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Research paper

Spray granulation: Importance of process parameters on *in vitro* and *in vivo* behavior of dried nanosuspensions

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ABSTRACT

The use of fluid bed granulation for drying of pharmaceutical nanoparticulates on micron-sized granule substrates is a relatively new technique, with limited understanding in the current literature of the effects of process parameters on the physical properties of the dried nanoparticle powders. This work evaluated the effects of spray mode, spray rate and atomizing pressure for spray granulation of drug nanosuspensions through a systematic study. Naproxen and a proprietary Novartis compound were converted into nanosuspensions through wet media milling and dried onto a mannitol based substrate using spray granulation. For naproxen, various physical properties of the granules, as well as the *in vitro* re-dispersion and dissolution characteristics of the nano-crystals, were measured. It was found that the spray mode had the most drastic effect, where top spray yielded smaller re-dispersed particle sizes and faster release rates of drug from granules than bottom spray. This was attributed to the co-current spraying in bottom spray resulting in denser, homogenous films on the substrate. Similar *in vitro* results were obtained for the proprietary molecule, Compound A. *In vivo* studies in beagle dogs with Compound A showed no significant difference between the liquid and the dried forms of the nanosuspension in terms of overall AUC, differences were observed in the t_{max} which correlated with the rank ordering observed from the *in vitro* dissolution profiles. These findings make spray granulation amenable to the production of powders with desired processing and handling properties, without compromising the overall exposure of the compound under investigation.

1. Introduction

A significant number of molecules currently under development [1] can be classified as BCS (Biopharmaceutical Classification System) Class II compounds [2], with dissolution rate limited solubility resulting in sub-optimal bioavailability. Recently, a revised classification system called the Developability Classification System (DCS) has been proposed, which further divides BCS Class II into two sub classes, IIa for dissolution rate limited and IIb for solubility rate limited compounds [3]. For compounds where dissolution is the rate limiting factor, production of nanoparticles and microparticles using wet media milling can improve the dissolution rate by increasing the surface area through particle size reduction based on the Noyes–Whitney equation [4]. This approach to delivering poorly water soluble compounds has been well proven, as several products manufactured using this technology are currently approved and available in the market [5].

Colloidal drug particles produced by wet media milling are typically stabilized against particle agglomeration using either steric (by means of polymers or nonionic surfactants) or electrostatic (by means of ionic surfactants) stabilization mechanisms, or sometimes a combination of both mechanisms which is referred to as electrosteric stabilization [6,7]. Although there is one instance of a nanosuspension product being marketed in the liquid form (Megace ES), conversion of a nanosuspension into a dried powder form that can be further filled into capsules or compressed into tablets is often desirable to ensure the maximum patient compliance. The drying step can be further necessitated by the fact that there could be possible stability issues, both physical (such as Ostwald ripening and agglomeration) and chemical (such as hydrolysis) associated with nanoparticles in their suspended form [8,9]. Additionally, for nanoparticles, there could also be a risk of crystallization upon storage if partial surface amorphization occurs during the wet milling process as has been reported by Sharma et al. [10]. However, it should be pointed out that it is also possible to

Abbreviations: HPC-EXF, hydroxypropylcellulose-EXF grade; SLS, sodium lauryl sulfate; DOE, design of experiment; ANOVA, analysis of variance; LOD, loss on drying; XRD, X-ray diffraction; FaSSGF, fasted state simulated gastric fluid; CMC, critical micelle concentration; FRI, flow rate index; ff_c, Jenike flow function; PDI, polydispersity index.

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generate an amorphous fraction in a dried nanosuspension, if the drug has some solid solubility in the stabilizer [11].

Freeze-drying and spray drying are the most common processes used to convert a nanosuspension into a solid form [12–16]. Both these processing methods result in powders that require further processing to improve the bulk density and flow properties prior to conversion into a tablet or a capsule dosage form. Granulation based approaches, which typically result in densification with improved flow properties of the powder, have also been used but only to a limited extent, with most studies focusing only on formulation parameters. For example, Basa et al. reported the layering of a ketoconazole nanosuspension formulation onto a lactose monohydrate substrate, although no evaluation of different formulation or process parameters was conducted [17]. Kayaert and co-workers studied the layering of naproxen and cinnarizine nanosuspensions onto sugar beads and focused on the effect of different formulation parameters of the nanosuspension, such as stabilizer type, concentration and free stabilizer, on coating efficiency and dissolution profiles [18]. Recently, we have shown the feasibility of a spray granulation based process to convert a nanosuspension of a proprietary Novartis compound into a solid dosage form, where formulation parameters such as the nanosuspension stabilizer, the substrate onto which the nanosuspension was coated and the drug loading were studied [19]. However, none of these studies have focused on the processing parameters that would be critical in drying a nanosuspension using a spray granulation based process. This lack of information on the critical process parameters of the spray granulation process for drying of nanosuspensions is a significant gap that needs to be investigated.

Naproxen has been widely used as a model compound to study wet media milling [8,18,20,21] and was selected as the model compound in this study to evaluate the processing parameters critical to drying of nanosuspensions using spray granulation. Naproxen is practically insoluble in water and has a melting point of 154 °C and a molar mass of 230.26 g mol^{-1} [22]. The critical parameters identified from the trials with naproxen were then confirmed with a Novartis developmental compound (further referred to as Compound A). Compound A is a poorly water soluble free base molecule with an equilibrium water solubility of 0.003 mg/mL at 25 °C, melting point of 263 °C, molecular weight of 388.4 g mol^{-1} and mean particle size of 15–20 μm [19].

A nanosuspension formulation of naproxen with demonstrated physical, chemical and dissolution stability for up to 9 months at 2–8 °C was used as the starting material for the process optimization trials. Process parameters such as nozzle atomization pressure and liquid spray rate, typically considered critical in a fluid bed spray granulation process [23], were investigated in this study. Additionally, two different spray modes were also evaluated. Powder properties, re-dispersibility, solid-state properties and the rate of dissolution were measured for the naproxen granulations obtained from the process optimization trials. The optimized process parameters from these trials were then used to manufacture dried nanosuspensions of Compound A. Two dried nanosuspension formulations of Compound A, with different *in vitro* release profiles, were compared in an *in vivo* study in fasted male beagle dogs against the coarse suspension and the parent nanosuspension formulations, to assess the implications of differences observed in *in vitro* dissolution profiles to *in vivo* exposure.

2. Materials and methods

2.1. Materials

Naproxen USP was purchased from Kalchem International (Lindsay, OK) and Compound A was provided by Novartis Pharmaceuticals Corporation. The sources of other materials used in the study were as follows: hydroxypropylcellulose-EXF grade (HPC-EXF) (Klucel) from Ashland Inc. (Covington, KY, USA), sodium lauryl sulfate (SLS) from TensaChem S.A. (Ougree, Belgium), mannitol (DC grade) from Roquette (Lestrem, France) and hard gelatin capsules (size 0) from Capsugel Inc. (Greenwood, SC, USA) respectively. Deionized water (18 MΩ) produced with a Millipore water system (Billerica, MA, USA) was used as the dispersion medium. Yttrium stabilized zirconium oxide beads with 0.2 mm diameter (Tosoh Corp., Tokyo, Japan) were used for the milling process. All other solvents and reagents used were of HPLC or analytical grade.

2.2. Preparation of nanosuspension

Formulations were composed of either 10% w/v of naproxen or Compound A, 2.5% w/v of HPC-EXF and 0.5% w/v of SLS. Suspensions were prepared by dissolving the excipients in an aqueous solution, into which the active was dispersed. The resulting suspension was wet milled with 0.2 mm grinding media using a Netzsch Labstar Mill (Exton, PA, USA) with a Zeta agitator in the recirculation mode. The following process parameters were used: pump speed of 250 rpm, agitator speed of 2000 rpm and milling time of 4–6 h. Particle size testing of in-process samples was carried out at regular time intervals throughout the duration of the milling process. The pH of naproxen and Compound A nanosuspensions after wet media milling was measured to be around 5.1 and 5.8, respectively.

2.3. Particle size measurement of nanosuspension

The particle size of the nanosuspension formulations was characterized using a Delsa Nano™ C particle size analyzer (Beckman Coulter, Brea, CA, USA). As per manufacturer claims, this instrument is capable of measuring particles in the size range of 0.6 nm to 7 μm through the use of dynamic light scattering; all samples measured were in the specified size range. A small amount (about 5 μL) of nanosuspension sample was added to a disposable cuvette and diluted with 5 mL of deionized water. The cuvette was manually shaken for about 10 s and placed inside the sample holder of the particle size analyzer. Once the intensity was within the permissible range, analysis was performed to obtain the particle size and the polydispersity index. All particle size measurements were performed in triplicate at 25 °C. All reported particle size data refer to intensity weighted size distributions.

2.4. Fluid bed granulation of nanosuspension

Fluid bed granulation of the nanosuspension was performed using a Huttlin Mycrolab (Huttlin GmbH, Schopfheim, Germany) fluid bed drier. The equipment can be operated such that the liquid feed can be sprayed from above into the fluidized bed (top spray) or from below up into the bed (bottom spray). The gas used was non-processed air heated to an inlet temperature between 70 and 80 °C and fed at a flow rate ranging from 8 to 15 m^3/h (adjusted for each granulation trial to maintain adequate fluidization). The inlet air temperature used in this study is within the range cited in the literature [24]. The mannitol substrate (82.5 g) was loaded onto the fluid bed prior to spraying and allowed to equilibrate to feed gas temperature. Sufficient fluidization of the substrate was maintained to ensure that there was no agglomeration of mannitol particles during the granulation process. The nanosuspension was sprayed onto the mannitol DC substrate to achieve a theoretical drug loading of 17% in the granulation using the process parameters (spray mode, liquid spray rate, and nozzle atomization pressure) listed in Table 1. The product temperature was maintained within the range of 37–42 °C throughout the process. At

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