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Thermally processed polymeric microparticles for year-long delivery of dexamethasone

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ABSTRACT

Dexamethasone-releasing poly(lactic-co-glycolic acid) (PLGA) microparticles were formulated using a solvent displacement technique with the addition of distillation aiming to increase drug delivery lifetime. Two PLGA copolymer ratios (50:50 and 75:25) were used to determine the influence of lactic acid and glycolic acid ratio on microparticle characteristics. The addition of distillation significantly slows the release of dexamethasone compared to traditional solvent removal via evaporation while still maintaining a therapeutic dosage. Microparticles formulated with PLGA 50:50 controllably release dexamethasone up to one year and 75:25 release up to two years in-vitro. The ratio of lactic acid to glycolic acid plays a significant role in microparticle stability, drug loading efficiency, and thermal properties. In all, this formulation technique offers new prospects for inflammation suppression in pediatric vascular and airway diseases.

1. Introduction

Vascular stenoses and tracheomalacia can be life-threatening conditions in pediatric patients. Common vascular obstructions are coarctation of the aorta and pulmonary artery stenosis presently treated with invasive surgery or metallic stents. Tracheomalacia is the abnormal softening of the trachea wall that leads to airway collapse during respiration under conditions in which extraluminal pressure exceeds intraluminal pressure. Currently, the modalities of treatment for tracheomalacia include positive pressure ventilation, surgical resection of the affected segment, external splinting, tracheopexy, aortopexy, or stenting [1,2]. Despite some technological advances airway stents are still controversial in regard to their success at internal reinforcement and biocompatibility [1]. The complications of tracheal stenting include over exuberant scar formation, accumulation of inflammatory cells surrounding the implanted stent, and high infection risk due to the stent being in contact with inhaled air [3–6]. Besides infection risk, vascular stents have the same long term complications. The inflammation associated with stenting has an acute onset estimated within one week after implantation with the chronic phase to follow up to six months. Pediatric patients have the additional issue of somatic growth, such that with time even efficacious stents will ultimately result in a fixed obstruction. Biodegradable stents offer the potential to eliminate this long-term limitation.

Dexamethasone is a corticosteroid anti-inflammatory agent prescribed for both adult and pediatric airway interventions and vascular stenoses [7,8]. Though effective, long-term systemic exposure of a corticosteroid such as dexamethasone can lead to side effects such as osteoporosis, dermal thinning, ophthalmological complications, and reduced growth velocity in children [9]. Local delivery of dexamethasone, such as a coating on a stent, could alleviate inflammation while limiting potential side effects from systemic exposure.

Technological advances in polymeric microparticle delivery systems may provide the long awaited solution to long-term therapeutic agent delivery as stent coatings. Using polymeric microparticles as a drug coating is advantageous because particle formulation methods are well documented, particle parameters (such as size and composition) are easily tunable, hydrophobic and hydrophilic active substances can be incorporated, and polymeric materials can degrade into non-toxic substances. One of the available techniques for microparticle formulation is a solvent displacement technique that is a fast, repeatable, and economical one-step process. Researchers have used microparticles produced from this technique for various drug delivery applications ranging from local inflammation suppression to implantable medical device systems [10,11].

To extend the lifetime of microparticles for long-term delivery, this study investigates the use of distillation for solvent extraction as a method of thermally processing microparticles. Thermal processing of

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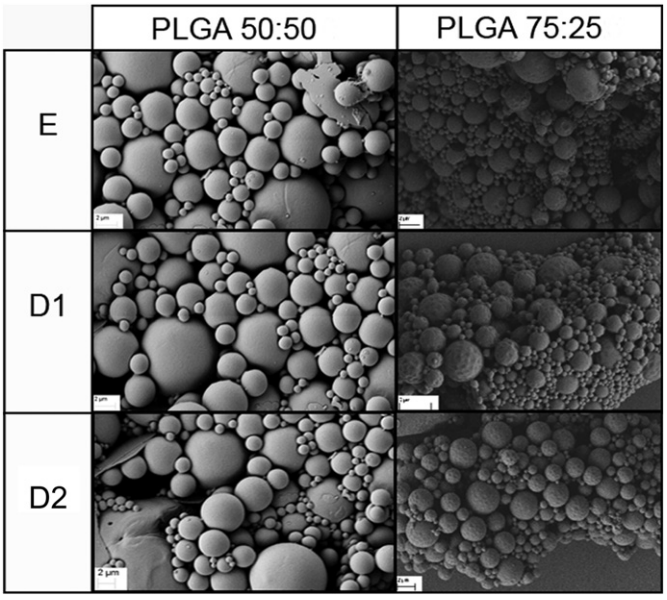

Fig. 1. Scanning Electron Microscopy image showing morphology of microparticle groups.

polymeric structures such as films, fibers, and biodegradable stents has been thoroughly researched [12–14]. Significant changes in molecular mobility, morphology, physical, and mechanical properties can be detected [12–14]. Using heat for structural relaxation of materials has been applied to metallic nanoparticles to alter grain size and influence shape [15]. To investigate the effect of distillation, microparticles loaded with dexamethasone were formulated from two copolymer ratios of PLGA; 50:50 and 75:25. In each copolymer ratio, two groups with distillation were examined and compared to a control group in with solvent removed via evaporation. Our hypothesis is that using distillation to remove solvent will prolong drug release lifetime and increase drug loading efficiency compared to evaporation. Additionally we hypothesize that PLGA 50:50 will produce microparticles of higher drug content and quicker drug release than PLGA 75:25. This study reports the effects of copolymer ratio and distillation on PLGA microparticles' size distribution, zeta potential, drug loading efficiency, glass transition temperature (T_g), and controlled release of dexamethasone.

2. Methods

2.1. Materials

PLGA 50:50 (153 kDa, PURAC, Netherlands), PLGA 75:25 (114 kDa, Evoniks Degussa Corp., USA), Tetrahydrofuran (THF), Pluronic F127 (hydrophilic non-ionic surfactant, also known as poloxamer 407), and Dexamethasone (Sigma Aldrich, USA).

2.2. Formulation of drug loaded PLGA microparticles

1 g PLGA was dissolved in 5 mL of THF via vortexing. Dexamethasone (12.5% w/w) was then added into this solution. 5 mL of 0.35% F127 solution was added to the PGLA solution and vortexed briefly then sonicated for 30 min. Solvent was then removed via evaporation (E) at room temperature for 3 h serving as the control. Two additional formulations with solvent removal via distillation were made. One distillation group (D1) was held at the solvent boiling point for 1 additional minute after solvent was removed and a second group (D2) held for 15 additional minutes. The particles were washed by centrifugation with distilled water at 1500 rpm for 5 min 3×. After final wash, particles were re-suspended in 10 mL distilled water and frozen until needed.

2.3. Particle morphology, effective hydrodynamic diameter and zeta potential

Particle morphology was assessed via Scanning Electron Microscopy (Zeiss Sigma, Zeiss, Switzerland) operating at 1–10 kV. Particle suspension was pipetted onto a glass coverslip and dried. Glass coverslip was mounted onto a metal stub by adhesion film and sputter coated in an Anatech Hummer VI (Anatech, Union City, CA) with gold/palladium. Effective hydrodynamic diameter and zeta potential were evaluated using Dynamic Light Scattering (DLS) (ZetaPALS, Brookhaven Instruments, Novata, CA, USA).

2.4. Drug loading efficiency

The drug loading efficiency was determined by the following equation:

$$\text{Drug loading efficiency } (\%) = \frac{\text{Loaded drug}}{\text{Initial drug}} \times 100. \quad (1)$$

The 0.25 mL of particle suspension for each group was dissolved in 1 mL of THF. The solutions were analyzed using Ultimate 3000 HPLC system (Thermo Fisher Scientific, Chicago, IL) and the Acclaim C30 column (Thermo Fisher Scientific, 3 μm, 3.0 × 150 mm). The mobile phase was 2% methanol, 30% THF, and 68% water (v/v) at 30 °C. A flow rate of 0.3 ml/min with an injection volume of 25 μL was used. The UV diode array detector was set at UV 240 nm for detection of dexamethasone [16].

2.5. Drug release and therapeutic potential

0.5 mL of drug-loaded microparticles suspended in distilled water (pH 7.38) was pipetted into 0.5 mL MINI Dialysis Device (Slide-A-Lyzer 10K MWCO, Thermo Scientific USA). Dialysis device was inserted into 2 mL tube filled with distilled water. Assembled dialysis apparatus was placed on a shaker in 37 °C incubator (n = 10 per group) and

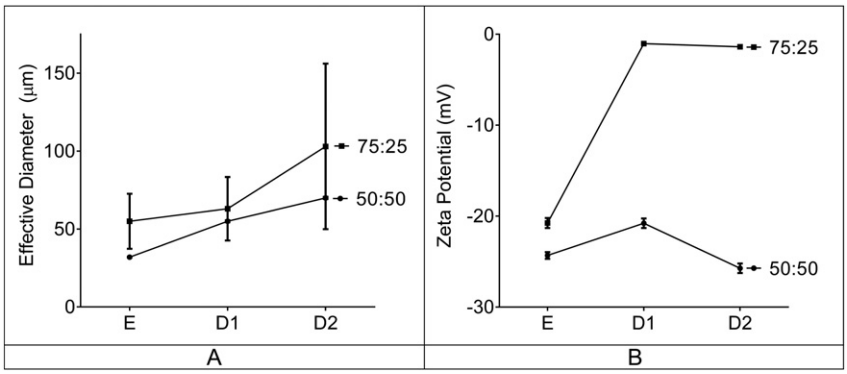

Fig. 2. Two-way ANOVA results for (A) effective diameter and (B) zeta potential of microparticle groups (data shown mean ± SEM, n = 5).

sealed. 1 mL of the solution from tube was removed 2, 4, and 7 days then weekly until end of release experiment. Any remaining water solution was decanted, fresh distilled water was added and the apparatus was resealed. The removed solutions were analyzed for drug concentration using the HPLC method described above.

2.6. Thermal properties

50 μL of the particle suspension was pipetted into a TZero aluminum pan and placed into a desiccator for 48 h. Pans were then sealed and analyzed via Q20 differential scanning calorimeter (TA Instruments, New Castle, DE). Samples without dexamethasone were equilibrated at 10 °C, ramped to 70 °C at 10 °C/min, held isothermal for 1 min then cooled to 10 °C at 50 °C/min. Samples containing dexamethasone followed same method ramped up to 285 °C. Samples followed their appropriate temperature sweep twice reporting the second curve. Heating curves were analyzed using TA Universal Analysis software (TA Instruments, New Castle, DE).

2.7. Statistical analysis

All experiments were performed with solvent removal via evaporation as the control. The two groups of distillation (D1 and D2) within each respective copolymer ratio were compared to the control (E). Statistical analysis was performed on the microparticle characterization data using Two-way ANOVA, respectively, with a Bonferroni multiple comparisons post-test ($\alpha = 0.05$) via GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla, California USA, www.graphpad.com. Cumulative drug release was compared between groups at each time point by Repeated-Measures ANOVA ($\alpha = 0.05$) using GraphPad. The mean plus or minus the standard deviation (SD) or standard error of the mean (SEM) is reported.

3. Results and discussion

3.1. Particle morphology, effective hydrodynamic diameter and zeta potential

3.1.1. The effect of distillation time on microparticle size

Microparticles were formulated and characterized using two copolymers of PLGA with three processing techniques. All particles regardless of copolymer ratio or processing technique were geometrically spherical (Fig. 1). For both copolymer ratios, effective diameter increases as distillation time increases. Microparticles formulated with PLGA 75:25 are larger in effective diameter than the PLGA 50:50 of the same processing conditions (Fig. 2A). The difference in diameter is likely due to the ratio of lactic acid to glycolic acid. Glycolic acid is more mobile in nature than lactic acid facilitating more side chain interactions resulting in tighter molecular packing. Additionally, in this distillation technique THF is removed at its boiling point 65 °C. This temperature is above the melting point of lactic acid (53 °C) but below the melting point of glycolic acid (75–80 °C) [17,18]. Therefore there is structural relaxation of lactic acid groups during distillation. Structural relaxation of polymers at or above T_g in thin films and other microparticle formulations has been reported [19–21]. In the presence of solvent being removed, the lactic acid relaxation can lead to aggregation and fusion of particles that are in close proximity. This phenomenon is further escalated due to the increased hydrophobicity of the lactic acid groups.

3.1.2. The effect of copolymer ratio on microparticle stability

The zeta potential of each group of microparticles formulated with PLGA 50:50 are not statistically different (Fig. 2B). However the zeta potential of the distillation groups with PLGA 75:25 are significantly different from their control. There is no significant difference in zeta potential between PLGA 50:50 and 75:25 control groups. The change in surface charge is likely attributed to the conformational changes of the carbonyl and hydroxyl groups of the lactic acid molecules. In the 50:50 formulation groups, there is no significant difference in zeta potential between groups, all of which can be categorized as moderately stable with values approximately −21 to −26 mV. The solvent removed via evaporation in 75:25 can be considered in the same stability. The zeta potential of the two 75:25 distilled groups significantly changes to approximately −1 mV which is classified as rapid coagulation or flocculation. A wide range of values for zeta potential (+14 to −73 mV) have been reported for PLGA microparticle formulations using a solvent displacement technique [22–24]. The variation in zeta potential is attributed to solvent and therapeutic agent choice. The effect of processing on the zeta potential of microparticles formulated from the same materials has not been adequately addressed in literature. The microparticles formulated in this method fall within reported ranges [22–24].

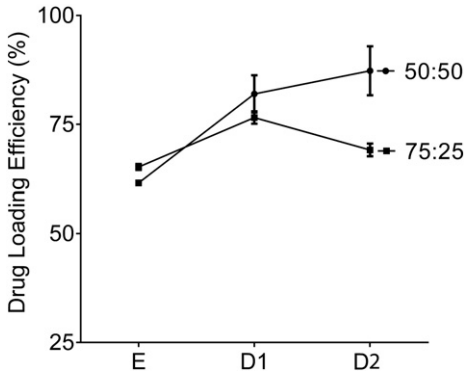

Fig. 3. Two-way ANOVA results for drug loading efficiency of microparticle groups (data shown mean ± SD, n = 10).

3.2. Drug loading efficiency

3.2.1. The effect of copolymer ratio on dexamethasone loading efficiency

Copolymer ratio and solvent removal technique both play a significant role in drug loading efficiency. In the statistical analysis an interaction was found in the Two-way ANOVA (Fig. 3) thus a pairwise One-way ANOVA was used instead (Table 1). Microparticle groups formulated from PLGA 50:50 with distillation have increased drug loading efficiencies compared to the control. There is no difference in drug loading efficiency between PLGA 75:25 distillation groups and control (Table 1).

Differences observed in drug loading efficiency values are likely attributed to the chemistry of the copolymer ratios. PLGA 50:50 contains a higher ratio of glycolic acid to lactic acid than PLGA 75:25. Glycolic acid is more mobile than lactic acid. Therefore PLGA 50:50 has a more mobile structure than 75:25 which allows for dexamethasone to bind to the backbone chain more efficiently. The addition of heat increases the relaxation of the backbone chain further increasing the binding efficiency. The higher ratio of lactic acid in PLGA 75:25 is responsible for the steric hindrance blocking dexamethasone from binding. Though heat is added during distillation, the backbone chain does not relax enough to overcome the steric hindrance.

Table 1
Pairwise One-way ANOVA of drug loading efficiency.

Polymer type	Pairwise comparisons		
	E vs. D1	E vs. D2	D1 vs D2
50:50	*	*	NS
75:25	NS	NS	NS

NS = not significant.
* Significant ($p < 0.05$).

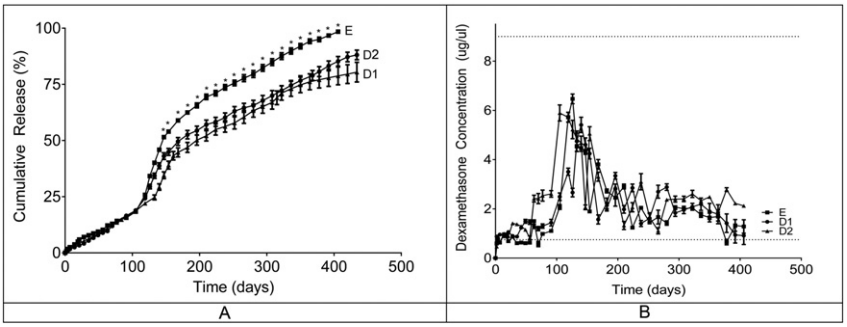

Fig. 4. PLGA 50:50 microparticle (A) cumulative dexamethasone release curves and (B) dexamethasone concentration released in adult therapeutic window 0.75–9 mg (data shown mean ± SEM, n = 10).

3.3. PLGA microparticle drug release

3.3.1. The effect of copolymer ratio on diffusion-controlled release rate

Drug release from PLGA follows diffusion-controlled release governed by copolymer composition. To release, drug molecules need to diffuse through the polymer matrix. The diffusion coefficient depends on the size of the drug molecules, pore size of the polymer matrix, drug hydrophobicity, and degradation rate of the polymer matrix [25]. In an aqueous environment, PLGA biodegrades by random hydrolytic chain scission of its ester linkages. Glycolic acid contains more of these ester linkages degrading more rapidly than the lactic acid that has an increased number of carbon–carbon linkages [26]. Lactic acid groups also are more hydrophobic due to a methyl side group, thus degrade more slowly than glycolic acid groups in water [27].

3.3.2. The effect of distillation on dexamethasone delivery

The different monomer ratios in PLGA 50:50 and 75:25 affect drug release kinetics in these microparticle formulations. Microparticles formulated with 50:50 release dexamethasone significantly faster than 75:25 (Figs. 4A, 5A). These results are in agreement with prior findings investigating lactic acid and glycolic acid ratio and particle drug release [23]. Within each copolymer ratio, dexamethasone releases significantly faster from microparticles with solvent removed by evaporation compared to distillation (Figs. 4A, 5A). According to manufacturer without any manipulation or processing PLGA 50:50 should degrade by three months and 75:25 by six months [28]. Solvent removal via distillation considerably increases the lifetime of the polymer product as a microparticle. As prior described, rapid solvent extraction during the formation of PLGA microparticles is analogous to thermal quenching and has been mathematically modeled [21,29]. When a microparticle is thermally quenched, particle density increases due to structural relaxation. The increase in density not only slows polymer degradation but limits the ability for dexamethasone to diffuse through the polymer matrix, slowing drug release.

3.3.3. The effect of distillation on microparticle longevity

The majority of PLGA microparticle developments deliver therapeutic agents for short-term applications ranging from a few weeks to months. For example, using an oil–water emulsion solvent evaporation technique, a two week release of dexamethasone was achieved from PLGA particles embedded into an alginate hydrogel for neural inflammation [23]. Using the same technique, a one month dexamethasone release was achieved for local inflammation and implantable medical devices [10,11]. A six week release formulation for cataract inflammation suppression was developed via oil–water solvent extraction method [30]. In the early exploration of microparticles formulation, it was anticipated that PLGA 75:25 microspheres may controllably release drug over one year using a spray drying technique [31]. Limited studies have been published with PLGA microparticles that last beyond several months. PLGA microspheres made by a double emulsion technique for delivery of a Hepatitis B vaccine successfully released antigen for 100 days [32]. Using a solvent displacement technique with evaporation a four month release of orntide was achieved from PLGA microspheres [33]. Few papers have reported immunological memory of antigens encapsulated in PLGA microparticles after one year but not necessarily successful delivery and release [34,35]. In our formulation, dexamethasone release at each time point for PLGA 50:50 formulation groups (Fig. 4B) fall into known adult dosage (0.75–9 mg) and PLGA 75:25 groups (Fig. 5B) fall into known pediatric dosage (0.3–2 mg) for airway disease intervention [7,36]. This formulation technique ensures therapeutic delivery of dexamethasone for long-term applications.

3.4. Polymer thermal properties

3.4.1. The relationship between T_g and microparticle degradation

This study focused on three factors than can generate a shift in the T_g of the polymer: dexamethasone incorporation, processing, and copolymer ratio. DSC has provided valuable insight on polymer behavior when manipulated with various processing conditions and techniques

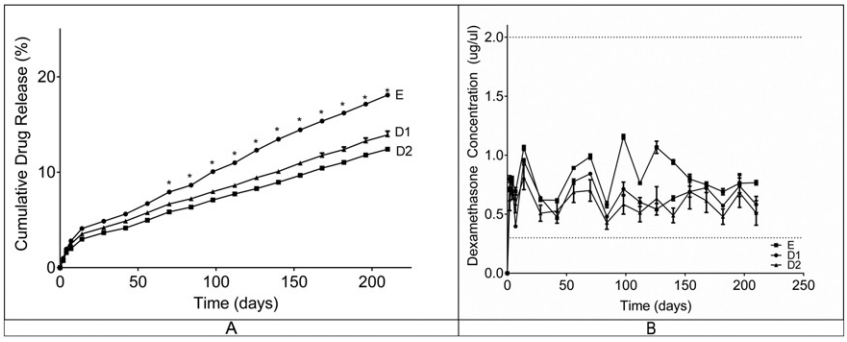

Fig. 5. PLGA 75:25 microparticle (A) cumulative dexamethasone release curves and (B) dexamethasone concentration released in pediatric therapeutic window 0.3–2 mg (data shown mean ± SEM, n = 10).

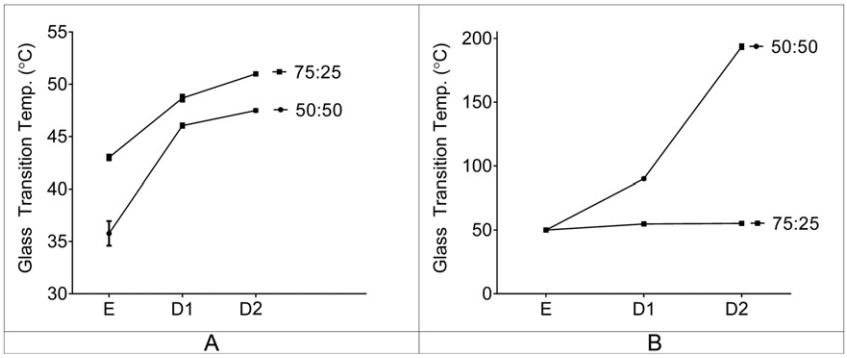

Fig. 6. Two-way ANOVA results for glass transition temperature (T_g) of microparticle groups formulation (A) without dexamethasone and (B) with dexamethasone (data shown mean ± SEM, n = 5).

[12–14,37]. The higher the T_g, the more hindered the motion of the chains around the polymer backbone. [38] Conversely, the lower the T_g the polymer side chains are more flexible providing ample motion leading to limited stability and thus faster degradation. With faster degradation comes the risk of adverse toxic effects which include increased local acidity, uncontrollable drug release kinetics, and decreased device lifetime due to increased degradation by autocatalysis. In polymeric drug delivery systems it is critical to strike a balance in which delivery vehicles degrade and therapeutic agents are released in a controlled manner leading to minimal side effects.

3.4.2. The effect of dexamethasone loading on polymer chain mobility

The incorporation of a drug into a polymer can significantly alter chain mobility, ergo shift T_g. There is a significant increase in T_g when dexamethasone is loaded into the microparticles compared to unloaded microparticles (Fig. 6). Results for dexamethasone-loaded microparticles reveal a significant increase in T_g in PLGA 50:50 microparticles with distillation but not PLGA 75:25. The incorporation of dexamethasone into PLGA 50:50 decreases chain mobility leading to a large increase in T_g. The mobility of PLGA 75:25 chains is limited; therefore dexamethasone does not further decrease mobility.

3.4.3. The effect of distillation on drug release

Processing technique is the most influential factor in particle T_g. An increase in T_g is observed in 50:50 as a function of time in which solvent removal via distillation occurs with a similar weak trend in 75:25 (Fig. 6B). Solvent extraction by distillation leads to molecular events similar to annealing. An increase in crystallinity and degradation time has been shown in annealed polyester fibers as a function of annealing temperature [37]. PLGA quenching via solvent removal is analogous to thermal quenching of pure amorphous polymer from a molten state [21]. It is reasonable that an increase in crystallinity or molecular stability by quenching occurs in particles formulated from this method. Polymer crystallinity by X-ray diffraction was not determined in this study but may provide further insight for future work regarding microparticle degradation kinetics. One major limitation of this research is that heat sensitive therapeutic agents (such as biomolecules) can be destroyed during the distillation step of this technique. Therefore this technique is limited to microparticle formulation with therapeutic agents that can resist heat up to 65 °C and retain their bioactivity.

3.4.4. The relationship between copolymer ratio and specific heat capacity

Copolymer ratio has an important role in specific heat capacity and T_g. Specific heat capacity is the amount of heat energy required to change the temperature of a substance. DSC curves reveal that these copolymer ratios have different specific heat capacities. In agreement with prior work, the specific heat capacity of PLGA 75:25 microparticles is greater than PLGA 50:50 (data not shown) [39]. This may explain why there is no associated change in T_g with distillation in dexamethasone-loaded 75:25 particles. PLGA 75:25 has the ability to store more energy than 50:50 and requires more energy to transition. The incorporation of dexamethasone into PLGA 75:25 does not alter its ability for energy storage but does into 50:50, hence the T_g shift.

4. Conclusion

Using this one-step solvent displacement technique with or without distillation can provide microparticles composed of PLGA with variable effective diameter, zeta potential, drug loading efficiency, and drug release lifetime. This method shows a simplified approach to modulate microparticle characteristics to tailor a specific drug release for patients. The study also demonstrated that the ratio of monomer groups is essential role in microparticle characteristics and therefore must be taken in consideration during formulation. This study was limited to the use of PLGA and dexamethasone via these techniques. Other polymers, therapeutic agents, and processing methods can also be investigated to provide similar effects for particle formulation. In future studies, these microparticles will be tested as a stent coating with intentions of designing a completely bioresorbable device system for the treatment of pediatric vascular stenosis and tracheomalacia. This drug delivery system may fulfill “unmet needs” of devices designed specifically for pediatrics. This formulation technique could provide an easy method for generating particles with a long drug release lifetime for numerous applications.

Acknowledgments

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