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>5</td>
<td>171.25</td>
<td>66.38</td>
</tr>
<tr>
<td>4</td>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>1</td>
<td>160.68</td>
<td>53.77</td>
</tr>
<tr>
<td>5</td>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>5</td>
<td>597.55</td>
<td>47.14</td>
</tr>
<tr>
<td>6</td>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>1</td>
<td>612.19</td>
<td>27.40</td>
</tr>
<tr>
<td>7</td>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>5</td>
<td>198.00</td>
<td>72.05</td>
</tr>
<tr>
<td>8</td>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>1</td>
<td>187.84</td>
<td>53.41</td>
</tr>
<tr>
<td>9</td>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>5</td>
<td>925.70</td>
<td>61.13</td>
</tr>
<tr>
<td>10</td>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>1</td>
<td>777.97</td>
<td>53.36</td>
</tr>
<tr>
<td>11</td>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>5</td>
<td>215.65</td>
<td>60.85</td>
</tr>
<tr>
<td>12</td>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>1</td>
<td>214.78</td>
<td>54.59</td>
</tr>
<tr>
<td>13</td>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>5</td>
<td>1071.34</td>
<td>41.09</td>
</tr>
<tr>
<td>14</td>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>1</td>
<td>774.25</td>
<td>25.27</td>
</tr>
<tr>
<td>15</td>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>5</td>
<td>129.67</td>
<td>52.25</td>
</tr>
<tr>
<td>16</td>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>1</td>
<td>116.27</td>
<td>42.14</td>
</tr>
</tbody>
</table>
Table 3.2. Estimated effect of nanosuspension formulation variables on particle size.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>1</td>
<td>10962</td>
<td>10962</td>
<td>10962</td>
<td>0.62</td>
<td>0.47</td>
</tr>
<tr>
<td>Excipient</td>
<td>1</td>
<td>27081</td>
<td>27081</td>
<td>27081</td>
<td>1.54</td>
<td>0.27</td>
</tr>
<tr>
<td>Ratio of drug to excipient</td>
<td>1</td>
<td>1718833</td>
<td>1718833</td>
<td>1718833</td>
<td>97.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Concentration of drug</td>
<td>1</td>
<td>24702</td>
<td>24702</td>
<td>24702</td>
<td>1.40</td>
<td>0.29</td>
</tr>
<tr>
<td>Drug*Excipient</td>
<td>1</td>
<td>20527</td>
<td>20527</td>
<td>20527</td>
<td>1.16</td>
<td>0.33</td>
</tr>
<tr>
<td>Drug*Ratio of drug to excipient</td>
<td>1</td>
<td>15727</td>
<td>15727</td>
<td>15727</td>
<td>0.89</td>
<td>0.39</td>
</tr>
<tr>
<td>Drug*Conc. of drug</td>
<td>1</td>
<td>5238</td>
<td>5238</td>
<td>5238</td>
<td>0.30</td>
<td>0.61</td>
</tr>
<tr>
<td>Excipient*Ratio of drug to excipient</td>
<td>1</td>
<td>9855</td>
<td>9855</td>
<td>9855</td>
<td>0.56</td>
<td>0.49</td>
</tr>
<tr>
<td>Excipient*Conc. of drug</td>
<td>1</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>0.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Ratio of drug to excipient*Conc. of drug</td>
<td>1</td>
<td>19509</td>
<td>19509</td>
<td>19509</td>
<td>1.11</td>
<td>0.34</td>
</tr>
<tr>
<td>Error</td>
<td>5</td>
<td>88142</td>
<td>88142</td>
<td>17628</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>1940590</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 132.772; R-Sq = 95.46% ; R-Sq(adj) = 86.37%
Table 3.3. Estimated effect of nanosuspension formulation variables on percent yield during spray drying.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>1</td>
<td>59.06</td>
<td>59.06</td>
<td>59.06</td>
<td>8.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Excipient</td>
<td>1</td>
<td>513.43</td>
<td>513.43</td>
<td>513.43</td>
<td>70.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Ratio of drug to excipient</td>
<td>1</td>
<td>609.67</td>
<td>609.67</td>
<td>609.67</td>
<td>83.66</td>
<td>0.00</td>
</tr>
<tr>
<td>Concentration of drug</td>
<td>1</td>
<td>560.78</td>
<td>560.78</td>
<td>560.78</td>
<td>76.95</td>
<td>0.00</td>
</tr>
<tr>
<td>Drug*Excipient</td>
<td>1</td>
<td>142.27</td>
<td>142.27</td>
<td>142.27</td>
<td>19.52</td>
<td>0.01</td>
</tr>
<tr>
<td>Drug*Ratio of drug to excipient</td>
<td>1</td>
<td>103.95</td>
<td>103.95</td>
<td>103.95</td>
<td>14.26</td>
<td>0.01</td>
</tr>
<tr>
<td>Drug*Conc. of drug</td>
<td>1</td>
<td>13.7</td>
<td>13.7</td>
<td>13.7</td>
<td>1.88</td>
<td>0.23</td>
</tr>
<tr>
<td>Excipient*Ratio of drug to excipient</td>
<td>1</td>
<td>218.63</td>
<td>218.63</td>
<td>218.63</td>
<td>30.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Excipient*Conc. of drug</td>
<td>1</td>
<td>71.77</td>
<td>71.77</td>
<td>71.77</td>
<td>9.85</td>
<td>0.03</td>
</tr>
<tr>
<td>Ratio of drug to excipient*Conc. of drug</td>
<td>1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.97</td>
</tr>
<tr>
<td>Error</td>
<td>5</td>
<td>36.44</td>
<td>36.44</td>
<td>7.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>2329.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = 2.69954   R-Sq = 98.44%   R-Sq(adj) = 95.31%

Table 3.4. Table showing reported values of melting and glass transition temperatures of indomethacin and naproxen.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Melting point (Tm)</th>
<th>Glass transition temperatures (Tg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td>152°C</td>
<td>7°C</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>158°C</td>
<td>42°C</td>
</tr>
</tbody>
</table>
Table 3.5. Table showing zeta potential values of different nanosuspension formulations A). Dowfax-2A1 and B). HPMC E15 containing formulations.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipient</th>
<th>Ratio of drug-to-excipient</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>-49.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>-59.8</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>10 to 1</td>
<td>-56.9</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Dowfax 2A1</td>
<td>2 to 1</td>
<td>-68.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>-23.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>-4.94</td>
</tr>
<tr>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>10 to 1</td>
<td>-19.8</td>
</tr>
<tr>
<td>Naproxen</td>
<td>HPMC E15</td>
<td>2 to 1</td>
<td>-5.25</td>
</tr>
</tbody>
</table>
Table 3.6. Particle sizes of different formulations after spray drying and storage stability.

**HPMC E15 containing formulations**

<table>
<thead>
<tr>
<th>Formulation No</th>
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**Dowfax 2A1 containing formulations**

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Table 3.7. Physical properties used for the $\chi$ (chi) calculations

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<td>HPMC E15</td>
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(Note: The density of the amorphous naproxen is unavailable due to the poor physical stability of its glass *i.e.* fast crystallizer, thus approximate value was used for calculations *i.e.* 3% less that the true density of its crystalline counterpart)
3.7. Figures:

**Figure 3.1.** Sotax® USP II adapted to hold the dialysis sacs. Top part of each vessel is attached with the plastic adapter to hold the dialysis sacs.

**Figure 3.2.** Assembly of the dialysis sac with the adapter in Sotax® USP II apparatus.
Figure 3.3. Schematic representation of the assembly of dialysis sac and its adapter within USP apparatus II.

Figure 3.4. Plot showing the effect of formulation variables on nanocrystal aggregation during
spray drying (y-axis showing mean particle size).

**Figure 3.5.** A plot of the interactions between formulation variables showing dependency of nanocrystal aggregation on drug-to-excipient ratio (drug to exp).

**Figure 3.6.** Plot showing the effect of formulation variables on the percent yield during spray drying (y-axis showing mean percent yield).
Figure 3.7. Plot showing interactions among formulation variables on the percent yield during spray drying.

Figure 3.8. Plot showing PXRD of all formulations containing indomethacin.
Figure 3.9. Plot showing PXRD of all formulations containing naproxen.

Figure 3.10. Dissolution profiles of indomethacin spray dried (SD) formulations containing: A) Dowfax-2A1; and B) HPMC E15 excipient. (Note: formulations 3 and 7 were less aggregated
compared to formulations 1 and 5, refer to Table 2).

**Figure 3.11.** Dissolution profiles of naproxen spray dried (SD) formulations: A) Dowfax 2A1; and B) HPMC E15 containing formulations. (Note: formulations 11 and 15 were less aggregated compared to formulations 9 and 13, refer to Table 2).
Figure 3.12. DSC plots of physical mixtures of naproxen (A) and indomethacin (B) with Dowfax 2A1.
Figure 3.13. DSC plots showing physical mixtures of indomethacin (A) and naproxen (B) with HPMC E15.
**Figure 3.14.** A plot showing interaction parameter values (slope values) of naproxen-HPMC E15 and indomethacin-HPMC E15 systems based on melting point depression approach.
Figure 3.15. Plot showing PXRD data of all the formulations after three month of storage stability.
3.8. References


Chapter 4

Bulking Agents to Prevent Nano-crystal Aggregation During

Spray or Freeze Drying

Abstract

In this study, the effect of small and high molecular weight bulking agents was evaluated during spray or freeze-drying of indomethacin nano-crystalline suspension (BCS class II) with Dowfax 2A1 as the stabilizer. Particle size, yields and crystallinity of the dried crystalline powders with or without bulking agents were determined. Interaction between the nanosuspension stabilizer (i.e. Dowfax 2A1) and bulking agents was investigated utilizing IR spectroscopy and contact angle measurement. All the formulations containing small molecular weight bulking agents were non-aggregating compared to those formulations containing polysaccharides during spray or freeze-drying processing of indomethacin nano-crystalline suspensions. In addition, higher crystalline powder yields were observed with formulations containing higher glass transition temperature bulking agents during spray drying. The bulking agents with low glass transition temperatures were sticking to the spray drier glass walls and thus resulted in lower yields. The small molecular weight bulking agents showed favourable or strong interactions with Dowfax 2A1 (via IR and contact angle) and this may be the reason of no or minimal nano-crystal aggregation during spray or freeze drying. A combination of bulking agent (i.e. small molecular weight and polysaccharides) may be utilized to achieve higher spray-drying
yields and non-aggregating nano-crystalline powders.

4.1. Introduction

Active pharmaceutical ingredients can be classified into four different categories (as per the Biopharmaceutics Classification System, Class I - IV) according to their solubility and permeability (1). BCS class I drugs have good solubility and permeability; class II drugs have good permeability but poor solubility; class III drugs have good solubility but poor permeability; and class IV drugs have poor solubility and permeability. In the last two decades, 30-40% of the newly discovered or synthesized chemical compounds have poor aqueous solubility and thus poor oral bioavailability (classified under BCS Class II/IV) (2-5). This problem poses a great challenge to formulators to design and develop appropriately soluble and thus orally bioavailable formulations of poorly soluble compounds. There are many formulation technologies utilized to increase solubility and/or dissolution rate to enhance oral bioavailability. One of the approaches utilized to increase dissolution rate is formulation of nano-crystalline suspensions (6, 7).

In recent years, the popularity of nano-technology has increased tremendously and many nano-based formulations are already on the market (8). Nano-crystalline suspensions can be described as colloidal dispersions of discrete drug crystals in stabilizer/s solutions of aqueous or non-aqueous media. The typical size range for pharmaceutical nanosuspensions is 100 - 1000 nm but most of the pharmaceutical nano-crystalline suspensions have sizes below 500 nm. There are two different approaches to formulate nano-crystalline suspensions: top-down (i.e. mainly grinding or milling from larger size crystals) and bottom-up (i.e. mainly precipitation or solvent evaporation from the drug solution).
reduction significantly increases the specific surface area (or surface area-to-volume ratio) and hence dissolution rate as described by the Noyes-Whitney equation (9). Accordingly, in case of “dissolution-rate limited” poorly water-soluble drugs, nano-crystalline suspensions can significantly enhance the drug dissolution and thus oral absorption.

Nano-crystalline suspensions can be formulated as solid-dosage forms (i.e. nano-crystalline powders) to improve the physical and chemical instabilities associated with liquid nano-crystalline suspension formulations such as, Ostwald ripening, aggregation etc. There are different methods utilized to formulate nano-crystalline powders such as, freeze or spray drying of nano-crystalline suspensions (10-16) and spraying of nano-crystalline suspensions on the carrier beads (such as sugar beads) followed by drying (17). In case of spray and freeze-drying, the material experiences thermal or freezing stress, respectively and the stress may affect product performance such as, dissolution performance etc. In addition, the drying process brings about concentration of the originally dispersed and dissolved materials and this may adversely affect both the physical and chemical stability of the formulation. For example, reduction in the solvent volume can lead to a decrease in the solubility of the surfactant or stabilizer, resulting in stabilizer precipitation and nano-crystal aggregation. One of the major problems associated with spray or freeze-drying of nano-crystalline suspensions is “nano-crystal aggregation” which leads to poor or inappropriate dissolution performance (10, 14, 15). It has been shown that the aggregation of nano-crystals during drying is dependent on the drug properties. For example, drugs with higher hydrophobicity such as, itraconazole and cinnarizine, resulted in higher agglomerates and were harder to disintegrate compared to drugs with lower hydrophobicity (14). Several auxiliary or bulking agents were tested and utilized during
spray and/or freeze-drying to prevent nano-crystal aggregation (15, 18). In one study, the authors have used un-conventional matrix formers such as, Avicel PH101, Fujicalin, Aerosol 200 and Intutec SP1 to prevent nano-crystal aggregation during spray drying (15). Typical matrix formers or bulking agents utilized in spray and/or freeze-drying of nano-crystalline suspensions are: sugars (such as, sucrose and trehalose); sugar alcohol (such as, mannitol); and polysaccharides (such as, maltodextrins). There are few reports available involving the use of these bulking agents against nano-crystal aggregation, but many cases exist where their ability to prevent nano-crystal aggregation is questioned (14, 15, 19-21). The aim of this study was to better understand the role of bulking agents or matrix formers to prevent nano-crystalline aggregation during the drying processes. In this study, several disaccharides, sugar alcohol and polysaccharides were investigated to prevent nano-crystal aggregation during spray or freeze-drying of nano-crystalline suspensions. Spray drying technology was investigated due to its economy and wide application in the pharmaceutical industry and academic settings, whereas freeze-drying was utilized for spray drying comparison purposes. Indomethacin (BCS class II) was selected as a model compound and Dowfax 2A1 (ionic surfactant, negatively charged) was utilized as the stabilizer for the nano-crystalline suspensions. The bulking agents were dissolved in the nano-crystalline suspensions and spray or freeze-drying was performed to evaluate their role in prevention of nano-crystal aggregation during the drying process.

4.1. Materials

Indomethacin USP, γ polymorph, was purchased from PCCA (Houston, TX). Dowfax 2A1 (alkyldiphenyloxide disulfonate) was generously gifted by Dow Chemical Company
(Midland, MI). HPLC grade acetonitrile (ACROS chemicals) was purchased from Fisher Scientific (Pittsburgh, PA). Hermetic pans and lids were purchased from TA instruments.

4.2. Methods

4.2.1. Preparation of indomethacin nanosuspensions

The indomethacin suspensions were prepared via the top-down approach using a Netzsch wet media mill. The required amount of indomethacin (1% w/v or 2%) was suspended in the stabilizer solution (Dowfax 2A1, 0.5% w/v or 0.1% w/v) and the suspension was stirred to achieve homogenous macro-suspensions. The macro suspensions were milled at a milling speed of 2000 rpm in the recirculation mode. The temperature of the suspensions was maintained below 25°C during the milling process to prevent any instability issues. The milling was performed for 90-120 minutes to achieve the required size of indomethacin nano-crystalline suspensions.

4.2.2. Spray drying of nano-crystalline suspensions

Indomethacin nano-crystalline suspension formulations were spray dried using a lab scale Buchi spray dryer B-190. Briefly, the spray dryer was pre-conditioned at the pre-set conditions of aspiration rate (-31 mbar); feed rate (9.3 mL/min) and inlet temperature (150°C) using 100 mL of distilled water. The optimized conditions were selected based on our previous study. Once the spray dryer was equilibrated, 100 mL of the prepared nano-crystalline suspension formulations with or without bulking agents were spray dried. Spray gas (atomizing air) was maintained at 40 mm Hg (air flow was approximately 600 L/hour) for all the formulations. The dried samples were removed from the collection chamber using a plastic scraper and evaluated for percent yield, particle size and polymorphic changes, if any.
4.2.3. Freeze drying of nano-crystalline suspensions

Indomethacin-Dowfax 2A1 nano-crystalline suspensions (2% w/v Indomethacin– 1% w/v Dowfax 2A1) were prepared as described above. Samples were prepared in 5 ml tubing glass vials (Wheaten Sciences Products) (2 mL fill volume) and freeze-dried in FTS system Lyostar™ II (SP scientific). Briefly, the indomethacin nano-crystalline suspensions (2% w/v, 1 mL) and different concentrations of bulking agent solutions (5% w/v, 10 w/v or 20% w/v, 1 mL) were added to these vials and vortexed to achieve homogenous mixing. The shelf temperature during primary drying was set at -40°C (freezing protocol was 5°C: 15 minutes; -5°C: 15 minutes and -40°C: 2 hours or 14 hours) and increased at 0.1°C to 40°C for secondary drying and held for 6 hours. All experiments were performed at a shelf temperature -40°C unless specified (-60°C in some cases). Chamber pressure throughout primary drying was set at 60 mTorr, and in all cases the product temperature was maintained below the collapse temperature. Vials were sealed in the chamber under vacuum and stored at -20°C until use.

4.3.4. Particle size analysis

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments) to determine the Z-average (at 90° scattering angle) and PDI of the nanosuspensions before and after the drying processes. Briefly, the dried samples were re-suspended in a saturated and filtered (0.2 µm membrane filter) solution of indomethacin in 30% glycerin solution to avoid any discrepancy from dissolution of nano-particles during measurements. The viscosity of this dispersant solution was measured using a Brookfield viscometer (Model DV-III) and this was used to calculate the particle size of the re-dispersed nano-suspension. Each sample was analyzed in triplicate and the results were
reported as the mean values of these runs.

4.3.5. Determination of percent yield

For calculation of percent yield, the drug amounts in liquid and dried nano-crystalline suspensions were determined using an HPLC-UV method (as described below). Briefly, the nano-crystalline suspension formulations were dissolved in the mobile phase and this solution was used to quantify the drug amount. The formula used for calculation of the % yield was:

$$\text{% Yield} = \frac{\text{Drug Mass Out} \times 100}{\text{Drug Mass In}} \%$$  \hspace{1cm} \text{Equation 4.1}

Note: Drug mass in and out represents, total amount of drug (in the nanosuspensions) that was spray dried and that was recovered (dried powder), respectively.

4.3.6. HPLC-UV method

The standard curve of indomethacin was generated using a Perkin-Elmer HPLC system (series 200) connected to a UV-Vis detector (Perkin-Elmer 785). The amount of indomethacin was quantified using a C-18 Zorbax® column (Waters Corporation, USA) and the mobile phase was a mixture of two phases (A/B) at a 50:50 v/v ratio. Phase A was a mixture of water and phosphoric acid (0.2% v/v) and phase B was acetonitrile. The flow rate was maintained at 1.3 ml/min and the UV absorbance was measured at 237 nm. Various dilutions were made in the mobile phase to prepare a standard curve. The concentration range for linearity was 0.005 mg/ml to 0.2 mg/ml with an $R^2$ value of 0.99.

4.3.7. Differential scanning calorimetry

DSC was performed using a TA Q1000 calorimeter (TA instruments, New Castle, DE,
USA) equipped with a refrigerated cooling accessory. The instrument was calibrated for enthalpy and heat capacity using indium and sapphire, respectively. Approximately 5-10 mg of the spray dried samples were sealed in hermetic pans and analyzed. The heating rate was maintained at 5°C/min from room temperature to 180°C. Nitrogen gas was used for purging at a flow rate of 50 ml/min. Data were analyzed using TA universal analysis software.

4.3.8. Powder X-ray diffraction (PXRD)

PXRD (powder X-ray diffraction) was used to study the crystallinity of the dried samples. X-ray diffraction patterns were obtained using an X-ray diffractometer (Model D5005, Bruker AXS Inc., Madison, WI) using Cu-\(\kappa\)\(\alpha\) radiation, a voltage of 40 kV, and a current of 40 mA. All the scans were performed at a scan rate of 2°/minute with steps of 0.02° from 5° to 50° at 2\(\theta\) angle.

4.3.9. Attenuated total reflectance- Fourier transform IR spectroscopy (ATR-FTIR)

IR spectroscopy was performed using a Nicolet FTIR (iS5 FTIR, Thermo Scientific) spectrometer attached with an attenuated total reflectance (ATR) accessory. All bulking agents were freeze-dried with or without Dowfax 2A1 solution (ratio of bulking agents-to-Dowfax 2A1: 10 to 1) as described in the freeze-drying section above. Freeze-dried powders were placed on the crystal window (Germanium) and compressed lightly using the pressure clamp. Spectra were recorded over a range of 400–4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\), for 128 parallel scans. Data analysis was performed on Omnic® 6.0a software (Thermo Nicolet Corporation).

4.3.10. Stability studies

All the spray-dried formulations (with and without auxiliary excipients) were stored at 4°C
and 25°C for 6 months in closed vials. The spray-dried samples were analyzed for particle size, powder XRD and DSC.

4.3.11. Contact angle measurement

All bulking agents were spray dried at a concentration of 10 % w/v and secondary vacuum drying was performed for 24 hours at room temperature. The spray-dried powders (approximately 150 mg) were compressed using a Carver press at a pressure of 5 tons for 2 minutes, after which the pellet was carefully removed from the die. The pellets were vacuum dried overnight at room temperature to remove any adsorbed moisture during processing. The dried pellets were utilized to measure contact angle with Dowfax 2A1 solution (approximately 40-50%) or distilled water. The contact angle was measured using a contact angle goniometer. A drop of 2.5 µl of Dowfax 2A1 or distilled water was selected for measurement. All measurements were performed in triplicate and the mean values were reported.

4.4. RESULTS

4.4.1. Effect of bulking agents on nano-crystal aggregation during spray drying

To evaluate the effect of different bulking agents (or matrix formers), spray drying was performed as described under the methods section. Different amounts of bulking agents were dissolved in the indomethacin nano-crystalline suspensions (1% w/v Indomethacin – 0.5% w/v Dowfax 2A1) before spray drying processing. The ratios of drug-to-bulking agent in the formulations were: 1 to 2.5, 1 to 5 or 1 to 10 w/w. As shown in Figure 4.1, all small molecular weight bulking agents (such as, disaccharides and sugar alcohol) were able to protect the nano-crystals from aggregating during spray drying with no statistical
difference over the concentration range investigated \((i.e. 2.5 – 10\% \text{ w/w})\). In the case of the high molecular weight bulking agents \((i.e. \text{ maltodextrins and Ficoll PM 70})\), large size nano-crystal agglomerates were observed (Figure 4.2). Nano-crystal aggregation of formulations containing Ficoll PM70 was comparatively low compared to those containing maltodextrins.

**4.4.2. Effect of bulking agents on spray-dried powder yields**

The recovery or percent yield of the spray-dried powder was calculated as described under the methods section. The yield of the spray-dried powders varied from 20\% \text{ w/w} to 65\% \text{ w/w} during spray drying processing. To understand the percent yield with different bulking agents, a plot of the glass transition temperatures (Tg) of the bulking agents (reported values) and spray drying percent yields was plotted as shown in Figure 4.3. A correlation was observed between the percent yield and the glass transition temperature. The formulations containing bulking agents with lower Tg (such as sucrose and maltose) had lower yields compared to those formulations containing bulking agents with higher Tg (such as lactose, trehalose, Ficoll PM70, Maltodextrin DE 4-7 and maltodextrin DE 16.5-19.5).

**4.4.3. Characterization of spray-dried nano-crystal powders**

**4.4.3.1. Differential scanning calorimetry**

All the bulking agents, except mannitol were amorphous after spray drying processing and no polymorphic changes were observed for indomethacin in any formulation (Figure 4.4).

**4.4.3.2. Powder-X-ray diffraction**

Powder X-ray diffraction was performed on the spray-dried nano-crystalline powders. Indomethacin remained crystalline before and after spray drying processing, whereas all
the bulking agents were amorphous, except mannitol as shown in Figure 4.5. In case of mannitol, the X-ray diffraction patterns correspond to the β form of crystalline mannitol.

4.4.4. Storage stability

Indomethacin nano-crystalline suspensions (approximately 180 nm) were prepared and spray drying was performed (ratio of drug-to-bulking agent 1 to 5 w/w) as described in the methods section. All spray dried formulations were stored at 4°C and 25°C for 6 months in closed vials. All the formulations containing small molecular weight bulking agents were stable and showed no further aggregation. In the case of maltodextrin containing formulations, further aggregation was observed at 25°C. No polymorphic changes were observed in any of the spray-dried powders (data not shown).

4.4.5. Freeze drying of indomethacin nano-crystalline powders

Two indomethacin nano-crystalline suspension formulations with different concentrations of Dowfax 2A1 were prepared and freeze-dried as described in the method section. As shown in Table 4.2 and Figure 4.6, the formulations containing small molecular weight sugars were non-aggregating compared to those containing polysaccharides. Indomethacin nano-crystal aggregation in the Ficoll PM70 containing formulation was low compared to those formulations containing maltodextrins. In addition, all the formulations were tested with different freezing steps (i.e. 2 hours and 14 hours) for the primary drying step. In case of 1% Indomethacin-0.1% Dowfax 2A1, the longer freezing step (i.e. 14 hours) resulted in larger aggregates compared to the shorter freezing step (i.e. 2 hours) as shown in Figure 4.7A. In the case of 1% w/v Indomethacin-0.5% w/v Dowfax 2A1, minimal aggregation was observed with or without all the bulking agents and no differences were observed with the different freezing steps (Figure 4.7B). In addition, all these formulations were tested
with an additional freeze-drying annealing step (- 10°C) or with a - 60°C freezing step but no difference in indomethacin nano-crystal aggregation was observed compared to freeze-drying performed at - 40°C (data not shown).

4.4.6. ATR-FTIR spectroscopy
Freeze drying of the stabilizer (Dowfax 2A1) and bulking agents was performed to understand any interactions between them. As shown in Figure 4.8, the C-H functional group IR region of the small molecule bulking agents with Dowfax 2A1 showed marked differences compared to those of the polysaccharides-Dowfax 2A1 freeze dried samples.

4.4.7. Contact angle measurement
To determine the interaction between the bulking agents and Dowfax 2A1, contact angle measurements were performed. As shown in Table 4.3, the contact angle between Dowfax 2A1 and polysaccharides was higher compared to small molecular weight bulking agents. In addition, the contact angle of the bulking agents with distilled water is given for comparison.

4.5. DISCUSSION
In this study, the role of bulking agents on the aggregation of nano-crystalline suspensions of the poorly soluble drug indomethacin during spray or freeze-drying processing was explored. All small molecular weight bulking agent-containing nano-crystalline formulations were non-aggregating during both spray and freeze-drying processing. The maltodextrin containing formulations showed aggregation during both spray and freeze-drying. However, Ficoll PM70 showed aggregation during spray drying (smaller aggregates compared to the maltodextrins) but no or minimal aggregation during freeze-
drying. FTIR spectroscopy was utilized to understand the interaction between Dowfax 2A1 and the bulking agents. As shown in Figure 4.8, small molecular weight bulking agents and Dowfax 2A1 mixtures (freeze-dried samples) showed changes in the C-H region, which suggests that these molecules have favorable interactions (such as hydrogen bonding, ionic interaction etc.), which might be responsible for nano-crystal size preservation. However, higher molecular weight bulking agents (such as maltodextrins or Ficoll PM70) with Dowfax 2A1 (freeze-dried sample) did not show any change in the C-H IR region, indicating no or minimal interaction and this may be responsible for the nano-crystal aggregation observed during spray or freeze-drying. In addition, as shown in Table 4.3, all small molecular weight bulking agents showed lower contact angle with Dowfax 2A1 compared to the polysaccharides. This suggests wetting of the small molecular weight bulking agents by Dowfax 2A1 solution is superior compared to that of the polysaccharides, which again may be due to favorable interactions (as observed via FTIR). Since, the indomethacin nano-crystals are surrounded by Dowfax 2A1 surfactant molecules, the interaction between Dowfax 2A1 and the bulking agent (or matrix former) appears to play an important role in nano-crystal preservation during the drying processes. Ficoll PM70 prevented nano-crystal aggregation only during freeze-drying (and there was no or minimal interaction with Dowfax 2A1 as indicated via IR spectroscopy and contact angle measurement). This suggests Ficoll PM70 has different mechanism of nano-crystal preservation during freeze-drying, which may be attributed to one or more of these properties: preferential exclusion, surface activity and/or increased solution viscosity limiting as reported by (20).

Over the Dowfax 2A1 concentration range investigated the extent of aggregation of the
freeze-dried formulations with no bulking agents and formulations containing maltodextrins showed dependency on the Dowfax 2A1 concentrations with less aggregation at higher Dowfax 2A1 concentration. Generation of new surfaces (ice crystal surface) during freeze-drying may cause surfactant molecules to re-distribute to these new interfaces. Such re-distribution may lead to reduced surfactant concentrations at the drug crystal surface in formulations containing low concentrations of Dowfax 2A1 thus resulting in higher aggregation.

In addition, it was observed that the spray-drying yield of the nano-crystalline powders was dependent on the glass transition temperature of the bulking agents (Figure 3). The reason for the lower yield for formulations containing low Tg bulking agent is considered to be due to sticking of the particles on to the spray dryer walls as a result of their rubbery state as previously reported.

4.6. CONCLUSIONS

Low molecular weight bulking agents are better in preventing nano-crystal aggregation during spray or freeze-drying processing due to favorable interactions between them and the stabilizer Dowfax 2A1, as observed via FTIR and contact angle measurement. All spray-dried nano-crystal formulations containing small molecular weight bulking agents were stable over a 6-month storage period. Higher spray-dried powder yields were observed for formulations containing bulking agents with higher glass transition temperatures due to less sticking inside the spray dryer glass walls. A combination of low molecular weight bulking agents and high molecular weight polysaccharides may be utilized to achieve higher yield and non-aggregating nano-crystals during spray drying.
Indomethacin nano-crystal formulations with low concentrations of Dowfax 2A1 resulted in higher aggregation during freeze-drying processing. Favorable interactions between bulking agent and nano-crystalline suspension stabilizers are required to achieve non-aggregated spray or freeze-dried nano-crystal powders.
4.7. Tables

Table 4.1. Particle size of spray-dried powders stored at different temperatures for 6 months.

<table>
<thead>
<tr>
<th>Indomethacin 1% w/v- Dowfax 2A1 0.5% w/v (Spray dried with bulking agents)</th>
<th>After spray drying</th>
<th>Spray dried powders stored for 6 months at 4°C</th>
<th>Spray dried powders stored for 6 months at 25°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Z average</td>
<td>PDI</td>
<td>Z average</td>
</tr>
<tr>
<td>No bulking agent</td>
<td>1473</td>
<td>0.424</td>
<td>1496</td>
</tr>
<tr>
<td>Sucrose</td>
<td>178.6</td>
<td>0.218</td>
<td>173.8</td>
</tr>
<tr>
<td>Trehalose</td>
<td>180.5</td>
<td>0.179</td>
<td>175.6</td>
</tr>
<tr>
<td>Lactose</td>
<td>184.7</td>
<td>0.211</td>
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<td>179.1</td>
<td>0.193</td>
<td>175.6</td>
</tr>
<tr>
<td>Mannitol</td>
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</tr>
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<td>0.697</td>
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<tr>
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<tr>
<td>Ficoll PM70</td>
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<td>0.244</td>
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Table 4.2. The effect of bulking agents on nano-crystal aggregation during freeze-drying.

<table>
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<tr>
<th>Bulking agent</th>
<th>Indomethacin 1% w/v - 0.5% Dowfax 2A1</th>
<th>Indomethacin 1% w/v - 0.1% Dowfax 2A1</th>
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<td></td>
<td>Before freeze drying</td>
<td>After freeze drying</td>
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<td>223.2</td>
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<td>227.7</td>
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<tr>
<td>Maltodextrin DE 4-7</td>
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<td>261.6</td>
</tr>
<tr>
<td>Maltodextrin DE 16.5-19.5</td>
<td>197.6</td>
<td>230.6</td>
</tr>
<tr>
<td>Ficoll PM70</td>
<td>197.6</td>
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Table 4.3. Contact angle between bulking agent (tablet) and Dowfax 2A1 (as solvent).

<table>
<thead>
<tr>
<th>Bulking agent</th>
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<th>Water (pure)</th>
</tr>
</thead>
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<tr>
<td>Sucrose</td>
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<td>Trehalose</td>
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<td>Mannitol</td>
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<td>98.15</td>
<td>31.5</td>
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<td>Maltodextrin DE16.5</td>
<td>74.35</td>
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<tr>
<td>Ficoll PM70</td>
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</table>
4.7. Figures

Figure 4.1. A plot showing the effect of bulking agents (small molecular weight) on indomethacin nano-crystal aggregation.

Figure 4.2. A plot showing the effect of bulking agents (polysaccharides) on indomethacin nano-crystal aggregation.
Figure 4.3. A plot showing the effect of bulking agents on spray-dried powder yields.
C. 2.5% Mannitol 
5% Mannitol 
10% Mannitol 

Indomethacin melting 
Mannitol melting 

D. 2.5% Sucrose 
5% Sucrose 
10% Sucrose 

Indomethacin melting 

E. 2.5% Trehalose 
5% Trehalose 
10% Trehalose 

Indomethacin melting
Figure 4.4. DSC thermograms showing the crystallinity of indomethacin and bulking agents (A: lactose; B: maltose; C: mannitol; D: sucrose; E: trehalose; F: maltodextrin DE16.5; G: maltodextrin DE4-7 and H: ficoll PM70 containing spray-dried powders).
Figure 4.5. PXRD of spray-dried powders (A: small molecular weight and B: polysaccharides containing formulations at drug-to-bulking agents: 1 to 5).
Figure 4.6. A plot showing the effect of bulking agents on nano-crystal aggregation during freeze-drying.

Figure 4.7A. A plot showing the effect of bulking agents on nano-crystal aggregation (1% w/v indomethacin-0.1% w/v Dowfax 2A1) with 2 or 14 hours of freezing step during freeze-drying.

Figure 4.7B. A plot showing the effect of bulking agents on nano-crystal aggregation (1% w/v indomethacin-0.5% w/v Dowfax 2A1) with 2 or 14 hours of freezing step during freeze-drying.
A. Sucrose and Dowfax 2A1 freeze dried
   Sucrose freeze dried

B. Lactose and Dowfax 2A1 freeze dried
   Lactose freeze dried

C. Maltose and Dowfax 2A1 freeze dried
   Maltose freeze dried

D. Trehalose and Dowfax 2A1 freeze dried
   Trehalose freeze dried
Figure 4.8. IR spectra of freeze-dried bulking agents with or without Dowfax 2A1 (A: sucrose; B: lactose; C: maltose; D: trehalose; E: mannitol; F: ficoll PM70; G: maltodextrin DE4-7 and H: maltodextrin DE16.5). Arrow indicates differences in the C-H IR region.
4.7. References:


Chapter 5

Wet Milling Induced Physical and Chemical Instabilities of Naproxen Nano-Crystalline Suspensions

Abstract

Wet milling is the most common approach to formulate nano-crystalline suspensions. The effect of high intensity wet milling on the physical and chemical stability of a poorly soluble drug was investigated. Naproxen (1% w/v) was suspended in two different stabilizers (*i.e.* HPMC E15 and Tween 80) and stabilizer concentrations (0.2% or 0.6% w/v) in distilled water. Wet milling was performed at two different speeds (*i.e.* 3400 rpm and 2000 rpm) for four continuous hours. The milled samples were analyzed for physical and chemical instabilities. Wet milling of naproxen-HPMC E15 at high milling intensity caused both physical and chemical instabilities as observed by particle size measurement and chemical analysis, respectively. The naproxen-Tween 80 formulations were stable regardless of milling intensity. Naproxen-HPMC E15 wet-milled samples, showed an IR peak shift suggesting strong bond formation or molecular interaction (*i.e.* amorphous phase). In addition, naproxen has a strong interaction with HPMC E15 as determined by MTDSC (*i.e.* melting point depression). The generation of amorphous phase at the naproxen-HPMC E15 crystal surface may be responsible for both aggregation and degradation during wet milling. Decarboxylated naproxen was identified as a degradation product. Milling intensity and/or selection of stabilizer/s are crucial for the stability of
nano-crystalline suspensions.

5.1. Introduction
Active pharmaceutical ingredients can exist in different solid-state forms: crystalline polymorphs, defected crystals and amorphous. These solid-state forms have different energetics that in turns lead to different physical and chemical properties [1, 2, 3, 4]. Crystalline polymorphs have different packing arrangements of the same molecules and amorphous solids have long-range three-dimensional packing disorders [4, 5]. Conversion from one solid-state to another (i.e. polymorph or amorphous transformation) requires passing through a thermodynamic energy barrier and involves a discontinuous change in free energy [5]. In addition, crystalline polymorphs have different energy states and the energy of the system increases or decreases depending on the solid-state transformation. Introduction of crystal defects and/or amorphous formation results in an increase in Gibbs’ free energy as well as an increase in free volume and thus results in thermodynamic instability.

The number of poorly soluble drug candidates coming out of drug discovery has increased tremendously over the past 20 years or so [6, 7, 8, 9, 10]. One of the approaches to increase solubility and thus oral bioavailability is to produce nano-crystalline suspensions. Milling (wet and dry) is the most common unit operation utilized to reduce particle size in pharmaceutical manufacturing. Milling induces defects and/or solid-state transformation, which in turn may affect material properties such as solubility and thus may alter bioavailability. There have been many literature reports on solid-state transformation...
during dry milling [11, 12, 13, 14] and also a few reports related to wet milling [15]. Wet media milling is widely utilized to form nano-crystalline suspensions to improve the dissolution rate of BCS (Biopharmaceutical classification system) class II/IV compounds. High intensity mills and relatively long milling processing times (sometimes up to 24 hours) are generally required [16] to produce nano-crystalline suspensions. During this process, mechanical energy is applied (i.e. collisions such as drug-drug, drug-chamber and drug-milling beads), which in turn generates strain on the crystal lattice and thus results in particle size reduction. The applied mechanical stress or strain can cause crystal defects and/or formation of amorphous phase. Amorphous regions or crystal defects are undesirable due to their high reactivity, which can lead to further chemical and/or physical instability such as nano-crystal aggregation, drug degradation, unstable formulations and polymorph conversion [17]. Solid-state transformation during the wet milling process may depend on the formulation (excipient/stabilizer, pH, buffering species etc.) and/or processing (processing time, temperature, milling intensity etc.) conditions. Appropriate stabilizer selection for wet milling is crucial for nano-crystalline suspension stability and performance. Not many studies are available which focuses on the effect of different stabilizer on the wet milling of poorly soluble drugs.

In this case study, physical and chemical instabilities of naproxen (a poorly soluble drug) are investigated following low and high wet milling intensity in the presence of HPMC-E15 (polymeric stabilizer) or Tween-80 (small molecular surfactant). Naproxen is categorized as a non-steroidal anti-inflammatory drug (NSAIDs). HPMC-E15 and Tween 80 are non-ionic stabilizers widely utilized for nano-crystalline suspension formulations. The influence of wet milling (i.e. Netzsch media mill) intensities in the presence of two
different excipients and concentrations were evaluated.

5.2. Materials

Crystalline naproxen was purchased from Fagron (Gallipot®). HPMC E15 was purchased from Dow Chemicals. Tween 80 and LC-MS grade solvents were purchased from Fisher Scientific (Pittsburgh, PA). Hermetic pans and lids were purchased from TA instruments.

5.3. Methods

5.3.1. Wet media milling

Briefly, naproxen (1% w/v) was suspended in the required amount of stabilizer solution in distilled water (either 0.2% w/v or 0.6% w/v) using a magnetic stirrer. The prepared macro-suspension was milled using a Netzsch® media mill at different processing conditions i.e. milling intensity. The chamber and milling media has a coating of zirconium oxide to provide high-energy efficient milling and low contamination from the metal parts during the attrition process. The macro-suspensions were milled either with HPMC E15 or Tween 80 at two different drug-to-stabilizer ratios i.e. 1:0.2 and 1:0.6. The concentration of the drug was kept constant at 10 mg/ml in macro-suspensions and milled for 4 hours either at low (i.e. 2000 rpm) or high (i.e. 3400 rpm) milling intensity. The milled samples were collected every 15 minutes and analyzed for change in particle size and chemical degradation.

5.3.2. Dry milling

5.3.2.1. Ball-milling
A Retsch MM330 ball mill was utilized. Briefly, 3 grams of sample (i.e. mixtures of naproxen-HPMC E15 at 1:0.6 ratio) was filled into the milling chamber. The sample was milled for 2 hours (30 milling minutes and 10 minutes wait time) at a frequency of 20/sec. The milled samples were analyzed for chemical degradation, powder X-ray diffraction and FTIR.

5.3.2.2. Cryo-milling

A Freezer mill (SPEX SamplePrep) 6750 was utilized. Approximately 3 grams of sample (i.e. naproxen-HPMC E15 at 1:0.6 ratio) was filled into the milling chamber. Liquid nitrogen was used as a cryogenic liquid. The milling chamber and the instrument were pre-chilled with the liquid nitrogen before milling processing. The sample was processed for 2 hours (2 minutes cooling and 2 minutes milling) at the highest frequency. The milled samples were analyzed for chemical degradation, powder X-ray diffraction and FTIR.

5.3.3. Solid-state characterization

5.3.3.1. Particle size analysis

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments). Briefly, the samples were suspended in a saturated and filtered (0.2 µm membrane filter) solution of naproxen in 30% glycerin. Each sample was analyzed in triplicate and the results were reported as the mean value.

5.3.3.2. Powder X-ray diffraction

PXRD (powder X-ray diffraction) was used to evaluate sample crystallinity. Diffraction patterns were obtained using an X-ray diffractometer (Bruker AXS Model D2 Phase diffractometer, Germany) using Cu-kα radiation (30 kV voltage and 10 mA current). All
scans were performed at 1°/min from 5° to 40° 2θ angles.

5.3.3.3. Attenuated total reflectance- Fourier transform IR spectroscopy (ATR-FTIR)

IR spectroscopy was performed using a Nicolet FTIR (iS5 FTIR, Thermo Scientific) spectrometer attached with an attenuated total reflectance (ATR) accessory. For solid samples (i.e. dry milled samples), powders were placed on the crystal window (Germanium) and compressed lightly using the pressure clamp. In case of liquid samples (i.e. milled suspension), the samples were centrifuged to separate the drug (as tiny pellets) from the distilled water. The supernatant was decanted off and drug pellets were vacuum dried and then analyzed as solid samples. Spectra were recorded over a range of 400–4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\), for 128 parallel scans. Data analysis was performed on Omnic® 6.0a software (Thermo Nicolet Corporation).

5.3.3.4. Modulated differential scanning calorimeter (MDSC)

A TA instrument Q1000 calorimeter (TA instruments, New Castle, DE, USA) connected to RCS90 cooling accessory was utilized for analysis. The instrument was calibrated for enthalpy and heat capacity using indium and sapphire, respectively. In case of wet milled samples, the suspension were centrifuged to separate the drug as pellet. The drug pellets were dried under vacuum and the pellets were utilized for DSC. About 5 - 10 mg of samples were hermetically sealed and analyzed. Nitrogen was used as a purging gas; at a flow rate of 50 ml/min. Samples were heated at 2°C/min at an amplitude of +/- 0.82°C with a period of 80 seconds. Data were analyzed using TA universal analysis software.

5.3.4. Chemical characterization
5.3.4.1. HPLC-UV analysis

To assess the chemical degradation following wet milling processing, an HPLC-UV method was developed. The concentration of naproxen was determined using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer) set at 270 nm. The mobile phase was acetonitrile/0.1% trifluoroacetic acid (50/50, v/v). A Waters® C18 symmetry column (4.6 mm×150 mm, 5 µm) was used with the flow rate set at 1 ml/min and the temperature set at 30°C. The chromatographs were analyzed using a PeakSimple™ Chromatography System.

5.3.4.2. LC-MS analysis

The detection was performed using a Shimadzu LCMS 2020 single quadrupole mass spectrometer system attached to a Shimadzu prominence Ultra Fast-LC. High purity nitrogen gas was used as the nebulizer gas at 10 liters/min. The mobile phase was a mixture of methanol and 0.1% formic acid containing water (90/10, v/v) at a flow rate of 0.2 µl/min. Electrospray ionization was operated in the positive ion mode. MS full ion.

5.4. Results

5.4.1. Naproxen wet milling with Tween 80 or HPMC E15

Wet milling was performed on naproxen-Tween 80 and naproxen-HPMC E15 macro-suspension formulations. Two different macro-suspension formulations with 1% w/v naproxen were prepared using either 0.2% w/v or 0.6% w/v excipient solutions. These formulation were milled at either low i.e. 2000 rpm or high i.e. 3400 rpm intensity for four hours continuously. During milling, suspension samples were collected from the milling
chamber and analyzed for particle size and chemical analysis. In the case of naproxen-Tween 80 milled suspensions, the formulations were both physically (particle size) and chemically (data not shown) stable at all excipient concentrations and milling intensities investigated. These naproxen-Tween 80 nano-suspension formulations were approximately 300 nm in size (Table 5.1).

In case of naproxen-HPMC E15 nano-suspension formulations, the particle size decreased to a minimum (approximately 300 nm) at or around 60 minutes. Prolonged milling (more than 60 minutes) caused an increase in particle size due to aggregation. Interestingly, the increase in particle size with naproxen-HPMC E15 nano-suspensions was only observed at the higher milling intensity i.e. 3400 rpm. Both drug formulations (i.e. 1% w/v drug-0.2% w/v HPMC E15 and 1% w/w drug-0.6% w/v HPMC E15) showed an increase in particle size with prolonged milling at 3400 rpm regardless of excipient concentration (Figure 5.1).

In addition, the naproxen-HPMC E15 wet milled samples (at 3400 rpm milling intensity) were chemically unstable as indicated by the presence of degradation peaks (Figure 5.2) when analyzed using HPLC. The percent degradation following four hours of continuous milling was approximately 6-8% w/w. These milled formulations were slightly yellow in color. The physical (aggregation) and chemical instabilities (drug degradation) associated with the milling process appear to be dependent on the HPMC E15 (i.e. stabilizer), as no instability issues were observed in case of Tween 80 containing formulations under the same milling conditions.

5.4.2. Dry milling of naproxen with HPMC E15

To further investigate naproxen degradation in the presence of HPMC E15 under aqueous
conditions (at high milling intensity), dry milling of naproxen and HPMC E15 were performed. The physical mixture of naproxen and HPMC E15 was prepared at the highest HPMC concentration (drug-to-HPMC ratio of 1:0.6) and high intensity ball or cryo-milling were performed. Following dry milling, the samples were analyzed for chemical stability. No drug degradation was observed with dry milling processing with or without HPMC E15 (data not shown). This suggests that the chemical instability associated with naproxen-HPMC E15 nano-suspensions during wet milling requires the presence of water.

**5.4.3. Characterization of wet milled formulations- Differential scanning calorimetry**

In the case of naproxen-Tween 80 wet milled formulations, a reduction in enthalpy was observed with increased milling time as shown in Figure 5.3A. The DSC thermograms were similar for naproxen-Tween 80 formulations milled at both intensities (*i.e.* 2000 or 3200 rpm) and excipient concentrations (the 3200 rpm milled sample data are shown in Figure 5.3A). In the case of naproxen-HPMC E15 formulations wet milled at 2000 rpm, the DSC results were similar to those of naproxen-Tween 80 formulations *i.e.* reduction in enthalpy with increased milling time (data not shown). Interestingly, in case of the naproxen-HPMC E15 formulation wet milled at the higher milling intensity (*i.e.* 3200 rpm), the drug melting peak shape was changed drastically with the milling time (Figure 5.3B) and low enthalpies were observed compared to Tween 80 formulations.

**5.4.4. Characterization of wet milled formulations- ATR-FTIR spectroscopy**

In order to obtain a control for comparisons with wet milled sample, ball milling of naproxen was performed. It was observed (Figure 5.4) that ball milling of naproxen alone caused small peak shifts (approximately 2 cm\(^{-1}\)) towards higher intensity in almost the
entire IR region (naproxen carboxyl stretching peak ~ 1726 cm$^{-1}$ after ball milling is also shown, magnified region in Figure 5.4). No further changes were observed with prolonged milling. The milled naproxen was utilized as a control for comparison with wet milled samples i.e. nano-crystalline suspension formulations.

FTIR analysis of the wet milled samples (i.e. naproxen-HPMC E15 and naproxen-Tween 80) was performed to understand drug degradation during the wet milling process. In the case of naproxen-HPMC E15 suspensions wet milled at higher wet milling intensity, a further peak shift towards higher IR intensity (i.e. blue shift, 1728 cm$^{-1}$ to 1730 cm$^{-1}$) was observed specially in the vibrational regions of the naproxen hydrogen bonded carboxylic acid moiety (1726 cm$^{-1}$ region) (Figure 5.5). In the case of naproxen-HPMC E15 suspensions wet milled at 2000 rpm, no peak shift was observed (Figure 5.6). FTIR spectra of naproxen-HPMC E15 wet milled at 2000 and 3200 rpm are shown in Figure 5.7 for comparison. No peak shift was observed for the naproxen-Tween 80 samples wet milled at higher and lower milling intensities (the sample milled at 3200 rpm is shown in Figure 5.8). To understand peak shifting in the case of naproxen-HPMC E15 wet milled at 3200 rpm, naproxen was ball-milled or cryo-milled (i.e. dry milling) with HPMC E15 at 1:0.6 w/w drug-to-HPMC ratio (highest excipient concentration) and the samples were analyzed by PXRD and FTIR. ATR-FTIR data of the dry milled samples with naproxen-HPMC E15 (as physical mixture) were similar (no change in peak position) to those of naproxen alone (Figure 5.9). Further, powder X-ray diffraction of all dry milled samples showed x-ray diffraction peaks of crystalline naproxen (Figure 5.10). No chemical degradation was observed in any samples (data not shown).
5.4.5. Interaction between naproxen and HPMC-E15: MTDSC

Modulated temperature DSC was performed to analyze the molecular interaction between naproxen and HPMC E15. As shown in Figure 5.11, increasing the concentration of HPMC E15 in naproxen (as physical mixtures) caused a depression in the naproxen melting point. This suggests strong interaction between naproxen and HPMC E15 at the molecular level.

5.4.6. Identification of naproxen degradation: LC-MS

To identify the degradation product during wet milling of naproxen-HPMC E15 suspension, LCMS analysis was performed. Wet milled samples (milled at higher intensity) were analyzed for chemical degradation. The degradation product peak appeared at 208 m/z ratio and the peak intensity of the degradation product increased with increasing milling time (Figure 5.12). The 208 m/z ratio may correspond to the sodium adduct of the decarboxylated product of naproxen as shown in Figure 5.13. Decarboxylation is the most common degradation pathway observed in the case of naproxen [18].

5.5. DISCUSSION

In this study, the effect of high intensity wet milling of naproxen was investigated with two different stabilizers i.e. Tween 80 or HPMC E15. The naproxen-HPMC E15 nanocrystalline suspension formulations milled at higher milling intensity (i.e. 3400 rpm) were not stable and particle size increased during the milling process compared to the Tween 80 containing formulations (Tables 5.1 and 5.2). Milling intensity was crucial for the stability
of the nano-crystalline suspensions, as instability was observed only at high milling speed in HPMC E15 containing formulations (i.e. 1% w/v drug-0.2% w/v HPMC E15 and 1% w/w drug-0.6% w/v HPMC E15). In addition, HPLC-UV analysis of naproxen-HPMC E15 wet milled samples (at 3400 rpm) showed the presence of degradation peaks (Figure 5.2), which were identified as decarboxylated naproxen (via LCMS). The naproxen-Tween 80 nano-crystalline formulations were both physically and chemically stable at both milling intensities investigated. This suggests that physical (aggregation) and chemical instabilities (drug degradation) associated with the milling process appear to be dependent on the presence of HPMC E15 as well as the milling intensity. Ball and cryo-milling (dry milling) of naproxen with or without HPMC E15 was performed and no drug degradation was observed. This suggests that naproxen degradation requires the presence of an aqueous environment.

In case of naproxen-HPMC E15 suspensions milled at 3200 rpm, the DSC thermograms of the wet milled samples suggested amorphous formation or surface interaction between naproxen and HPMC E15. In addition, naproxen-HPMC E15 wet milled samples prepared at higher intensity, showed blue shifts in the vibrational regions of the naproxen hydrogen bonded carboxylic acid moiety (1728 cm\(^{-1}\) region) as shown in Figures 5.5 and 5.7. These shifts occur as a result of increase in the stretching force constant and indicate the formation of strong bonds. This can be explained by considering the effect of intermolecular interaction between the amorphous naproxen and the HPMC E15 under aqueous conditions. Crystalline naproxen exists as a catemer (unlike its other family members such as ibuprofen that exist as a dimer), where one naproxen molecule interacts with two other neighboring molecules via intermolecular hydrogen bonding [19]. High
milling intensity and/or specific interaction with excipients (such as, HPMC-E15) may induce surface amorphization, which may lead to disruption of the naproxen crystal lattice. The amorphous phase (or co-amorphous naproxen-HPMC E15) has higher reactivity that can lead to chemical degradation. In addition, the DSC thermograms showed the melting point depression of naproxen to lower temperatures with increasing concentrations of HPMC E15 (in physical mixtures) (Figure 5.11). These results suggest strong molecular interaction between naproxen and HPMC E15. This strong interaction and the higher milling intensity may cause naproxen amorphization and thus physical and/or chemical instabilities. Sharma et al. have observed similar effects in the case of indomethacin-PVP nano-crystalline suspensions [15]. Moreover, intermolecular interaction between naproxen and HPMC E15 would stabilize the amorphous phase present at the nano-particle surface. The increase in naproxen-HPMC E15 nano-suspension particle size and the peak shift following wet milling can be explained when surface stabilizer is involved in solid-state transformation (*i.e.* molecular interaction). Accordingly, the stabilizer is not able to protect against aggregation. Kayaert et al. [20] have observed similar solid-state transformation effects with a different grade of HPMC (HPMC 5 mPa s) and naproxen during spray drying but not during wet milling. Although these researchers did not observe solid-state transformation under wet milling, this could be a result of the lower viscosity HPMC (5 mPa s compared to 12-18 mPa s in the current study) and the lower intensity mill used in the Kayaert study.

In the case of naproxen-Tween 80 nano-suspensions (Figure 5.8) milled at both intensities (*i.e.* 2000 or 3200 rpm), no aggregation, chemical degradation or blue shift in FTIR region were observed suggesting naproxen remained crystalline during the wet milling process.
and was therefore stable.

5.6. CONCLUSIONS

In conclusion, selection of excipient and milling intensity are very crucial for the stability of nano-crystalline suspensions during high intensity wet media milling. Any strong interaction between the drug and excipient such as hydrogen bonding and ionic interaction can lead to instability during the wet milling process via drug solubilization (or amorphization) in the excipient mixture. Modulated temperature DSC and FTIR can be routinely utilized to investigate potential molecular interactions between the drug and the excipients to carefully select appropriate stabilizers for nano-crystalline suspensions.
5.7. Tables

Table 5.1. Particle size analysis during media milling of naproxen with Tween 80 determined using a Malvern Zetasizer ZS90.

<table>
<thead>
<tr>
<th>Drug concentration (% w/v)</th>
<th>Stabilizer concentration (% w/v)</th>
<th>Milling speed (rpm)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2000</td>
<td>314</td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>2000</td>
<td>322</td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
<td>3200</td>
<td>273</td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>3200</td>
<td>378</td>
</tr>
</tbody>
</table>

Table 5.2. Particle size analysis during media milling of naproxen with HPMC E15 stabilizer determined using a Malvern Zetasizer ZS90.

<table>
<thead>
<tr>
<th>Drug concentration (% w/v)</th>
<th>Stabilizer concentration (% w/v)</th>
<th>Milling speed (rpm)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2000</td>
<td>231.1</td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>2000</td>
<td>267.6</td>
</tr>
<tr>
<td>1</td>
<td>0.2</td>
<td>3200</td>
<td>207.6</td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>3200</td>
<td>210</td>
</tr>
</tbody>
</table>
5.8. Figures

**Figure 5.1.** The milling profile of naproxen-HPMC E15 nanosuspensions at high milling intensity (i.e. 3400 rpm) determined using a Malvern Zetasizer ZS90.

**Figure 5.2.** Appearance of degradation peaks during wet milling of Naproxen-HPMCE15 nanosuspensions analyzed *via* HPLC-UV.
Figure 5.3A. DSC thermograms of naproxen-Tween 80 formulations.

Figure 5.3B. DSC thermograms of naproxen-HPMC E15 formulations (milled at 3200 rpm).
Figure 5.4. A FTIR plot showing the effect of ball milling on naproxen (naproxen carboxyl stretching peak $\sim 1726 \text{ cm}^{-1}$ after ball milling is shown in the magnified region).

Figure 5.5. ATR-FTIR analysis of naproxen-HPMC E15 wet milled samples milled at 3200 rpm.
Figure 5.6. ATR-FTIR analysis of naproxen-HPMC E15 wet milled samples milled at 2000 rpm.

Figure 5.7. Comparison of ATR-FTIR analysis of naproxen-HPMC E15 wet milled at both 2000 and 3200 rpm for 4 hours.
Figure 5.8. ATR-FTIR analysis of naproxen-Tween 80 wet milled samples milled at 3200 rpm.

Figure 5.9. Dry milling of naproxen with or without HPMC E15.
Figure 5.10. Powder XRD showing x-ray diffraction pattern of dry milled samples.

Figure 5.11. MTDSC showing naproxen crystal melting point depression with an increase in
concentration of HPMC E15.

Figure 5.12. LC-MS data showing the presence of a degradation peak at 208 m/z ratio during wet milling (A. un-milled and B. wet milled samples of Naproxen-HPMC E15 nanosuspensions).

Figure 5.13. Proposed degradation product of naproxen observed during wet milling with HPMC E15.
5.9. REFERENCES


Chapter 6

Optimization and Dissolution Performance of Different Sized Spray-Dried Naproxen Nano-Crystals

Abstract

The purpose of this study was to investigate the in vitro dissolution performance of the different sized spray-dried nano-crystalline powders of naproxen. A DoE approach was used to formulate and optimize nano-crystalline suspension. The critical wet milling operation parameters were i.e. drug concentration, drug-to-stabilizer ratio, stabilizer type (HPMC E15 or Tween 80) and milling intensity. The nano-crystalline suspensions were optimized for size and physical stability and then spray-dried to obtain nano-crystalline powders. Trehalose and lactose were investigated as spray-drying auxiliary excipients to achieve non-aggregating powders. Particle size, DSC and PXRD were utilized for characterization of powder formulations. A modified USP apparatus II was utilized to determine the in vitro dissolution of powder formulations. The size of the nano-crystalline suspensions was dependent on drug concentration and milling intensity. HPMC E15 containing formulations were better in terms of the spray-dried powder yield compared to Tween 80 containing formulations. Trehalose was selected to formulate non-aggregating nano-crystalline powders. No polymorphic changes were observed following the wet milling and spray-drying processes. Size dependent in vitro dissolution profiles, utilizing a
dialysis sac method were obtained for the crystalline powders.

6.1. Introduction

In the recent decades, high throughput screening (HTS) methodologies and in silico approaches have been utilized for the identification of drug candidates with the result that most drugs coming of drug discovery have poor aqueous solubility, high hydrophobicity and high molecular weight (1-4). It has been estimated that more than 40% of drug candidates are poorly soluble. In order for such drug candidates to progress in the drug development pipeline, it is essential to improve their aqueous solubility and therefore their oral bioavailability.

Nano-crystalline suspensions offer a great advantage in terms of increasing the dissolution rate and/or solubility of poorly soluble drugs (5-8). Pharmaceutical nano-crystalline suspensions can be defined as sub-micron colloidal dispersions of discrete drug crystals, ranging from 100 to 1000 nm. These nano-sized systems are thermodynamically unstable due to higher Gibbs’ free energy (higher surface-to-volume ratio) (9). However, they can be kinetically stabilized on pharmaceutically relevant timescales, using stabilizers such as small molecule surfactants and/or polymers. According to the Noyes-Whitney equation, dissolution rate is directly proportional to surface area. Accordingly, drug crystals with smaller size have a higher surface-to-volume ratio and therefore a faster dissolution rate (10). It should be noted that particle size has a great impact on not only the dissolution rate but also solubility. As described by the Ostwald-Freundlich (Kelvin) equation, smaller crystal size can lead to a higher surface pressure and thus increased drug solubility (11). However, the size impact on drug solubility was only observed for drug particles smaller
than 100 nm (5, 12).

Two different approaches are generally utilized to formulate nano-crystalline suspensions, i.e. bottom-up and top-down (13). For the bottom-up approach, drug crystals are milled (preferably wet milling) or passed through channels (such as high pressure homogenization) to achieve nano-crystalline suspensions, whereas for the top-down approach, drug solutions are precipitated using an anti-solvent or via solvent evaporation (such as spray drying) to achieve nano-sized drug crystals. Nano-crystalline suspensions are susceptible to both physical instability (i.e. crystal growth and agglomeration) and chemical instability (i.e. drug degradation) compared to solid dosage forms. Accordingly, several drying approaches such as spray- and freeze-drying have been employed to obtain solid nano-crystalline powders (14-16).

In this study, a DoE approach has been applied to understand the process of wet milling used to achieve nano-crystalline suspensions. In addition, spray drying was utilized to achieve a non-aggregating powder formulation. Naproxen, a poorly soluble drug, was chosen as the model compound. Two different stabilizer types (i.e. polymeric (HPMC E15) and small molecule surfactant (Tween 80) were utilized to formulate the naproxen nano-crystalline suspensions. Four critical operation parameters of the wet milling process were identified: drug concentration, milling intensity, drug-to-stabilizer ratio and type of stabilizer. Following optimization of the wet milling process, a spray dryer (Buchi B-290, bench-top) was utilized to produce nano-sized crystalline powders. Two different auxiliary excipients were used to prevent physical and chemical instabilities (such as aggregation and/or degradation) during the spray-drying process. Three different size crystalline
powders were obtained and *in vitro* dissolution testing was performed using our previously
developed dialysis sac method. This study demonstrated that a systematic approach can be
used to achieve non-aggregating nano-crystalline powders with high dissolution rate. This
approach may be applicable to other poorly soluble drugs.

6.2. Materials

Crystalline naproxen was purchased from Fagron (Fagron, St. Paul, MN). HPMC E15 was
purchased from Dow Chemical Company (Midland, MI). HPLC grade acetonitrile
(ACROS chemicals), trifluoroacetic acid and Tween 80 were purchased from Fisher
Scientific (Pittsburgh, PA). Hermetic pans and lids were purchased from TA instruments.
Dialysis sacs were purchased from Spectrum Labs (Spectrum Laboratories, Inc.).

6.3. Methods

6.3.1. Preparation of naproxen nano-crystalline suspensions

Briefly, naproxen was suspended in aqueous stabilizer solution (*i.e.* HPMC E15 or Tween
80 solutions), using a magnetic stirrer. The suspensions were stirred for 30 minutes to
completely wet the drug to obtain macro-suspensions. The prepared macro-suspensions
were milled using a Netzsch media mill (Netzsch, Exton, PA), as described under the DoE
section below. All suspension formulations were milled for four hours and analyzed for
particle size and polymorphic changes, if any. The temperature of the mill was maintained
below 28°C using two cooling bath re-circulators (one attached to the milling chamber and
the other attached to the suspension re-circulation chamber).
6.3.2. Spray drying of nano-crystalline suspensions

Naproxen nano-crystalline suspension formulations were spray dried using a lab scale Buchi spray dryer B-290 (Buchi Labortechhnik AG, Switzerland). Briefly, the spray dryer was pre-conditioned using 100 ml of distilled water. Once the spray dryer was equilibrated, 100 ml of the nano-crystalline suspensions were spray dried using a 0.5 mm spray nozzle. All nano-crystalline suspension formulations were spray dried at a feed rate of 7.5 ml/min and an inlet temperature of 150°C. Atomizing spray air pressure was kept constant at 40 mm Hg (approximate air flow 600 l/hour) for all the formulations studied. The dried samples were scraped off the collection chamber using a plastic scraper and further evaluated for moisture content, particle size as well as crystallinity.

6.3.3. Particle size analysis

Particle size measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments). The Z-average (at 90° scattering angle) and the PDI (polydispersity index) of the nano-crystalline suspensions were determined. Briefly, the samples were suspended in a saturated and filtered (0.2 µm membrane filter) solution of naproxen in 30% glycerin solution to avoid any discrepancy arising from drug dissolution during measurement. The viscosity of this dispersant solution was measured using a Brookfield viscometer (Model DV-III) and utilized in calculating the particle size of re-dispersed or liquid nano-crystalline suspensions. Each sample was analyzed in triplicate and the results were reported as the mean value of the runs.

6.3.4. Differential scanning calorimeter (DSC)
The DSC (TA Q1000 calorimeter) was calibrated using indium and sapphire for temperature and enthalpy, respectively. Approximately 5 - 10 mg of spray dried or liquid samples were sealed in hermetic pans and analyzed. The heating rate was kept constant at 5°C/min. Nitrogen at 50 ml/min was used as the purging gas. Data was analyzed using TA universal analysis software.

6.3.5. HPLC-UV analysis

Naproxen concentration was determined using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer) set at 270 nm. The mobile phase was a mixture of acetonitrile and 0.1% trifluoroacetic acid (50/50, v/v). A Waters® C18 symmetry column (4.6 mm×150 mm, 5 µm) was used with 1 ml/min flow rate and the column temperature was set at 30°C. The chromatograms were analyzed using PeakSimple Software.

6.3.6. Powder X-ray diffraction (PXRD)

PXRD was used to determine the crystallinity of the spray-dried samples. X-ray diffraction patterns were obtained using an X-ray diffractometer (Model D5005, Bruker AXS Inc., Madison, WI) with Cu-κα radiation, a voltage of 40 kV, and a current of 40 mA. All scans were performed at a rate of 2°/minute with steps of 0.02° from 5° to 40° 2θ ranges.

6.3.7. In vitro dissolution testing

In vitro dissolution testing was performed in USP apparatus II (AT7 smart, Sotax AG, Switzerland) using a previously reported dialysis sac method. The spray-dried crystalline powders were re-suspended in distilled water and 1 ml of re-suspended suspension was
placed inside the dialysis sac. Briefly, dialysis sacs containing crystalline suspension formulations were secured in the dissolution chamber (as shown in Figure 1) and *in vitro* dissolution testing was performed. All the dissolution tests were conducted at 37°C in 900 ml (sink condition) of phosphate buffer (50 mM, pH 6.8) with 50 rpm paddle speed. At pre-determined time intervals, 1 ml of sample was withdrawn from the dissolution chamber (outside the dialysis sacs) and replenished with 1 ml of fresh phosphate buffer. All samples were analyzed using HPLC.

**6.3.8. Design of Experiment (DoE)**

A preliminary study was conducted to identify the critical factors in the media milling process and to select the appropriate range of these factors for the DoE study. It was observed that milling intensity and drug concentration (drug load) were critical for the milling efficiency *i.e.* rate of particle size reduction. In addition, type of stabilizer and drug-to-stabilizer ratio were important for the stability of the nano-crystalline suspensions. Based on these preliminary results (data not shown), four critical operation parameters were selected: 1) milling speed (rpm); 2) drug concentration (% w/v); 3) type of stabilizer (polymeric versus small molecule); and 4) ratio of drug-to-stabilizer (w/w). The ranges of these four critical parameters (Table 6.1) were selected based on successful processing of the nano-crystalline suspensions. A full factorial design was utilized to understand the process and formulation parameters for particle size reduction. The critical quality attributes (CQAs) of the liquid crystalline suspension formulations were: particle size and drug crystallinity. As shown in the full factorial design space (Table 6.2), a total of 22 experiments were performed. All formulations were milled for four hours and every other
hour wet milled samples were collected and analyzed.

6.4. Results

6.4.1. Wet milling of naproxen nano-crystalline suspensions

In the case of Tween 80 containing suspension formulations, the drug-to-stabilizer ratio had a minimal effect on the particle size of naproxen nano-crystalline suspensions (Figure 6.1). In addition, an increase in drug concentration from 1% (w/v) to 5% (w/v) resulted in decreased size of the nano-crystals during wet milling. Interestingly, the higher milling speed resulted in larger nano-crystal size compared with the lower milling speed as shown in Figure 6.1. As shown in the contour plots (Figure 6.2), the smallest nano-crystals were obtained at the highest drug concentration (i.e. 5% w/v) and the lowest milling speed (i.e. 2000 rpm) at all drug-to-stabilizer ratios studied.

Similar results were obtained with HPMC E15 (polymeric stabilizer) containing naproxen nano-crystalline suspensions. The ratio of drug-to-stabilizer had no or minimal effect on the particle size of naproxen nano-crystals (Figure 6.3). Low milling speed (i.e. 2000 rpm) and high drug concentration (i.e. 5%, w/v) resulted in small naproxen nano-crystalline suspensions (Figures 6.3 and 6.4). As shown in the surface plots (Figure 6.5), the slowest milling speed (i.e. 2000 rpm) and the highest drug concentration (i.e. 5%, w/v) resulted in the smallest naproxen nano-crystalline suspensions for both small molecule surfactant (Tween 80) and large molecular weight polymeric (HPMC E15) stabilizers.

6.4.2. Optimization and characterization of naproxen nano-crystalline suspensions

Based on a DoE study, 5% w/v of naproxen (with 0.2% w/v stabilizer concentration) and
lower milling speed *i.e.* 2000 rpm were chosen to formulate all the naproxen nano-crystalline suspension formulations. Milling was performed at these optimized conditions and the samples withdrawn during the milling were analyzed for particle size and crystallinity. In case of the Tween 80 containing formulations, the particle size remained constant after approximately 90 minutes of continuous milling and no further particle size reduction was observed (Figure 6.6). In case of the HPMC E15 containing formulations, longer milling time was required to achieve small nano-crystals (approximately 240 minutes) as shown in Figure 6.6. The naproxen nano-crystals prepared with HPMC E15 were significantly smaller compared to those prepared with Tween 80. In addition, no polymorphic changes were observed during the milling process (Figure 6.7A and 6.7B). A slight lowering of the naproxen melting temperature was observed with the nanosuspension formulations.

**6.4.3. Spray drying to formulate non-aggregating nano-crystalline powders**

Spray drying was utilized to prepare dried powders of naproxen nano-crystalline suspensions. It was observed that spray-dried powders could not be obtained for Tween 80 containing naproxen nano-crystalline formulations. Most of the naproxen nano-crystalline powder was stuck on the drying chamber wall of the spray dryer, resulting in very low recoveries (less than 5% w/w). Accordingly, HPMC E15 was chosen for spray drying processing of the naproxen nano-crystalline suspensions. Based on other literature and our previous results, disaccharides (*i.e.* lactose and trehalose) were utilized to prevent nano-crystal aggregation during spray drying processing. Two formulations of naproxen-HPMC E15 crystalline suspensions were wet milled at drug-to-stabilizer (HPMC E15) ratios of 1:0.2 (w/w) and 1:0.5 (w/w), respectively. These wet milled formulations were spray dried
with either lactose or trehalose at the drug-to-disaccharide ratio of 1:3 w/w. As shown in Figure 6.8, disaccharides (bulking agent) can effectively prevent against nano-crystal aggregation. Trehalose was selected for naproxen nano-crystalline powder formulations (with 0.5% w/v HPMC E15 as stabilizer) since trehalose resulted in a higher yield of spray-dried powder compared to lactose.

6.4.4. In vitro dissolution testing

Three different sized (i.e. approximately 240 nm, 640 nm and un-milled) naproxen-HPMC E15 crystalline suspensions were formulated based on the above DoE and spray drying results. All these formulations were characterized for particle size (before and after spray drying), moisture content and crystallinity. All the formulations had less than 3% moisture content (data not shown) as determined by Karl-Fisher titrimetry. The particle sizes of all the liquid and spray-dried formulations are shown in Table 6.3. It was noted that there was a slight increase in the particle size and PDI following the spray drying process. No polymorphic changes were observed for any of the formulations (Figure 6.9). A previously developed dialysis sac method in combination with USP apparatus II, was utilized for in vitro dissolution testing of the naproxen crystalline suspensions. The in vitro dissolution testing was performed under both acidic (pH 1.2) and neutral conditions (pH 6.8) (Figure 6.10A and 6.10B). As shown in Figure 13, the dialysis sac method was able to discriminate among the different sized naproxen crystalline suspension formulations (i.e. milled and un-milled suspensions) at both pH values investigated.

6.4. Discussion

It was observed that the smallest nano-crystal formulations were obtained at the lowest
milling speed and the highest drug concentrations. Over the concentration range studied (1-5% w/v), high drug concentration is expected to increase the number (or frequency) of collisions (either among the drug particles or between the drug particles and the zirconia beads) and therefore result in smaller sized crystals. Since higher milling speed should result in a reduction in particle size, the large particle size observed at 3400 rpm must be a result of formulation destabilization and aggregation. The naproxen nano-crystals obtained at 2000 rpm using HPMC E15 as the stabilizer were smaller than those prepared using Tween 80 as a stabilizer and took a longer milling time to achieve constant particle size (Figure 6). This may be due to the higher viscosity of the HPMC E15 solution compared to Tween 80. It has been previously reported by Kumar et al. that there is a strong interaction between naproxen and HPMC E15. Accordingly, HPMC E15 will provide better surface coverage at the nano-crystal surface compared to Tween 80 resulting in smaller crystal size (17). The naproxen-Tween 80 formulations could not be successfully spray dried due to the powder sticking on the spray dryer walls. This is considered to be a result of the very high water solubility and very low melting temperature (Tm) of Tween 80 (approximately -20°C). This can be compared to HPMC E15 which has a glass transition temperature of approximately 150°C. All the formulations were spray dried at 150°C (inlet temperature) and since there is such a large differential between the Tm of Tween 80 and the spray drying inlet temperature, Tween 80 will be in a rubbery/melted state resulting in the observed stickiness of the formulations. Trehalose containing formulations had better yields compared to lactose containing formulations. This is considered to be due to the higher Tg of trehalose (114°C) compared to lactose (101°C). As discussed above the lower Tg lactose will be more rubbery compared to trehalose and therefore will cause more
sticking of the particles to the spray dryer walls. Our previously developed dialysis sac adapter in conjunction with USP apparatus II was able to distinguish three crystalline naproxen formulations based on their sizes. This study shows the importance of particle size on dissolution rate and therefore the importance of the nano-sized formulation to enhance dissolution.

6.5. Conclusions
A DoE approach was utilized to understand the process and optimization of wet milling of naproxen nano-crystalline suspensions followed by spray drying. The milling intensity and the drug concentration were the most critical factors to achieve stable nano-crystalline formulations. Small sized nano-crystals were obtained with slow milling speed and high drug concentration. The ratio of drug-to-stabilizer had no or minimal impact on size under the ranges investigated. Nano-crystal aggregation was observed at the highest milling speed i.e. 3200. The size of the HPMC E15 containing nano-crystalline suspension was smaller compared to Tween 80 formulations, which may be due to the better surface coverage provided by HPMC E15. In addition, it was not possible to spray dry the Tween 80 containing formulations due to the low melting temperature of Tween 80, which resulted in particle aggregation and sticking to the spray dryer walls. Three different sized (wet milling and un-milled) formulations were optimized and spray drying was performed to achieve non-aggregating crystalline formulations. A discriminatory dissolution method was developed to distinguish crystalline formulations based on their size. This method can be utilized for the testing of other dosage forms such as microspheres, liposomes, emulsions etc.
Table 6.1. Critical operation parameters for wet media milling.

<table>
<thead>
<tr>
<th>Critical parameters</th>
<th>Levels</th>
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<tr>
<td>Milling speed/intensity (rpm)</td>
<td>2000 (low), 2700 and 3400 (high)</td>
</tr>
<tr>
<td>Drug concentration (% w/v)</td>
<td>1 (low), 3 and 5 (high)</td>
</tr>
<tr>
<td>Type of stabilizer</td>
<td>HPMC-E15 (high molecular weight polymer) and</td>
</tr>
<tr>
<td></td>
<td>Tween-80 (small molecule surfactant)</td>
</tr>
<tr>
<td>Ratio of drug-to-stabilizer (w/w)</td>
<td>1:0.2 (low), 1:0.4 and 1:0.6 (high)</td>
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</table>
Table 6.2. Full factorial design space and particle size following wet milling processing.

<table>
<thead>
<tr>
<th>Run Order</th>
<th>Type of stabilizer</th>
<th>Conc. of drug</th>
<th>Ratio of drug to stabilizer</th>
<th>Milling intensity (rpm)</th>
<th>Z average (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 80</td>
<td>1</td>
<td>0.6</td>
<td>3400</td>
<td>327</td>
<td>0.25</td>
</tr>
<tr>
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<tr>
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<td>0.4</td>
<td>2700</td>
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<tr>
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<td>3400</td>
<td>283.5</td>
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</tr>
<tr>
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<tr>
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<tr>
<td>22</td>
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<td>0.2</td>
<td>3400</td>
<td>668.5</td>
<td>0.27</td>
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</table>
Table 6.3: Particle size measurement (intensity average) before and after spray drying of naproxen crystalline suspensions.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Particle size (nm) Before SD</th>
<th>Particle size (nm) After SD</th>
<th>PDI (nm) Before SD</th>
<th>PDI (nm) After SD</th>
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<tr>
<td>Nano-crystalline formulation (~ 240 minutes milled)</td>
<td>243.3</td>
<td>282.0</td>
<td>77.8</td>
<td>114</td>
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<td>Milled formulation (~2 minutes)</td>
<td>641.7</td>
<td>664.2</td>
<td>265</td>
<td>327.4</td>
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<tr>
<td>Un-milled formulation</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
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6.7. Figures

Figure 6.1. A plot showing the effect of the critical operation factors on the particle size of naproxen nano-crystals using Tween 80 as a stabilizer.
Figure 6.2. Contour plots showing the effect of milling speed and drug concentration on the particle size (nm) of naproxen nano-crystals with Tween 80 (top: drug-to-stabilizer ratio of 1:0.2 and bottom: drug-to-stabilizer ratio of 1:0.6).
Figure 6.3. A plot showing the effect of the critical factors on the particle size of naproxen nano-crystals using HPMC E15 as a stabilizer.
Figure 6.4. Contour plots showing the effects of milling speed and drug concentration on the particle size (nm) of naproxen nano-crystals with HPMC-E15 (top: drug-to-stabilizer ratio of 1:0.2 and bottom: drug-to-stabilizer ratio of 1:0.6).
Figure 6.5. 3D surface plots showing the effect of drug concentration and milling speed on particle size reduction of naproxen crystalline suspensions after 4 hours of continuous milling (top: Tween 80 as a stabilizer and bottom: HPMC E15 as a stabilizer).
Figure 6.6. A plot showing size reduction during wet media milling at optimized conditions (i.e. 2000 rpm and 5% w/v drug concentration) using different stabilizers (i.e. Tween 80 and HPMCE15).

Figure 6.7A. DSC thermogram showing the effect of milling time on the crystallinity of naproxen.
suspensions using Tween 80 as a stabilizer.

**Figure 6.7B.** DSC thermogram showing the effect of milling time on the crystallinity of naproxen suspensions using HPMC E15 as a stabilizer.

**Figure 6.8.** A plot showing the effect of auxiliary excipients (or bulking agent) on nano-crystal
aggregation following spray-drying processing.

**Figure 6.9:** Powder X-Ray diffraction of spray dried naproxen crystalline powders.

**Figure 6.10A.** *In vitro* dissolution profiles of spray dried naproxen crystalline formulations (re-
suspended liquid form) in 50 mM phosphate buffer (pH 6.8).

**Figure 6.10B.** *In vitro* dissolution profiles of spray dried naproxen crystalline suspensions (re-suspended liquid form) in 0.1N HCl (pH 1.2).
6.8. References:


7.1. Summary and conclusions

In recent years, the number of poorly soluble drug entities (Biopharmaceutics Classification System, BCS class II/IV) coming out of drug discovery has increased significantly. Nano-crystalline suspension is one of the commonly utilized approaches to increase the dissolution rate and hence oral bioavailability of poorly soluble drugs. One of the major challenges with formulation of nano-crystalline suspensions is the preservation of their physical and chemical stability in aqueous medium (i.e. distilled water). Nanosuspensions are susceptible to both physical instability (crystal growth and agglomeration) and chemical instability (degradation) compared to solids. Spray and freeze-drying of nano-crystalline suspensions are preferred but not many exhaustive studies are available to understand the process of drying. Hence, the goal of this research was to provide critical information on: 1) effect of critical drying formulation and process variables on the performance of nano-crystalline powders, and 2) understand the effect of wet milling in combination with spray drying on the stability of dried powder of nano-crystalline formulations.

The effect of spray drying processing variables of nano-crystalline suspensions was investigated. It was observed, that formulations dried at higher temperatures had less moisture content compared to formulations dried at lower temperatures. The
nanosuspension percent yield was mainly dependent on the flow and aspiration rates. The inlet temperature was identified as the only critical parameter (p value <0.05) affecting particle aggregation during nanosuspension spray drying due to crystal melting or amorphization at or above the melting temperature (indomethacin melting temperature 158°C). Particle aggregation increased with increasing temperature due to surface amorphization/crystal defects that lead to unstable formulations. Accordingly, It was concluded that spray drying of nano-crystalline suspensions should be performed below the drug melting temperature with high flow and aspiration rates for maximum recovery with minimal nano-crystal aggregation.

After optimization of spray drying processing variables, the effect of nano-crystalline formulation variables were investigated during spray drying. A DoE approach was utilized to understand the critical formulation variables. The aggregation tendency of spray-dried nano-crystal powders showed a dependency on the drug-to-stabilizer ratio. The nanocrystal yield during spray drying showed dependency on all factors studied i.e. drug, excipient, drug to stabilizer ratio and drug concentration. The Tg and charge of the formulation played a dominant role during the spray drying process. In addition, we have applied chi ($\chi$) interaction parameter to the stability of polymer-stabilized nano-sized systems (i.e. nanocrystalline formulations). The stability of the spray-dried powder correlated with the interaction parameter (calculated using the melting point depression approach) between the drugs (i.e. naproxen and indomethacin) and HPMC E15 (high molecular weight polymer). A dissolution method utilizing dialysis sacs with USP apparatus II was able to distinguish aggregated versus non-aggregated nano-sized formulations.
After spray drying process and formulation optimization, the effect of matrix formers or bulking agents was investigated on nano-crystal aggregation. All the formulations containing small molecular weight bulking agents were non-aggregating compared to those formulations containing polysaccharides during spray or freeze-drying processing of indomethacin nano-crystalline suspensions. In addition, higher crystalline powder yields were observed with formulations containing higher glass transition temperature bulking agents during spray drying. The bulking agents with low glass transition temperatures were sticking to the spray drier glass walls and thus resulted in lower yields. The small molecular weight bulking agents showed favourable or strong interactions with Dowfax 2A1 (via IR and contact angle) and this may be the reason for no or minimal nano-crystal aggregation during spray or freeze drying. A combination of bulking agent (i.e. small molecular weight and polysaccharides) may be utilized to achieve higher spray-drying yields and non-aggregating nano-crystalline powders.

In addition, the effect to high intensity wet milling on the physical and chemical stability of a poorly soluble drug was investigated. Wet milling of naproxen-HPMC E15 at high milling intensity caused both physical and chemical instabilities as observed by particle size measurement and chemical analysis, respectively. The naproxen-Tween 80 formulations were stable regardless of milling intensity. Naproxen-HPMC E15 wet-milled samples, showed an IR peak shift suggesting strong bond formation or molecular interaction (i.e. amorphous phase). In addition, naproxen has a strong interaction with HPMC E15 as determined by Modulated temperature DSC (i.e. melting point depression). The generation of amorphous phase at the naproxen-HPMC E15 crystal surface may be
responsible for both aggregation and degradation during wet milling. Decarboxylated naproxen was identified as a degradation product. Milling intensity and/or selection of stabilizer/s are crucial for the stability of nano-crystalline suspensions.

The QbD (or specifically DoE) studies performed in this research are examples of how this approach can be used to obtain design space for spray-dried nano-crystalline powders of poorly soluble drugs. In addition, the selection criteria utilizing DSC (*i.e.* melting point depression approach) can be utilized for stabilizer/s selection for nano-crystalline suspension formulation as well during spray drying processing. From an industrial perspective, this study provides an in-depth understanding of the parameters involved in nano-crystalline formulation and processing *via* wet milling and spray drying.

### 7.2. Future studies

Further investigation should be performed to fully understand the interaction between bulking agents and stabilizers. In particular, Raman, IR and AFM microscopy can be performed to evaluate the distribution of drug crystals within the stabilizer matrix. In addition, ss-NMR can be utilized to understand the interaction between them.

The developed spray-dried nano-crystalline formulations were superior in their dissolution performance compared to the un-milled formulation. In addition, *in vivo* studies should be performed in combination with *in vitro* dissolution, to evaluate and perform IVIVC for the spray-dried nano-crystalline powders.