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Research paper

Comparison of powder produced by evaporative precipitation into aqueous solution (EPAS) and spray freezing into liquid (SFL) technologies using novel Z-contrast STEM and complimentary techniques

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Abstract

The objective of this study was to compare the properties of particles formed by nucleation and polymer stabilization (e.g. evaporative precipitation into aqueous solution (EPAS)) versus rapid freezing (e.g. spray freezing into liquid (SFL)). Powders formed by EPAS and SFL, composed of danazol and PVP K-15 in a 1:1 ratio, were characterized using X-ray powder diffraction, modulated differential scanning calorimetry (MDSC), contact angle determination, dissolution, scanning electron microscopy (SEM), environmental scanning electron microscopy (ESEM), BET specific surface area, and Z-contrast scanning transmission electron microscopy (STEM). Large differences in particle morphologies and properties were observed and explained in terms of the particle formation mechanisms. Both techniques produced amorphous powders with high T_g and low contact angle values. However, STEM analysis showed highly porous bicontinuous nanostructured 30 nm particles connected by narrow bridges for SFL versus aggregated 500 nm primary particles for EPAS. The combination of STEM and other characterization techniques indicates solid solutions were formed for the SFL powders consistent with rapid freezing. In contrast, the EPAS particle cores are enriched in hydrophobic API and the outer surface is enriched in the hydrophilic polymer, with less miscibility than in the SFL powders. Consequently, dissolution rates are faster for the SFL particles, although both techniques enhanced dissolution rates of the API.

Keywords: Particle engineering processes; Precipitation; Freezing

1. Introduction

The influence of active pharmaceutical ingredient (API) solubilization on bioavailability has become increasingly important in the pharmaceutical industry. Many APIs are poorly water soluble with high mucosal permeability [1]. These compounds are classified as biopharmaceutical classification system II compounds for which their maximum bioavailability is limited by their rate of dissolution [2]. Improving the dissolution rate of these compounds is achieved through an increase in surface area available for dissolution by decreasing the particle size of the API and optimizing its wetting characteristics [3] or through complexation with cyclodextrins [4]. Several methods have been used to impose these characteristics on poorly soluble APIs. Milling and solution based techniques have been reviewed extensively [5–7]. Our laboratories have introduced two technologies for enhancing the dissolution rate of a poorly water soluble API by creating nanostructured particles using spray freezing into liquid (SFL) [8–17] and evaporative precipitation into aqueous solution (EPAS) techniques [18–21].

The SFL process creates micronized powders with enhanced dissolution rates. This is a particle engineering

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process that utilizes the atomization of a feed solution containing an API and dissolution enhancing excipient(s) directly into a cryogenic liquid, such as nitrogen. The resulting dried powder is composed of discrete microparticles where the API is molecularly dispersed with a polymer in a porous matrix. This molecular dispersion is achieved by rapid freezing in liquid nitrogen, which prevents phase separation. In previous studies, it was found that enhanced dissolution is due to the amorphous nature of the produced powder, high surface area and enhanced wettability of the SFL nanostructured particles [17]. A schematic representation of the SFL apparatus has been reported [12]. During the SFL process, the API loaded organic solvent is pressurized and atomized via a poly ether–ether ketone (PEEK) nozzle below the surface of liquid nitrogen. Because of the rapid flow rate, liquid–liquid impingement and marked temperature drop in the jet, the emitted solvent is atomized into fine, high surface area microdroplets that are frozen rapidly. The suspension of frozen microdroplets is lyophilized to remove solvent, resulting in an amorphous, micronized powder.

In the EPAS process, the API precipitates due to evaporation of the organic solvent near or above the boiling point and contact with an aqueous solution. A schematic representation of the EPAS process has been reported [21]. The API loaded organic solvent is pressurized, heated and atomized through an elliptical conical stainless steel nozzle into a heated water bath containing stabilizing excipients. The large pressure drop across the small nozzle orifice creates intense atomization with rapid evaporation of the primary organic solvent due to the high temperature of the feed solution and aqueous receiving solution. The rapid evaporation of the feed solvent results in supersaturation, nucleation and precipitation of the API. The excipients within the organic feed solution and/or aqueous receiving vessel stabilize the particles by preventing particle growth and recrystallization of the API precipitates. In addition, the excipients also enhanced the API particle dissolution rate and long term storage stability [21].

The morphology and performance of a nanostructured powder is expected to be highly dependent upon the particle formation process. Solid solution formation can be achieved through co-precipitation or co-melting [3,22]. Co-precipitation and co-melting require supersaturation of the API within the matrix to be limited in time by rapid cooling and rapid removal of solvent so as to inhibit agglomeration of API particles. Solid dispersion formation is achieved through supersaturation and precipitation of the API within the matrix similar to solid solution formation. However, solid dispersions are formed during processes where nucleation and growth of the particles occurs to varying extents or through fusion of the component particles [23].

Because of the nanoscale size of API domains achieved through EPAS and SFL processing, it is difficult to evaluate the particle size and morphology of the powders. Techniques used to evaluate products formed by particle engineering technologies typically include dissolution, X-ray powder diffraction, particle size analysis (laser light scattering or dynamic light scattering), contact angle and surface area analysis. More recently, several authors have utilized atomic force microscopy (AFM) to evaluate nanoparticles adsorbed on to the surface of glass or a film [24–27]. This technique, although useful for assessment of particles, requires that a dispersion be formed and surrounding excipients be removed from the API. This limits the technique for assessment of colloidal dispersions and other dispersible solids. Also, transmission electron microscopy (TEM), in conjunction with staining materials, has been used to evaluate the presence and size of API domains within an excipient matrix [8,28–30]. Z-contrast TEM or STEM dark-field imaging is a novel method for high resolution viewing of API/excipient mixtures without the use of electron density staining or dispersion formation and drying. This tomography process has been used in the semiconductor industry and requires the use of a high-angle annular dark field (HAADF) detector [31]. The high-angle scattering is associated with electron interaction close to the nucleus of the atom. For this reason, the detector is very sensitive to compositional changes and thickness within the specimen. It has not been used previously on pharmaceutical formulations and could prove to be a valuable asset to researchers investigating and characterizing nanoparticles where dispersion in a solvent or staining can remove or mask important morphological features.

The objective of this study was to evaluate and compare particle morphologies produced by the two technologies, SFL and EPAS, in order to assess how morphology impacts the enhancement of the dissolution rate of a poorly water soluble API. The particles were evaluated based on degree of crystallinity, thermal characteristics, dissolution rates and morphologies. The high magnification in Z-contrast STEM provides a clearer view of the morphology at the primary particle level than SEM. STEM micrographs and other particle properties were analyzed in context of the particle formation mechanisms to assess the degree of miscibility of the API and polymer. In the organic SFL solution, both polymer and API nucleate and grow to form amorphous domains during the rapid freezing [10]. In contrast, the presence of an organic–water interface and an aqueous external phase in EPAS provides a driving force during nucleation and growth for the core of the particles to be enriched in hydrophobic API and the outer surface to be enriched in the hydrophilic polymer [21]. Only small amounts of polymer are required at the outer surface to stabilize the particles [19]. API/stabilizer ratios have been as high as 93% for particles with surface areas of 3 m^2/g. It is hypothesized that the differences in the particle formation mechanisms for the EPAS and SFL processes are likely to produce differences in polymer API miscibility and subsequent API bioavailability.