International Journal of Pharmaceutics 473 (2014) 10–19

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A new and improved method for the preparation of drug nanosuspension formulations using acoustic mixing technology

Dennis H. Leung [a,*], David J. Lamberto [a], Lina Liu [a], Elizabeth Kwong [b], Todd Nelson [a], Timothy Rhodes [a], Annette Bak [a]

[a] Merck & Co., Inc., Rahway, NJ, United States
[b] Kwong Eureka Solutions, Montreal, Quebec, Canada

ARTICLE INFO

Article history:
Received 13 January 2014
Received in revised form 19 March 2014
Accepted 2 May 2014
Available online 28 June 2014

PubChem classification:
Naproxen CID: 156391

Keywords:
Formulation
Nanoparticles
Nanotechnology
High throughput technologies
Particle size
Milling

ABSTRACT

Drug discovery and development is a challenging area. During the drug optimization process, available drug compounds often have poor physicochemical and biopharmaceutical properties, making the proper *in vivo* evaluation of these compounds difficult. To address these challenges, drug nanoparticles of poorly soluble compounds have emerged as a promising formulation approach. Herein, we report on a new drug sparing technology utilizing low shear acoustic mixing to rapidly identify optimized nanosuspension formulations for a wide range of compounds with dramatically improved material and time efficiencies. This approach has several key advantages over typical methods of preparing nanoparticles, including miniaturization of the milling process, the ability to evaluate multiple formulation conditions in a high throughput manner, and direct translation to optimized formulation scale-up for *in vivo* studies. Furthermore, there are additional benefits obtained with this new approach resulting in nanosuspension formulations with significant stability and physical property enhancements over those obtained using traditional media milling techniques. These advantages make this approach highly suitable for the rapid evaluation of potential drug candidates in the discovery and development space.

1. Introduction

The discovery and development of new drugs is a complex activity. Despite increasing investments in research and development, the number of new drug approvals has not increased while the attrition rate of new drug candidates has increased (PhRMA, 2011; Munos, 2009; Scannell et al., 2012). Many of these difficulties are due to failures in drug development in the clinic due to lack of effective predictions of therapeutic and toxicological responses (Kola and Landis, 2004). In order to improve the quality of drug candidates progressing into the clinic for late stage development, one strategy is to pursue an appropriate evaluation of the pharmacokinetics and pharmacodynamics of drug candidates in animal models using translatable formulation approaches to improve early understanding of efficacy and toxicology. One approach to address this issue is to increase collaboration between discovery and development groups to minimize the number of drug candidates with development risks (Kwong et al., 2011; Venkatesh and Lipper, 2000).

However, early in drug discovery and development the availability of chemical matter can be limited. In addition, during the drug optimization process, available active pharmaceutical ingredients (APIs) often have poor physicochemical and biopharmaceutical properties with low aqueous solubility, metabolic instability, and unoptimized potency (Gleeson, 2008; Gleeson et al., 2011; Kwong et al., 2011; Lipinski, 2002; Venkatesh and Lipper, 2000; Wenlock et al., 2003). As a result, it can be a significant challenge to deliver the drug substance to the target receptor in order to provide an early evaluation of *in vivo* efficacy and toxicity. Typical conventional formulations of these drug candidates such as suspensions may result in poor absorption and bioavailability making it difficult to interpret the results of these studies (Fahr and Liu, 2007; Stegemann et al., 2007).

To address these challenges, drug nanoparticles of poorly soluble compounds have emerged as a promising formulation approach (Kesisoglou et al., 2007; Merisko-Liversidge and Liversidge, 2011; Merisko-Liversidge and Liversidge, 2008; Pu et al., 2009; Rabinow, 2004). Nanosuspension formulations consisting of sub-micron sized nanocrystalline drug particles of generally <400 nm diameter suspended in aqueous solution can

* Corresponding author at: Merck & Co., Inc., 126 East Lincoln Ave., P.O. Box 2000, Rahway, NJ, United States. Tel.: +1 732 594 6982; fax: +1 732 594 4858.
E-mail address: dennis_leung@merck.com (D.H. Leung).

http://dx.doi.org/10.1016/j.ijpharm.2014.05.003
0378-5173/

significantly improve the oral bioavailability of some APIs. For oral administration, the first step in absorption is dissolution of the API in the gastrointestinal region. The small size and large exposed surface area of the nanoparticles can increase the rate of dissolution of the drug compound (Noyes and Whitney, 1897). This can result in improved rate of absorption, increased bioavailability, faster onset of action, minimized risk of food effect, and lower variability for some compounds, particularly those where absorption is limited by dissolution rate (Kesisoglou et al., 2007; Merisko-Liversidge and Liversidge, 2011). In addition, these nanosuspensions typically offer relatively high drug loading, making them an efficient method of drug formulation (Muller et al., 2011). Furthermore, drug nanoparticles are also amenable to a variety of additional administration routes such as allowing for direct systemic drug delivery *via* parenteral or inhalation administration, thus bypassing potential issues with oral absorption inherent to the compound (Muller et al., 2011; Sun and Yeo, 2012). This makes nanosuspension formulation approaches particularly attractive in the discovery space due to the potential to facilitate the rapid evaluation of early drug candidates with poor biopharmaceutical properties, such as carrying out critical *in vivo* proof of biology studies without spending extensive resources optimizing the chemical series. This can often lead to an early go/no go decision for pursuing specific targets, saving time and resources.

This nanoparticle formulation strategy has recently been used successfully in the development and commercialization of several drug products including Rapamune® and Emend® (Oliver et al., 2007; Shen and Wu, 2007; Wu et al., 2004), among others (Merisko-Liversidge and Liversidge, 2011). However, this process has been difficult to translate into the drug discovery space to support the evaluation of multiple drug candidates due to the often prohibitive amount of material and time resources required to identify a suitable nanosuspension formulation. While there have been some efforts in miniaturizing the nanomilling technique (Cooper et al., 2004; Van Eerdenbrugh et al., 2009a), the development of a general and rapid process to significantly improve the efficiency of this approach remains challenging and would be of considerable value.

Herein, we report on a new drug sparing technology utilizing acoustic mixing to rapidly identify optimized nanosuspension formulations for a wide range of compounds with dramatically improved material and time efficiencies. This approach has several key advantages over typical methods including miniaturization of the milling process, the ability to evaluate multiple formulation conditions in a high throughput manner, and direct translation to optimized formulation scale-up for *in vivo* studies. Furthermore, there are additional benefits obtained with this new approach resulting in nanosuspension formulations with significant stability and physical property enhancements over those obtained by using traditional media milling techniques. These advantages make this approach highly suitable for the rapid evaluation of potential drug candidates in the discovery and development space.

2. Materials and methods

2.1. Materials

Reagents and solvents were obtained from commercial sources and used as received unless otherwise noted. Compounds **1** and **2** were obtained from the Process Chemistry Department (Merck Research Laboratories, Rahway, NJ, USA) as crystalline free bases and used as received (Szewczyk et al., 2011). Loadings and concentrations are reported as weight percent (wt%) unless otherwise noted.

2.2. Experimental techniques

2.2.1. Particle size analysis

Particle size measurements of the nanosuspensions were obtained using a Wyatt DynaPro™ Plate Reader Dynamic Light Scattering instrument. The nanosuspension samples were diluted to approximately 1 mg/mL concentration and 25 μL was dispensed into a Corning® low volume black polystyrene 384-well plate for analysis. The particle size of each sample was reported as an average of 10 acquisitions with an acquisition time of 3 s at 25 °C. The autocorrelation curves obtained were fitted and the average particle diameter, D50, and D90 were determined using the cumulants or regularization model when appropriate. The normalized polydispersity (%Pd) was calculated as the polydispersity divided by the estimated hydrodynamic radius from the cumulant fit of the autocorrelation function multiplied by 100.

2.2.2. Optical microscopy visualization

Optical micrographs of the nanosuspensions were obtained using a Carl Zeiss Axiovert 200M inverted microscope equipped with AxioCam and AxioVision software connected to a desktop computer. A drop of the nanosuspension formulation was placed on a microscope slide and under a cover slip. The sample was analyzed under transmitted-light bright field mode using a 50× power objective to determine risk of formation of large aggregates.

2.2.3. Residual metal analysis

Quantification of residual zirconium, yttrium, and silicon concentrations in the formulations was determined using a PerkinElmer Elan 6000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Approximately 10 mg of sample was dissolved with 10 mL of 80% nitric acid for the analysis.

2.2.4. Viscosity measurement

Viscosity measurements of the nanosuspensions were determined using a PAC VISCOlab 3000 Laboratory Viscometer. The temperature was maintained at 25 °C using a recirculating water jacket. Viscosity measurements were taken on a sample until the viscosity of the sample stabilized and a standard deviation of $<0.2\%$ in the measurements was obtained.

2.3. Typical procedure for preparation of nanosuspensions using acoustic mixing

A suitable container was charged with 500 μm YTZ® Grinding Media from Tosoh (50% by volume). The API was then added as a solid. In a separate container, an aqueous solution of water and the appropriate amount of polymer and surfactant was prepared. Stock solutions of 1–5% w/w polymer or surfactant were used. The aqueous mixture was then added to the sample container containing the drug and grinding media. The slurry was stirred gently using a spatula to ensure that the drug solid was suitably wetted, resulting in a mixture containing the drug slurry and grinding media with a total volume of ~90% of the volume of the container. The container was sealed and then mixed on the Resodyn LabRAM ResonantAcoustic® mixer at 40% intensity (~50 G acceleration) for up to 90 min. Aliquots of the suspension were taken for particle size analysis every 30 min. The nanosuspension formed was recovered and separated from the grinding media using a syringe equipped with a 25 gauge syringe needle or filtration through a 200 μm stainless steel mesh and characterized using the procedures above.