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Development of *in vitro-in vivo* correlation of parenteral naltrexone loaded polymeric microspheres

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

[a] Department of Pharmaceutical Sciences, 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

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ABSTRACT

Establishment of *in vitro-in vivo* correlations (IVIVCs) for parenteral polymeric microspheres has been very challenging, due to their complex multiphase release characteristics (which is affected by the nature of the drug) as well as the lack of compendial *in vitro* release testing methods. Previously, a Level A correlation has been established and validated for polymeric microspheres containing risperidone (a practically water insoluble small molecule drug). The objectives of the present study were: 1) to investigate whether a Level A IVIVC can be established for polymeric microspheres containing another small molecule drug with different solubility profiles compared to risperidone; and 2) to determine whether release characteristic differences (bi-phasic *vs* tri-phasic) between microspheres can affect the development and predictability of IVIVCs. Naltrexone was chosen as the model drug. Three compositionally equivalent formulations of naltrexone microspheres with different release characteristics were prepared using different manufacturing processes. The critical physicochemical properties (such as drug loading, particle size, porosity, and morphology) as well as the *in vitro* release characteristics of the prepared naltrexone microspheres and the reference-listed drug (Vivitrol®) were determined. The pharmacokinetics of the naltrexone microspheres were investigated using a rabbit model. The obtained pharmacokinetic profiles were deconvoluted using the Loo-Riegelman method, and compared with the *in vitro* release profiles of the naltrexone microspheres obtained using USP apparatus 4. Level A IVIVCs were established and validated for predictability. The results demonstrated that the developed USP 4 method was capable of detecting manufacturing process related performance changes, and most importantly, predicting the *in vivo* performance of naltrexone microspheres in the investigated animal model. A critical difference between naltrexone and risperidone loaded microspheres is their respective bi-phasic and tri-phasic release profiles with varying burst release and lag phase. These variations in release profiles affect the development of IVIVCs. Nevertheless, IVIVCs have been established and validated for polymeric microspheres with different release characteristics.

1. Introduction

Owing to their advantages such as improved patient compliance and longer duration of action, extended release drug delivery systems have attracted great attention in the past several decades, resulting in the successful commercialization of various types of extended release drug products [1]. Parenteral polymeric microspheres, particularly poly (lactic-*co*-glycolic acid) (PLGA) and poly(lactic acid) (PLA) based microspheres have been one of the most effective non-oral extended release drug products on the market [2]. This is due to the fact that the PLGA/PLA-based microsphere drug products are biodegradable and biocompatible with the ability to sustain the delivery of various therapeutics (*e.g.* small molecules and biologics) over long periods of time [3–6]. These microsphere drug products often contain a substantial amount of potent therapeutics, which makes them "high-risk" drug products since any unexpected change in bioavailability may result in severe side effects or toxicity [7]. Moreover, the critical physicochemical properties of polymeric microspheres (such as drug loading, particle size and porosity) are sensitive to minor changes in the manufacturing processes, which in turn may affect drug release characteristics and hence product performance [8]. Accordingly, it is crucial to assure the performance and safety of such drug products.

In vitro drug release testing can provide extensive insight into the release rate as well as drug release mechanism(s) [9,10]. Therefore, it is an important tool to not only ensure consistent product performance and safety, but also assist in product development. When a correlation

* 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 address: d.burgess@uconn.edu (D.J. Burgess).
[1] Current address: University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, USA.

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between *in vitro* and *in vivo* drug release is established, the *in vitro* release method may potentially be used as a surrogate for bioequivalence studies that would otherwise be required for any scale-up and post-approval changes (SUPAC). The establishment of *in vitro-in vivo* correlations (IVIVCs) minimizes the need for animal studies and clinical trials, and therefore reduces the cost and duration of generic microsphere drug product development.

In vitro-in vivo correlation (IVIVC) is a predictive mathematical model describing the relationship between an *in vitro* property (*e.g.* rate or extent of drug release) of a dosage form and a relevant *in vivo* response (*e.g.* plasma drug concentration or amount of drug absorbed) [11]. The U.S. FDA has categorized four main levels of IVIVC: Levels A, B, C, and multiple level C. A Level A IVIVC represents a point-to-point correlation between the *in vitro* and *in vivo* input rates (*e.g.* the *in vivo* dissolution). It is considered the most informative, and is recommended by the U.S. FDA. A Level A IVIVC is also the only level of IVIVC that can be used to obtain a bio-waiver. In general, the U.S. FDA recommends the use of two or more formulations with different release characteristics in order to develop a reliable IVIVC.

Unlike oral dosage forms, the establishment of an IVIVC for complex parenteral microsphere drug products has been very challenging. This is due to not only their complex characteristics (such as multiphase drug release profiles) but also the lack of a standard/compendial *in vitro* release testing method, which can mimic and predict their *in vivo* performance to the maximum extent possible [12–15]. Until now, literature reports on the establishment of IVIVCs for complex parenteral microsphere drug products have remained sparse. Most of the reported literature is on “proof-of-concept” research demonstrating the possibility of developing IVIVCs using one or two microsphere formulations with different release characteristics [16–22]. Recent research has demonstrated that a reliable Level A IVIVC can be developed for compositionally equivalent parenteral PLGA microspheres containing water insoluble small molecule therapeutics (such as risperidone) with manufacturing differences [8]. The drug loading as well as the burst release of microspheres containing water soluble therapeutics tends to be highly variable with minor manufacturing changes. For example, the solvent exchange/evaporation rates during the microsphere solidification process are prone to vary, which in turn may alter drug loading and the drug release characteristics [23]. This is a very critical issue for the development of generic microsphere products. In addition, this makes it difficult to obtain two or more compositionally equivalent microsphere formulations with manufacturing differences for the development and validation of IVIVCs that would be useful for generic drug product manufacturers. Due to the solubility differences in aqueous and organic solvents, the release characteristics of such compounds from microspheres may significantly differ from that of water insoluble compounds (such as risperidone). For example, burst release is typically higher for water soluble compounds as a result of drug migration to the microsphere surfaces during preparation [23,24]. As reported earlier, differences in the burst release affects the predictability of developed IVIVC [8]. Until now, a Level A IVIVC has not been reported for parenteral PLGA microspheres containing therapeutics that are soluble in water and have bi-phasic release characteristics.

The objectives of the present study were to demonstrate whether a Level A IVIVC can be established using compositionally equivalent PLGA microspheres containing a small molecule with different solubility profiles compared to risperidone, and to investigate whether the differences in the release characteristics (bi-phasic *vs* tri-phasic) have an impact on the predictability of IVIVCs. Naltrexone (marketed in the microsphere form as Vivitrol®) was chosen as the model therapeutic. Three compositionally equivalent naltrexone PLGA microspheres with manufacturing differences were prepared. The *in vitro* release characteristics of the prepared naltrexone microsphere formulations and the reference listed drug (RLD) product Vivitrol® were determined using a previously developed USP apparatus 4 method. The pharmacokinetic profiles of the naltrexone microspheres were investigated using a rabbit model, and compared with the obtained *in vitro* release profiles to establish an IVIVC and investigate its predictability.

2. Materials and methods

2.1. Materials

PLGA (7525 DLG7E) 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), benzyl alcohol (BA) and the reference standard (*i.e.* naltrexone-D3) 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). LC-MS grade 0.1% v/v formic acid water and methanol were purchased from VWR (Radnor, PA). Milli-Q® water (Barnstead, Dubuque, IA) was used for all studies. All other chemicals were obtained commercially as analytical-grade reagents.

2.2. Preparation of naltrexone microspheres

PLGA (7525 DLG7E, Mw > 100KD) with similar molecular weight as that used in the commercial product Vivitrol® was used to formulate compositionally equivalent naltrexone microspheres using different manufacturing processes. Briefly, 250 mg of PLGA was dissolved in organic solvent (*i.e.* ethyl acetate (16.7%, w/v) or methylene chloride (25%, w/v)). Naltrexone is poorly soluble in both methylene chloride and ethyl acetate and accordingly, a co-solvent (benzyl alcohol) was used to facilitate dissolution of naltrexone. The polymer solution was added to the naltrexone (~167 mg) solution in benzyl alcohol (30%, w/v). The organic phase containing both the polymer and drug was then dispersed into a 1% (w/v) PVA solution (5 mL, 0.22 μm membrane filtered), and an oil-in-water (o/w) emulsion was prepared by employing size reduction techniques such as homogenization (3200 rpm for 60 s) (IKA® Works, Inc.) and magnetic stirring (600 rpm for 15 min). The PVA solution was saturated with organic solvents (*i.e.* 1.8 and 8.5% (v/v) in the case of methylene chloride and ethyl acetate, respectively) in order to prevent abrupt precipitation of the polymer during emulsification. The resultant o/w emulsion was added to water (125 mL) and stirred at 220 rpm for 15 h to allow microsphere solidification. The organic solvents were then removed 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 a 25 μm sieve were collected and washed using an aqueous ethanol solution (25% (v/v), < 5 °C). Lyophilization was used to dry the microspheres.

2.3. Characterization of naltrexone microspheres

2.3.1. High performance liquid chromatography (HPLC) analysis

The quantification of naltrexone was conducted using a PerkinElmer HPLC system (series 200) with a UV absorbance detector (PerkinElmer, Shelton, CT) set at 210 nm. The mobile phase was 10 mM phosphate buffer (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 and 50 μL for *in vitro* release testing sample analysis. The chromatographs were analyzed using a PeakSimple™ Chromatography System (SRI instruments, Torrance, CA).

2.3.2. Drug loading

The naltrexone microspheres (~4 mg) were weighed and transferred into 10 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 solutions were filtered (Millex® HV, 0.22 μm PVDF syringe filter) and the

naltrexone concentrations were determined *via* the validated HPLC assay as described above. Drug loading was calculated as:

$$\text{Drug Loading (\%)} = \frac{\text{weight of drug entrapped}}{\text{weight of microspheres analyzed}} \times 100$$

2.3.3. Particle size and size distribution

Particle size and particle size distribution of the naltrexone microspheres were measured using an AccuSizer autodiluter particle sizing system (Nicomp, Santa Barbara, CA). Briefly, the microspheres were dispersed in a filtered 0.1% (w/v) PVA solution in water to ensure good dispersion, and then particle size analysis was conducted.

2.3.4. 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.

2.3.5. Morphology

The morphology of the commercial product Vivitrol® and the prepared naltrexone microspheres was analyzed using scanning electron microscopy (SEM). Briefly, dry microspheres were mounted on carbon taped aluminum stubs and sputter coated with gold under an argon evaporator and high vacuum. The samples were then observed using SEM (NanoSEM 450, Nova).

2.3.6. Porosity

The porosity of the commercial product and the prepared naltrexone microspheres was determined using a Mercury Porosimeter (AutoPore IV 9500, Micromeritics). Briefly, approximately 200 mg of 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.

$$\text{Porosity (\%)} = \left(1 - \frac{\text{Bulk density}}{\text{Apparent (skeletal)density}}\right) \times 100$$

2.4. In vitro release studies

In vitro release testing of the prepared naltrexone microspheres and the commercial product was conducted using a previously developed modified USP apparatus 4 method at 37 °C [25]. Briefly, 10 mg of microspheres were mixed with glass beads (1 mm) and placed in the USP apparatus 4 dissolution cells. 50 mL of phosphate buffered saline (PBS) (10 mM, pH 7.4) containing 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, one mL of samples were withdrawn and replenished with fresh media. The obtained samples were analyzed *via* HPLC. The release medium was replaced with fresh release medium every five days to avoid drug degradation. Media replacement during release testing was taken into account in the calculation of the fraction release. All drug release tests were conducted in triplicate and the results are reported as the mean % cumulative release ± SD.

2.5. In vivo release studies

The *in vivo* release characteristics of the prepared naltrexone microspheres and the commercial product were investigated using a rabbit model. Briefly, male rabbits (New Zealand White) weighing approximately 3 to 4 kg were randomly assigned to each treatment group (n = 6). The naltrexone microspheres were suspended in the diluent used for dispersion of the commercial product Vivitrol®, and injected into the rabbit hind leg thigh muscles at a dose of 11.69 mg/kg. Blood samples were collected from the marginal ear veins at predefined time intervals. In addition, a pharmacokinetic study of the naltrexone solution in saline (dose: 0.11 mg/kg, *i.v.*) was conducted (n = 6). The collected blood samples were centrifuged at 14,500 rpm for 5 min to separate out the plasma. The plasma was collected and stored at − 80 °C until analysis. The animal study protocol was reviewed and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC) prior to the beginning of the experiments.

2.6. Plasma sample analysis

Naltrexone was extracted from plasma samples using tert-butyl methyl ether by a liquid-liquid extraction method. Naltrexone D-3 was used as the internal standard. Briefly, the internal standard solution (100 ng/mL, 20 µL) was added to 100 µL plasma samples. Samples were vortex-mixed for 5 min followed by addition of tert-butyl methyl ether (1 mL). Then the samples were vortex-mixed again for 15 min followed by centrifugation (4 °C) at 14,500 rpm for 5 min. The supernatants were collected in polypropylene centrifugation tubes and the solvent was evaporated under nitrogen flow. The dry residues were then reconstituted with methanol (150 µL) and injected into a LC-MS/MS system for sample analysis.

The LC-MS/MS system consisted of an Agilent HP-1100 LC system and a TSQ Quantum Ultra Mass Spectrometer (Waters) with an electrospray ionization (ESI) ion source. Chromatographic separations were carried out on a Kinetex HILIC column (50 × 2.1 mm, 2.6 µm, 100 Å) through an isocratic mobile phase (0.1% (v/v) formic acid in water/methanol (20/80, v/v)) at 30 °C. The following MS detection parameters were used: 4000 V electrospray voltage, 360 °C capillary temperature, collision pressure 3.0, and 30 V collision energy. Detection of daughter ions was conducted in the positive-ion selected reaction monitoring mode with the following transitions in a single reaction monitoring (SRM) mode: m/z 342 (naltrexone) to m/z 324.2 (dehydronaltrexone, daughter ion), and m/z 345.2 (naltrexone-D3) to m/z 327.2 (dehydronaltrexone-D3, daughter ion). The injection volume was 15 µL. The data acquisition was ascertained by Xcalibur software. Calibration curves were established on each day when analysis was conducted, and showed good linearity with correlation coefficients > 0.99. The lowest limit of quantification (LLOQ) for naltrexone was 0.5 ng/mL and the mean recovery of plasma samples from low to high concentrations of naltrexone was > 90%. The inter- and intra-day variations of the three different concentrations of naltrexone (0.5, 10, and 20 ng/mL) were < 15%.

2.7. Pharmacokinetic analysis and the development of an IVIVC

The development of IVIVC for the prepared naltrexone PLGA microspheres was performed following the same principles as detailed in the U.S. FDA guidance on development of IVIVC for extended release oral dosage forms [11]. Briefly, the *in vivo* plasma profiles of naltrexone PLGA microspheres were deconvoluted using the Loo-Riegelman method [14,26]. Standard errors are not shown in the deconvoluted *in vivo* absorption profiles because the average plasma concentration values were used. The fraction absorbed *in vivo* was calculated as below:

$$\frac{Ab}{Ab_{\infty}} = \frac{C_p + C_t + K_{10}(AUC)_0^t}{K_{10}(AUC)_0^{\infty}}$$

C_p, C_t, K_{10} and AUC are the drug concentration in the central compartment (plasma), apparent tissue compartment concentration, elimination rate constant and area under the plasma *vs* time curve, respectively. The distributive and elimination micro rate constants (k_{12}, k_{21} and k_{10}) that are necessary for calculating C_t, and the total fraction

absorbed at time t, were calculated using WinNonlin® 6.4 (Pharsight, Certara corporation St. Louis, USA) based on the plasma concentrations of naltrexone after intravenous administration of the naltrexone solution.

The development and validation of the IVIVC for the naltrexone microspheres were performed using WinNonlin® 6.4 (Pharsight, Certara corporation St. Louis, USA).

2.8. Statistical data analysis

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

3. Results and discussion

3.1. Physicochemical properties of naltrexone microspheres

It has been reported that the critical physicochemical properties (*e.g.* particle size, and porosity) of parenteral PLGA microspheres containing a water insoluble model compound (risperidone) are sensitive to minor changes in manufacturing processes (such as solvent systems and particle size reduction technique) [8]. In order to understand the effect of manufacturing processes on the physicochemical properties of parenteral PLGA naltrexone microspheres that were prepared using different manufacturing processes, three compositionally equivalent naltrexone microspheres were obtained: 1) Formulation 1_stirring (methylene chloride and benzyl alcohol solvent system); 2) Formulation 2_stirring (ethyl acetate and benzyl alcohol solvent system); and 3) Formulation 3_homogenization (ethyl acetate and benzyl alcohol solvent system).

The physicochemical properties of the prepared naltrexone microspheres with manufacturing differences and the Vivitrol® are presented in Table 1. As shown in Table 1, all naltrexone microsphere formulations had similar drug loading (*ca.* 29%, w/w), and the Vivitrol® had a slightly higher drug loading (33.5%). It was observed that maintaining high drug loading of the naltrexone microspheres was very challenging, since water soluble compounds can leak into the larger outer aqueous phase during the microsphere solidification process, resulting in low drug loading. Lower temperature (4 °C) was used throughout the preparation process to minimize drug diffusion from the inner organic phase droplets into the aqueous phase. It has previously been reported that the polymer precipitation rate facilitates entrapment of most of the drug inside the core and hence, is critical to achieve high drug loading for hydrophilic drugs [23]. In the present study, solvent removal and consequent, polymer precipitation were controlled by continuously applying a vacuum at a constant pressure (25–30 in. Hg) to achieve fast solvent evaporation and therefore fast microsphere solidification, to minimize drug loss into the aqueous phase during preparation. Particle size and particle size distribution of all three prepared naltrexone microsphere formulations and Vivitrol® are shown in Fig. 1. Despite the fact that all the formulations were prepared using different manufacturing processes, the prepared naltrexone microspheres showed similar D50 values in terms of population distribution (*ca.* 50 μm), which were similar to that of Vivitrol®. Whereas, in the case of volume distribution, Formulation 3 showed a smaller D50 value (*ca.* 68 μm) compared to Vivtrol® and the other two prepared formulations (*ca.* > 100 μm) ($p < 0.05$). In addition, the span value of Formulation 3 was the lowest compared to that of Vivitrol® and Formulations 1 and 2, which indicates narrow size distribution. The microspheres prepared using the stirring technique (*i.e.* Formulations 1 and 2) showed a large variation in the particle size distribution, while the microspheres prepared using the homogenization method (*i.e.* Formulation 3) showed comparatively smaller but uniform particle size. This can be explained by the fact that the homogenization process provided a stronger emulsification force, thus leading to a smaller particle size with a narrower size distribution compared to the stirring process.

Table 1
Physicochemical properties of naltrexone microspheres investigated.

Sample	Solvent	Preparation method	Drug loading (%, w/w)	Porosity (%, w/w)
Formulation 1	DCM & BA	Magnetic stirring	28.74 ± 1.64	49.83
Formulation 2	EA & BA	Magnetic stirring	29.7 ± 1.11	58.32
Formulation 3	EA & BA	Homogenization	29.57 ± 1.75	65.08
Vivitrol®	–	–	33.50 ± 1.43	50.21

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

Differences in the manufacturing processes (such as different solvents, and rates of solvent diffusion and evaporation) have been reported to affect the inner structure and/or the porosity of PLGA microspheres [27,28]. As shown in Fig. 2, all the prepared naltrexone microspheres were polydispersed, and of a spherical shape with the presence of a few large pits on their surfaces. The presence of large pits on the microsphere surfaces may be due to shrinkage during the solvent evaporation process under vacuum. Furthermore, the naltrexone microspheres prepared using methylene chloride as the solvent (*i.e.* Formulation 1) had a lower percentage porosity (49.83%) compared to those microspheres prepared using ethyl acetate & benzyl alcohol as the solvent system (58.32% and 65.02% for Formulations 2 and 3, respectively) (Table 1). Since ethyl acetate is relatively more miscible with water compared to methylene chloride, water inclusion during microsphere solidification led to the formation of a porous core structure and hence higher porosity, which is consistent with our recent research on risperidone PLGA microspheres [29]. No significant differences were observed in the T_g of the naltrexone microspheres.

Overall, it was observed that the critical quality attributes of the naltrexone microspheres (such as drug loading, particle size and porosity) are very sensitive to manufacturing differences such as the solvent system, the solvent removal rate, temperature and the particle size reduction technique. It was anticipated that the differences in these critical quality attributes of naltrexone microspheres may affect their *in vitro* and *in vivo* performance.

3.2. In vitro release characteristics of naltrexone microspheres

One of challenges in developing an IVIVC for complex parenteral drug products (such as microspheres) is the lack of compendial *in vitro* release testing methods. Various methods have been used to investigate *in vitro* release profiles of parenteral polymeric microspheres, including sample-and-separate, dialysis and USP apparatus 4 methods [10,30–32]. The 2004 AAPS-EUFEPS workshop recommended a dissolution method using USP apparatus 4 for microspheres [12]. An effort has recently been made to develop a suitable *in vitro* release testing method for naltrexone microspheres [25]. However, due to instability issues associated with naltrexone, frequent media replacement was determined to be necessary during long-term release testing to avoid oxidative degradation. Based on recent research, a USP apparatus 4 method with excellent reproducibility was used to investigate the *in vitro* release characteristics of the naltrexone microspheres [25].

As shown in Fig. 3, the developed "real-time" USP apparatus 4 method was able to discriminate between the *in vitro* release profiles of all the prepared naltrexone microsphere formulations with manufacturing differences and also Vivitrol®. Unlike the previously reported risperidone microspheres [8], the naltrexone microspheres investigated showed bi-phasic release profiles, indicating naltrexone release from these microspheres may be governed by a combination of drug diffusion and polymer degradation. Drugs that are soluble in water can more easily diffuse into the release media compared to water insoluble compounds such as risperidone. Hence, there may be continuous release of drug from microsphere pores even in the absence of polymer

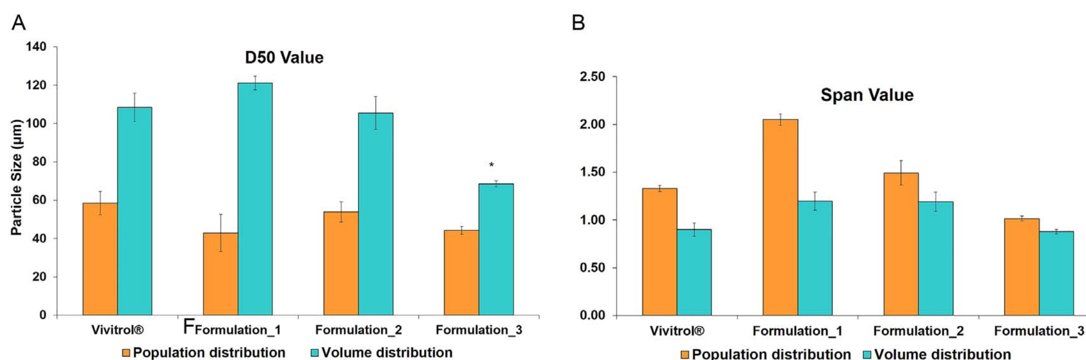

Fig. 1. Particle size and particle size distribution of Vivitrol® and the naltrexone microspheres prepared using different manufacturing processes. (A) D50 value; and (B) span value. All values are expressed as mean ± SD (*n* = 3 batches) (* represents statistically different particle size compared to Vivitrol®).

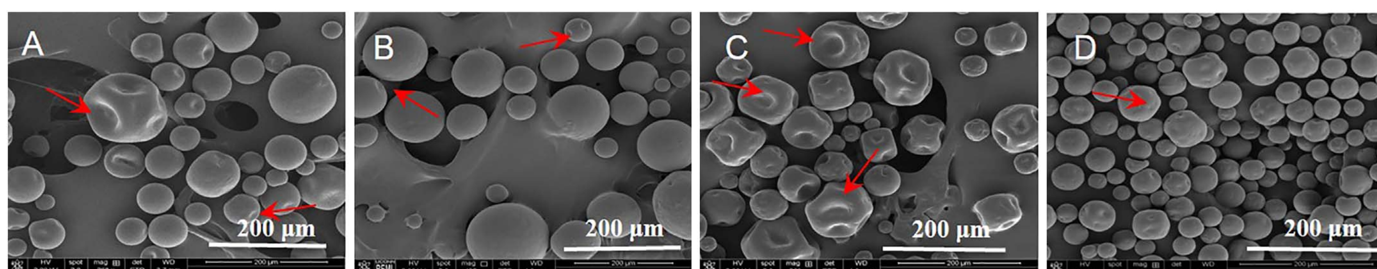

Fig. 2. SEM micrographs of the commercial product, Vivitrol® (A); Formulation 1 (B); Formulation 2 (C); and Formulation 3 (D). Symbol: The red arrows point to the pits of the microsphere surfaces.

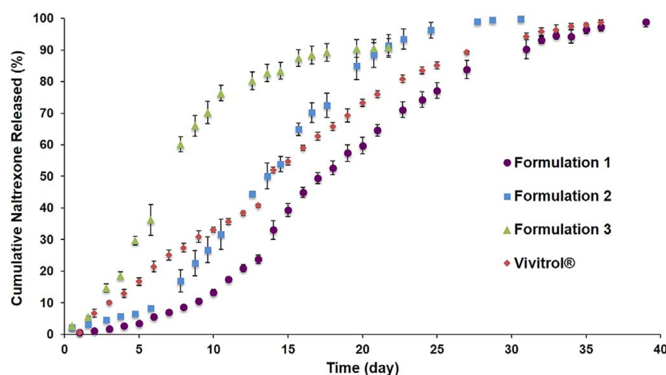

Fig. 3. *In vitro* release profiles of compositionally equivalent naltrexone microsphere formulations (with manufacturing differences) and Vivitrol®. The developed USP apparatus 4 method was used at 37 °C in 10 mM PBS (pH 7.4) containing 0.02% (w/v) Tween 20 and 0.02% (w/v) sodium azide (*n* = 3). The release media was replaced every five days.

degradation. Consequently, such drugs encapsulated in PLGA microspheres may have bi-phasic release profiles with no or very short lag phase.

Formulations 1 and 2 reached > 90% release at around day 40 and day 30, respectively, and had initial lag phases of approximately five days compared to Vivitrol® and Formulation 3, which did not have a lag phase (Fig. 3). The naltrexone microspheres prepared using the homogenization method (Formulation 3) which had a small and uniform particle size distribution as well as a highly porous structure, exhibited a much faster release rate and plateaued at around day 20. This can be compared to Formulations 1 and 2, which had larger particle size and longer release duration. Small microspheres have a shorter diffusion distance and the highly porous structure of Formulation 3 also facilitates diffusion. In addition, the increased water penetration leads to faster polymer degradation, which also facilitates drug release. Among the three prepared naltrexone microsphere formulations, Formulation 2 had an intermediate release rate due to its larger particle size compared to Formulation 3, and its higher porosity compared to Formulation 1. Formulation 1 showed the slowest release profile. This is considered to be a result of the low porosity and large particle size of Formulation 1 (Table 1). Interestingly, all the prepared naltrexone microsphere formulations showed low burst release. This was achieved by the use of low temperature (4 °C) and high vacuum (25–30 in. Hg) throughout the microsphere preparation process, which increases the polymer precipitation rate causing the drug to be entrapped within the microsphere matrix, resulting in a low burst release percentage. Furthermore, the *in vitro* release profile of the Vivitrol® was initially similar to Formulation 3 with no lag phase, however the release rate and duration were closest to Formulation 1. The Vivitrol® may be prepared *via* a different manufacturing process and/or with a different PLGA polymer. These results reaffirm, using another small molecule model compound, that the *in vitro* release characteristics of PLGA microspheres are very sensitive to changes in the manufacturing processes.

3.3. *In vivo* release characteristics of naltrexone microspheres

The pharmacokinetic profiles of the naltrexone solution following single intravenous administration (*i.v.*), and of the naltrexone microspheres following intramuscular administration (*i.m.*) in rabbits are shown in Fig. 4A and B, respectively. The *in vivo* profiles of all naltrexone microspheres (Fig. 4B) were determined to have good correlation with their respective *in vitro* release profiles (Fig. 3). Formulation 1 showed a slower absorption peak (T_{max}, Day 5) with the longest absorption/release duration (30 days) compared to the other naltrexone microspheres investigated. Formulation 2 had an intermediate absorption peak (T_{max}, Day 4) as well as an overall intermediate absorption/release duration (22 days), while Formulation 3 had the earliest absorption peak (T_{max}, Day 2) with the shortest absorption/release duration (15 days). These results are in line with the *in vitro* release profiles and with the differences in the porosity and mean particle size among the three formulations, as discussed above.

Key pharmacokinetic parameters of the naltrexone solution following *i.v.* administration were analyzed using WinNonlin® 6.4 (Pharsight,

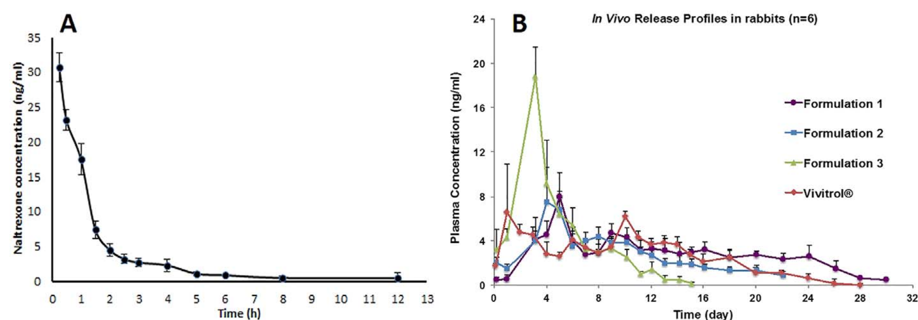

Fig. 4. Mean plasma concentration-time profiles of naltrexone in rabbits following: (A) intravenous administration of the naltrexone solution at a single dose of 0.11 mg/kg; and (B) intramuscular administration of naltrexone PLGA microspheres at a single dose of 11.69 mg/kg (mean ± SD, n = 6).

Table 2
Pharmacokinetic parameters of the naltrexone solution following intravenous administration (n = 6).

A	B	α (h^{-1})	β (h^{-1})	K_{10} (h^{-1})	K_{12} (h^{-1})	K_{21} (h^{-1})
38.446 ± 7.35	1.982 ± 0.820	1.124 ± 0.169	0.118 ± 0.047	0.795 ± 0.119	0.280 ± 0.058	0.168 ± 0.067

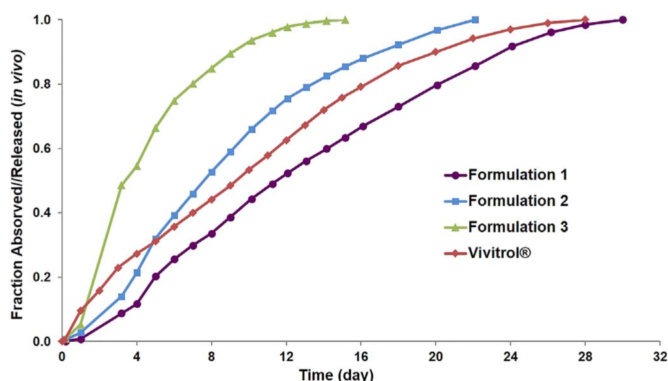

Fig. 5. *In vivo* fraction absorbed/released profiles (*i.m.*) of naltrexone microspheres prepared with manufacturing differences and Vivitrol® (deconvoluted using the Loo-Riegelman method from the data presented in Fig. 4).

Certara corporation, St. Louis, USA) and are shown in Table 2. These pharmacokinetic parameters were used to deconvolute the pharmacokinetic profiles (*i.m.*) of the naltrexone microspheres investigated using the Loo-Riegelman method [26]. As shown in Fig. 5, the deconvoluted *in vivo* profiles of the Vivitrol® and the prepared naltrexone microspheres followed the same rank order as their *in vitro* release profiles: Formulation 3 > Formulation 2, Vivitrol® > Formulation 1 (Fig. 3). It is also significant to note, that both Vivitrol® and Formulation 3 do not show evidence of a lag phase *in vivo* and this is consistent with their *in vitro* release profiles, whereas Formulations 1 and 2, which showed a lag phase in their *in vitro* release profiles also showed evidence of a change in the release rate *in vivo* around day four.

The deconvoluted *in vivo* release profiles appeared to be faster compared to their respective *in vitro* release profiles (Fig. 3). As reported previously, this might be due to enhanced polymer degradation as a result of local acidic pH [18,20] and the presence of other biological components (such as enzymes [33]) accelerating polymer degradation and thereby drug release *in vivo*. Moreover, the *in vivo* drug release profile of Vivitrol® in rabbits appeared to have a slightly shorter duration with a faster release rate compared to the clinical data reported in literature [34]. This is consistent with our recent research on risperidone microspheres, where the rabbit pharmacokinetic data were significantly faster than the clinical data [8]. It is suspected that the differences in the local environment (such as interstitial fluid volume, blood flow and the presence of other biological components) between the rabbit hind leg thigh muscle and human gluteal muscle as well as differences in drug metabolism may be responsible for the interspecies differences in the pharmacokinetics of both naltrexone and risperidone and their respective microsphere formulations [35].

It was demonstrated that despite all the prepared naltrexone microspheres being compositionally equivalent, they had different *in vitro* and *in vivo* drug release characteristics due to the differences in their physicochemical characteristics resulted from different manufacturing processes.

3.4. IVIVC

The "Guidance for industry, extended release oral dosage forms" recommends the use of a minimum of two, preferably three or more formulations with different release rates to develop an IVIVC if the *in vitro* release of the formulations is dependent on the release testing conditions [11]. It has been previously demonstrated that naltrexone release from the prepared microsphere formulations was dependent on the release testing conditions (such as pH, medium additives and temperature) [25]. Accordingly, all three prepared naltrexone microsphere formulations with different *in vitro* and *in vivo* drug release characteristics (Figs. 3 and 4B) were used to develop and validate an IVIVC. The fraction absorbed/released *in vivo* of any combination of two formulations out of the three prepared naltrexone microsphere formulations was plotted against the time-shifted fraction released *in vitro* at the respective time points to determine the correlation if any. The time-shifting factor (5.2) was kept the same for all naltrexone microspheres investigated. As shown in Fig. 6 (A, C and E), an affirmative IVIVC (*i.e.* a Level A, point-to-point correlation, as per U.S. FDA guidance) between the fractions released *in vitro* and fractions released/absorbed *in vivo* was observed for all combinations (correlation coefficients > 0.94). All the developed IVIVCs were comparable as manifested by similar slopes and intercepts.

The IVIVC equation obtained using two internal formulations was used to predict the *in vivo* performance of the third external formulation from its "real-time" *in vitro* release profile. The predicted *in vivo* profile of the third external formulation was compared with its deconvoluted *in vivo* profiles obtained in rabbits. As shown in Fig. 6 (B, D and F), the predicted *in vivo* profiles of Formulations 1 and 2 were similar to the observed *in vivo* profiles with no significant difference. However, that of Formulation 3 did show differences, particularly within the first 8 days. This may be due to the dissimilarity in the drug release profile of Formulation 3 compared to Formulations 1 and 2, in that Formulation 3 does not exhibit a lag phase.

The *in vivo* release profile of the commercial product Vivitrol® was also predicted from its "real-time" *in vitro* release profile using all three

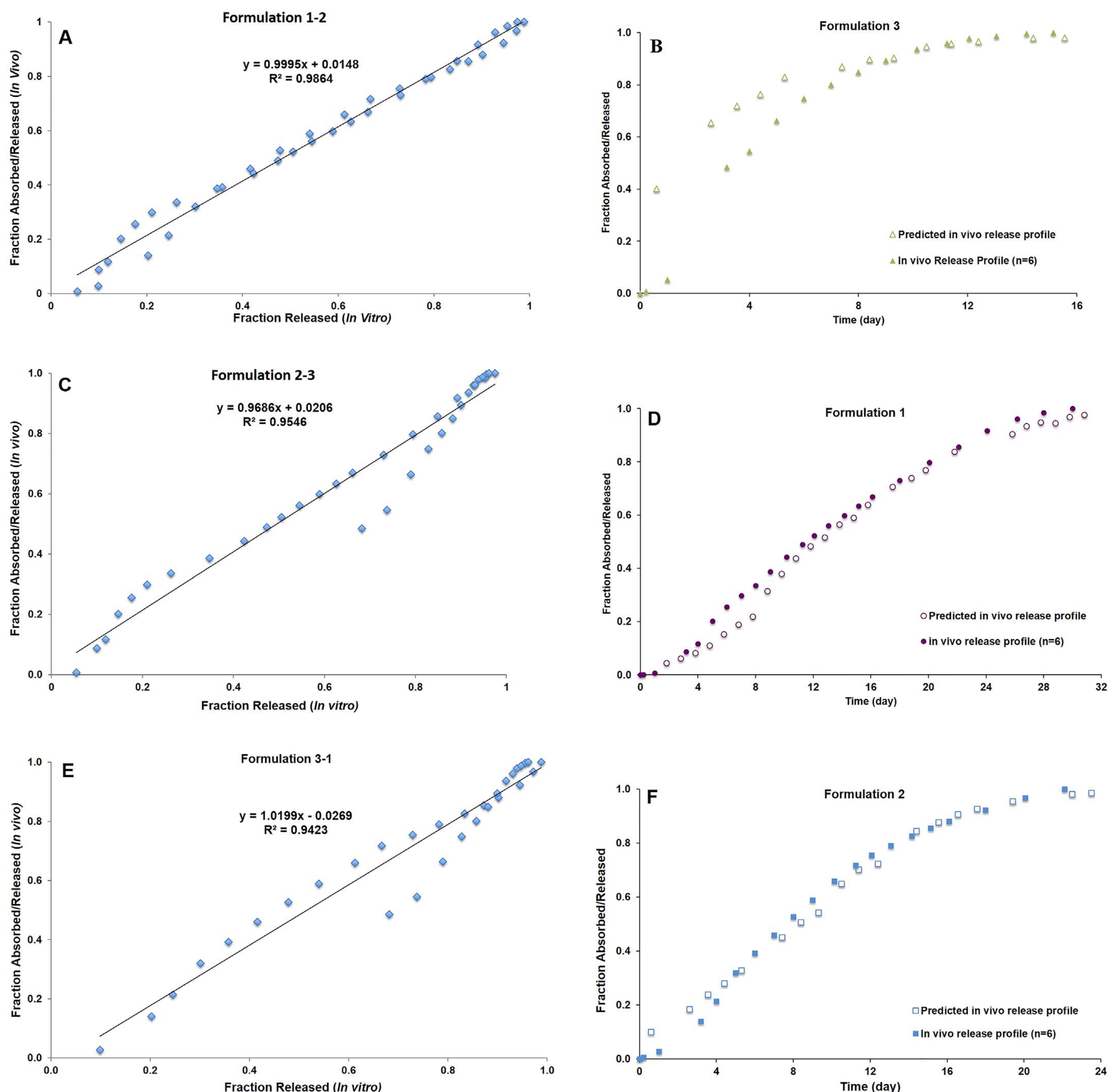

Fig. 6. Level A IVIVC developed for naltrexone microspheres using the Loo- Reigelman method (time shifting factor: 5.2). (A) IVIVC_1 (developed using Formulations 1 and 2). (B) Experimental and predicted *in vivo* release profiles of Formulation 3. (C) IVIVC_2 (developed using Formulations 2 and 3). (D) Experimental and predicted *in vivo* release profiles of Formulation 1. (E) IVIVC_3 (developed using Formulations 3 and 1). (F) Experimental and predicted *in vivo* release profiles of Formulation 2.

developed IVIVCs, and compared with its observed *in vivo* profile (deconvoluted) obtained in rabbits. As shown in Fig. 7, all three predicted *in vivo* release profiles were similar to the observed *in vivo* profiles, irrespective of which developed IVIVC was used. However, during the initial release period (of approximately four to five days), the predicted release profile of Vivitrol® was slightly higher than that observed. It would appear that microsphere formulations with and without a lag phase are not ideal predictors of one another. A similar observation has been reported for compositionally equivalent risperidone microsphere formulations with manufacturing differences, where formulations with high burst release were not ideal predictors for formulations with low burst release. However, for the compositionally equivalent risperidone microspheres, the developed IVIVCs showed acceptable predictability in terms of % prediction error of $AUC_{0\text{-last}}$ and C_{max} as per U. S. FDA guidance irrespective of differences in burst release.

The internal validation of the developed IVIVC model was accomplished by convolving the *in vitro/in vivo* release data of Formulations 1 and 3, since Formulations 1 and 3 had the slowest and fastest release rates. Table 3 represents the %prediction error (PE) values for the Cmax and AUC of these two formulations. The PE% values of the Cmax and AUC of Formulation 1 were − 1.68 and − 12.16, respectively. The PE% values of the Cmax and AUC of Formulation 3 were − 22.24 and − 1.92, respectively. The average absolute %PE value of Cmax and AUC were 11.96 and 7.04, respectively. As per the U.S. FDA guidance [11], the predictability can be acceptable when the average absolute %PE

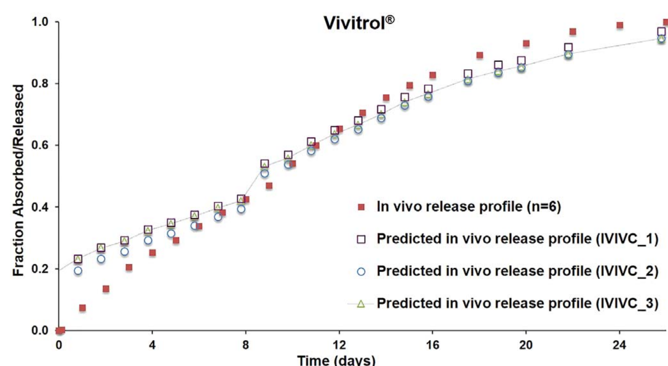

Fig. 7. Experimental and predicted (time shifting factor: 5.2) *in vivo* release profiles of Vivitrol® using the developed IVIVCs. IVIVC_1 developed using Formulations 1 and 2. IVIVC_2 developed using Formulations 2 and 3. IVIVC_3 developed using Formulations 3 and 1.

Table 3
Validation and % prediction error (PE) of the developed IVIVC for naltrexone microspheres.

	C_{max} (μg/L)			$AUC_{0\text{-last}}$ (μg/L* day)		
	Obs.	Pred.	%PE	Obs.	Pred.	%PE
Internal validation						
Formulation1	7.98	7.84	− 1.68	80.82	70.99	− 12.16
Formulation 3	18.78	14.61	− 22.24	72.17	70.79	− 1.92
Average internal			11.96			7.04
External validation						
Formulation 2	7.49	7.74	3.38	62.78	69.14	10.13
Prediction						
Vivitrol®	7.54	6.84	− 9.27	74.60	81.70	9.53

values are of 10% or less and the %PE value for each formulation should not exceed 15%. In the present study, the average absolute internal %PE for the AUC (7.04%) was within the recommended range of 10% or less. However, the average absolute internal %PE for C_{max} (11.96%) was slightly > 10%, suggesting the internal predictability of the developed IVIVC for C_{max} was inconclusive. The FDA guidance states that if the criteria for internal validation are not met, the external predictability should be evaluated as the final determination of the IVIVC model. Accordingly, the evaluation of external predictability of the IVIVC was performed. It can be seen in Table 3 that the external %PE for C_{max} and $AUC_{0\text{-last}}$ were 3.38% and 10.13%, respectively, which is in accordance with the recommended external predictability evaluation (%PE of 10% or less). These results indicate that the developed IVIVC has good external predictability, thus could be used as a surrogate for bioequivlance in rabbits. Furthermore, the predictability of the developed IVIVC for Vivtrol® was also investigated. Both %PEs for C_{max} (− 9.27%) and $AUC_{0\text{-last}}$ (9.53%) were below 10%. These results confirmed that the developed IVIVC for naltrexone microspheres can be used to predict not only the prepared compositionally equivalent formulations with manufacturing differences but also a microsphere formulation with relatively similar composition and drug loading.

4. Conclusions

The present manuscript describes for the first time that a Level A IVIVC based on a compendial dissolution apparatus (USP apparatus 4) can be developed for compositionally equivalent PLGA microspheres containing a therapeutic that is water soluble (*i.e.* naltrexone), and for PLGA microspheres with bi-phasic release characteristics. The critical quality attributes of the naltrexone microspheres (such as drug loading, particle size, and porosity) were very sensitive to manufacturing differences such as solvent system, solvent removal rate, and temperature as well as the particle size reduction technique. It was shown that solvent removal, drug diffusion and polymer precipitation during microsphere solidification must be closely controlled in order to maintain consistently high drug loading for microspheres containing hydrophilic therapeutics. The developed USP apparatus 4 method was able to detect *in vitro* performance changes resulting from manufacturing processes differences and most importantly, predict *in vivo* performance of the naltrexone microspheres. Due to the instability issues associated with naltrexone, frequent medium replacement was implemented during long-term release testing to prevent oxidative degradation of naltrexone. Together with our previous research on developing a Level A IVIVC for polymeric microspheres containing a water insoluble therapeutic (*e.g.* risperidone) with tri-phasic release characteristics, it can be concluded that Level A IVIVCs can be developed for parenteral microsphere drug products using USP apparatus 4 in a rabbit model. However, considering the interspecies differences between animals and humans, further investigation is necessary to determine whether an IVIVC can be developed using clinical data. In addition, it would appear that care should be taken in developing and applying IVIVCs to formulations with significant differences in burst release and lag phases.

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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