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Beyond Q1/Q2: The Impact of Manufacturing Conditions and Test Methods on Drug Release From PLGA-Based Microparticle Depot Formulations

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ABSTRACT

Drug-loaded polymeric microparticles have been used as long-acting injectable (LAI) depot formulations. To obtain U.S. Food and Drug Administration approval, a generic LAI depot product needs to be qualitatively (Q1) and quantitatively (Q2) the same in terms of inactive ingredients as its reference-listed drug. However, Q1/Q2 sameness as the reference-listed drug does not guarantee the same *in vitro* drug release profile and *in vivo* performance, especially when the manufacturing methods are different. There is little consensus on how the *in vitro* testing needs to be done to examine the release profiles of LAI depot formulations. This study examined the manufacturing differences in making risperidone-loaded poly (lactide-co-glycolide) microparticles and their impact on the release kinetics. It also examined the impacts of *in vitro* testing methods on the drug release profiles. Two in-house manufactured risperidone poly(lactide-co-glycolide) microparticles and Risperdal Consta® were used in the study. Of the *in vitro* release methods tested, the orbital agitation method provided the most reproducible release profiles. The results indicate that the *in vitro* release kinetics depend not only on manufacturing procedures but also on the *in vitro* testing conditions, such as the agitation speed, vessel-dimensions, solid beads, media exchange volume, and other parameters both under real-time and accelerated testing conditions. In the current case, the *in vitro* experimental condition seemed to affect the drug release kinetics more than the manufacturing differences. The developed orbital agitation release testing method is simple, robust, and reproducible, which allows the comparison of *in vitro* release profiles of formulations that are prepared with manufacturing differences.

Introduction

Microparticles (or microspheres) made of biodegradable poly(lactide-co-glycolide) (PLGA) have been used for developing long-acting injectable (LAI) depot formulations for the last 3 decades. These PLGA-based microparticle formulations present significant advantages for drugs which have either low oral bioavailability (e.g., poorly water-soluble Biopharmaceutics Classification System class IV drugs[1]) or poor patient compliance (e.g., addiction treatment medications such as naltrexone[2]). The mechanisms of drug release from PLGA-based microparticles are complicated involving multiple factors, such as drug diffusion and polymer degradation. After administration, PLGA degrades into nontoxic components, lactic and glycolic acids, which are either metabolized or excreted from the body.[3] The degradation opens more water-accessible space leading to the interior of the particle.[4] In addition, accumulation of lactic acid and glycolic acid during degradation could lead to a substantial decrease in the interior pH, which can be as low as 3.[5] Such a low pH subsequently causes accelerated degradation, as the acid catalyzes enhanced hydrolysis of PLGA molecules.[6] The autocatalytic degradation can result in accelerated drug release.

Generic LAI formulations are required to qualitatively (Q1) and quantitatively (Q2) match reference-listed drug products in terms of inactive ingredients.[7] Many parameters, both in formulation and in the manufacturing method, affect the release profile of drugs

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from PLGA microparticles.[1] Some of these parameters, such as polymer composition and relative content of inactive ingredients including the polymer are well captured by the Q1/Q2 requirements. The Q1/Q2 requirement, however, does not capture other parameters, such as the manufacturing process, drug distribution within the particles, drug crystallinity, and the *in vitro* testing method.[8] The effects of the manufacturing process on microparticle properties can be examined using various methods, such as scanning electron microscopy, atomic force microscopy, infrared Raman microscopy, and differential scanning calorimetry.[8-13] The effects on drug release kinetics, however, can be best examined by measuring *in vitro* drug release profiles.

In vitro drug release testing has been routinely used as a quality control tool.[14,15] In addition to that, some *in vitro* drug release testing methods can also be used to predict *in vivo* performance of a product (*in vitro–in vivo* correlation).[16-19] For LAI drug products, there are also benefits to developing an accelerated drug release testing method.[20] There are several *in vitro* test methods available for measuring the drug release.[21] Commonly used methods include rotating basket (U.S. Pharmacopeia [USP] type I apparatus), paddle-type (USP type II apparatus), and reciprocating cylinder (USP type III apparatus) methods which were developed specifically for oral dosage forms.[22] For injectable microparticle formulations, there are no compendial methods for *in vitro* drug release testing. To date, there are literature reports on using a USP Apparatus II,[23,24] a USP Apparatus IV (flow-through method),[25] and an orbital agitation method.[26-28] Each of these methods has its own advantages and shortcomings. For short and intermediate term studies, USP apparatus IV have shown excellent reproducibility and discriminatory power. However, USP apparatus IV are relatively more expensive than other conventional apparatuses and may not be readily available in many laboratories. In addition, long-term use of USP apparatus IV (e.g., 1~6 months) could be challenging as continuous flow-through (open loop) of release medium over an extended period of time could present many challenges. In comparison, USP apparatus II and orbital agitation methods may be more suitable for long-term use. Unlike USP apparatus II, which is a standardized method in terms of set up, the testing conditions of orbital agitation methods, such as volume, agitation rates, and container type, vary widely from study-to-study.[29-31] In addition, the impact of these testing conditions on *in vitro* drug release testing of LAI microparticle formulations and its discriminatory power have not been systemically evaluated.

The goal of this study was to examine whether the drug release profiles from compositionally equivalent PLGA microparticles with manufacturing differences can be distinguished by *in vitro* release tests. Risperidone-loaded PLGA microparticles were prepared under different manufacturing conditions and their *in vitro* release properties were compared. Risperdal Consta® was also used as a reference to examine the *in vitro* release under different experimental conditions. The manufactured batches were designed to have Q1/Q2 sameness between each other yet had different processes applied to their preparation. *In vitro* risperidone release kinetics was examined under various testing conditions, which have significant impacts on the release profiles.

Experimental Section

Materials

Risperidone was obtained from Jubilant Life Pharma. PLGA (75:25, 100,000 Da; PolyVivo AP125) was obtained from the PolySciTech division of Akina, Inc. Risperdal Consta® 25 mg (Janssen Pharmaceuticals, Inc.), was purchased through Purdue University Retail Pharmacy. Poly(vinyl alcohol) (PVA, Mowiol 4-88, ~31,000 Da), phosphate-buffered saline with 0.05% Tween 20 (PBST), and dioxane were purchased from Sigma-Aldrich. Dichloromethane (DCM) was purchased from Fisher. Ethyl acetate was purchased from Macron Chemicals. Poly(divinylbenzene) (DVB) was purchased from Jordi Labs. A cluster of 22.6-mm flow-through cells were purchased from Sotax, Inc. Centrifuge tubes (part number 352098; Falcon™) with a diameter of 7 mm and a height of 114 mm were purchased from Fisher Scientific. Glass test tubes measuring 22 mm by 150 mm were obtained from Fisher Scientific. Glass flasks (part number FG5021-100) with a diameter of 50 mm at the base and a height of 75 cm were purchased from Southern Labware. Glass jars (part number GLC-01672; Qorpak) measuring 71 mm by 90 mm were purchased from General Laboratory Supply. Glass beads (unwashed, ≤106 μm; Sigma-Aldrich) were washed with deionized water and acetone and dried before use. All other chemicals were of reagent grade and used as received.

Methods

Preparation of Risperidone Microparticles

Several batches of risperidone microparticles were prepared by an oil-in-water emulsion method. PLGA and risperidone were dissolved in DCM (polymer:drug = 3:2, w/w). Ethyl acetate was added to the polymer/drug solution and the final solution had a DCM:ethyl acetate ratio of 2:1. The concentration of polymer and drug in the solution was 23.3%. The polymer/drug solution was emulsified in 1.0% PVA (containing 1.5% DCM) with an homogenizer (IKA, Ultra-turrax) for 30 min, with additional 1% PVA added after 15 min of mixing. The microparticles underwent solvent extraction in 2 L of 1% PVA for 1.5 h. The prepared microparticles were sieved between 7 and 105 μm using nylon mesh and washed with deionized water. The microparticles were collected and freeze-dried (Formulations 1-1 and 1-2).

In another method, PLGA and risperidone were dissolved in DCM (polymer:drug = 3:2, w/w). The concentration of polymer and drug in the solution was 23.3%. The polymer/drug solution was emulsified in 1.0% PVA with an homogenizer for 30 min, with additional 1% PVA added after 15 min of mixing. The microparticles underwent solvent extraction in 2 L of 1% PVA for 24 h. The prepared microparticles were sieved between 7 and 105 μm using nylon mesh and washed with deionized water. The microparticles were collected and freeze-dried (Formulation 2-1 and 2-2). The use of the microparticles in the 7-105 μm range for all samples allowed testing of the effect of manufacturing conditions and *in vitro* drug release methods on the drug release kinetics.

High Performance Liquid Chromatography Analysis

Risperidone was quantified using a Varian ProStar high performance liquid chromatography (HPLC) system equipped with a model 210 isocratic pump, a model 410 autosampler, and a model 335 photodiode array detector. The mobile phase was water:acetonitrile:trifluoroacetic acid, 75:25:0.1 (v:v:v). The system was equipped with an Agilent C18 column (4.6 × 150 mm, 5 μm, 180 Å) and the flow rate was set to 1 mL/min. The injection volume for drug loading samples and release samples was 100 μL. The chromatographs were analyzed with Galaxie software at 275 nm. Standard curves were generated with a concentration range of 0.2 to 20 μg/mL.

Drug Loading Determination

Ten milligrams of risperidone microparticles were weighed into a glass vial. Dioxane (2 mL) was added to the vial and the vial was vortexed until the microparticles dissolved. The entire solution was carefully transferred into a 50-mL volumetric flask. The solution was diluted to approximately 80% of the total volume with 0.1-M

hydrogen chloride (HCl) and sonicated for 15 min. The flask was then filled to volume with 0.1-M HCl to extract out the risperidone from the PLGA solution. The sample was diluted to a concentration within the standard curve range with 0.1-M HCl and filtered through a 0.45-μm polyvinylidene fluoride (PVDF) syringe filter before HPLC analysis. Percent drug loading was calculated as follows: (mass of risperidone/microparticle mass) ×100. Drug loading was performed in triplicate and the results are reported as the average ± standard deviation.

Modified Flow-Cell Apparatus

A modified flow-cell apparatus was built in our laboratory. The cluster of 22.6-mm flow-through cells were modified in the following manner. The inlet on the bottom chamber was threaded to accommodate a ¼-28 UNF tubing adapter. A cylinder with a slightly larger diameter than the bottom chamber of flow-through cells was mounted onto metal brackets. The bottom chamber of the flow-through cell was placed in the mounted cylinder. The upper chamber of the flow-through cell was placed on top of the lower chamber and secured with a laboratory-manufactured spring-loaded clamp. Four flow-through cells were mounted vertically in this manner. The wetted dimensions, cell orientation, and temperature control of the apparatus was harmonized to the USP IV standards.[32] The assembly was placed in an incubator (model 12-140; Quincy Labs) and secured to the incubator base. Media tubing (Masterflex 96400-14) was connected to the flow-through cells in a closed-loop configuration and placed on a 4-channel peristaltic pump (model 07522-20; Masterflex® L/S®). Media bottles were placed in the incubator and sampling ports were constructed in the bottle lids. The incubator temperature was set and monitored with a thermometer mounted inside the incubator at the same height as the flow-through cells (Fig. 1).

In Vitro *Drug Release Methods*

Release Method 1. The use of flow-cell methods for measuring release of risperidone from PLGA microspheres has a strong precedent in literature.[25] We utilized these methods, with slight modifications, for this study as follows. A 5-mm glass bead was placed in the bottom chamber of the 22.5-mm flow-through cells, followed by 5.0 g of 1-mm glass beads. A nylon mesh with 10-μm pore size was placed on top of the glass beads and held in place with an acrylonitrile butadiene styrene (ABS) ring. Approximately 14.5 g of 1-mm glass beads were added on top of the nylon mesh.

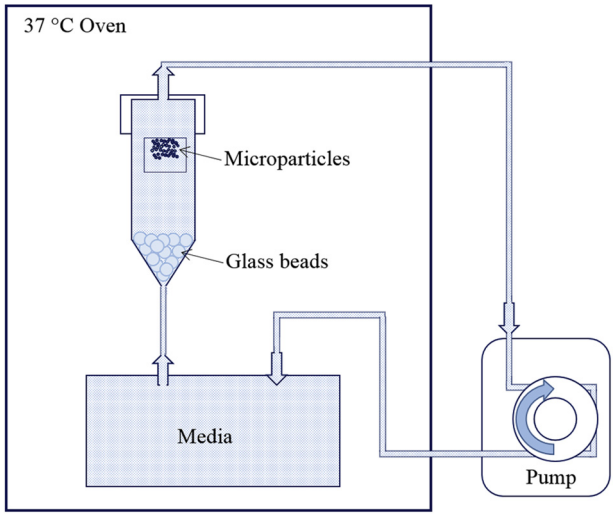

Figure 1. Schematic flow chart of flow-cell apparatus.

One vial of Risperdal Consta® 25 mg was dispersed in manufacturer-supplied diluent. Approximately 0.5 mL of dispersed microparticles were injected into the 1-mm glass beads above the nylon mesh of each flow-through cell with a 21 gauge, 1″ needle. A nylon mesh with 10-μm pore size was placed on top of the glass beads and held in place with an ABS ring. In some release tests, the nylon mesh was incorporated between 2 ABS rings with cross-arms for additional stability of the nylon mesh. The flow-through cells were placed in the modified flow-cell apparatus as aforementioned. Media bottles containing 1 L of PBST with 0.1% sodium azide were placed in a 45°C incubator and the media tubing was connected to the flow-through cells and peristaltic pump. The temperature 45°C was selected to correlate to previously validated accelerated conditions.[33] The flow rate was set to 4 mL/min. Five-milliliter samples were removed through the sampling ports and replaced with 5 mL of fresh PBST with 0.1% sodium azide. The sample was filtered through a 0.45-μm PVDF syringe filter and analyzed for risperidone content via HPLC. All release testing was performed in quadruplicate unless otherwise noted, and the results are reported as the average ± standard deviation.

Release Method 2. Approximately 25 mg of risperidone microparticles were weighed into a release vessel. PBST (40 mL) was added to the vessel and the vessel was placed in an incubator capable of orbital agitation (model IncuShaker Mini; Southwest Science) or a static incubator (model 12-140; Quincy Lab, Inc.). The temperature was set to 37°C or 45°C for real-time or accelerated conditions, respectively.[33] The agitation was set to 100 rpm in the orbitally agitating incubator, which is a common agitation rate in literature sources.[31,34] At predetermined time intervals, 30 mL of media was removed, taking care to avoid removing microparticles. The removed media was replaced with fresh PBST at room temperature, and the vessel was returned to testing conditions. The sample was filtered through a 0.45-μm PVDF syringe filter and analyzed for risperidone content via HPLC. The media replacement was considered in the calculation of the cumulative percent drug release. All release testing was performed in triplicate unless otherwise noted, and the results are reported as the average ± standard deviation.

Release Method 3. Solid beads were added with the microparticle sample. The solid beads, either hydrophobic DVB or hydrophilic glass beads, were weighed into the release vessel. Ethanol (100 μL) was added to pre-wet the solid beads, especially DVB beads. PBST (20 mL) was added to the vessel. The microparticle sample was weighed and transferred to the vessel with an additional 20 mL of PBST. The vessel was placed in an incubator capable of orbital agitation (model IncuShaker Mini; Southwest Science) or a static incubator (model 12-140; Quincy Lab, Inc.). The temperature was set to 37°C or 45°C. The agitation was set to 100 rpm in the orbitally agitating incubator. At predetermined time intervals, 30 mL of media was removed, taking care to avoid removing microparticles. The removed media was replaced with fresh PBST at room temperature, and the vessel was returned to testing conditions. The sample was filtered through a 0.45-μm PVDF syringe filter and analyzed for risperidone content via HPLC. The media replacement was considered in the calculation of the cumulative percent drug release. All release testing was performed in triplicate unless otherwise noted, and the results are reported as the average ± standard deviation.

Release Method 4. Twenty-five milligrams of risperidone microparticles were weighed into a release vessel. PBST (40 mL) was added to the vessel and the vessel was placed in an incubator capable of orbital agitation (model IncuShaker Mini; Southwest Science). The temperature was set to 37°C or 45°C and the agitation

was set to 100 rpm. At predetermined time intervals, 1 mL of media was removed from the release vessel. The removed media was replaced with fresh PBST at room temperature, and the vessel was returned to testing conditions. The sample was filtered through a 0.45-μm PVDF syringe filter and analyzed for risperidone content via HPLC. All release testing was performed in triplicate unless otherwise noted, and the results are reported as the average ± standard deviation.

Gel Permeation Chromatography

Risperidone microparticles were analyzed for PLGA molecular weight, number, and polydispersity index by gel permeation chromatography (GPC) with a Waters system equipped with a model 1515 isocratic pump, a model 2707 autosampler and a model 2414 refractive index detector. Samples were dissolved in DCM, filtered through a 0.45-μm syringe filter, and eluted with 1 mL/min flow of DCM across 3 GPC columns (300 × 7.8 mm) in sequence. Polystyrene standards were used to generate a calibration curve. Data collection and analysis was performed using Waters software.

PLGA Degradation Study

Risperidone release from microparticles was measured at 37°C. Release samples were taken as described for Release Methods 2 and 3, and at predetermined time points the media was removed from the vessel and the microparticles were freeze-dried. The dried microparticles were dissolved in DCM and the PLGA was analyzed via GPC.

Risperidone Solubility Study

Lactic acid was dissolved in water to make 0.1%, 0.5%, and 1.0% solutions. HCl solutions of 0.1% and 1.0% were prepared as controls, as well as PBST. The pH of each solution was measured. Risperidone was added in excess to each solution and was allowed to equilibrate overnight with shaking at room temperature. The mixture was filtered through a 0.45-μm PVDF membrane to remove the excess risperidone. The resulting filtrate was analyzed for risperidone content by HPLC.

Risperidone Absorption to DVB and Glass Bead

The uptake and absorption of risperidone with the solid-release modifiers, DVB, and glass beads, was tested for each one as follows. A selected quantity (20 mg) of each bead type was loaded into a glass vial and prewetted with 100 μL of ethanol. A solution of risperidone in PBST (20 mL) was added to the vial. The solid-release modifiers remained in the solution of risperidone in PBST at room temperature overnight. The supernatant was removed, diluted if necessary, and filtered through a 0.45-μm polytetrafluoroethylene syringe filter. The concentration of risperidone in the supernatant of each vial, as well as the concentration of risperidone in a control PBST solution was determined by HPLC. The amount of risperidone absorbed by the solid-release modifiers was calculated by subtracting the amount of risperidone in the supernatant from the amount of risperidone in the control solution.

Statistical Analysis

Means and standard deviations were calculated using Microsoft Excel. For direct comparison between data sets, GraphPad Software was utilized to perform t-test.

Results

Drug Loading

Four batches of microparticles were manufactured. The risperidone loading of the PLGA microparticles produced for release testing was quantified by HPLC and is shown in Table 1. The batches produced with an organic phase consisting of DCM only had a slightly lower risperidone content than those with DCM and ethyl acetate. The differences in loading between each of these batches were statistically significant, except for the 2 DCM batches (formulations 2-1, 2-2: $p > 0.05$; rest $p < 0.05$).

Table 1
Drug Loading of Risperidone Microparticles ($n = 3$)

Sample	Formulation	Drug Loading (%)
Formulation 1-1	DCM/ethyl acetate	29.49 ± 0.30
Formulation 1-2	DCM/ethyl acetate	30.74 ± 0.45
Formulation 2-1	DCM	26.59 ± 1.76
Formulation 2-2	DCM	27.29 ± 0.51

Flow-Cell Apparatus Release Test

The purpose of building the modified flow-cell apparatus was to develop a more-flexible alternative to the commercially available USP apparatus IV to assay release results under conditions similar to that in the USP apparatus type IV. In initial tests, the glass bead packing material was contained between nylon mesh that was sandwiched between ABS rings with cross-arms. The release test was performed twice and the profile is shown in tests 1 and 2 of Figure 2. The flow-cell apparatus release test showed large variability between the samples. The standard deviation between samples in the same test was very high in both tests. The lag phase of the Risperdal Consta® also varied from 2 to 6 days between the 2 tests. In both tests, 100% risperidone release was not obtained before the drug concentration in the media-started plateauing or dropping. The release test was repeated with the nylon mesh held in place by ABS rings to eliminate poor media flow as the cause of the poor reproducibility. Test 3 of Figure 2 shows the release profile generated from this test. The standard deviation between the 4 samples was very large once the steady state release phase was reached. The cumulative percent drug release was also lower than expected. In this test, the lag phase was 15 days before the steady-state drug release phase was reached. The delay period was twice as long as the delay period in test 2 in Figure 2. Numerous changes to the flow-cell apparatus were made in attempts to improve the reproducibility, however, none of the release tests generated from the apparatus showed tighter standard deviation between samples. The poor reproducibility limited the use of the flow-cell apparatus as a tool to distinguish differences in release methods and manufacturing conditions. This failed, negative result highlights the

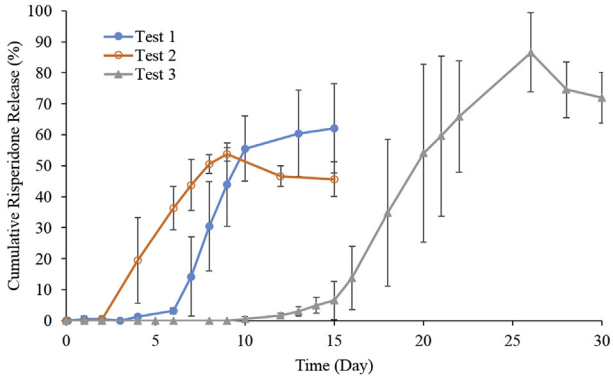

Figure 2. Accelerated *in vitro* release profile of Risperdal Consta® 25 mg in the modified flow-cell apparatus at 45°C, replicate testing (test 1, $n = 2$; test 2, $n = 4$; test 3, $n = 4$).

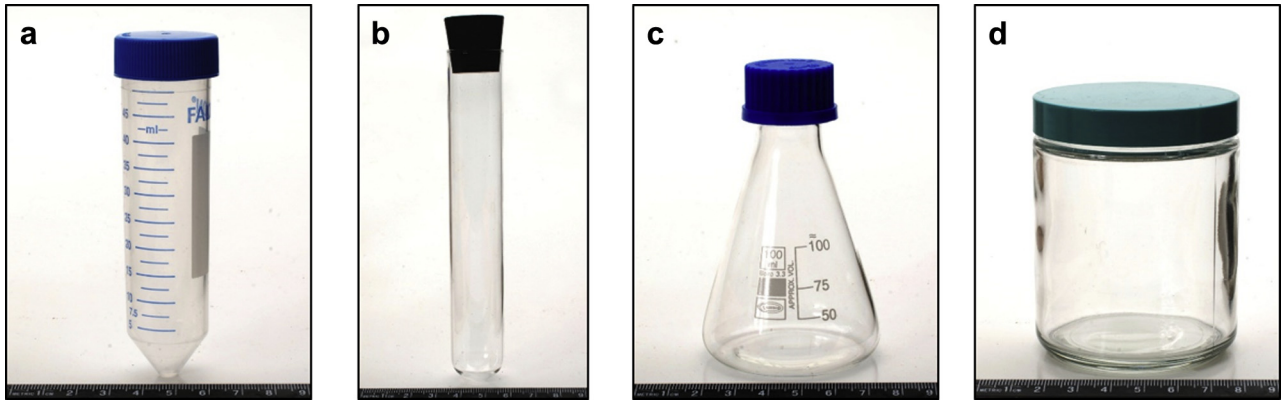

Figure 3. Vessels for risperidone microparticle release testing: centrifuge tube (a), centrifuge tube with glass beads (not pictured), test tube (b), glass flask (c), and glass jar (d).

exacting needs for conditions to be harmonized precisely to allow for comparison of data.

Vessel Study

Four vessels were chosen to determine the effect of vessel type on the *in vitro* drug release profile. Figure 3 shows the types of vessels chosen for drug release testing. The diameter of the vessels ranged from 7 mm with a steep conical shape (centrifuge tube) to 65 mm with a flat surface (glass jar). Formulation 1-1 risperidone microparticles were tested following release method 2 at 45°C in an incubator with orbital agitation.

As shown in Figure 4, the accelerated *in vitro* release profile varied according to the diameter of the release vessel. In the vessels with a narrow diameter, that is, centrifuge tube and glass tube, the risperidone release profile had a lower burst release and a slower rate of release over the course of the test than the vessels with a larger diameter. This effect can be seen strongly in the release at Day 4 and the glass jar/glass flask have significantly higher release than either the glass tube or the centrifuge tube (p <0.05). The risperidone microparticles in the centrifuge tubes with and without glass beads exhibited substantial aggregation in the vessels after approximately 2 days in the orbitally agitating incubator.

The microparticles in the centrifuge tubes aggregated into one large piece during the test and did not achieve 100% drug release, possibly due to the risperidone being unable to completely diffuse from the clumped microparticles. The microparticles in the glass tube showed aggregation also, but did not form into one large clump. These microparticles formed several large aggregates on the bottom of the glass tube. The microparticles in the glass flask and glass jar did not show noticeable aggregation. Thus, the experimental condition should be set to eliminate microparticle aggregation for reproducibility and 100% drug release.

The *in vitro* release profile of Risperdal Consta® was also examined in 2 release vessels at 45°C in an incubator with orbital agitation, as seen in Figure 5. In this test, the release of risperidone in the smaller diameter vessel (centrifuge tube) was significantly slower than the release in the glass flask. The difference becomes significant after Day 4 (p <0.05). Significant aggregation was seen in the microparticles contained in the centrifuge tube. The microparticles in the flask did not show noticeable aggregation. While the risperidone release kinetics in Figures 4 and 5 are different, both show the high impact of microparticle aggregation on the risperidone release profiles. The aggregation of microparticles not only slowed down the diffusion of risperidone but also affected the risperidone solubility in the microenvironment around microparticles due to lowered local pH.

Solid Beads Study

Risperdal Consta® microparticles were used to examine the effect of the addition of solid beads to the vessel. The uptake assay showed that glass beads did not uptake risperidone from the solution at all, and this was expected as the adsorption of hydrophobic risperidone to the glass surface is expected to be minimal. DVB was found to uptake 16.7 ± 0.3% risperidone by weight. Release testing was performed using the glass flask at 45°C with orbital agitation. Varying amounts of modifier were tested, corresponding to the amount of DVB needed to adsorb 10%, 30%, and 50% of risperidone

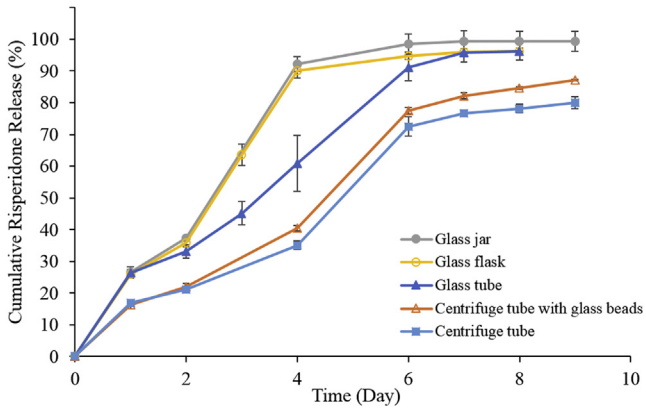

Figure 4. Accelerated *in vitro* release of risperidone microparticles at 45°C in various vessels (formulation 1-1; n = 3).

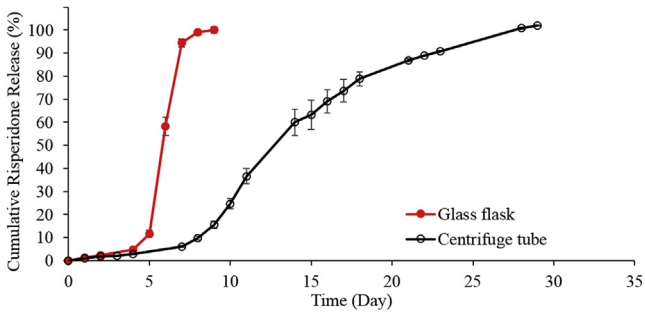

Figure 5. Accelerated *in vitro* release profile from Risperdal Consta® in flasks (n = 3) and centrifuge tubes at 45°C (n = 2).

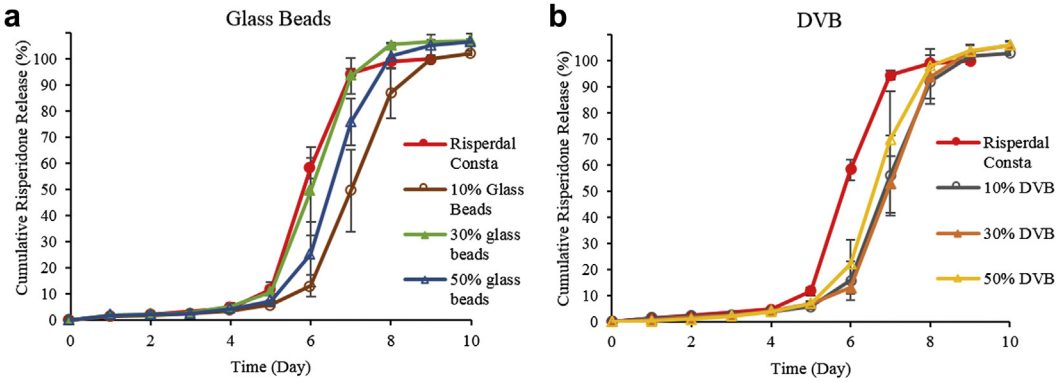

Figure 6. Risperdal Consta® *in vitro* release profile at 45°C in the presence of glass beads (a) and DVB (b) ($n = 3$).

in each flask. As shown in Figure 6, the addition of the solid beads affected the lag phase of the Risperdal Consta® release profile.

In the release test with glass beads, the general trend is that the lag phase can be lengthened by 1 day. This effect was more prominent in the tests with all 3 amounts of DVB. The release kinetics observed with DVB during Days 6-8 is similar to that of Risperdal Consta® during Days 5-7. There is no statistical difference between release at Day 6 for Risperdal Consta and any DVB condition at Day 7 ($p > 0.05$) which indicates the delay of a single day. This indicates that the risperidone release from PLGA microparticles remains the same. Thus, the extended delayed release from 5 to 6 days may be due to absorption of released risperidone to DVB particles. This absorption hypothesis, however, cannot explain why glass beads, which do not absorb risperidone at all, also showed extended delayed release at least at the 10% concentration. The lack of difference between glass beads and DVB under these conditions indicates that absorption is not a significantly contributing factor in these tests. Thus, mechanical impact by glass beads may also play a role. It is possible that glass beads may collide with PLGA microparticles during the orbital shaking process, and such mechanical collision may disturb the PLGA microparticle surface. As shown in Figure 6a, the impact of glass beads on release kinetics is not consistent. This suggests that risperidone release is not directly affected (e.g., diffusion is altered), but through another variable that may affect risperidone solubility as well as diffusion. This effect will require further study, such as measuring the effect of the presence of glass beads and DVB without agitation or scanning electron microscopy imaging to check for microparticle impacts during the test, to understand the mechanism at play.

Sampling Volume Study

Risperidone microparticles from formulations 2-1 and 2-2 were used to determine the effect of sampling volume during the release test at 37°C in an incubator with orbital agitation. Glass flasks were used as the release test vessels. The release profiles in Figure 7 show that a sampling volume of 1 mL (out of the total 40 mL) lengthens the lag phase significantly when compared to a 30-mL sampling volume. Statistically significant ($p < 0.05$) differences are observed between 1-mL and 30-mL release profiles starting from Day 3 in formulation 2-1 and Day 5 in formulation 2-2.

The observed difference in the lag phase between the release tests could be attributed to 2 possible factors: (1) minimized particle aggregation. The applied agitation (100 rpm) possibly was not sufficient to prevent particle aggregation. The microparticles that underwent release testing with a 30-mL sampling volume undergo additional agitation every time a media sample was removed and replaced with fresh PBST. The release test with 1-mL sampling volume did not expose the microparticles to agitation of the same nature as the release test with 30-mL sampling volume. The aggregated particles with reduced surface area to volume ratio had a lower drug release rate due to slower drug diffusion. (2) Violation of the sink condition. The risperidone release at the steady state is faster with 30-mL media change, and this indicates that the 1-mL media change may have caused a non-sink condition.

Agitation Study

Risperdal Consta® microparticles were used to examine the effect of agitation from the orbital agitator on the release profile. A 30-mL sampling volume was used in both release tests, but one test

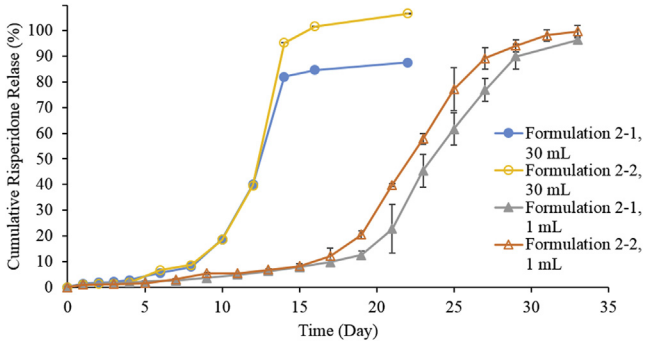

Figure 7. Effect of sampling volume on *in vitro* release profile of risperidone microparticles at 37°C (1 mL, $n = 3$; 30 mL, $n = 2$).

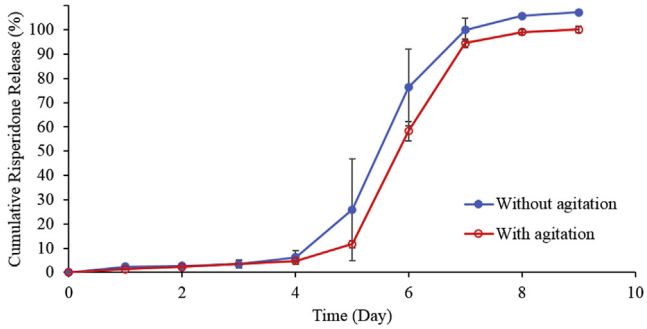

Figure 8. Effect of agitation from incubator on the accelerated *in vitro* release profile of Risperdal Consta® ($n = 3$) at 45°C.

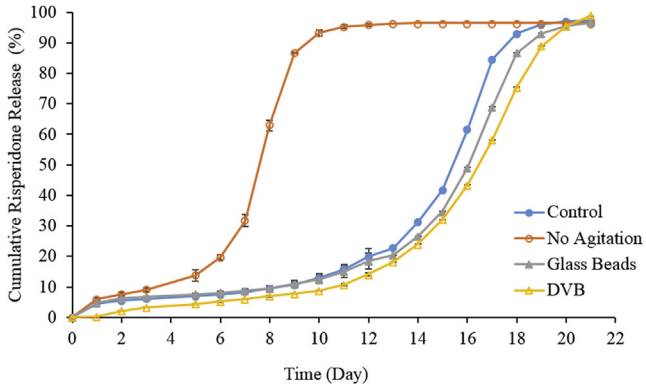

Figure 9. *In vitro* risperidone release profiles from microparticles at 37°C (formulation 1-2; $n = 3$).

was performed in an incubator set at 37°C with orbital agitation at 100 rpm and 1 test was performed in an incubator set at 45°C without any agitation. Figure 8 shows that the release of risperidone is slightly faster without any agitation from the incubator than it is in the orbitally agitating incubator. However, the difference is not statistically significant. This observation contradicts the expectations of increased speed of release with agitation due to improved flow and mixing of release media around microspheres. Similar to the results from Figure 6 for glass beads, this indicates that there must be other factors involved in the system.

PLGA Degradation Study

To further understand the effect of solid beads and agitation, the molecular weight of PLGA was determined by GPC during release tests. The release testing was done at 37°C with risperidone microparticles from formulation 1-2. The sampling volume for all release tests was 30 mL. As seen in Figure 9, the release profile trends were similar to previous tests. The release profile without agitation was faster than the test with orbital agitation. The addition of DVB caused a lower initial release and lengthened the lag phase slightly. The addition of glass beads lengthened the lag phase slightly as well. The impact of the presence of glass beads and DVB beads is the same as that observed at 45°C in Figure 6. The faster release of risperidone in the absence of agitation was puzzling. To understand this, PLGA degradation was examined during the risperidone release study. It was hypothesized that the lack of agitation affected PLGA degradation, which may subsequently affect the risperidone release kinetics.

The changes in PLGA properties are shown in Table 2. The PLGA in the release test without agitation showed the fastest decrease in PLGA molecular weight at Day 5, and at Day 21 the molecular weight was undetectable by GPC. The microparticles that underwent testing with glass beads and DVB showed slightly slower PLGA degradation than the control sample at Day 5; however, the molecular weight was similar to the control sample throughout the remainder of the test. The molecular weight changes in the samples corresponded with the release rate of risperidone in the release tests.

The aforementioned lack of agitation could cause particle aggregation, which not only slows down drug diffusion but also slows down the diffusion of generated acids. The accumulation of acids inside the particle aggregates could increase the internal osmotic pressure and decrease local pH. The increased osmotic pressure may cause particle collapse, which explains the observed faster drug release. In addition, the observed accelerated PLGA degradation indicated that there could be an accumulation of acids. An attempt was made to perform an additional study to examine the solubility of risperidone in acidic conditions. Lactic acid solutions were examined as lactic acid constitutes 75% of the PLGA polymer used in this study (formulations 1 and 2) as well as in Risperdal Consta®. As seen in Table 3, the solubility of risperidone drastically increases as the solution becomes acidic. The risperidone solubility in lactic acid solutions increased as the lactic acid concentration increased to 1.0%. The pH of the 1.0% lactic acid solution is 2.31, and yet the risperidone solubility is higher than at pH 1.68 achieved by HCl. A pH decrease by lactic acid from 2.85 to 2.31 corresponded to a greater than 5 times increase in risperidone solubility.

Discussion

The drug release methods examined in the current manuscript identified several important factors affecting the drug release rates of risperidone from microparticle formulations. The release vessel shape, sampling volume, and agitation rate all impacted the risperidone release to different extents. The release vessel shape is important for testing microparticle formulations, since it affects the tendency of microparticles to aggregate. The simple effect of the dimensions of the container under which the release assay is performed has a pronounced effect on the obtained release rate (Figs. 4 and 5). Typically, the release from wider-bottomed containers is faster as these improve both aqueous access and have reduced aggregation of the particles. The aggregation of the microparticles in the bottom of cone/conical shaped containers has a drastic effect on the release of drug from the particles. This occurs during degradation as the glass transition of the particles decreases below that of the test method and the particles adhere strongly onto one another forming a solid clump which has a relatively low surface area to volume ratio relative to the mass of particles which formed it. This aggregation is important as it drastically increases the diffusional path length of the drug from the particles at the interior of the aggregates relative to what it would have been for the loose particles. This greatly reduces the measured release rate. It was also observed that the sampling volume could play a role in drug release kinetics. The larger volume of sampling resulted in faster release, and this effect may occur through maintaining the sink condition with fresh medium and disturbing the microparticles during sampling. To fully understand the impact of sampling volume on the release kinetics, however, more studies are needed.

Two opposite effects of agitation rate on the release profile have been observed. When the agitation rate is high enough to fully prevent particle aggregation, the drug release rate is higher than

Table 2
Changes in Molecular Weight of PLGA in Risperidone Microparticles Under Various Release Conditions (Formulation 1-2; $n = 1$)

Time (Day)	No Agitation		Control		Glass Beads		DVB	
	Molecular Weight (Da)	PDI	Molecular Weight (Da)	PDI	Molecular Weight (Da)	PDI	Molecular Weight (Da)	PDI
0	84,832	1.66	84,832	1.66	84,832	1.66	84,832	1.66
5	38,553	2.29	42,150	2.84	51,247	1.87	52,873	1.89
12	26,523	2.88	30,034	2.27	26,891	3.16	36,508	1.79
21	Undetectable	–	24,679	4.06	24,391	4.08	32,707	1.79

Table 3
Risperidone Solubility and pH of Various Acidic Solutions ($n = 3$)

Solution	pH	Risperidone Solubility (mg/mL)
0.1% lactic acid	2.85	6.91 ± 0.15
0.5% lactic acid	2.52	19.41 ± 0.22
1.0% lactic acid	2.31	38.16 ± 0.22
0.1% HCl	1.68	12.62 ± 0.23
1.0% HCl	0.80	57.26 ± 0.92
PBST	7.45	0.291 ± 0.001

the rate determined using a lower agitation rate. However, with further decreases in agitation rate or without agitation, the drug release can be accelerated compared with the condition where aggregation is fully prevented. Intuitively, it is expected to see a slowed drug rate when lacking agitation due to particle aggregation. However, it should be noted that when aggregation occurs, not only drug diffusion slows down but also the diffusion of generated acidic degradants.[35-38] When the degradation rate of PLGA is faster than the diffusion rate of the generated acids, they start to accumulate inside the particle. The accumulation of acids could cause reduced local pH. In case of risperidone, it is highly soluble in this low pH solution, resulting in high concentrations of risperidone in the local area of the microparticles leading to increased concentration gradient between the particle and bulk media and thus an increased drug release rate. This observed impact of local pH change on risperidone release rate is more pronounced at 37°C (real-time release; Fig. 9) relative to that observed at 45°C (accelerated release; Fig. 8) as solubility of risperidone is also temperature dependent. Many studies have been conducted to investigate the impact of local pH on PLGA degradation, but little has been done on how local pH affects risperidone solubility. It is reasonable to expect that this effect does not directly translate to formulations which have an active pharmaceutical ingredient that is insensitive toward pH in terms of solubility.

Another interesting observation here is that the presence of solid particles physically blended in with the microparticles has a slight retardation effect on the measured release rate in accelerated testing, regardless of the type of media used, either glass beads or DVB (Figs. 6 and 9). The driving parameter here is the simple addition of the solid media adds mechanical agitation and space between the particles, which reduces the development of acidity. This also is observed in a reduced early stage degradation of the polymer chains (Table 2) for both samples containing DVB or glass beads.

The exact methodology for orbital agitation in the *in vitro* release assay varies widely between various labs and reports,[26-31] but the method is critical. As can be seen from the release study data here, the exact methodology of incubation greatly influences the release rate obtained for various formulations. The methodology details overrule the manufacturing parameters. As shown in Figure 5, testing of Risperdal Consta® in a centrifuge tube nearly doubles the delay period relative to that of testing the same formulation in a glass jar from 5 days to roughly 10 days under accelerated conditions. Similarly, the lack of agitation for a manufactured batch being tested under real-time conditions nearly halves the delay period from about 12 to 6 days (Fig. 9). Given that the methodology parameters for the test can change the apparent delay parameters of a formulation by a factor of 2, these parameters must first be set and harmonized to be applicable for observing manufacturing differences. The fact that experimental parameters affect the *in vitro* drug release kinetics has a few implications. One can adjust the experimental conditions to obtain the same *in vitro* release kinetics. Thus, one *in vitro* release testing alone does not guarantee that the 2 PLGA microparticle formulations were prepared under the same/similar manufacturing conditions. It is recommended that drug release kinetics be tested under a few different experimental conditions. For this reason, it is difficult to determine which experimental condition is better than the others. It becomes even more important to describe in detail the experimental conditions used in the *in vitro* release kinetics to avoid any impact resulting from an experimental parameter itself.

Conclusions

Since equipment for orbital shaking is widely available, cost friendly, easy to set up, and suitable for long-term use, the orbital shaking method is an attractive way for performing *in vitro* drug release testing of LAI products. However, a lack of consensus on the methodology hinders the ability to correlate results from one *in vitro* release study to another due to the differences in container, media, agitation rate, and other parameters from lab to lab. The same event, such as aggregation, could have totally opposite effects on the drug release kinetics. In addition, the effects of these methodology differences are so pronounced that they overwhelm the release differences due to manufacturing parameters. When establishing comparability of *in vitro* drug release profiles for equivalence evaluation, methodology parameters must be validated and harmonized for easy comparison of the results. Optimally, these parameters must be established before using the *in vitro* release data for establishing an appropriate *in vitro-in vivo* correlation.

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