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The effect of PLGA molecular weight differences on risperidone release from microspheres

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ABSTRACT

The objective of the present study was to investigate the effect of molecular weight differences of poly (lactic-*co*-glycolic acid) (PLGA) on the *in vitro* release profile of risperidone microspheres. Four different PLGA molecular weights were investigated and all the microsphere formulations were prepared using the same manufacturing process. Physicochemical properties (particle size, drug loading, morphology and molecular weight) as well as *in vitro* degradation profiles of the prepared microspheres were investigated in addition to *in vitro* release testing. The *in vitro* release tests were performed using a previously developed flow through cell (USP apparatus 4) method. The particle size of the four prepared microsphere formulations varied, however there were no significant differences in the drug loading. Interestingly, the *in vitro* release profiles did not follow the molecular weight of the polymers used. Instead, the drug release appeared to be dependent on the glass transition temperature of the polymers as well as the porosity of the prepared formulations. The catalytic effect of risperidone (an amine drug) on PLGA during manufacturing and release testing, minimized the differences in the molecular weights of the four formulations, explaining the independence of the release profiles on PLGA molecular weight.

1. Introduction

Poly (lactic-*co*-glycolic acid) (PLGA) based microspheres is one of the most successful complex parenteral drug products on the market. In addition to their ability to deliver drugs in a controlled manner over periods of weeks to months (Mitragotri et al., 2014; Hoffman, 2008), PLGA is known as an attractive polymer due to its biocompatibility as well as its ability to modulate drug release characteristics by varying its composition, molecular weight (Mw) and chemical structure. Currently, there are six U.S. FDA approved parenteral PLGA microsphere drug products (such as Risperdal® Consta®, Lupron Depot® and Sandostatin® LAR) (Jain et al., 2016; FDA Approved Drug Products) on the market. The therapeutic indications for these parenteral microsphere products include cancer, schizophrenia and alcohol dependence, and they bring significant benefit to public health. On the other hand, these products are considered "high-risk" because they contain substantial amounts of potent therapeutic drugs aiming to continuously release drug over long periods of time. Thus, any unanticipated changes in their drug release profiles could lead to severe toxicity (Burgess et al., 2002; Martinez et al., 2007). Consequently, it is essential to understand the extent of the impact of changes in the materials as well as the manufacturing processes on drug product release profiles. In vitro release profiles help to assure the *in vivo* therapeutic performance as well as the safety of these drug products, and this is an area where more research is necessary. Such research is also important to promote the development of generic drug products, especially since no generic complex drug products have so far appeared on the market.

To evaluate the *in vitro* release profiles of controlled release parenteral products such as PLGA microspheres prepared with small differences (*e.g.*, changes in the manufacturing equipment, manufacturing processes, polymer Mw and supplier of polymers), validated *in vitro* release testing methods with good discriminatory ability and reproducibility are necessary (Shen et al., 2016; Bao et al., 2017). However, no compendial *in vitro* release testing method for these controlled release parenteral products has been developed until now. Previously, Rawat et al. have developed accelerated and real-time *in vitro* release testing

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methods based on a compendial dissolution apparatus (*i.e.*, USP apparatus 4, flow-through cell) (Rawat et al., 2011). Recently there have been reports of Level A (FDA, 1997) IVIVCs for PLGA microspheres with a variety of molecules such as risperidone, naltrexone and leuprolide (Shen et al., 2015; Andhariya et al., 2017; Andhariya et al., 2019). Additionally; it has been reported (Shen et al., 2015) that the critical physicochemical properties of microspheres (such as particle size) are sensitive to minor manufacturing differences, which has potential to alter their *in vitro* and *in vivo* release profiles.

The objective of the present study was to investigate the effect of Mw differences of PLGA on its *in vitro* release profiles. Risperidone (the active ingredient of Risperdal® Consta®) was selected as a model drug and microspheres were prepared with four different PLGA Mws using the same manufacturing process. Physicochemical properties as well as *in vitro* degradation profiles of the prepared microspheres were investigated in addition to the *in vitro* release characteristics. In vitro release tests were performed using the flow-through cell (USP apparatus 4) method previously reported (Rawat et al., 2011).

2. Materials and methods

2.1. Materials

PLGA polymers (lactide/glycolide:75/25) with different Mws were purchased from Evonik (Birmingham, AL) (E1 and E2), Polyscitech (West Lafayette, IN) (P) and Lactel (Birmingham, AL) (L). Risperidone was purchased from AK Scientific (Union City, CA). Poly (vinyl alcohol) (PVA, MW 30–70 kDa), Phosphate buffered saline (PBS) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). Methylene chloride, Tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO, ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Nanopure quality water (Barnstead, Dubuque, IA) was used for all studies. All other chemicals used in all studies were purchased as analytical-grade reagents.

2.2. Methods

2.2.1. Preparation of risperidone microspheres

PLGA polymer with similar monomer ratio but different Mw than that used in the commercial product Risperdal® Consta® was used to prepare compositionally equivalent risperidone microspheres using the manufacturing method previously developed by our group (11). Briefly, PLGA polymers were dissolved in ethyl acetate (EA) at 16.7% w/v and risperidone was dissolved in benzyl alcohol (BA) at 24% w/v concentration. The two solutions were mixed quickly and then emulsified with a 1% (w/v) PVA solution saturated with EA to form an oil-in-water (o/w) emulsions using homogenization at 3400 rpm for 30 s. The resulting emulsion was dispersed into the 2.5% (v/v) EA in water and stirred at 450 RPM using a magnetic stirrer. After overnight extraction and microsphere solidification at 4 °C, the residual organic solvents were removed under vacuum at room temperature for 3 h, followed by washing using 25% ethanol (v/v). The sieves (25 μm and 212 μm) were used to remove small particles or aggregates during washing and the collected microspheres were freeze-dried.

2.2.2. Polymer viscosity

Five milligrams of the polymers were weighed and dissolved in chloroform (1 mL). The viscosity of the solution was determined with a DV2T viscometer (Brookfield, Canada), spindle CP-40 at 100 rpm. Inherent viscosity was calculated based on the equation below:

$$\text{Inherent viscosity (dL/g)} = \ln(\eta/\eta_s)/c$$

where η is the viscosity of the solution at 100 rpm, η_s is the viscosity of chloroform and c is the mass concentration of the polymer (g/dL).

2.2.3. Differential scanning calorimeter (DSC) analysis

A differential scanning calorimeter (DSC) (TA Instruments Q1000) was used to determine the glass transition temperature (T_g) of the PLGA polymers and the prepared microspheres. Around 5 mg of samples sealed in the standard aluminum sample pans was heated from 0 °C to 190 °C and then cooled from 190 °C to 0 °C at a ramp rate of 20 °C/min, followed by a second heating from 0 °C to 190 °C. The T_g was determined as the midpoint in the second heating cycle thermogram.

2.2.4. Gel permeation chromatography (GPC)

GPC (Waters, USA) with an evaporative light scattering detector was used to determine the Mw of the PLGA polymer as well as the PLGA in the prepared microsphere formulations. The samples were dissolved in tetrahydrofuran (THF) and filtered through 0.45 μm filters.

2.2.5. Drug loading

Drug loading was determined by dissolving ~5 mg of the prepared risperidone microspheres in the 2.5 mL dimethyl sulfoxide (DMSO), sonicated and then diluted with methanol up to 10 mL. The solution was filtered using 0.22 μm filters and analyzed using previously developed method (Mobile phase: acetonitrile/water/TFA (30/70/0.1, v/v/v); Kinetex C18 column (250 × 4.6 mm, 5 μm, 100 Å); detection wavelength: 275 nm and flow rate:1 mL/min). The drug loading was calculated as described below:

$$\text{Percent drug loading} = (\text{weight of risperidone inside microspheres}/\text{weight of microspheres analyzed}) \times 100.$$

2.2.6. Particle size and particle size distribution

An AccuSizer 780A (Nicomp, Santa Barbara, CA) was used to determine the particle size and the particle size distribution of the prepared risperidone microspheres. Approximately 7 mg of the microspheres were dispersed in 350 μL of filtered 0.1% (w/v) PVA solution, and sonicated. 100 μL samples was used for each measurement.

2.2.7. Morphology

Scanning electron microscopy (SEM, NanoSem 450, Nova) was used to evaluate the morphology of the prepared risperidone microspheres. Briefly, dry microspheres were placed on the carbon taped aluminum stubs and then sputter coated with gold.

2.2.8. Porosity

A mercury intrusion porosimetry (MicroActive AutoPore V9600, Micromeritics) was used to determine the % porosity and the average pore diameter. Briefly, approximately 100 mg samples of the microspheres were tested at a mercury filling pressure of 0.53 psi. Total % porosity and average pore diameter were recorded.

$$\text{Porosity (\%)} = (1 - B/A) \times 100$$

where A is the bulk density and B is the apparent skeletal density

2.2.9. In vitro release testing

A previously developed and validated USP apparatus 4 method (Rawat et al., 2011) was used to perform the real-time *in vitro* release testing for the prepared risperidone microspheres at 37 °C. Briefly, ~10 mg of the risperidone microspheres were mixed with 1 mm glass beads to prevent aggregation and placed in flow through cells. Two different release media was used: (1) PBS (10 mM, pH7.4) with 0.01% (w/v) sodium azide (250 mL) and; (2) HEPES (10 mM, pH7.4) with 0.02% (w/v) sodium azide, 99 mM sodium chloride and 0.02% (v/v) Tween 20. At the pre-determined time intervals, 1 mL samples were withdrawn and replaced with 1 mL of fresh media. Risperidone concentration in the samples were determined using a previously developed HPLC assay method (Shen et al., 2016). All drug release testing was conducted in triplicate and the results are reported as mean ±

standard deviation.

2.2.10. In vitro degradation studies

Approximately 26 mg of the risperidone microspheres were dispersed in 26 mL of 10 mM HEPES buffer with 0.02% (w/v) sodium azide, 99 mM sodium chloride and 0.02% (v/v) Tween 20 using a screw capped bottle. These samples were incubated in a water shaker bath (C76, New Brunswick Scientific, Edison, NJ) at the agitation speed of 100 rpm and 37 °C. Samples were collected at pre-determined time intervals, washed with water three times and freeze dried. The dried samples were analyzed using SEM, GPC and DSC methods as describe above.

2.2.11. Statistical data analysis

A paired student *t*-test was used to evaluate significant differences between the properties of the prepared microsphere formulations. The level of significance accepted was at $p < 0.05$.

3. Results

3.1. Physicochemical properties of PLGA polymers

PLGA (lactide/glycolide:75/25, ester end capped) with different Mws (E1, E2, P and L) were obtained from several manufacturers. To compare the physicochemical properties of each of the polymers using the same methodology, their viscosity, T_g and Mw were determined *via* DV2T viscometer (Brookfield, Canada), DSC (TA Instruments Q1000) and GPC (Waters), respectively. As shown in Table 1, the E2 polymer has the lowest Mw, followed by E1, P and L in that order. The results of the inherent viscosity calculated based on the viscosity data were consistent with that of MWs obtained *via* GPC, whereas the rank order of the T_g values was different from that of the MWs. In addition, the appearance of each of the polymers was different. E1 and E2 had similar appearances (white dispersed powder), P was in the form of white chunks and L was a brown crystalline solid. The analytical data provided by the manufacturers showed that L had higher residual monomers and Tin compared to E1 or E2. This difference in the purity (*e.g.* residual monomers and Tin) may have resulted in the different appearance of the polymers (data not shown).

3.2. Physicochemical properties of risperidone microspheres

The physicochemical properties (*e.g.*, drug loading, particle size as well as porosity) of the prepared risperidone microspheres are shown in Tables 2 and 3. The same manufacturing process was used for all four polymers and the yield was from 34 to 48% (w/w). The drug loadings of the prepared formulations were around 36% (Table 2) and there were no significant differences between the formulations prepared using the different Mw polymers. Formulation_E2 had the smallest particle size and Formulation_L had the largest particle size. Formulation_E1 and Formulation_P showed similar average particle size values in terms of both population (*ca.* 67 μm) and volume (*ca.* 110 μm) ($p > 0.05$). The rank order of the particle size of the prepared microspheres was consistent with that of the polymer Mw. Interestingly, even though the four formulations were prepared with the same processing method, they had different morphologies and porosities. As can be seen in Fig. 1, Formulations_E1, E2 and L were mixtures of particles with smooth and wrinkled surfaces whereas most particles in Formulation_P had smooth surfaces. As for the porosity, Formulation_P had a higher porosity percentage (73.15%) compared to the other formulations.

Table 2
Physicochemical properties of the risperidone microsphere formulations with different polymers (mean ± S.D., n = 3).

Sample	Polymer	Drug Loading (%, w/w)	Particle size (Population, μm)	Particle size (Volume, μm)
Formulation_E1	E1	36.16 ± 0.36	67.63 ± 0.76	111.01 ± 3.79
Formulation_E2	E2	35.50 ± 0.81	64.11 ± 1.17	89.85 ± 3.62
Formulation_P	P	36.27 ± 1.97	65.56 ± 1.91	121.37 ± 12.17
Formulation_L	L	36.80 ± 0.58	73.35 ± 1.21	129.74 ± 7.89

Table 3
Porosity of the risperidone microsphere.

Sample	Polymer	Porosity%	average pore diameter (nm)
Formulation_E1	E1	57.46	0.12
Formulation_E2	E2	65.92	0.15
Formulation_P	P	73.15	0.17
Formulation_L	L	58.50	0.16

3.3. In vitro release characteristics of risperidone microspheres

Real-time *in vitro* release testing of the prepared risperidone microspheres were conducted using the real-time modified USP apparatus 4 (flow-through cell) method. Rawat et al. previously developed a reproducible and discriminatory method (Rawat et al., 2011) and Shen et al. reported a 1:1 linear, level A IVIVC (rabbit model) for risperidone PLGA microspheres (Shen et al., 2015). A level A IVIVC is the most effective type of IVIVC; with the potential to be used as biowaivers tool for *in vivo* studies if developed using clinical data. In the current study, both PBS (pH7.4) with 0.01% (w/v) sodium azide and HEPES buffer (pH7.4 with sodium chloride, Tween 20 and sodium azide, as recommended in the FDA's draft guidance on risperidone PLGA microspheres (FDA Product-Specific Guidance for Generic Drug Development) were used as release media. The risperidone release profiles were similar in both release media (Fig. 2). However, the release rate was slightly faster in the HEPES buffer, which may be due to the presence of the surfactant (*i.e.*, Tween 20) that can facilitate wetting of the PLGA microspheres and hence, faster buffer penetration into the microspheres during release testing (Shen et al., 2015).

All of the prepared formulations showed very low burst release percentage (< 5%). Surprisingly, the *in vitro* release profiles of the prepared risperidone microspheres were inconsistent with the expected results based on their polymer Mws. Formulation_P had a significantly shorter lag phase (*ca.* 10 days) than the other formulations (*ca.* over 20 days). The time to reach a plateau in the release profile of the prepared risperidone microspheres was around 25 days for Formulations_P and L, whereas for Formulations_E1 and E2, it took around 35 days to reach a plateau. Formulation_L, which was prepared with the largest Mw polymer, had the fastest release rate, followed by Formulations_E1, E2 and P. This rank order of the release rate was the same in both

Table 1
Physicochemical properties of polymers (mean ± S.D., n = 3).

Polymer	Manufacturer	Mw (kDa)	Polydispersity	Inherent Viscosity (dL/g)	Tg (°C)
E1	Evonik	70.12 ± 0.26	1.42 ± 0.00	0.64 ± 0.12	49.40 ± 1.01
E2	Evonik	56.55 ± 0.20	1.42 ± 0.02	0.49 ± 0.06	48.82 ± 0.25
P	Polyscitech	86.11 ± 1.14	1.71 ± 0.02	0.78 ± 0.08	45.96 ± 2.84
L	Lactel	103.71 ± 10.29	1.50 ± 0.14	0.91 ± 0.03	45.31 ± 0.88

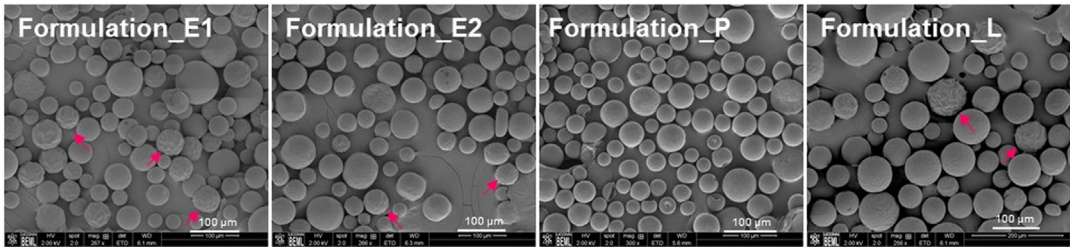

Fig. 1. SEM micrographs of the prepared risperidone microsphere formulations: scale bar: 100 µm. The arrows indicate some examples of wrinkled particles.

release media.

3.4. In vitro degradation studies of risperidone microspheres

In vitro degradation testing of the risperidone microspheres prepared with the different Mw polymers was investigated. Fig. 3 and 4 show the result of the changes in Mw of the polymers over the period of time during the *in vitro* degradation studies. All the microspheres prepared using polymers of different Mw exhibited first order degradation kinetics (Fig. 3) for the hydrolytic degradation of ester bonds in the PLGA. The rate constants calculated from the slopes of the Ln (Mw) versus time graph were 0.0275, 0.0247, 0.0167 and 0.0353 for Formulations_E1, E2, P and L, respectively. The rate of decrease in the Mw for Formulation_P was slower compared to that of the other microsphere formulations and the fastest polymer degradation rate was observed with Formulation_L. These results correlated with the observed release rate tested using USP apparatus 4 (*i.e.* Formulation_P and Formulation_L had the slowest and the fastest release rates, respectively).

The morphological changes observed in the prepared microsphere formulations with time during release testing are shown in Figs. 4 and 5. Prior to the degradation studies, some wrinkled particles were observed in Formulations_E1, E2 and L as well as smooth spherical particles whereas most of the particles in Formulation_P had smooth surfaces. The degradation of all the prepared formulations appeared to follow the inside-out mechanism, as typically observed with PLGA microspheres (Vert et al., 1994). In the case of Formulation_E1 and Formulation_L, pores and channels were observed by day 15 (Fig. 4, arrow), even though it was still in the lag phase according to the results of the real-time *in vitro* release testing studies. Polymer erosion occurred rapidly after day 15, especially for Formulation_L and no individual microspheres existed at day 25 for Formulation_L and at day 30 for Formulation_E1. In the case of Formulation_E2 and Formulation_P, pores and channels were observed by day 20. No individual microspheres existed at day 35 for both these formulations. When the prepared formulations were compared at the same sampling point (Day 20), more degraded particles were observed in Formulation_L, followed by Formulations_E1, E2 and P and this rank order of degradation rate based on morphology correlated with that of the drug release rates from the real-time *in vitro* release testing data. These results indicated that risperidone microspheres prepared with different Mw polymers (*i.e.,* 57–104 kDa) had different degradation rates which did not depend on the polymer Mw.

4. Discussion

Drug release from high Mw PLGA microspheres is normally governed by a combination of polymer erosion as well as drug diffusion mechanisms (Makadia and Siegel, 2011; Zolnik et al., 2006; Faisant et al., 2002). Accordingly, these PLGA microspheres often exhibit complex drug release characteristics (such as bi- or tri-phasic release profiles with and without lag phase). These *in vitro* release profiles could be altered by the physicochemical properties of the polymer (such as Mw, crystallinity, monomer ratio and sequence) and the encapsulated drug as well as critical quality attributes of microspheres (such as porosity, particle size, morphology and drug loading) (Dunne et al., 2000; Luan et al., 2006; Panyam et al., 2003). Risperidone, the drug used in these studies, is a tertiary amine with a pKa of 8.18 (20 °C) and has been reported to catalyze hydrolytic degradation of PLGA (Fig. 3) (Shen et al., 2016; Rawat et al., 2011; Selmin et al., 2012). Since the risperidone microspheres were prepared using PLGAs with the same copolymer ratio, the polymer Mw and crystallinity as well as the physicochemical properties of microspheres were responsible for the differences in the degradation rates, drug diffusion and hence, the *in vitro* release characteristics.

It was evident that the risperidone-loaded microspheres with different Mw PLGA polymer had different particle size (Table 2). On the other hand, there were no significant differences in the drug loading of the prepared formulations. As was observed at Day 0 of the *in vitro* degradation studies (Table 4), the prepared formulations had smaller Mws than the polymers themselves. This is due to the presence of free risperidone in solution during microsphere preparation, which catalyzes the polymer degradation (Selmin et al., 2012).

All the risperidone microspheres were prepared by the same processing method using EA and BA as the solvent system. Since EA and

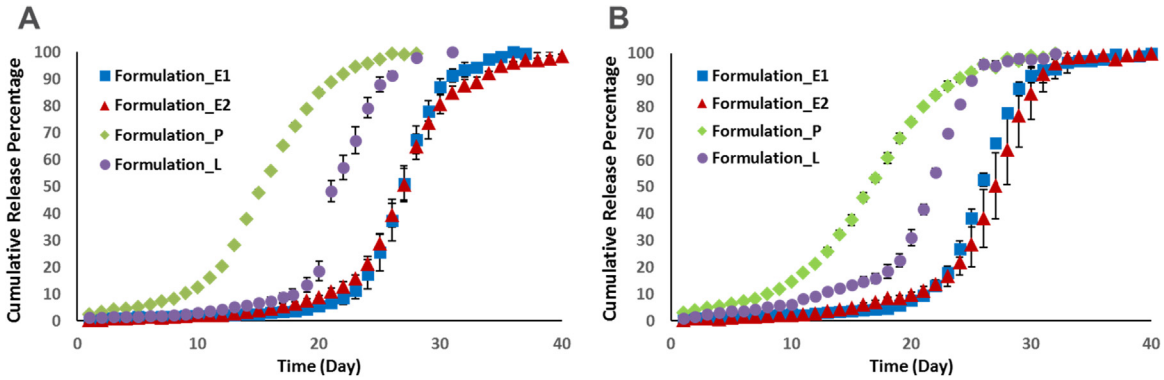

Fig. 2. In vitro release profiles of risperidone microsphere formulations obtained using the flow-through cell method ("real-time" conditions) with (A) PBS and (B) HEPES buffer. (■) Formulation_E1, (▲) Formulation_E2, (♦) Formulation_P, and (●) Formulation_L.

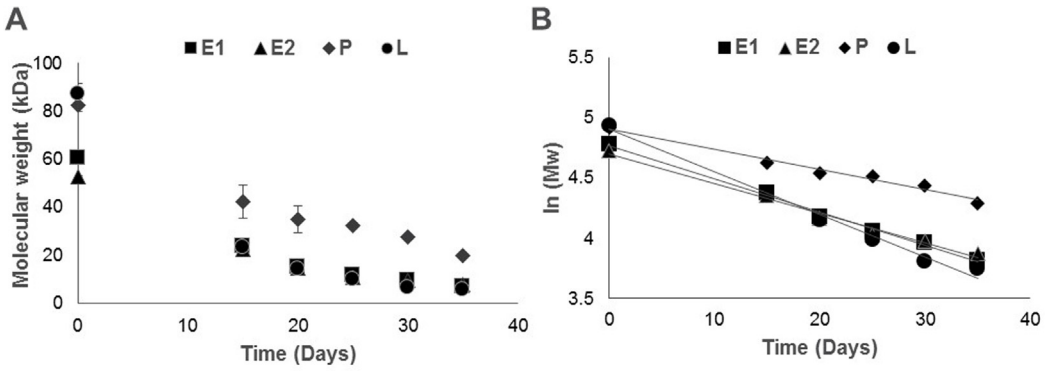

Fig. 3. (A) Changes in molecular weight of PLGA in the prepared risperidone microspheres after exposure to 10 mM HEPES (pH 7.4 with sodium chloride, Tween 20 and sodium azide) at 37°C. (B) shows the same data as A) but in a semi-logarithmic plot: (■) Formulation_E1, (▲) Formulation_E2, (♦) Formulation_P and (●) Formulation_L.

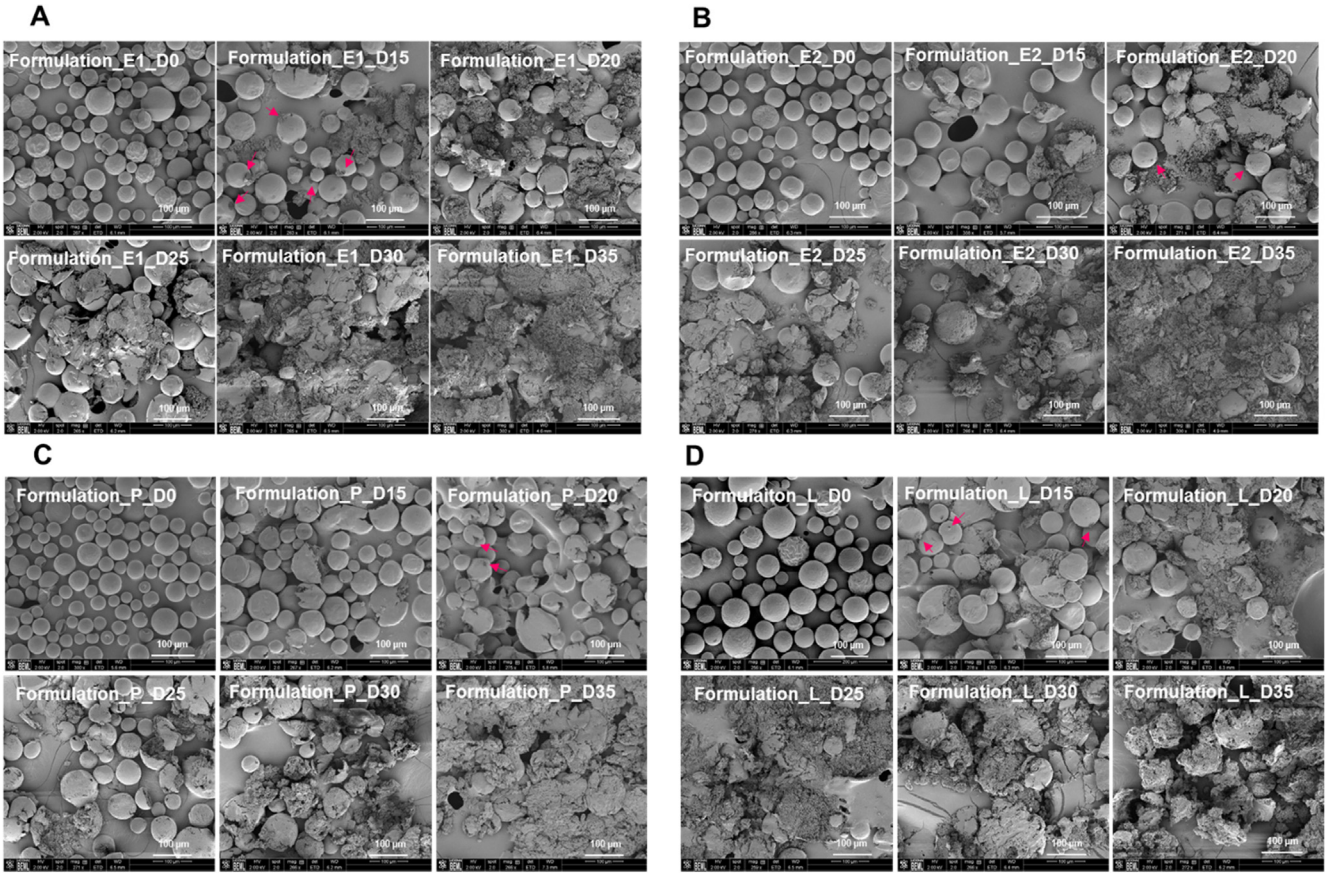

Fig. 4. SEM micrographs of: (A) Formulation_E1, (B) Formulation_E2, (C) Formulation_P and (D) Formulation_L after exposure to 10 mM HEPES (pH 7.4 with sodium chloride, Tween 20 and sodium azide) over a period of 35 days: scale bar: 100 μm. The arrows indicate the formation of pores and channels.

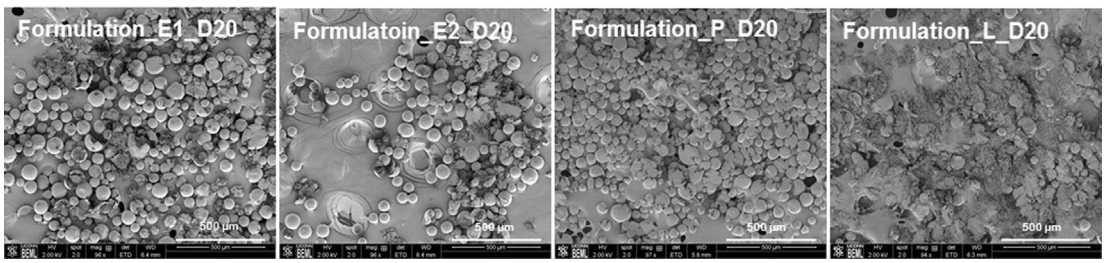

Fig. 5. SEM micrographs of the prepared formulations with different molecular weight polymers after exposure to 10 mM HEPES (pH 7.4 with sodium chloride, Tween 20 and sodium azide) for 20 days: scale bar 500 μm.

Table 4
Changes in molecular weight (kDa) of risperidone-loaded microsphere formulations after exposure to 10 mM HEPES (pH 7.4) at 37°C.

Sample	Day 0	Day 15	Day 20	Day 25	Day 30	Day 35
Formulation_E1	60.23 ± 0.70	23.67 ± 0.98	15.16 ± 0.27	11.39 ± 0.20	9.27 ± 0.03	6.63 ± 0.23
Formulation_E2	52.64 ± 0.49	22.48 ± 1.58	14.69 ± 0.15	10.68 ± 0.47	9.56 ± 0.13	7.49 ± 0.22
Formulation_P	82.48 ± 2.63	42.28 ± 6.93	34.82 ± 5.71	32.42 ± 1.25	27.49 ± 1.34	19.68 ± 0.41
Formulation_L	87.05 ± 4.58	23.33 ± 1.39	14.15 ± 1.92	9.82 ± 0.25	6.41 ± 0.40	5.61 ± 0.15

water are partially miscible (Sah, 1997); dynamic exchange of EA and water during the microsphere solidification and solvent extraction may result in a relatively high amount of water included inside the microspheres. When this entrapped water escapes from the microspheres, during the drying process, it may result in a porous structure inside the microspheres. The morphology (porosity and smoothness of the surface) were different between the prepared formulations (Table 3, Fig. 1). This may be a result of the different polymer sources, since the synthesis and purification methods used by the different manufacturers may result in different monomer sequence, type and extent of residual solvent. Differences in the manufacturing/purification methods were also implied by their different appearances (data not shown) and T_g values. The rank order of the T_g values was not consistent with the Mw of the polymers (Table 1). To elucidate the reason for the porosity differences, additional studies are required.

As shown in Fig. 2, all four prepared formulations in the studies exhibited a tri-phasic release pattern similar to Risperdal® CONSTA® (Rawat et al., 2012). Generally, initial burst release of drug is related to the drug on the surface of the particles, in contact with the medium (Makadia and Siegel, 2011). EA has relatively higher water solubility resulting in rapid polymer precipitation, which in turn limits the movement of drug onto the surface or outer layer of the of the microspheres. Therefore, risperidone may be mostly entrapped inside the microspheres with limited amount of surface associated drug resulting in the observed low burst release (Sah, 1997; Lu et al., 2014).

Before the release started, all of the prepared formulations exhibited a lag phase for 10 to 20 days. Once the microspheres were immersed in the aqueous medium, the water penetration into the microspheres starts followed by the polymer hydrolytic degradation to soluble oligomers and monomers. The high porosity of Formulation_P makes it easy for water to access the ester linkage of the polymers and for drug to escape from the microspheres, which may be the major reason for the shorter lag phase of this formulation (Fig. 2). On the other hand, the less porous formulations (*i.e.,* Formulations_E1, E2 and L) showed a longer lag phase because of the reduced water accessibility. Compared to Formulations_E1 and E2, Formulation_L had a shorter lag phase and this may be explained by the T_g of the polymers (Karavelidis et al., 2010). The T_g of L was significantly lower than E1 and E2 (Table 1) which means more flexibility of the polymer chains in Formulation_L. This flexibility resulted in higher water accessibility and a shorter lag phase for Formulation_L in spite of its relatively lower porosity (Table 3). During the lag phase, the random chain scission process occurred resulting in a significant polymer Mw decrease (Fig. 3 and Table 4). As mentioned above, risperidone, a tertiary amine drug, catalyzes hydrolytic degradation of PLGA resulting in a drastic decrease in polymer Mw during release testing. This would reduce the differences in the Mw of the four polymers and therefore minimize the effect of polymer Mw on drug release rates. These results are comparable with a previous report using different monomer ratio PLGA polymers where it was shown that expected differences in the degradation rate among the polymers were mitigated in the presence of risperidone (Selmin et al., 2012).

In the secondary release phase, the release rate showed a rank order different from that of the polymer Mw. Formulation_L which was prepared using the largest Mw polymer exhibited the fastest release, followed by Formulations_E1, E2 and P. This rank order appeared to correlate with the polymer degradation rate (*i.e.*, decrease of Mw) (Fig. 3) as well as the changes observed in SEM images (Fig. 5) of samples from the *in vitro* degradation studies. This may be explained by the extent of degradation products trapped inside the microspheres, which are affected by the porosity, particle size and T_g of the polymers. It has been reported that PLGA degrades *via* heterogeneous mechanisms, *i.e.*, the degradation proceeds more rapidly in the center than at the surface (Vert et al., 1994; Grizzi et al., 1995). This leads to the accumulation of the acidic degradation product with carboxylic acid end groups inside the microspheres and hence, acidic conditions inside the microspheres. This acidic environment autocatalytically accelerates further polymer degradation resulting in the faster degradation of microspheres in the center compared to the surface. (Fu et al., 2000; Shenderova et al., 1999; Brunner et al., 1999). The porosity may affect not only water accessibility but also the extent of the autocatalytic polymer ester hydrolysis. Since Formulation_P had the highest porosity and the largest average pore diameter compared to the other formulations (Table 3), the generated degradation products (oligomers or monomers) may be easily released from these microspheres into the aqueous medium and the aqueous medium may more easily penetrate into the microspheres (Fu et al., 2000). Thus; mitigating acidification, by reducing the autocatalytic hydrolysis effect and resulting in the slower release rate of Formulation_P. The reduced accumulation of degradation products in Formulation_P was also evident in the DSC thermograms of the samples from the *in vitro* degradation studies, which showed endothermic peaks around 120–150 °C in some samples (Fig. 6, arrow). These peaks appeared to be due to the crystallization of the degradation products accumulated inside the microspheres (Park, 1995; Göpferich, 1996; Schliecker et al., 2003; Erbetta et al., 2012). There are random ester bonds in each polymer, linking the glycolic acid (G) and the lactic acid (L). As a result of the inherent higher reactivity with water and more hydrophilic glycolic unit than with the more hydrophobic lactic unit, the glycolic acid unit (G-G; or G-L) linked ester may be preferentially cleaved compared to the lactic-lactic acid (L-L) linked ester. Thus, the remaining lactic acid rich oligomers become crystallized. The melting temperature of the monomer D,L lactide is 125 °C. If the crystallization of D,L lactide happens, the peaks of melting for the oligomers with different Mw which have different melting points will be observed around 100–170 °C (Park, 1995; Schliecker et al., 2003). Since the oligomers or monomers released into the aqueous media were removed before analysis, these peaks indicated the existence of the degraded products inside of the microspheres of Formulations_E1, E2 and L. However, there were no or very small peaks observed in Formulation_P, which may be because the degradation products easily release into the aqueous media. In addition, Formulation_P showed larger mass loss% than the other formulations during the *in vitro* degradation studies (data not shown), which also supports the hypothesis of faster release of degradation products from this formulation.

In spite of having the largest polymer Mw and similar porosity to Formulation_E1, Formulation_L showed fastest release rate. The reason for this fastest release rate may be attributed to the larger particle size of Formulation_L. In the larger particles the degradation products (relatively high Mw oligomers) have a longer path to the surface making it relatively more difficult for diffusion into the aqueous phase compared to smaller particles (*i.e.*, Formulations_E1 and E2) (Dunne et al., 2000; Grizzi et al., 1995). Accordingly, more degradation products will be

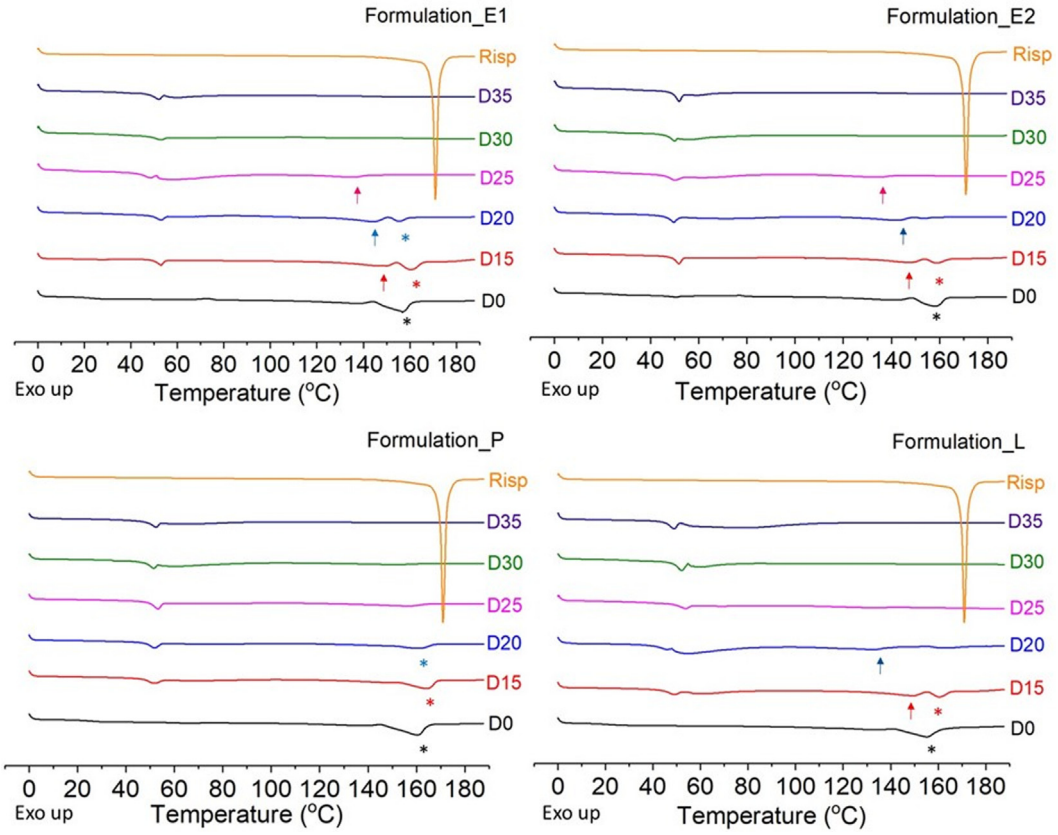

Fig. 6. DSC thermograms (1st cycle) of prepared formulations after exposure to 10 mM HEPES (pH 7.4) over a period of 35 days. The arrow indicates the crystallization of degradation products entrapped in the microspheres. The star indicates the melting of risperidone.

entrapped within Formulation_L and therefore the autocatalytic process will be faster.

5. Conclusions

The results suggest that the drug release rate from PLGA microspheres is not only dictated by the manufacturing process and the polymer properties, but also by any potential polymer-drug interactions and other critical formulation parameters. Therefore, it is important to understand all critical formulation properties and critical processing parameters that can have an impact on the critical quality attributes of the final drug products such as the drug release rate. Specifically, in this research, the T_g of the polymers as well as the microsphere porosity and particle size (which affect the water accessibility and autocatalytic process) had a greater effect than the polymer Mw in the formulations investigated. This is a consequence of risperidone interacting with PLGA, which has a catalytic effect on PLGA thus minimizing the contribution of polymer Mw on the drug release rate. It is interesting that differences in the microsphere porosities were observed in spite of the processing parameters for the four formulations being the same (EA and BA solvent system). This is speculated to be due to differences in the polymer sources, and accordingly differences in the synthesis and purification methods, which may result in different monomer sequences, as well as the type and extent of residual solvent. Additional studies are required to elucidate these critical differences in the polymers.

The knowledge from these studies may help to establish specifications for PLGA polymers to be used in the development of bioequivalent microsphere drug products.

CRediT authorship contribution statement

Moe Kohno: Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Janki V. Andhariya:** Conceptualization, Investigation, Writing - review & editing. **Bo Wan:** Investigation, Writing - review & editing. **Quanying Bao:** Methodology, Writing - review & editing. **Sam Rothstein:** Writing - review & editing, Project administration. **Michael Hezel:** Writing - review & editing, Project administration. **Yan Wang:** Writing - review & editing, Project administration. **Diane J. Burgess:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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