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Evaluation of nanoparticle formation feasibility upon wet milling based on physicochemical properties of drug molecules

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ABSTRACT

Nanosuspension formulations have been widely used in drug discovery settings to improve *in vivo* exposure of poorly water-soluble compounds. Wet milling in bench scale is the most applied method in the industry to prepare nanosuspensions for discovery support due to its ease of preparation, high yield, and cost effectiveness. Despite the popularity of nanosuspensions, there is little information about the system's overall performance and what range of particle sizes this bench method can provide. Several parameters such as compound surface energy have been speculated to be a key attribute of the final particle size, but there is a lack of strong evidence. The objective of this study was to identify potential critical properties that may dictate the particle size reduction limit upon wet milling using six poorly soluble compounds with different physicochemical properties as model drugs. It was found that the efficiency of particle size reduction by this popular wet milling method differs from compound to compound. The results of this study have shown that higher melting enthalpy and lower solubility generally resulted in a lower particle size distribution at steady state upon wet milling. These two properties may be used as a preliminary evaluation of the feasibility of forming nanoparticles after milling.

Keywords: *Nano suspension, Wet-milling,enthalpy, solubility.*

1. INTRODUCTION

Nanosuspensions have been widely used to improve *in vivo* exposure of poorly water-soluble compounds, especially in drug discovery settings when large numbers of potential candidates are screened for efficacy and toxicity. Nanosuspension is the dispersion of active pharmaceutical ingredient (API) with sub-micron particle size in an aqueous medium. It can be prepared by comminution of larger particles (top-down approach) or by precipitation of dissolved drugs (bottom up approach) [1, 2]. Compared to amorphous solid dispersions, the other popular enabling formulation technology, nanosuspension requires less material and a shorter time for development. It also allows higher drug loading for more efficient drug delivery. Therefore, it has many applications in supporting the profiling of large numbers of new chemical entities (NCE) resulting from high throughput screening drug discovery process.

Bench-scale wet milling has been used in the industry to prepare nanosuspensions for discovery support for years [3-5]. Although the particle size of some of the compounds studied can be effectively reduced by the wet milling procedure, it is also found that certain materials have higher size limits under the same milling conditions. Despite the popularity, little information is available on the overall performance of the system and most importantly what range of particle sizes this bench method can provide. Most importantly, how to predict the overall efficiency of wet milling on new compounds is still necessary. Several parameters such as compound surface energy or Ostwald ripening have been speculated to be the key drivers for the final particle size, however, evidences are lacking [6, 7]. It is our belief that more predictive parameters are needed for researchers to estimate the efficiency of particle size reduction so the system can be better utilized In this study, we have attempted to identify potential critical properties that may dictate the particle size reduction limit upon wet milling using six poorly soluble compounds (Celebrex, DCU, Phenytoin, Fluticasone Propionate, Griseofulvin, Naproxen, and Nifedipine) with different physicochemical properties as model drugs. The results of the study may be used as a guideline in the evaluating the feasibility of forming a nanosuspension formulation.

2. EXPERIMENTAL SECTION

2.1. Materials.

HPLC grade acetonitrile was obtained from Burdick & Jackson (Muskegon, MI); reagent grade formic acid was obtained from EM Science (Gibbstown. NJ), Celebrex, 1, 3-dicyclohexyl urea (DCU), Diphenylhydantoin, Fluticasone Propionate, Griseofulvin, Naproxen, Nifedipine, and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO).

Lead free glass beads (0.5-0.75 mm) were purchased from Glen Mill (NJ) and pre-conditioned in house. The water purification system used was a Millipore Milli-Q system. The particle size distribution of regular suspension and nanosuspension were measured by using Mictrotrac®S3500 and Nanotrac® (PA, USA) instrument. The particle size distribution was calculated based on the general purpose (normal sensitivity) analysis model

and the following refractive indices: particle RI: 1.58, absorption: 1.0 and dispersant RI: 1.38.

2.2. PXRD andDSC Analysis for Melting point and Melting Enthalpy Determination.

The PXRD pattern was recorded at room temperature with a Rigaku (Texas, USA) MiniFlex II Desktop X-ray Powder Diffractometer. Radiation of Cu Kα at 30 KV -15 mA was used with 2θ increment rate of 3°/min. The scans run over a range of 2-40° 2θ with a step size of 0.02° and a step time of 2s. The pre and post milling samples were placed on a flat Silicon Zero Background sample holder to obtain the PXRD pattern. Differential scanning calorimetry was performed on a TA instruments Q-100 modulated DSC to determine measure melting point (MP) and melting enthalpy of each model compounds. Samples were initially equilibrated at 25°C then were heated to 350°C at 10 °C/min.

2.3. Solubility Determination.

The solubility of the model compounds in the suspending vehicles was measured. Each compound was added in excess amount to 4 mL glass vials containing 1 mL of the vehicle. The samples were shaken for 24 hours at room temperature to allow the system to reach equilibrium before subject to HPLC or LC/MS analysis.

2.4. HPLC Method.

A gradient reversed-phase HPLC method was developed to quantify the amount of drug dissolved for most of compounds studied except for DCU. The HPLC system composed of an Agilent HP diode array (DAD) detector, a quaternary solvent delivery system (Palo Alto, CA), a C8 (5 µm, 4.6 * 150 mm) column. Mobile phase solvent line A containing acetonitrile with 0.1% TFA (v/v), solvent line B containing Milli-Q water with 0.1% TFA (v/v).

2.5. LC/MS/MS analysis.

Due to lack of UV chromophores, the solubility DCU was determined by LC/MS analysis. The method was described as follows. The HPLC separation was performed on an Accela pump and Accela autosampler. The analytes were separated on a 3.0 x 150 mm ACE C18 column (3 um in particle size). The composition of mobile phase A is 0.1% acetic acid in water. The mobile phase B is Acetonitrile. The gradient program was: 0 min, 10% B; 7min, 90% B; 10 min, 10% B. The flow rate was 0.5 ml/min. The injection volume was 5 ul.

The subsequent MS analysis was carried out on a triple stage quadpole mass spectrometer (TSQ Quantum Access Max, Thermo Fisher Scientific). The mass spectrometer was equipped with a heated-ESI interface that was operated in positive ion mode. For DCU, m/z transition was 225.1à100.1, at collision energy of 23eV.

2.6. Surface Energy Measurements.

Surface energy measurements were carried out on an SEA Surface Energy Analyzer (Surface Measurement Systems Ltd., London, UK). Samples were packed into silanised glass columns (30 cm long, 6-mm OD2mm ID).Prior to measurement the sample was pre-treated at 60ºC for 60 min to allow for equilibrium to be reached between the samples and gas carrier. The samples were then measured at 25ºC with carrier gas flow rate of 10 ml/min. The dispersive contribution of the surface energy were determined using Decane, Nonane, Octane, and heptane as the probe molecules while Ethanol, Ethyl acetate, and Dichloromethane were used to measure specific free energies representing the interaction between polar probes and the samples.

2.7. Wet Milling.

A bench scale wet milling devise described previously [3-5] was used for particle size reduction.All the model compounds are suspended in 0.05 % (w/w) Tween 80 solution at concentration of 50 mg/mL. Corresponding amount of glass beads (1.5 times weight by weight of the final formulation) was added into the suspensions of each model compounds. The mixtures were then stirred at 1200 rpm for a period of 72-96 hours. Particle sizes of each preparation at a couple of time points were determined until steady state was achieved. For the second part of study, the stirring speed was increased to 1600 rpm to investigate the grinding force effects on the steady-state particle size (if any). For solubility effects on particle size at steady state, nifedipine and celebrex were wet-milled in 0.1N HCl /0.05% Tween 80 and 0.1 N NaOH/0.05% Tween, respectively.

3. RESULTS SECTION

There are various patents and publications of nanoparticulate drug preparations and applications [8-23]. In practice, nanoparticles can be produced by two general approaches. These are: 1) constructing particles from their molecular state, such as fast precipitation or rapid expansion; or 2) by breaking large particles, such as by milling. Because of practical reasons, the method of wet milling for fabricating nanoparticles is a more popular choice in the industry for the drug discovery setting. However, the approach of making and maintaining a stable nanoparticulate system is not free of problems. Challenges such as solid form changes and physicochemical stability of the formulations need to be addressed.

The most common physical stability issues include sedimentation, agglomeration, crystal growth and change of crystallinity state [24].The potential for particle agglomeration has been addressed by researchers and summarized in great detail (8, 16). In theory, the new surface area generated by particle size reduction ΔA, is based on a ΔG (Gibbs free-energy) and H (Enthalpy) cost and it is defined by;

$dG = -dWnet$: where *Wnet* is the surface energy

$\Delta G = \gamma_{s/l} * \Delta A$: where $\gamma_{s/l}$ is the interfacial tension.

$H' = G' - T(\partial G'/\partial T)_p = \gamma - T(\partial\gamma/\partial T)_p$ where *H'* is the enthalpy of the surface per unit.

The ΔG increase due to the increase in surface area by milling or precipitation will create a less stable system. Such a system will have a tendency to offset the increase in surface area and thereby reduce ΔG by agglomeration or limiting particle size. This phenomena can be effectively controlled by introducing surfactants (reduce $\gamma_{s/l}$) and maintaining good particle size control. The addition of surfactants can provide stabilization at longer times due to an increased energy barrier and by preventing particles from coming close enough to cause agglomeration [8]. It has been well recognized that there is a close relationship between

solubility and interfacial tension between a solid and a liquid. The relationship was firstly described by Ostwald- Freundlich equation [25].Despite the fact that a considerable amount of work has been done to verify the above, there is little unambiguous experimental evidence confirming their validity and it is generally believed to be more complicated [25] and less predictive. In order to investigate this, we selected 6 compounds represents a wide variety of properties for investigation. The physicochemical properties of model compounds: Celebrex, DCU, Phenytoin, Fluticasone Propionate, Griseofulvin, Naproxen, and Nifedipine were listed in Table 1 and their particle sizes at steady state upon wet milling were presented in Table 2. No form change was found by DSC and PXRD by comparing pre and post milling samples.

The evaluated properties were molecular weight, acid dissociation constant, partition coefficient (logP), melting point (MP), melting enthalpy, solubility, and surface energy. Of them, MP, melting enthalpy, solubility, and surface energy were determined empirically while the rest of properties were referred to literature values. The parameters evaluated in this study are often used as indications of hydrophobicity, crystalline lattice energy, and surface activities of drug molecules.

Previous investigators have reported a direct correlation with logP and melting enthalpy on the feasibility of preparing stable nanosuspension formulation when they were studying the stabilization mechanism of polymer stabilizers as a function of drug properties [24]. They have concluded that potential candidate for wet comminution was a drug substance with a high enthalpy and hydrophobicity. However, in this study, we found compounds with higher enthalpy generally gave larger particle size distribution at steady state in the system without a stabilizer.

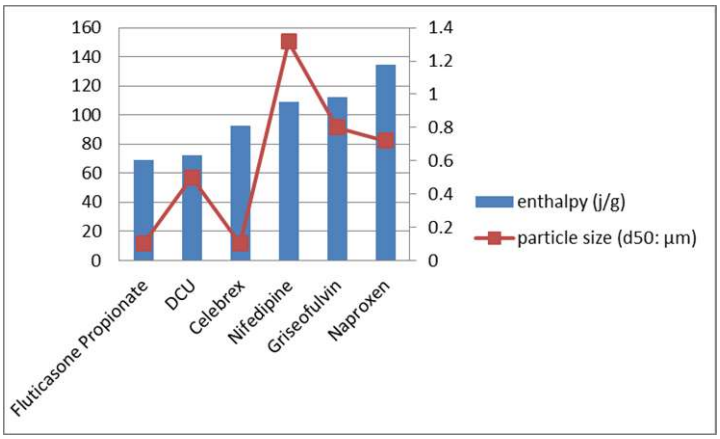

Figure 1. Melting Enthalpy versus the Mean Particle Size of Model Compounds at Steady State.

Generally speaking, as illustrated in Figure 1, lower enthalpy resulted in smaller particle size when no stabilizing excipients were present in the formulation. Of the properties evaluated in our work, drug solubility also displayed a certain degree of correlation with the particle size measured at steady state. Furthermore, increasing stirring speed (providing more energy) doesn't seem to change the final particle sizes distribution significantly. This finding agrees with our hypothesis, that the steady-state particle size is more dependent on the system's free energy. Appling more energy to the system may not result in further reduction of the particle size. Since no impacts of grinding energy on particle size at steady state were observed, no further investigations were conducted on comminution effects.

Figure 2 shows compounds with solubility at single micrograms per milliliter range are more likely to form particles with lower sub-micron size upon wet milling than those with higher solubility.

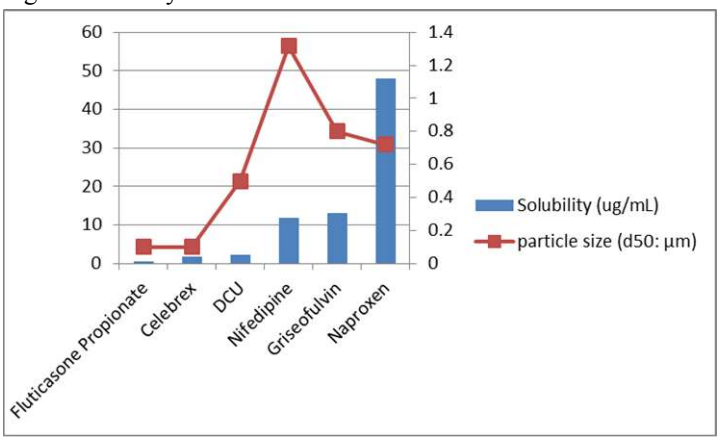

Figure 2. Solubility versus The Mean Particle Size of Model Compounds at Steady State

The solubility effect on the nanoparticle formability is most likely due to Ostwald ripening phenomena in which small particles in solution dissolve and deposit on the surface of large particles and it is known Ostwald ripening is driven by a concentration gradient [26]. We hypothesize that since the local concentration around smaller particles is larger than the average concentration around larger particles, a net flux of molecules flowing from smaller particle to larger particles lead to shrinking of the small particle and growth of the large particle. As a result, the system would have a larger mean diameter of a particle size distribution (PSD). The Ostwald ripening phenomena may be less prominent for poorer soluble compounds due to potential lower concentration gradient. Therefore, compounds with lower solubility can more easily form man-sized particles. The observations of this study are also consistent with the proposed theory. In order to further examine the solubility effects, we have used vehicles with higher solubilizing capacity for Nifedipine and Celebrex as wet milling suspending agent in another set of experiment.

As shown in table 1A and table 2, the solubility of Nifedipine in 0.1 N HCl/0.05% Tween 80 was 170.4 µg/with corresponding steady state particle size of approximately19.8µm (D_{50}) while that of Celebrex in 0.1 N NaOH/0.05% Tween 80 was 50.6 µg/mL with corresponding steady state particle size of approximately 10 µm (D_{50}).The data is in agreement with our hypothesis that lower solubility is required to achieve better particle size reduction upon wet milling. The result is not surprising since a higher concentration will give higher re-crystallization rate due to more frequent collision between molecules. As a result, there is a greater tendency for particle size to grow.

No direct correlations can be drawn between particle size and other properties evaluated. Wang et al has reported that the surface energy of drug and polymer stabilizer is an important but not only factor in determining the steady state value of the nanosuspension particle size resulted from wet milling (6). However, our finding didn't demonstrate the same relationship. According to our analysis, the surface energy of a drug starting

material did not significantly impact the particle size distribution at steady state after wet milling in a drug/surfactant system compared to that observed for drug/polymer stabilizer systems.

In summary, the result of our study have shown melting enthalpy and solubility of drug candidates have the highest correlation with the final particle size distribution at steady state of wet milled suspensions. These two properties may be used as preliminary assessment on the feasibility of forming nanoparticles upon wet milling. Further study is needed to further understand the impacts of enthalpy and solubility on particle size distribution of wet milled suspensions.

Table 1A. Physicochemical Properties of the Model Compounds.

Drug	M.W.	pKa	log P	Tm (°C)	enthalpy (j/g)	Solubility (ug/mL)	Surface Energy (mJ/m^2)
Celebrex	381.4	11.1	3.9	158	92.6	1.8	47.8
DCU	224.3	13.89	5.3*	224	72.15	2.3	42.3
Diphenylhydantoin	252.3	8.3	2.2	298.4	138.9	20.9	77.4
Fluticasone Propionate	500.6	12.55	3.4	288.7	69.02	0.51	48.0
Griseofulvin	352.8	17.69*, -4.3*	2.2	220	112.3	13.1	43.1
Naproxen	230	4.15	3.2	157.1	134.6	47.9	47.9
Nifedipine	346.3	3.93	2.3	174.6	108.8	11.8	51.1

Table 1B. Solubility of Nifedipine in 0.1 N HCl/0.05% Tween 80 and Celebrex in 0.1 N HCl/0.05% Tween 80.

Drug	Solubility (ug/mL)
Nifedipine	170.4
Celebrex	50.6

Table 2. The Mean Particle Size of Model Compounds at Steady State Upon Wet Milling.

Drug	Particle Size (d50 in μm)	
	Without 0.1 N NaOH	With 0.1 N NaOH
Celebrex	0.1	10.93
DCU	0.5	
Diphenylhydantoin	0.8	
Fluticasone Propionate	0.1	
Griseofulvin	0.8	
Naproxen	0.7	
	Without 0.1 N HCl	With 0.1 N HCl
Nifedipine	1.3	19.82

4. CONCLUSIONS

The bench scale wet milling device can effectively prepare nanosuspensions for compounds with appropriate properties. Of the physicochemical properties studied, higher melting enthalpy and lower solubility generally rendered lower particle size distribution at steady state upon wet milling. Surface energy and other properties have found has little or no effects on the particle size of wet milled suspensions.

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