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Validation of USP apparatus 4 method for microsphere *in vitro* release testing using Risperdal® Consta®

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ABSTRACT

The current manuscript addresses the need for a validated *in vitro* release testing method for controlled release parenteral microspheres. A USP apparatus 4 method was validated with the objective of possible compendial adaptation for microsphere *in vitro* release testing. Commercial microspheres (Risperdal® Consta®) were used for method validation. Accelerated and real-time release tests were conducted. The accelerated method had significantly reduced test duration and showed a good correlation with the real-time release profile (with limited number of sample analysis). Accelerated conditions were used for method validation (robustness and reproducibility). The robustness testing results revealed that release from the microspheres was not flow rate dependent and was not affected by minor variations in the method (such as cell preparation technique, amount of microspheres, flow-through cell size and size of glass beads). The significant difference in the release profile with small variations (±0.5 °C) in temperature was shown to be due to a change in risperidone catalyzed PLGA degradation in response to temperature. The accelerated method was reproducible as changing the system/equipment or the analyst did not affect the release profile. This work establishes the suitability of the modified USP apparatus 4 for possible compendial adaptation for drug release testing of microspheres.

1. Introduction

Controlled release parenteral drug products have been developed to deliver drugs for the treatment and prevention of a variety of diseases (Adler-Moor and Proffitt, 2005; Martin, 2005; Tracy, 2005). The number of these drug products is expected to further increase since: (1) new peptide and protein drugs are being discovered that are unstable and exhibit poor permeability in the gastro-intestinal tract; (2) gene medicine is moving from research into the clinic and these products require parenteral delivery; (3) drug targeting is becoming more common (to avoid toxic side effects, to localize expensive drugs at the site(s) of action and to deliver drugs to otherwise inaccessible sites in the body).

Controlled release parenteral drug products are available in several dosage forms, including microspheres, liposomes, implants, and drug eluting stents. These products are mostly given to patients *via* subcutaneous (SC) or intramuscular (IM) routes (intra-cornory delivery in the case of drug eluting stents) with drug release *in vivo* for weeks or months. Appropriate tests are needed to ensure the safety and efficacy of controlled release parenteral products. Unlike conventional dosage forms, there is a lack of standard pharmacopoeial/regulatory tests for controlled release parenteral drug products, constraining their development. In particular, there is no standard method(s) to test *in vitro* release for the purposes of: routine assessment of product quality; formulation optimization in product development; as well as for development of *in vitro–in vivo* correlation/relationships (IVIVC/R) (Burgess et al., 2004; Martinez et al., 2008; Siewert et al., 2003). The currently used methods such as 'sample and separate' and 'dialysis sac' do not use an official USP dissolution/release apparatus (Cleland et al., 1997; D'Souza and DeLuca, 2006). This makes inter-laboratory comparisons and regulatory approval difficult. The need of guidance for *in vitro* release testing methods of controlled release parenteral drug products has been emphasized in the workshops organized by American Association of Pharmaceutical Scientist (AAPS), the International Pharmaceutical Federation (FIP), the European Federation of Pharmaceutical Scientists (EUFEPS), the Controlled Release Society (CRS), the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the US Food and Drug Administration (FDA) and the European Agency for Evaluation of Medicinal Products (EMEA) (Burgess et al., 2004, 2002; Gray, 2002; Martinez et al., 2008; Siewert et al., 2003). Siewert et al. (2003) (FIP/AAPS

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guidelines) have suggested the use of compendial methods or their modification for the *in vitro* release testing of novel/special formulations wherever possible.

Poly(lactide-co-glycolide) (PLGA) microspheres have emerged as one of the most promising controlled release parenteral products. Since 1989, FDA has approved many parenteral PLGA microsphere products (*e.g.* Lupron Depot®, Sandostatin LAR®, Nutropin Depot®, Trelstar Depot® and Resperdal® Consta®). A variety of methods such as 'sample and separate', 'dialysis sac' and 'continuous flow' are being used for *in vitro* release testing of microspheres. However, the *in vitro* release profiles vary depending upon the test conditions used (Cleland et al., 1997; D'Souza and DeLuca, 2006; Zolnik et al., 2005). Therefore, there is a need of a standard *in vitro* release testing method for these products that can be used to minimize variations in release profiles and make inter and intra laboratory comparisons possible.

Zolnik et al. have developed a modified USP apparatus 4 (flow-through cell) method for *in vitro* release testing of microspheres under real time and accelerated testing conditions and have demonstrated the advantages of the USP apparatus 4 method over conventional 'sample and separate' methods (Voisine et al., 2008; Zolnik et al., 2006, 2005). Besides geometrical/dimensional and operational (*i.e.* flow rate, temperature, *etc.*) accuracy, the modified USP apparatus 4 (flow-through cell) method offers the advantages of: (1) separation of microspheres from the release medium; (2) flexibility of using different release media volumes (as needed); (3) ease of operation; (4) minimum evaporation of the media; (5) flexibility of monitoring release *via in situ* fiber optics; (6) automation. However, validation of the modified USP apparatus 4 method needs to be performed to ensure its suitability for the intended use (*e.g.* as a standard *in vitro* release testing method for quality control of microspheres). ICHQ2A guidelines on 'Analytical Method Validation' defines robustness of a method as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage" (ICH-Q2A, March 1995). Robustness testing involves determining parameters that are critical for method reproducibility. Method reproducibility is determined by performing the *in vitro* release test on different days, using different equipment and having the tests performed by different analysts (Brown, 2005).

Controlled release parenteral PLGA microspheres are designed to release drug over long periods of time (*i.e.* days to months). Therefore, accelerated *in vitro* release tests are needed for quality control and formulation development. However, a relationship between the real time and accelerated method should be established in order to demonstrate the suitability of the test method to be used in routine quality control testing. Accelerated drug release from PLGA microspheres can be achieved by increasing PLGA degradation at elevated temperature or extreme pH conditions (acid or alkali catalyzed PLGA degradation) (Zolnik and Burgess, 2007; Zolnik et al., 2006). It is desired that the mechanism of drug release does not change during accelerated release testing so that ideally a 1:1 correlation can be established between accelerated and real time release profiles. However, it is possible that the release mechanism changes under accelerated test conditions (extreme pH or temperature). Such a method showing at least a rank order correlation between different formulations can be used for quality control purposes (Martinez et al., 2008; Zolnik and Burgess, 2007; Zolnik et al., 2006).

The present work introduces a meaningful *in vitro* release testing method for controlled release microspheres. Real time and accelerated release methods were developed using modified USP apparatus 4 (flow-through cell). The accelerated method was validated for robustness and reproducibility and the method parameters critical for reproducibility of release profiles obtained using modified USP apparatus 4 were identified. The commercial PLGA microspheres: Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) were used for this work.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) polymer, PLGA 65:35 (MW: 95 kDa) was a gift from Birmingham Polymers, Inc. Methylene chloride, tetrahydrofuran (optima grade) and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Pittsburg, PA). Risperidone was purchased from Sigma–Aldrich (St. Louis, MO) and Tecoland Corporation (Edison, NJ). Poly(vinyl alcohol) (PVA) (MW: 30–70 kDa), trifluoroacetic acid were obtained from Sigma–Aldrich (St. Louis, MO). Nanopure™ quality water (Barnstead, Dubuque, IA) was used for all studies. The commercial microspheres Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) (batch numbers: 176921, 168800, 169941, 9BA231, 9BA237, 8MA071, 9MA575 and 8JA816; Ortho McNeil-Janssen) were purchased from University of Connecticut, Student Health Services pharmacy.

2.2. Methods

2.2.1. Preparation of microspheres

Risperidone loaded PLGA microspheres were prepared by oil-in-water (o/w) emulsion solvent extraction/evaporation technique. One gram of PLGA was dissolved in four ml methylene chloride. 300 mg risperidone was dissolved in the PLGA solution using a homogenizer (PowerGen 700 D, Fisher Scientific) at 10,000 rpm for 30 s. This organic phase was then added slowly to 20 ml of a 1% (w/v) aqueous poly(vinyl alcohol) (PVA) and homogenized at 10,000 rpm for 2 min. This emulsion was added to 250 ml of a 0.1% (w/v) aqueous PVA solution and stirred at 600 rpm under vacuum for 4 h at 25 °C. The resulting microspheres were filtered (Durapore® membrane filter, 0.45 μm, Fisher Scientific), washed three times with de-ionized water and vacuum dried for 24 h. Microsphere formulations were prepared in triplicate.

2.2.2. Characterization of drug and microspheres

2.2.2.1. High performance liquid chromatography (HPLC). The concentration of risperidone was determined using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer) set at 275 nm. The mobile phase was acetonitrile: water: trifluoroacetic acid (25:75:0.1%, v/v/v). Agilent C18 stable bond (4.6 mm × 15 cm) column was used with the flow rate set at 1 ml/min. The chromatographs were analyzed using PeakSimple™ Chromatography System (SRI Instruments, Torrace, CA). An injection volume of 30 μl was used for drug release samples. This validated method is a stability indicating HPLC assay. A standard curve was prepared to include 80–120% (0.1–20 μg/ml) of the expected unknown (drug) concentration. The precision, accuracy and linearity were determined in the range 0.5–20 μg/ml.

2.2.2.2. Determination of ionization constant (pK_a) of risperidone. Ionization constants of risperidone were determined by potentiometric titration using a glass electrode (Fisher Scientific pH meter) at 25, 40 and 45 °C (Albert and Serjeant, 1984). A 0.001 M solution of risperidone was prepared in de-ionized water. 25 ml of risperidone solution (0.001 M) was titrated with 0.01 N hydrochloric acid (HCl). The pH and volume at the half neutralization point was determined and ionization constants (pK_a) of risperidone at different temperatures were calculated using Handerson–Hasselbach equation. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.3. Drug loading. Five mg of microspheres were dissolved in 10 ml tetrahydrofuran (THF). The solution was filtered (Millex® HV, PVDF 0.45 μm syringe filter) and the risperidone concentration was determined *via* HPLC as described before using an injection volume of 2.5 μl. Drug loading was determined as: percent drug loading = (weight of drug entrapped/weight of microspheres used) × 100. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.4. Particle size analysis. An AccuSizer 780A autodiluter particle sizing system was used to determine the mean particle diameter. About 50 mg of microspheres were dispersed in 2 ml of 0.1% (w/v) PVA solution. 200 μl of the dispersion was used for particle size analysis. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.5. Glass transition temperature. The glass transition temperature of the microspheres was analyzed using a TA instrument Q100 differential scanning calorimeter (DSC). Samples were heated from −40 °C to 100 °C, cooled to −40 °C and heated again to 100 °C at a rate of 20 °C/min. The first cycle of the thermograms was used to determine the glass transition temperature (Tg) of the microspheres. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.6. Molecular weight determination. The molecular weight of the microspheres was determined by gel permeation chromatography (GPC; Waters) with an evaporative light scattering detector (ELSD). The mobile phase was THF with a flow rate of 2 ml/min at 40 °C. Ten mg microspheres were dissolved in 10 ml tetrahydrofuran (THF) and filtered through 0.45 μm filters for GPC analysis. The data collection and analysis was performed using Waters Millenium software. Polystyrene standards (2000, 900, 824, 400, 200, 110, 43, 18.80, 17.60, 6.93, 2.61, 0.98 kDa) were used for calibration and weight average molecular weights (Mw) were calculated. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.7. Microsphere degradation. 10 mg of blank and risperidone microspheres were placed in 6 ml phosphate buffer saline pH 7.4 in round bottom glass tubes. The tubes were incubated in water bath maintained at 40 and 45 ± 0.2 °C. Three tubes each of blank and risperidone loaded microspheres were removed every 24 h up to 4 days. The tubes were vacuum dried for 24 h after decanting the buffer. The change in molecular weight of the microspheres was monitored using GPC as described in Section 2.2.2.6.

2.2.2.8. In vitro release. The USP apparatus 4 (Sotax CE7 smart with CY 7 piston pump, Sotax, Horsham, PA) was modified by packing the flow-through cells (12 mm diameter) with 1 mm diameter glass beads (Zolnik et al., 2005). The modified USP apparatus 4 (flow-through cell) was operated in a closed system configuration. Flow-through cells were prepared by filling 1/3rd of the cells with 1 mm diameter glass beads. About 10 mg of microspheres were weighed and divided into three approximately equal parts. First part was placed over the glass beads in the cells and one small spoon (Sotax, catalogue number: 5609) of glass beads was added thereafter. This process was repeated for the second and third parts of the microspheres. The cells were filled with the remaining glass beads up to the brim. An anti-static gun was used to neutralize static charge on glass beads, microspheres and spatula to facilitate sample preparation. 250 ml of 0.05 M phosphate buffer saline pH 7.4 with 0.1% sodium azide was circulated through the flow-through cells (fitted with regenerated cellulose filter 0.45 μm) at a flow rate of 8 ml/min. To evaluate the robustness of the method, higher flow rate (16 ml/min), larger glass beads (2.4–2.9 mm diameter) and larger flow-through cell (22.6 mm diameter) were used. In addition, the flow-through cells were prepared in alternative ways by: (1) adding microspheres in two parts instead of three; (2) increasing the amount of microspheres by 50%. Alternative pH values investigated were pH 6.9 and 7.9. The temperature of the Sotax USP apparatus 4 was maintained at 37 ± 0.1 °C and 45 ± 0.1 °C for real time and accelerated tests, respectively. The temperature (in the flow through cells) was varied by ±0.5 °C in the accelerated tests. The release medium was de-aerated using the USP method (USP General Chapter <711>) before starting the release test. De-aeration during the test was performed using helium sparging in the media bottles. One ml samples were withdrawn and replaced with fresh media at suitable time intervals. The samples were analyzed using HPLC as described before using an injection volume of 30 μl. Release medium was replaced with fresh media at appropriate time intervals to maintain sink conditions. Media replacement during the release study was taken into account in the calculation of fraction release. All drug release tests were conducted in triplicate and the results are reported as the mean ± SD.

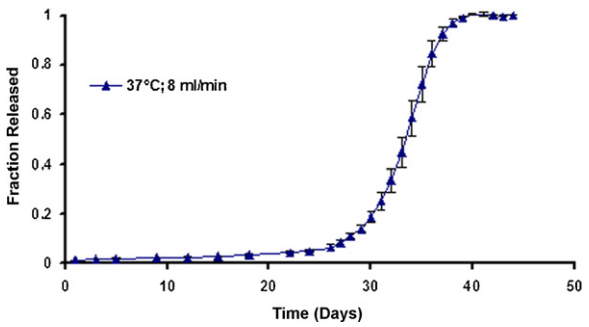

Fig. 1. *In vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 37 °C ($n = 3$).

3. Results

Commercial microspheres (Risperdal® Consta®) were used for the accelerated and real time *in vitro* release testing with the modified USP apparatus 4 (flow-through cell) method. The drug loading and particle size range reported for these microspheres were ∼38% w/w and 25–150 μm, respectively (Ramstack et al., 2002). The drug loading of the microspheres determined experimentally in the present work was 39.74 ± 0.67% (w/w).

3.1. Real time and accelerated release profiles

The *in vitro* release of the commercial microspheres (Risperdal® Consta®) performed under real time conditions (37 °C) showed an initial burst release (24 h release) of 1.6% followed by a lag phase of about 24 days. The lag phase was followed by a drug release phase from day 24 to 40 (Fig. 1). Accelerated *in vitro* release testing was performed by elevating the temperature from 37 to 45 °C. A burst release (24 h release) of approximately 2% was observed under accelerated conditions. The lag phase was significantly reduced from approximately 24 days at 37 °C to 4 days at 45 °C. The lag phase of 4 days under accelerated conditions was followed by a rapid drug release phase for up to approximately 7 days. The overall duration of drug release from the microspheres was reduced from approximately 40 to 7 days (Fig. 2).

The real time and accelerated release profiles were compared on the same axis by time scaling (scaling factor: 6.5) the real time release profile. As shown in Fig. 2, the real time and accelerated release profile overlapped after time scaling. Scaling factor was determined as the ratio of time to 50% drug release under real time

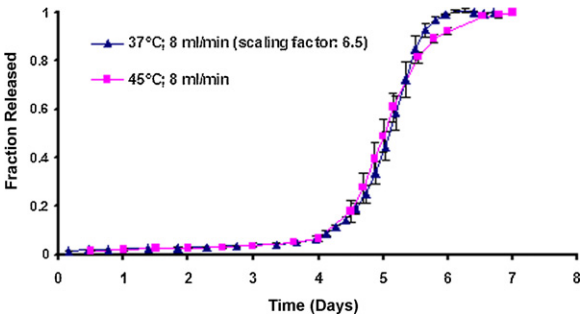

Fig. 2. *In vitro* release profiles of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 37 °C (time scaled) and 45 °C ($n = 3$).

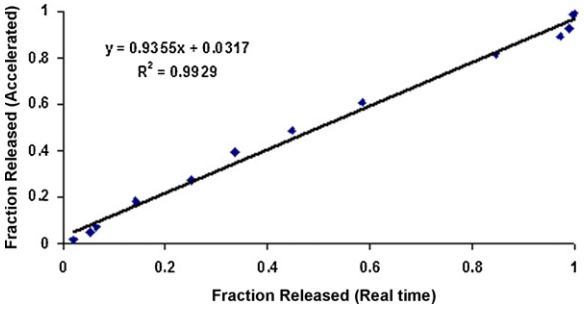

Fig. 3. Correlation between real time (37 °C) and accelerated (45 °C) fraction released.

and accelerated release conditions. A good correlation (with the limited sample analysis, $n = 3$) between fraction released under real time and accelerated conditions was obtained with a correlation coefficient of 0.9929 (Fig. 3).

3.2. Robustness evaluation of the accelerated drug release method

Robustness testing of the original accelerated release conditions was performed using the commercial microspheres (Risperdal® Consta®). Robustness of the accelerated drug release method was evaluated by making deliberate changes to the method parameters: (1) flow rate; (2) sample preparation technique; (3) size of the flow-through cell; (4) amount of microspheres; (5) size of glass beads; (6) pH of the release medium; (7) temperature. As shown in Fig. 4, the *in vitro* release of drug from the microspheres was not affected by the flow rate (8 and 16 ml/min). As explained in Section 2.2.2.8, the flow through cells were prepared for release testing by adding microspheres in three approximately equal parts in between the glass beads. The effect of cell preparation technique was evaluated by adding microspheres in two approximately equal parts instead

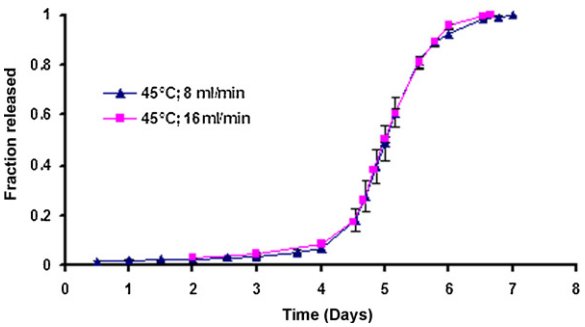

Fig. 4. Effect of flow rate on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

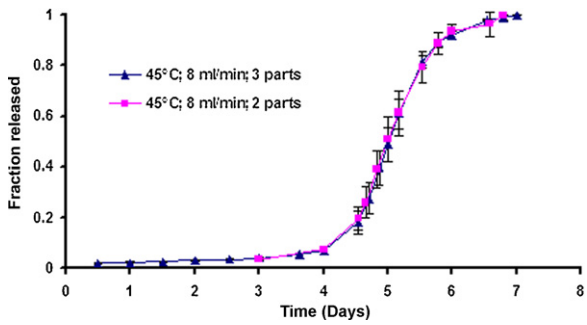

Fig. 5. Effect of cell preparation technique on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

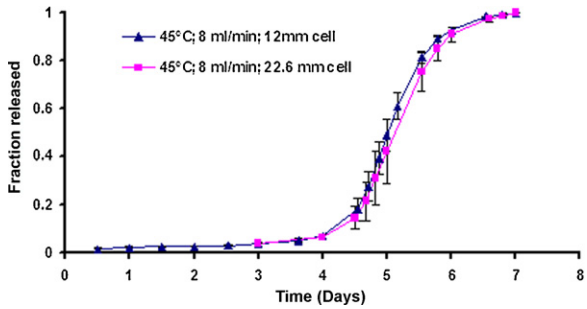

Fig. 6. Effect of flow-through cell size on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

of three. However, this change in cell preparation technique had no affect on the release profile of the microspheres (Fig. 5). The results were not affected by change in the size of the flow-through cell (12–22.6 mm diameter) as shown in Fig. 6. Increasing the amount of microspheres by 50% did not have any affect on the release profile (Fig. 7). There was no change in the release profiles when size of the glass beads was increased from 1 mm to 2.4–2.9 mm (Fig. 8). A slightly slower release profile was observed when the pH of the release medium (phosphate buffer saline) was decreased by 0.5 units (pH 7.4–6.9) whereas the release profile was faster when pH was increased by 0.5 units (pH 7.4–7.9) (Fig. 9). The release profile of the microspheres was significantly affected by change in release test temperature by ±0.5 °C as shown in Fig. 10.

3.3. Temperature sensitivity of risperidone loaded PLGA microspheres

Blank and risperidone microspheres were prepared (as described in Section 2.2.1) in order to further investigate the

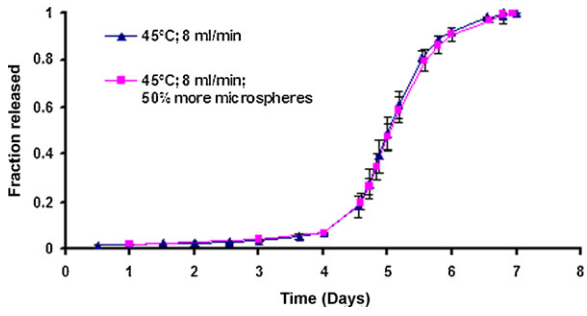

Fig. 7. Effect of amount of microspheres on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

Table 1
Properties of commercial Risperdal® Consta® 25 mg long acting injection and risperidone loaded (and blank) PLGA microspheres prepared in the present work.

Parameters	Risperdal® Consta® 25 mg long acting injection	Risperidone loaded PLGA microspheres	Blank microspheres
Molecular weight and co-polymer ratio (kDa)	~90[a]	~95 (65:35)	~95 (65:35)
Particle size (μm)	25–150[a]	6.7 ± 0.62	7.97 ± 0.91
Drug loading (% w/w)	39.74 ± 0.67	14.23 ± 1.42	–
Burst release (%)	1.6 ± 0.10	1.22 ± 0.42	–
Glass transition temperature (°C)	48.84 ± 0.34	40.56 ± 2.30	38.4 ± 0.52

[a] Values from literature (Ramstack et al., 2002).

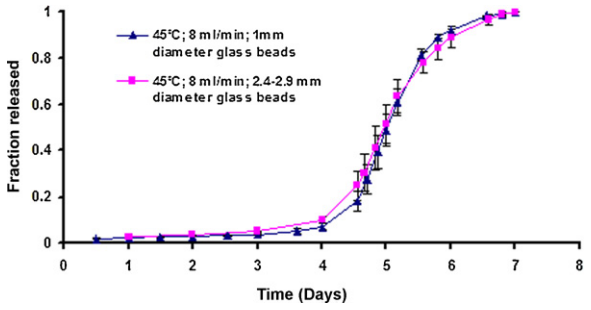

Fig. 8. Effect of size of glass beads on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

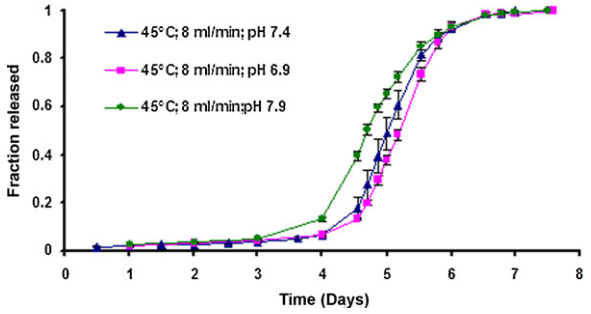

Fig. 9. Effect of pH on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 ± 0.5 at 45 °C ($n = 3$).

temperature sensitivity (Fig. 10) of the commercial microspheres (Risperdal® Consta®). 95 kDa (65:35 co-polymer ratio) PLGA was used for microsphere preparation. As shown in Table 1, the characteristics of the commercial and the prepared risperidone microspheres were different. The average particle size and drug loading of the prepared risperidone microspheres were approximately 7 μm and 14% (w/w), respectively whereas the reported particle size and drug loading of the commercial microspheres

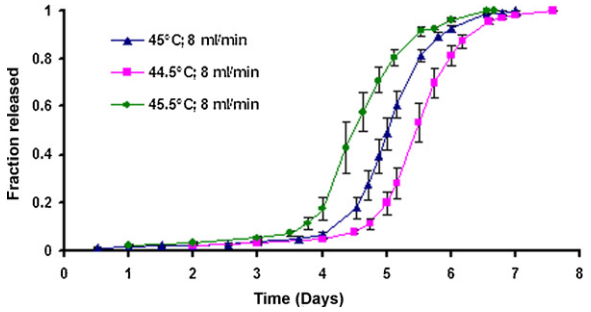

Fig. 10. Effect of temperature on the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 ± 0.5 °C ($n = 3$).

(Risperdal® Consta®) were 25–150 μm and 38% (w/w), respectively (Ramstack et al., 2002). The glass transition temperature of the commercial microspheres (~48 °C) was higher than the prepared blank and risperidone microspheres (~40 °C). However, the burst release from the two formulations (commercial and the prepared risperidone microspheres) was similar (24 h release determined as described in Section 2.2.2.8) (Table 1).

The prepared risperidone microspheres degraded faster when compared to the corresponding blank microspheres as evident from the change in molecular weight with time in Fig. 11. The change in molecular weight in response to the temperature difference of 5 °C (40 and 45 °C) was also higher in the case of prepared risperidone microspheres as compared to the blank microspheres.

3.4. Reproducibility testing

The *in vitro* release tests were performed (using the commercial microspheres, Risperdal® Consta®) by: (1) the same analyst using different USP apparatus 4 systems and (2) by different analysts using different USP apparatus 4 systems to determine method reproducibility (Figs. 12 and 13). There was no change in the release profiles indicating that the modified USP apparatus 4 (flow-through cell) was reproducible for *in vitro* release testing of microspheres.

4. Discussion

The commercial microspheres (Risperdal® Consta®) exhibited a small burst release (approximately 1.6% within the first 24 h) which may be due to the diffusion of surface associated drug. The prolonged lag phase (~3 weeks) observed in the real time *in vitro* release is considered to be due to the polymer erosion needed to generate sufficient microsphere porosity to facilitate drug diffusion and subsequent release. A change in polymer molecular weight

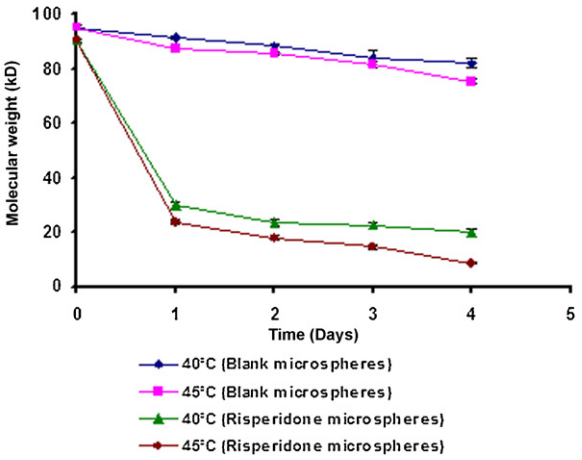

Fig. 11. Change in molecular weight of blank and risperidone loaded PLGA microspheres at 40 and 45 °C.

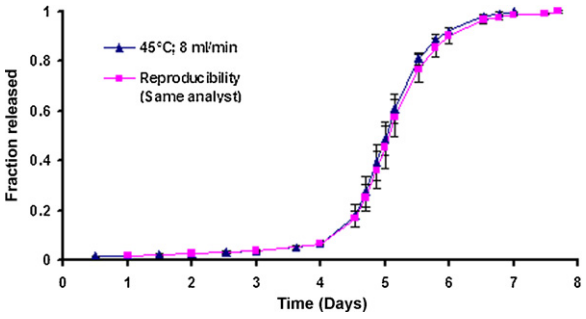

Fig. 12. Reproducibility (same analyst and different equipment) of the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

from 90 to 20 kDa during the lag phase has been reported for these microspheres (Ramstack et al., 2002). A combination of polymer erosion and drug diffusion has been explained as the possible drug release mechanism for higher molecular weight PLGA microspheres (Zolnik et al., 2006). Accordingly, the drug release phase of the commercial microspheres (Risperdal® Consta®) (weeks 4–6) is considered to be due to a combination of polymer erosion and drug diffusion. The polymer molecular weight decreases from 20 kDa to less than 10 kDa during the drug release phase of the commercial microspheres (Risperdal® Consta®) as reported by Ramstack et al. (2002).

Accelerated release testing significantly reduced the duration of drug release from the commercial microspheres (Risperdal® Consta®). The overlap of real time (37 °C) and accelerated release profiles (45 °C) after time scaling (Fig. 2) indicated that accelerated release testing at elevated temperature did not change the mechanism of drug release from the microspheres. This observation is supported by the work of Zolnik et al. (2006) where the accelerated tests at elevated temperatures followed an erosion controlled release mechanism and were predictive of the secondary zero order release phase at 37 °C.

The accelerated *in vitro* release test method was validated (robustness and reproducibility). The robustness testing showed that drug release from the commercial microspheres (Risperdal® Consta®) was not flow rate dependent (Fig. 4) indicating that the accelerated drug release is controlled by erosion of the polymer. Similar results have been reported previously where flow rate (USP apparatus 4) had no affect on dexamethasone release from high molecular weight (25, 28 and 70 kDa) PLGA microspheres as the drug release mechanism was erosion controlled. Flow rate dependent release was observed from low molecular weight PLGA microspheres (*i.e.* 5 kDa) due to the diffusion controlled drug release mechanism (Zolnik et al., 2006).

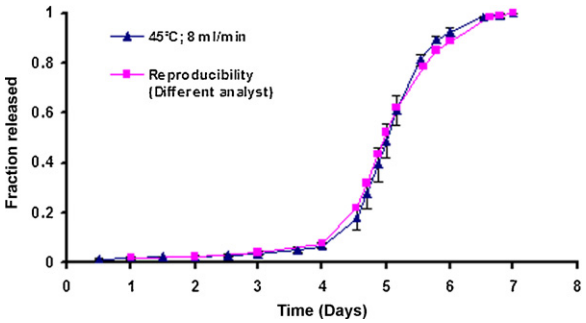

Fig. 13. Reproducibility (different analysts and different equipments) of the *in vitro* release profile of Risperdal® Consta® 25 mg long acting injection (risperidone microspheres) in 0.05 M PBS pH 7.4 at 45 °C ($n = 3$).

The effect of slight change in cell preparation technique and the amount of microspheres added to the flow through cell were evaluated as these parameters are likely to vary with analysts and experiments (performed at different times). The method was shown to be robust with respect to the cell preparation technique (dividing into two or three parts) and the amount of microspheres added to the flow through cell (Figs. 5 and 7). The two flow-through cell sizes (*i.e.* 12 mm and 22.6 mm diameter) were evaluated in case there was a need of specifying any one particular cell size for microspheres release testing. Flow-through cell size did not have any effect on the release profile of the microspheres (Fig. 6). Flow-through cell size may be selected depending upon the amount of microspheres to be used for release testing. One mm diameter glass beads are commonly used with USP apparatus 4. It was pertinent to evaluate the effect of larger glass beads on the release since glass beads size can modify the distribution of microspheres in the cell (change in void volume) as well as the flow pattern around the microspheres. Increasing the glass beads size from 1 mm to 2.4–2.9 mm had no effect on the release profile (Fig. 8). Glass beads less than 1 mm diameter were not evaluated as they can block the inlet tubing in the flow through cell. Change in flow-through cell size at a particular flow rate can affect hydrodynamics inside the cell. However, this is not expected to affect the release profile of erosion controlled microspheres such as those evaluated in the present work.

PLGA degradation is catalyzed by both alkaline and acidic pH (Makino et al., 1985; Zolnik and Burgess, 2007). A faster release was observed when the pH of medium (PBS) was increased by 0.5 units (pH 7.4 to 7.9). This is considered to be due to the increase in hydroxyl ion concentration and hence, faster PLGA degradation. The release profile was slower when the pH was decreased by 0.5 units (pH 7.4 to 6.9) as pH 6.9 is close to the neutral pH and hence PLGA degradation is expected to be slower (Fig. 9).

The release profile of the microspheres was sensitive to a change of ±0.5 °C in temperature (Fig. 10). The temperature was maintained within an accuracy of 0.1 °C using the Sotax USP apparatus 4 system (refer Section 2.2.2.8). The permissible temperature range for *in vitro* release/dissolution testing is ±0.5 °C (USP general chapter on dissolution <711>). However, this temperature range (±0.5 °C) is not suitable for *in vitro* release testing of products such as the commercial microspheres (Risperdal® Consta®) that are temperature sensitive. Therefore, there is a need to control the temperature within accuracy of ±0.1 °C for reproducibility of *in vitro* release profiles.

Amine-catalyzed PLGA hydrolysis and a consequent faster polymer degradation has been observed for microspheres encapsulating amine drugs such as thioridazone, methadone and quinine (Cha and Pitt, 1988; Maulding et al., 1986; Wischke and Schwendeman, 2008). Oster et al. synthesized amine modified branched PLGA to achieve rapid polymer degradation for DNA delivery and found that the degradation rate increased with increase in amine substitution of the polymer. This effect has been attributed to the increased water uptake and acid catalyzed degradation of PLGA ester linkages by protonated amine groups (Oster et al., 2004). Maulding et al. have explained thioridazone (amine drug) catalyzed PLGA degradation to be due to the formation of N-acylammonium ion intermediate which readily hydrolyzes *via* ester bond cleavage (Fig. 14) (Maulding et al., 1986).

Risperidone catalyzed PLGA hydrolysis has been reported for the commercial microspheres (Risperdal® Consta®) (Chue, 2003). Risperidone, being an amine drug, has a pK_a of 8.18 (at 20 °C) associated with the piperidine nitrogen as shown in Fig. 15 (Jug et al., 2009). Therefore, protonation of the piperidone nitrogen under physiological conditions (pH 7.4) may be responsible for risperidone catalyzed PLGA degradation (acid catalyzed degradation). The

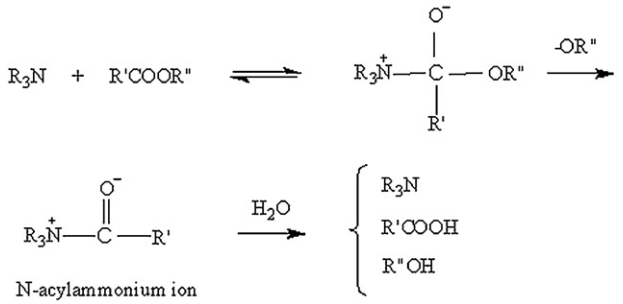

Fig. 14. Amine catalyzed PLGA hydrolysis *via* N-acylammonium ion intermediate formation (Maulding et al., 1986).

pK_a values of risperidone, determined experimentally (potentiometric titration; refer Section 2.2.2.2), were 7.89 ± 0.01, 7.64 ± 0.02 and 7.56 ± 0.01 at 25, 40 and 45 °C, respectively. Therefore, approximately 59% of the piperidine nitrogen groups in risperidone are expected to be protonated during *in vitro* release at 45 °C (release medium pH 7.4; pK_a 7.56 at 45 °C). Accordingly, significant risperidone catalyzed PLGA degradation is possible under *in vitro* release testing conditions considering the high drug loading of the microspheres (~38%, w/w). It was speculated that the rate of this catalysis reaction increases many times in response to a small rise in temperature. To confirm this hypothesis, blank and risperidone microspheres were prepared using emulsion/solvent evaporation method (refer Section 2.2.1). PLGA with a co-polymer ratio of 65:35 and average molecular weight (Mw) 95 kDa was used which was similar to the PLGA molecular weight reported for the commercial microspheres (Risperdal® Consta®) (Ramstack et al., 2002). However the co-polymer composition of PLGA used in the commercial microspheres (Risperdal® Consta®) was not available. Higher glass transition temperature (Tg) of the commercial microspheres (~48 °C) when compared to the prepared blank and risperidone microspheres (~40 °C) indicates higher lactide content in the PLGA of commercial microspheres. As shown in Table 1, the particle size (approximately 7 μm) and drug loading (14%, w/w) of the prepared microspheres were lower than the commercial microspheres. This may be due to the difference in formulation/processing conditions and the polymer used. The initial burst release was similar in both commercial and prepared risperidone microspheres. Low burst release (~1–2%) may be a result of risperidone solubility in the polymer phase (PLGA in methylene chloride) which results in a homogenous drug distribution.

The observation that molecular weight of the prepared risperidone microspheres decreased significantly faster when compared to blank microspheres at 40 and 45 °C was consistent with the literature reports of amine-catalyzed PLGA degradation as explained earlier (Cha and Pitt, 1988; Chue, 2003; Maulding et al., 1986; Oster et al., 2004; Wischke and Schwendeman, 2008). The lower initial molecular weight (~90 kDa on day 0) of the prepared risperidone microspheres compared to the blank microspheres (~95 kDa on day 0) (Fig. 11) indicates that risperidone catalyzed PLGA degradation occurred during microsphere preparation. The increase in degradation rate with increase in temperature (40–45 °C) for both the prepared blank and risperidone microspheres was due to the temperature dependence of PLGA hydrolysis (Wu, 1995). The greater decrease in the molecular weight of the prepared risperidone microspheres in response to a 5 °C increase in temperature (40–45 °C) supports the hypothesis that PLGA degradation catalyzed by the drug risperidone increases significantly in response to increase in temperature (Fig. 11). The degradation of the prepared risperidone microspheres was faster at 45 °C in spite of the slight decrease in the percent of protonated groups from approximately 63 (at 40 °C) to 59% (at 45 °C) due to a decrease in pKa from 7.64 ± 0.02 (40 °C) to 7.56 ± 0.01 (45 °C). It appears that the effect of an approximately 4% decrease in protonation was offset by the increase in the catalytic degradation rate with increase in temperature. The degradation studies (at 40 and 45 °C) were performed using the prepared risperidone microspheres with approximately 14% (w/w) drug loading. The effect of temperature on PLGA degradation is expected to be more pronounced in the commercial microspheres (Risperdal® Consta®) due to their higher drug loading (~38%, w/w). This behavior is likely to be responsible for the significant difference observed in the release profile of the commercial microspheres (Risperdal® Consta®) with a temperature difference of ±0.5 °C (Fig. 10).

The pH and the temperature of the release medium were identified as the parameters critical for achieving reproducible *in vitro* release profiles for the commercial microspheres (Risperdal® Consta®) using the USP apparatus 4 method. Hence, these parameters should be controlled precisely in the *in vitro* release test. However, pH and temperature effects may vary depending upon the encapsulated drug and polymer characteristics such as molecular weight, crystallinity and hydrophobicity.

In addition to pH and temperature, dissolved air was observed to be a critical factor affecting *in vitro* release results (data not shown). While initial de-aeration of the release/dissolution medium is useful in dissolution testing of immediate release drug products, the release medium usually re-aerates in long term *in vitro* release testing and this can affect the results. It was speculated that entrapment of microspheres in the air bubbles (in the flow-through cell) resulted in a slower drug release profiles. De-aeration during the *in vitro* release test was useful in avoiding this variability.

Robustness testing results were useful in successfully reproducing the *in vitro* release profile of the commercial microspheres (Risperdal® Consta®). The method was reproducible as changing the system/equipment or the analyst did not affect the release profile (Figs. 12 and 13). Validation helped in establishing suitability of the modified USP apparatus (flow-through cell) as a standard method for *in vitro* release testing of microspheres.

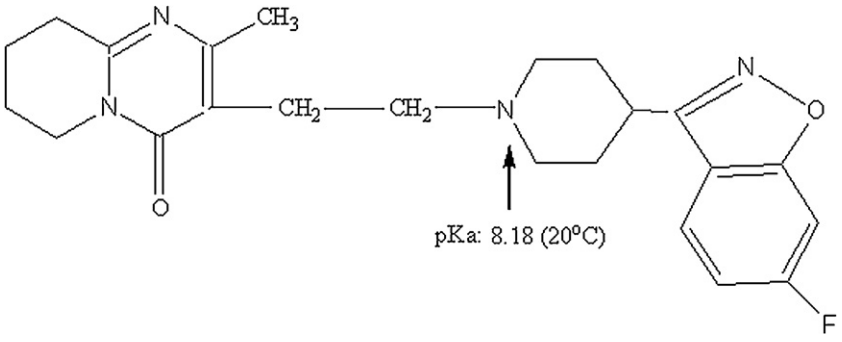

Fig. 15. Chemical structure of risperidone.

5. Conclusions

The modified USP apparatus 4 method was validated for robustness and reproducibility and was shown to be an appropriate method for *in vitro* release testing of microspheres. This method appears to be suitable for possible compendial adaptation following appropriate method transfer investigation. A one-to-one linear correlation was observed for the accelerated and real time release profiles of the commercially available Risperdal® Consta® microspheres. The work emphasizes the need of selecting appropriate accelerated test conditions such that the method is predictive of the real time release profile in addition to reducing the test duration. The method was shown to be robust (with respect to the small changes in the method parameters) and reproducible (no effect of changing the equipment or analyst on the release profile). The validation results demonstrate that the method can be adapted easily in laboratories involved in the *in vitro* release testing of microspheres. The work also provides important information about the temperature sensitivity of the commercial microspheres (Risperdal® Consta®) which has not been reported earlier. The temperature sensitivity of these microspheres was shown to be due to the increase in risperidone catalyzed PLGA degradation in response to temperature. This resulted in significantly different release profiles when the test temperature was changed by $\pm 0.5\,^{\circ}C$. Accordingly, this study emphasizes the need for controlling the temperature of this *in vitro* release test for the commercial microspheres (Risperdal® Consta®) within accuracy of $\pm 0.1\,^{\circ}C$. Such tight temperature control is not anticipated to be necessary for other microsphere systems unless the incorporated drug has similar reactivity with PLGA. Such a method will help in product development; quality assurance; and the regulatory approval process of microsphere formulations.

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References

Adler-Moor, J.P., Proffitt, R.T., 2005. Case study: AmBiosome—a development case study of a liposomal formulation of the antifungal agent amphotericin B. In: Burgess, D.J. (Ed.), Injectable Dispersed Systems. Taylor and Francis, Boca Raton, pp. 481–541.

Albert, A., Serjeant, E.P., 1984. Determination of ionization constants by potentiometric titration using a glass electrode. In: Albert, A., Serjeant, E.P. (Eds.), The Determination of Ionization Constants: A Laboratoty Manual. , third ed. Chapman and Hall, New York, pp. 14–38.

Brown, C.K., 2005. Dissolution method development: an industry perspective. In: Dressman, J., Kramer, J. (Eds.), Pharmaceutical Dissolution Testing. Taylor and Francis, pp. 351–372.

Burgess, D.J., Crommelin, D.J.A., Hussain, A.S., Chen, M.-L., 2004. Assuring quality and performance of sustained and controlled release parenterals: EUFEPS workshop report. AAPS PharmSci. 6, 1–12.

Burgess, D.J., Hussain, A.S., Ingallinera, T.S., Chen, M.-L., 2002. Assuring quality and performance of sustained and controlled release parenterals: workshop report. AAPS PharmSci. 4, 1–11.

Cha, Y., Pitt, C.G., 1988. A one week subdermal delivery system for L-methadone based on biodegradable microcapsules. J. Control. Release 7, 69–78.

Chue, P., 2003. Risperidone long-acting injection. Expert Rev. Neurother. 3, 435–446.

Cleland, J.L., Duenas, E., Daugherty, A., Marian, M., Yang, J., Wilson, M., Celniker, A.C., Shahzamani, A., Quarmby, V., Chu, H., Mukku, V., Mac, A., Roussakis, M., Gillette, N., Boyd, B., Yeung, D., Brooks, D., Maa, Y.-F., Hsu, C., Jones, A.J.S., 1997. Recombinant human growth hormone poly(lactic-co-glycolic acid) (PLGA) microspheres provide a long lasting effect. J. Control. Release 49, 193–205.

D'Souza, S.S., DeLuca, P.P., 2006. Development of a dialysis *in vitro* release method for biodegradable microspheres. AAPS PharmSci. Tech. 6, E323–E328.

Gray, V.A., 2002. AAPS/FDA workshop on dissolution/*in vitro* release testing and specifications for special dosage forms. Diss. Technol. 9, 3 (article).

ICH-Q2A, March, 1995. Guidelines for Industry. In: Text on Validation of Analytical Procedures.

Jug, M.M., Kos, I., Bećirević-Laćan, M., 2009. The pH-dependent complexation between risperidone and hydroxypropyl-b-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. 64, 163–171.

Makino, K., Arakawa, M., Kondo, T., 1985. Preparation and in vitro degradation properties of polylactide microcapsules. Chem. Pharm. Bull. 33, 1195–1201.

Martin, F.J., 2005. Case study: DOXIL the development of pegylated liposomal doxurubicin. In: Burgess, D.J. (Ed.), Injectable Dispersed Systems. Taylor and Francis, Boca Raton, pp. 427–480.

Martinez, M., Rathbone, M., Burgess, D., Huynh, M., 2008. *In vitro* and *in vivo* considerations associated with parenteral sustained release products: a review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting. J. Control. Release 129, 79–87.

Maulding, H.V., Tice, T.R., Cowsar, D.R., Fong, J.W., Pearson, J.E., Nazareno, J.P., 1986. Biodegradable microcapsules: acceleration of polymeric excipient hydrolytic rate by incorporation of a basic medicament. J. Control. Release 3, 103–117.

Oster, C.G., Wittmar, M., Unger, F., Barbu-Tudoran, L., Schaper, A.K., Kissel, T., 2004. Design of amine-modified graft polyesters for effective gene delivery using DNA-loaded nanoparticles. Pharm. Res. 21, 927–931.

Ramstack, J.M., Grandolfi, G.P., Mannaert, E., D'Hoore, P., Lasser, R.A., 2002. Risperdal® CONSTA™: prolonged-release injectable delivery of risperidone using Medisorb® microsphere technology. Abstr. Am. Assoc. Pharm. Sci. (AAPS).

Siewert, M., Dressman, J., Brown, C., Shah, V.P., 2003. FIP/AAPS guidelines for dissolution/*in vitro* testing of novel/special dosage forms. Diss. Technol., 6–15.

Tracy, M.T., 2005. Case study: biodegradable microspheres for the sustained release of proteins. In: Burgess, D.J. (Ed.), Injectable Dispersed Systems. Taylor and Francis, Boca Raton, pp. 571–581.

Voisine, J.M., Zolnik, B.S., Burgess, D.J., 2008. *In situ* fiber optic method for long-term in vitro release testing of microspheres. Int. J. Pharm. 356, 206–211.

Wischke, C., Schwendeman, S.P., 2008. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. Int. J. Pharm. 364, 298–327.

Wu, X.S., 1995. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: Wise, D.L., Trantolo, D.J., Altobelli, D.E. (Eds.), Encyclopedic Handbook of Biomaterials and Bioengineering. Marcel Dekker, New York, p. 1015.

Zolnik, B.S., Burgess, D.J., 2007. Effect of acidic pH on PLGA microsphere degradation and release. J. Control. Release 122, 338–344.

Zolnik, B.S., Leary, P.E., Burgess, D.J., 2006. Elevated temperature accelerated release testing of PLGA microspheres. J. Control. Release 30, 293–300.

Zolnik, B.S., Raton, J.-L., Burgess, D.J., 2005. Application of USP apparatus 4 and in situ fiber optic analysis to microsphere release testing. Diss. Technol., 11–14.