



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



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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.

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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

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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:

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

2.2.2.3. Particle size analysis. Particle size and particle size distribution of the naltrexone microspheres were measured using an AccuSizer autodiluter based on the single particle optical sizing technique (Nicomp, Santa Barbara, CA). Briefly, microspheres were dispersed in a filtered 0.1% (w/v) PVA solution in water to ensure good dispersion for the particle size analysis.

2.2.2.4. Differential scanning calorimeter (DSC) analysis. The glass transition temperatures (T_g) of the naltrexone microspheres were analyzed using a modulated temperature differential scanning calorimeter (MTDSC) (TA Instruments Q2000). Briefly, experiments were performed in hermetically sealed pans using a 2 °C/min heating rate and a modulation amplitude of ± 0.82 °C with an 80 s modulation period. The weight of each sample was ~ 5 –6 mg. The T_g was determined as the glass transition midpoint in the reversing signal. The crystallinity of naltrexone was also investigated.

2.2.2.5. Porosity. Porosity of the prepared naltrexone microspheres was determined using a Mercury Porosimeter (AutoPore IV 9500, Micromeritics). Briefly, approximately 200 mg of the naltrexone microspheres were introduced into the porosimeter and tested at a mercury filling pressure of 0.53 psi. Total intrusion volume, total pore area as well as porosity (%) were recorded.

$$\% \text{ porosity} = 1 - \frac{\text{bulk density}}{\text{apparent (skeletal) density}} \times 100$$

2.2.3. In vitro drug degradation study

An exhaustive study was performed in order to understand whether naltrexone was stable under different *in vitro* release testing conditions such as phosphate buffered saline with different pH (i.e. 4.5, 6.0, 7.4, and 9.0), and temperature (i.e. 37 °C and 45 °C). 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide were added to the testing media since they are included in the FDA recommended dissolution medium (Rawat et al., 2012). In addition, the effect of antioxidant (i.e. sodium ascorbate) on the prevention of drug degradation was investigated.

2.2.4. “Real-time” in vitro release study

Vivitol[®] was used as the testing microsphere drug product to develop suitable *in vitro* release testing method(s) for naltrexone microspheres. Both sample-and-separate and USP apparatus 4 methods were investigated. In the case of the sample-and-separate method, 10 mg of microspheres were suspended in 50 mL of phosphate buffered saline (PBS, 10 mM, pH 7.4) with 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide and incubated in a water shaker bath at 100 rpm at 37 °C. At pre-determined time intervals, one mL of release samples were withdrawn and centrifuged at 3000 rpm for 3 min. Supernatants (0.9 mL) were filtered through 0.22 μ m filters and analyzed via HPLC. Fresh medium (0.9 mL) was mixed with the pellets (if any) and transferred back to the testing vessels. In the case of the USP apparatus 4 method, 10 mg of microspheres were mixed with glass beads (1 mm) and placed in the USP apparatus 4 dissolution cell. The method of mixing the microspheres and the glass beads was as previously reported (Zolnik et al., 2006). 50 mL of PBS (10 mM, pH 7.4) with 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide was circulated through the flow through cells at a flow rate of 8 mL/min at 37 °C. At pre-determined time intervals, 1 mL of samples were withdrawn and replenished with fresh media. The obtained samples were analyzed via HPLC. 50 mL round amber media storage bottles were used for release testing. The release medium was replaced with fresh release media every five days to avoid drug degradation

during testing. Media replacement during the release testing was taken into account in the calculation of the fraction released. All drug release tests were conducted in triplicate, and the results were reported as the mean% cumulative release \pm SD.

2.2.5. Accelerated in vitro release study

Extreme release testing conditions (such as high temperature and pH) were investigated in order to develop a suitable accelerated *in vitro* release testing method. Due to the instability of naltrexone under extreme pH conditions, an elevated temperature accelerated *in vitro* release method at 45 °C was developed. Similar *in vitro* release testing procedures as described in 2.2.4. were used. Sodium ascorbate (SA) (0.0625%, w/v) was added to the release medium to avoid media replacement during accelerated *in vitro* release testing.

2.2.6. Statistical data analysis

Statistical analysis was performed to evaluate significant differences between different microsphere formulation characteristics using a paired student *t*-test. The level of significance was accepted at $p < 0.05$.

3. Results and discussion

3.1. In vitro drug degradation study

It has been reported in the literature that naltrexone base is prone to pH and temperature dependent oxidative degradation (Abdelkader et al., 2011). In order to select suitable media for *in vitro* release testing of naltrexone microspheres, the stability of naltrexone under different release testing conditions (such as different pH, and temperature) was investigated. As shown in Fig. 1, naltrexone was relatively stable (<5% degraded over 30 days) at low pH (i.e. pH 4.5 and pH 6.0) at 37 °C. Whereas, naltrexone degradation was dramatically increased at higher pH (i.e. pH 7.4 and pH 9.0) at 37 °C, particularly at pH 9.0 ($\sim 25\%$ degraded by day 5). At pH 7.4, naltrexone appeared to be relatively stable since at day 5 only $\sim 3\%$ degraded at 37 °C. In addition, naltrexone degradation was accelerated at pH 7.4 under elevated temperature (45 °C) resulting in more than 10% degradation at day 5 (Fig. 2). The degradation product was collected and analyzed using LC-MS/MS. A peak with an *m/z* value of 374.14 was observed (data not shown), suggesting the presence of an *N*-oxide product of naltrexone (C₂₀H₂₄NO₆) (as shown in Fig. 3). The basic drug naltrexone deprotonates under alkaline conditions and therefore, the drug may be more susceptible to electron loss and hence oxidative degradation. It was observed that the addition of an antioxidant (0.0625% (w/v) sodium ascorbate) in the medium (PBS, pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide, can

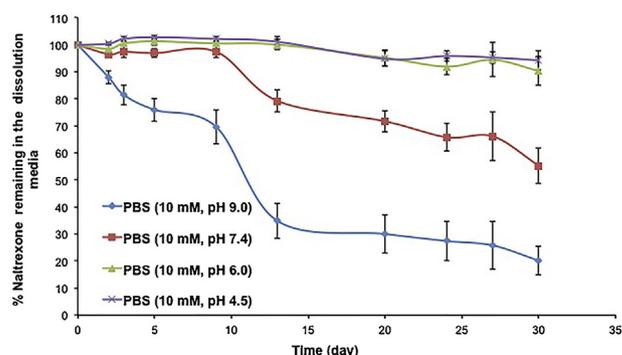


Fig. 1. Effect of pH on the stability of naltrexone at 37 °C ($n = 3$). All the media contain 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide.

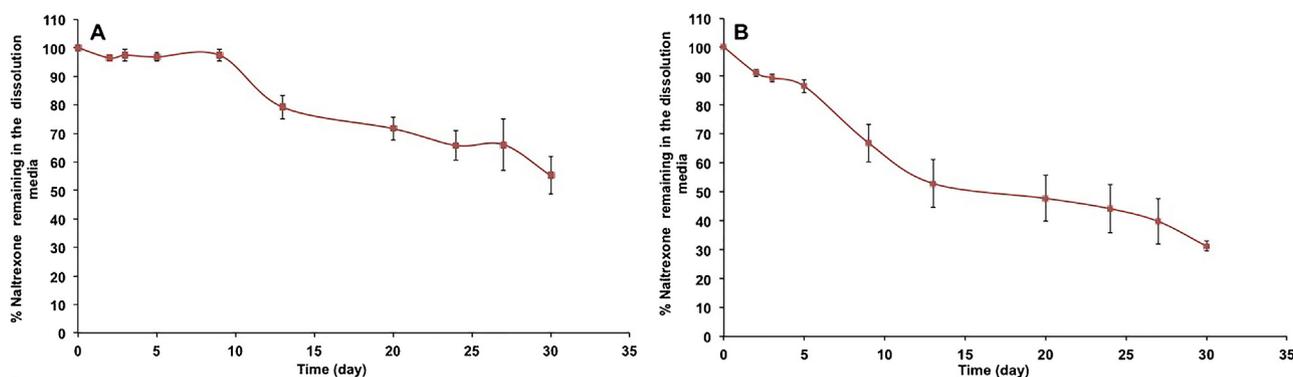


Fig. 2. Effect of temperature on the stability of naltrexone in PBS buffer (10 mM, pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide ($n = 3$). (A) 37 °C and (B) 45 °C.

protect naltrexone against oxidation up to 10 days at 37 °C (~4% degraded by day 10) (data not shown). This suggested that 0.0625% (w/v) sodium ascorbate may be used to prevent naltrexone degradation during elevated temperature release testing at 45 °C, whereas media replacement may be necessary for “real-time” release testing. Accordingly, “real-time” *in vitro* release testing of naltrexone microspheres at 37 °C was performed via frequent media replacement (every five days) to avoid oxidative drug degradation at pH 7.4.

3.2. “Real-time” *in vitro* release testing of naltrexone microspheres

The commercially available naltrexone microsphere product Vivitrol® was used as the testing formulation for the development of reproducible and discriminatory *in vitro* release testing method (s). Since there is a lack of compendial *in vitro* release testing methods available for parenteral microsphere drug products, the two most commonly used methods (*i.e.* sample-and-separate and USP apparatus 4) for such products were investigated. As shown in Fig. 4, sustained naltrexone release from Vivitrol® with a very low initial burst release (*ca.* 0.5%) was observed, indicating that most of the drug was possibly entrapped inside the microsphere core during preparation. It was observed that more than 90% naltrexone was released from Vivitrol® by day 35 at 37 °C, which is consistent with naltrexone release from Vivitrol® *in vivo* (over a period of 40 days) (Negroni et al., 2001). Since the release medium was replaced every five days, no major degradation peak(s) in the HPLC chromatograms were observed throughout testing, confirming that frequent media replacement can be implemented to avoid naltrexone degradation in the release media (10 mM PBS, pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide. Reproducibility of the “real-time” USP apparatus 4 method was also investigated. It can be seen from Fig. 5 that the *in vitro* release profiles of Vivitrol® (different batches, A and B) obtained at

different testing dates overlapped, indicating the USP apparatus 4 method was very reproducible under the “real-time” testing conditions (37 °C).

In vitro release testing of the two batches of Vivitrol® (Batch A and B) was also performed using the sample-and-separate method. As shown in Fig. 6, less than 90% naltrexone release was observed by day 27. However, the *in vitro* release profiles of the two batches of Vivitrol® differed from one another when the sample-and-separate method was used. Since sample separation as well as media replacement via centrifugation was performed during release testing, variable amounts of sample loss during sample collection as well as media replacement might have occurred, thus resulting in irreproducible results. Although release media containing 0.02% v/v Tween 20 was used to facilitate microsphere wetting as well as to minimize aggregation, microsphere aggregation was observed during testing. Compared to the sample-and-separate method, no sample separation and hence microsphere loss occurred when the USP apparatus 4 method was used. In addition, the addition of glass beads in the USP apparatus 4 dissolution cells minimizes microsphere aggregation, as well as facilitates laminar flow of the release medium throughout the dissolution cells. Lastly, the USP apparatus 4 method is based on compendial dissolution apparatus with well-defined geometry and hydrodynamic conditions, which can facilitate inter-laboratory comparisons. Taken together, the USP apparatus 4 method appears to be a suitable *in vitro* release testing method for naltrexone microspheres.

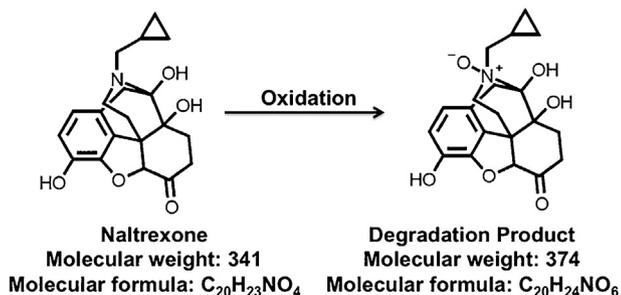


Fig. 3. Possible oxidative degradation pathway of naltrexone under the *in vitro* release testing conditions investigated.

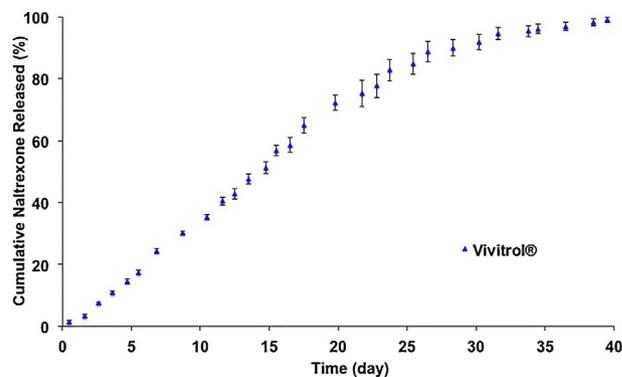


Fig. 4. *In vitro* release profile of Vivitrol® in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide at 37 °C obtained using the USP apparatus 4 method ($n = 3$). The dissolution media was replaced every five days.

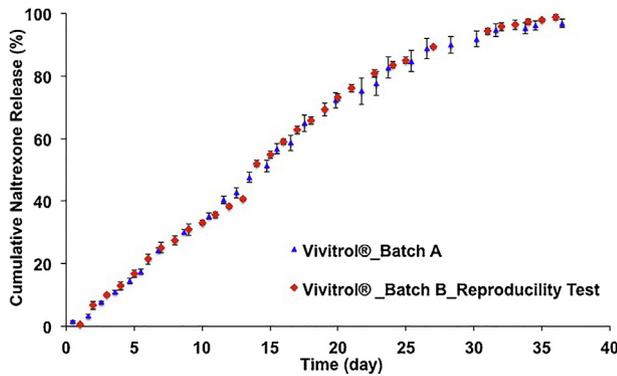


Fig. 5. *In vitro* release profiles of different batches of Vivitrol[®] in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide obtained using the USP apparatus 4 method ($n=3$) at 37 °C. The dissolution medium was replaced every 5 days.

3.3. Accelerated *in vitro* release testing of naltrexone microspheres

Different strategies (such as extreme pH, and elevated temperature) have been utilized to accelerate drug release from PLGA microspheres (Shen and Burgess, 2012a; Faisant et al., 2002, 2006). Elevated temperature conditions can increase polymer erosion (Zolnik et al., 2006) as well as drug diffusion by increasing molecular mobility (Duda and Zielinski, 1996), consequently resulting in accelerated drug release within a short period of time. However, it has been shown in Fig. 2 that elevated temperature (45 °C) resulted in increased naltrexone degradation. In order to develop a suitable fast quality control method for naltrexone microspheres, both frequent media replacement (every 12 h) as well as the use of 0.0625% (w/v) sodium ascorbate in the release medium (10 mM PBS, pH 7.4) without media replacement were investigated using USP apparatus 4. As shown in Fig. 7, naltrexone release from Vivitrol[®] was dramatically accelerated at 45 °C, and the release testing duration was significantly reduced from 35 to 40 days ("real-time", at 45 °C) to around 6 days at 45 °C in both cases. Moreover, the addition of 0.0625% (w/v) sodium ascorbate in the release medium successfully prevented naltrexone from degradation during accelerated release testing. The accelerated *in vitro* release profile obtained in the release medium containing 0.0625% (w/v) sodium ascorbate without media replacement was almost identical to that obtained with frequent media replacement. Based on these results, 0.0625% (w/v) sodium ascorbate was added to the release media during the elevated temperature accelerated release testing at 45 °C. The

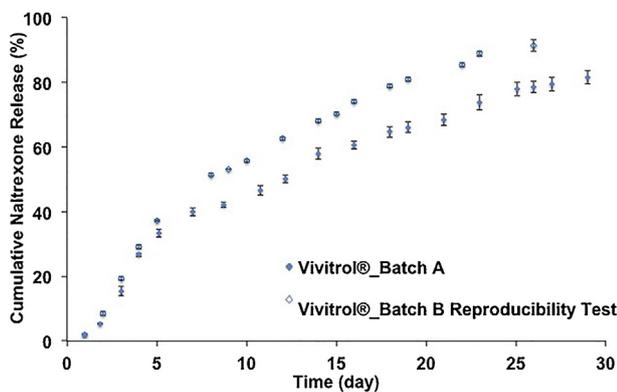


Fig. 6. *In vitro* release profiles of different batches of Vivitrol[®] in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide obtained using the sample-and-separate method at 37 °C ($n=3$). The dissolution medium was replaced every 5 days.

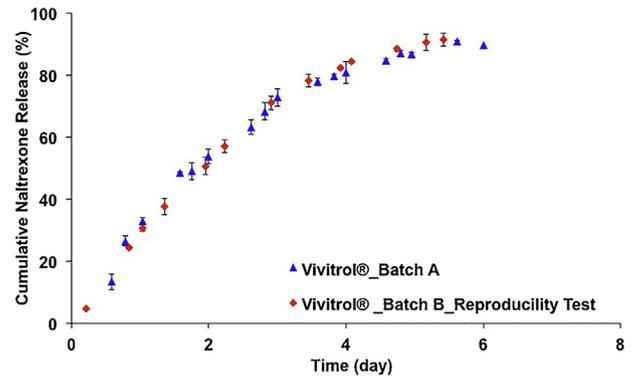


Fig. 8. *In vitro* release profiles of different batches of Vivitrol[®] in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20, 0.02% (w/v) sodium azide and 0.0625% (w/v) sodium ascorbate obtained using USP apparatus 4 at 45 °C ($n=3$).

reproducibility of the developed accelerated USP apparatus 4 method was also investigated. As shown in Fig. 8, *in vitro* release profiles of Vivitrol[®] (Batches A and B) obtained at different testing dates almost overlapped, indicating that the developed accelerated USP apparatus 4 method was reproducible.

For sustained release PLGA microspheres, "real-time" release testing requires extended periods of time, which may affect the time for batch release of product as well as delay the product development process. Accordingly, an accelerated *in vitro* release method with good correlation with "real-time" *in vitro* release is essential. As shown in Fig. 9, the time-scaled "real-time" release profile overlapped with the respective accelerated release profile obtained using USP apparatus 4. A linear correlation between the fractions released under "real-time" (time-scaled, scaling factor: 6.5) and accelerated conditions was observed for the USP apparatus 4 method with a regression coefficient (R^2) above 0.99. This suggested that the drug release mechanisms (the combination of polymer erosion and drug diffusion) at both temperatures may be similar.

It has been previously reported that manufacturing changes (such as solvent systems, and emulsification processes) can affect microsphere physicochemical characteristics, which in turn can affect their *in vitro* and *in vivo* performance (Shameem et al., 1999). Accordingly, it is essential to develop *in vitro* release testing methods that are capable of detecting changes in *in vitro* release characteristics resulting from changes in the manufacturing process. The capability of the developed accelerated USP apparatus 4 method to differentiate naltrexone microspheres with

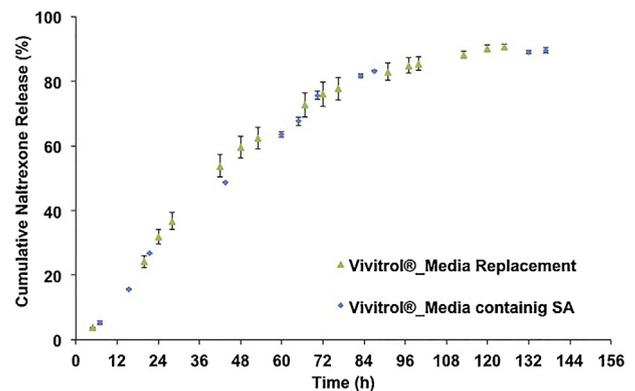


Fig. 7. *In vitro* release profiles of Vivitrol[®] in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20 and 0.02% (w/v) with or without an antioxidant (sodium ascorbate) obtained using USP apparatus 4 at 45 °C ($n=3$). The medium without sodium ascorbate was replaced every 12 h while the medium with sodium ascorbate was not replaced during testing.

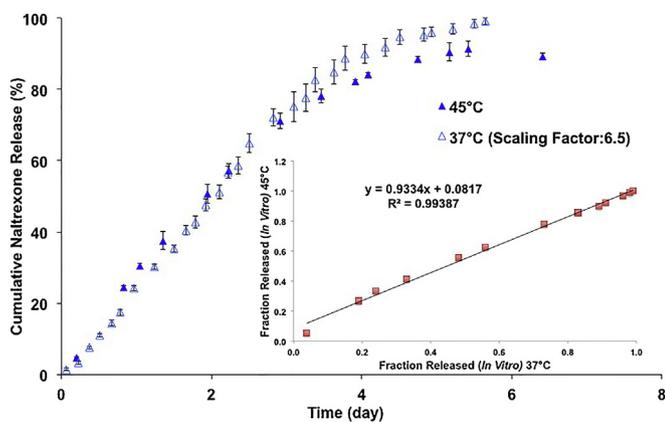


Fig. 9. *In vitro* release profiles of Vivitrol[®] in 10 mM PBS (pH 7.4) at 37 °C (time-scaled) and at 45 °C using the developed USP apparatus 4 method ($n=3$). Insert figure shows linear correlation between “real-time” (time-scaled, scaling factor: 6.5) (37 °C) and accelerated (45 °C) fraction of naltrexone released.

Table 1
Physicochemical properties of naltrexone microsphere formulations ($n=3$).

Sample	Solvent	Drug loading (% w/w)	Porosity (% w/w)	D50 (Mean ± SD) (μm)	
				Population	Volume
Formulation 1	DCM&BA	28.74 ± 1.64	49.83	42.93 ± 9.69	121.11 ± 3.61
Formulation 2	EA&BA	29.7 ± 1.11	58.32	53.93 ± 5.27	105.49 ± 8.63
Formulation 3	EA&BA	29.57 ± 1.75	65.08	44.32 ± 2.07	68.56 ± 1.52
Vivitrol [®]	–	33.50 ± 1.43	50.21	58.46 ± 6.01	108.49 ± 1.52

* BA: benzyl alcohol, EA: ethyl acetate, DCM: methylene chloride.

manufacturing differences was investigated. Compositionally equivalent naltrexone microspheres were prepared using different manufacturing processes. It can be seen in Table 1 that all the prepared naltrexone microspheres had similar drug loading (~29% w/w), despite different manufacturing processes. Since different particle size reduction techniques were used, the prepared naltrexone microspheres had different particle size and particle size distribution. Furthermore, the prepared naltrexone microspheres showed different porosity, indicating that these microspheres may have different internal structures as a result of differences in the dynamic solvent exchange processes during microsphere solidification. No significant differences were observed in the T_g of the microspheres, and the physical mixture of

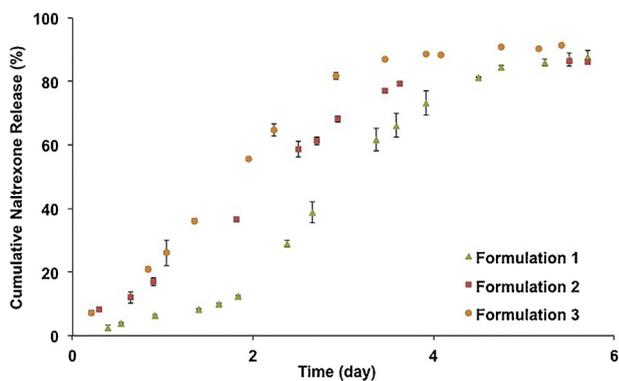


Fig. 10. *In vitro* release profiles of the prepared naltrexone microspheres in 10 mM PBS (pH 7.4) containing 0.02% (v/v) Tween 20, 0.02% (w/v) sodium azide and 0.0625% (w/v) sodium ascorbate using USP apparatus 4 at 45 °C ($n=3$).

the drug and the PLGA polymer (ca. 42 °C). In addition, the crystallinity of drug did not change during microsphere preparation (data not shown). Due to the differences observed in their critical quality attributes (such as particle size and porosity), these microsphere formulations possessed different “real-time” *in vitro* release characteristics (Chu et al., 2006). The discriminatory ability of the developed elevated temperature USP apparatus 4 method was assessed using these microsphere formulations with different *in vitro* release characteristics.

As shown in Fig. 10, the accelerated USP apparatus 4 method was capable of discriminating the prepared compositionally equivalent naltrexone microspheres with manufacturing differences. Drug release from the prepared microspheres reached a plateau around day 6 (Formulations 1 and 2) and day 4

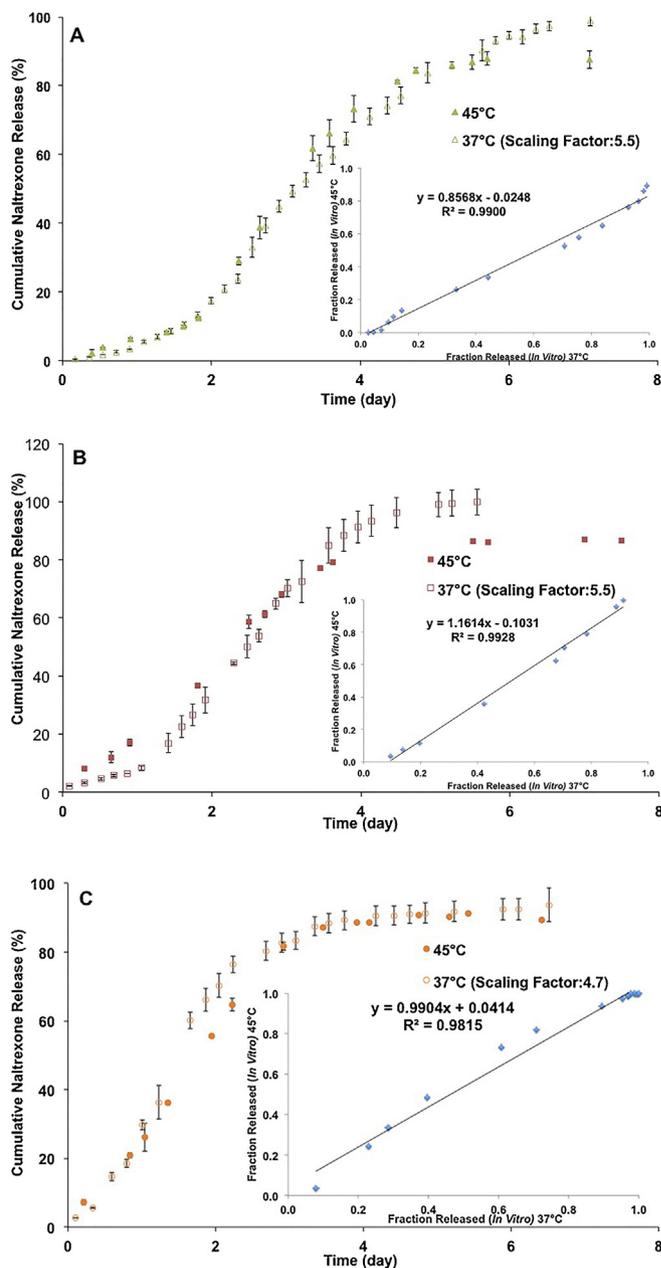


Fig. 11. *In vitro* release profiles of Formulations 1, 2 and 3 in 10 mM PBS (pH 7.4) at 37 °C (time-scaled) and at 45 °C using the USP apparatus 4 method ($n=3$). Insert figures show linear correlations between “real-time” (time-scaled) (37 °C) and accelerated (45 °C) fractions of naltrexone released. (A) Formulation 1; (B) Formulation 2; and (C) Formulation 3.

(Formulation 3). Accelerated release profiles of all the prepared naltrexone microsphere formulations followed the same rank order as their “real-time” release profiles. Release testing duration was reduced from 40 days (Formulations 1 and 2) and 30 days (Formulation 3) to within 6 days under accelerated conditions. The fraction released under accelerated testing conditions were compared with that under “real-time” testing conditions (after time scaling) for all the prepared naltrexone microspheres. As shown in Fig. 11, the time-scaled “real-time” release profiles overlapped with their respective accelerated release profiles for all the prepared naltrexone microsphere formulations investigated. A linear correlation was obtained between the time-scaled “real-time” and accelerated fraction released with a regression coefficient (R^2) of >0.98. This suggested that the release mechanisms at both temperatures may be similar. These results demonstrated the developed accelerated USP apparatus 4 method is both discriminatory and reproducible. Accordingly, it can be used as a suitable fast quality control tool for naltrexone microspheres.

4. Conclusion

A reproducible accelerated release testing method with discriminatory ability was developed using USP apparatus 4 for compositionally equivalent naltrexone loaded polymeric microspheres with manufacturing differences for the first time. The degradation of naltrexone was prevented using frequent media replacement and anti-oxidant containing media during “real-time” and accelerated release testing, respectively. The accelerated *in vitro* release profiles of naltrexone microspheres correlated well with their respective “real-time” release profiles, indicating that the drug release mechanism(s) may be similar under both release testing conditions. Overall, the developed accelerated USP apparatus 4 release testing method has demonstrated the potential of being a fast quality control tool that will assure the product performance as well as assist the product development of parenteral naltrexone polymeric microspheres.

Disclaimer

This article reflects the views of the authors and should not be construed to represent FDA’s views or policies.

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