Microencapsulation of luteinizing hormone-releasing hormone agonist in poly (lactic-co-glycolic acid) microspheres by spray-drying

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ABSTRACT

A spray drying technique was developed to prepare injectable and biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres encapsulating a model luteinizing hormone-releasing hormone agonist (LHRHa)-based peptide, leuprolide. Various spray drying parameters were evaluated to prepare 1-month controlled release formulations with a similar composition to the commercial Lupron Depot\textsuperscript{®} (LD). A single water-in-oil emulsion of aqueous leuprolide/gelatin solution in PLGA 75/25 acid capped (13 kDa Mw) dissolved in methylene chloride (DCM) was spray-dried before washing the microspheres in cold ddH\textsubscript{2}O and freeze-drying. The spray-drying microencapsulation was characterized by: particle size/distribution (span), morphology, drug/gelatin loading, encapsulation efficiency, and residual DCM and water content. Long-term release was tested over 9 weeks in PBS + 0.02% Tween 80 + 0.02% sodium azide pH 7.4 (PBST) at 37°C. Several physical-chemical parameters were monitored simultaneously for selected formulations, including: water uptake, mass loss, dry and hydrated glass transition temperature, to help understand the related long-term release profiles and explore the underlying controlled-release mechanisms. Compared with the commercial LD microspheres, some of the in-house spray-dried microspheres presented highly similar or even improved long-term release profiles, providing viable long-acting release (LAR) alternatives to the LD. The in vitro release mechanism of the peptide was shown to be controlled either by kinetics of polymer mass loss or by a second process, hypothesized to involve peptide desorption from the polymer. These data indicate spray drying can be optimized to prepare commercially relevant PLGA microsphere formulations for delivery of peptides, including the LHRHa, leuprolide.

1. Introduction

Long-acting-release products (LARs) are an important strategy to microencapsulate small and large biomacromolecules (e.g., peptides, proteins and DNA/RNA) for long-term controlled release. A number of LARs have resulted in successful pharmaceutical products including Lupron Depot\textsuperscript{®} (LD), Zoladex\textsuperscript{®}, Trenantone\textsuperscript{®}, Eligard\textsuperscript{®}, Decapetyl\textsuperscript{®} and Profact\textsuperscript{®} [1]. Biodegradable poly(lactic-co-glycolic acids) (PLGAs) are the most commonly used biocompatible and biodegradable polymers [2]. It has been well accepted that long term release kinetics of microspheres is generally dominated by three mechanisms as follows [3]: (i) diffusion-controlled release through the polymer matrix or through water-filled pores; (ii) hydration-controlled release through swelling of polymers, new pore formation and osmotic pumping; (iii) erosion-controlled release by generating new pores in the polymer matrix after polymer degradation and mass loss. We have proposed additionally the role of polymer pore healing [4] and peptide desorption [5] as important for affecting controlled release from PLGA. These release mechanisms may separately or jointly govern the drug release in different release stages.

The pulsatile release of luteinizing hormone-releasing hormone (LHRH) from the hypothalamus plays a critical role in the normal function of the hypothalamic-pituitary-ovarian axis [6]. In women and men, desensitization of pituitary gonadotropin and silence of gonadal

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function can be pharmacologically achieved by continuous LHRH treatment and long-term LHRH agonist (LHRHa) administration [7]. In the past thirty years, various LHRHAs have been applied in the therapy of prostate cancer, endometriosis, precocious puberty and uterine fibroids via parenteral administration [8]. LHRHAs such as leuprolide or goserelin have short half-lives due to peptidase degradation, and are commonly formulated as implants and administered parenterally. Leuprolide and goserelin have been formulated into FDA-approved long-acting-release (LAR) injectable depot products (e.g., LD, Eligard® and Zoladex®) using microspheres and implant platforms [9]. Despite success in the delivery of LHRHAs, some challenges remain. LHRHa commercial microsphere products (e.g., LD) are typically made by conventional emulsion/solvent evaporation methods, often resulting in a substantial initial burst release [10]. Generic entry into the LAR market has also been a challenge [11]. Therefore, it would be beneficial to explore alternative manufacturing approaches in the development of LHRHa-loaded biodegradable long-acting injectable controlled release microspheres.

Spray drying is attractive as an energy efficient and scalable drying and formulation strategy for pharmaceutical processing compared to other manufacturing methods [12]. Spray drying is a controllable continuous process and can convert various solutions into dried micron- and even nano-sized particles in a relatively short time by forming an atomized spray in a high-temperature gaseous environment [13]. The spray drying technique has been frequently applied in the field of food and chemistry and quickly expanded to other fields such as fabrics, cosmetics, and electronics. In the pharmaceutical field, spray drying is often employed to formulate small molecule drugs. However, there is an increasing trend towards processing of biomacromolecules and biopharmaceuticals into various carriers such as microspheres, microcapsules and nanoparticles [14]. Spray drying is suitable for different feedstocks including emulsions, slurries, melts and pastes, and is applicable to heat-insensitive and heat-sensitive compounds. However, despite these merits, the heat and mass transfer and fluid dynamics are complicated in terms of their temporal and spatial diversity [15], which has an important impact on the final formulation properties. The spray drying process includes typical steps such as atomization, drying and separation [14,16]. First, the feedstock solutions are prepared and pumped into the atomizer chamber of an atomizing nozzle. Then, droplets are generated from the nozzle tip and are atomized in the drying chamber in the presence of the hot drying gas. Second, atomized wet particles are dried through solvent evaporation by a hot gas stream, where process settings and the device dimensions may influence the residence time and drying efficiency. Finally, the dried products are achieved by separating them from the drying gas flow using a cyclone principle and are collected in the product receiver device [15]. Spray-drying process parameters may affect the heat/mass transfer and other thermodynamic behaviors, and lead to different physicochemical properties of particles produced. These processing parameters include feed solution properties, inlet air temperature, drying gas flow rate, pump rate and atomization from the nozzle (airflow or size). These processing variables have had a critical impact on yield, particle size and distribution, morphology, drug loading and encapsulation efficiency, residual solvent and moisture content in previous studies involving amorphous solid dispersions [13,17,18] and are therefore expected to impact the glass transition (Tg), long-term release profiles and mechanisms of engineered microparticles especially when peptides (e.g. LHRHAs) are loaded in LAR microspheres. Current understanding of these aspects is insufficient and a deeper knowledge of their relationships is needed.

The current study provides a systematic presentation and basic understanding on the characterization of PLGA microspheres loaded with LHRHAs-based peptides when employing spray-drying technology. This study should be useful to aid in the development of new and generic microsphere formulations. Leuprolide is a synthetic nine amino acid sequence (p-Glu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt) hormone that is similar to the natural hormone LHRH in the brain, and was selected as a representative of LHRHAs. Leuprolide is approximately 40-fold higher in potency, is much less polar and can desensitize pituitary gonadotropin receptors at a far lower dose than the natural LHRHAs [19]. The 1-month LD, is a commercial PLGA microsphere product for the encapsulation and sustained release of leuprolide acetate [20]. After its FDA-approval in 1989, the LD has been a benchmark in the study of LAR biodegradable microsphere formulations. For development of generic injectable PLGA LAR formulations, the proposed products are required to be qualitatively (Q1) and quantitatively (Q2) the same as the reference-listed drug products for approval in an Abbreviated New Drug Application (ANDA) according to the 505 (j) pathway [21]. From previous work in our lab on reverse-engineering the 1-month LD [21], PLGA 75/25 (Mw ~13 kDa), type-B gelatin and leuprolide acetate were established as main components to match the composition of the 7.5 mg LD for 1-month administration. A ProCepT 4 MB-TriX spray dryer with a two-fluid nozzle and co-current airflow pattern was adopted. Nozzle, atomizing and feed thermodynamics as well as process settings of spray drying were considered in microparticle formation engineering including inlet air temperature (Ti), polymer (i.e., PLGA) concentration (Ci), airflow in nozzle (An), Ratio (R) between inlet atomization air flow (Af) and the liquid mass flow rate (LF), nozzle size (Sn) and initial sample (feedstock) temperature (Tf) at different levels. Microparticle formation was evaluated by characterizing physicochemical properties including yield, particle size (D10, D50, D90) and size distribution (span), drug loading and encapsulation efficiency, gelatin loading and encapsulation efficiency, morphology of particles, residual methylene chloride content, residual moisture content, dry Tg and hydrated Tg.

Long-term release profiles and mechanisms were evaluated based on kinetics of long-term cumulative release, initial burst release, water uptake, mass loss, the change/loss in molecular weight, and change in dry Tg and hydrated Tg.

2. Materials and methods

2.1. Materials

The 7.5 mg leuprolide dose for 1-month administration Lupron Depot® (AbbVie Inc., North Chicago, IL, USA) was purchased from the pharmacy at the University of Michigan Health System. Type B gelatin derived from bovine bone with bloom number 250 was supplied from Nitta Gelatin Inc. (Osaka, Japan). Wako PLGA 7515 polymer (13 kDa, acid terminated, LA/GA:75/25, type-B) with a 0.6–1.2 mm bifluid nozzle (ProCepT, Zelzate, Belgium) was purchased from Waters (Waters Corporation, Milford, MA, USA). All solvents used were HPLC grade and were purchased from Fisher Scientific.

2.2. Preparation of microspheres by spray-drying

LD-like microparticles (Table 1) were prepared using a spray-drying method. The water phase consisted of a suitable amount of leuprolide and gelatin in 1 mL of water and was maintained at 60 °C for 5 min. Drug and gelatin dissolved completely. Poly(lactic-co-glycolic) acid (PLGA) 75/25 was dissolved in methylene chloride (DCM) at a final concentration of 50–300 mg/mL. The overall batch size was 6 mL. The water phase and the oil phase were mixed by vortexing for 20 s and then emulsified for 2 min using a homogenizer at a speed of 15,000 rpm (Virtis Tempset I.Q.® Homogenizer, Sentry Microprocessor, Kent City, Michigan) to form a W/O emulsion. The obtained W/O emulsion was cooled in an ice-bath for 1 min to decrease the evaporation of methylene chloride in the emulsion. As shown in Fig. 1, the W/O emulsion was subjected to spray drying by a ProCepT 4 MB-TriX spray dryer (ProCepT, Zelzate, Belgium) with a 0.6–1.2 mm bifluid nozzle (Sn)
and a 7–16L/min airflow in the nozzle (AF<sub>nozzle</sub>). Cyclone airflow (AF<sub>cyclone</sub>) was open at a value of 0.12m<sup>3</sup>/min and cooling airflow (AF<sub>cooling</sub>) was shut off. The emulsion was fed (LF<sub>feed</sub>) at a rate of 50–200mL/min and atomized at an inlet airflow of 0.4–0.7m<sup>3</sup>/min (AF<sub>inlet</sub> at 50–80°C (T<sub>inlet</sub>). The spray-dried powder was collected by the device product receiver. The spray-dried crude powder was rinsed with 50mL of cold ddH<sub>2</sub>O at 4°C and centrifuged at a speed of 4000 rpm, and the procedure was performed three times to wash off the unencapsulated drug from the microsphere surface. The microspheres were freeze-dried under reduced pressure for at least 48h. Blank microsphere formulations were prepared using the Basic Formulation (Table 1) but without leuprolide acetate.

### 2.3. Determination of yield and particle size/distribution

The yield of microspheres was recorded by the ratio of the weight of samples received in the product receiver and the initial sample weight. Microspheres were dispersed in ddH<sub>2</sub>O and particle size distribution was analyzed three times using a laser diffraction (Master Sizer 2000, Malvern Instruments Ltd, Malvern, UK). The D<sub>10</sub>, D<sub>50</sub>, D<sub>90</sub> and span of microspheres were recorded to determine the particle sizes and distribution. The span of the microsphere size distribution can reflect the dispersity of particles and was calculated as follows:

\[
\text{Span} = \frac{D_{90} - D_{10}}{D_{50}}
\]  

(1)

where D<sub>90</sub>, D<sub>50</sub> and D<sub>10</sub> are the microsphere diameters measured at the 90th, 50th and 10th percentiles of undersized microparticles, respectively. Sizes are expressed by volume-average values.

### 2.4. Determination of leuprolide loading and encapsulation efficiency

Leuprolide loading was determined by two-phase extraction followed by ultra performance liquid chromatography (UPLC) analysis. Briefly, 5mg of leuprolide-loaded PLGA microspheres were dissolved with 1mL of methylene chloride by vortexing for 5min. Then, 2mL of 0.03mM phosphate buffer pH 6.0 was added and vortexed for 5min to guarantee sufficient extraction. After centrifugation at 4000rpm for 5min, 1mL of the aqueous phase was collected. The amount of leuprolide in aqueous phase was determined using UPLC (Acquity, Waters, Milford, MA, USA) composed of a BEH C18 column (Waters, Milford, MA, USA) and a UV detector with an absorption wavelength of 280nm and an injection volume of 2μL. A gradient of acetonitrile with 0.1% (v/v) trifluoroacetic acid (TFA) (solvent A) and ddH<sub>2</sub>O (solvent B) at a flow rate of 0.5mL/min was performed as follows: 0min (25:75, A:B), 2min (35:65, A:B) and 2.5min (25:75, A:B) for a 1min recovery to initial conditions. A new standard curve was established during each UPLC run for determination of drug loading. Based on control recovery experiments we have verified that efficiency of extraction is complete. Drug encapsulation efficiency was calculated by the determined drug loading divided by the theoretical loading.

### 2.5. Determination of gelatin loading and encapsulation efficiency

Amino acid analysis was used to determine gelatin content in the spray-dried formulations. The amino acid alanine was used to determine the gelatin content and norleucine was added to the samples during the derivatization as the internal standard. About 15mg of microspheres were weighed into hydrolysis tubes and 1.0mL of 6N HCl constant boiling solution (Fisher Chemical, Fair Lawn, NJ, USA) was added. The headspace gas of the tubes was reduced under vacuum to prevent breakage of the vessel during heating for hydrolysis. After incubation, the solution was frozen with liquid nitrogen and lyophilized under vacuum at room temperature. Then, 400–800μL of 20mM HCl was added into each tube to reconstitute the samples. Standards were prepared by dilution of hydrolyzed gelatin samples. Derivatization
analysis were performed using Waters AccQ∙Tag Chemistry kit. Briefly, hydrolyzed amino acids were derivatized using the borate buffer (<5% sodium tetraborate in water) with the Waters AccQ∙Fluor reagent (6-aminooquinolyl-N-hydroxysuccinimidyl carbamate). The derivatized samples were separated by reverse phase UPLC using a C18 column (AccQ∙Tag Ultra C18, 1.7 μm (Millipore Corporation, Milford, MA)) and a gradient elution of 5% solution of Waters AccQ∙Tag Eluent A concentrate (19 wt% sodium acetate, 6–7 wt% phosphoric acid and 1–2% wt% triethylamine) in water (solvent A) and 2% formic acid in acetonitrile solution (solvent B) at a flow rate of 0.5 mL/min as follows: 0 min (99.9% A), 1 min (98.5% A), 11.5 min (78% A), 13.5 min (40% A) and 15 min (99.9% A), followed by a 2 min recovery with initial conditions. The urea derivatives yielded during the derivatization were detected at 250/395 nm excitation/emission wavelengths by fluorescence detection. The ratios of peak area of the alanine and the internal standard, norleucine, were plotted against the concentrations of gelatin (mg/mL) to obtain the standard curve. The concentration of gelatin in the samples was determined using their ratio of peak area of alanine to the internal standard, norleucine, and finally, the % gelatin in the sample based on the originally weighed out mass of the sample. Gelatin encapsulation efficiency was calculated by the determined gelatin loading divided by the theoretical loading.

2.6. Scanning electron microscopy

The surface morphology of microspheres was observed via a Hitachi S3200 N scanning electron microscope (SEM) (Hitachi, Tokyo, Japan). The various microspheres including spray-dried microspheres and commercial LD were fixed on a brass stub using double-sided carbon adhesive tape and the samples were generated electrically conductive by coating with a thin layer of gold for 120 s at 40 W under vacuum. Commercial LD microspheres were rinsed with 5 mL of cold water at 4 °C and centrifuged at a speed of 4000 rpm, and the procedure was performed three times to remove lyoprotectants. Then, microspheres were freeze-dried under reduced pressure for at least 48 h. LD microspheres with or without washing were used as controls.

2.7. Determination of residual solvent

Residual solvent (methylene chloride) in the spray-dried formulations and in LD was determined by gas chromatography using a ThermoSci Trace 1310 gas chromatograph. Approximately 10 mg of microspheres and 1 mL of DMSO were weighed into a vial and sealed. Each sample was run in triplicate using headspace injection with the following GC conditions: nitrogen gas was used as the carrier solvent at a flow of 25 mL/min; air flow was 350 mL/min and hydrogen flow was 35 mL/min; the front detector temperature was 240 °C and the front
inlet pressure was a constant flow at 2 mL/min. Each sample was agitated for 20 min at 80 °C and 1 mL of the headspace sample was injected into the front inlet with the temperature of 140 °C, split flow of 40.0 mL/min, and a split ratio of 20. The GC column temperature was initially set at 40 °C for 15 min, then increased at 10 °C/min to 240 °C and held at 240 °C for 2 min. A standard curve was prepared by adding methylene chloride to DMSO at 1, 10, 25, 50, 75 and 100 ppm.

2.8. Determination of residual moisture

Residual water content in spray-dried microsphere formulations and in the LD were determined by Karl Fischer (KF) titration (Metler Toledo, C20 Coulometric Titrator, Columbus, OH). Approximately 10 mg of microspheres and 1 mL of anhydrous, septum-sealed, DMSO (Fisher Scientific, AS0811, Hampton, NH) were weighed into a vial and sealed with a septum cap. The samples (n = 3) were injected into the KF for titration and blank DMSO (n = 3) injections were subtracted from the test samples to account for contribution of solvent moisture. All septum-sealed DMSO and sample vials were kept under anhydrous conditions by purging with nitrogen gas while removing liquid.

2.9. Long-term release

Ten mg of leuprolide-loaded spray-dried microspheres or 11.8 mg of LD microspheres (~1 mg leuprolide equivalence) were dispersed in 1 mL 10 mM phosphate-buffered saline with 0.02% tween 80 (PBST) (containing 0.02% sodium azide) at pH 7.4. Then, the microspheres were incubated at 37 °C with agitation at 240 rpm (KS 130 basic, IKA Works Inc., Wilmington, NC, USA) and after centrifugation at 8000 rpm for 5 min, the supernatant (~0.8 mL) were collected and replaced with fresh media at days 1, 3, 7 and every 7 days up to day 63. The amount of leuprolide released was determined by UPLC as described in Section 2.4.

2.10. Determination of Molecular weight (Mw) decline

Each formulation was incubated in the same manner used for conducting the long-term release study including replacement of media. After collection from the release media, microspheres were washed with ddH2O and dried at room temperature under reduced pressure. Tetracyhdrofurann (THF) was added to dissolve the microspheres to obtain approximately 10 mg/mL polymer concentration and then supernatants were subjected to gel permeation chromatography (GPC) using two styragel columns (HR 1 and HR 0.5 columns, Waters, Milford, MA, USA) with Waters 1525 HPLC system. Samples were eluted with THF at 1.0 mL/min and monitored by refractive index detection. Breeze software was used to obtain weight-averaged molecular weight (Mw). The Mw was calculated using monodisperse polystyrene standards ranging from 2 k to 30 kDa.

2.11. Quantification of microsphere water uptake and mass loss

Microspheres were incubated as in the release kinetics evaluation and collected on pre-weighed nylon membrane filters under vacuum and washed off with ddH2O. Then, the surface water was removed by further filtration under vacuum for 5 s and the wet weight of the microspheres was immediately measured. The samples were dried at room temperature under reduced pressure and then the dry weight was recorded. To correct for the interparticle water, dry microspheres were dispersed in each buffer solution at 4 °C and the wet and dry weights were measured after immediate collection at 20 °C. The water uptake of microspheres at time (Wtu) was thus assessed by:

\[ W_{tu} = (W_{wet}^t - W_{dry}^t - W_{dry}^t) / W_{dry}^t \]

where \( W_{wet}^t \) and \( W_{dry}^t \) are the weights of wet microspheres and dry microspheres, respectively, after immediate collection at \( t = 0 \). The water uptake of microspheres at time \( t \) was calculated according to:

\[ W_{tu} = (W_{wet}^t - W_{dry}^t) / W_{dry}^t \]

where \( W_{wet}^t \) and \( W_{dry}^t \) are the wet and dry microsphere weights after incubation at 37 °C in the release media at time \( t \). The percent mass loss was calculated according to:

\[ \text{Mass loss} = \left( \frac{W_0 - W_{dry}}{W_0} \right) \times 100 \]

where \( W_0 \) is the initial weight of dry microspheres.

2.12. Determination of dry glass transition temperature (dry Tg)

The Tg of microspheres was determined with a modulated differential scanning calorimeter (mDSC) (Discovery, TA instruments, New Castle, DE). Both fresh microspheres and microspheres collected at various time points during the long-term release testing were used as samples. Approximately 0.5–3 mg of microspheres were sealed in DSC aluminum pans and lids. Briefly, temperatures were ramped between –20 °C and 90 °C at 3 °C/min. All samples were subjected to a heat/cool/heating cycle. The results were analyzed by TA TRIXOS software.

2.13. Determination of hydrated glass transition temperature (hydrated Tg)

Approx. 0.5–1 mg of microspheres were placed into DSC aluminum pans and 20 μL ddH2O was added, and sealed with aluminum hermetic lids. Both fresh microspheres and microspheres collected at various time points during the long-term release testing were used as samples. The hydrated samples were incubated for 24 h at room temperature. The hydrated Tg was measured with a modulated differential scanning calorimeter (mDSC) (Discovery, TA instruments, New Castle, DE). Briefly, temperatures were ramped between 5 °C and 75 °C at 3 °C/min. All samples were subjected to a heat/cool/heating cycle. The results were analyzed by TA TRIXOS software.

2.14. Statistical analysis

Statistical analysis was performed using Excel software. Data were expressed as the mean ± standard deviation (SD) and statistical analysis was performed using an unpaired Student's t-test. Data were considered significantly distinct from controls at \( p < .05 \). Moreover, \( p < .01 \) or \( p < .001 \) was also given to reflect the degree of significance. Excel was used to analyze and obtain time for 50% release (t50, release), 50% mass loss (t50, erosion) and 50% Mw loss (t50, degradation).

3. Results and discussion

3.1. Process, parameters and yield in spray drying

There are several types of atomization methods and mass fluid patterns in spray drying. The common atomization devices include rotary atomizers, hydraulic (pressure) nozzles, pneumatic nozzles and ultrasonic nozzles [16,22]. Mass fluid patterns involve co-current airflow, counter-current airflow and mixed (combined) fluid according to the direction of droplet and drying gas from the top of the chamber to the bottom [23]. Gas types are also important for atomization and drying processes and can impact droplet sizes, mass fluid density, velocity and separation process [16]. Here, a ProCePT Matrix 4 MB-Trix spray dryer was used. The spray dryer is equipped with a pneumatic nozzle with a two-fluid (liquid fluid and gas liquid) channel and a co-current mass fluid pattern in the atomizing chamber. Air was selected as the drying gas fluid because of its practicality. As shown in Fig. 1, there are five units for this spray dryer, including the atomizing device, cyclone apparatus, product receiver, control panel and pump...
system. The control panel can control the inlet air flow speed ($AF_{inlet}$), inlet/outlet air temperature ($T_{inlet}$ or $T_{outlet}$), cooling air flow ($AF_{cool}$) and cyclone air flow ($AF_{cyclone}$). The pump speed (i.e. liquid mass flow rate, $LF_{feed}$) and airflow in nozzle ($AF_{nozzle}$) can be regulated by the pump system. Before spray drying, the primary emulsion was prepared as the feedstock by homogenizing the mixture of PLGA/DCM organic solution and leuprolide/gelatin solution. 7.5 mg once-monthly LD was adopted as a commercial product control. The current spray-drying process consisted of the following fundamental stages: (i) preparation of the feedstock by homogenizing the mixture of PLGA/DCM organic solution and leuprolide/gelatin solution; (ii) transport of the feedstock from the initial position to the atomizing chamber through the pump system and atomization of the liquid feed by the atomizing nozzle; (iii) drying of spray in hot air flow and formation of dry microparticles; and (iv) separation from the circulating drying air and cyclone device, and collection of dry particles in the product receiver.

As indicated in Fig. 2, the feed, atomization, drying and product recovery stage with various critical process parameters were chosen for consideration: (A) the $T_{inlet}$ was varied from 80°C to 50°C at atomization and drying stages; (B) PLGA concentration ($C_{feed}$) was from 5% to 30% at feed stage; (C) $AF_{nozzle}$ was from 7.0 to 16.0 L/min at the atomization stage; (D) the Ratio ($Ratio_{A/L}$, also termed ALR) between atomization gas flow rate ($AF_{inlet}$) and the liquid mass flow rate ($LF_{feed}$) was from 2.0 to 10.0 at feed, atomization and separation stages; (E) $S_{nozzle}$ was from 0.6 mm to 1.2 mm for nozzle size at atomization stage; (F) initial sample temperature ($T_{feed}$) was from 4°C to 37°C at feed stage. Since high initial burst release (IBR) commonly exists in commercial and conventional microsphere formulations and can be affected by loosely bound drug on the surface [3], cold water-washing and freeze-drying was used as additional post-treatment procedures to remove surface-bound peptide and minimize the IBR effect. The drug loading and gelatin loading in LD is 10% and 1.7%, respectively. From these standard values, the basic formulation was established as the standard formulation for adjusting the composition of other spray-dried formulations. Results of pilot studies indicated that: (i) Compared with $T_{inlet}$ outlet temperature ($T_{outlet}$) has relatively lower impact on the formation of droplets, thus $T_{outlet}$ was set as a fixed value (60°C); and
(ii) Instrument equilibrium time ($T_{\text{eq}}$) requires at least 30 min before initiating the spray drying process after setting parameters. Detailed compositions of designed formulations and representative process parameters are summarized in Table 1.

It should be noted that yield strongly relies on the scale of work when employing a conventional spray dryer. In lab scale production, the yield is far below optimal, and is in the range of 20–70% [24]. Yield can be considered as the external result of internal heat/mass transfer process in spray drying. Generally, there are two main factors resulting in low yields. First, the design of the cyclone separation device cannot capture particles with diameters <2 μm [23,25], and these fine particles usually pass through with the exhaust air. Second, unsuitable process variables can cause particles to deposit and adhere on the wall of the spray dryer. For the spray dryer we used, cooling airflow is unfavorable to separate particles and can induce particle adhesion on the nearby surface and result in a lower yield, so cooling airflow was turned off (i.e., $AF_{\text{cyclone}} = 0$). $AF_{\text{cyclone}}$ is helpful to separate particles, but there was no obvious effect on yield when the parameter was varied, so $AF_{\text{cyclone}}$ was set at a constant value, ~0.12 m$^3$/min. In addition, after each use, it is crucial to wash the spray dryer using organic solvent (e.g., methylene chloride) to avoid the use of soap that can induce static electricity and result in remarkable particle absorption on the wall of the chamber and cyclone, and decrease yield significantly. Yield (%) was defined here in the normal way as the % weight fraction of the amount of materials originally contained in feedstock that could be collected from the product receiver and particles on the inside wall of the cyclone were not calculated as a part of the yield.

As indicated in Fig. 3, particle yields were in the range 30–60%. It was determined that a higher $T_{\text{inlet}}$ was favorable to improve yield and the lowest $T_{\text{inlet}}$ at 50 °C led to the lowest yield (~43.5%). The temperature in the atomizing chamber could also influence atomization and drying efficiency of generated droplet/particles. Higher $T_{\text{inlet}}$ can accelerate the formation of droplets and solidifying of particles in the chamber and subsequent drying/transfer from the chamber. Moreover, higher $T_{\text{inlet}}$ can reduce droplet/particle deposition on the wall of the atomizing chamber because it can shorten the formation time (~milliseconds [12]) of the core-shell structure of particles when atomizing droplets to convert into particles. The feed concentration, $C_{\text{feed}}$, has a remarkable impact on the viscosity of the feedstock which is critical for the generated atomizing state and the appearance of droplet/particles. For example, the highest $C_{\text{feed}}$ (30%) caused a serious decline in the yield (~33.4%), whereas a $C_{\text{feed}}$ of 5%~20% maintained yields of 48%~60%. Also, lower yield (<40%) was observed with a lower nozzle air flow, $AF_{\text{nozzle}}$ (7.0 L/min), or higher, $AF_{\text{nozzle}}$ (16.0 L/min). Too high of an $AF_{\text{nozzle}}$ enhanced the loss of some liquid droplets onto the wall of the chamber. Too low of an $AF_{\text{nozzle}}$ tended to generate insufficient forces of liquid atomization and makes it difficult to achieve a large amount of micron size particles. When $AF_{\text{inlet}}$ was at the lowest level (i.e., $Ratio_{A/L}$ 2.0), collection of particles was noticeably reduced (~37.5% yield) because of ineffective separation ability. Higher $Ratio_{A/L}$ (3.0, 4.0, 6.0 and 8.0) caused an obvious increase of yield (>50%), with a slight decrease in yield (~47.7%) with the highest $Ratio_{A/L}$ of 10.0. The nozzle size, $S_{\text{nozzle}}$, at 1.2 mm and 0.6 mm resulted in slightly lower yields (~45%~48%), while 1.0 mm and 0.8 mm of $S_{\text{nozzle}}$ enhanced yields beyond 50%. However, despite the minor influence of $S_{\text{nozzle}}$ on yield, $S_{\text{nozzle}}$ should be considered an important factor because it can directly affect the formation routes of atomizing and impact shapes or morphology of droplets/particles. $T_{\text{feed}}$ is usually ignored in previous published studies [12,13,16], despite it being a crucial variable affecting feed properties, and impacting the atomization process and droplet geometry in particle formation engineering. $T_{\text{feed}}$ at 4 °C had the highest yield (~52.6%), suggesting that lower $T_{\text{feed}}$ is beneficial to minimize evaporation of the feedstock and maintain a lower viscosity and optimal mobility for subsequent atomization.

### 3.2. Microsphere size distribution and morphology analysis

Variables of atomization, feed and nozzle thermodynamics were adjusted to identify their effect on particle sizes and distributions. The commercial LD was used as a control for particle size analysis. For air thermodynamics, $T_{\text{inlet}}$ and $Ratio_{A/L}$ were selected as two important variables. Fig. 4 depicts the particle size of the microspheres. As shown in the figure, altering $T_{\text{inlet}}$ from 80 °C to 50 °C seemed to have less of an effect on the change of sizes, $D_{10}, D_{90}$; spans were similar under
different $T_{\text{inlet}}$, with the exception that the size of smaller particles ($D_{10}$) significantly increased under lower $T_{\text{inlet}}$ ($p < .01$ or $p < .001$). Decreased $T_{\text{inlet}}$ led to the size increase of small-scale particles. However, for low $\text{Ratio}_{A/L}$ at 2.0, 3.0 and 4.0, particle sizes were significantly lower ($p < .001$ or $p < .05$). Increasing $\text{Ratio}_{A/L}$ led to the occurrence of large particles. Higher $\text{Ratio}_{A/L}$ represents a faster airflow during atomization and too high of an inlet airflow (Ratio$_{A/L}$ 10.0) caused insufficient drying and even agglomeration, adhesions and caking of powders. Feed thermodynamics were investigated in terms of two crucial parameters, feed concentration ($C_{\text{feed}}$) and feed temperature ($T_{\text{feed}}$). The highest level of $C_{\text{feed}}$ (PLGA 30%) caused a significant increase in $D_{10}$ ($p < .001$), $D_{50}$ ($p < .001$), $D_{90}$ ($p < .01$) and span ($p < .05$), and resulted in the generation of larger particles, probably owing to the higher polymer solution viscosity. Nozzle properties such as the airflow in nozzle and nozzle size are other critical influencing factors for particle sizes. At the lowest $A_{\text{nozzle}}$ (7.0 L/min), particle sizes of $D_{10}$, $D_{50}$ and $D_{90}$ were the largest. Particle size was gradually reduced with increasing $A_{\text{nozzle}}$. The airflow of the nozzle can influence the atomization process and high $A_{\text{nozzle}}$ may enhance the atomization effect and form smaller droplets through a faster breakthrough from liquid sheet into a droplet shape during the atomizing process. $\text{Ratio}_{A/L}$ 10.0 (i.e. high inlet airflow) induced higher span and dispersity, probably due to too high flowability of particles and insufficient drying and separation. Larger particle sizes and span were significantly found in nozzle sizes of 1.0 mm and 1.2 mm relative to those of 0.6 and 0.8 mm ($p < .001$, $p < .01$ or $p < .05$). $T_{\text{feed}}$ had a minor impact on $D_{10}$, $D_{50}$, $D_{90}$ and span. Different $T_{\text{feed}}$ resulted in similar values of particle sizes and span. Generally, $C_{\text{feed}}$, $A_{\text{nozzle}}$, $\text{Ratio}_{A/L}$, $S_{\text{nozzle}}$ had significant influence on particle sizes and dispersity.

The morphology of microspheres is shown in Fig. 5. Almost all microspheres prepared by spray drying were more spherical than the commercial product, except the PLGA 30% Formulation (Fig. 5E). Higher $C_{\text{feed}}$ (PLGA 30%) likely made it difficult to form droplets during atomization. Higher $C_{\text{feed}}$ could result in over-strengthened surface enrichment because of a high evaporation rate/low diffusion rate, probably leading to the appearance of irregular particles. The surface of spray-dried microspheres generally was slightly rough and wrinkled, and had some irregular pores. The appearance of LD particles is also...
spherical when removing lyoprotectants and there are some pores on the surface (Fig. 5R and S).

### 3.3. Loading and encapsulation efficiency of drug and gelatin

During the evolution of droplet/particles, the component migration and radial distribution occurs, accompanied with solvent evaporation, usually resulting in homogenous and heterogeneous re-orientation of components on the surface, in the formation of the internal matrix or the core of microspheres [13,26,27]. It is possible that there are differences in performance (e.g., loading, residuals and release behavior) derived from microscopic heterogeneity (uneven component distribution) despite similar macroscopic appearance (e.g., particle size). During the drying of droplets (Fig. S2), there is a known tendency for solvents to move towards the surface during phase separation and solvent evaporation from the surface [28,29]. Drug loading is an external outcome of internal drug re-construction or re-arrangement.

Fig. 6 describes the drug loading (Fig. 6A), drug encapsulation efficiency (EE) (Fig. 6B), gelatin loading (Fig. 6C) and gelatin EE (Fig. 6D) of various microspheres. Commercial LD with 10% drug loading and 1.7% gelatin loading was used as a standard control.

As shown in Fig. 6A and B, a decrease in T\textsubscript{inlet} slightly decreased drug loading (~9–10%). However, the effect of T\textsubscript{inlet} on drug loading is minor. Notably, C\textsubscript{feed}, AF\textsubscript{nozzle}, Ratio\textsubscript{A/L}, S\textsubscript{nozzle} had a remarkable impact on drug loading and EE relative to other two parameters. C\textsubscript{feed} had a pronounced impact on the drug loading and EE (p < .001), the higher C\textsubscript{feed}, the higher the drug loading/drug EE. The lowest C\textsubscript{feed} (10% PLGA or 5% PLGA) caused reduced drug loading (~7% or ~5%, respectively, p < .001) and drug EE (~58% or ~40%, respectively, p < .001). These results suggest that significant levels of drug distribute on the particle surface leading to reduced drug content after washing. A lower concentration of feed solution would take longer for the drug to reach the solidification point during the particle formation process [29]. The slowed deposition caused by a low C\textsubscript{feed} might decelerate the translocation of drug towards the inner space of the droplets and cause the relative increase in drug on the surface. A low C\textsubscript{feed} would be expected to decrease the evaporation rate and inversely increase the evaporation or drying time. The decrease in C\textsubscript{feed} was unfavorable and might lead to smaller size and over-enrichment of the drug at droplet surface due to divergent phase behavior. Another influencing parameter is the nozzle airflow (AF\textsubscript{nozzle}). The highest drug loading (~11.4%) and drug EE (~88.6%) were found in the formulation with the lowest AF\textsubscript{nozzle} (7.0 L/min) (p < .01). The lower the nozzle airflow, the larger the droplet/particle size, due to the enhanced feed flow rate (LF\textsubscript{feed}). Under these conditions, larger droplets provide a lower surface area/mass ratio and therefore less surface drug is expected to be present and susceptible to washing off. Microspheres with the highest Ratio (Ratio\textsubscript{A/L} 10.0) caused a significant decrease (p < .05) in drug loading (~8.8%) and drug EE (68.5%), which can be accounted for in the fact that too high AF\textsubscript{inlet} might induce insufficient migration of drug towards the inner particle space and enhance its translocation to the surface. Similarly, with the effect of airflow in nozzle, the increase of nozzle size tends to promote the formation of larger droplets/particles and relatively lower the drug distribution to surface. S\textsubscript{nozzle} (0.8 mm and 0.6 mm) showed lower drug loading (~9.1%) and drug EE (~70%) relative to a higher S\textsubscript{nozzle} (1.2 mm and 1.0 mm) (p < .05 or p < .01). As for the impact of the feed temperature, all T\textsubscript{feed} values investigated did not cause differences in drug loading and EE, with values around ~9–10% and ~70–80%, respectively, and was not a dominant factor in controlling drug loading and EE. T\textsubscript{inlet} and T\textsubscript{feed} had relatively little impact on drug/gelatin loading and EE. Among the process parameters tested, it was obvious that the feed concentration was the most critical factor affecting drug loading and EE and must be strictly controlled [27]. Generally, post-treatment washing causes a decrease in drug loading and EE due to the loss of surface peptide. For example, after washing, the basic formulation resulted in 2.35% and 18.35% decrease.

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Fig. 5. SEM images of various formulations including three different magnifications (1–3) for the following variables (see Table 1): (A) Basic Formulation; (B) Inlet Air Temp 70°C; (C) Inlet Air Temp 60°C; (D) Inlet Air Temp 50°C; (E) PLGA 30%; (F) Airflow in Nozzle 13.0 L/min; (G) Airflow in Nozzle 16.0 L/min; (H) Ratio\textsubscript{A/L} 2.0; (I) Ratio\textsubscript{A/L} 3.0; (J) Ratio\textsubscript{A/L} 6.0; (K) Ratio\textsubscript{A/L} 8.0; (L) Nozzle Size 1.2 mm; (M) Nozzle Size 0.8 mm; (N) Nozzle Size 0.6 mm; (O) Initial Sample Temp 13°C; (P) Initial Sample Temp 25°C; (Q) Initial Sample Temp 37°C; (R) LD without washing; (S) LD control with washing; and (T) Empty microspheres.
of drug loading and EE, respectively (see Fig. S1). However, this procedure is essential to achieve viable or more practical microspheres that are intended for commercialization.

Among all prepared microspheres, the ones with 9–11% drug loading were selected for further evaluation as they had similar drug loading as the 1-month LD. In addition to drug loading, gelatin loading and gelatin EE must also be investigated because the control of long-term drug release mainly derives from the introduction of gelatin [10,30]. Microspheres equivalent in terms of drug/gelatin loading are prerequisites for further comparison of other attributes with the commercial product. Fig. 6C and D show the gelatin loading and gelatin EE of candidate microspheres. The results revealed that most microspheres had ~1.7% gelatin loading, comparable to the LD (1.7%), except some formulations such as RatioA/2, 6.0 Formulation (~1.47%), Nozzle size (1.2 mm) Formulation (~1.48%) and Nozzle size (0.6 mm) Formulation (~1.53%). For most microsphere formulations, gelatin is expected to have a more homogenous radial distribution relative to drug distribution since gelatin is more resistant against water washing and has a much higher EE relative to the drug. The possible reason is that gelatin is expected to be distributed in the interior pores of the polymer matrix, whereas the peptide is expected to mostly exist as a salt in the polymer phase (and to a limited extent on or near the particle surface) while ion-paired with the carboxylate terminus of polymer chains, oligomers, and monomers.

3.4. Drying kinetics, residual solvent and conventional/hydrated $T_g$

During the solvent evaporation process in the formation of droplets/particles, there are four typical drying stages as outlined below (Fig. S2) [27,30]: (i) the initial heating stage of the droplets commences with the beginning of droplet formation. Droplets are rapidly heated to the atomizing chamber temperature, accompanied with solvent evaporation and slight reduction in droplet sizes (from $D_0$ to $D_1$); (ii) a constant drying period when temperature becomes constant and droplet mass decreases, accompanied with droplet shrinkage ($D_1 > D_{12} > D_2$); (iii) the falling drying stage (i.e., the drying stage during which solvent evaporation rate declines) where wet particles consist of a dry outer layer and an inner wet core. The dry outer layer hinders the drying of the inner wet core and solvent evaporation rate declines. The outer layer becomes thick and formatting matrix is gradually consolidated.
(D₂ > D₃ > D₄); and (iv) in the final sensible heating stage, the solvent content decreases to a minimal possible value and a dry non-evaporating solid sphere is generated. Particle temperatures increase without a remarkable size change (D₄) [31].

The effect of parameters on residual solvents should be considered during these basic drying stages. As shown in Fig. 7A and B, for residual methylene chloride (DCM) or water, the increase in inlet air temperature reduced the residual content of both solvents. It is reasonable that the increase of \( T_{\text{inlet}} \) would accelerate the formation of the outer crust layer and hinder the solvent evaporation from the wet matrix or core at drying stage ii. We did not detect any residual DCM content in LD or empty microspheres. The introduction of drug (leuprolide acetate) seems to slow down the organic solvent removal due to the interaction of the drug and polymer/gelatin [32]. Similarly, water content was also low in the LD (~0.14% w/w) and empty microspheres (~0.47%) because of this reason. It should be noted that thick feed concentration (higher \( C_{\text{feed}} \), PLGA 30%) resulted in a dramatic increase in residual DCM (~0.076%) and water (1.66%) relative to the PLGA 20% formulation. The higher \( C_{\text{feed}} \) may generate dry particles at an enhanced density that might be a crucial factor at drying stage iii and stage iv that impedes the removal of organic solvent and water at drying. The higher nozzle airflow noticeably lowered the residual DCM and there was no detectable residual DCM in formulations with \( AF_{\text{nozzle}} \) 13.0 L/min and \( AF_{\text{nozzle}} \) 16.0 L/min. A higher \( AF_{\text{nozzle}} \) would generate smaller particles, which is beneficial to remove organic solvents. However, water content was similar in formulations under varying levels of \( AF_{\text{nozzle}} \), at around ~1.5%. Because of differing boiling points of DCM and water, there are different diffusion/evaporation rates during the formation of droplets/particles, leading to the differences in residuals. For smaller \( S_{\text{nozzle}} \) (0.8 mm and 0.6 mm), there was no detectable residual DCM, while higher sizes resulted in a higher residual DCM. Larger particles formed under a larger \( S_{\text{nozzle}} \) resulted in appreciable residual DCM. A higher \( T_{\text{feed}} \) resulted in a lower residual DCM, mainly due to the shortened time during the initial drying stage (stage i). Feed temperature has a significant impact on the removal of DCM and accelerates the drying process through enhancing mass transfer rates in terms of the lower boiling point (~39°C) of DCM. However, the residual water content was higher in formulations under all feed temperatures (\( T_{\text{feed}} \)). It is
possible that evaporation of organic solvent might have a competitive effect on the removal of water during drying stages (especially stages iii and iv) because both solvents probably use the same evaporation pathway from inside to outside (especially at drying stages iii and iv) when solid outer crust, matrix or core is generated. Because of the enhanced airflow speed, higher inlet air flow (Ratio A/L 6.0 and Ratio A/L 8.0) obviously shortened drying time at drying stages i-iv and led to decreased residual DCM. Nozzle airflow, Ratio A/L, and nozzle size had no obvious impact on residual water content. Generally, most spray-dried drug-loaded microspheres had higher residual DCM or water content than the commercial product or empty microspheres, indicating that further work may be necessary to optimize final drying conditions during process scale-up.

The thermal behavior of microspheres is very informative to determine the effects of excipients, residual solvents, and processing on the final microspheres. Due to their amorphous nature, PLGA polymers and PLGA-based microspheres may generate distinctive glass transition temperature (Tg) values. The relationship between Tg and storage temperature is known to affect the process of physical aging of polymers or microspheres due to the underlying enthalpy of relaxation [33,34]. Because spray drying is a rapid heating-drying process combined with complicated heat and mass transfer, Tg profiles of products should be evaluated. As shown in Fig. 7C, unprocessed PLGA 75/25 had the lowest Tg (~38.4°C, p < .001) and the spray-drying process increased Tg values to various levels. Empty microspheres had a Tg of ~44°C. It is obvious that the introduction of leuprolide significantly elevated Tg levels (p < .001), owing to the previously reported peptide-polymer interaction [35]. Spray-dried microspheres resulted in similar Tg values, around 47.5–48.5°C, which were significantly lower than the Tg of commercial LD (49.2°C, p < .001). These microspheres made by spray drying had a higher Tg beyond room temperature and could be stable under storage conditions. The effect of hydration on Tg of PLGA 75/25, commercial and spray-dried products was investigated to better understand different performances during release incubation. As indicated in Fig. 7D, water caused a Tg decrease of all samples below physiological temperature, as has been reported extensively [34] and as predicted by the Gordon-Taylor equation and Tg of water (~135 K) [35,36]. Water decreased Tg values of PLGA 75/25 to similarly low values (~25°C, p < .05). A more noticeable decrease of hydrated Tg was found in commercial LD and empty microspheres during plasticization, reaching ~30°C. However, all spray-dried microspheres exhibited a slightly higher hydrated Tg value (~30–32°C) than the LD. This finding suggests that spray-dried microspheres would be expected to be more resistant to water-induced hygrothermal aging, degradation or erosion of internal amorphous structure during long-term release process.

3.5. Release kinetics and erosion behavior

The controlled release behaviors of spray-dried microsphere formulations were evaluated in comparison to the commercial LD (Fig. 8). The initial burst release (IBR) observed after the first day of incubation was significantly higher for LD relative to all other samples (~29.5%, p < .01) (Fig. S3A). Microspheres with higher Tinlet (80°C) exhibited lower IBR (~18%, p < .01), likely due to formation of a denser outer crust layer. The higher Cseed (PLGA 30%) formulation had a higher IBR compared with PLGA 20% formulation (p < .01). Increased AFnozzle (13.0 and 16.0 L/min) resulted in higher IBR relative to AFnozzle...
Fig. 9. Kinetics of physical-chemical properties of the microspheres accompanying release during normal incubation in PBST (pH 7.4) for selected formulations. (A) Long-term release of leuprolide is replotted for comparison compared with kinetics of: (B) water uptake \((n = 3)\); (C) microsphere mass loss \((n = 3)\); (D) Mw decline \((n = 3)\); (E) dry \(T_g\) \((n = 1–3)\); and (F) hydrated \(T_g\) \((n = 1–2)\). Symbols represent mean ± S.D.
Higher AF_{inlet} (i.e. higher Ratio_{A/L}) resulted in an enhanced IBR (p < .05, p < .01 or p < .001), which is probably attributed to decreased hardness of microspheres caused by insufficient drying processes. Smaller nozzle size resulted in an increased IBR effect, as the generated microspheres under this condition had increased available surface area for release. Formulations made using different T_{feed} values had similar IBR (~17%), which was lower compared to LD.

Following the initial burst release, slow and continuous release was observed throughout 63 days (Fig. 8). From day 0 to day 42, the LD presented a higher release than spray-dried microspheres. It was determined that microspheres prepared with T_{feed} 13 °C, 25 °C and 37 °C (especially 25 °C) had relatively lower overall release tendencies (Fig. 8). A T_{inlet} of 80 °C led to the lower release from microspheres during the initial 28 days compared with the lower inlet temperatures (Fig. 8A), probably due to a higher density or harder outer crust of microspheres fabricated under a higher T_{inlet}. More irregular structures and fragments in higher C_{feed} (PLGA 30%) may have contributed to its higher release relative to PLGA 20% formulation (Fig. 8B). Other spray-dried microsphere formulations generally resulted in slower long-term release relative to commercial product (Fig. 8C and D). The slow release (Fig. 8F) under high T_{feed} might derive from the possibility that increased T_{feed} could cause the formation of denser microparticles due to the viscosity increase of feed solution, and these particles would be more resistant against the erosion in the release media. If the one-day IBR effect is removed from consideration for mechanistic analysis, an IBR-deduced long-term release for 62 days can be generated, as shown in Fig. S4. The LD and PLGA 30% had a slightly higher release during the initial 34 days and then had a similar release thereafter (Figs. S4A and C). Spray-dried microspheres under different T_{inlet} (Fig. S4B), AF_{inlet} (Fig. S4D), Ratio_{A/L} (Fig. S4E) and S_{nozzle} (Fig. S4F) conditions had a slower release especially from 0 to 34 days. However, most of the spray-dried microspheres released more slowly during the entire release period. It is worth noting that T_{feed} had a crucial impact on long-term release and remarkably lowered the release compared with the commercial product (Fig. S4G). For microspheres prepared under different T_{feed} values, despite having similar release from days 0 to 27, a higher T_{feed} obviously delayed the latter release (day 27 to day 62). After initial burst, 50% release time (t_{50,release}) of all microspheres were calculated according to Table 2. Characteristic times of release (after initial burst), erosion (after initial burst) and degradation (after initial burst), t_{50,release}, t_{50,erosion} and t_{50,degradation} (in days).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>t_{50,release} (day)</th>
<th>t_{50,erosion} (day)</th>
<th>t_{50,degradation} (day)</th>
<th>Ratio_{50,release}/Ratio_{50,erosion}</th>
<th>Ratio_{50,release}/Ratio_{50,degradation}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupron Depot^a</td>
<td>26.45 ± 1.69</td>
<td>35.17 ± 0.71</td>
<td>27.00 ± 0.29</td>
<td>0.75 ± 0.04</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td>Basic formulation</td>
<td>31.74 ± 0.29</td>
<td>43.34 ± 0.76</td>
<td>36.69 ± 0.31</td>
<td>0.73 ± 0.01</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>PLGA30% formulation</td>
<td>27.27 ± 1.71</td>
<td>41.86 ± 1.75</td>
<td>35.42 ± 0.13</td>
<td>0.65 ± 0.06</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio 2.0 formulation</td>
<td>39.58 ± 1.28</td>
<td>41.20 ± 1.40</td>
<td>33.35 ± 0.51</td>
<td>0.96 ± 0.03</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>Ratio 3.0 formulation</td>
<td>41.58 ± 0.87</td>
<td>38.58 ± 2.23</td>
<td>31.19 ± 0.82</td>
<td>1.03 ± 0.03</td>
<td>1.33 ± 0.05</td>
</tr>
<tr>
<td>Ratio 8.0 formulation</td>
<td>36.61 ± 0.24</td>
<td>38.82 ± 0.49</td>
<td>32.48 ± 2.39</td>
<td>0.94 ± 0.01</td>
<td>1.13 ± 0.09</td>
</tr>
<tr>
<td>Initial Sample Temp 37°C</td>
<td>40.89 ± 0.41</td>
<td>39.66 ± 0.16</td>
<td>28.64 ± 0.57</td>
<td>1.04 ± 0.00</td>
<td>1.43 ± 0.04</td>
</tr>
</tbody>
</table>

^a Group 1 and Group 2 are designated as without (1) and with (2) erosion control, respectively.

The sudden T_g increase at day 56 was found for Initial Sample Temp 37 °C and the Ratio_{A/L} 2.0 formulations. It is possible that a rapid re-release of sequestered acid could have resulted in less plasticization over this period. LD was less resistant to the water-mediated degradation from the observation of its faster decline of hydrated T_g relative to spray-dried microspheres. In comparison, the basic formulation had a stronger capacity to resist the erosion and resulted in the slower decline of hydrated T_g. Diffusion/pore-mediated release played an important role at the latter release stage besides mass loss (i.e. erosion). Moreover, changes of these profiles versus cumulative leuprolide release percentage are summarized in Fig. S6.

3.6. Analysis of mechanisms in long-term release of peptide

For the microspheres selectively investigated, 50% mass loss time (t_{50,erosion}), 50% release time (t_{50,release}) and 50% Mw decline time (t_{50,degradation}) after initial burst release were calculated according to
Fig. 10. Mechanistic analysis of leuprolide release from spray-dried and Lupron Depot® (LD) formulations. Release after initial burst is plotted vs. mass loss (A) and Mw loss (B). Separation of the data sets in A and B according to Group 1 (C and D) with faster release than mass loss and similar release to Mw loss; and Group 2 (E and F) with similar release to mass loss and slower release to Mw loss. Symbols represent mean ± S.D. (n = 3).
corresponding curves, as shown in Table 2. For formulations that \( \frac{T_{90,\text{release}}}{T_{90,\text{erosion}}} \) is close to 1, an erosion-controlled release mechanism is implicated. An erosion-control trend with \( \frac{T_{90,\text{release}}}{T_{90,\text{erosion}}} \) ranging from 0.96–1.04 was observed for the four formulations at the bottom of the table (Group 2). By contrast, in Group 1 formulations, the ratio was \( \leq 0.01 \), namely 0.75 ± 0.04 for the LD, 0.73 ± 0.01 for the basic formulation, and 0.65 ± 0.06 for the PLGA 30% formulation. Separation of these groups was also evident when release was plotted against dry \( T_g \) for the eroded microspheres, as shown in Fig. 10. For example, the Group 1 formulations showed superimposable behavior reaching 70% release at just a \( T_g \) of \( \sim 47 \) °C, whereas Group 2 formulations had much lower \( T_g \) (42–45°C) at this stage. The latter behavior is reasonable considering that the Group 1 formulations release more drug from the polymer before the polymer had reached its very low Mw form. There are a couple of possibilities to explain the more rapid release behavior observed for Group 1. The most plausible explanation is that the peptide is bound as a salt to the polymer, giving rise to the elevated \( T_g \) \[35\]. The peptide under suitable conditions is expected to desorb from the polymer, for example, when water-soluble monomers are released. The water-soluble monomers, which have carboxylic acids with only slightly higher pKa’s than the carboxylic acid moieties on longer PLGA chains \[37\], would be expected to exchange peptide ionically bound with long chains of PLGA, to form a soluble salt of peptide and glycolic or lactic acids to leave the polymer. Consistent with this hypothesis, it was observed that leuprolide can rapidly form a salt with, and absorb in, solid particles of low Mw PLGAs from aqueous solution at 37 °C \[30\]. Desorption could also theoretically occur if there is some frequency of cycling of the leuprolide-PLGA salt to the polymer surface where the drug could then exchange with cations (e.g., \( \text{Na}^+, \text{H}^+ \)) in the release medium. Other possibilities for the more rapid drug release than mass loss could be due to osmotically induced mechanisms \[3\]. The underlying factor(s) responsible for the slightly different release mechanisms from Group 1 and Group 2 formulations is not known at this time. Possible reasons include variations in: a) rate and extent of clumping of microspheres in the centrifuge tubes, which may reflect differences in polymer surface and particle size, and b) other factors relatively affecting rates of peptide desorption vs. mass loss such as microsphere size and morphology (i.e., shape, density and depth of pores on the surface, or irregular structures) \[4\].

4. Conclusions

Spray drying of formulations for controlled release of peptides and larger molecules remains a developing field yet the technology is extremely promising in terms of extensive applications and versatility. By systematically altering a series of parameters based on nozzle, atomizing and feed thermodynamics, we designed a spray-drying process capable of preparing LD-equivalent composition formulations that behave similarly or different to the one-month LD. Decreasing \( T_{\text{inlet}} \) results in generation of small-scale particles and reduced residual solvent levels; however, \( T_{\text{inlet}} \) has relatively little impact on drug/gelatin loading and EE. \( C_{\text{feed}} \) has a critical influence on the microsphere formation and performance. Higher \( C_{\text{feed}} \) leads to a larger particle size and wider size distribution. Decreased \( C_{\text{feed}} \) causes a significant decline in drug loading and noticeable residual solvent due to prolonged solidifying time. The highest \( C_{\text{feed}} \) (30% PLGA) generates irregular particles and triggers accelerated long-term release, similar to the LD. Therefore, the \( C_{\text{feed}} \) parameter should be critically controlled in practice. The increase in \( A_P/\text{nozzle} \) induces the production of smaller particles, and reduces drug loading/EE. Elevated \( R_{\text{ratio}}/A \) results in larger particles. The highest \( R_{\text{ratio}}/A \) (10.0) tested causes a significant decline in drug loading/EE. Large values of \( S_{\text{nozzle}} \) generated larger particles and enhanced drug loading/EE. \( T_{\text{feed}} \) is a key factor to control residual moisture content and influence the long-term release profiles/mechanisms. By contrast, \( T_{\text{feed}} \) affects less particle size/distribution, drug loading/EE or gelatin loading/EE. Higher \( T_{\text{feed}} \) causes a delayed long-term release. Generally, except with the higher \( C_{\text{feed}} \) formulation (PLGA 30%), most of spray-dried microspheres present a slower controlled release behavior than commercial LD. There is no detectable residual DCM for LD and some of spray-dried particles. All spray-dried microspheres displayed higher moisture content than the LD. Spray-dried particles display similar dry \( T_g \) values and higher hydrated \( T_g \) compared with the LD. The presence of drug in microspheres elevated the dry \( T_g \). There is a decreasing trend of dry \( T_g \) and hydrated \( T_g \) over the long-term release period. There are multiple underlying release mechanisms for these spray-dried microspheres. Some spray-dried microspheres display more sustained long-term release profiles and offered viable long-acting release (LAR) alternatives to commercial products. For certain spray dried and LD particles, after the initial burst release proceeds erosion kinetics according to a hypothesized desorption mechanism. For other slower releasing formulations, release and erosion kinetics are superimposable, indicating erosion-controlled release. The current study provides a systematic presentation and basic understanding on the characterization of PLGA microspheres loaded with LHRHa-based peptides when employing the spray-drying technology. This study should be useful to aid developing new and generic microsphere formulations.

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Appendix A. Supplementary data

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References


