International Journal of Pharmaceutics 566 (2019) 532–540

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Effect of minor manufacturing changes on stability of compositionally equivalent PLGA microspheres

Janki V. Andhariya[a], Jie Shen[a,1], Yan Wang[b], Stephanie Choi[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

ARTICLE INFO

Keywords:
PLGA
Microspheres
Storage stability testing
Manufacturing changes
Risperidone

ABSTRACT

The physicochemical properties and drug release characteristics of Q1/Q2 equivalent microspheres are sensitive to minor manufacturing changes, which may alter their stability under different storage-conditions. This may be undesirable due to the presence of a substantial amount of drug in microsphere products. Hence, the objective of the present work was to investigate the impact of minor manufacturing changes on the stability of Q1/Q2 equivalent microspheres under various storage conditions. Two Q1/Q2 equivalent risperidone microsphere formulations prepared with minor manufacturing changes (solvent system *etc.*) showed differences in their physicochemical properties (size, morphology, porosity *etc.*), drug release characteristics and hence, storage stability. Overall, both formulations were stable under long-term storage conditions (4 °C/ambient humidity). However, under the intermediate storage conditions (25 °C/ambient humidity), only formulation 1 was stable while formulation 2 showed significant polymer degradation, particle aggregation and alteration in the drug release characteristics. Lastly, under accelerated storage conditions (40 °C/ambient humidity *vs* 75% RH), the extent of polymer degradation, morphological changes and alteration of drug release characteristics of formulation 2 was significantly higher compared to that of formulation 1. Thus, minor manufacturing changes have the potential to significantly alter the storage stability and, hence, the quality and performance of complex drug products such as microspheres.

1. Introduction

Storage stability testing is an integral part of pharmaceutically acceptable drug product development for three main reasons: (1) safety of patients; (2) legal requirements concerned with the identity, strength, purity and quality of the drug; and, (3) to prevent the economic repercussions of marketing an unsuitable product (Bajaj et al., 2012; Puthil and Vavia, 2009). Stability testing provides evidence of how the quality and therapeutic performance of a drug product varies with time under the influence of a variety of factors to establish a product shelf life and recommended storage conditions (ICH harmonised tripartite guideline, 2003). The most important factors that influence the stability of any drug product are environmental factors such as temperature and humidity as well as product-related factors such as the physiochemical properties of the dosage form (Zolnik et al., 2006; Jog et al., 2016; Jog et al., 2016). Accordingly, it is essential to evaluate the impact of differences in the physicochemical properties on the stability and, hence, quality and performance of a drug product under various storage conditions.

During the past few decades, PLGA microspheres have emerged as one of the most successful complex parenteral drug products on the market owing to their biodegradability, biocompatibility, and their capability to deliver drugs in a controlled manner over periods of weeks to several months (Hoffman, 2008; Andhariya and Burgess, 2016; Wang et al., 2012; Mitragotri et al., 2014). However, PLGA microspheres are considered “high-risk” products since they usually contain substantial amounts of potent therapeutics, and any unanticipated changes in their *in vivo* drug release characteristics may lead to severe toxicity (Mao et al., 2012; Burgess et al., 2002). Accordingly, it is necessary to investigate the stability of microspheres under various storage conditions, which may lead to unintended changes in their performance over time. The impact of storage conditions on the stability and hence, quality (physicochemical properties) and performance (release characteristics) of microspheres have been evalauted (Burgess et al., 2002; Martinez

* 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.

https://doi.org/10.1016/j.ijpharm.2019.06.014
Received 13 April 2019; Received in revised form 30 May 2019; Accepted 6 June 2019
Available online 07 June 2019
0378-5173/

et al., 2007). The impact of various storage conditions on qualitatively (Q1) and quantitatively (Q2) equivalent microspheres with differences in their physiochemical properties has not been previously reported. It has been reported that minor changes in manufacturing processes can impact the physicochemical properties of Q1/Q2 equivalent microspheres (Wang and Burgess, 2013; Rawat and Burgess, 2011), a situation that may be encountered during post approval changes of innovator products as well as during generic drug product development. Therefore, it is critical to evaluate the stability of such products under various storage conditions.

Accordingly, the objective of the present work was to investigate the impact of minor manufacturing changes on the stability and, hence, quality and performance of Q1/Q2 equivalent microsphere formulations under various storage conditions. Two Q1/Q2 equivalent risperidone microsphere formulations prepared with minor manufacturing changes were used as model products. The stability of these formulations was evaluated by monitoring alteration of their critical physicochemical properties (such as moisture content, glass transition temperature, polymer molecular weight, size and morphology) and drug release characteristics under long-term (4 °C/ambient humidity), intermediate (25 °C/ambient humidity) and accelerated (40 °C/ambient humidity, 40 °C/75% RH) storage conditions.

2. Material and method

2.1. Materials

PLGA (7525 DLG 6E) was purchased from Evonik (Birmingham, AL). Risperidone was purchased from Jai Radhe, India. Poly(vinyl alcohol) (PVA, MW 30–70 kDa), sodium chloride (ACS grade), phosphate buffer saline powder (pH 7.4), sodium azide and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). Methylene chloride, ethyl acetate, benzyl alcohol, dimethyl sulfoxide (DMSO, ACS grade), methanol (HPLC-grade), acetonitrile (HPLC-grade) and tetrahydrofuran (THF, HPLC-grade) were purchased from Fisher Scientific (Pittsburgh, PA). Milli-Q® water was used for all studies. All other chemicals were obtained commercially as analytical-grade reagents.

2.2. Methods

2.2.1. Preparation of risperidone microspheres

PLGA (7525 DLG 6E) with similar molecular weight as that used in the commercial product Risperdal® Consta® was used to prepare compositionally equivalent risperidone microspheres with manufacturing differences (*e.g.*, homogenization, vortex mixing, and solvent system). Briefly, when methylene chloride (DCM) was used as the solvent, both PLGA and risperidone were dissolved in DCM (polymer/drug, 4/3 (*w/w*)). The polymer/drug solution was then dispersed into an aqueous PVA solution (1%, *w/v*) saturated with DCM to form an oil-in-water (o/w) emulsion *via* homogenization (3400 rpm, 2 min) (IKA® Works, Inc.). The microparticles were hardened *via* solvent extraction and evaporation at room temperature for 3 h and then the solvent was further removed under vacuum. The resulting microspheres were collected and washed using distilled water and then lyophilized. Different sieving procedures using 25 µm and 212 µm sieves (*i.e.*, wet sieving (pre-lyophilization) and dry sieving (post-lyophilization)) were used. When ethyl acetate (EA) and benzyl alcohol (BA) were used as the solvent system, PLGA was dissolved in EA (16.7%, *w/w*) and risperidone was dissolved in BA (24%, *w/w*), respectively. The polymer and the drug solutions were then mixed and transferred to the 1% (*w/v*) PVA solution (saturated with EA) to form o/w emulsions *via* vortex mixing (1200 rpm, 10 s). The resulting emulsions were transferred to a solvent extraction medium (2.5% (*v/v*) EA in water) and the solvent was extracted overnight at 4 °C. Following solvent extraction, residual organic solvents were removed under vacuum at room temperature, following which the microspheres were collected and washed using an aqueous alcoholic solution (25% ethanol, *v/v*). The resulting microspheres were sieved using 25 µm and 212 µm sieves and then lyophilized.

2.2.2. Microsphere incubation for storage stability testing

The prepared risperidone loaded PLGA microsphere formulations were placed in tightly closed scintillation vials in vacuum desiccators and incubated at 4, 25 and 40 °C and ambient humidity to study storage temperature effects. In addition, the prepared formulations were also stored at 40 °C and 75% RH to study the effect of accelerated storage conditions under high humidity conditions as per the ICH guidelines (ICH harmonised tripartite guideline, 2003). Samples were incubated in open vials over saturated salt solutions in vacuum chambers to achieve 75% RH. Microspheres were sampled at pre-defined time points to determine any changes in various critical physicochemical properties (such as drug loading, particle size and size distribution, moisture content, Tg and MW) as well as the drug release characteristics. The microsphere samples collected at day 0 were used as a control.

2.2.3. Characterization of the prepared microspheres

2.2.3.1. High performance liquid chromatography (HPLC) analysis. The quantification of risperidone was conducted using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector set at 275 nm. A mobile phase consisting of acetonitrile/water/TFA (30/70/0.1, v/v/v) at a flow rate of 1 ml/min and a Kinetex C18 column (250 × 4.6 mm, 5 µm, 100 Å) column were used. The sample injection volume was 10 µL for drug loading and 50 µL for *in vitro* release testing sample analysis. The chromatographs were analysed using a PeakSimple™ Chromatography System (SRI instruments, Torrance, CA). This method is a stability indicating HPLC assay (Shen et al., 2015).

2.2.3.2. Drug loading. Five milligrams of the risperidone microspheres were weighed and transferred into a 10 ml volumetric flask. DMSO (2.5 ml for risperidone and 2 ml for LA) was added into the volumetric flasks and the samples were sonicated until all particles were dissolved. Methanol was added up to the 10 ml mark to dilute the sample. The solution was filtered (Millex® HV, 0.22 µm PVDF syringe filter) and the drug concentration was determined with a validated HPLC assay method as described above. All the measurements were conducted in triplicate and the results are reported as the mean ± SD. Drug loading was calculated as:

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

2.2.3.3. Particle size and particle size distribution. Particle size and particle size distribution of the risperidone microspheres were measured using an AccuSizer autodiluter particle sizing system (Nicomp, Santa Barbara, CA). Briefly, microspheres were dispersed in 0.1% (w/v) PVA solution in water to ensure good dispersion, and then particle size analysis was conducted. All the measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.3.4. Morphology. The morphology of the commercial product Risperdal® Consta® and the prepared risperidone microspheres was characterized using scanning electron microscopy (SEM). Briefly, dry microspheres were mounted on carbon taped aluminium stubs and sputter coated with gold. The samples were analysed using SEM (NanoSEM 450, Nova).

2.2.3.5. Porosity. The porosity of the risperidone microspheres was determined using a Mercury Porosimeter (AutoPore IV 9500, Micromeritics). Briefly, approximately 200 mg samples of the microspheres were introduced into the porosimeter and tested at a mercury filling pressure of 0.53 psi. Total % porosity and average pore diameter were recorded.

Table 1
Manufacturing differences of the prepared risperidone loaded PLGA microspheres.

Formulation	Solvent system	Emulsification method	Solvent removal method	Solvent removal temperature (°C)
Formulation 1	DCM	Homogenization	Evaporation	Room temperature
Formulation 2	EA & BA	Vortex	Extraction/Evaporation	4 °C

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

2.2.3.6. Molecular weight (MW). 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 tetrahydrofuran (THF) with a flow rate of 2 ml/min at 40 °C. Sample solutions in tetrahydrofuran (THF) at a concentration of 1 mg/mL were filtered through a 0.45 µm filter (Millipore, USA) before injection into the GPC system. The data collection and analysis were 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.

2.2.3.7. Moisture content analysis. The residual water/absorbed moisture content in the prepared risperidone microspheres was determined using a Karl Fischer (KF) Autotitrator (model 831 KF Coulometer, Metrohm, UK) based on Karl-Fischer Titration Method. Briefly, dehydrated methanol was titrated to the electrometric end-point with the KF reagent as the control. The risperidone microspheres were dispersed in the dehydrated methanol and carefully transferred to the titration vessel and then titrated using the KF reagent till the characteristic end-point.

2.2.3.8. Differential scanning calorimeter (DSC) analysis. The glass transition temperatures (T_g) of the risperidone microspheres, as well as a physical mixture of the blank microspheres and risperidone were analysed using a modulated temperature differential scanning calorimeter (MTDSC) (TA Instruments Q2000). Briefly, weighed quantity (~5–6 mg) of each sample was transferred to standard aluminum pans and sealed with aluminum lids. The DSC experiment was performed using a 2 °C/min heating rate and a modulation amplitude of ± 0.82 °C with an 80 s modulation period. All samples were subjected to a heat/cool/heat cycle. The results were analysed using TA analysis software, and the T_g was determined as the glass transition midpoint in the reversing signal. The crystallinity of risperidone was also investigated.

2.2.3.9. In vitro release studies. *In vitro* release testing of the risperidone microspheres was investigated using the previously developed and validated USP apparatus 4 methods (Martinez et al., 2007). Briefly, the microspheres (10 mg) were mixed with glass beads (1 mm) and placed in the USP apparatus 4 dissolution cells. PBS (10 mM, pH 7.4, 250 ml) with 0.01% (*w/v*) sodium azide was circulated through the flow through cells at a flow rate of 8 ml/min at 37 °C (real-time) and 45 °C (accelerated). At pre-determined time intervals, 1 ml samples were withdrawn and replenished with fresh media. The release samples were analyzed *via* HPLC. Measurements were conducted in triplicate and reported as mean ± SD.

Release profiles of microspheres stored under various storage conditions were compared based on differences in the % release at each time point, % total cumulative release as well as the release duration. In addition, the difference (f1) and similarity (f2) factors were also calculated using the following formulae for model independent comparison of the release profiles.

$$f_1 = \{[\sum^{|} R_t - T_t|]/[\sum^{R_t}]\} \times 100$$

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum^{(R_t - T_t)^2}\right]^{-0.5} \times 100\right\}$$

where n = number of time points, Rt = % drug dissolved from reference profile at time point t, Tt = % drug dissolved from test profile at time point t.

The f1 values up to 15 (0–15) and f2 greater than 50 (50–100) were used as an indication of sameness or equivalence of the two release profiles.

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

3. Results and discussion

3.1. Characterization of the prepared risperidone microspheres

Two Q1/Q2 equivalent risperidone loaded PLGA microsphere formulations were prepared using minor differences in the manufacturing processes as shown in Table 1.

As shown in Table 2, both the prepared risperidone microsphere formulations had similar drug loading showing that they are Q1/Q2 equivalent. Moreover, formulations 1 and 2 showed similar particle size (Table 2) despite the use of different emulsification methods *i.e.,* homogenization *vs* vortex mixing. However, significant differences in the morphology, % porosity, moisture content, Tg and MW of the formulations were observed. As shown in Table 2, % porosity of formulation 1 was lower than formulation 2, which may be due to differences in the solvent systems used (Shen et al., 2015). Briefly, differences in the solvent removal rate (DCM *vs* EA&BA) influence dynamic solvent exchange during microsphere solidification, which ultimately affects the porosity of the prepared microspheres (Shen et al., 2015). In addition, this resulted in differences in the surface morphology of the prepared microspheres. As shown in Fig. 1, formulation 1 resulted in spherical particles with smooth surfaces while formulation

Table 2
Physicochemical properties of the prepared risperidone loaded PLGA microspheres.

Formulation	%Drug loading (Mean ± SD)	Particle size (µm, mean ± SD)		% Porosity (w/w)	T_g (°C)	Moisture content (Mean, %W/W)
		Population	Volume			
Formulation 1	37.25 ± 0.79	52.59 ± 6.64	106.16 ± 5.48	46.04 ± 2.90	42.16	0.07
Formulation 2	35.59 ± 0.11	58.00 ± 7.66	108.67 ± 6.65	54.98 ± 1.25	27.63	0.14

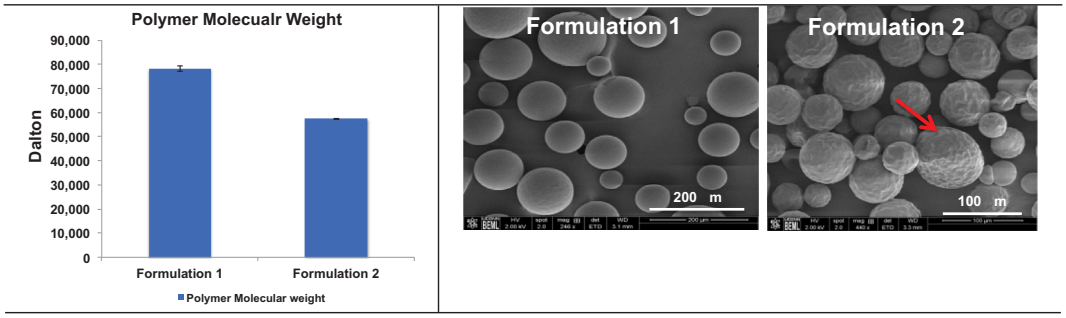

Fig. 1. Polymer molecular weight (mean ± SD, n = 3) and SEM images of the morphology of the prepared risperidone microspheres. Symbol: the red arrow points to wrinkled surface of the microspheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2 resulted in particles of irregular shape with wrinkled surfaces (Shen et al., 2015; Andhariya et al., 2017). Moreover, the differences in the porosity may be responsible for the observed differences in the moisture content of both formulations (Table 2). Although the total moisture content of both formulations was very low (< 0.2%, w/w), formulation 2 had slightly higher moisture content compared to formulation 1. This may be due to more moisture absorption in the relatively more porous structure of formulation 2 compared to formulation 1. Also, the Tg of formulation 2 was lower compared to formulation 1, which may be due to differences in the moisture content and/or residual solvent content of both formulations. As a result of the higher boiling point of BA compared to DCM, BA may not be completely removed during the solvent removal process from formulation 2. The residual solvent and moisture content may exert a plasticization effect on the polymer chains and hence, decrease the Tg of formulation 2 compared to formulation 1.

As shown in Fig. 1, significant differences in the polymer MW of both formulations were observed ($p < 0.05$), with formulation 2 having lower polymer MW compared to formulation 1. This is due to the presence of PLGA microspheres in the aqueous phase for a longer duration (18 h) during the preparation of formulation 2 compared to formulation 1 (4 h), resulting in more hydrolytic degradation of the PLGA in formulation 2 compared to formulation 1. Similar reductions in the polymer molecular weight were observed for the polymers incubated in the aqueous phase for the same duration as for the manufacture of Formulations 1 and 2.

An accelerated release testing method was used to investigate the release profiles of the prepared formulations due to the long-term real-time release nature of these formulations (~35 days at 37 °C) compared to accelerated release duration ((~7 days at 45 °C) (Shen et al., 2015). As shown in Fig. 2, typical *in vitro* release profiles with low/no burst release followed by a lag phase and a fast releasing phase, were observed for both formulations. However, significant differences were observed in the drug release characteristics (overall release rate and lag phase duration) between the two formulations. Formulation 1 had a longer lag phase of ~3 days while that of formulation 2 was shorter *i.e.*, ~2 days, under accelerated release testing conditions. This may be due to the lower porosity and higher polymer MW of formulation 1 compared to formulation 2. Despite the differences in release rate and lag phase duration, the total % cumulative release (~95%) and release duration (~6 days) of both formulations were similar.

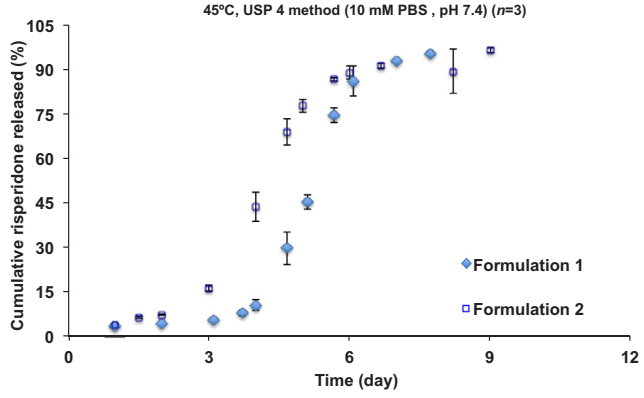

Fig. 2. Accelerated *in vitro* release profiles of risperidone microsphere formulations in 10 mM PBS (pH 7.4) at 45 °C.

3.2. Storage stability testing

The prepared Q1/Q2 equivalent risperidone microsphere formulations with different physicochemical properties were used for storage stability testing.

3.2.1. Long term storage stability testing (4 °C/ambient humidity)

Both formulations were stored at 4 °C and ambient humidity for a period of 12 months. Critical physicochemical properties and drug release characteristics of the stored samples were investigated at predetermined time points (0, 3, 6 and 12 months). As shown in Table 3, no significant changes in the drug loading of both formulations were observed following 12-months storage at 4 °C/ambient humidity. This indicates that the drug was stable and did not undergo degradation under the storage conditions. Statistically significant differences ($p < 0.05$) in the moisture content of samples from each formulation were observed following 12-months storage at ambient humidity. However, total moisture content of both formulations was still low *i.e.*, < 1%.

Differences in the T_{gs} of the formulations were observed at the end of the 12-month storage period (Table 3), which may be the result of a combination of the plasticization effect of the absorbed moisture and the physical aging of the polymer during long-term storage (Rawat and Burgess, 2011; Shen et al., 2015; Ania et al., 1989). In the case of physical aging, structural relaxation of the polymer chains leads to a decrease in the free volume and, hence, polymer chain mobility, which in turn would be expected to increase the T_g of the polymer. On the other hand, the plasticization effect of the absorbed moisture would be

Table 3
Critical physicochemical properties of the prepared risperidone microspheres stored at 4 °C/ambient humidity.

Formulation	Time point (Months)	%Drug loading (Mean ± SD)	Moisture content (Mean, %W/W)	Tg (°C)
Formulation 1	0	37.25 ± 0.79	0.07	42.91
	3	36.97 ± 0.80	0.37	41.80
	6	37.01 ± 0.04	0.37	43.71
	12	37.81 ± 0.74	0.54	46.04
Formulation 2	0	35.59 ± 0.11	0.14	27.63
	3	35.60 ± 0.77	0.14	24.04
	6	35.35 ± 0.24	0.17	26.06
	12	36.84 ± 1.60	0.41	26.27

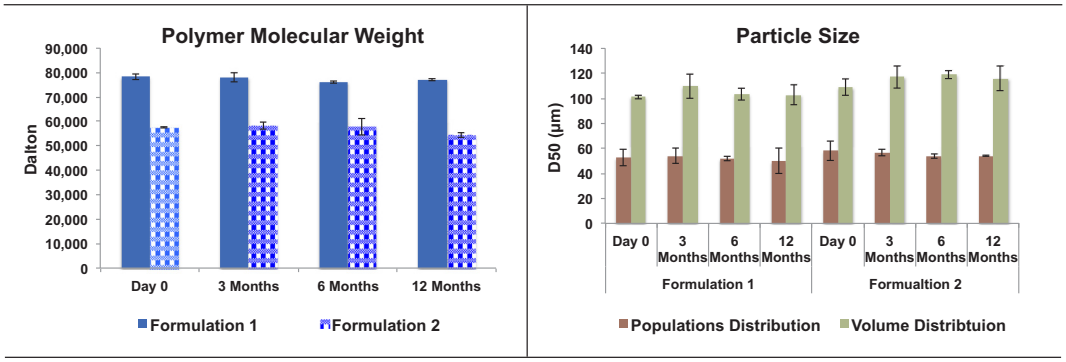

Fig. 3. Polymer molecular weight and particle size of the prepared risperidone microspheres over the storage period of 12 months at 4 °C/ambient humidity. All data are presented as mean ± SD (n = 3).

expected to decrease the T_g of the polymer. Accordingly, the increase in the T_g of formulation 1 indicates that the physical aging has a greater influence compared to plasticization. While the T_g of formulation 2 decreased initially (3 months) due to the plasticization effect and then increased close to its initial T_g value due to the physical aging effect observed over long-term storage. Thus, overall a very slight difference was observed in the T_g of formulation 2 at the end of the 12-month storage period compared to formulation 1. This may be due to differences in the porosity and polymer MW of the two formulations (Table 2). Formulation 2 had higher porosity and lower polymer MW, which may provide: *1)* more free volume for structural relaxation and, hence, less physical ageing; and *2)* increased mobility of the polymer chains resulting in a more pronounced plasticization compared to formulation 1.

Despite slight increase in the moisture content, no significant differences were observed in the polymer MW and particle size of both formulations at the end of 12 months (Fig. 3). Similarly, no significant changes in the morphology of both formulations such as physical aggregation or shape deformation (Fig. 4) were observed for both the formulations, which is in agreement with the particle size results.

Following the physicochemical characterization, accelerated *in vitro* release testing of the formulations was conducted to determine whether the observed minor changes in the physicochemical properties (moisture content, Tg) have the potential to alter the drug release characteristics and hence microsphere performance. As shown in Fig. 5, typical bi-phasic *in vitro* release profiles were observed with no significant changes in the respective lag phase, % cumulative release and release duration of both formulations over the 12-month storage period. The f1 (< 15) and f2 (~100) factors indicated the sameness of the release profiles at all time points for formulation 1. However, a statistically significant difference ($P < 0.05$) in the release rate of the fast releasing phase (around day 5, 12-month sample, Fig. 6) of formulation 2 was observed in terms of the slope of the linear regression. This may be due to the accelerated release testing temperature (45 °C) being significantly higher than the Tg of formulation 2 (Table 2). Accordingly, "real-time" *in vitro* release testing of formulation 2 was conducted at 37 °C to confirm the differences in the release profiles of these samples (Shen et al., 2015). As shown in Fig. 7, no significant changes in the release rate of the different phases, % cumulative release and total release duration of formulation 2 were observed following 12-month storage at 4 °C. In addition, the f1value of ~4 and f2 value of ~100 also indicated the sameness of these profiles.

3.2.2. Intermediate storage stability testing at 25 °C/ambient humidity

Storage stability testing of the prepared risperidone microspheres was also investigated at 25 °C/ambient humidity. As shown in Table 4, no significant change in the drug loading was observed for either formulation. Again, a slight increase in the moisture content of formulation 2 was observed, which may be due to its relatively more porous structure compared to formulation 1. As shown in Table 4, the Tg values of formulations 1 and 2 increased significantly following one-month storage at 25 °C/ambient humidity ($p < 0.05$), which indicates a more pronounced effect of physical ageing of the polymer compared to that at 4 °C. This may be a result of increase in polymer chain mobility and the structural relaxation rate at higher temperature (Rawat and Burgess, 2011).

As shown in Fig. 8, significant changes in the polymer MW of formulation 2 (~31% change, $p < 0.05$) were observed compared to formulation 1. This may be due to the initial lower polymer MW of formulation 2 compared to formulation 1, which may degrade faster than the high MW polymer of formulation 1. In addition, formulation 2 has relatively high moisture content, which may further facilitate its

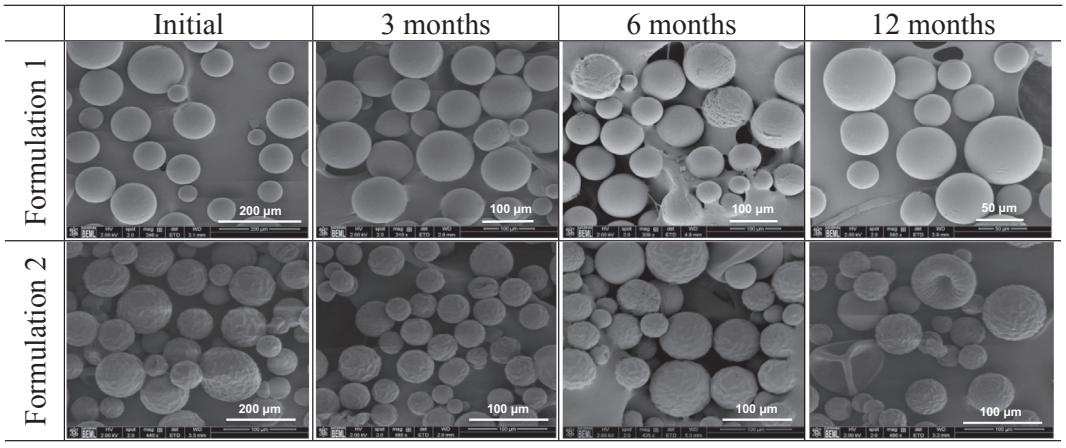

Fig. 4. SEM images of the morphology of the prepared risperidone microsphere formulations stored at 4 °C/ambient humidity.

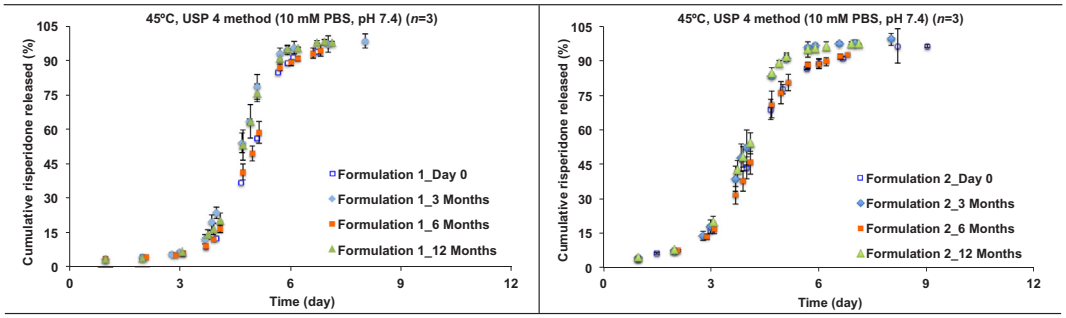

Fig. 5. Accelerated *in vitro* release profiles of risperidone microspheres stored at 4 °C/ambient humidity.

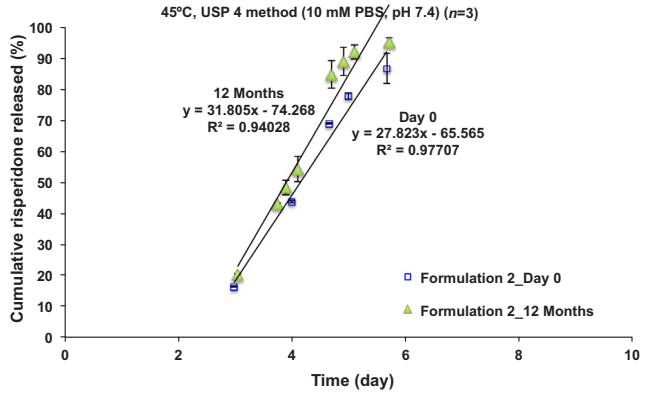

Fig. 6. Comparison of the fast release phase of the accelerated *in vitro* release profiles of risperidone microspheres following 12 months of storage at 4 °C/ ambient humidity.

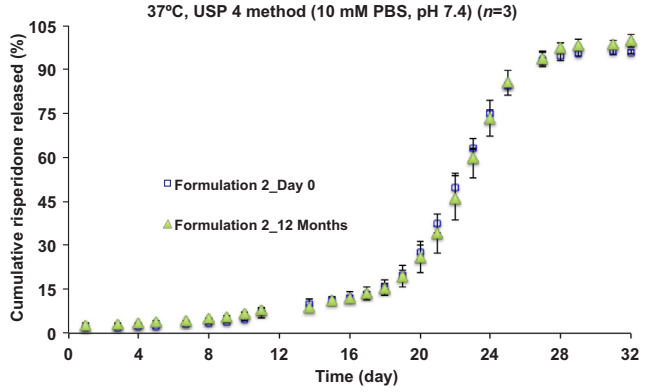

Fig. 7. Real-time *in vitro* release profiles of formulation 2 stored at 4 °C/ambient humidity.

Table 4
Critical physicochemical properties of the prepared risperidone microspheres stored at 25 °C/ambient humidity.

Formulation	Time point (Month)	% DL (Mean ± SD)	Moisture content (Mean % W/W)	T_g (°C)
Formulation 1	0	37.39 ± 1.04	0.42	42.90
	1	38.58 ± 0.92	0.38	47.59
Formulation 2	0	35.81 ± 1.77	0.47	28.50
	1	34.70 ± 1.02	0.50	31.36

hydrolytic degradation compared to formulation 1. The higher storage temperature of 25 °C compared to 4 °C will accelerate polymer degradation (Grayson et al., 2005).

As shown in Fig. 8, formulations 1 and 2 did not show any significant changes in particle size in terms of population distribution. Whereas in terms of volume distribution, formulation 2 showed a significant increase in particle size compared to formulation 1 (Fig. 8). This may be due to physical aggregation of the formulation 2 microspheres as a result of their high moisture content and low Tg compared to formulation 1. This was confirmed by investigation of the morphology of the stored samples. As can be seen in Fig. 9, formulation 2 showed physical aggregation while no significant changes were observed in formulation 1.

The accelerated *in vitro* release profiles of formulation 1 following 1-month storage at 25 °C/ambient humidity are shown in Fig. 10. The release profiles of formulation 1 following 1-month storage were not significantly different in terms of lag phase, % cumulative release and release duration. In addition, the f1 (~9) and f2 (~100) factors indicated the sameness of the release profiles. The real-time *in vitro* release profiles of formulation 2 following 1-month storage at 25 °C/ ambient humidity are shown in Fig. 11A. Please note that "real-time" *in vitro* release testing of formulation 2 was conducted at 37 °C to investigate the differences in the release profiles of these samples since the accelerated release testing temperature (45 °C) is significantly higher than the Tg of formulation 2 (Table 2). Formulation 2, despite physical aggregation as noted above, showed a significantly shorter release duration (24 days) following 1-month storage at 25 °C/ambient humidity compared to the control sample (28 days) (Fig. 11A). This difference in the total release duration was a result of a shorter lag phase following 1-month storage at 25 °C/ambient humidity (12 days compared to 16 days). There was no significant difference in the fast releasing rate ($p > 0.05$) in terms of the slope of linear regression (Fig. 11B)). This can be attributed to the significant decrease in the polymer MW of formulation 2 during storage (Table 4) with the result that the critical chain length where the fast releasing phase begins is achieved earlier. The f1 factor was high (~30) confirming significant difference in the release profiles.

3.2.3. Accelerated storage stability testing (40 °C/ambient humidity and 40 °C/75%RH)

As shown in Table 5, no significant changes in the drug loading were observed under both accelerated storage conditions. As shown in Fig. 12, the moisture content of both formulations increased significantly under both accelerated storage conditions, with relatively higher moisture content for the samples stored at high humidity conditions. Moreover, formulation 2 had overall relatively high moisture content compared to formulation 1.

The T_g of formulation 1 increased by 5 °C (Table 5) under both accelerated storage conditions, which may be due to more pronounced physical aging compared to the plasticization effect of the absorbed moisture under these storage conditions. Whereas, there was no significant change in Tg of formulation 2 under either of the accelerated storage conditions. This is probably a consequence of the low initial Tg of formulation 2.

As shown in Fig. 12, significant reduction in the polymer MW of both formulations was observed under both accelerated storage

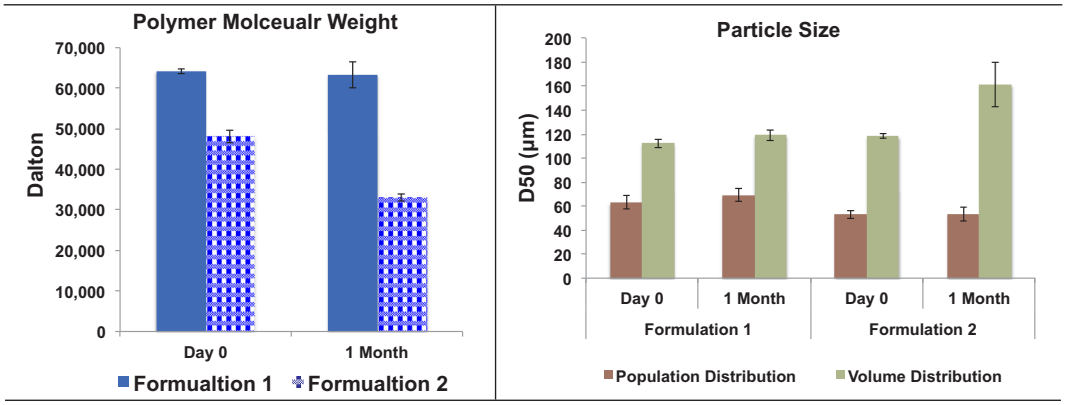

Fig. 8. Polymer molecular weight and particle size of the prepared risperidone microspheres stored at 25 °C/ambient humidity. All data are presented as mean ± SD (n = 3).

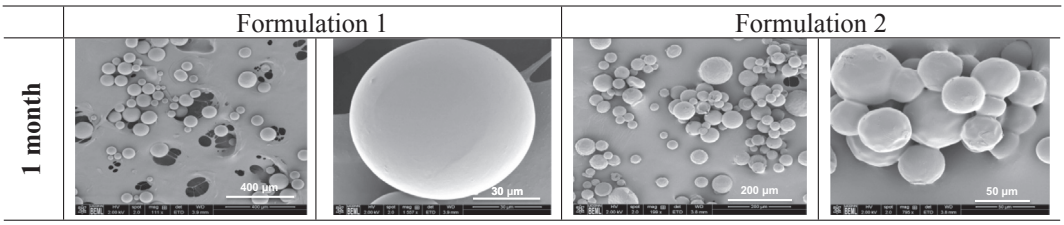

Fig. 9. SEM images of the prepared risperidone microspheres stored at 25 °C/ambient humidity.

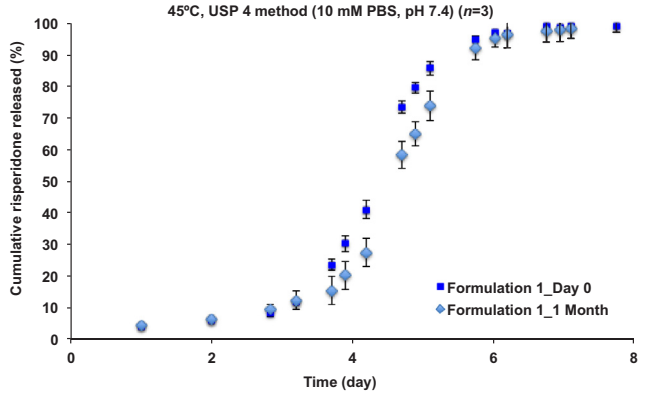

Fig. 10. Accelerated *in vitro* release profiles of formulation 1 stored at 25 °C/ ambient humidity.

conditions, which is due to the accelerated hydrolytic degradation of the polymer. The change in the polymer MW of formulation 2 was significantly greater compared to formulation 1, which is likely due to the lower initial polymer MW of formulation 2.

Table 5
Critical physicochemical properties of the prepared risperidone microspheres stored under accelerated storage conditions.

Formulation	Time point (Day)	% DL (Mean ± SD)	T_g (°C)
Formulation 1	0	36.91 ± 0.37	40.51
	8 (A)	36.70 ± 0.95	45.10
	8 (B)	36.99 ± 0.09	45.94
Formulation 2	0	35.52 ± 0.21	23.95
	8 (A)	35.60 ± 0.77	22.71
	8 (B)	35.44 ± 0.39	22.79

A: 40 °C/ambient humidity, B: 40 °C/75%RH.

As shown in Fig. 13, the surface morphology of formulation 1 changed from smooth to an indented surface (as indicated by the white arrows) while maintaining spherical shape and no physical aggregation was observed under both accelerated storage conditions. Whereas, formulation 2 showed significant shape deformation and physical aggregation resulting in the formation of polymer blocks of irregular shape, which might be due to the Tg of formulation 2 being lower (Table 5) than the storage temperature (40 °C). The observed differences in the morphology of the formulations may be due to the more

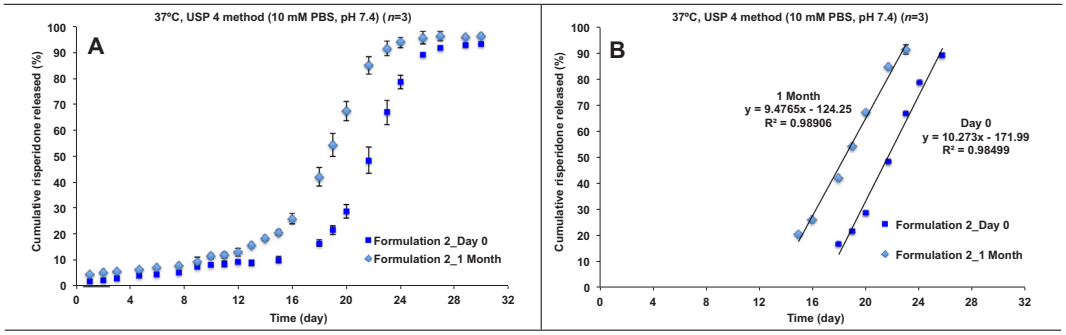

Fig. 11. (A) *In vitro* release profiles of formulation 2 stored at 25 °C/ambient humidity; and (B) Comparison of the fast release phase of the *in vitro* release profiles of formulation 2 freshly prepared (control) and following storage at 25 °C/ambient humidity.

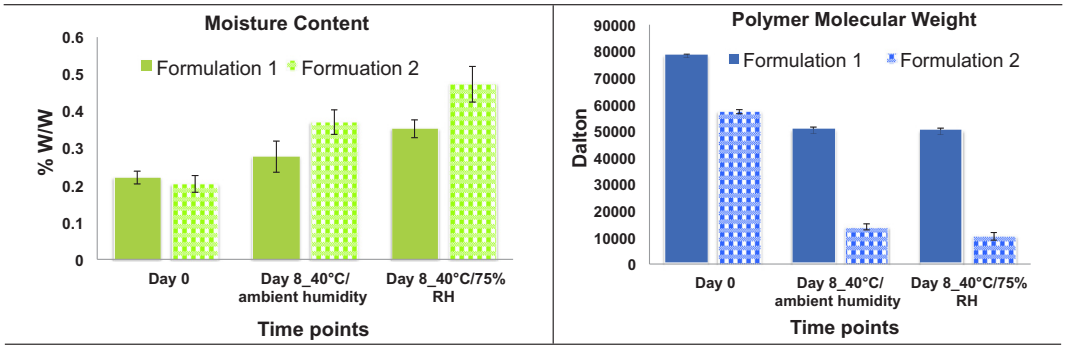

Fig. 12. Moisture content and polymer molecular weight of the prepared risperidone microsphere formulations stored under accelerated storage conditions. All data are presented as mean ± SD (n = 3).

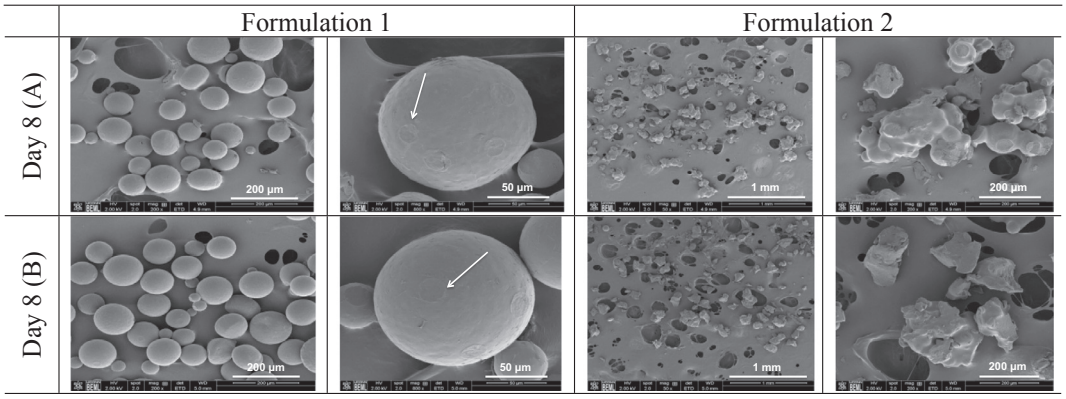

Fig. 13. SEM images of the prepared risperidone microspheres stored at: (A) 40 °C/ambient humidity; and (B) 40 °C/75% RH. Symbol: White arrows indicate indented surface.

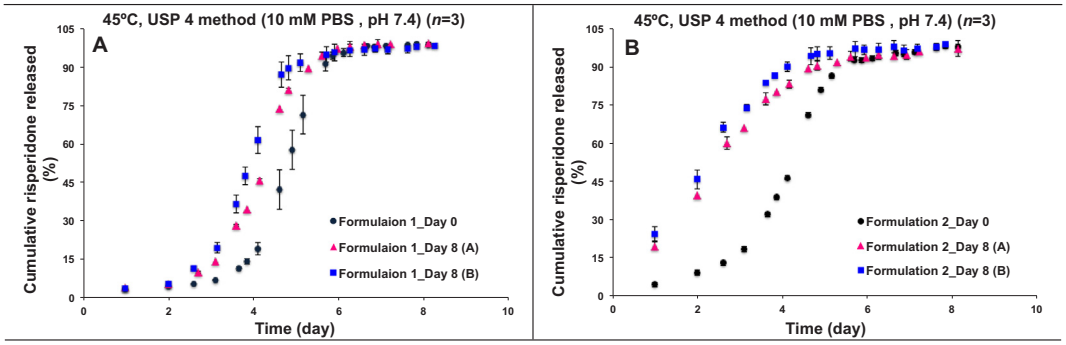

Fig. 14. Accelerated *in vitro* release profiles of formulations 1 and 2 stored at: (A) 40 °C/ambient humidity; and (B) 40 °C/75% RH.

porous structure, higher moisture content and lower T_g of formulation 2 compared to formulation 1.

The observed changes in the physicochemical properties under both accelerated storage conditions resulted in significant differences in the accelerated release profiles of both formulations (Fig. 14). The observed faster release may be due to the significant decrease in polymer MW of both formulations under the accelerated storage conditions resulting in faster polymer erosion and hence faster drug release rates. Moreover, the significantly higher polymer degradation observed for formulation 2 under both storage conditions, resulted in a change in the accelerated release profile from biphasic to first order.

Compared to ambient humidity conditions, significantly high moisture content was observed in samples from both formulations under high humidity (75%RH) conditions (Fig. 13). Despite this, the changes observed in the polymer MW of both formulations (Fig. 13) stored under the two different humidity conditions were not significantly different. Similarly, the changes observed in the other physicochemical properties such the Tg and morphology as well as the drug release characteristics of both formulations under the two different humidity conditions were not significantly different. These results together indicate that the high storage temperature (40 °C) had a greater influence on the stability of both formulations compared to the high humidity (75% RH) conditions.

Summary table: impact of minor manufacturing changes on the stability of prepared risperidone microsphere formulations under various storage conditions.

Parameters	Storage conditions		
	Long-term	Intermediate Formulation 1	Accelerated
Drug loading	✓	✓	✓
Particle size	✓	✓	✓
Morphology	✓	✓	X

Tg	X	X	X
MW	✓	✓	X
Moisture content*	X	X	X
Release characteristics	✓	✓	X
Formulation 2			
Drug loading	✓	✓	✓
Particle size	✓	X	NA
Morphology	✓	X	X
Tg	✓	X	✓
MW	✓	X	X
Moisture content*	X	X	X
Release characteristics	✓	X	X

✓: No change, X: Change, * the moisture content was lower than the 1% under all conditions.

4. Conclusions

Differences in the physicochemical properties of Q1/Q2 equivalent microspheres prepared with minor manufacturing changes had a significant influence on their stability under various storage conditions. Microsphere physicochemical properties such as morphology, particle size, polymer molecular weight, moisture content, glass transition temperature were identified as the critical quality attributes (CQA) that should be investigated to evaluate the impact of manufacturing changes on the stability of Q1/Q2 equivalent microspheres during storage stability testing. Overall, both Q1/Q2 equivalent risperidone microsphere formulations prepared with minor manufacturing changes were stable over 12 months storage at 4 °C/ambient humidity despite differences in their physicochemical properties. However, the influence of minor manufacturing changes on the stability of microspheres stored at temperatures higher than 4 °C highlights the need to establish product specific storage and distribution conditions for microsphere products. This also suggests that cold chain technology should be implemented to prevent adverse effects of high temperature and humidity on the quality and performance of microsphere products.

Overall, this research is very helpful to the pharmaceutical industry as well as the regulatory authorities to understand the impact of minor manufacturing changes, which may occur during post approval changes or generic drug product development, on the stability of microspheres under various storage conditions and to facilitate the development of appropriate preventive strategies.

Declaration of Competing Interest

None

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2019.06.014.

References

Bajaj, S., Singla, D., Sakhuja, N., 2012. Stability testing of pharmaceutical products. J. Appl. Pharm. Sci. 02 (03), 129–138.

Puthil, S., Vavia, P.R., 2009. Stability studies of microparticulate system with piroxicam as model drug. AAPS PharmSciTech 10 (3), 872.

ICH harmonised tripartite guideline, Stability testing of new drug substances and products, Q1A (R2), 2003.

Zolnik, B.S., Leary, P.E., Burgess, D.J., 2006. Elevated temperature accelerated release testing of PLGA microspheres. J. Control. Release 112 (3), 293–300.

Jog, R., Unachukwu, K., Burgess, D.J., 2016. Formulation design space for stable, pH sensitive crystalline nifedipine nanoparticles. Int. J. Pharm. 514 (1), 81–92.

Jog, R., Kumar, S., Shen, J., Jugade, N., Tan, D.C.T., Gokhale, R., Burgess, D.J., 2016. Formulation design and evaluation of amorphous ABT-102 nanoparticles. Int J. Pharm. 498 (1–2), 153–169.

Hoffman, A.S., 2008. The origins and evolution of "controlled" drug delivery systems. J. Control. Release 132, 153–163.

Andhariya, J.V., Burgess, D.J., 2016. Recent advances in testing of microsphere drug delivery systems. Expert Opin. Drug Deliv. 13 (4), 593–608.

Wang, Y., Burgess, D.J., 2012. Microsphere technologies. In: Wright, J.C., Burgess, D.J. (Eds.), Long Acting Injections and Implants. Springer, pp. 167–194.

Mitragotri, S., Burke, P.A., Langer, R., 2014. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. Nat. Rev. Drug Discov. 13, 655–672.

Mao, S., Guo, C., Shi, Y., Li, L.C., 2012. Recent advances in polymeric microspheres for parenteral drug delivery—part 1. Expert Opin. Drug Deliv. 9, 1161–1176.

Burgess, D.J., Hussain, A.S., Ingallinera, T.S., Chen, M.L., 2002. Assuring quality and performance of sustained and controlled release parenterals: AAPS Workshop Report, co-sponsored by FDA and USP. Pharm. Res. 19, 1761–1768.

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

Wang, Y., Burgess, D.J., 2013. Influence of storage temperature and moisture on the performance of microsphere/hydrogel composites. Int. J. Pharm. 454 (1), 310–315.

Rawat, A., Burgess, D.J., 2011. Effect of physical ageing on the performance of dexamethasone loaded PLGA microspheres. Int. J. Pharm. 415, 164–168.

Shen, J., Choi, S., Qu, W., Wang, Y., Burgess, D.J., 2015. In vitro-in vivo correlation of parenteral risperidone polymeric microspheres. J. Control. Release 218, 2–12.

Andhariya, J., Shen, J., Choi, S., Wang, Y., Zou, Y., Burgess, D.J., 2017. Development of in vitro-in vivo correlation of parenteral naltrexone laoded polymeric microspheres. J. Control. Release 255, 27–35.

Grayson, A.C., Cima, M.J., Langer, R., 2005. Size and temperature effects on poly(lactic-co-glycolic acid) degradation and microreservoir device performance. Biomaterials 26 (14), 2137–2145.

Ania, F., Martinez-Salazar, J., Baltá Calleja, F.J., 1989. Physical ageing and glass transition in amorphous polymers as revealed by microhardness. J. Mater. Sci. 24 (8), 2934–2938.