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Complex sameness: Separation of mixed poly(lactide-*co*-glycolide)s based on the lactide:glycolide ratio

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ABSTRACT

Poly (lactide-*co*-glycolide) (PLGA) has been used for making injectable, long-acting depot formulations for the last three decades. An in depth understanding of PLGA polymers is critical for development of depot formulations as their properties control drug release kinetics. To date, about 20 PLGA-based formulations have been approved by the U.S. Food and Drug Administration (FDA) through new drug applications, and none of them have generic counterparts on the market yet. The lack of generic PLGA products is partly due to difficulties in reverse engineering. A generic injectable PLGA product is required to establish qualitative and quantitative (Q1/Q2) sameness of PLGA to that of a reference listed drug (RLD) to obtain an approval from the FDA. Conventional characterizations of PLGA used in a formulation rely on measuring the molecular weight by gel permeation chromatography (GPC) based on polystyrene molecular weight standards, and determining the lactide:glycolide (L: G) ratio by ^{1}H NMR and the end-group by ^{13}C NMR. These approaches, however, may not be suitable or sufficient, if a formulation has more than one type of PLGA, especially when they have similar molecular weights, but different L:G ratios. Accordingly, there is a need to develop new assay methods for separating PLGAs possessing different L:G ratios when used in a drug product and characterizing individual PLGAs.

The current work identifies a series of semi-solvents which exhibit varying degrees of PLGA solubility depending on the L:G ratio of the polymer. A good solvent dissolves PLGAs with all L:G ratios ranging from 50:50 to 100:0. A semi-solvent dissolves PLGAs with only certain L:G ratios. Almost all semi-solvents identified in this study increase their PLGA solubility as the L:G ratio increases, i.e., the lactide content increases. This lactoselectivity, favoring higher L:G ratios, has been applied for separating individual PLGAs in a given depot formulation, leading to analysis of each type of PLGA. This semi-solvent method allows a simple, practical benchtop separation of PLGAs of varying L:G ratios. This method enables isolation and identification of individual PLGAs from a complex mixture that is critical for the quality control of PLGA formulations, as well as reverse engineering for generic products to establish the Q1/Q2 sameness.

1. Introduction

Parental depot formulations are designed to deliver drugs for prolonged durations ranging from a week to 6 months, which can be useful to increase patient compliance and reduce potential side effects. These formulations are administered into patients, typically by intramuscular or subcutaneous injection. Most of the depot formulations involve the use of a delivery vehicle (e.g., polymer or lipid), where the active pharmaceutical ingredient (API) is embedded or encapsulated. To date, one of the most widely used excipients for long-acting depot formulations is poly(lactide-*co*-glycolide) (PLGA) [1–5]. PLGAs hydrolyze into lactic acid and glycolic acid post administration. In general, critical properties of PLGA polymers and PLGA microparticles include, but may not be limited to, molecular weight [6,7], lactide:glycolide (L:G) ratio [8,9], monomer sequence [10], polymer structure [11–15], end group [16,17], crystallinity [6,18,19], and glass transition temperature [19,20]. Although each of these properties can affect drug release mechanisms of a formulation, the magnitude of each impact may vary. In addition, the properties of PLGA can be altered during manufacturing processes, as it may undergo degradation and/or a certain fraction of the polymer may be removed. Therefore, depending on the processing condition, the PLGA in the finished drug product may no longer be

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representative of the original PLGA in terms of certain key characteristics, such as molecular weight, molecular weight distribution, and L:G ratio.

The first PLGA-based depot formulation approved by the U.S. Food and Drug Administration (FDA) in 1989 was Lupron Depot® delivering leuprolide acetate for 1 month [21]. Since then, about 20 long-acting injectable PLGA formulations have been developed in the form of microparticles (Lupron Depot®, Sandostatin LAR® Depot, Nutropin Depot®, Trelstar®, Somatulin Depot®, Arestin®, Risperidal Consta®, Vivitrol®, Bydureon®, Lupaneta Pack®, Signifor® LAR, and Zilretta®), in situ forming implants (Atridox®, Eligard®, Sublocade®, and Perseris®), or solid implants (Zoladex Depot®, Ozurdex®, and Propel®). Despite their three decades of use, none of these brand drugs have generic counterparts on the market yet, in part, due to difficulties in reverse engineering of these drug products. To obtain approval under Section 505(j) of the Federal Food, Drug, and Cosmetic Act, an injectable generic PLGA drug product must contain the same inactive ingredients and in the same concentration as the reference listed drug (RLD). However, an applicant may seek approval of a drug product that differs from the RLD in preservative, buffer, and antioxidant provided that the applicant identifies and characterizes the differences and provides information demonstrating that the differences do not affect the safety or efficacy of the proposed drug product. Unlike other compendial or simple excipients, demonstrating Q1/Q2 sameness of the PLGA can be challenging considering the inherent heterogeneity of PLGA as a random copolymer.

The PLGA from a final formulation can be determined by extraction of PLGA from the formulation, purification to remove an API and other excipients, and characterization of the polymer using analysis techniques, such as gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) [3,22]. These methods, however, are unsuitable for formulations which include more than one type of PLGA in a formulation. For these complex formulations, different PLGAs must first be separated from one another, and then each can be characterized separately. GPC cannot separate different PLGAs having similar molecular weights, but different L:G ratios. Gradient polymer elution chromatography (GPEC) has been suggested as a tool to separate copolymers based on the composition, e.g., PLGAs with different L:G ratios, through application of a gradient of eluents from a poor solvent for the PLGAs to a good solvent [23]. An eluent of methanol and tetrahydrofuran was used to obtain a linear relationship between the retention time and the L:G ratio. Although the retention time of individual PLGAs was shortened, as the L:G ratio increased from 50:50 to 100:0, the peaks were wide and overlapped, making it difficult to separate unknown PLGAs from a mixture.

This study identifies different solvents that can separate PLGAs with different L:G ratios. Different PLGAs can be separated by a unique solubility of each PLGA in different solvents based on the L:G ratio. It is common to distinguish solvents for PLGA as a good solvent (e.g., dichloromethane or acetone) or a poor solvent (or non-solvent) (e.g., hexane or water). The Hansen solubility parameters are commonly used to study the solvent quality of polymers, including PLGAs [24–27]. The quantitative comparison of PLGAs with different L:G ratios, however, is rather difficult due to qualitative determination, usually binary yes/no tests, of the solvent quality. Many solvents, however, are able to dissolve certain PLGAs depending on their L:G ratio [28,29], and these solvents are referred to as “semi-solvents” [30]. Most semi-solvents show a preference of high lactide content, i.e., lacto-selectivity. The lacto-selective semi-solvent effects were employed to separate and characterize PLGAs of different L:G ratios used in a Trelstar® 22.5 mg formulation.

Table 1
PLGA polymers used for testing solvent solubility.

#	L:G ratio (by ^{1}H NMR)	Molecular weight (M_w by GPC-ES)[a]	Manufacturer (Cat#)[b]
1	50:50	60,889 Da	Evonik (RG504H, Lot# D160400525)
2	50:50	77,954 Da	Evonik (RG504H, Lot# R140800515)
3	50:50	30,327 Da	PolySciTech (AP041)
4	51:49	87,580 Da	PolySciTech (AP154)
5	57:43	87,391 Da	PolySciTech (AP155)
6	60:40	26,199 Da	PolySciTech (AP196)
7	65:35	93,068 Da	PolySciTech (AP220)
8	69:31	65,608 Da	PolySciTech (AP226)
9	75:25	17,503 Da	PolySciTech (AP165)
10	75:25	72,176 Da	Evonik (RG755S)
11	78:22	89,288 Da	PolySciTech (AP018)
12	80:20	73,202 Da	PolySciTech (AP207)
13	81:19	25,300 Da	PolySciTech (AP084)
14	84:16	64,967 Da	PolySciTech (AP087)
15	85:15	75,554 Da	Lactel (A15–015)
16	88:12	80,885 Da	PolySciTech (AP085)
17	94:6	23,063 Da	PolySciTech (AP120)
18	100:0	30,747 Da	PolySciTech (AP156)
19	100:0	78,819 Da	PolySciTech (AP002, Lot# 41028SMS)

[a] Weight average molecular weight by GPC using polystyrene external standards (GPC-ES).

[b] The characterization information of PLGAs manufactured by PolySciTech can be found on https://akinainc.com/polyscitech/products/polyvivo/polyesters.php.

2. Experimental

2.1. Materials

PLGAs used in this project were obtained from Evonik (Essen, Germany), Lactel (Birmingham, AL), and PolySciTech (West Lafayette, IN, U.S.A.). Unless otherwise specified, all references to ‘lactide’ in this manuscript, either as a homopolymer or as a copolymer with glycolide, are for the “DL” form of this monomer. The polymers used in this study are listed in Table 1. The molecular weights in Table 1 were measured by GPC using polystyrene external standards (EasiCal from Agilent) (GPC-ES). Acetone, dichloromethane (DCM), hexane, tetrahydrofuran, and xylenes were purchased from Fisher Scientific (Chicago, IL). Ethanol (200 proof) was obtained from Decon Laboratories, Inc. (King of Prussia, PA). Benzyl alcohol (analytical reagent grade) was obtained from Mallinckrodt Chemicals (St. Louis, MO). Butyl acetate (anhydrous, ≥99%), isopentyl acetate (reagent grade, 98%), methanol, *n*-methyl-2-pyrrolidone, 2-pentanone (Ultrapure grade, ≥99%), 2-butanone (MEK, ACS reagent grade, ≤99.0%) and poly(vinyl alcohol) (PVA, Mowiol 4–88, ~31,000 Da) were purchased from Sigma-Aldrich (St. Louis, MO). Toluene (extra dry, 99.85%) was purchased from Acros Organics (Pittsburgh, PA). Deionized water was obtained from a Barnstead Easypure II system (Chicago, IL). Mannitol (USP grade) was obtained from SPI Pharma (Grand Haven, MI). Polysorbate 80 was from Fluka (St. Louis, MO). Sodium carboxymethyl cellulose (CMC, Walocel™ CRT 1000PA) was from Dow Chemical (Kankakee, IL). Unless otherwise specified, all other chemicals were of reagent grade and used as received. Trelstar® 3.75 mg (Actavis Pharma, Inc., lot # 14–005656), 11.25 mg (Actavis, lot #14–006140), and 22.5 mg (Allergan USA, lot#16–008386) formulations were purchased through the Purdue University Pharmacy to examine the PLGA properties, and additional Trelstar® 22.5 mg doses (Allergan USA, lot#14–005911) were purchased through We Pharma (Morrisville, NC).

2.2. PLGA solubility in solvents

The PLGA solubility was measured in a variety of solvents using PLGAs with the L:G ratios ranging from 50:50 to 100:0 as listed in Table 1. Each polymer (100 mg) was placed in a tared 20 mL glass vial and 4 mL of the test solvent was added to each polymer at a fixed concentration of 25 mg/mL. The vials were agitated in an orbital-agitating incubator at 100 rpm at 30, 40, or 50 °C for 16–24 h. Subsequently, the vials were carefully decanted to remove solvent and dried to a constant mass in a vacuum oven (Temperature 40–50 °C, −780 mmHg) prior to weighing. The solubility was calculated by percent mass dissolved by measuring the initial mass and the final mass of the polymer remaining in the vial after incubation with a solvent.

2.3. Monomer sequence distribution by nuclear magnetic resonance (NMR)

Each PLGA sample (5–10 mg) was dissolved in deuterated chloroform ($CDCl_3$) (0.8 mL) and pipetted into a 7 in × 5 mm NMR tube. NMR scanning was performed using a Bruker AV-III-500-HD NMR spectrometer running TopSpin software (version 3.2) equipped with 5 mm Broad Band Fluorine Observation *Z*-gradient cryoprobe Prodigy™ (the PINMRF group, Purdue University) for ^{1}H NMR. These spectra were used to determine an L:G ratio as previously described [3]. Briefly, the integrated areas of the peak at 5.2 ppm (1H) and at 4.8 ppm (2H) were compared to directly confirm the exact L:G ratio of each PLGA. The mole fraction of lactide (M_L) was calculated using the integrated areas of the peaks of lactide (P_L) and glycolide (P_G): $M_L = P_L/(P_L + (P_G/2))$. The monomer sequence distribution, i.e., glycolide-glycolide (G-G) or glycolide-lactide (G-L) blockiness were determined by ^{13}C NMR according to a previously described method [9]. Briefly, the spectra were collected from 50 mg/mL polymer solutions in $CDCl_3$ using a Bruker Avance-III-800 MHz spectrometer. This was done by using a pulse sequence without NOE enhancement (Bruker pulse program zgig30), and employing an ca. 30-degree ^{13}C observe pulse, a 4.6 s relaxation delay, and a 0.4 s acquisition time.

2.4. Molecular weight of PLGAs by gel-permeation chromatography-external standard (GPC-ES) and gel-permeation chromatography-quadruple detector (GPC-4D)

The molecular weights of the polymers were determined as previously described [3]. Briefly, a Breeze-2 Water's GPC system comprising of a model 1515 isocratic pump, model 2707 autosampler, and model 2414 RI detector had tetrahydrofuran (THF) mobile phase pumped over three sequential GPC columns. These were a Phenogel 5 µm packed 50 Å pore-size (300 mm × 7.5 mm), a Phenogel 5 µm packed 1 µm (10e4 Å) pore-size (300 mm × 7.5 mm, Phenomenex), and an Aglient Resipore 3 µm mixed pore-sizes (300 mm × 7.5 mm) columns. The injection volume was 100 µL of a 2 mg/mL solution in DCM. Agilent EasiCal polystyrene external standards were used to calibrate the system, and the number average molecular weight, weight average molecular weight, and polydispersity were determined using Water's Empower software.

The molecular weights of PLGAs extracted from Trelstar® were determined by a GPC-4D system, consisting of an Agilent 1260 Infinity II HPLC system connected to Dawn Heleos II (MALLS) coupled to Dynapro Nanostar DLS via an optical cable, Optilab T-rEX (RI detector) and Viscostar III viscometer operated by Astra 7 software (Wyatt). Separation was performed with a linear gradient column (Tosoh Bioscience LLC, TSKgel GMH_{HR}-L, 7.8 mm × 30 cm). Samples were prepared in acetone at a concentration of ~2.5 mg/mL. Samples were analyzed with a flow rate of 0.6 mL/min of acetone using triplicate 50 µL injections.

2.5. Hansen solubility parameters of PLGAs

The solvent quality for a PLGA is reflected by the solubility of the polymer in the solvent. However, the solubility of polymers is difficult to determine experimentally because of their high solution viscosity. The solvent quality for polymers has been reflected empirically by qualitative solubility parameters, such as visual observation of swelling and turbidity, making it difficult for quantitative comparison of PLGAs with different L:G ratios [28]. In these yes/no solubility tests, if the polymer swells, the solvent has been treated as a good solvent, even though the polymer is not fully dissolved [27]. In this study, the solubility of PLGAs in different solvents was measured by testing whether a solvent dissolves a PLGA at the concentration of 25 mg/mL. In calculating the Hansen solubility parameters, only those solvents that dissolve PLGAs at least 17.5 mg/mL (70% of 25 mg/mL) and above are treated as good solvents. The threshold of 17.5 mg/mL was chosen, because it allows distinction of “soluble” from “partially soluble” or “swellable”, making it easier to separate PLGAs with different L:G ratios using different solvents as described below in Sections 3.2. PLGA solubility in solvents and 3.3 Hansen solubility parameters. The calculation was done using the Hansen Solubility Parameters in Practice (HSPiP) program [31]. The HSPiP program contains all the solubility parameters of various solvents [24–26].

2.6. PLGA separation and purification from microparticles made of known PLGAs

To confirm that the semi-solvent approach can be used to separate PLGAs from the microparticles, mixtures of microparticles made of different PLGAs were prepared. PLGAs used for making microparticles were #1, #5, #11, #14, #15, and #19 in Table 1. The molecular weight of these PLGAs ranges from 61,891 Da to 89,288 Da. These molecular weights were chosen, as our analysis of Trelstar® indicated that its weight average molecular weight was in the range of 70,000–90,000 Da. The L:G ratio of these PLGAs are 50:50, 57:43, 69:31, 78:22, 84;16, 85:15, and 100:0. When the two PLGAs with different L:G ratios were mixed, the mixture is identified by the lactide content of each PLGA, e.g., 50 L/85 L indicates mixture of PLGA 50:50 and PLGA 85:15.

Microparticles of each PLGA were prepared by a conventional emulsion technique. This was done by initially dissolving PLGAs in DCM at 18% (*w/v*) concentration. Polylithic microparticles were generated by co-dissolving two different PLGAs in DCM at 9% (w/v) concentration for each to yield an 18% w/v total solids in DCM. Separately, a 0.5% w/v PVA (Mowiol 4–88, Aldrich) was prepared in deionized (DI) water. Mixing was performed in a 4 L beaker (United, BG1000) with propeller paddle (3 bladed, 19 mm long × 18 mm wide paddles 34° angle) was placed in beaker ¾ of the way deep (18–22 cm) and slightly off-center at 1/3 distance from wall (4–6 cm). The beaker was filled with 4 L of 0.5% PVA and stirred at 1000 RPM by an overhead stirrer (Southwest Science model SOS20). The PLGA-DCM solution (45 mL) was quickly injected via 1.5-in.-long/18 gauge needle into the middle of the beaker with the tip beneath the surface of the rapidly stirring water solution. Stirring proceeded for 5 h at room temperature under a fume hood to remove any solvent, subsequently, stirring stopped and the microparticle solution was passed through a #20 mesh (850 µm) stainless steel sieve and then through a 7 µm nylon mesh (component supply) to collect the particles. The particles were rinsed with deionized water, collected into 50 mL centrifuge tubes with extra deionized water, vortexed to mix and then centrifuged at 2000 RPM to collect. The supernatant was discarded and the washing process repeated two more times for a total of three washings. The washed particles were dried in a vacuum desiccator over calcium sulfate (Drierite) under reduced pressure (75 Torr, KNF vacuum pump) for 3 days to remove residual water. In another approach, microparticles were prepared using individual PLGA, and different microparticles were mixed to generate different

Table 2
Microparticle formulations used for validation of the benchtop separation method.

Formulation	Polymer 1 (mg)	Polymer 2 (mg)	Mannitol (mg)	Na CMC (mg)	Polysorbate 80 (mg)
50 L/78 L	50 L (1,125.8)	78 L (1,126.5)	916.8	323.6	22.0
50 L/85 L	50 L (974.1)	85 L (971.9)	790.6	273.0	25.6
50 L/100 L	50 L (1,122.4)	100 L (1,122.7)	915.6	325.1	22.3
57 L/78 L	57 L (564.1)	78 L (562.3)	456.2	160.5	24.2
57 L/84 L[a]	57 L (1,124.9)	84 L (1,126.0)	916.0	321.1	26.4
57 L/85 L	57 L (1,123.7)	85 L (1,129.0)	917.5	314.2	23.8
78 L/84 L	78 L (562.1)	84 L (562.1)	455.6	159.0	20.0
78 L/100 L	78 L (564.6)	100 L (562.9)	457.2	160.3	13.8
84 L/100 L	84 L (562.8)	100 L (563.8)	455.1	159.9	14.4

[a] Polylithic microparticles, and all others were mixtures of individual PLGA microparticles.

formulations consisting of two different L:G ratios. Thus, PLGA microparticles containing two different L:G ratios were prepared either by making polylithic microparticles or by mixing microparticles made of each PLGA. The microparticles generated by this methodology were assayed by GPC-ES to determine molecular weight post-formulation.

Microparticles consisting of two different PLGAs with different L:G ratios were prepared using the excipients used in Trelstar® as shown in Table 2. Each formulation was carefully mixed and split into vials containing 283 mg of the mixture. The formulations in Table 2 were separated first by dissolving in DCM followed by hexane precipitation. The solid PLGA was treated with a sequence of semi-solvents, e.g., xylene, isopentyl acetate, isobutyl acetate, and chlorobenzene. The semi-solvent that dissolves PLGAs with higher L:G ratio was used first. The polymers extracted were analyzed for their mass, molecular weight, and L:G ratio, and the obtained values were compared with those of the original PLGAs.

2.7. PLGA separation and purification from Trelstar® 22.5 mg

The three dosage forms (3.75 mg, 11.5 mg and 22.5 mg) of Trelstar® underwent testing as previously described to assay the PLGA contained in each product [3]. One vial of Trelstar® 22.5 mg was opened and the contents of the vial transferred into two tared 15 mL glass centrifuge tubes containing a 3 mm glass bead. The vial of Trelstar® 22.5 mg was rinsed with 4 mL of DCM and the rinse was transferred to the 15 mL tubes containing the Trelstar® aliquots. The centrifuge tubes were vortexed and placed in a 30 °C shaking incubator set to shake at 100 rpm overnight. The next day, the tubes were centrifuged for 2 min at 3400 rpm. The supernatant was removed and filtered through a 0.22 μm PTFE syringe filter into hexane to precipitate the polymers. The hexane was contained in 15 mL glass centrifuge tubes with a 3 mm glass bead that had been previously tared. The tubes were capped and placed at 4 °C overnight. The next day, the tubes were centrifuged for 5 min at 3400 rpm and the supernatant removed. The tubes containing the polymers were dried under deep vacuum at 55 °C. After drying, a small portion (5–10 mg) of the polymers was removed for H-NMR analysis to determine the L:G ratio of the polymers.

The centrifuge tubes containing the purified polymers were massed and the amount of polymer in each tube was calculated. A solvent that dissolves PLGA with a higher L:G ratio was added first to each tube at a volume to obtain a polymer concentration of 25 mg/mL. Each tube was vortexed for at least 30 s and then the tubes were placed in a 30 °C shaking incubator set to shake at 100 rpm overnight for about 20 h. The next day, the centrifuge tubes were centrifuged for 2 min at 3400 rpm. The supernatant was transferred to a tared scintillation vial and dried by rotary evaporation, followed by deep vacuum drying at 55 °C for at least 48 h. The polymer in the vial was massed and analyzed by ^{1}H NMR for the L:G ratio and ^{13}C NMR for blockiness. After NMR analysis was completed, the sample was re-dried for GPC-TD analysis to determine molecular weight. The polymer in the glass centrifuge tubes was re-dissolved in a small quantity of DCM and rotary evaporation was applied to dry the polymer as a thin layer along the walls of the tubes. The tubes were dried under deep vacuum at 55 °C, and the mass of the dried tube was determined prior to the addition of the next solvent. This process was repeated with the other solvents that dissolve PLGAs with lower L:G ratios.

3. Results

3.1. The glycolide blockiness: glycolide sequence distribution

When the molecular weight and the polymer concentration were in the similar range, the factors that may affect PLGA solubility in different solvents include the L:G ratio and the glycolide sequence distribution, commonly known as the glycolide blockiness. As the blockiness of the glycolide increases, the PLGA solubility in solvents decreases. The blockiness was determined using the glycolide carbonyl group located at 166–167 ppm, as shown in Fig. 1 [9]. It was calculated by dividing the peak intensity of the glycolide carbonyl adjacent to another glycolide unit ($I_{G\text{-}G}$, upfield G-G peak at 166.33 ppm) by the peak intensity of the glycolide carbonyl adjacent to a lactide unit ($I_{G\text{-}L}$, downfield G-L peak at 166.41 ppm). This ratio of the two carbonyl peaks is described as the R_c value:

$$R_c = \frac{I_{G-G}}{I_{G-L}}.$$

The higher R_c value indicates the higher the degree of blockiness of PLGA. As the blockiness increases, more glycolides are aggregated by themselves and less interfaced with lactides, leading to higher heterogeneity. The R_c value can be a useful parameter for comparing glycolide sequence distribution of PLGAs, as the PLGA microstructure can affect its physicochemical properties, such as solubility and degradability [9]. In fact, the blockiness value provides an additional piece of information that is critical in determining the composition of PLGAs based on the L:G ratios. (See below Section 3.6. Identification of PLGAs Present in Trelstar® 22.5 mg formulation).

3.2. PLGA solubility in solvents

Various PLGAs with different L:G ratios were tested for their solubilities in organic solvents. Each polymer set was carefully incubated at pre-determined temperatures overnight at 100 rpm to allow ample time to reach equilibrium solubility. Afterwards, the liquid part was removed by decantation and the remaining solid was dried to a constant mass and weighed. Table 3 shows an example of solubility data for selected organic solvents to show that the solubility is a function of the temperature. The temperature effect on solubility is pronounced as shown by PLGA 50:50 in benzyl alcohol, PLGA 75:25 in n-butyl acetate and toluene, and PLGA 88:12 in 2-Heptanone. The dissolution of PLGAs in these solvents was measured at 30 °C, because it was easy to control the exact temperature using ovens. Most ovens do not have a temperature control set to 25 °C.

The solubility data in Table 3 provides insight into the selection of

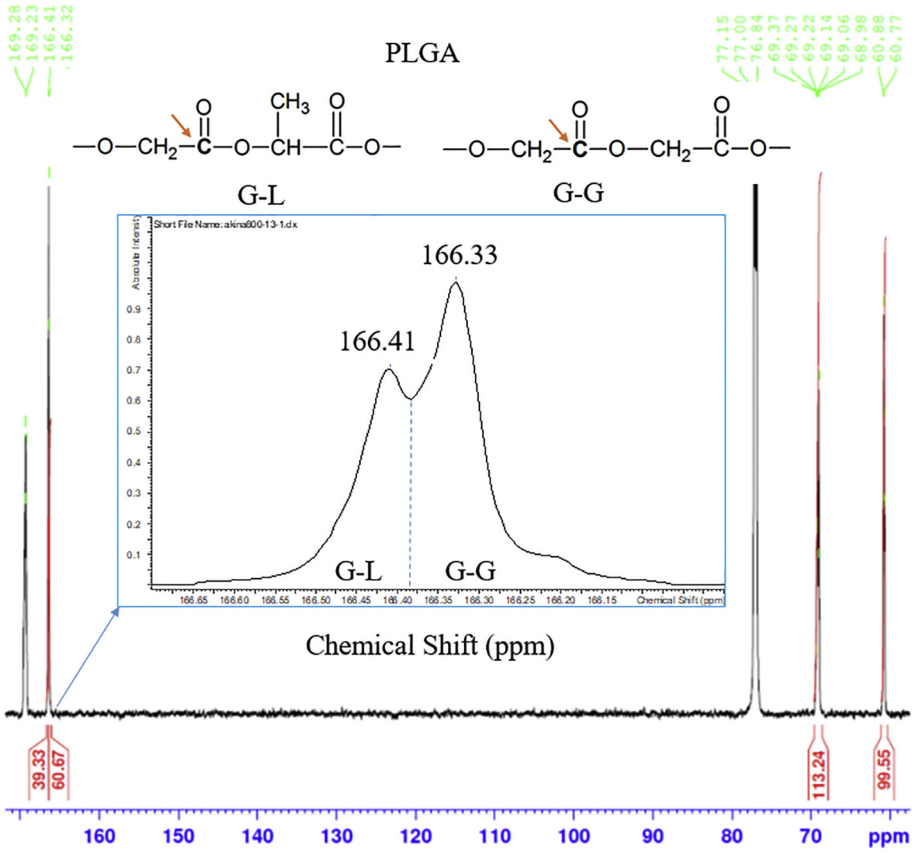

Fig. 1. Peak assignments for determining G-L and G-G from ^{13}C NMR of an example spectra obtained for PLGA 50:50 (RG504H).

Table 3
Solubility of PLGAs with different L:G ratios in solvents. The polymer concentration for testing was 25 mg/mL.

Solvent	L:G Ratio	Percent (% in *w*/w) Dissolved		
		30 °C	40 °C	50 °C
Ethyl Acetate	50:50	99 ± 0	98 ± 0	97 ± 3
Acetone		98 ± 1	97 ± 2	98 ± 3
Anisole		97 ± 0	99 ± 0	98 ± 0
Benzyl alcohol		6 ± 1	54 ± 2	98 ± 0
2-Hexanone		3 ± 1	3 ± 1	4 ± 1
Toluene		1 ± 0	1 ± 1	1 ± 2
Trichloroethylene		0 ± 2	2 ± 0	−6 ± 6
Benzyl alcohol	75:25	99 ± 1	97 ± 1	98 ± 0
Trichloroethylene		98 ± 1	99 ± 0	99 ± 0
n-Butyl acetate		53 ± 5	79 ± 7	97 ± 1
Toluene		25 ± 0	35 ± 0	47 ± 1
2-Hexanone		16 ± 1	29 ± 1	48 ± 1
Xylenes		0 ± 0	−3 ± 0	−2 ± 1
Trichloroethylene	88:12	99 ± 0	98 ± 0	97 ± 0
Toluene		94 ± 3	95 ± 1	95 ± 1
2-Hexanone		83 ± 0	90 ± 1	95 ± 0
2-Heptanone		27 ± 0	54 ± 2	73 ± 2
Xylenes		15 ± 2	33 ± 2	49 ± 3
2-Octanone		17 ± 3	17 ± 1	22 ± 1

solvents to separate PLGAs from complex formulations. Benzyl alcohol and trichloroethylene dissolve PLGA 75:25 very well, but not PLGA 50:50. Thus, they can be used to separate PLGA 75:25 from PLGA 50:50. 2-Hexanone dissolves PLGA 88:12 much better than PLGA 75:25, and thus, it can be used to separate PLGA 88:12 (or 85:15) from PLGAs with lower L:G ratios, but 2-hexanone still dissolves PLGA 75:25 at the concentration of 4 mg/mL. Thus, for separation of PLGA 85:15 from PLGA 75:25, xylene appears to be a valuable solvent as it does not dissolve PLGA 75:25 at all.

The results of the solubility of PLGAs with different L:G ratios in 20 solvents are shown in Fig. 2. Several solvents have been identified which are so-named 'full' solvents that dissolve PLGA regardless of the L:G ratio. These include acetone, acetonitrile, anisole, chloroform, DCM, dimethylformamide, dimethylsulfoxide, dioxane, ethyl acetate, formic acid, *n*-methyl-2-pyrrolidone, and triacetin. Separately, solvents including castor oil, ethanol, decanol, diethyl ether, hexane, lactic acid, methanol, and water were determined to be non-solvents which do not dissolve any PLGA. Only one solvent, PEG 400, showed a preference to dissolve PLGA with low L:G ratio over high L:G ratio.

Fig. 2 shows that solvents have a selectivity in dissolving PLGAs of a certain L:G ratio and above preferentially, but some can still dissolve a fraction of PLGAs with lower L:G ratios. For convenience, this phenomenon is termed "spillover dissolution". None of the solvents used in Fig. 2 dissolves PLGA 50:50 to 25 mg/mL. Benzyl alcohol is a good solvent that can distinguish PLGA 65:35 from PLGA 50:50. Chlorobenzene and pentanone dissolve PLGA 75:25, but not PLGA 65:35. Hexanone, 4-methyl-2-pentanone, isopentyl acetate, and pentyl acetate are able to selectively dissolve PLGA 85:15 against PLGA 75:15. Heptanone and xylenes can separate PLGA 100:0 from PLGA 80:20. As the data in Fig. 2 shows, solvents tend to dissolve PLGAs with high L:G ratios better, i.e., semi-solvents have a lacto-selectivity. The solvents with lacto-selectivity tend to have chemical structures with more hydrophobic carbon chains. As the L:G ratio decreases, the PLGA solubility decreases. Thus, separation of different PLGAs can be done more effectively, if those solvents that dissolve PLGAs with higher L:G ratios are used first. For example, xylenes can be used first to dissolve all PLGAs with higher than an 85:15 ratio. Then, isopentyl acetate can be used to dissolve the remaining PLGAs higher than 80:20. Subsequently, toluene, butyl acetate, and pentanone can be used to separate PLGAs with 75:25, 70:30, and 65:35, respectively. Any sequence of solvents in

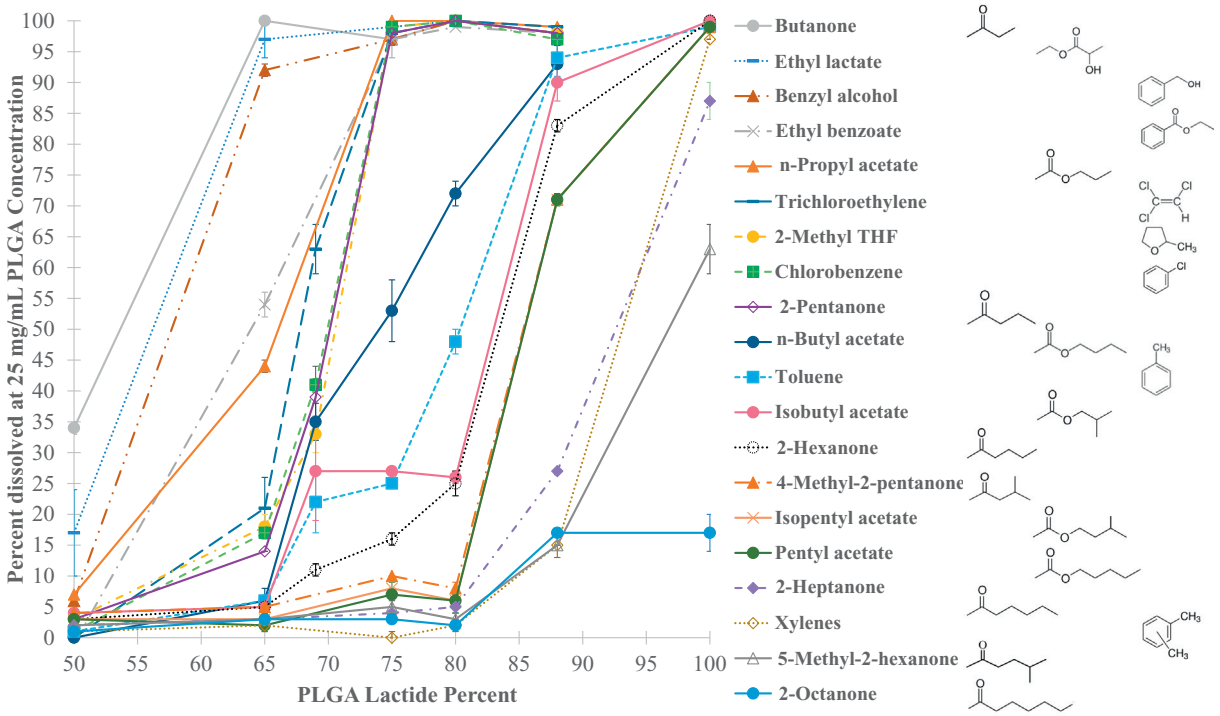

Fig. 2. Dissolution of PLGAs in solvents as a function of the lactide content (or L:G ratio) at 30 °C. The 100% dissolution indicates complete dissolution at the concentration of 25 mg/mL.

Fig. 2 can be used to dissolve PLGAs based on the lacto-selectivity.

3.3. Hansen solubility parameters

Hansen solubility parameters (HSP) are designed to consider whether a solvent is good or bad, and the HSPiP considers only a score of 1 (good) or 0 (bad). This binary option may be acceptable in identifying solvents that interact with the polymer of interest, in this case PLGAs with different L:G ratios. As shown in Fig. 2 above, the same solvent may have different solubility depending on the L:G ratio. Thus, even though the solvent may be considered good in the Hansen score, it may actually be not good enough to dissolve a PLGA to the 25 mg/mL concentration. A good solvent needs to be distinguished from a not-good-enough solvent. Those solvents that dissolve PLGA of different L:G ratios at ≥17.5 mg/mL were chosen to be good solvents, because they allow separation of PLGAs with different L:G ratios, as shown in Fig. 2. The Hansen solubility parameters of the solvents used in Fig. 2 are listed in Table 4. If a solvent is a good solvent for PLGA with a low L:G ratio, it is also a good solvent for PLGAs with higher L:G ratios, but not vice versa. For example, n-pentanone is a good solvent for PLGA with the L:G ratio of 75:25 and higher, but it is a poor solvent for PLGAs 65:35 and 50:50.

The Hansen solubility sphere of each PLGA was calculated using HSPiP. Fig. 3 shows the solubility spheres of 5 different L:G ratios using good solvents listed in Table 4. The good solvents for PLGAs with lower L:G ratios are also good solvents for the PLGAs with higher L:G ratios. For example, butanone (= methyl ethyl ketone), ethyl lactate, and benzyl alcohol are good solvents for PLGA 65:35, and thus, they are also good solvents for PLGA 75:25 and higher L:G ratios. Since no solvents used in Fig. 2 dissolve PLGA 50:50 > 17.5 mg/mL, dichloromethane and *N*-methyl-2-pyrrolidone were used as good solvents. As the L:G ratio increases, more solvents are able to dissolve PLGAs and thus, the radius (R) of the sphere increases. PLGA with each L:G ratio has unique Hansen solubility parameters. However, they can be changed if more solvents are used in the calculation, although they will remain close to the original values. The important information in Fig. 3 is that many more solvents can dissolve PLGA with higher L:G ratios, and only a limited number of solvents can dissolve PLGA 50:50.

Table 4
Hansen solubility parameters of the solvents (from the HSPiP program [31]).

Solvent	δD	δP	δ_H	Good solvents for
Methylene dichloride (= dichloromethane)[a]	17.0	7.3	7.1	PLGA 50:50
N-methyl pyrrolidone	16.8	2.8	6.7	
Butanone (= methyl ethyl ketone)	16.0	9.0	5.1	PLGA 65:35
Ethyl lactate	16.0	7.6	12.5	
Benzyl alcohol	18.4	6.3	13.7	
Ethyl benzoate	17.9	6.2	6.0	PLGA 75:25
n-Propyl acetate	15.3	4.3	7.6	
Trichloroethylene	18.0	3.1	5.3	
2-Methyl tetrahydrofuran	16.9	5.0	4.3	
Chlorobenzene	19.0	4.3	2.0	
2-Pentanone (= methyl n-propyl ketone)	16.0	7.6	4.7	
n-Butyl acetate	15.8	3.7	6.3	PLGA 85:15
Toluene	18.0	1.4	2.0	
Isobutyl acetate	15.1	3.7	6.3	
2-Hexanone (= methyl butyl ketone)	15.3	6.1	4.1	
4-Methyl-2-pentanone (=methyl isobutyl ketone)	15.3	6.1	4.1	
Isopentyl acetate (= isoamyl acetate)	15.3	3.1	7.0	
Pentyl acetate (= amyl acetate)	15.8	3.3	6.1	
2-Heptanone (= Methyl n-amyl ketone)	16.2	5.7	4.1	PLGA 100:0
Xylenes	17.8	1.0	3.1	
5-methyl-2-hexanone (= methyl isoamyl ketone)	16.0	5.7	4.1	Limiting solvents for PLGA 100:0[c]
3-Octanone (= Ethyl amyl ketone)[b]	16.2	4.5	4.1	
Ethanol	15.8	8.8	19.4	Non-solvents
Water	15.5	16.0	42.3	

[a] The names inside parentheses are the ones used in the HSPiP.
[b] 2-Octanone was used in this study, but its Hansen solubility parameters are not available, and thus, the values for 3-octanone were used instead.
[c] Limiting solvents only partially dissolve PLGAs.

Previous studies measured the Hansen solubility parameters of

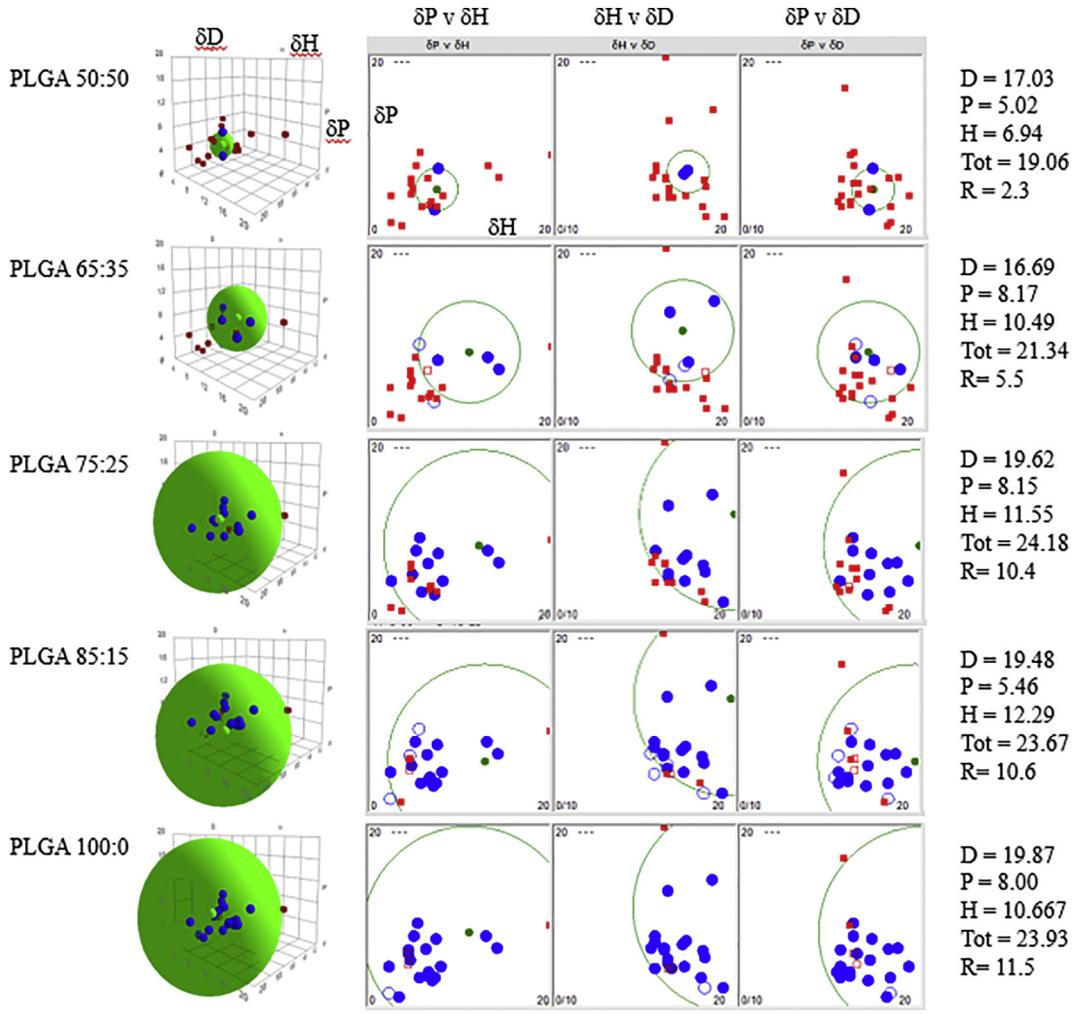

Fig. 3. 3D and 2D plots of the Hansen solubility spheres of PLGAs with different L:G ratios. The x-, y-, and z-axes represent dispersion (δD), polar (δP), and hydrogen bonding (δ_H) components, respectively, of the Hansen solubility parameters. The closed blue circles and red squares represent good solvents and poor solvents, respectively. The open blue circles and red squares represent good solvents out of the sphere (wrong outs) and poor solvents inside the sphere (wrong ins), respectively.

PLGAs, and the parameter values are different from ours [28,29]. This is due to the use of a different set of solvents and qualitative observation, such as swelling and turbidity, to determine good solvents. If limiting solvents are included in the calculation of the solubility sphere, the radius of the sphere increases more than those shown in Fig. 3 for each L:G ratio. The HSPiP separates good solvents that are not included in the solubility sphere as "wrong-out" solvents, and the poor solvents that are included as "wrong-in" solvents. Depending on the number of wrong-in and wrong-out solvents in the calculation of the solubility sphere, the radius either increases or decreases. This makes exact calculation of the solubility sphere of each L:G ratio difficult. The solubility spheres of different L:G ratios, however, can be used to find more solvents that can selectively dissolve PLGA of a specific L:G ratio. More importantly, the solubility sphere allows creation of new solvents by mixing known solvents in different ratios [32].

3.4. Validation of separation of PLGAs from known mixtures of microparticles

PLGA microparticle formulations were prepared for validation of the L:G ratio-based separation using semi-solvents. Eight different formulations in Table 2 were used for the separation study. Because the separations involved PLGAs with L:G ratios close to each other, e.g., 78:22 and 84:16 (or 78 L and 84 L), each solvent was added to the polymer at a volume to produce a 25 mg/mL polymer concentration. A sample of the polymer mixture was removed after treatment with each solvent and analyzed for the L:G ratio (or the lactide%) by ^{1}H NMR and the molecular weight by GPC-ES. Masses were carefully monitored and the weight ratios of the two different PLGAs in each formulation were determined. The results from these separations of microparticles are shown in Table 5.

The results in Table 5 indicate that the semi-solvent approach was able to separate PLGAs based on their lactide content, and the L:G ratio of a mixture of two PLGAs is pretty much the average of the two L:G ratios. The L:G ratio of Polymer 1 (i.e., lower lactide% fraction of the mixture) separated from the mixture remains close to the original L:G ratio. In general, the PLGAs with lower lactide% show slightly increased L:G ratios, and the PLGAs with higher lactide% have slightly reduced L:G ratios. This is probably due to a distribution of the L:G ratio of a given PLGA. The lactide% of PLGA 50:50 after separation ranges from 52.1% to 54.7%. On the other hand, the lactide% of PLGA 84:16 ranges from 83.4% and 84.6%. The overall trend in Table 5 indicates that the L:G ratio distribution may be wider for PLGA 50:50 than that for PLGA with higher L:G ratios. The narrower L:G ratio distribution for PLGAs with higher lactide% is understandable.

The molecular weights of the same PLGAs were also measured after each PLGA was made into microparticles and then separated from the prepared microparticles. The changes in the molecular weights before

Table 5
Separation of PLGAs based on the lactide% (L:G ratio) from microparticles mixtures.

Formulation (Polymer 1/ polymer 2)	Lactide% of mixture (polymers 1 + 2)	Lactide% of Polymer 1 (M_w by GPC-ES)	Lactide% of Polymer 2 (M_w by GPC-ES)	Weight ratio (polymer 2/ polymer 1)
50 L/78 L (50:50/78:22)	65.6 ± 0.7	53.3 ± 2.6 (63,826 ± 3246 Da)	76.2 ± 0.6 (89,652 ± 2835 Da)	1.14 ± 0.14
50 L/85 L (50:50/85:15)	68.9 ± 1.3	52.1 ± 2.3 (57,809 ± 1325 Da)	83.8 ± 0.2 (83,488 ± 2843 Da)	1.12 ± 0.25
50 L/100 L (50:50/100:0)	73.8 ± 0.4	54.7 ± 2.3 (54,583 ± 1056 Da)	97.4 ± 0.2 (119,843 ± 2023 Da)	1.3 ± 0.2
57 L/78 L (57:43/78:22)	64.9 ± 0.1	53.8 ± 0.4 (50,477 ± 1028 Da)	76.7 ± 0.4 (89,593 ± 990 Da)	1.08 ± 0.10
57 L/84 L[a] (57:43/84:16)	68.7 ± 0.1	55.6 ± 0.9 (48,405 ± 519 Da)	82.1 ± 0.7 (41,204 ± 336 Da)	1.05
57 L/85 L (57:43/85:15)	70.9 ± 1.5	56.1 ± 1.4 (53,450 ± 1112 Da)	83.1 ± 0.2 (85,528 ± 2671 Da)	1.00
78 L/84 L[b] (78:22/84:16)	81.5 ± 0.6	78.2 ± 0.3 (87,152 ± 528)	83.4 ± 0.1 (40,421 ± 503 Da)	1.21 ± 0.07
78 L/100 L (78:22/100:0)	88.7 ± 0.1	78.7 ± 0.5 (89,846 ± 1008 Da)	98 ± 0.1 (109,813 ± 6746 Da)	1.17 ± 0.14
84 L/100 L[a] (84:16/100:0)	92.0 ± 0.07	84.6 ± 0.3 (43,889 ± 643)	98.2 ± 0.1 (105,976 ± 1383 Da)	1.18 ± 0.01

[a] Polylithic microparticles, and all others were mixtures of individual PLGA microparticles.
[b] Isopentyl acetate was used for these mixtures, while isobutyl acetate was used for all other mixtures.

Table 6
Weight average molecular weights of PLGAs before and after making microparticles.

#	L:G Ratio (by ^{1}H NMR)	M_w before making microparticles by GPC-ES	M_w after separation from microparticles by GPC-ES
1	50:50	60,889 Da	61,891 Da
5[a]	57:43	87,391 Da	48,568 Da
7	65:35	93,068 Da	85,720 Da
11	78:22	89,288 Da	89,641 Da
14[a]	84:16	64,967 Da	42,452 Da
15	85:15	75,554 Da	85,720 Da
19	100:0	78,819 Da	101,116 Da

[a] The molecular weight of PLGA separated from polylithic microparticles of Polymers #5 and #14 is 44,643 Da.

and after making microparticles are shown in Table 6. It is noted that the molecular weight of PLGAs separated from microparticles changed from the original molecular weight of feed-stock (i.e., before making microparticles). Such changes in the molecular weight during the manufacturing process may be due to a variety of factors, including incomplete dissolution in solvents and removal of a certain polymer fraction during processing. The molecular weight may become smaller or higher after microparticle preparation, and the extent of changes also varies widely. Thus, it is important to characterize PLGAs after microparticles are prepared. After all, the Q1/Q2 sameness applies to the final microparticle formulations, not the raw PLGAs.

3.5. Initial analysis of PLGAs used in Trelstar® formulations

Three different Trelstar® formulations were used to isolate PLGAs in each formulation for characterization. As shown in Table 7, the PLGA used in the 3.75 mg formulation was very different from the PLGAs used in the 11.25 mg and 22.5 mg formulations. The L:G ratio of 57:43 is understandable, as most 1 month long-acting depot formulations use PLGA 50:50, which degrades relatively faster than other PLGAs with higher L:G ratios. It is also understandable that the 3-month 11.25 mg formulation is made of PLGA 78:22, which is close to the ratio of 75:25 frequently used in 3-month formulations. However, it is a bit surprising that the molecular weight and L:G ratio of the 22.5 mg formulation (for 6-month delivery) appear to be very close to those of the 11.25 mg formulation. The L:G ratio and molecular weight of the PLGA for 22.5 mg formulation is not much different from the one for the 11.25 mg formulation. This cannot explain the difference in the duration of drug release by 3 months.

Table 7
Characterization of PLGAs obtained from Trelstar® formulations by GPC-ES.

Sample	M_w (Da)	M_n (Da)	End Group	L:G Ratio (Molar)
Trelstar® 3.75 mg	85,207	25,191	Ester	57:43
Trelstar® 11.25 mg	72,286	47,214	Acid	78:22
Trelstar® 22.50 mg	74,042	46,368	Acid	80:20

According to the Trelstar® package insert, 11.25 mg and 22.5 mg formulations contain 118 mg and 182 mg PLGA, respectively. The difference in the PLGA quantity in the two formulations should not allow extension of drug release for another 3 months. The larger amount of the drug will simply result in a higher initial burst release with about the same duration of drug release, and thus, efficacy. The only logical explanation with the information on PLGA in Table 7 is that there is more than one type of PLGA in the 6-month 22.5 mg formulation, and the average of different PLGA properties results in the L:G ratio being similar to that of the 3-month 11.25 mg formulation. If this is indeed the case for the 22.5 mg formulation, then it will be extremely difficult to determine what types of PLGAs were used in the Trelstar® 22.5 mg formulation. Consequently, establishing Q1/Q2 sameness of the formulation will not be easy without a new assay method for isolating different PLGAs used in the 22.5 mg formulation and characterizing them. Thus, this study focused on developing a new assay method of fractionating PLGAs based on their molecular properties, such as molecular weight and L:G ratio.

Our initial study on the molecular weights of Trelstar® formulations using GPC-ES showed a single peak, indicating that the molecular weights of different PLGAs may be similar. In general, the higher the molecular weight, the less soluble in a given condition. The same PLGA is soluble in a solvent, if its molecular weight is low, e.g., 50,000 Da, but not soluble with higher molecular weights, e.g., 200,000 Da. In this study, the molecular weights of PLGAs were limited to 100,000 Da or less, as the known molecular weights of PLGAs used in Trelstar® ranged from 70,000–90,000 Da (Table 7). In addition, the concentrations of PLGAs tested in the solubility studies were limited to 25 mg/mL to eliminate the impact of polymer concentration on the solubility. This is also practical, since the concentration of PLGAs isolated from 22.5 mg Trelstar® formulations for characterization will not be higher than 25 mg/mL.

3.6. Identification of PLGAs present in Trelstar® 22.5 mg formulation

Since the composition of PLGAs with different L:G ratios in the Trelstar® 22.5 mg formulation was unknown, a series of solvents known to dissolve PLGAs with certain L:G ratios were used to survey which combination of solvents are most useful in separating PLGAs from Trelstar® 22.5 mg. Based on the solubility properties of solvents in Fig. 2, six different solvents were chosen to selectively dissolve PLGA with different L:G ratios, as listed in Table 8.

The L:G ratio of the xylenes-soluble fraction of Trelstar® 22.5 mg formulation was 84.0:16.0. This indicates the original PLGA ratio of the fraction was not above 85:15, since xylene dissolves PLGA 100:0, if present. The L:G ratio of the isopentyl acetate-soluble fraction was

Table 8
The L:G ratio and masses of solvent-separated PLGA fractions of Trelstar®. Each solvent was used to dissolve PLGA fractions by applying in sequence from the top of the table.

Sequence	Solvent	L:G Ratio of PLGA	Mass of PLGA (mg)	% of Total PLGA
1	Xylenes	84.0:16.0	9.3	6.3
2	Isopentyl acetate	82.9:17.1	24.8	16.4[b]
3	Toluene	82.9:17.1	37.3	24.6
4	n-Butyl acetate	74.3:25.7	19.3	12.7
5	2-Pentanone	72.6:27.4	22.0	14.5
6	Butanone[a]	70.9:29.1	38.2	25.2
	Butanone residual	70.9:29.1	0.5	0.3
			Total: 151.4[b]	Total:100.0

[a] Butanone = methyl ethyl ketone.
[b] The weight of the PLGA obtained after dissolution of Trelstar 22.5 mg in DCM followed by precipitation in hexane was 148.4 mg. The total weight of 151.4 mg is 2% higher than the initial weight, and this is most likely due to the experimental error resulting from repeated dissolution and precipitation of the PLGA in Trelstar 22.5 mg.

82.9:17.1. The lactide ratio may be lowered slightly from 85:15, because isopentyl acetate can dissolve about 2 mg/mL of PLGA 75:25. The L:G ratio of the toluene soluble fraction was also 82.9:17.1, which is the same as the isopentyl acetate-soluble portion, indicating that PLGA 85:15 was not completely removed by xylenes and isopentyl acetate. Toluene can dissolve a significant amount of PLGA 75:25 (Fig. 2), but since the L:G ratio did not drop significantly from the previous solvent determinations, the toluene appears to have preferentially dissolved the remaining PLGA 85:15. The L:G ratio of the butyl acetate-soluble fraction was 74.3:25.7. This is lowered from the previous fractions, indicating the 85% lactide PLGA was removed by the previous solvents. Butyl acetate can dissolve a significant amount of PLGA 80:20 and 75:25, but very little PLGA 65:35 and not any PLGA 50:50. Considering the fact, however, that butyl acetate can dissolve about 9 mg/mL of PLGA 70:30, the L:G ratio of the butyl acetate-soluble fraction at 74.3:25.7 requires an explanation. The PLGA 70:30 solubility of about 9 mg/mL in butyl acetate is when only it is dissolved in the solvent in the absence of other PLGAs. Thus, it appears that each solvent may preferentially dissolve a PLGA with higher L:G ratio from the mixture. The L:G ratio of the pentanone-soluble fraction was 72.6:27.4. This is lowered slightly from the previous fraction due to spillover dissolution, as pentanone can dissolve a significant amount of PLGA 75:25 and a small amount of PLGA 65:35. The small amount of dissolved third PLGA may have decreased the L:G ratio from 74.3% to 72.6%. Finally, the determined L:G ratio of the butanone-soluble fraction was 70.9:29.1. This is a significant change from the previous two fractions. Butanone dissolved almost all the remaining PLGA in the tubes. A final ^{1}H NMR analysis of the polymer remaining in the tubes also indicated a L:G ratio of 70.9:29. The solvent survey indicates a mixture of three polymers, by analyzing the abilities of the solvents used to dissolve the PLGAs, and the three polymers are most likely PLGA 85:15, 75:25, and 70:30. There is no particular reason why the L:G ratio has to be one of the three, but most commercially available PLGAs are produced with such L:G ratios. The information in Table 8, however, is still not conclusive to determine the L:G ratios of three PLGAs.

To further characterize the polymer fractions, the solvent-separated PLGA fractions underwent ^{13}C NMR analysis to calculate each fraction's R_c value. The fractions underwent further analysis by GPC-4D to determine weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity, as shown in Table 9. The R_c value of the PLGA fractions increased as the glycolide content of the PLGA increased. The R_c values of the xylenes, isopentyl acetate, and toluene fractions are about the same, indicating that the three fractions may have the same L:G ratio of 85:15. A large difference in the R_c value was seen between the toluene and n-butyl acetate fractions. The 2-pentanone fraction also has the R_c value similar to that of the n-butyl acetate fraction, suggesting that the two fractions have the same L:G ratio of 75:25. Another big change in the R_c value was seen between the 2-pentanone and butanone fractions, but the difference was not as large since the change in glycolide content is smaller between these two fractions. The data in Tables 8 and 9 collectively indicate that the three PLGAs used in Trelstar® 22.5 mg formulation have the L:G ratios of 85:15, 75:25, and 70:30.

The GPC-4D analysis showed some differences in the molecular weight of the PLGA fractions. The majority of the 85% lactide PLGA was extracted with toluene. This fraction showed a molecular weight of approximately 51,000 Da. The other two 85% PLGA fractions that were extracted with xylenes and isopentyl acetate had lower molecular weights. The molecular weight of the PLGA 75:25 extracted with n-butyl acetate and 2-pentanone was approximately 26,000–37,000 Da. The molecular weight of the 70% PLGA was approximately 55,000 Da. For the L:G 85:15 fraction, the lower molecular weight PLGA is dissolved first, followed by higher molecular weight, e.g., 17,552 in xylenes to 28,339 in isopentyl acetate, and to 51,260 in toluene. The same trend is also observed in the L:G 75:25 fraction. Thus, it appears that the PLGAs can be separated first by the L:G ratio followed by the molecular weight.

4. Discussion

4.1. Selection of solvents based on the Hansen solubility parameters

One important factor in successful separation of different PLGAs is selecting the right solvents which have preference towards lacto-selectivity or vice versa. Dissolution of a polymer in a solvent requires the energy (E_{vap}) to break all intermolecular interactions in a unit volume, which is known as the cohesive energy density (*CED*). The square root of the *CED* is the Hildebrand solubility parameter, or total solubility parameter, δ_t. The solubility parameters of polymers are difficult to obtain, because there is no measurable value of ΔH_{vap} or boiling point for polymers. Thus, indirect methods are used to obtain the values [33–35]. The classical method of determining Hansen solubility parameters of a polymer involves testing of polymer solubility in different solvents with known partial solubility parameters, or Hansen's three solubility parameters. The Hansen solubility is a theoretical and experimental extrapolation of polymer-solvent interaction based on energy contributions from dispersion interactions (δ_d), polar interactions (δ_p), and hydrogen bonding interactions (δ_H) [24–26]. The partial solubility parameters of solvents that interact with the given solvent (i.e., either dissolves the polymer or the polymer swells in the solvent) are plotted in three planes of the $2\delta_d$, δ_p and δ_H axes to form circles of radius R, which is the radius of solubility sphere, or interaction sphere. The coordinates of the center of the spheres represent Hansen solubility parameters of polymers [35]. This classical method has a shortcoming that good solvents may not be included in the interaction sphere or poor solvents may be included in the sphere. This shortcoming was overcome by calculating the maximum separation distance and using it as the diameter of the interaction sphere to find the coordinates of its middle as the Hansen parameters [36]. The limitation of this method is that the position of the middle is not well defined, if there exist several equal maximum separation distances. In addition, this method treats good and limiting solvents of a polymer with equal weight, i.e., there is no mechanism of distinguishing the good from the limiting solvents. Limiting solvents are those that have limited interactions, e.g., the polymer may not dissolve, but can swell in them [35].

Solubility parameters are frequently used to predict compatibility between polymers and solvents. The dissolution rate and extent of polymer dissolution in a solvent depend on the thermodynamic compatibility of the polymer with the solvent and the T_g of the polymer

Table 9
^{13}C NMR and GPC-4D analysis of solvent-separated PLGA fractions of Trelstar®.

	Solvent	L:G Ratio of PLGA	R_c	Weight average Mol Wt (Da)	Number average Mol Wt (Da)	Polydispersity
1	Xylenes	84.0:16.0	0.342	17,552 ± 333	15,616 ± 266	1.124 ± 0.002
2	Isopentyl acetate	82.9:17.1	0.425	28,339 ± 60	24,568 ± 113	1.148 ± 0.003
3	Toluene	82.9:17.1	0.464	51,260 ± 81	45,027 ± 189	1.138 ± 0.004
4	n-Butyl acetate	74.3:25.7	0.802	26,121 ± 184	22,690 ± 179	1.151 ± 0.001
5	2-Pentanone	72.6:27.4	0.874	37,178 ± 90	32,489 ± 171	1.144 ± 0.004
6	Butanone	70.9:29.1	1.00	55,256 ± 430	49,312 ± 408	1.120 ± 0.003

[37], the nature of solvent (solubility parameters), temperature, chemical composition and molecular structure of the polymer, and crystalline form of solid polymorphism [33]. The accuracy of the calculated polymer solubility parameters depends to a great extent on the data available [26]. Basically, the calculated values are empirical and cannot really predict the extent of solubility for the polymer of which data are not available. The solubility sphere calculated in Fig. 3 may become different, if a different set of solvents are used. The usefulness of the Hansen solubility parameters, however, is in the ability of mixing different solvents to create a new solvent that may have very different solubility parameters. The Hansen solubility parameters of a new solvent can be calculated by the volume fractions of different solvents [32]. Thus, for better separation of PLGAs with different L:G ratios, new solvents can be created of which position in the solubility sphere is between the two spheres. The Hansen solubility parameters are also useful in understanding and predicting drug distribution in PLGA microparticles [38].

It is difficult to describe the solubility of PLGAs with a simple binary (yes/no) solubility profile for a series of solvents. A PLGA may dissolve, but to different extents ranging from > 0 to < 100%. In traditional Hansen solubility testing, the binary classification, i.e., yes for even a partially soluble solvent, may have been adequate, but separation of PLGAs based on the L:G ratios requires a more definite criterion. The effect of the L:G ratio on the PLGA solubility in a range of solvents has been determined in the current study. Due to a lack of existing nomenclature, such semi-solvents are referred to as “lacto-selective” solvents given the strong preference for dissolution of PLGA with high L:G ratio over the low lactide content. Trends in lacto-selectivity were observed, especially for ketones and esters. For ketones, each incremental addition of a methyl unit led to an increased requirement for lactide content to enable solubility. The simplest ketone, acetone, is a full solvent for all PLGAs. Adding a single methyl generates methyl ethyl ketone (=butanone) which has poor solubility for PLGA 50:50, but does dissolve PLGA 60:40 and above. Pentanone exhibits good dissolution (> 17.5 mg/mL) starting from 75:25, hexanone exhibits good dissolution starting from 85:15 lactide and heptanone exhibits good dissolution only at 100:0, i.e., poly(D,L-lactide). Likewise, esters exhibit a similar profile of increasing lacto-selectivity with increasing length (ethyl acetate < propyl acetate < butyl acetate < pentyl acetate). In most cases, isomers exhibit higher lacto-selectivity, and lower solubility, than their straight-chain equivalent (i.e., isobutyl acetate vs. butyl acetate, hexanone vs. 4-methyl-2-pentanone), although there is little difference in the case of pentyl acetate versus isopentyl acetate.

4.2. Semi-solvents for lacto-selectivity

The effect of temperature seems most prevalent at the ‘edges’ of lacto-selectivity, that is, PLGAs which possess just barely enough lactide to enable partial solubility in a given solvent. The effect generally followed expectations in which increasing temperature did lead to an increase in solubility. Increased temperature, however, could not overcome the effects of lacto-selectivity. For example, PLGA 50:50 exhibits nearly complete insolubility in toluene at all tested temperatures. To test this effect, we attempted dissolution of PLGA 50:50 under an extreme temperature of boiling-reflux conditions overnight. Even under this condition, the toluene failed to dissolve the PLGA 50:50. Conversely, PLGA with high L:G ratios are readily soluble in toluene, even at room temperature.

There are many practical applications for the semi-solvent effect, one of which is the sequential dissolution of PLGAs with varying L:G ratios from a clinical formulation and analysis of each polymer individually. To investigate this potential, we first investigated the effect of lacto-selectivity on microparticles. The microparticles were observed to obey the same trends as the bulk materials in that high-lactide microparticles dissolved readily in semi-solvent toluene while low-lactide microparticles remained undissolved. Subsequently, a series of test formulations with known mixtures of different types of PLGAs were generated and made into test-samples spiked with other excipients and contaminants which would commonly be found in a clinical formulation. Polymer extraction and purification techniques were applied successfully to obtain PLGAs separated from other excipients. The formulations were subsequently separated into their constituent PLGAs by use of a lacto-selective solvent for further characterization. Separation was easily achieved both for dry-mixes of separate PLGA powders, as well as for microparticles comprised of multiple types of PLGA. This separation was accomplished using conventional bench-top laboratory techniques and represents a fairly straight-forward and practical means towards de-formulation and assay of clinical products.

The exact, underlying mechanism which causes lacto-selectivity remains to be elucidated. One factor may be the formation of glycolide blocks, i.e., blockiness, within the PLGA chains. Polyglycolide tends to exhibit very poor solubility due to the formation of a crystalline structure [39]. Glycolide blocks within PLGA copolymers can arrange preferentially, thus forming crystalline domains that are difficult for solvent molecules to enter. Measurement of blockiness, R_c, indicated increasing presence of glycolide-glycolide linkages with increasing glycolide content within the PLGAs. This is likely a contributing factor to the overall reduced solubility of PLGA 50:50 in the identified semi-solvents in this study. The R_c values provide critical information supplementing the data on the L:G ratio of separated PLGA fractions.

Considering the fact that commercially available PLGAs are based on the L:G ratios of 50:50, 60:40, 65:35, 75:25, 85:15, 90:10, and 100:0, most PLGAs currently used in commercial products can be separated. It is possible that a formulation scientist chooses to use PLGAs with close L:G ratios, e.g., 55:45 and 60:40, or 75:25 and 80:20, but the two PLGAs may have very similar properties, and thus, the separation of those PLGAs may not be necessary. The reason for separating PLGAs with different L:G ratios is to identify them and understand the final behavior of the mixed PLGAs in general for quality control, as well as reproducibility of a formulation. More studies are needed to identify solvent systems that can separate PLGAs with very close L:G ratios. However, the current solvents used in Fig. 2 that can separate PLGAs with the L:G ratios of 50:50, 65:35, 75:25, 85:15, and 100:0 will be suitable for separation and characterization of PLGAs in products currently in clinical use. Further studies are necessary to find semi-solvents that can separate PLGAs with the incremental difference in the lactide content of 5, e.g., the L:G ratio of 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10, 95:5, and 100:0.

The semi-solvent approach for isolating PLGAs with different L:G ratios presents advantages over other approaches, including gradient

polymer elution chromatography (GPEC) [23]. In our preliminary study using GPEC based on universal non-solvent (hexane) to semi-solvent (toluene), both PLGA 100:0 and 50:50 were eluted under semi-solvent conditions. The peak retention times were different by 1.7 min, but the extremely broad peak, with peak-widths (baseline) of 4.78 min for PLGA 100:0 and 6.27 min for PLGA 50:50, made it difficult to separate PLGAs based on the L:G ratio. Compared with the GPEC method, the semi-solvent approach is easy to carry on in any laboratory without any specialized equipment. The semi-solvent method could be used as a routine method for comparative analysis of PLGAs between RLDs and proposed generics, as well as quality control for PLGA products.

5. Conclusion

Assay of an FDA approved drug formulation comprised of multiple types of PLGA requires the ability to separate one type of PLGA from another. This can be achieved using semi-solvents exhibiting various degrees of solubility towards PLGA with respect to its L:G ratio. This lacto-selectivity of semi-solvents makes it possible to deconstruct and analyze complex PLGA formulations made of more than one type of PLGA. Semi-solvents can be used as a tool for quality control, as well as for reverse engineering of a drug product containing a mixture of PLGA polymers.

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