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ABSTRACT

A PRELIMINARY INVESTIGATION OF THE STERILE-FILTERABILITY OF BCS CLASS II DRUG NANOSUSPENSIONS PREPARED VIA WET STIRRED MEDIA MILLING

by
Parul Ohri

Drug nanoparticles can achieve targeting capabilities, enhanced dissolution rates and improved bioavailability when injected intravenously. Sterile filtration of drug nanoparticle suspensions (nanosuspensions) is critically needed for administration by intravenous delivery route. Avoiding gamma irradiation and high temperatures, sterile filtration could be an effective process to sterilize drug nanosuspensions. On the other hand, two major challenges must be tackled: drug particles must at least be smaller than the filter pore size and minimum amount of non-toxic stabilizers must be used to prevent side effects like pain on the injection site. The aim of this study is to prepare naproxen (NPX) nanosuspensions via wet stirred media milling using various polymers and surfactants as stabilizers and to assess their physical stability with the ultimate goal of achieving the sterile filterability of such produced suspensions. An intensified milling process was investigated for fast production of sub-200 nm drug particles. In the second part, various formulations to stabilize NPX nanoparticles were screened. Lastly, the suspensions with NPX particle size less than 220 nm were filtered through a sterile 0.22 μm disposable capsule filter. Laser diffraction, scanning electron microscopy, rheometry, and surface tension measurement were used to evaluate the breakage kinetics and storage stability. Although NPX nanosuspensions were stabilized using stabilizers acceptable for injection, their sterile filtration was not successful, indicating a critical need for further research regarding the use of acceptable stabilizers and filter type/processing.

A PRELIMINARY INVESTIGATION OF THE STERILE-FILTERABILITY OF BCS CLASS II DRUG NANOSUSPENSIONS PREPARED VIA WET STIRRED MEDIA MILLING

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

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

January 2016

APPROVAL PAGE

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The thesis work was dedicated to my beloved family.

ACKNOWLEDGMENT

I would like to express my deepest appreciation to my thesis advisor, Dr. Ecevit Bilgili whose irrepressible quest for research enlivened me to the accomplishment of my Master's Thesis. Besides my advisor, I would like to thank the rest of my thesis committee, Dr. Costas G Gogos, Dr. Sagnik Basuray and Dr. Nicolas Ioannidis for their invaluable support and uninterrupted participation in my thesis.

I would like to extend my sincerest thanks to Meng Li, who mentored me from the scratch and backed me at every step of my research work. One simply could not wish for a friendlier supervision from her. I am extremely grateful for the technical advice of my fellow lab mates, Dr. Mohammad Azad, Anthony Quarato and Liang Chen, which helped me in reaching my goals.

Finally, a special recognition goes out to my mom and brother, for their support, encouragement and cooperation during my pursuit of Masters of Science. I thank both of you for your patience and love you more than you will ever know.

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CHAPTER 1

INTRODUCTION

1.1 Objectives

The goal of this exploratory and preliminary study is to assess the sterile filterability of Biopharmaceutical Classification System (BCS) Class II drug nanosuspensions, which are prepared via wet stirred media milling (WSMM). To achieve this goal, the thesis has the following specific objectives: (a) to explore the impact of process parameters on the final product nanosuspensions, (b) to study the impact of various stabilizers, i.e., polymers and surfactants, to impart physical stability against aggregation of drug nanoparticles present in the milled aqueous suspension, and (c) to filter the drug nanosuspensions which have D90 less than 220 nm through a sterile vacuum filter using four different membranes.

1.2 Background Information

1.2.1 Bioavailability Enhancement of Poorly Water-Soluble Drugs

The bioavailability enhancement of BCS Class II (poorly water soluble) drugs can be achieved by (a) increasing the surface area of drug crystals by particle size reduction (Noyes and Whitney, 1897); (b) use of pro-drug and drug derivatives such as strong electrolyte salt forms that usually have higher rate of dissolution (Liu et al. 2006); (c) microemulsions which have been employed along with incorporation of proteins to increase solubility of drugs (Ashwini et al. 2014); (d) micellar solubilization, which involves use of surfactants to lower surface tension and improve the dissolution performance of poorly soluble drug products (Carvalho et al. 2010); (e) complexation of

drugs, which has been used to enhance aqueous solubility and drug stability (Meyer et al. 1998); (f) decreasing crystallinity of drug substance through formation of solid solutions/amorphous solids (Kim et al. 2008, Shen et al. 2010, Zhang et al. 2006); and (g) formation of water-soluble complexes (Jansook et al. 2010).

1.2.2 Methods for Producing Drug Nanosuspensions

Among various methods for enhancing the bioavailability of BCS Class II drugs, size reduction of the drug crystals to the nanometer scale has been identified to be a promising approach (Kondo et al. 1993, Liversidge et al. 1996). According to the Nernst–Brunner equation, particle size reduction increases the specific surface area enhancing the dissolution rate, which in turn improves the bioavailability of poorly water-soluble BCS class II drugs (Noyes and Whitney, 1897, A. Dokoumetzidis et al. 2006). As smaller particles dissolve faster, it is expected that drug nanoparticles with very large surface area could significantly enhance the dissolution rate, thus allowing for sufficiently high bioavailability for some of the BCS Class II drugs.

There has been a growing interest in the production of drug particles in the size range 50-200 nm in a reproducible manner (Niwa et al. 2011, Juhnke et al. 2010). The manufacturing of a drug nanosuspension leads to the formation of nanoparticles with higher surface area and interface (Eerdenbrugh et al. 2008). Preparation of nanosuspensions involves adoption of two general approaches: bottom-up approach (forming nanoparticles from molecules) and top-down approach (size reduction). The best example of a bottom-up approach is anti-solvent precipitation/crystallization (Sinha et al. 2013). Anti-solvent precipitation involves the addition of drug solution to a solvent

precipitating the drug (the solvent known as non-solvent), which further controls the crystallization of drugs by presence of certain stabilizers in the non-solvent phase (Thorat et al. 2013). Due to the constraints in the bottom-up approach during scale-up, the top-down approach has been commonly used as a promising technique for producing nanosuspensions (Ghosh et al. 2011). In the top-down approach, various wet milling techniques such as media milling, high-pressure homogenization (HPH), etc. have been used. Among these techniques, size reduction of drug crystals to a nanometer scale via wet stirred media milling has been determined to be a promising approach to boost bioavailability (Kondo et al. 1993, Liversidge et al. 1996). Typically, 100–500 nm particles were prepared by several hours of milling with relatively high-energy consumption (Bose et al. 2012, Cerdeira et al. 2010, Knieke et al. 2013). The process of media milling consists of mechanical attrition of drug particles using milling media such as yttrium stabilized zirconium oxide beads of definite size range (Van Eerdenburgh et al. 2008). High-pressure homogenization has also been used because of its reduced product contamination (Keck and Muller, 2006). The mean particle size of nanosuspensions prepared by HPH is usually between 400 nm and 1000 nm (Lou et al. 2011, Wang et al. 2011, Xiong et al. 2008).

Wet stirred media milling (WSMM) has proved to be a robust top-down approach for producing nanosuspensions of poorly water-soluble drugs (Bhakay et al. 2011, Bruno et al.1996, Merisko-Liversidge et al. 2003) due to its universal applicability to all BCS Class II drugs, solvent-free/environmentally benign operation, capability to handle high drug loading, and scalability (Afolabi et al. 2014). Unlike other milling methods, WSMM can produce drug nanoparticles down to 50-200 nm particles or larger in a

pharmaceutical industry (Sinha et al. 2013). A cursory review of recent literature, which is not intended to be comprehensive, on finely milled BCS Class II drugs via WSMM is presented in Table 1.1. The data support the commonly held notion that fast preparation of drug suspensions with a D90 particle size below 220 nm particle size is extremely challenging.

Table 1.1 Recent Literature on Drug Nanosuspensions Prepared via Wet Stirred Media Milling (WSMM)

Drug	**Drug Loading** (% w/w)	**Batch Size** (ml)	**Bead Size** (μm)	**Milling Time** (min)	**Final Median Size**, d_{50} (nm)	**Final Particle Size**, d_{90} (nm)	**References**
Naproxen	5	12[b]	300	240	NR[a]	200	Bitterlich et al. 2015
Naproxen	10	400[b]	200	64	143	238	Monteiro et al. 2013
Naproxen	1	10[c]	NR[a]	60	NR[a]	207	Sumit et al. 2014
Naproxen	5	NR[a]	200	60	NR[a]	<500	George et al. 2013
Iodipamide	15	1000	800-1000	5-10[d]	98	<220	Zheng et al. 1997
Indomethacin	20	10	1000	21	NR[a]	345	Liu et al. 2011
Indomethacin	NR[a]	50	NR[a]	30	200	2370	Sharma et al. 2009
Fenofibrate	2.5	200	400	60	460	960	Knieke et al. 2013
Naproxen	10	200	400	90	144	230	Sievens et al. 2012
Griseofulvin	10	200	400	64	163	211	Bilgili et al. 2012

[a]Not Reported.
[b]Volume of water (ml) used in the suspension.
[c]Mass (mg) of the suspension.
[d]Days of ball milling.

Wet stirred media milling (WSMM) involves the use of micron-sized drug particles and media (beads) in an aqueous solution of dissolved stabilizers usually polymers and/or surfactants, which are mixed by a stirrer (rotor) at a very high speed. The micron-sized drug particles are captured between the colliding beads due to repeated stressing caused by milling continued for adequate time. Production of nanosuspensions creates new interfaces resulting in positive Gibbs free energy. As a result, these nanosuspensions are thermodynamically unstable and undergo aggregation of particles, which leads to decrease in interfacial tension (Wu et al. 2011, Van Eerdenbrugh et al. 2008). This problem can be dealt by wetting the hydrophobic surfaces of the drug particles on addition of stabilizers, which increases the activation energy of the aggregation process (Verma et al. 2009). Therefore, proper selection of stabilizers is mandatory. Stabilizers are added to prevent the aggregation of milled drug particles and to inhibit particle growth (ripening) during milling/storage. Dissolution and in vivo performance may be affected by aggregation of the milled drug particles, which can be prevented by addition of stabilizers (Ghosh et al. 2011, Kesisoglou et al. 2007). An optimal stabilizer type/concentration, which ensures proper short- and long-term physical stability of a drug nanoparticle suspension, is usually obtained by stabilizer screening studies at the bench-scale (Kesisoglou et al. 2007, Van Eerdenbrugh et al. 2008).

Nanosuspensions are stabilized via steric and/or electrostatic mechanisms imparted by the use of various stabilizers. Steric stabilization is attained by adsorbing polymers onto the drug particle surface; whereas electrostatic stabilization is achieved by adsorbing charged molecules, both ionic surfactants and charged polymers, onto the particle surface (Van Eerdenbrugh et al. 2009). Thus, the mechanism of stabilization is

contributed by both the physical properties of stabilizers and surface properties of the drug (Figure 1.1). The application of a proper stabilizer considers several factors:(a) polymer length and molecular weight of a polymer achieves a thermodynamic driving force for physical adsorption on the surface of the particle, (b) molecular weight of a polymeric stabilizer is inversely proportional to the rate of adsorption, (c) high concentration of long change polymers may lower the dissolution rate. Surfactants should be sparingly used in the pharmaceutical applications to lessen or diminish the unfavorable impacts:

- Aggregation of drug nanoparticles in suspension during milling or storage above critical concentration (Cerdeira et al. 2010).

- Micellar solubilization (Seedher et al. 2008), and size growth during Ostwald ripening (Knieke et al. 2013, Verma et al. 2011).

- Toxicity (Liversidge et al. 2005) caused if used in excess especially in inhalation products (Lebhardt et al. 2011, Suzuki et al. 2000).

- Causing gastric and pulmonary irritation (Oberle et al. 1995).

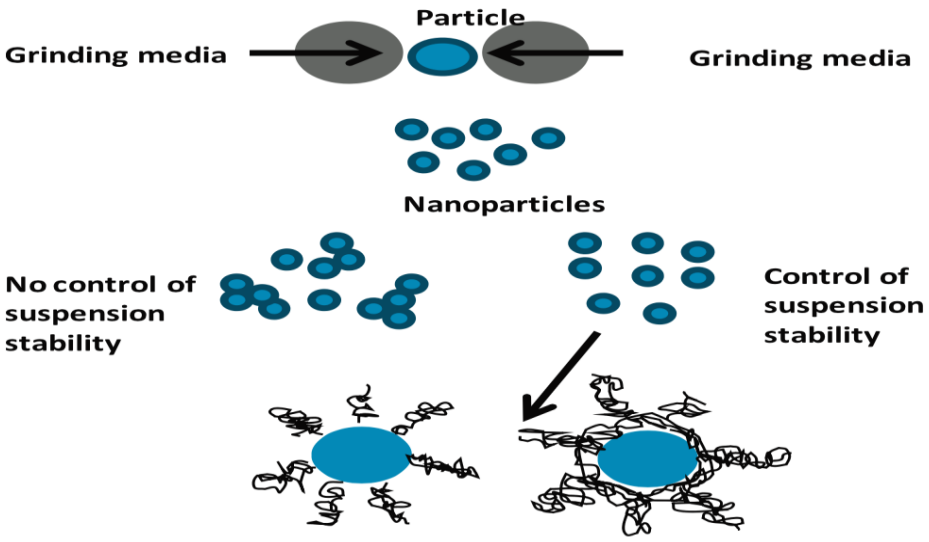

Figure 1.1 The mechanism of stabilization of the milled drug particles during the media milling process.
Source: George, M. & Ghosh, I. (2013), *European Journal of Pharmaceutical Sciences*

1.2.3 Applications of Nanosuspensions in Intravenous Administration Route

Nanosuspension is a carrier-free nanoparticle system containing only pure drug crystal and minimum surfactant and/or polymer dissolved in water for stabilization (Keck & Müller, 2006). Nanosuspension could greatly increase drug dissolution rate; this important feature renders it an excellent strategy to deal with BCS Class II and IV drugs (Müller et al. 2001, Rabinow, 2004, Kesisoglou et al., 2007). Routes of administration of drugs are generally classified as enteral and parenteral. Enteral route of administration deals with the GI tract and includes oral, buccal, and rectal route. Parenteral routes of drug delivery commonly refers to injectable such as intravenous (IV), intramuscular (IM), and subcutaneous (SC) but could also include topical and inhalation. Drug undergoes either first-pass metabolism or is not absorbed through the gastrointestinal tract in enteral route of administration. Consequently, the bioavailability after oral administration can be poor and very often below the therapeutic level. Intravenous

administration could provide greater bioavailability and is an alternative to oral administration (Xiong et al. 2008). All other categories of injections except IV must cross one membrane, involving an absorption process in the administration. As compared to other dosage forms, IV administration route offers many advantages:

- Quick onset of action in case of emergency.
- Reduction in dose of the drug and the ability to target the drug quickly to the desired site of action, especially in the case of severe infections.
- Control over dose and rate allows more predictable pharmacokinetic profiles.
- Control of plasma concentration.
- Bioavailability is generally 100% as the whole dose is delivered to the blood stream.
- Larger doses of poorly soluble drugs may be given in larger volume by IV infusion over an extended time.

There is a considerable limitation in use of intravenous route due to harmful solvent and excipients, which can cause serious side effects other than the drug itself (Wang et al. 2011, Rabinow et al. 2007). Moreover, a prerequisite for the IV injection of suspensions is a small particle size, i.e. preferentially in the nanometer range with little content of microparticles (Muller et al. 1998). The microparticles lead to toxic effects and ultimately to emboli when they exceed a critical level in the administered dose (Davis and Traube, 1978, Schroeder et al. 1978, Slake et al. 1981). Thus, size range of ≤100 nm is preferred for parenteral nanocrystals (Jinno et al. 2006). Under such circumstances, nanocrystals could be considered as the ideal candidates for intravenous delivery provided their formulation does not employ excess use of such harmful excipients.

1.2.4 Process Intensification

Process intensification of WSMM process targets for faster production of particles below 220 nm of BCS Class II drug particles. Drug particle sizes less than 220 nm may allow for successful sterile filtration. Despite previous experimental studies focusing on the impact of various process and formulation parameters on the milled particle size (Afolabi et al. 2014, Cerdeira et al. 2011, Ghosh et al. 2011, 2012, 2013, Monteiro et al. 2013, Singare et al. 2010, Singh et al. 2011), only Li et al. (2015) reduced milling time and energy consumption, while keeping media contamination low via intensification of process parameters such bead loading, rotor speed, and suspension flow rate upon use of optimal bead size.

1.2.5 Sterilization of Drug Nanosuspensions

Sterilization of drug nanosuspensions is critically intended for administration by intravenous injectables. This can be achieved by termination sterilization (e.g., autoclaving, sterile filtration, and gamma irradiation) of finished products or aseptic processing that is very costly. Autoclaving can lead to particle aggregation and thermal degradation of the drug due to use of high temperatures (Torchilin et al. 2006). Similarly, gamma irradiation can degrade the stabilizing polymers leading to particle aggregation and generate impurities along with the sterilization validation concerns (Torchilin et al. 2006). Therefore, autoclaving and gamma irradiation are non-preferred processes for sterilization of drug nanosuspensions. While drug solutions can be sterilized commonly using the techniques mentioned here, only few drug nanosuspensions have been sterilized using these techniques, mainly the aseptic processing (Alekha et al. 2014). A platform technology for sterilizing drug nanosuspensions besides the costly aseptic processing

does not exist. Hence, assessment of the sterile filtration of drug nanosuspensions is warranted, and is the goal of the present study.

Filtration is aimed at sterilizing a drug solution or nanosuspension by removing microorganisms, which is followed by aseptic packaging. Unfortunately, drug suspensions pose a particular challenge: particles larger than the pore openings cannot pass through the filter and are retained on the surface of the filter (Grace H. P., 1956). An appropriate sterilizing grade filter is one that reproducibly removes all microorganisms from the process stream, producing a sterile effluent (Zheng et al.1997). Generally, capsule configurations of vacuum sterilizing grade filters, which are sterilized by gamma irradiation, are used. The total time for product filtration should be limited to established maximum to prevent microorganisms from penetrating the filter and to prevent a significant increase in upstream bioburden and endotoxin load (S. Niazi et al. 1949). Factors that can affect filter performance commonly include (a) viscosity of the material to be filtered, (b) pH, (c) compatibility of the material or formulation components with filter itself, (d) pressures, (e) flow rates, (f) maximum use time, (g) temperature, (h) osmolality, (i) and the effect of hydraulic shock (Jornitz et al. 2006).

Most applications make use of filters made of cellulose esters, polyvinyl fluoride, polytetrafluoroethylene, nylon and other polymeric materials (Mckinnon et al. 1993). Membrane filters constitute of two types of membranes:

- Hydrophobic (‘water-disliking’) for use with gas filtration, in which compounds are repelled by water and are usually neutral, and

- Hydrophilic (‘water-liking’) for use with liquid filtration, in which compounds have affinity to water and are usually charged or have polar side groups to their structure that will attract water.

The membrane filters must be fully compatible with the chemical characteristics of the nanosuspensions due to filter membranes containing non-toxic wetting agents that may interfere with some applications. Moreover, other membranes may bind proteins or other macromolecules, which may lead to premature filter clogging or loss of valuable samples. Therefore, it is very important to understand their characteristics and the potential effects filter membranes can have on the solutions they contact. The varying membranes used in sterile filters are as follows:

- Cellulose Acetate (CA) membranes have low binding affinity, low chemical resistance, and are naturally hydrophobic. Furthermore, these membranes have small amounts (less than 1%) of non-toxic wetting agents to ensure proper wetting of the membrane (Corning Storage Bottles Selection and Use Guide).
- Polyethersulfone (PES) membranes have faster flow rates. These membranes are without wetting agents and have low chemical resistance (Corning Filtration Guide).
- Polyvinylidene fluoride (PVDF) membrane has high flow rates; ultra-low binding properties, and broad chemical and temperature resistance (Polyvinylidene Fluoride (PVDF) Membrane).

- Polyamide (PA) membrane is mechanically very strong and exhibits excellent wet strength and dry strength. Also, these are hydrophilic making them suitable for aqueous and organic solutions (Polyamide Membranes).

1.3 Organization of Thesis

Chapter 2 describes the experimental details of the study, including methods and materials for suspension and different filter membranes used, as well as methods for product characterization. Results and discussion of the impact of process parameters, different polymer and surfactant concentrations, different polymers and surfactants, and filtration studies are presented in Chapter 3. Chapter 4 presents a summative assessment of potential polymers and surfactants, stabilizing NPX nanosuspensions with an objective to achieve a particle size under 220 nm. Chapter 5 discusses potential future work.

CHAPTER 2

EXPERIMENTAL

The methods of preparing and characterizing naproxen nanosuspensions are given in this chapter. Naproxen nanosuspensions were prepared via wet stirred media milling and their particle size, viscosity, and surface tension were characterized. The milled drug nanosuspensions with a final D90 particle size below 220 nm were sterile filtered using capsule filter with four different membrane materials.

2.1 Preparation of Naproxen Nanosuspensions

2.1.1 Materials

WSMM experiments were carried out on the poorly water-soluble drug naproxen (NPX). Naproxen is a proprionic acid derivative related to the arylacetic acid group of nonsteroidal anti-inflammatory drugs (FDA). Figure 2.1 shows the chemical structure of NPX, which has a molecular weight of 230.26 Da and is practically insoluble in water. NPX is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Naproxen is rapidly and completely absorbed from the gastrointestinal tract with an *in vivo* bioavailability of 95%; hence, it serves as a model BCS Class II drug.

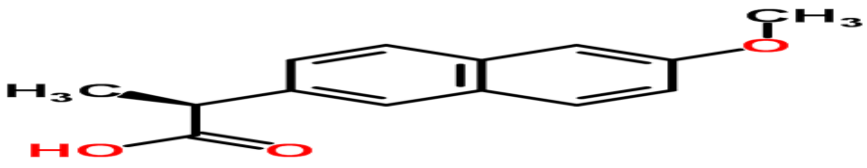

Figure 2.1 Chemical structure of naproxen.
Source: www.chemspider.com

The absorption of drugs with poor aqueous solubility like NPX is dissolution rate limited

and therefore, they exhibit poor bioavailability resulting in multiple dosing of drug as well as fluctuation in blood concentrations (Medina et al. 2015).

To stabilize the drug particles during milling and storage, different stabilizers were used in the suspensions. The physicochemical properties of different stabilizers are presented in Table 2.1

Table 2.1 Properties of Stabilizers Used in Wet Stirred Media Milling

Stabilizers	**Solubility at 25 ºC** (mg/mL)	**Molecular weight** (Da)	**Melting Point** (ºC)
PVP 12	0.17	2000-3000	120
PVP 17	0.17	7000-11000	126
P188	>10%	7680-9510	52
P407	>10%	9840-14600	56
Tween 20	100	1225	56-58
Tween 80	50-100	1310	-21
HPC SL	20	100000	180-220
SDS	150	288.38	206
Soluplus	0.03	118000	166
HPMC E3	50	86000	190-200

2.1.2 Preparation Methods

Table 2.2 presents the suspension formulations used in this study. Feed NPX suspensions were prepared using a shear mixer (Cat#. 14-503, Fisher Scientific, Pittsburgh, PA, USA) running at a fixed speed of 300 rpm. To find an efficient process condition for fast production of drug particles, in Runs 1-3, HPC (SL grade)-SDS combination which provides sufficient stabilization for various drug nanoparticles such as griseofulvin, was selected based on our previous work (Bilgili & Afolabi, 2012). Firstly, 2.5% HPC SL was added to deionized water in a beaker gradually for 15 min while the mixer ran at a fixed speed of 300 rpm for 15 more min. Then, 0.5% SDS was added to the HPC SL solution gradually for 5 min and led to mix for 10 more min. All percentages (%) used throughout the preparation refer to w/w with respect to deionized water. The final HPC SL–SDS solution was further mixed for 15 min to ensure proper dissolution of HPC SL and SDS particles. For formulations wherein a single stabilizer was used (Runs 4-20), the stabilizer was added in 15 min and allowed to mix for another 15 min to ensure that the stabilizer is fully dissolved. Desired amount of NPX powder (10%) was weighed and added to the stabilizer solution gradually for 30 min while mixing continued.

Table 2.2 Stabilizer Percentages for Each Run in WSMM

Run	**Stabilizer**	**Concentration** (% w/w)
4	PVP 17	0.5
5	PVP 17	2.5
6	PVP 12	2.5
7	PVP 17	5
8	PVP 17	10
9	HPC SL	2.5
10	HPMC E3	2.5
11	Soluplus	2.5
12	P188	0.5
13	P188	2.5
14	P188	5
15	P188	10
16	P407	2.5
17	Tween 20	2.5
18	Tween 20	5
19	Tween 80	2.5
20	Tween 80	5

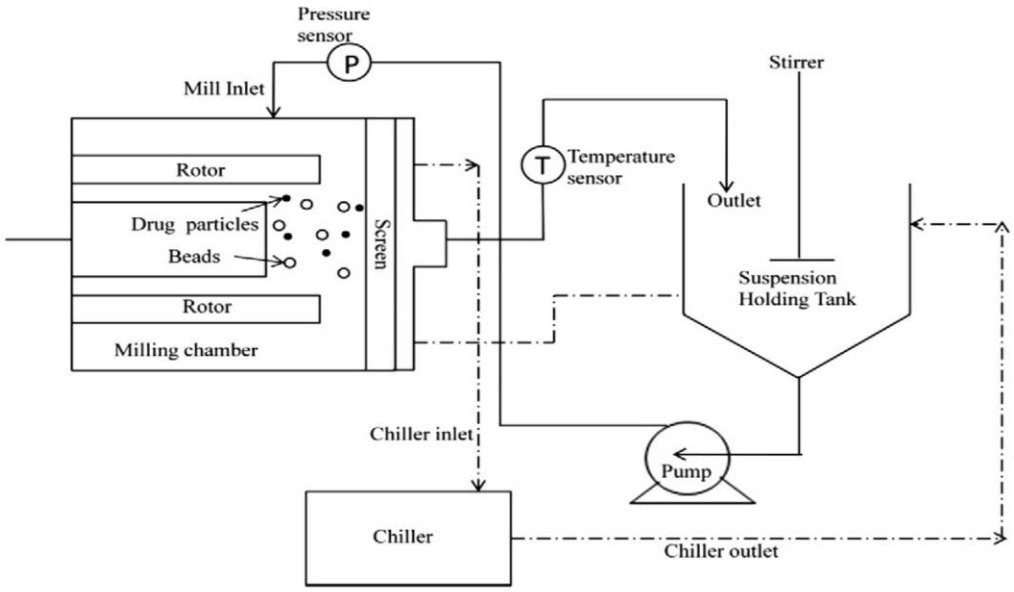

Figure 2.2 Schematic of the Netzsch stirred media mill (Model: Microcer) operating in the recirculation mode. P and T stand for Pressure Gauge and Thermocouple, respectively.
Source: Bhakay, A., Davé, R., & Bilgili, E. (2013), Powder Technology.

Drug suspensions were subsequently milled in a Netzsch wet media mill (Microcer, Fine Particle Technology LLC, and Exton, PA, USA). The wet stirred media milling process is depicted in Figure 2.2. The milling chamber is lined with zirconia and has a volume V_m of 80 ml. In this so-called recirculation mode, each feed suspension was poured into the holding tank and was recirculated between the holding tank and milling chamber at a constant volumetric flow by a peristaltic pump. The suspensions were milled for 64 min, which allowed sufficient time for preparation of NPX nanoparticles. Milling media (beads) are inside the milling chamber and set into motion by the rotation of the rotor. A turbulent motion was induced in the suspension by the high speed rotor, and turbulent energy dissipates during frequent bead–bead collisions (Eskin et al. 2005), causing extensive breakage of drug particles captured between the beads (Bhakay et al. 2011, Bilgili et al. 2006). Yttria-stabilized zirconia beads with a nominal size of 100 μm

were used as the milling media. A screen with 50 μm nominal opening size, located at the outlet of the milling chamber, retained the zirconia beads, while allowing the passage of the drug suspension. Both the milling chamber and the holding tank are equipped with a chiller unit (model number M1-25A-11HFX, Advantage Engineering, Green-wood, IN, USA) which kept the suspension temperature in the holding tank below 35°C, as a maximum. The stirrer was stopped occasionally followed by intermittent cooling when the temperature reached 35°C due to high heat generation rate. Samples were taken from the outlet of the milling chamber at several intervals of milling. The final suspensions (after 64 min milling) were tested for density and shear viscosity, and they were refrigerated at 8°C for a period of 7 days. Particle sizes right after milling and after 7 days of storage were compared to assess the physical stability of the suspensions.

Based on the process intensification approach proposed by Li et al. (2015), the process parameters were varied from the baseline (Run 1) first by reducing the bead size (Run 2) and then increasing the rotor tip speed, bead loading, and suspension flow rate simultaneously (Run 3), with the objective of achieving fast production of drug particles less than 200 nm in D90 (refer to Table 2.3 for the parameters). The process parameters for Run 3, the intensified process, were then adapted for the rest of this study, where the impact of various stabilizers was investigated under the most intense milling conditions.

Table 2.3 Effect of Process Parameters Investigated in the Wet Milling Experiments

Run	**Milling Speed** (rpm)	**Bead Loading**, (g)	**Bead Size** (μm)	**Pump Speed** (mL/min)
1	3200	196	400	126
2	3200	196	100	126
3	4000	261	100	343

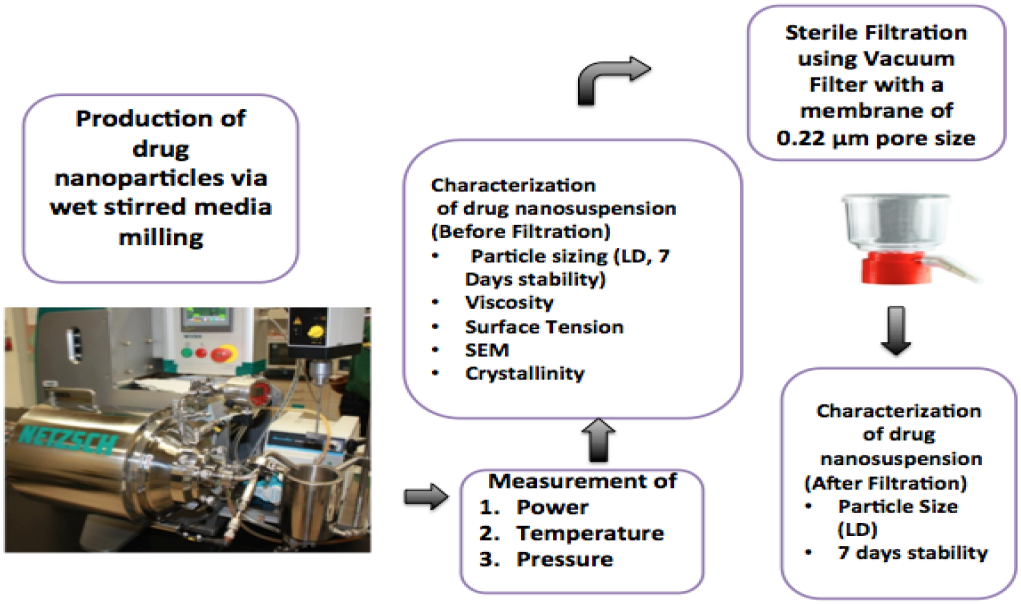

Figure 2.3 Experimental set-ups for the sterile filtration process.

2.2 Sterile Filtration of Nanosuspensions

Filtration experiments of stabilized NPX nanosuspensions were performed at room temperature using polyvinylidene difluoride (PVDF), polyethersulfone (PES), cellulose acetate (CA), and polyamide (PA) vacuum membrane filters (Corning Incorporated Life Sciences, MA, USA) as shown in Figure 2.4, having a pore diameter of 0.22 μm;

membrane area of 19.6 cm^2; and volume of 250 ml. A sterile filter is connected to the vacuum line and the nanosuspension was poured from the top on the filter membrane.

30 g NPX nanosuspension was passed through sterile filters with four different membranes. All the drug particles larger than the surface opening or pore size are retained at or near its surface.

Figure 2.4 Sterile vacuum filters used for sterile filtration of NPX nanosuspensions.
Source: Innovative Products for Filtration and Ultrafiltration" *Corning Filtration Guide.*

2.3 Characterizations

2.3.1 Particle Size Distribution

Particle size analysis of the milled suspensions was performed by laser diffraction using a Beckmann Coulter LS230. A polarized intensity differential scattering (PIDS) obscuration water optical model was employed. The PIDS was maintained between 40% and 50% while the obscuration was maintained 8% for all particle size measurements. A refractive index (RI) value of 1.61 for the NPX particles (Kean WF et al. 1989) and 1.33 for the measurement medium (DI) water were used. Prior to the size measurement, milled suspension samples (~2 ml) were diluted with 5 ml of stabilizer solution. The refrigerated

suspension samples after 7-day storage were stored in room temperature for 30 min and then mixed via a digital vortex mixer (Thermo Fisher Scientific Inc., USA) at 1500 rpm for 1 min. ~2 ml samples were taken and diluted for particle size measurement using the same stabilizer solution as in the suspensions. Suspensions after filtration process were also tested for particle sizes immediately after filtration and after 7-day storage following the procedures above.

2.3.2 Apparent Shear Viscosity

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

2.3.3 Surface Tension

The surface tension of the final HPC–SDS solution or HPC solution and milled suspensions was measured using Attension Sigma 700 (Biolin Scientific, Linthicum, MD, USA). The Attention calculates surface tension from force measurements of interaction of a probe (Wilhelmy plate) at the boundary between air and a liquid.

2.3.4 Scanning Electron Microscopy (SEM)

Particle size and morphology of the as-received and milled drug particles were examined via SEM with a LEO 1530 SVMP (Carl Zeiss, Inc., Peabody, MA, USA). About 0.1 ml of the milled suspension was diluted with 30 ml de- ionized water, and a drop was placed on a silicon chip (Ted Pella, Inc., Redding, CA, USA), dried, sputter coated, and observed in SEM.

2.3.5 X-Ray Power Diffraction (XRD)

The crystallinity of the as-received drug, unmilled physical mixture (overnight dried aqueous suspension with as-received drug and PVP 17), and overnight dried, milled suspensions was analyzed using X-ray Powder Diffraction (XRD, PANalytical, West-borough, MA, USA), provided with Cu K_α radiation (λ = 1.5406 Å). The samples were scanned for 2θ ranging from 5° to 40° at a scan rate of 0.165 s^{-1}.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Impact of Process Intensification

3.1.1 Impact of Bead Size

NPX particles were wet-milled using beads with two different nominal sizes: 400 and 100 μm in Runs 1-2. Understanding the impact of bead size is crucial for subsequent process intensification of the most milling process (Li et al. 2015). 90% passing size (d_{90}) obtained in Run 2 with 100 μm beads was 195 nm, which is slightly smaller than the one obtained in Run 1 with a particle size of 229 nm with 400 μm beads (Table 3.1). Smaller beads have higher frequency of bead-bead collisions and drug particle compressions despite potentially decreased maximum contact pressure (Li et al. 2015). Besides, wear of 100 μm beads is expected to be lower than 400 μm beads, causing lower contamination in the milled drug suspensions (Li et al. 2015). Overall, it is suggested that the use of 100 μm beads can be advantageous for the fast production of finer NPX particles and should be used for process intensification.

3.1.2 Impact of Increase in Bead Loading, Rotor Speed, and Flow Rate

Bead loading of 196 g was used in Runs 1-2, which produced a d_{90} of 195 nm in Run 2 at the lowest. The process was intensified in Run 3 with an increase in bead loading, rotor speed, and flow rate. The intensified milling condition was based on previous work Li et al. (2015). With the intensified process, drug particle size was reduced to 185 nm after milling, as shown in Table 3.1. Final particle sizes were approximately attained after 16 min. An intensified milling process increased the apparent breakage rate and led to

smaller final particle size. There was a decrease in clearance between the beads upon increasing bead loading. The increase in rotor speed further led to a dramatic increase in the bead–bead collisions and drug particle compressions. When a higher suspension flow rate was used, the breakage rate was further increased due to tighter residence time distribution of the suspension in the mill chamber (Monteiro et al. 2012). The milling conditions in Run 3 led to the fastest NPX breakage and were therefore used in the following formulation studies.

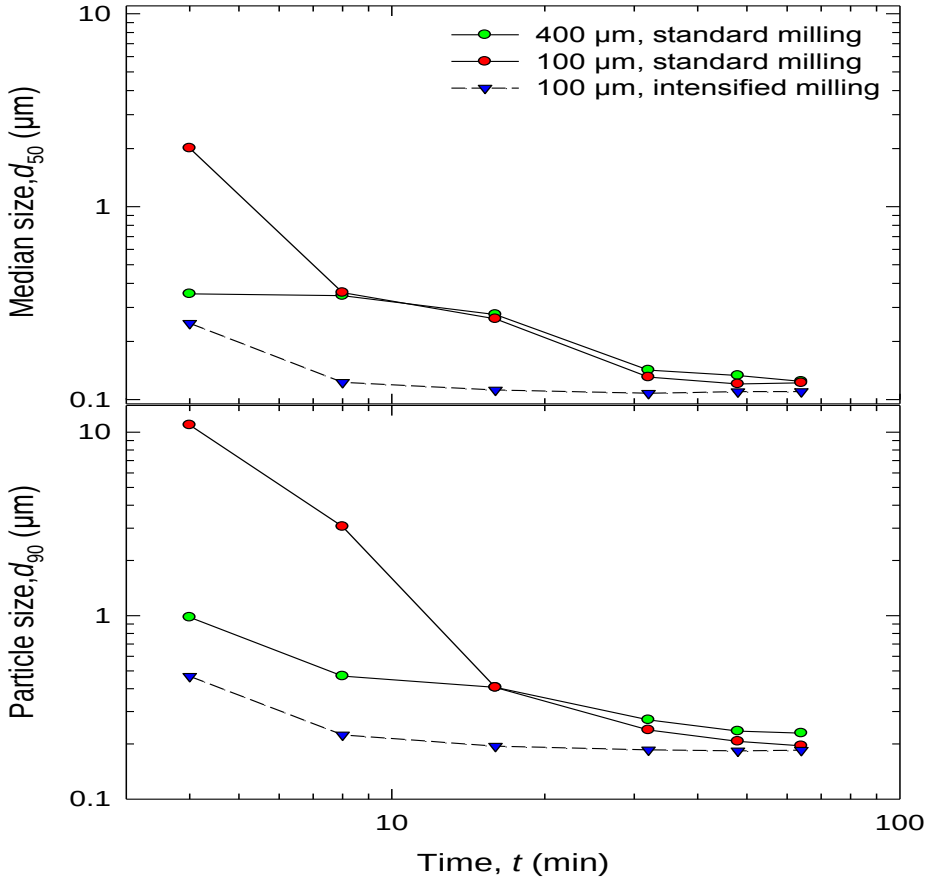

Figure 3.1 Impact of process parameters: (a) the time-wise variation of the median size, (b) the final particle size of NPX during milling. Runs 1, 2, and 3 refer to milling of NPX with 400, and 100 µm YSZ beads respectively at the baseline process conditions (Q = 126 ml/min), and intensified process conditions (Q = 343 ml/min). At t = 0 min, NPX particles have d_{50} = 15.08 ± 0.15 µm and d_{90} = 37.59 ± 0.01 µm.

Table 3.1 Particle Size and Standard Deviation (SD) Obtained From Laser Diffraction (LD) for Runs 1-3 Suspensions After Milling and After 7 days Storage

Run	d_{50} ± SD (µm)		d_{90} ± SD (µm)	
	After Milling	7-Day Storage	After Milling	7-Day Storage
1	0.124 ± 0.000	0.127 ± 0.001	0.229 ± 0.070	0.226 ± 0.007
2	0.122 ± 0.060	0.114 ± 0.002	0.195 ± 0.002	0.214 ± 0.015
3	0.110 ± 0.000	0.111 ± 0.001	0.185 ± 0.001	0.196 ± 0.005

A rheological characterization of the milled suspensions was performed because the injectable formulations should have ideally low viscosity (preferably below 100 cP), yet being physically stable. Fig. 3.2 shows that all milled suspensions had less than 50 cP apparent shear viscosity, which is highly desirable for injectables. An addition of SDS to an HPC SL solution increased the viscosity significantly, which can be attributed to the formation of HPC–SDS aggregates or micelle-like SDS clusters bound to the polymer. Such HPC–SDS interactions are expected to result in a synergistic electrostatic stabilization (Bilgili and Afolabi, 2012).

The lowest apparent shear viscosity of 6.7 cP was obtained at 1000 (1/s) for Run 3 in Table 3.2. The pre-suspension of 2.5% HPC SL and 0.5% SDS before milling showed the highest values of apparent shear viscosity of 162.7 cP at 1000 (1/s) shear rate. This and the ranking of Runs 1-3 suspensions (Table 3.2) can be explained by the fact that suspensions with smaller particle size exhibit lower viscosity. Similar observation was also made by Winnik and Winnik (1990), Evertsson and Nilsson (1997), and Berglund et al. (2003). Also, the viscosity of a milled suspension was lower than that of the stabilizer solution (Table 3.2) because of relatively well-dispersed nature of the NPX suspensions with HPC–SDS and the reduced concentration of HPC–SDS in the bulk solution of the NPX suspensions due to enhanced HPC SL adsorption (Bilgili et al. 2012).

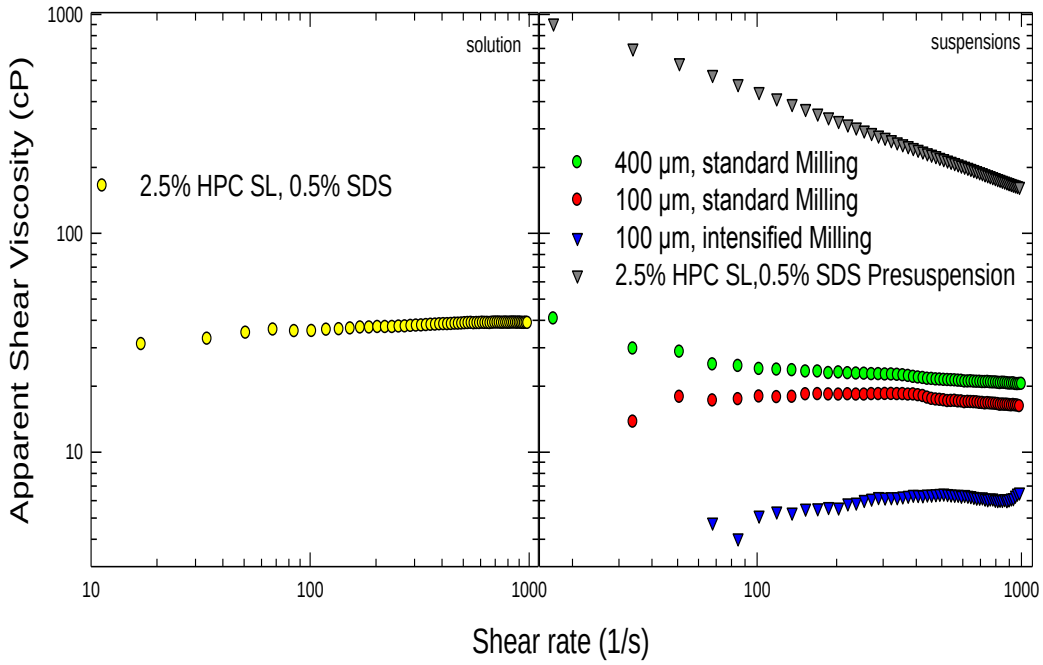

Figure 3.2 Log–log plots for apparent shear viscosity versus shear rate for (a) the HPC SL solutions; (b) milled NPX suspensions.

Table 3.2 Apparent Shear Viscosity of the HPC SL-SDS-based Solution and Suspensions at 25 °C and 1000 (1/s) Shear Rate

Run	**Apparent Shear Viscosity** (cP)
Pre-suspension (unmilled)	162.7
Stock stabilizer solution	44.5
1	20.5
2	16.2
3	6.7

Table 3.3 shows that the HPC SL–SDS solution has higher surface tension than water (34.363 ± 0.111 mN/m) due to formation of micelle like SDS clusters bound to HPC SL. On the other hand, the surface tensions of milled suspensions were slightly lower than those of the corresponding stabilizer solutions because of the reduced bulk concentration of the stabilizers in the milled suspensions. HPC SL imparts poorer wettability to drugs in water relative to SDS even though it reduces surface tension of water (Dalvi et al. 2010, Rasenack et al. 2003).

Table 3.3 Surface Tension for HPC SL-SDS Suspensions

Run	**Solution** (mN/m) Mean ± SD	**Milled Suspension** (mN/m) Mean ± SD
1	37.868 ± 0.064	34.363 ± 0.111
2	37.786 ± 0.055	36.638 ± 0.377
3	37.838 ± 0.09	36.517 ± 0.564

3.2 Impact of Polymer Concentration

3.2.1 Particle Size of Milled Suspensions

In the absence of any stabilizer, NPX particles alone could not be milled since it is very hydrophobic and a foam appeared during the milling and milling could not be continued. Thus, stabilizers are needed to reduce surface tensions and prevent aggregation. Figure 3.3 shows the evolution of the NPX particle size with different PVP concentrations during milling. HPC-SDS combination was used as a comparative baseline, which provides sufficient stabilization for NPX drug particles. However, HPC-SDS combination cannot be used for injectables; hence, acceptable stabilizers like PVP are being investigated here. In general, NPX nanosuspensions were successfully prepared in the presence of PVP. The particle size decreased and attained a plateau in time; there was no significant increase in particle size during milling and storage (Table 3.4). Hence, PVP successfully suppressed the aggregation during the milling and storage. The effect of PVP concentration on particle size is relatively weak; the suspensions were stable even at low PVP concentration. A slight optimum concentration of 2.5% exists for PVP 17, in view of the 7-day stability data (Table 3.4), while 2.5% HPC SL in presence of SDS produced the lowest median size (d_{50}) and 90% passing size (d_{90}), i.e., 110 nm and 185 nm respectively, due to synergistic stabilization imparted by HPC-SDS combination. Overall, these results suggest that stable NPX nanosuspensions can be prepared using polymers acceptable for injectable applications; however, the sterile filterability of such suspensions is yet to be assessed below.

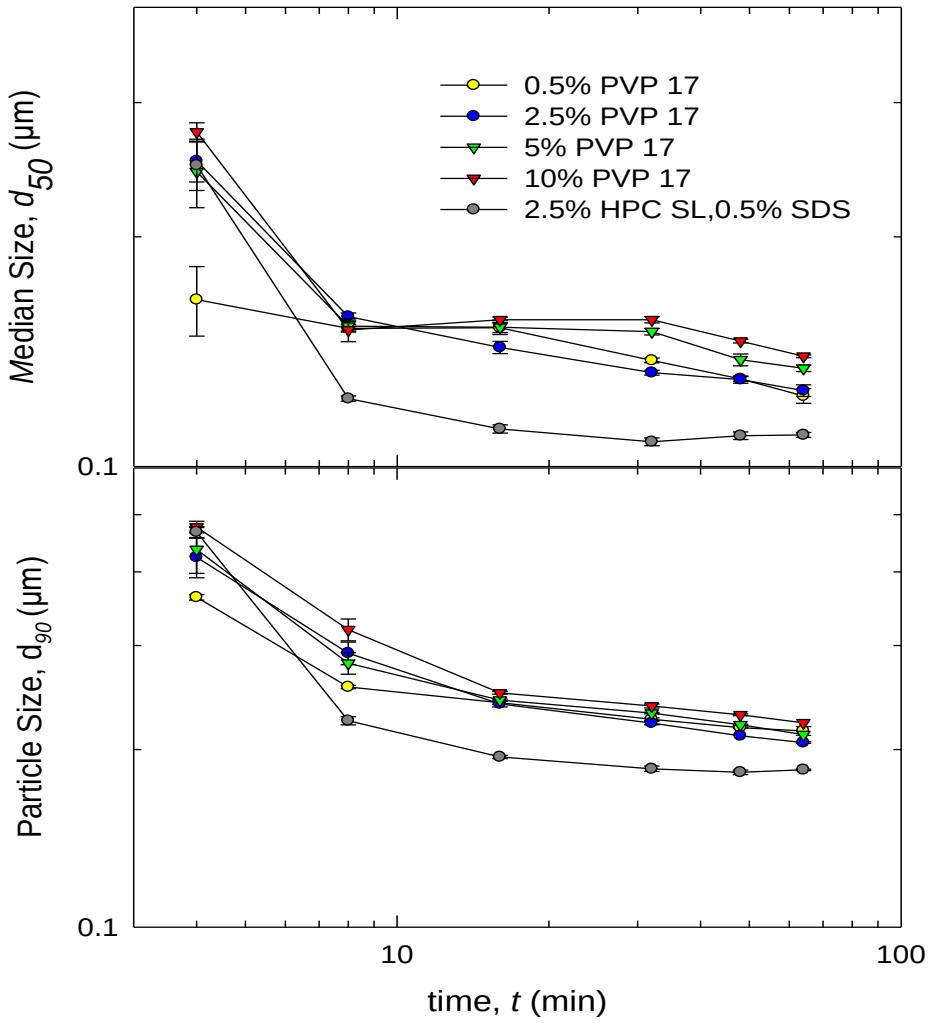

Figure 3.3 Impact of polymer concentration: (a) the time-wise variation of the median size (d_{50}), (b) 90% passing size (d_{90}) of NPX during milling.

Table 3.4 Particle Size and Standard Deviation (SD) Obtained from Laser Diffraction (LD) for NPX Suspensions After Milling and After 7 days Storage

Run	**Formulation**	d_{50} (µm) ± SD		d_{90} (µm) ± SD	
		After Milling	7-Day Storage	After Milling	7-Day Storage
3	2.5% HPC SL, 0.5% SDS	0.110 ± 0.000	0.111 ± 0.001	0.185± 0.001	0.196± 0.005
4	0.5% PVP 17	0.124 ± 0.003	0.128 ± 0.000	0.215± 0.004	0.219± 0.000
5	2.5% PVP 17	0.126 ± 0.002	0.130 ± 0.003	0.206± 0.000	0.209± 0.000
7	5% PVP 17	0.135 ± 0.001	0.135 ± 0.001	0.212± 0.000	0.214± 0.001
8	10% PVP 17	0.139 ± 0.001	0.139 ± 0.001	0.222± 0.000	0.225± 0.001

3.2.2 Apparent Shear Viscosity

In order to investigate the impact of PVP 17 concentration on the suspension rheology, the apparent shear viscosity was obtained as a function of shear rate (Figure 3.4). HPC-SDS was used as a baseline formulation to assess the performance of PVP at different concentrations. All of the polymer solutions and the milled NPX suspensions has apparent shear viscosity less than 6 cP at the maximum (Table 3.5), except the case of HPC-SDS solution. Low viscosity (below 100 cP, preferably 50 cP) is critical for injectable suspensions in mitigating the side effects and ensuring proper injections. The slight shear thickening behavior may be due to inaccuracy of the instrument at low viscosity region.

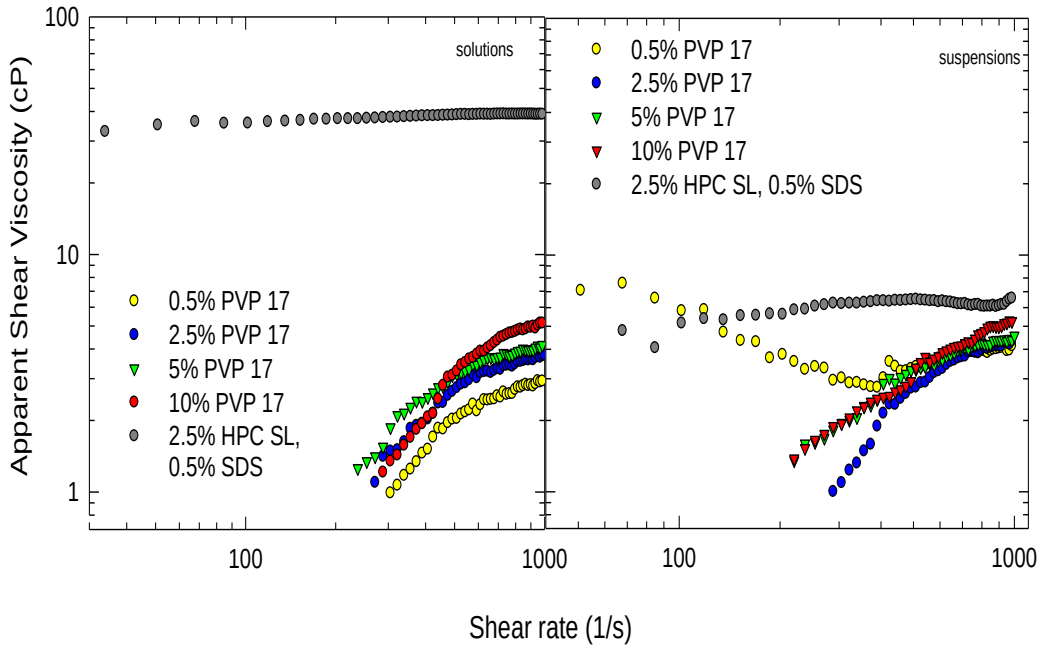

Figure 3.4 Log–log plots for apparent shear viscosity versus shear rate for (a) the PVP 17 solutions; (b) milled NPX suspensions.

Table 3.5 Apparent Shear Viscosity of Different Concentrations of PVP 17 Suspensions at 25 °C and 1000 (1/s) Shear Rate

Run	Formulation	**Apparent Shear Viscosity** (cP) (solution)	**Apparent Shear Viscosity** (cP) (milled suspension)
3	2.5% HPC SL, 0.5% SDS	44.5	6.7
4	0.5% PVP 17	3.6	4.9
5	2.5% PVP 17	3.8	4.3
7	5% PVP 17	4.1	4.4
8	10% PVP 17	5.2	5.3

3.2.3 Surface Tension

In general, surface tension of the milled suspensions is higher than the stock solution for all PVP 17 concentrations, as shown in Table 3.6. The higher surface tension in the milled suspensions is probably due to the presence of a hydrophobic drug, absence of a surfactant, and PVP adsorption on drug particle surfaces, so that less amount of PVP is available in solution to reduce the suspension surface tension. Due to the presence of SDS, the solution and the suspension had similar surface tension values for NPX suspensions with HPC–SDS formulation, which had much lower surface tension than PVP formulation.

Table 3.6 Surface Tension for Different Concentrations of PVP 17 Suspensions

Run	Formulation	**Solution** (mN/m) Mean ± SD	**Milled Suspension** (mN/m) Mean ± SD
3	2.5% HPC SL, 0.5% SDS	37.838 ± 0.090	36.517 ± 0.564
4	0.5% PVP 17	47.763 ± 0.133	57.218 ± 0.213
5	2.5% PVP 17	44.541 ± 0.115	52.027 ± 0.081
7	5% PVP 17	51.933 ± 0.255	56.751 ± 0.216
8	10% PVP 17	46.783 ± 0.044	55.255 ± 0.056

3.3 Impact of Different Polymers

3.3.1 Particle Size

Impact of different polymers on the stabilization of NPX drug particles was studied in current section. The polymer concentration was fixed at 2.5% based on the optimized PVP concentration. HPC-SDS was again used as a baseline. The evolution of both D50 and D90 exhibited a monotonic decrease for all polymers (Figure 3.5), except Soluplus. When Soluplus was used as a stabilizer, the drug particle size fluctuated during milling and the final particle size of D90 was above 1 μm. This suspension exhibited severe aggregation. The final particle sizes shown in Table 3.7 show that PVP 12, PVP 17, HPC SL, and HPMC E3 were all able to stabilize NPX nanoparticles without the use of a surfactant. On the other hand, only PVP 12 and PVP 17 grades were acceptable for injectable applications.

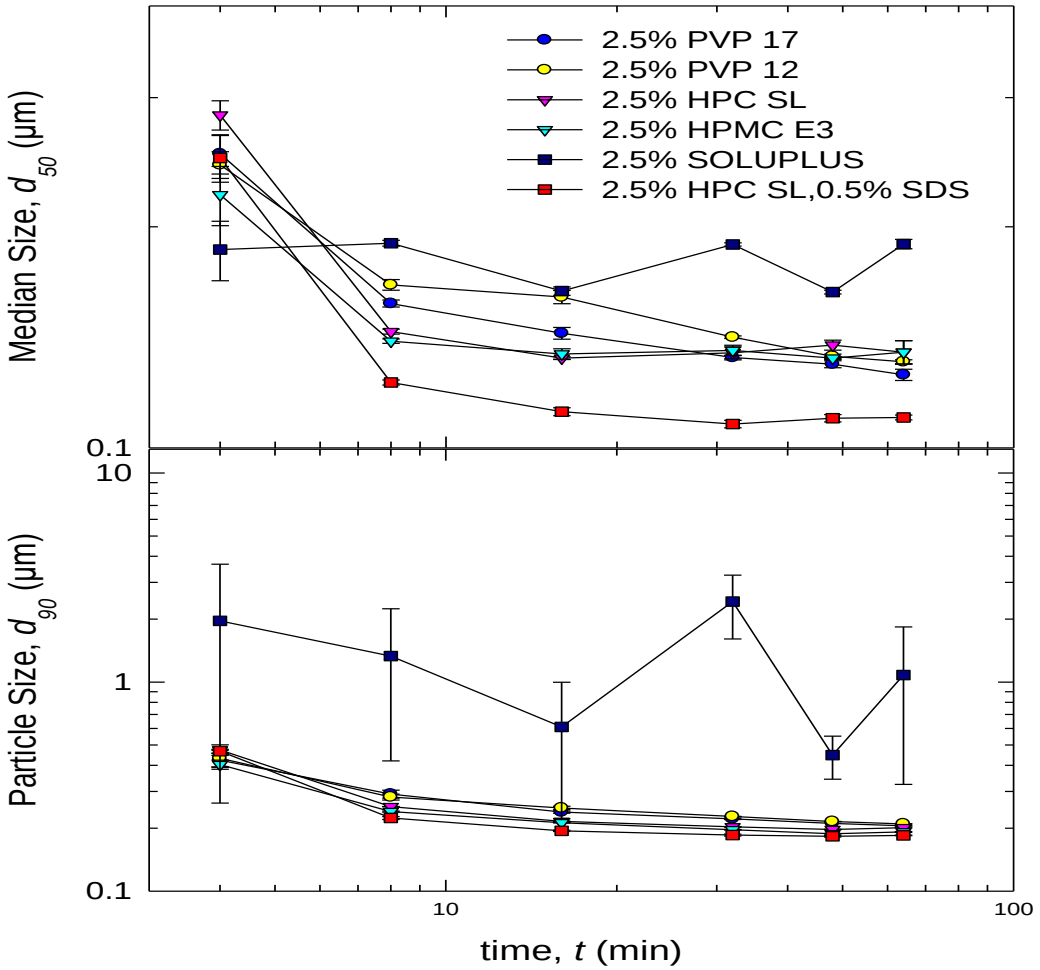

Figure 3.5 Impact of different polymers on: (a) the time-wise variation of the median size (d_{50}), (b) the 90% passing size (d_{90}) of NPX during milling.

Table 3.7 Particle Size and Standard Deviation (SD) Obtained from Laser Diffraction (LD) for Different Polymer Suspensions After Milling and After 7 days Storage

Run	**Formulation**	d_{50} ± SD (µm)		d_{90} ± SD (µm)	
		After Milling	7-Day Storage	After Milling	7-Day Storage
3	2.5% HPC SL, 0.5% SDS	0.110 ± 0.000	0.111 ± 0.001	0.185 ± 0.001	0.196 ± 0.005
5	2.5% PVP 17	0.126 ±0.002	0.130 ± 0.003	0.206 ± 0.00	0.209 ± 0.000
6	2.5% PVP 12	0.131 ±0.000	0.131 ±0.001	0.210 ± 0.001	0.213 ± 0.001
9	2.5% HPC SL	0.135 ± 0.004	0.130 ± 0.000	0.201 ± 0.001	0.195 ± 0.001
10	2.5% HPMC E3	0.135 ± 0.005	0.118 ±0.001	0.192 ± 0.001	0.189 ± 0.001
11	2.5% SOLUPLUS	0.189 ± 0.020	0.135 ± 0.001	1.08 ± 0.756	0.213 ± 0.001

3.3.2 Apparent Shear Viscosity

Viscosity measurement was conducted to all polymer solution and milled suspensions. All the polymer solutions and milled suspensions show viscosity less than 8 cP at the maximum, except the case of HPC-SDS solution (Table 3.8).

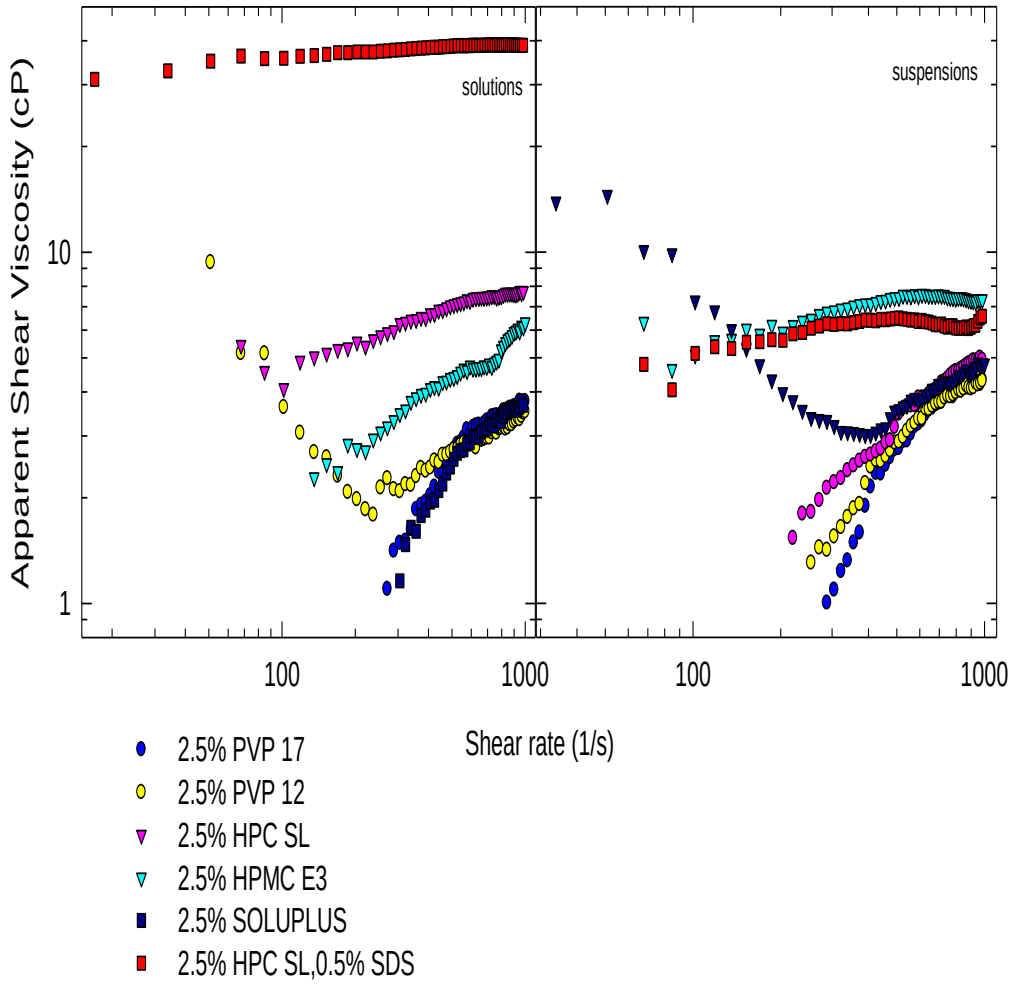

Figure 3.6 Log–log plots for apparent shear viscosity versus shear rate for (a) the solutions; (b) milled NPX suspensions.

Table 3.8 Apparent Shear Viscosity of Different Polymer Suspensions at 25 °C and 1000 (1/s) Shear Rate

Run	**Formulation**	**Apparent Shear Viscosity** (cP) (Solution)	**Apparent Shear Viscosity** (cP) (Milled Suspension)
3	2.5% HPC SL, 0.5% SDS	44.5	6.7
5	2.5% PVP 17	3.8	4.3
6	2.5% PVP 12	3.5	4.3
9	2.5% HPC SL	7.6	4.9
10	2.5% HPMC E3	6.3	7.3
11	2.5% SOLUPLUS	3.7	4.9

3.3.3 Surface Tension

Table 3.9 shows that the surface tension of the milled suspension was higher than the stock solution, where polymer was used as the sole stabilizer. The increase in suspension surface tension is due to the polymers adsorption on drug particle surfaces, so that less amount of polymer is available in solution to reduce the suspension surface tension. In the cases of PVP 12 and PVP 17, it seems polymer with lower molecular grade (PVP 12) is less capable in reducing surface tension.

Table 3.9 Surface Tension for Different Polymer Suspensions

Run	**Formulation**	**Solution** (mN/m) Mean ± SD	**Milled Suspension** (mN/m) Mean ± SD
3	2.5% HPC SL, 0.5% SDS	37.838 ± 0.090	36.517 ± 0.564
5	2.5% PVP 17	44.540 ± 0.115	52.027 ± 0.081
6	2.5% PVP 12	52.559 ± 0.854	57.567 ± 0.292
9	2.5% HPC SL	42.227 ± 0.057	43.216 ± 0.088
10	2.5% HPMC E3	41.902 ± 0.087	46.266 ± 0.138
11	2.5% SOLUPLUS	43.231 ± 0.016	49.191 ± 0.366

3.4 Impact of Surfactant Concentration

3.4.1 Particle Size

This part of the study focuses on the impact of an injection-acceptable surfactant, i.e., Poloxamer concentration on NPX nanoparticle stabilization. HPC-SDS was again used as a baseline condition. Figure 3.7 shows that an optimum Poloxamer concentration exists to stabilize NPX nanoparticles, which is 2.5%. At 0.5%, severe aggregation took place, leading to the formation of coarse aggregates as large as 14 μm. Above 2.5%, an increase in Poloxamer concentration increased the final NPX particle size achieved (Table 3.10). However, all D90 values for the milled suspensions stabilized by Poloxamer were above 0.3 μm (Table 3.10), suggesting that Poloxamer was not as effective as PVP in stabilizing the NPX particles. Particle sizes slightly increased after 7 days storage, especially for Poloxamer with the lowest concentration. Therefore, 2.5% was selected as an optimum concentration and applied to the rest of surfactant studies.

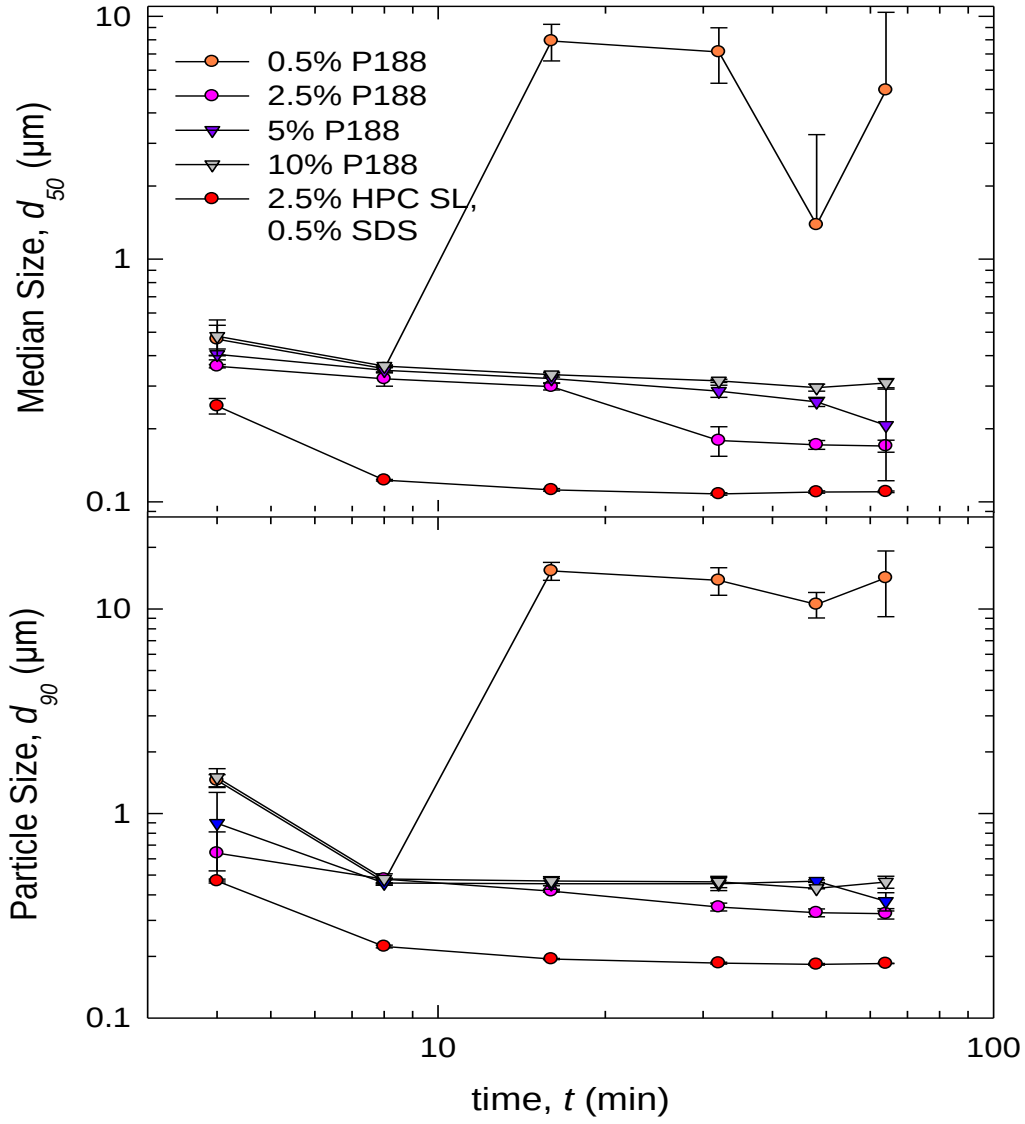

Figure 3.7 Impact of surfactant concentration: (a) the time-wise variation of the median size (d_{50}), (b) the 90% passing size (d_{90}) of NPX during milling.

Table 3.10 Particle Size and Standard Deviation (SD) Obtained from Laser Diffraction (LD) for Surfactant Concentration After Milling and After 7 Days Storage

Run	**Formulation**	d_{50} ± SD (µm)		d_{90} ± SD (µm)	
		After Milling	7-Day Storage	After Milling	7-Day Storage
3	2.5% HPC SL, 0.5% SDS	0.110 ± 0.000	0.111 ± 0.001	0.185 ± 0.001	0.196±0.005
12	0.5% P188	4.965 ± 5.422	6.251 ± 1.311	14.185 ± 5.017	18.487±0.815
13	2.5% P188	0.169 ± 0.009	0.162 ± 0.003	0.324 ± 0.019	0.315±0.006
14	5% P188	0.207 ± 0.085	0.248 ± 0.004	0.372 ± 0.038	0.398 ± 0.003
15	10% P188	0.308 ± 0.014	0.312 ± 0.003	0.462 ± 0.031	0.475 ± 0.026

3.4.2 Apparent Shear Viscosity

Viscosity measurement was conducted on all surfactant solutions and milled suspensions. All the surfactant solutions and milled suspensions show viscosity less than 7 cP at the maximum, except the case of HPC-SDS solution (Table 3.11). An increase in Poloxamer concentration increased the apparent shear viscosity in the presence or absence of the milled NPX particles (Figure 3.8).

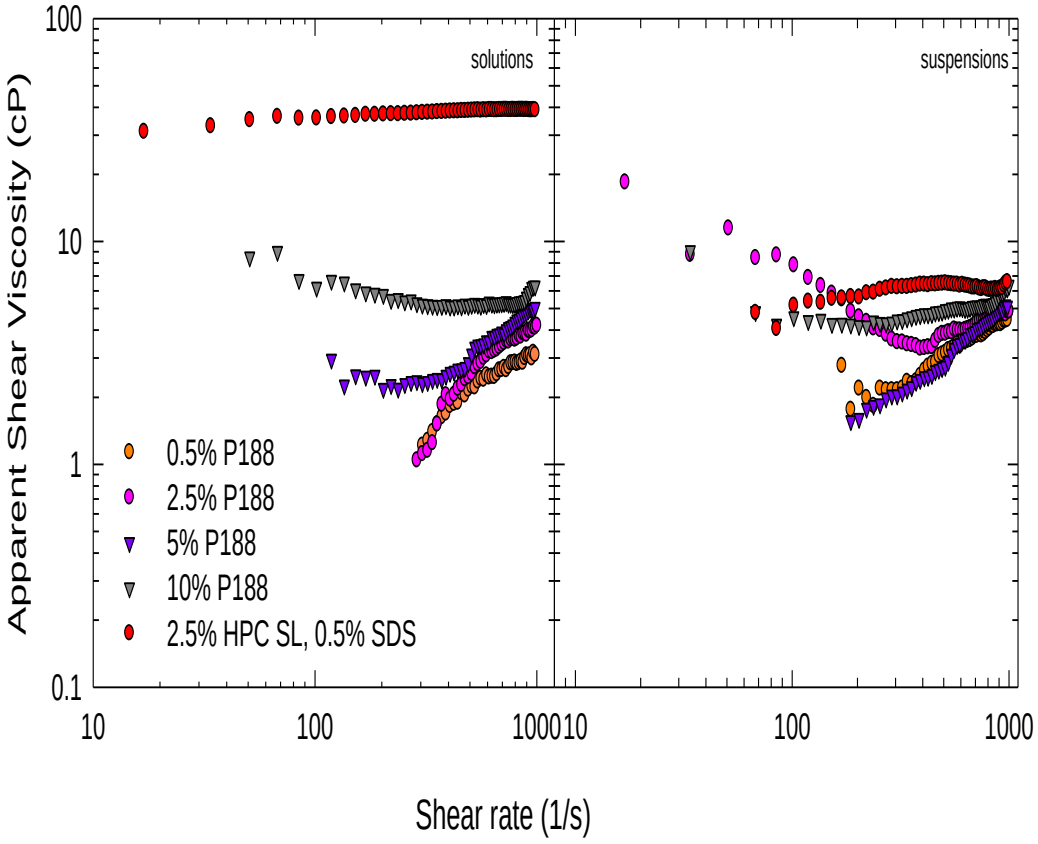

Figure 3.8 Log–log plots for apparent shear viscosity versus shear rate for (a) the P188 solutions; (b) milled NPX suspension

Table 3.11 Apparent Shear Viscosity of Surfactant P188 Suspensions at 25 °C and 1000 (1/s) Shear Rate

Run	**Formulation**	**Apparent Shear Viscosity** (cP) (Solution)	**Apparent Shear Viscosity** (cP) (Milled Suspension)
3	2.5% HPC SL, 0.5% SDS	44.5	6.7
12	0.5% P188	3.1	4.4
13	2.5% P188	4.2	4.9
14	5% P188	5.1	5.1
15	10% P188	6.3	6.4

3.4.3 Surface Tension

Surface tensions of the milled suspension were higher than the stock solution in all the cases (Table 3.12), where Poloxamer was used as the sole stabilizer. The increase in suspension surface tension is due to the surfactant adsorption on drug particle surfaces, so that less amount of surfactant is available in solution to reduce the suspension surface tension. An increase in concentration of Poloxamer 188 consistently decreased the surface tension of the stabilizer solutions and milled suspensions.

Table 3.12 Surface Tension for Different Concentrations of P188 Suspensions

Run	**Formulation**	**Solution** (mN/m) Mean ± SD	**Milled Suspension** (mN/m) Mean ± SD
3	2.5% HPC SL, 0.5% SDS	37.838 ± 0.090	36.517 ± 0.564
12	0.5% P188	44.344 ± 0.147	68.691 ± 2.727
13	2.5% P188	44.524 ± 0.122	45.600 ± 0.131
14	5% P188	42.563 ± 0.046	43.260 ± 0.218
15	10% P188	41.279 ± 0.078	42.782 ± 0.128

3.5 Impact of Different Surfactants

3.5.1 Particle Size

The particle sizes in the nanosuspension for various injection-acceptable surfactants were plotted as a function of milling time as shown in Figure 3.9. While unacceptable for injection, HPC-SDS combination was used as a baseline formulation to stabilize NPX drug nanoparticle with a minimum amount of aggregates in the system. Two FDA approved injectable surfactants with two different molecular weights were investigated. All formulations with surfactant as the sole stabilizer showed monotonic decrease in particle sizes initially, followed by a light increase or decrease in D50 after 48 min. It is shown that Poloxamer 188 outshines other surfactants at 2.5%, but it is still not sterile filterable as it has a final particle size D90 above 220 nm (Table 3.13). The 7-day stability values demonstrated the particle size growth of NPX particles, which can be attributed to simple aggregation and possibly Ostwald ripening (Zu et al. 2014). Particle sizes after 7-day storage slightly increased, especially for Tween 20 and Tween 80.

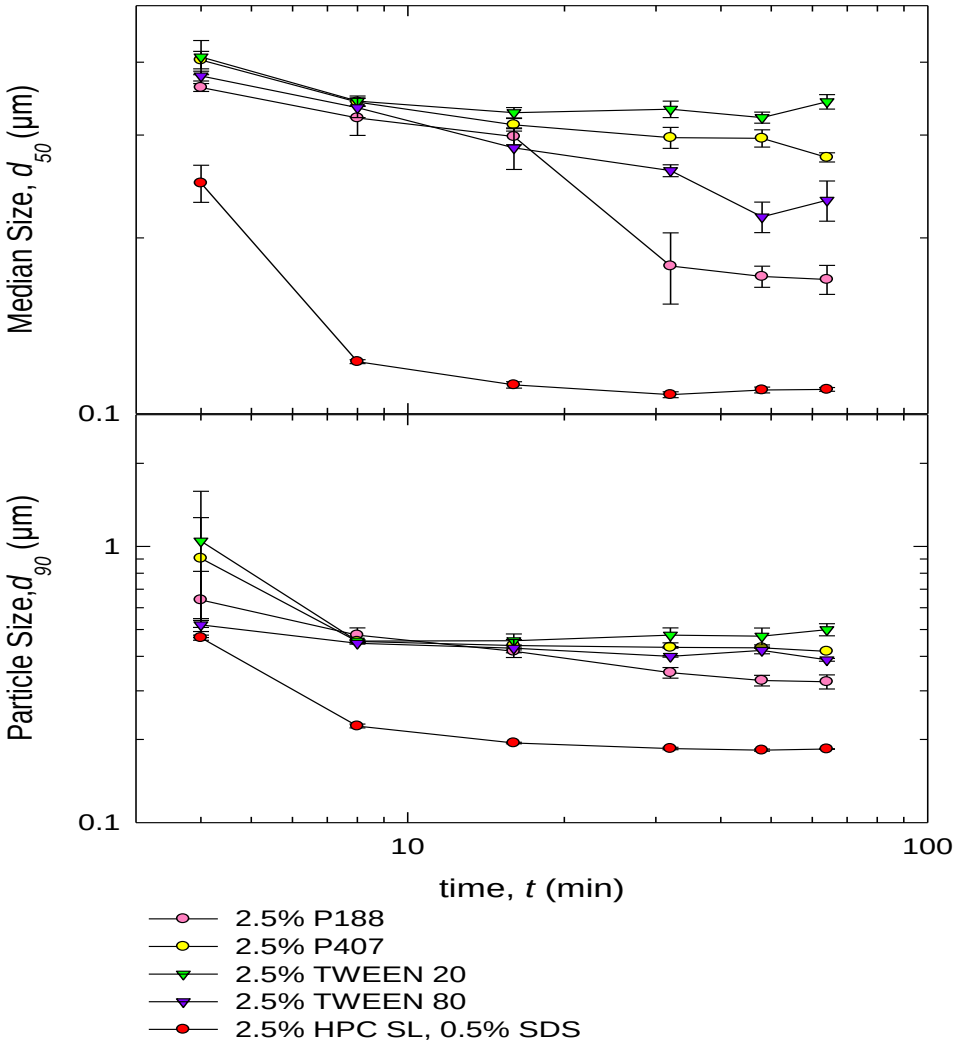

Figure 3.9 Impact of different surfactants on: (a) the time-wise variation of the median size (d_{50}), (b) the 90% passing size (d_{90}) of NPX during milling.

Table 3.13 Particle Size and Standard Deviation (SD) Obtained from Laser Diffraction (LD) for Different Surfactants After Milling and After 7 Days Storage

Run	**Formulation**	d_{50} ± SD (μm)		d_{90} ± SD (μm)	
		After milling	7-Day storage	After milling	7-Day storage
3	2.5% HPC SL, 0.5% SDS	0.110 ± 0.000	0.111 ± 0.001	0.185 ± 0.001	0.196 ± 0.005
13	2.5% P188	0.169 ± 0.009	0.162 ± 0.003	0.324 ± 0.019	0.315 ± 0.006
16	2.5% P407	0.275 ± 0.005	0.292 ± 0.006	0.417 ± 0.000	0.431 ± 0.001
17	2.5% Tween 20	0.342 ± 0.009	0.364 ± 0.004	0.499 ± 0.025	0.537 ± 0.007
19	2.5% Tween 80	0.164 ± 0.018	0.179 ± 0.069	0.389 ± 0.005	0.518 ± 0.075

3.5.2 Apparent Shear Viscosity

Viscosity measurement was conducted on all surfactant solutions and milled suspensions. All the surfactant solutions and milled suspensions show viscosity less than 7 cP at the maximum, except the case of HPC-SDS solution (Table 3.14).

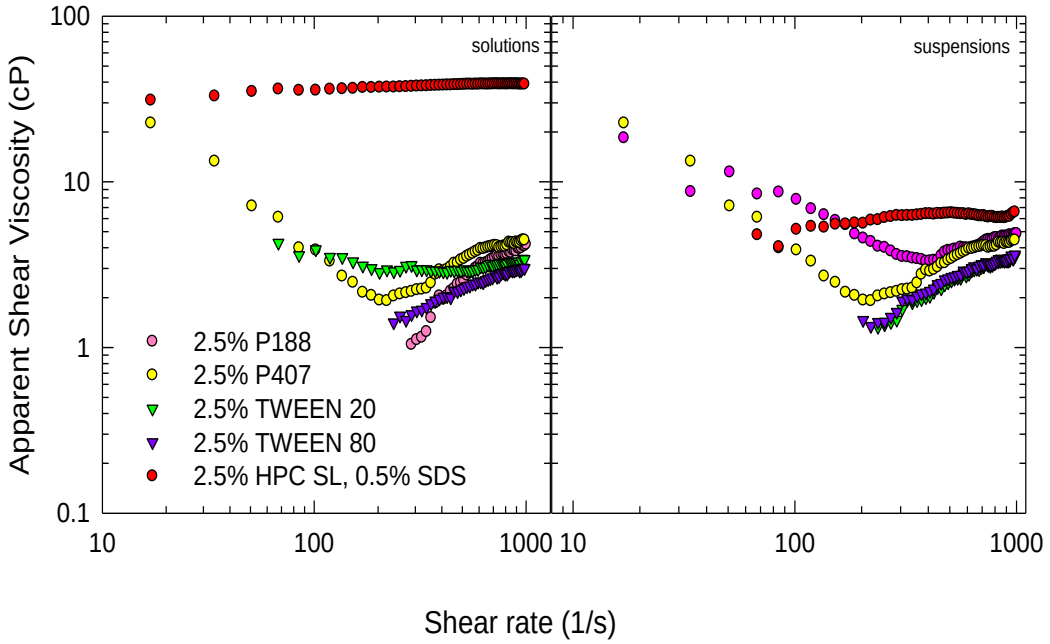

Figure 3.10 Log–log plots for apparent shear viscosity versus shear rate for (a) different surfactant solutions; (b) milled NPX suspensions.

Table 3.14 Apparent Shear Viscosity of Different Surfactant Suspensions at 25°C and 1000 (1/s) Shear Rate

Run	Formulation	**Apparent Shear Viscosity** (cP) (Solution)	**Apparent Shear Viscosity** (cP) (Milled Suspension)
3	2.5% HPC SL, 0.5% SDS	44.4	6.7
13	2.5% P188	4.2	4.9
16	2.5% P407	4.4	4.5
17	2.5% Tween 20	3.4	3.6
19	2.5% Tween 80	3.0	3.6

3.5.3 Surface Tension

Surface tensions of the milled suspension were higher than the stock solution in all the cases (Table 3.15). The increase in suspension surface tension is due to the surfactant adsorption on drug particle surfaces, so that less surfactant is available in solution to reduce the surface tension. Tween was more effective than Poloxamers in reducing the surface tension.

Table 3.15 Surface Tension for Different Surfactant Suspensions.

Run	**Formulation**	**Solution** (mN/m) Mean ± SD	**Milled Suspension** (mN/m) Mean ± SD
3	2.5% HPC SL, 0.5% SDS	37.838 ± 0.090	36.517 ± 0.564
13	2.5% P188	44.524 ± 0.122	45.6 ± 0.131
16	2.5% P407	37.539 ± 0.028	37.938 ± 0.059
17	2.5% Tween 20	35.554 ± 0.273	35.132 ± 0.262
19	2.5% Tween 80	34.891 ± 0.442	34.58 ± 0.665

3.6 Sterile Filtration

Several stabilizers were identified to produce NPX nanoparticles less than 220 nm in D90. The feasibility of sterile filtration was assessed here. Four membranes, cellulose acetate (CA), polyethersulfone (PES), polyvinylidene fluoride (PVDF), and polyamide (PA) were tested. The comparison of filtration results with four membranes is given in Table 3.16. For sterile-filterable suspensions, no significant change in particle size in the filtrate vs. 7-day stored suspension was observed after filtration. For example, 2.5% HPC SL-0.5% SDS formulation, which is a baseline formulation, is sterile filterable; however HPC SL and SDS are not admissible by FDA (US Food and Drug Administration) for injectable formulations. Therefore, this formulation cannot be used in intravenous injectables. On the other hand, the highly versatile polyvinylpyrrolidone PVP 17 is pharmaceutically acceptable by FDA and has diverse properties including its solubility in water and in a broad range of liquid media, high chemical and thermal resistance, and unique wetting, binding, and film-forming properties. Interestingly, the NPX nanosuspension with 2.5% PVP 17 cannot be filtered; drug particles could not pass through the membrane despite the fact that the nanosuspension had a D90 below 220 nm.

Considering the failure of 2.5% PVP 17 to prove its sterile filterability, 0.5% SDS as a favorable surfactant, was added to the same formulation for the filtration studies. However, even a combination of 2.5% PVP 17-0.5% SDS could not allow passage of the NPX nanoparticles through any of the membranes. NPX nanopaticles with HPC SL alone could be sterile filtered through only two membranes (PES and PVDF). Such failures have also been observed in previous literature because of various limitations listed below:

- The particles of the membrane filters approximate the pore size of the filter surface because of their surface-retention mechanism. Such particles stop up the pores and prevent fluid flow (S.S. Block, 2001).
- Not all the particles smaller than its pore size pass through the membrane filter. Some of these particles are collected on the membrane surface, and some are trapped in the tortuous capillaries themselves. If there are a sufficient number of these smaller particles, a rapid buildup in pressure differential results (S.S. Block, 2001).

Table 3.16 Filtration Studies in Different Membranes for: a) 2.5% HPC SL-0.5% SDS, b) 2.5% K17, c) 2.5% HPC SL

Membrane (0.22 μm)	d_{50} ± SD (μm)		d_{90} ± SD (μm)	
	After Filtration	7-Day Storage	After Filtration	7-Day Storage
a) 2.5% HPC SL-0.5% SDS				
Cellulose Acetate (CA)	0.105 ± 0.001	0.107± 0.001	0.175 ± 0.002	0.177 ± 0.001
Polyethersulfone (PES)	0.116 ± 0.002	0.111 ± 0.002	0.177 ± 0.001	0.18 ± 0.002
Polyvinylidene fluoride (PVDF)	0.106 ± 0.001	0.107 ± 0.001	0.175 ± 0.001	0.177 ± 0.001
Polyamide (PA)	0.104 ± 0.003	0.108 ± 0.003	0.175 ± 0.001	0.176 ± 0
b) 2.5% K17				
Cellulose Acetate (CA)	NF	NF	NF	NF
Polyethersulfone (PES)	NF	NF	NF	NF
Polyvinylidene fluoride (PVDF)	NF	NF	NF	NF
Polyamide (PA)	NF	NF	NF	NF
c) 2.5% HPC SL				
Cellulose Acetate (CA)	NF	NF	NF	NF
Polyethersulfone (PES)	0.131 ± 0.000	0.133 ± 0.001	0.190 ± 0.000	0.190 ± 0.000
Polyvinylidene fluoride (PVDF)	0.128 ± 0.003	0.135 ± 0.001	0.192 ± 0.001	0.192 ± 0.001
Polyamide (PA)	NF	NF	NF	NF

NF-Non-Filterable.

3.7 SEM

Figure 3.11 shows the SEM image of as-received NPX particles and NPX particles after milling (Run 5). SEM images of the unmilled and milled NPX particles confirm the breakage of the NPX particles and formation of 50–250 nm primary particles. NPX particles become smaller and more rounded upon milling. A comparison of particles sizes based on laser diffraction measurement and SEM images suggests that NPX nanoparticles were formed by breakage, but they aggregated to various extents in the suspensions depending on the stabilizer used. Stabilizers allow proper wetting of the hydrophobic drug surfaces, which can help to disperse aggregates formed during the milling process (Kissa et al. 1999). Hence, both the laser diffraction and the SEM imaging suggest that the PVP 17 stabilized suspension had relatively small extent of aggregation and the dominant mechanism during the milling was breakage and not aggregation.

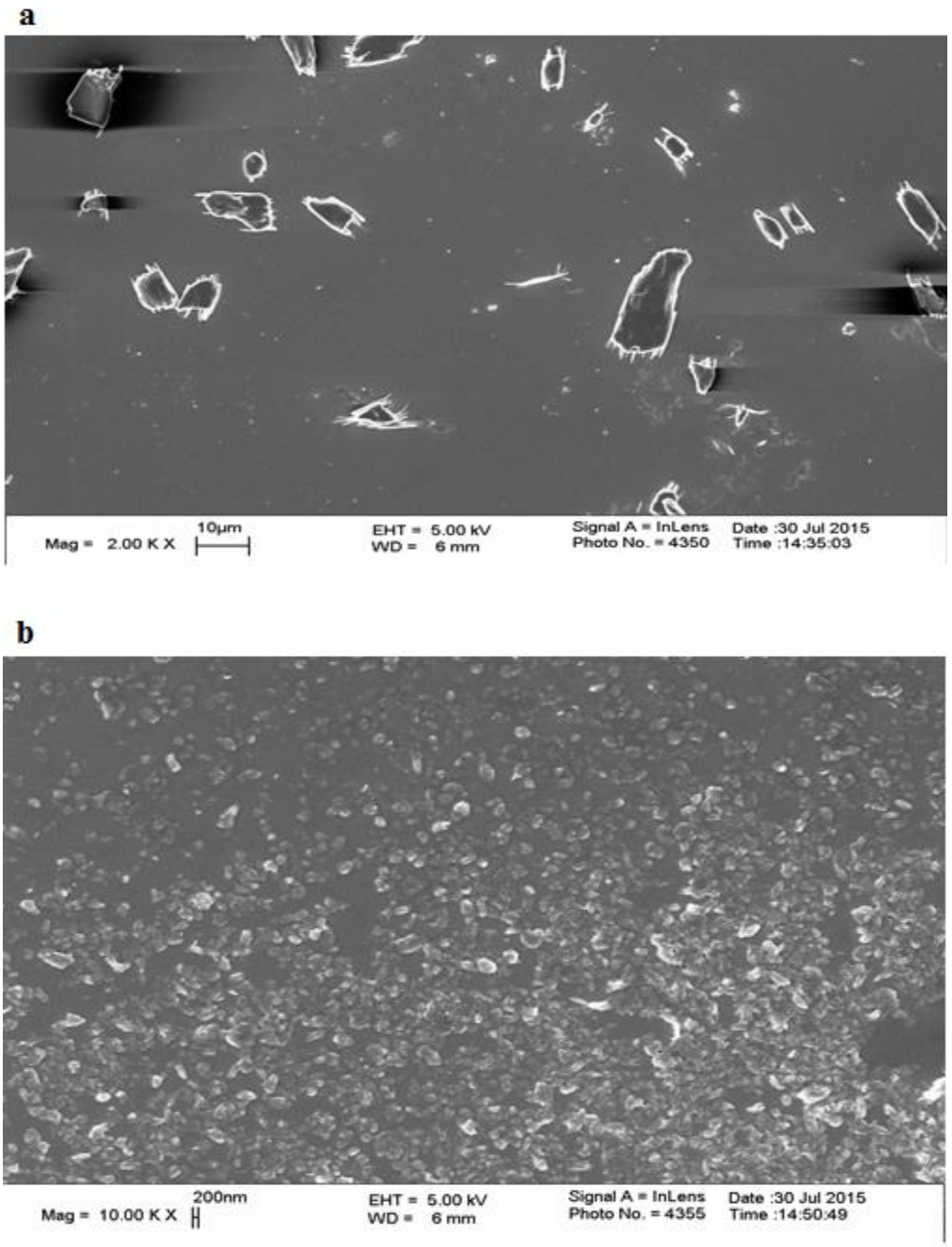

Figure 3.11 SEM images showing the evolution of NPX particle size and morphology during Run 5: (a) as received (b) After 64 min milling. Run 5 refer to the use of 100 µm YSZ beads at the intensified process conditions (tip speed: 11.7 m/s, and flow rate: 343 ml/min). Initially, the NPX particles have d_{50} = 14.645 ± 0.465 µm and d_{90} = 31.868 ± 1.143 µm (marker size: 200 nm, 10.00 k magnification).

3.8 XRD

One concern for the wet media milling process is that the energetic process may lead to transition in the crystalline state of drugs. Figure 3.12 presents the XRD diffractograms of as-received NPX, physical mixture of NPX and 2.5% PVP 17, as well as milled suspension of NPX and 2.5% PVP 17 after overnight drying. The characteristic peaks of NPX appeared in all diffractograms without a broad halo after milling. As compared to the as-received NPX pattern, a slight reduction in the NPX peak intensities in the unmilled physical mixture is seen, which is due to dilution and surface coverage of NPX particles by PVP 17. On comparing dried, milled suspension's pattern with that of the physical mixture, we note that the peak positions remained the same despite a reduction in peak heights after milling, which can be attributed to defect formation and accumulation during milling as well as the aforementioned dilution effect (Monteiro et al. 2013). While XRD cannot detect minor amount of amorphous phase due to indirect inference, crystal orientation effects, and instrument-related intensity variations (Venkatesh et al. 2001), the aforementioned XRD results overall suffice to show that the crystalline state of NPX was largely preserved after milling.

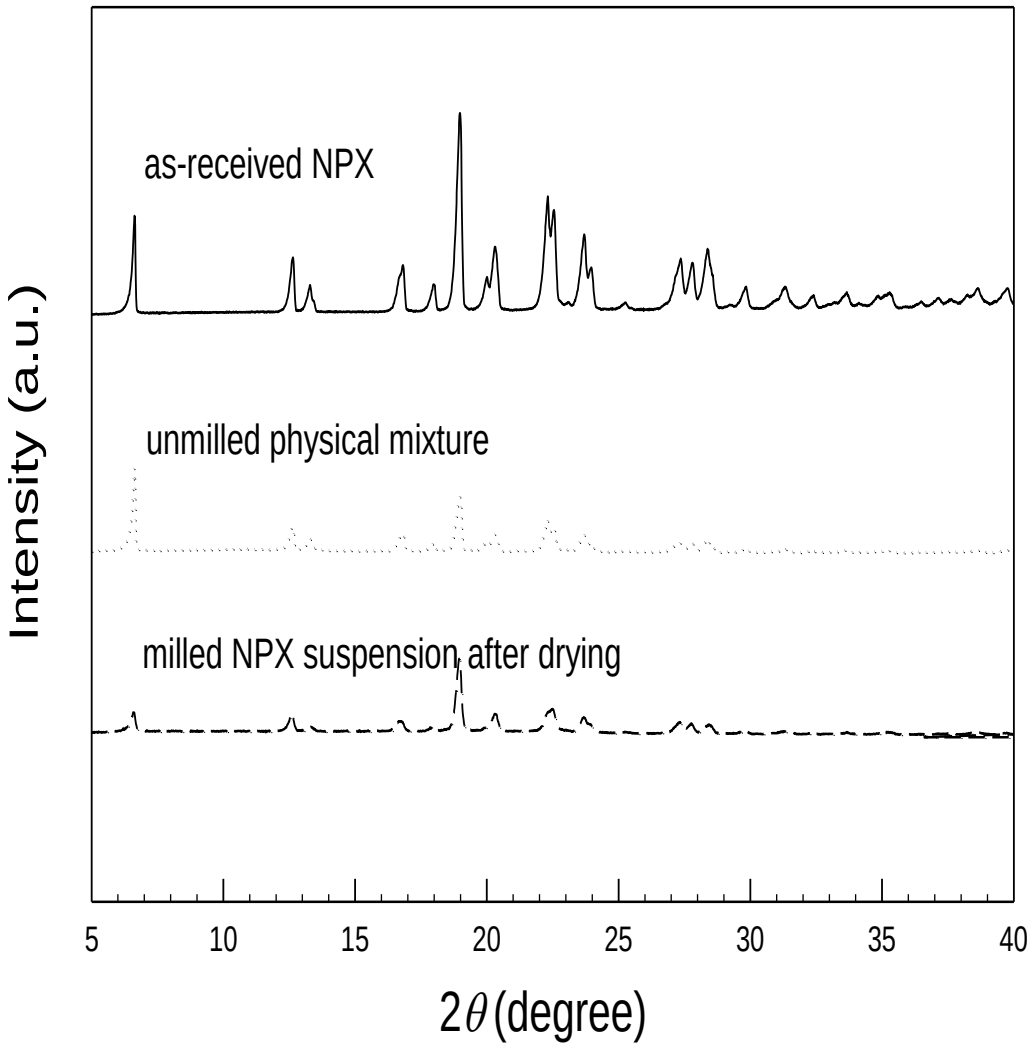

Figure 3.12 XRD diffractograms of as-received NPX, and unmilled physical mixture (NPX and 2.5% PVP 17), and dried, milled suspensions prepared with 100 μm YSZ beads for 64 min milling at the intensified process conditions (u= 11.7 m/s, and Q= 343 ml/min).

CHAPTER 4

SUMMARY AND CONCLUSION

Naproxen (NPX) suspensions with various stabilizers have been produced via wet stirred media milling with the goal of achieving sterile-filterable drug nanosuspensions. Process intensification with smaller beads led to formation of NPX nanoparticles faster. The intensified process was then used to assess the impact of various stabilizers on the aggregation and physical stability. A baseline stabilizer formulation, HPC-SDS, was used as a baseline comparison, which led to D90 below 220 nm although it cannot be used for injectables. While HPC, HPMC, and PVP were more effective than various surfactants in stabilizing the NPX nanosuspensions, only PVP is acceptable for injectable application by FDA. Severe aggregation was observed when injection-acceptable surfactants were used especially at low concentration. The rheological characterization of the milled NPX suspensions suggests that most milled suspensions had relatively low viscosity (less than 10 cP), which is highly desirable for injectables. The NPX nanosuspensions with D90 below 220 nm were filtered through four different types of membranes. The concept of sterile filtration has been demonstrated with the HPC-SDS and HPC alone formulations; yet they cannot be used in injectable applications. While PVP allowed for NPX suspensions to have D90 below 220 nm, the respective nanosuspension cannot be sterile-filtered through any of the membrane materials. Hence, we found that while D90 below 220 nm is a necessary condition for sterile-filterability, but it is not sufficient. It is clear that NPX nanoparticles and PVP interact with membrane surfaces in such a way to cause blocking of the filters. Further research is required to understand such interactions toward designing a filter/process which ensures successful sterile filtration of nanosuspensions.

CHAPTER 5

FUTURE WORK

The current study has dealt with various challenges to render NPX nanosuspensions sterile-filterable. The following topics or aspects are of major interest for future work:

- A thorough literature search for identifying the FDA approved polymers–surfactants and their concentration range
- A thorough literature search for identifying the current and potential applications of sterile-filterable drug nanosuspensions such as long-acting parenteral suspensions
- A thorough literature search for factors controlling the ultrafiltration process adopted in other chemical process industries
- Combination of various polymers and surfactants to stabilize NPX nanosuspensions and assessment of the impact of this combination on sterile filterability
- Use of multiple drugs to generalize the approach adopted in this study
- Pre-filtration of the drug suspensions to remove coarse aggregates prior to sterile filtration with a 220 nm pore membrane filter
- Impact of heat treatment of the filter/suspensions on the sterile filterability

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