International Journal of Pharmaceutics 520 (2017) 79–85

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Accelerated *in vitro* release testing method for naltrexone loaded PLGA microspheres

Janki V. Andhariya[a], Stephanie Choi[b], Yan Wang[b], Yuan Zou[b], Diane J. Burgess[a], Jie Shen[a,1,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, CT, 06269, USA
[b] Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, FDA, Silver Spring, MD, 20993, USA

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 24 January 2017
Accepted 25 January 2017
Available online 30 January 2017

Keywords:
PLGA microspheres
Naltrexone
Compositionally equivalent
USP apparatus 4
Accelerated *in vitro* release testing

ABSTRACT

The objective of the present study was to develop a discriminatory and reproducible accelerated release testing method for naltrexone loaded parenteral polymeric microspheres. The commercially available naltrexone microsphere product (Vivitrol®) was used as the testing formulation in the *in vitro* release method development, and both sample-and-separate and USP apparatus 4 methods were investigated. Following an *in vitro* drug stability study, frequent media replacement and addition of anti-oxidant in the release medium were used to prevent degradation of naltrexone during release testing at "real-time" (37 °C) and "accelerated" (45 °C), respectively. The USP apparatus 4 method was more reproducible than the sample-and-separate method. In addition, the accelerated release profile obtained using USP apparatus 4 had a shortened release duration (within seven days), and good correlation with the "real-time" release profile. Lastly, the discriminatory ability of the developed accelerated release method was assessed using compositionally equivalent naltrexone microspheres with different release characteristics. The developed accelerated USP apparatus 4 release method was able to detect differences in the release characteristics of the prepared naltrexone microspheres. Moreover, a linear correlation was observed between the "real-time" and accelerated release profiles of all the formulations investigated, suggesting that the release mechanism(s) may be similar under both conditions. These results indicate that the developed accelerated USP apparatus 4 method has the potential to be an appropriate fast quality control tool for long-acting naltrexone PLGA microspheres.

1. Introduction

Biodegradable polymeric microsphere based parenteral controlled release drug products have been widely used for long-term controlled delivery of small molecule therapeutics as well as biologics such as peptides and proteins owing to their various clinical advantages such as low dosing frequency and hence improved patient compliance, as well as their ability to maintain effective therapeutic concentrations over extended periods of time, thus enhancing product safety and efficacy (FDA Guidance for Industry, 1997; Burgess et al., 2004a). As the improved therapy of these controlled release drug products is rooted in the optimum drug concentration/time profiles at the site of action in the body, it is essential to understand drug release characteristics of these types of drug products to ensure product performance and safety.

"Real-time" *in vitro* release testing is typically conducted to characterize drug release characteristics under physiological conditions (Burgess et al., 2002; Mitra and Wu, 2010). However, "real-time" *in vitro* release testing of controlled release formulations often runs over a long period of time ranging from weeks to months, or even years (Hoffman, 2008; Mao et al., 2012; Wang and Burgess, 2012; Mitragotri et al., 2014), which if applied to batch release testing would result in reduced effective product shelf-life. Consequently, there is a need to develop fast and reliable quality control tool(s) to assure product performance as well as batch-to-batch reproducibility for consistent pharmacological effect. An accelerated *in vitro* release testing method, which increases drug

* Corresponding author at: Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, 69 North Eagleville Road U3092, Storrs, CT, 06269-3092, USA.
E-mail addresses: jie.shen@uconn.edu, jie_shen@uri.edu (J. Shen).
[1] Current address: University of Rhode Island, 7 Greenhouse Road, Kingston, RI, 02881, USA.

http://dx.doi.org/10.1016/j.ijpharm.2017.01.050
0378-5173/

release rate and hence reduces the testing duration, can aid in fast production batch release as well as speed up product development.

Various conditions such as high temperature (Shen and Burgess, 2012a), extreme pH (Faisant et al., 2002, 2006), organic solvent as well as addition of surfactant (Xie et al., 2015), have been used to accelerate drug release from PLGA microspheres (Shameem et al., 1999). All these approaches speed up the drug release rate *via* different mechanisms such as increased molecular mobility of the drug as well as the polymer chains and increased water/media penetration, all of which lead to fast drug diffusion and enhanced polymer degradation rate. It is known that drug release from polymeric microspheres is mainly controlled by a combination of drug diffusion and polymer erosion (Zolnik et al., 2006). Selection of an appropriate accelerated approach depends on various factors such as the polymer glass transition temperature (Faisant et al., 2002; Aso et al., 1994; Agrawal et al., 1997), and the drug stability under various stress testing conditions (Kim and Burgess, 2002). Ideally, accelerated *in vitro* release profiles should have 1:1 linear correlation with "real-time" release profiles after time scaling/shifting (Zolnik et al., 2006). A 1:1 linear correlation shows that the exposure of polymeric microspheres to such stress conditions does not significantly alter the underlying drug release mechanism(s) (Xie et al., 2015). However, the use of extreme conditions to accelerate drug release may change the mechanism (s) of drug release. Nevertheless, the accelerated drug release profiles should follow at least the same rank order as the "real-time" release profiles (Burgess et al., 2004b). Most importantly, the accelerated *in vitro* release testing method should be able to differentiate any significant variation in drug release characteristics of formulations in order to be used as a quality control tool, *i.e.* the method should show good discriminatory ability (Shen and Burgess, 2012b). This is because minor manufacturing process changes may result in alteration of the release characteristics (Shen et al., 2016).

Owing to the complexity of these controlled release drug products, currently there is no compendial method available for parenteral polymeric microspheres. Various *in vitro* release testing methods have been used to investigate "real-time" and accelerated *in vitro* release characteristics of parenteral polymeric microspheres, including dialysis, sample-and-separate and USP apparatus 4 methods (Shen and Burgess, 2012a; Rawat et al., 2011; Amatya et al., 2013; D'Souza et al., 2014). The sample-and-separate method is simple, and provides reasonably accurate assessment of *in vitro* drug release, which makes it useful during the initial stages of product development. On the other hand, the USP apparatus 4 (continuous flow through) method utilizes a compendial apparatus with well-defined geometry and hydrodynamic conditions and hence, offers various advantages such as better reproducibility (Andhariya and Burgess, 2016). The U.S. FDA has recommended that a dissolution method using USP apparatus 4, and, if applicable, USP apparatus 2 (Paddle) or any other appropriate method, should be developed for comparative *in vitro* release evaluation of such drug products (FDA-Recommended Dissolution Method Database). Accordingly, both sample-and-separate and USP apparatus 4 methods were investigated in the present study. The aim of the present work was to develop a reproducible and discriminatory accelerated *in vitro* release testing method for compositionally equivalent polymeric microspheres with manufacturing differences. Naltrexone (the active pharmaceutical ingredient in the commercial microsphere product Vivitrol®) was chosen as a small molecule model therapeutic. Compositionally equivalent polymeric microspheres were prepared using different manufacturing processes. Both sample-and-separate and USP apparatus 4 methods were investigated. Furthermore, the reproducibility and discriminatory ability of the developed accelerated release method were assessed.

2. Materials and methods

2.1. Materials

PLGA (7525 DLG7E, MW > 100 kDa) was purchased from Evonik (Birmingham, AL). Anhydrous naltrexone base was purchased from Mallinckrodt Pharmaceuticals (St. Louis, MO). Poly (vinyl alcohol) (PVA, MW 30–70 kDa), trifluoroacetic acid (TFA), and benzyl alcohol (BA) were purchased from Sigma-Aldrich (St. Louis, MO). Methylene chloride (DCM), ethyl acetate (EA), and dimethyl sulfoxide (DMSO, ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Milli-Q® water (Barnstead, Dubuque, IA) was used for all studies. All other chemicals were obtained commercially as analytical-grade reagents.

2.2. Methods

2.2.1. Preparation of naltrexone microspheres

PLGA with similar molecular weight as that in the commercial product Vivitrol® was used to formulate naltrexone microspheres using an oil-in-water (o/w) emulsion solvent extraction/evaporation technique. Briefly, 250 mg of PLGA (7525 DLG7E) was dissolved in organic solvent (*i.e.* ethyl acetate or methylene chloride). Naltrexone was dissolved in benzyl alcohol (30%, w/v) and added to the polymer solution. The organic phase was added to a 1% (w/v) PVA solution pre-saturated with organic solvent used (to prevent abrupt precipitation of the polymer during preparation), and an o/w emulsion was then prepared by employing droplet size reduction techniques such as homogenization or magnetic stirring. The resultant o/w emulsion was added to the aqueous phase and stirred at 220 rpm for 15 h to allow microsphere solidification and solvent removal under vacuum at room temperature. Microspheres were then removed from vacuum and sieved using two sieves, a 212 μm sieve on the top and 25 μm sieve on the bottom. The microspheres retained on the 25 μm sieve were collected, washed using an aqueous ethanol solution (25% (v/v), <5 °C), and lyophilized.

2.2.2. Characterization of physicochemical parameters of naltrexone microspheres

2.2.2.1. High performance liquid chromatography (HPLC) analysis. The quantification of naltrexone was conducted using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer, Shelton, CT) set at 210 nm. The mobile phase was phosphate buffer (10 mM, pH 6.6)/methanol (35/65, v/v) and the flow rate was 1 mL/min. A Zorbax® C18 column (150 × 4.6 mm, 5 μm; Agilent technologies) was used as the stationary phase. The sample injection volume was 10 μL for drug loading determination and 50 μL for *in vitro* release testing sample analysis. The chromatographs were analyzed using a PeakSimple™ Chromatography System (SRI instruments, Torrance, CA).

2.2.2.2. Drug loading. The naltrexone microspheres (~4 mg) were weighed and transferred into 5 mL volumetric flasks. DMSO (2.5 mL) was added into the volumetric flasks and the samples were sonicated until all particles were dissolved. Methanol was used to dilute the sample. The solution was filtered (Millex® HV, 0.22 μm PVDF syringe filter) and the naltrexone concentration was determined with a validated HPLC assay. Drug loading was calculated as:

$$\%\ drug\ loading = \frac{weight\ of\ drug\ entrapped}{weight\ of\ microspheres\ analyzed} \times 100$$