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Fabrication of biodegradable spheroidal microparticles for drug delivery applications

Michael J. Heslinga, Eric M. Mastria, Omolola Eniola-Adefeso *

Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Particle shape, in addition to size, is becoming increasingly recognized as important in the design of drug carriers for *in vivo* use. However, few methods exist for fabricating non-spherical particles from biodegradable polymers. This work describes for the first time the fabrication of biodegradable spheroidal microparticles using the simple oil-in-water emulsion solvent evaporation technique (O/W ESE). Unloaded and paclitaxel-loaded spheroids were fabricated from poly(lactic-co-glycolic acid) (PLGA), and the shape and size of fabricated spheroids were manipulated by controlling fabrication process parameters including stir speed, aqueous and oil phase viscosity, aqueous phase pH, and the polymer molecular weight and end group. The presented data show that high aqueous phase viscosity, basic aqueous phase pH and hydrophilic polymer side chains and end groups are all conditions that favor the formation of spheroidal particles. The described technique is advantageous over methods currently described in the literature in its simplicity in setup, high particle yield and adaptability to a wide range of biodegradable polymers and therapeutics.

1. Introduction

Biodegradable spherical particles are typically proposed for use as drug carriers for localized delivery of therapeutics in many human diseases due to their ease of fabrication and well-defined model for degradation and drug release [1–6]. The need for a paradigm shift away from spherical carriers for localized drug delivery, however, has become evident in recent literature suggesting that non-spherical particles may be optimum for *in vivo* drug delivery [7]. In one study, Champion and Mitragotri showed that macrophages can easily internalize spherical particles having diameters up to 15-μm, but the internalization of spheroids (rod-like particles) is unachievable when macrophage attacks the rods on their major axis [8]. Similarly, Desimone et al. in their recent publication showed that HeLa cells internalized rod-like particles at a higher rate than spheres of the same volume [9]. Also, Decuzzi and Ferrari using a theoretical model found that oblate (disk-like) particles were more effective in adhering to cell surface in laminar flow than spherical particles of the same volume [10], and Muzykantov et al. showed that immunotargeted disks displayed longer blood circulation *in vivo* than spheres of similar volume [7]. Finally, Shapiro and Gavze presented a theoretical model for particle motion in shear flow near a wall which suggested that rod-like particles preferentially drift towards the wall (over spherical particles) due to the complex hydrodynamic forces and torques acting on them, thereby suggesting spheroidal-shaped carriers would possess a higher affinity for the vascular wall than their spherical counterparts [11]. Thus, the use of non-spherical carriers for localized drug (or gene) delivery can lead to enhanced *in vivo* efficacy of therapeutics (e.g. higher cell transfection) and hence improve the treatment of many human diseases.

Overall, the increasing interest in non-spherical particles for use as drug carriers highlights the critical need for practical methods of fabricating these particles from biodegradable polymers in a manner that allows for easy loading and release of therapeutics. To date, two methods have been described for fabricating drug-loaded, non-spherical particles from biodegradable polymers for potential application in drug delivery. One method utilizes heat/solvent stretching of monodisperse spherical particles in polymer templates to achieve monodisperse non-spherical shapes [12,13]. While this method can achieve a narrow distribution of particle size, heating of particles can denature/degrade drug cargo and the use of solvent to render particles stretchable can lead to leaching of drug content — thus making this method not particularly suitable for drug delivery applications [12]. A second method utilizes imprint lithographic techniques called PRINT to produce diversely shaped particles [14]. While particles are fabricated under relatively mild conditions in the PRINT method, this process can be expensive to set up and is currently not well proven for use with a wide range of biodegradable polymeric material or therapeutics. Others have also described microfluidic-based methods for fabricating non-spherical particles that have focused on the use of photoactive polymers but have yet to demonstrate the ability to load therapeutics into these particles for drug delivery applications [15,16]. Described herein is the fabrication of biodegradable prolate spheroids for potential drug delivery applications using the simple oil-in-water (O/W) emulsion/solvent evaporation (ESE) technique that has been

* Corresponding author. Department of Chemical Engineering, University of Michigan, 2300 Hayward Street, 3074 H. H. Dow Building, Ann Arbor, MI 48109, USA. Tel.: +1 734 936 0856; fax: +1 734 764 1761.
E-mail address: lolaa@umich.edu (O. Eniola-Adefeso).

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extensively described for fabricating spherical drug carriers loaded with a wide range of therapeutics [17,18]. Specifically, by simple manipulation of the droplet dynamics and the diffusion process during the emulsification and solidification (evaporation) stages of particle formation, spheroidal microparticles can be fabricated from poly(lactic-co-glycolic acid) (PLGA) using the ESE method. Particle minor axis length and aspect ratio were found to be functions of the physical properties of the oil (droplet) and aqueous (continuous) phases, monomer ratio of lactic to glycolic acid, and stir speed. The presented data demonstrate the ability to fabricate spheroidal particles with average minor axes down to 2.5 μm — a size that is significantly smaller than the average luminal diameter in human capillaries and thus these spheroids may easily traverse these vessels. Also, it is shown that spheroids can be fabricated loaded with paclitaxel as proof-of-concept that the described method can allow for the encapsulation of therapeutic agents. Overall, the described ESE method for fabricating spheroidal particles has the advantages of using a proven microparticle fabrication technique that is simple in setup and operation, inexpensive, amendable to a range of biodegradable polymers, and provides favorable surface characteristics that permit the attachment of ligands/antibodies for targeted drug delivery [19].

2. Materials and methods

2.1. Microparticle fabrication

Microparticles were fabricated via the oil-in-water (O/W) solvent evaporation method as previously described [19] with some modifications (high surfactant concentration in the presence of Tris base). Briefly, 50 mg of PLGA polymer of choice was dissolved in 10 ml methylene chloride. The relevant characteristics of the different types of PLGA used in this study are summarized in Table 1. The polymer solution (oil phase) was injected into 100 ml of an aqueous buffer continuously stirred (1800 rpm) using a Lightnin' mixer (model L1U08F) fitted with a glass propeller (Beckman Coulter — shaft diameter = 2.8 cm). Unless otherwise stated, the aqueous buffer consisted of the surfactant polyvinyl alcohol (PVA; M_W 30,000–70,000) at 1% w/v and Tris base (Trizma) at 1.2% w/v in deionized (DI) water and maintained at pH 8.40 via titration with hydrochloric acid (base aqueous formulation). This composition of the aqueous buffer permitted the formation of stretchable oil droplets, and the high concentrations of PVA allowed for slowed solvent evaporation that is necessary for droplets to solidify in the stretched form. The emulsion was stirred for 1 h in order to produce the necessary shear force for droplet stretching and facilitate solvent evaporation upon stretching. The resulting microparticles were collected and washed (DI water) via centrifugation at 750 rpm prior to freeze-drying in a Labconco lyophilizer. The resultant powder was stored at −20 °C until use. For drug-loaded spheroids, 2.5 mg paclitaxel and 47.5 mg PLGA polymer were dissolved in 10 ml methylene chloride to form the oil phase. Similar to unloaded spheroids, the oil phase was injected into the aqueous phase and the emulsion stirred at 1800 rpm.

2.2. Microparticle characterization

Physical/surface characteristics of microparticles were studied using a Nikon TE 2000-S optical microscope and a Philips XL30FEG scanning electron microscope (SEM). Samples were prepared for light microscopy and SEM by suspending microparticles in DI water and pipetting the suspension onto a microscope slide or double-sided carbon tape fastened to an SEM stub. Prior to loading on the SEM for analysis, the particle suspension on carbon tape was air-dried and particles were subsequently gold-coated in a SPI-Module sputter coater. Particle size and aspect ratio were obtained from microscopy and SEM images via Metamorph analysis software. Unless otherwise stated, data is reported as average of at least 3 batches ± standard error between batches. Significance in data between different process variables was assessed using *all* data points obtained over multiple batches via student's *t*-test and one-way Anova with post-test. p value < 0.05 was considered significant.

2.3. Characterization of particle drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency for paclitaxel-loaded microparticles were determined by dissolving dried paclitaxel-loaded microparticles in methylene chloride. The concentration of paclitaxel in the polymer-drug solution was measured via UV absorption at 232 nm, where absorption data was converted into concentration of paclitaxel using a calibration curve generated from solutions with known concentration of the drug. Background absorbance of PLGA was subtracted using a similar calibration curve. Drug loading was calculated to be the mass of entrapped paclitaxel divided by the mass of dry microparticles. Encapsulation efficiency was calculated as the ratio of entrapped drug mass fraction to that of the drug mass fraction present in the oil phase during fabrication.

2.4. In vitro release studies

The *in vitro* release of paclitaxel-loaded microparticles was measured in 10 mM Dulbecco's phosphate-buffered saline (DPBS with Ca^{2+} and Mg^{2+}, Invitrogen) at pH 7.4 and 37 °C. Specifically, 7.5 mg of paclitaxel-loaded (2.7 wt.%) spheroids was suspended in 15 ml of DPBS in a screw capped polypropylene centrifuge tube. Tubes were placed on an orbital shaker bath maintained at 37 °C. At desired time points, tubes were centrifuged at 1000 *g* for 5 min. The supernatants were removed completely and pellets resuspended in fresh DPBS to maintain sink conditions for release as done in previous publications [20–23] and returned to the water bath. 5 ml of dichloromethane was added to collected supernatants and the mixture shaken for 30 s to facilitate drug extraction. After 30 min of allowing the oil and water to phase separate, the drug-rich dichloromethane was collected using a glass pipet and analyzed for drug content using a spectrophotometer as previously described (Section 2.3). Release studies were done in at least triplicate.

Table 1
PLGA polymers used in fabricating spheroidal particles ("–" information not available).

Polymer	Co-monomer ratio (lactic:glycolic acid)	Average molecular weight (M_w in Da)	IV (dl/g)	End group	Manufacturer
A	50:50	66,000	–	–COOH	Birmingham Polymer
B	50:50	47,000	0.36	–COOH	Lakeshore Biomaterials
C	50:50	55,200	0.47	–COOH	
D	50:50	~84,000	0.57	–COOH	
E	65:35	~65,000	0.48	–COOH	
F	75:25	~61,000	0.46	–COOH	
G	85:15	~65,000	0.48	–COOH	
H	50:50	47,000	0.37	$-(-CH_2-CH_2-O)_a-H$	
I	50:50	51,000	0.41	$CH_3(CH_2)_{11}O$	

3. Results

3.1. Fabrication of spheroidal particles

PLGA prolate spheroids were fabricated using the oil-in-water solvent evaporation method [18,19]. The minimum aqueous phase viscosity required for achieving spheroidal shape for an oil phase containing 5% w/v polymer A occurred at 1.0% w/v of PVA in an aqueous phase containing 1.2% w/v Trizma at pH 8.40 (base formulation) and stirred at 1800 rpm. Particles obtained from this base formulation were ~95% spheroidal and had average minor and major axis lengths of 4.0 ± 0.2 μm and 24.2 ± 2.7 μm ($n = 3$), respectively. The average particle aspect ratio (AR — ratio of major to minor axes) was 7.0 ± 1.0 (average of individual particle aspect ratios between batches ± SE). All PLGA spheriodal particles fabricated using the base aqueous formulation displayed a fairly smooth surface morphology as shown in Fig. 1. Overall, spheroids were produced at a rate 10^7–10^8 particles/h using the modified oil-in-water solvent evaporation method. The centrifugation process did not affect the percentage of spheroid in collected samples since pre- and post-centrifugation samples showed the same level of spheroids (e.g. 91% pre-centrifugation versus 94% post-centrifugation at base formulation). However, the average axis lengths were affected by centrifugation as expected; thus, all average parameter reported were based on samples collected at a fixed centrifugation speed of 750 rpm.

3.2. Effect of PVA concentration on spheroid size and aspect ratio

In some experiments, the concentration of PVA in the aqueous phase at a fixed Trizma concentration of 1.2% w/v was varied from 1–4% w/v. This increase in PVA concentration resulted in an increase in buffer viscosity from 1.6 to 5.5 mPa-s as measured by a Cannon–Fenske viscometer (size 50). Fig. 2 shows plots of the spheroid physical characteristics and the percentage of spheroids in recovered particles as functions of PVA concentration. Spheroids were produced at all PVA concentrations tested, and the major and minor axes of the recovered spheroids decreased as the PVA concentration increased from 1 to 4% w/v. The percentage of spheroids in the recovered particles was between 90 and 96% for PVA concentrations between 1 and 2%. However, as the PVA concentration increased beyond 2%, the spheroidal recovery rate decreased significantly — dropping to 30% at 4% PVA. The average particle aspect ratio was constant at ~7.0 for particles fabricated at PVA concentrations between 1 and 2.5%, but significantly decreased as PVA concentration increased to 4.0% (i.e. particles tended towards a spherical shape).

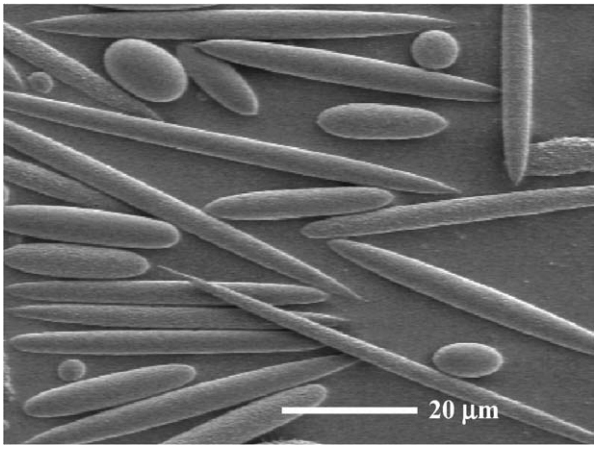

Fig. 1. SEM picture of polymer A microparticles. PVA = 1.0% (w/v). Tris base = 1.2% (w/v). Stir rate = 1800 rpm. Buffer pH = 8.40.

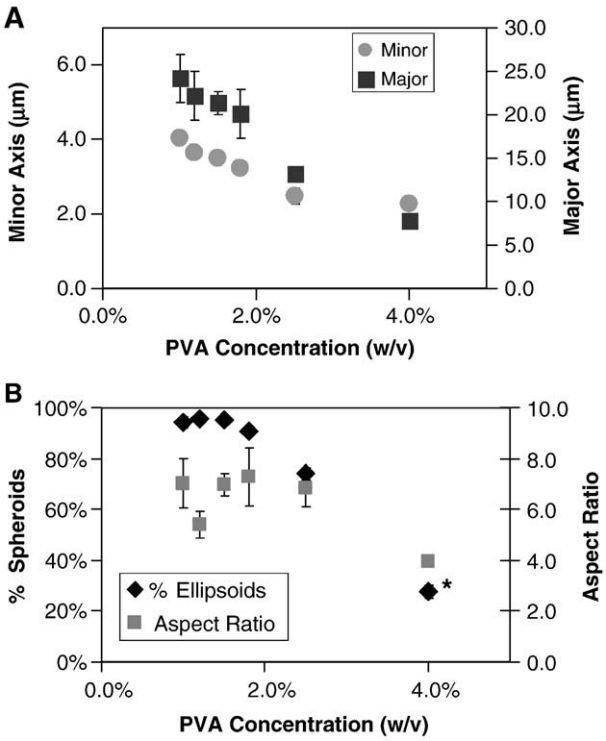

Fig. 2. Graph of (A) major (■) and minor (●) axis lengths, and (B) the percentage of spheroids in recovered particles (◆) as a function of the aqueous phase PVA concentration for microparticles fabricated from polymer A as functions of PVA concentration. Trizma = 1.2% w/v. Stir rate = 1800 rpm. Aqueous phase pH = 8.40. *$p < 0.05$ versus 1.0% PVA.

3.3. Effect of Tris base (Trizma) on microparticle shape, size and surface morphology

The effect of Trizma concentration in the aqueous (continuous) phase on microparticle shape was investigated. For these experiments the PVA concentration in the aqueous phase was held constant at 2.0% (w/v) with the other fabricating conditions the same as the base formulation. A minimum concentration of 0.3% (w/v) Trizma was necessary in order to observe particle deformation (spheroids), but the optimum Trizma effect occurred at a concentration of 0.5% (w/v). As the Trizma concentration increased from 0.5% to 2.0% (w/v), the fraction of collected particles that were spheroids decreased significantly from 90% to 42% (Fig. 3A), and the spheroid particle aspect ratio decreased from 7.2 to 3.9. When the PVA concentration in the buffer was reduced to 1%, a high level of spheroid recovery (>90%) was observed up to 1.2% (w/v) Trizma (Fig. 2B). Beyond this concentration, the percent spheroids produced also decreased with increasing Trizma concentration (~80 and ~60% spheroids at 1.5 and 2.0% Trizma in 1.0% PVA). Overall, particles fabricated with 1.2% (w/v) Trizma or higher had smooth surface characteristics for all PVA concentration tested as previously shown (Fig. 1) while particles fabricated with low Trizma concentration (<1.2% w/v) and in high PVA concentration (>1.2%) buffers displayed a rough surface morphology as shown in Fig. 3B. In general, particles fabricated with low concentrations of Trizma (<1.2%) in the aqueous buffer at a fixed concentration of PVA were more homogeneous in their major axis length and smaller on their minor axis than their high Trizma fabricated counterparts (Fig. 1).

3.4. Effect of aqueous phase pH on microparticle shape

To study the potential effect of pH on the shape and size of fabricated spheroids, the pH of the aqueous phase was varied between

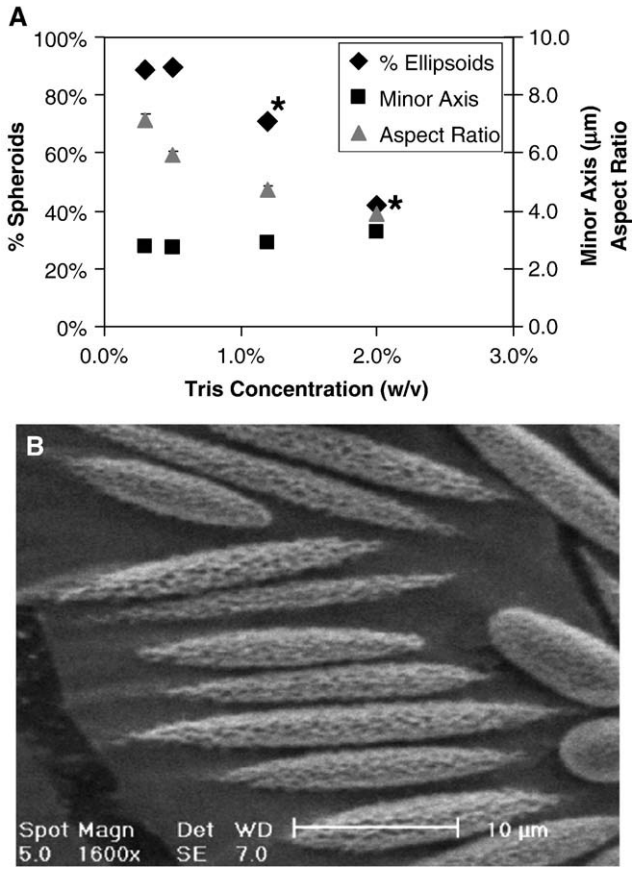

Fig. 3. (A) Graph of percent spheroids (◆), minor axis (■) and aspect ratios (triangle symbol) of polymer A microparticles as functions of Trizma concentration in the aqueous phase with PVA concentration = 1.0% (w/v) and (B) SEM picture of polymer A microparticles with Trizma concentration = 0.3% (w/v) and PVA concentration = 1.5% (w/v). Stir rate = 1800 rpm. Aqueous phase buffer pH = 8.40. $p<0.05$ compared to 0.5% Trizma.

5.0 and 10.0 from the base pH of 8.4 in buffers containing 1.0, 1.8 and 2.5% PVA at fixed concentration of 1.2% Trizma (1.2%) by adjusting the amount of hydrochloric acid added to the aqueous phase. As the aqueous phase pH decreased from 8.4 to 5.0, the percentage of spheroids in the recovered sample (Fig. 4) and the average particle aspect ratio (Table 2) decreased significantly for all PVA concentrations studied. An increase in pH beyond 8.4 did not result in any significant change in the percentage of spheroids for all PVA concentration studied as shown in Fig. 4.

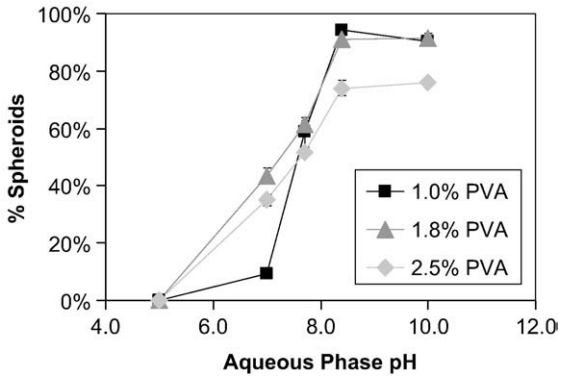

Fig. 4. Graph of percent spheroids of polymer A microparticles as functions of aqueous phase pH. Stir rate = 1800 rpm. Trizma = 1.2% w/v.

Table 2
Physical characteristics of polymer A particles as functions of pH at 1% PVA.

pH	Aspect ratio	Major axis (μm)	Minor axis (μm)
5.0	–	6.0 ± 0.1	6.0 ± 0.1
7.0	2.7 ± 0.1	11.8 ± 0.4	4.9 ± 0.1
7.7	3.9 ± 0.1	13.3 ± 0.4	3.9 ± 0.1
8.4	7.0 ± 1.0	24.2 ± 2.7	4.0 ± 0.1
10.0	7.7 ± 0.5	23.5 ± 0.9	3.6 ± 0.1

Trizma = 1.2% w/v.

3.5. Effect of mechanical stir speed on microparticle shape

The effect of mechanical stir speed on particle size and shape was studied for aqueous buffers containing 0.3 and 1% w/v PVA. At 1% PVA, the percentage of spheroids in the recovered particles was not affected when stir speed was decreased from 1800 to 1200 rpm while keeping other system parameters constant (Table 3). A further decrease in mixing speed down to 1000 rpm at 1.0% PVA did not result in particle formation since solvent evaporation at this speed was drastically hindered. However, the effect of reduced stir speed on particle shape was more significant when the aqueous phase contained 0.3% w/v PVA — where a decrease in speed from 1800 rpm to 1200 rpm yielded an increase from 11% to 86% spheroids. Further reduction in stir speed to 800 rpm at 0.3% w/v PVA did not significantly change the percentage of spheroids in the recovered particles, however, particle aspect ratio increased from 6.1 at 1200 rpm to 12.9 due to the major axis length increasing from 28.1 to 41.6 μm. Overall, decreasing the stirring speed increased particle aspect ratio for both PVA concentrations tested. Mixing speeds higher than 1800 rpm were not explored due to mixer limitation.

3.6. Effect of end group on microparticle shape

The effect of three different PLGA end groups: acid (polymer B), poly (ethylene-glycol) (PEG, M_W 1500; polymer H) and ester (polymer I), on microparticle characteristics was studied using the base aqueous phase formulation (at 1800 rpm). Fig. 5A shows a plot of average particle axis lengths as functions of polymer end group. Particles fabricated from polymer B had the highest percentage (~90%) of spheroids and the largest spheroid particle aspect ratio (6.0) at the conditions studied. The fraction of spheroids decreased to ~25% (mostly spheres) and the average aspect ratio of stretched particles decreased to 4.0 when particles where fabricated from polymer H. Particles fabricated from polymer I exhibited no elongation (aspect ratio ~1). When the particle fabrication speed was decreased from 1800 to 1200 rpm while keeping other aqueous phase conditions constant, the polymer H spheroidal particle yield increased to 80% and the particle aspect ratio increased to 5.6 as shown in Fig. 5B. Particles fabricated from polymer I remained spherical at all stirring speeds explored.

3.7. Effect of oil phase viscosity and lactic-to-glycolic acid monomer ratio on microparticle shape

The effect of the oil (droplet) phase viscosity on microparticle shape and size was studied by varying the molecular weight of the

Table 3
Physical characteristics of polymer A particles as functions of emulsion stir rate.

PVA (% w/v)	Stir speed (rpm)	%Ellipsoid	Aspect ratio	Major axis (μm)	Minor axis (μm)
0.3	1800	11 ± 2.2	2.4 ± 0.1	12.3 ± 0.6	5.6 ± 0.3
0.3	1200	86 ± 0.8	6.1 ± 0.5	28.1 ± 1.4	5.5 ± 0.2
0.3	800	88 ± 1.0	12.9 ± 2.2	41.6 ± 8.5	3.9 ± 0.3
1.0	1800	94 ± 1.0	7.0 ± 1.0	24.2 ± 2.7	4.0 ± 0.1
1.0	1200	90 ± 2.1	13.1 ± 1.0	41.0 ± 3.0	3.6 ± 0.0

pH = 8.40. Trizma = 1.2% w/v.

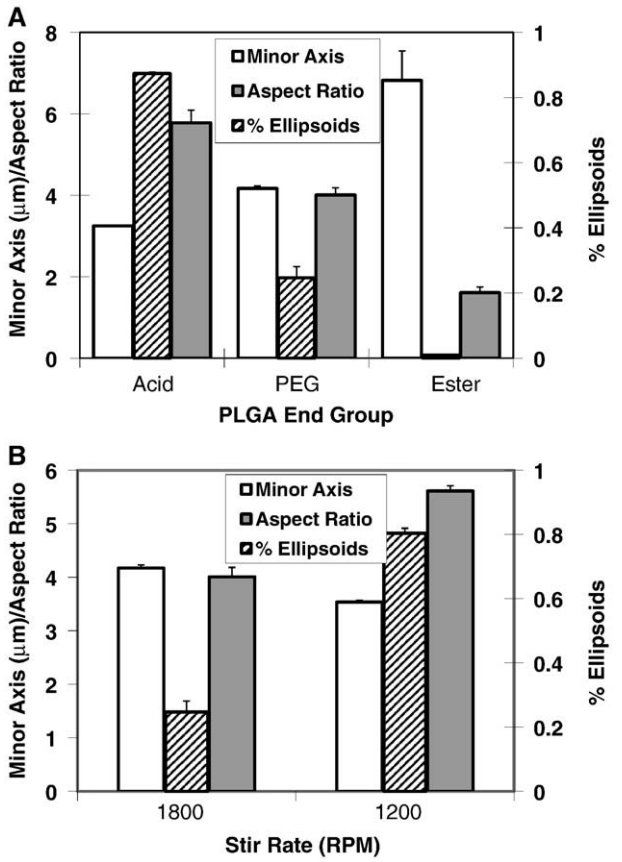

Fig. 5. Graph of (A) percent spheroids, major and minor axis lengths as functions of polymer end groups for 50/50 PLGA microparticles at 1800 rpm stir speed and (B) percent spheroids and aspect ratio as functions stir speed for microparticles fabricated using polymer H. PVA = 1.0% (w/v). pH = 8.40. $p<0.05$ compared to 1800 rpm.

PLGA polymer, i.e. increasing polymer molecular weight corresponds to increase in the oil phase viscosity. The percentage of spheroids in the recovered particles was 87%, 81%, and 64% for an oil phase with polymer B, C and D respectively. The corresponding average particle aspect ratios were 5.8, 5.4, and 3.9.

When particles were fabricated from PLGA polymers having different co-monomer ratios at a constant molecular weight (matched by manufacturer inherent viscosity — IV) and process parameters, there was no clear trend in the percentage of spheroids in the recovered particles as the ratio (mol%) of lactic acid in the polymer increased from

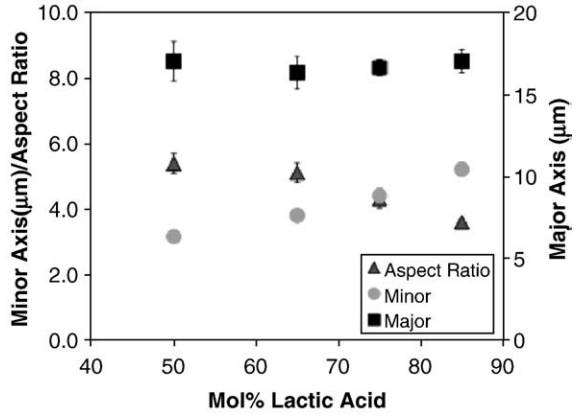

Fig. 6. Graph of major (■) axis, minor (●) axis and aspect ratio (▲) for PLGA–acid microparticles as functions of percent lactic acid. Stir rate = 1800 rpm. PVA concentration = 1.0% (w/v). Buffer pH = 8.40. Trizma = 1.2% w/v.

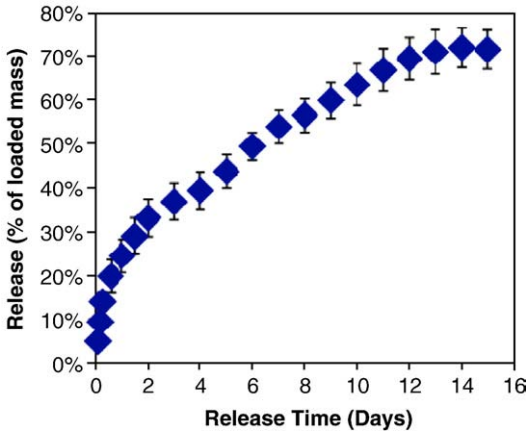

Fig. 7. Pacitaxel release from polymer A spheroids (fabrication at 1800 rpm, 1.0% w/v PVA, and pH 8.40) in DPBS at pH 7.4. Value = mean ± SD ($n=6$).

50 to 85% (polymer C, E–G). Similarly, data presented in Fig. 6 show no significant effect of co-monomer ratio on the particle average major axis length. However, the average particle minor axis length significantly increased and thus resulted in a decrease in the average aspect ratio as the percent lactic acid increased from 50% to 85%.

3.8. Paclitaxel-loaded PLGA spheroids

Prolate spheroids were fabricated in the presence of paclitaxel to demonstrate the viability of this method for application in drug delivery. Paclitaxel-loaded polymer A microparticles fabricated at the base aqueous phase condition (compare to data in Section 3.1) and with 2.5 mg paclitaxel in the oil phase resulted in particles that were ~90% spheroids with average aspect ratio and minor axis of 8.2 (±0.7) and 3.0 (±0.1) μm, respectively. These particles exhibited an average drug loading of ~2.7 wt.% (±0.4%) with an average encapsulation efficiency of 50.7% (±4.2%). Overall, paclitaxel-loaded spheroids exhibit the same smooth surface morphology seen with unloaded spheroids fabricated at the base aqueous phase condition. A preliminary release study was conducted with paclitaxel-loaded spheroids. As shown in Fig. 7, loaded spheroids show an initial burst release of paclitaxel followed by a linear release over time similar to previous reports in the literature [21,24,25].

4. Discussion

Particle shape in addition to size is becoming increasingly recognized as important in the design of drug carriers for use *in vivo* [7,8]. This increasing attractiveness of non-spherical particles for use in drug delivery applications gives rise to the need for practical and adaptable methods for the manufacture of such particles from biodegradable polymers like PLGA. Presented here is a modified emulsion/solvent evaporation (ESE) method that enables the fabrication of prolate spheroidal particles from biodegradable polymers via manipulation of droplet dynamics and solvent diffusion during particle solidification. Specifically, with the understanding that microparticle formulation in the ESE method can be divided into three stages: (1) droplet formation or emulsification, (2) solvent removal (solidification) and (3) drying; and that microparticle solidification can be further divided into three states: (i) solvent, (ii) gel and (iii) glass, the continuous phase can be manipulated such that the rate of solvent removal by diffusion is slowed, leading to oil droplets being sustained in the gel state long enough to permit stretching by high-shear mixing. The improved mass transfer upon stretching allows for rapid solvent evaporation that leads to the solidification of particles in their stretched state. The presented data show that

spheroids (unloaded and drug loaded) can be fabricated at a rate of 10^7–10^8 particles/h (per 50 mg of polymer in 100 ml of aqueous buffer) via the modified ESE method by employing a high aqueous phase PVA concentration (>1% w/v PVA) in the presence of Tris base (Trizma) to achieve low diffusion rate of the polymer solvent, dichloromethane. Furthermore, spheroidal shape and size are shown to be functions of the stir speed during fabrication, viscosity of the aqueous and oil phases, pH of the continuous phase, co-monomer ratio of the PLGA polymer and the type of end group present on the polymer chain.

The effects of the above-mentioned process parameters on microparticle characteristics can best be understood via droplet dynamics. In the ESE system, the oil droplet (dispersed phase) may be deformed and/or broken up by the viscous forces acting upon it. Droplet deformation (stretching) and breakup are controlled by two dimensionless groups: (1) the viscosity ratio of the droplet phase to continuous phase ($M=\eta_d/\eta_s$) and (2) the capillary number (Ca), which is the ratio of viscous to capillary forces as defined in Eq. (1) below [26]:

$$\mathrm{Ca} = \gamma\eta_s\, a/\Gamma \tag{1}$$

where γ is the shear rate, η_s is the viscosity of the solvent (continuous) phase, a is the droplet size, and Γ is the interfacial tension between the droplet and the continuous phase. In general, a high capillary number (high viscous forces or low interfacial tension) and/or a low viscosity ratio result in emulsion conditions that favor droplet deformation. Intermediate values of the viscosity ratio ($M\approx 1$) at a fixed capillary number lead to severe droplet breakup and high values of M result in no droplet deformation or breakup [26].

Thus, in the described spheroid fabrication method, the high concentration of PVA served a dual role: (1) it increased the aqueous phase viscosity to slow solvent removal and (2) higher aqueous phase viscosity produced a low M and high capillary number that favor droplet deformation. As such, increasing PVA concentration to the critical value of 1% w/v while fixing other aqueous phase conditions permitted the deformation of oil droplets, which resulted in a high yield of spheroidal particles (~95% spheroids, Fig. 2) that was maintained up to 2% w/v PVA. Similarly, particle deformation, as defined by particle aspect ratio, did not change in this PVA concentration window. The decrease seen beyond 2% w/v PVA for the percentage of spheroids in the recovered sample and the average particle aspect ratio can be explained by the competing effects of higher viscosity — (1) higher shear forces acting on droplets that favor deformation (i.e. high Ca #) and (2) smaller "initial" droplet size formed during emulsification that trend towards no deformation. Thus, for a given viscosity ratio ($M=\eta_d/\eta_s$) there is a critical capillary number such that further increases in viscous forces result in smaller droplets and reduced droplet stretching [26]. Hence, the constant aspect ratio and percentage of particles recovered that are spheroids seen between 1 and 2% w/v PVA suggest a constant capillary number that is due to a balance between the effects of decreasing droplet size and increasing viscous forces on droplet deformation. Beyond 2% w/v PVA, the initial droplet breakup upon insertion of the oil into the aqueous phase resulted in increasingly smaller droplets — falling below the critical droplet size required for stretching at constant interfacial conditions.

It is also likely that the low spheroid yield observed at high PVA concentrations is due to PVA interfering with the interfacial effect of Trizma in the aqueous phase. Though PVA was the surfactant necessary for droplet stability and stretching in the described ESE method, the presence of Trizma in the aqueous phase was shown to be necessary for the successful fabrication of spheroids. When Trizma was absent in the aqueous phase, only spherical particles were produced at all PVA concentrations. The addition of a moderate amount of Trizma (0.3–0.5% w/v) to the aqueous phase led to the formation of stretched particles. While Trizma itself is not a surfactant, its presence in the aqueous phase provided the necessary interfacial environment, via its "surface-active" hydrophilic groups, for droplet deformation over breakup, i.e. the hydrophilic groups on Trizma interacted with the hydrophilic groups on the polymer chain to lower interfacial tension [27]. Thus, it is plausible that at a fixed Trizma concentration, a high PVA buffer composition may crowd out the effect of Trizma on particle deformation. However, when the concentration of Trizma in the aqueous phase increased beyond a critical value at fixed PVA concentrations (0.5% w/v for a 2.0% PVA aqueous phase), the percentage of spheroids in recovered particles significantly decreased with increasing Trizma concentration (Fig. 3). This is likely a result of the increased amount of electrolytes present in the aqueous phase at higher Trizma concentrations due to the increased amount of hydrochloric acid needed to maintain pH at 8.4 [27]. Indeed, when the aqueous phase with 2.0% w/v Trizma in 2.0% w/v PVA was used for particle fabrication without a pH adjustment down to 8.4, the percent of particles that were ellipsoids significantly increased (~80%) as compared to a similarly composed buffer at pH 8.4 (~40% spheroids; Fig. 3).

Overall, the pH of the aqueous phase in the ESE particle fabrication method is of great importance to drug loading [28,29]. The fact that spheroidal particles are preferentially produced at basic pH at constant PVA and Trizma concentrations is likely due to the effect of pH on the interfacial environment provided by Trizma. Specifically, the percentage of spheroids produced was high when the aqueous phase pH was greater than the p*K*a of Trizma (p*K*a ~8.1). When the pH was below 8 and the lone pair on the amine group was largely protonated, the formation of spheroids was not favored. However, it is possible that PVA also affects interfacial tension in response to pH since previous publications have shown that PVA exhibits lower interfacial tension at higher pH [30,31].

The emulsification speed proportionally affects the shear rate in the aqueous phase where increasing stir rate increases the viscous force acting on droplets. Decreasing the stir rate from 1800 rpm to 1200 rpm at a low PVA concentration of 0.3% w/v where *few* spheroids were formed at 1800 rpm caused the formation of a higher fraction of stretched particles (Table 3). This observation is due to a lower stir speed producing larger oil droplets during the initial emulsification as been previously reported for microsphere fabrication via the ESE method [18,32–34], and a larger droplet size favors deformation (Eq. (1)). While the effect of stirring speeds higher than 1800 rpm was not studied due to equipment limitation, the authors speculate that increasing the stirring speed beyond 1800 rpm in this system would move particles towards a more spherical shape due to a combination of higher droplet breakup and increased mass transfer that promotes rapid droplet solidification at higher stir rates.

PLGA chains often terminate in end groups that can have a large effect on the characteristics of the polymer [35–37]. In this system, spheroids were easily formed from acid-terminated PLGA chains at the base fabrication condition, while PLGA having ester or PEG end groups mostly produced spheres. The observation with PLGA–acid and PLGA–ester is not surprising; the hydrophilic acid end groups in the PLGA–acid are available to interact with the continuous phase, creating low interfacial tension that favors droplet deformation. However, the ester end groups are hydrophobic, increasing the interfacial tension (i.e. no possibility of hydrophilic interaction between oil and aqueous phase) to a point where the shearing forces cannot overcome the capillary forces keeping the droplets together. Previous publications have shown similar effect of the hydrophobic resistance of PLGA–ester in emulsification, where PLGA–ester used in the ESE method form larger particles (resisted droplet breakup) than PLGA–acid because of the increased interfacial forces [38]. While the PEG chain groups on PLGA–PEG are hydrophilic in nature, its size (~1500 Da) will tend to promote entanglement and polymer chain entanglement favors less deformation (i.e. higher droplet viscosity). However, when PLGA–PEG particles were fabricated at a lower stir speed, the resultant particles were mostly spheroids since the lower shear force reduced droplet breakup and produced droplets large

enough to overcome the higher interfacial tension produced by PEG chain entanglement.

The characteristics of PLGA polymer such as molecular weight and co-monomer ratio are known to directly impact the physical characteristics and encapsulation efficiency of spherical drug carriers fabricated using the emulsion/solvent evaporation method [38,39]. In the described system, the decrease in particle aspect ratio (less deformation) with higher ratios of lactic acid in the polymer chain is likely due to the more hydrophobic nature of lactic acid (extra methyl side group) that causes a higher interfacial tension to exist between the oil droplet and the continuous phase [38]. Similarly, the percent of spheroids in the recovered sample and their corresponding aspect ratio decreased with increasing PLGA molecular weight, i.e. less deformation because an increase in molecular weight directly corresponds to an increase in polymer chain length that produces higher chain interaction and entanglement. Furthermore, a longer polymer chain translates to a lower concentration of the hydrophilic end groups that produce the low interfacial tension necessary for droplet deformation.

5. Conclusions

In this work, spheroidal-shaped microparticles were fabricated using the oil-in-water emulsion solvent evaporation (ESE) method. It was shown that the size and shape of these non-spherical microparticles can be controlled by careful manipulation of key process parameters that include the speed of emulsification, aqueous viscosity and pH, oil phase viscosity, polymer molecular weight and the end group on the polymer chain. The data showed that a high aqueous phase viscosity, a basic aqueous phase pH and hydrophilic end groups on the polymer chain are all conditions that promote formation of spheroidal particles. Furthermore, spheroids were successfully fabricated loaded with the therapeutic drug paclitaxel as a proof of concept for their potential application in drug delivery. Paclitaxel-loaded spheroids showed a similar release profile to ones previously reported for pacitaxel-loaded microspheres, where an initial burst release is seen within hours of degradation followed by a linear (constant) release over several days. Overall, this method of fabricating spheroidal particles via the emulsion/solvent evaporation technique is advantageous over methods currently described in the literature due to its simplicity in setup, high particle yield and adaptability to different biodegradable polymers and therapeutics. Based on previous publications, spheroidal particles are potentially advantageous over spherical particles for *in vivo* drug delivery. The simple fabrication method presented herein will allow many investigators quick and inexpensive access to these particles and thus rapidly advance their potential for *in vivo* application. Detailed degradation and drug release characteristics of spheroidal particles relating to a variety of loaded therapeutics would be the subject of another publication.

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