International Journal of Pharmaceutics 602 (2021) 120627

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Analysis of semi-solvent effects for PLGA polymers

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ARTICLE INFO

Keywords:
PLGA
Semi-solvent
L:G ratio
Comonomer sequence
Long-acting depot
Esters
Ketones

ABSTRACT

Poly(lactide-co-glycolide) polymers (PLGAs) have been used in many clinical formulations of injectable, long-acting formulations. Frequently, PLGAs having different lactide:glycolide (L:G) ratios, molecular weights (MWs), end-groups, and molecular structures have been used individually or in mixtures. To understand the properties of existing formulations made of PLGAs and to develop new formulations, understanding PLGA properties is essential. Yet, the separation of individual PLGA components from a mixture and their characterization has been challenging due to an incomplete understanding of PLGAs.

This study focuses on separating PLGAs based on their molecular properties, such as L:G ratio, molecular weight, and comonomer sequence. The separation of PLGAs exploits the use of semi-solvents that dissolve only PLGAs having lactide contents (L%) above a certain threshold. More semi-solvents have been identified that show a specific transition L% between 50 and 100%. The mechanism study of semi-solvents indicates that semi-solvents, in general, prefer PLGAs with relatively higher L%, lower molecular weight, and higher G-L sequences as opposed to G-G sequences. The examination of a series of esters and ketones indicates that a solvent with lower molar volume is more effective as a semi-solvent. At a similar molar volume, esters are more effective than ketones in dissolving PLGAs with the same L:G ratio. The ability to separate and identify PLGA fractions allows better characterization of existing formulations and higher flexibility in designing new injectable, long-acting PLGA formulations.

1. Introduction

Poly(lactide-co-glycolide) (PLGA) polymers have been commonly used to make injectable, long-acting formulations. There are currently more than 20 clinically approved drug products on the market using PLGA (Park et al., 2021a). Some reference listed drug (RLD) formulations use more than one type of PLGA having different molecular weights (MWs) and/or lactide:glycolide (L:G) ratios, and others use non-linear PLGA molecules. Analyzing exact PLGA compositions is critical for quality control purposes and for developing generic counterparts of RLD formulations. Separation and characterization of different PLGA components have been challenging due to the lack of established methods. Recently, a new gel-permeation chromatography (GPC) approach was used to identify the structure of branched glucose-PLGA molecules (Hadar et al., 2019), and a semi-solvent method has been developed for the separation of PLGAs based on their L:G ratios (Skidmore et al., 2019). The term "semi-solvent" implies a conditional solubility (i.e., insoluble, partially soluble, and soluble) depending on the solvent and the L:G ratio (or the lactide percentage, L%). Each semi-solvent has a threshold L:G ratio for dissolving PLGAs (Skidmore et al., 2019). While various semi-solvents were identified, the mechanisms of the semi-solvent effect have not been fully understood.

As a hydrophobic, linear polyester, PLGAs dissolve in various organic solvents. However, not all PLGAs dissolve in all solvents. While the name "PLGA" is used as a general term, PLGAs with different L:G ratios are physicochemically different polymers from the solubility point of view. For example, PLGA 50:50 and PLGA 75:25 are both made of the same two monomers, but benzyl alcohol dissolves only PLGA 75:25 (Park et al., 2019; Skidmore et al., 2019). The different monomer ratios make the two PLGAs exhibit vastly different properties. Solvents for PLGAs can be generally arranged into three classes. These include non-solvents (or poor solvents) that do not dissolve PLGA at any lactide content (e.g.,

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https://doi.org/10.1016/j.ijpharm.2021.120627
Received 8 March 2021; Received in revised form 14 April 2021; Accepted 17 April 2021
Available online 27 April 2021

hexane and ethanol), good solvents (or full solvents) that dissolve PLGA regardless of the commonly used lactide contents (e.g., dichloromethane, dimethylformamide, and hexafluoroisopropanol), and semi-solvents that exhibit a lactide-dependent solubility (e.g., benzyl alcohol, n-butyl acetate, 2-pentanone, and toluene). All semi-solvents characterized to date have exhibited an increasing solubility with higher L%. The only exception has been PEG 400 that shows higher solubilities for PLGAs of lower L% (Hadar et al., 2017).

Although PLGA is typically considered a random copolymer, the comonomer sequence of PLGA can be varied because the lactide and glycolide components have different properties affecting their reaction rates. Compared to lactide, glycolide has a higher ring-opening polymerization enthalpy (Sedush et al., 2014), a lower melting point (86 °C vs. 124.5 °C of D,L-lactide (Weast, 1984)), and better solubility in the PLGA-melt phase (Gao et al., 2002). Thus, under the conventional, tin-catalyzed melt-polymerization of PLGA, the glycolide monomer initially reacts faster than the lactide monomer, forming glycolide-rich segments. These are subsequently randomized by transesterification between the polymer chains (Qian et al., 2011). Because these processes are related to the reaction temperature and shear, the degree of blockiness (the G-G sequences) varies depending on the manufacturing conditions. The synthesis of less blocky PLGAs is possible, but it generally requires costly components or labor-intensive techniques impractical for industrial manufacturing of a commercially viable product (Dong et al., 2000; Li et al., 2011; Qian et al., 2011). For this reason, the PLGAs found in clinical formulations carry some degree of blockiness. The variations in the blockiness can affect PLGA properties, such as interactions with solvents (Gao et al., 2002) and degradation rates (Li et al., 2011).

The exact driving mechanisms behind the semi-solvent phenomenon remain unclear but are suspected to be related to the self-crystallization of G-G blocks and glycolide-rich regions, which renders these areas resistant to dissolution due to self-affiliation. Thus, the L:G ratio is a factor in determining the solubility in semi-solvents. This study examines the relative impacts of the L% on blockiness and the impact of solvent isomerization on its semi-solvent performance.

2. Materials and methods

2.1. Materials

Lactide and glycolide monomers were obtained from Ortec, Inc. Stannous octoate catalyst was obtained from Aldrich, purified by vacuum distillation, and stored over calcium sulfate (Drierite) desiccant. PLGA polymers were from Polyscitech (polyscitech.com), Evonik (Essen, Germany), and Durect (Birmingham, AL). Deuterated chloroform (stabilized with silver foil) was purchased from Aldrich. NMR tubes were purchased from Wilmad. Mobile phase solvents (tetrahydrofuran, acetone) of liquid-chromatography grade were purchased from Fisher Scientific and vacuum filtered through a $\leq$ 0.45 μm PVDF or PTFE filter before use. Methyl cyclobutyl-carboxylate, ethyl lactate acetate, and butane-2,3-dipropionate were obtained from Polyscitech. All other chemicals were of reagent grade or higher and used as received unless otherwise specified.

2.2. Synthesis of PLGAs with varying L:G ratios and MW

PLGA polymers were synthesized, as previously described (Garner et al., 2015). Both lactide and glycolide monomers were first dissolved in dichloromethane (DCM) at 50 °C and then vacuum distilled to obtain a uniform mixture of both monomers. Lactide and glycolide monomers at different molar ratios, initiator (lactic acid for acid endcap, decanol for ester endcap), and stannous octoate (1:200 molar equivalent to monomers dissolved in anhydrous toluene at 10% w/v) were charged into a round-bottom flask equipped with an oval stir bar and a stopcock attachment. The mixture was vacuum purged (Duoseal vacuum pump, Welch) to remove solvents and moisture. The mixture was subsequently sealed off and reacted at 150–170 °C for 8 h with magnetic stirring to perform ring-opening polymerization. The resultant crude polymer was rinsed with ethanol, dissolved in dichloromethane, filtered to remove insoluble portions into stirring hexane to precipitate. The collected precipitate was dried under vacuum (-30 in.-Hg) at 50–70 °C until reaching a constant mass (1–3 weeks). By varying the feed ratios of initiator, lactide, and glycolide, various PLGAs could be generated with a range of MWs and L:G ratios. Because ring-opening polymerization and transesterifications are random processes, the polymers require characterization to establish their parameters before proceeding.

2.3. Semi-solvent fractionation of PLGAs

To fractionate each PLGA and collect portions of discrete lactide contents, the PLGAs were solubilized or washed with semi-solvents. Each PLGA (~10 g) was diluted up to 50 mL with a designated semi-solvent in a centrifuge tube. These were dissolved in a shaking incubator (Southwest Science) with 30 °C/100 RPM agitation overnight (16–24 h). The next day the polymer solution was centrifuged at 3,000 RPM for 3 min to force insoluble PLGA portions to the bottom of the tube. The precipitate was collected as the semi-solvent insoluble fraction and supernatant as the soluble fraction. After fractionation, each portion was repurified by dissolving in acetone, followed by vacuum filtering (Chemrus, Aldrich), precipitating in 200 proof ethanol (Decon Laboratories), and vacuum drying of the collected precipitates at 50–60 °C to a constant mass.

The semi-solvent used for fractionation was matched to the PLGA lactide% (L%). For PLGAs with L% <85%, 2-pentanone was used as the primary separation semi-solvent, and xylene was applied to remove high-lactide content portions as needed. For PLGAs with L% >85%, toluene was used as the separating solvent. Each fraction was characterized individually by NMR and GPC to confirm their properties. The fractions that did not dissolve in selected semi-solvents were still soluble in deuterated chloroform, acetone, and tetrahydrofuran used in the characterization processes.

2.4. ^{13}C NMR and ^{1}H NMR characterization of PLGAs

NMR analysis of polymers was performed to obtain properties relevant to the PLGA structure and sequencing. Each sample (5–10 mg) was dissolved in 0.8 mL of $CDCl_3$ and transferred into a 7-inch NMR tube. The spectra were collected by Purdue Interdepartmental NMR Facility (PINMRF, pinmrf.purdue.edu). The spectra were analyzed as previously described (Garner et al., 2015; Skidmore et al., 2019). Briefly, the ^{1}H NMR peaks at 5.2 ppm (1H, lactide, P_L) and 4.8 ppm (2H, glycolide, P_G) were used to calculate the molar ratio of lactide (M_L) in the PLGA according to $M_L = P_L/(P_L+(P_G/2))$.

The comonomer sequence (CMS) distribution was determined following the glycolide carbonyl shift in ^{13}C NMR, which occurs depending on the monomer type adjacent to the glycolide unit (Hausberger and DeLuca, 1995). In this case, the peak intensity ~ 166.4 ppm corresponds to a glycolide adjacent to a lactide ($I_{G\text{-}L}$), and the peak intensity ~ 166.3 ppm corresponds to a glycolide adjacent to another glycolide ($I_{G\text{-}G}$). The ratio of the comonomer sequence ($R_{cms} = I_{G\text{-}L}/I_{G\text{-}G}$) was used to describe the non-blockiness of the two-monomer distribution. The inverse of R_{cms} describes the blockiness of G-G sequences ($R_c = 1/R_{cms} = I_{G\text{-}G}/I_{G\text{-}L}$) (Skidmore et al., 2019). The higher the R_{cms} value is, the less blocky the PLGA is in its comonomer sequence.

2.5. Gel-permeation chromatography (GPC)

Conventional GPC using external standard MWs (GPC-ES) was performed as previously described (Hadar et al., 2020; Hadar et al., 2019; Skidmore et al., 2019). Briefly, MW properties were determined using a Waters Breeze-2 system with tetrahydrofuran (THF) at a flow rate of 1 mL/min across three sequential columns with detection by refractive

index. The peaks were calibrated against commercially available polystyrene standards (Agilent) to determine weight average, number average, and polydispersity index.

GPC with quaternary detection (GPC-4D) that measures the absolute MWs was also performed as previously described (Hadar et al., 2020; Hadar et al., 2019; Skidmore et al., 2019). Briefly, PLGA samples (2 mg/ml, 100 µl injection) were analyzed using the GPC-4D system consisting of an Agilent 1260 Infinity II HPLC connected to Dawn Heleos II (MALLS) with Dynapro Nanostar (DLS) attached via an optical cable, Optilab T-rEX (RI detector) and Viscostar III viscometer. The system was operated by Astra 7 (Wyatt) software. The separation was performed with a linear gradient column (Tosoh Bioscience LLC, TSKgel GMHHR-L, 7.8 mm × 30 cm) at 0.6 mL/min flow of acetone. The dn/dc parameter was input to the software based on NMR determined L:G ratio for each sample and according to the correlation between L:G ratio and dn/dc.

2.6. Determination of PLGA solubility in semi-solvents

The PLGA solubility in semi-solvents was determined gravimetrically. Using a 20 mL glass scintillation vial, 100 mg of PLGA with a weight average MW of 80 ± 20 kDa and an NMR-determined L:G ratio was added along with 4 mL solvent to be tested. The vials were shaken at 30 °C/100 RPM overnight (16–24 h) and were subsequently decanted. The remaining solid was vacuum dried to a constant mass and weighed to determine the percent solubilized by comparing the initial and the final masses. This was performed in triplicate unless otherwise specified.

The semi-solvent properties were quantified by determining the lactide content (L%) at the solubility crossover at 10 mg/mL (corresponding to the 40% solubility of the 25 mg/mL solubility of good solvents). The crossover solubility was determined using the two closest data points to the 10 mg/mL solubility. Additionally, the slope of the line at this transition was recorded as a relative measure of the sharpness of the transition.

2.7. Impact of isomer-size on semi-solvent capacity

For solvents of esters or ketones with either six or seven carbons, the molar volume was calculated by reproducing the molecular structure in Chemsketch (ACDLabs, 2015) and generating the software predicted molar volume. Each solvent's molar volume was plotted against the lactide content at 10 mg/ml crossover solubility to evaluate a correlation between these parameters.

3. Results

3.1. PLGA fractionation

Semi-solvents prefer dissolution of PLGAs with higher lactide contents (higher L:G ratios), and thus, they are lacto-selective solvents (Skidmore et al., 2019). While many semi-solvents are available, more semi-solvents need to be discovered and the semi-solvent mechanisms need to be understood. For testing the semi-solvent effect, various solvents and solvent mixtures are evaluated according to a standardized solubility test at 30 °C. The percent solubility (of 25 mg/ml) was determined as detailed in Section 2.6. Fig. 1 shows the semi-solvent properties of four solvents. The three semi-solvents, 2-pentanone, toluene, and xylenes, have been routinely used as the first-line semi-solvents for separating PLGAs with different L%, or L:G ratios, as they are readily available, inexpensive, and relatively volatile. Fig. 1 also shows that ethyl lactate can effectively separate PLGAs with the L% between 50 and 60. However, its boiling point of 154 °C makes the drying process challenging and time-consuming. Thus, while ethyl lactate is a highly useful semi-solvent, it is not practical for daily use.

PLGAs with various L:G ratios were first washed with semi-solvents before they were characterized. The initial goal of washing PLGAs with those semi-solvents was to remove impurities, but they turned out

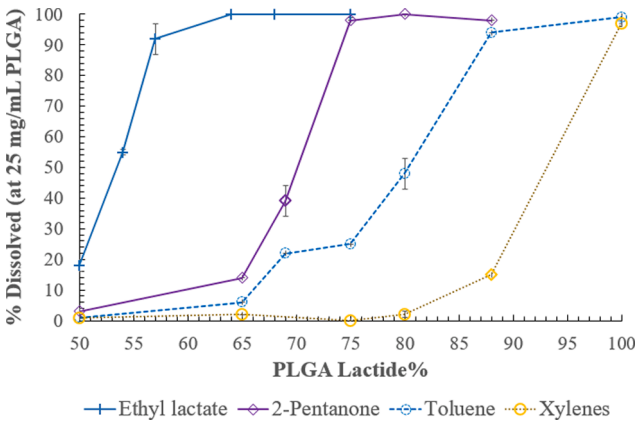


Fig. 1. Dissolution of PLGAs in semi-solvents as a function of the lactide content (L%) at 30 °C. The 100% dissolution indicates complete dissolution of PLGAs at the concentration of 25 mg/mL. Thus, 40% dissolution means the dissolution to make the final PLGA concentration of 10 mg/mL.

to extract a PLGA fraction with a higher L%. PLGAs with L% lower than 80 were treated with 2-pentanone to separate higher lactide% fractions first. Toluene or xylenes were used as the first semi-solvent for PLGAs with L%>80.

The PLGA polymers used in this study are listed in Table 1. Selected polymers were put through semi-solvent fractionation to separate into their component portions. The resultant fractions were evaluated to obtain their lactide content (L%) by ^{1}H NMR and non-blockiness (R_{cms}) by ^{13}C NMR. Additionally, the fractions were evaluated by GPC-4D to obtain their exact MWs. Table 1 summarized the properties of all collected fractions. These fractions, along with previously described PLGAs, were utilized to evaluate the semi-solvent property.

Upon fractionation by semi-solvents, the PLGA fraction that remains soluble has a lower MW, a higher lactide content, and a higher R_{cms} than

Table 1
Fractionation of PLGA polymers using semi-solvents and their characterization.

PLGA (Initial)		PLGA (After Semi-Solvent Fractionation)					
L:G	End Group	Semi-Solvent Fraction	GPC-4D			NMR	
			M_n	M_W	I.V.	L:G	R_{cms}
48:52	A	2P-I	9,096	11,290	16.7	49:51	0.64
51:49	E	2P-I	9,328	11,899	17.3	49:51	0.63
53:47	E	2P-I	38,686	48,706	37.5	52:48	0.63
57:43	A	2P-I	28,371	36,143	33.5	54:46	0.66
61:39	A	2P-I	32,340	41,764	35.3	57:43	0.73
64:36	A	2P-I (0.88)*	26,139	32,750	31.8	64:36	1.22
		2P-S (0.12)*	11,757	15,313	20.8	67:33	1.25
68:32	A	2P-I (0.94)*	34,843	45,681	38.0	68:32	1.12
		2P-S (0.06)*	14,398	18,280	22.3	69:31	1.21
69:31	E	2P-I	2,317	4,182	8.3	65:35	1.23
75:25	E	2P-I (0.71)*	39,739	49,563	41.0	71:29	1.29
		2P-S + X-I (0.29)*	25,260	34,871	31.2	76:24	1.57
78:22	A	2P-I (0.23)*	45,485	57,221	46.7	77:23	1.47
		2P-S (0.77)*	47,792	59,454	47.6	78:22	1.57
81:19	A	T-I	23,349	29,509	29.9	83:17	1.88
84:16	E	T-I	21,960	28,415	28.8	78:22	2.01
88:12	E	T-I (0.50)*	38,789	54,348	40.3	76:24	1.25
		T-S (0.50)*	25,108	33,658	29.5	92:8	1.78
90:10	A	T-I	39,273	50,281	41.2	75:25	1.43
95:5	E	T-I	30,123	37,581	34.0	95:5	1.38
		T-S	26,644	33,467	31.4	95:5	2.92

*Relative mass quantity of the total in the insoluble or soluble fractions. A = acid endcap, E = ester endcap, M_n = Number average MW, M_w = weight average MW, I.V. = inherent viscosity (mL/g), 2P-I = 2-pentanone-insoluble, 2P-S = 2-pentanone-soluble, T-I = toluene-insoluble, T-S = toluene-soluble, X-I = xylene-insoluble. The semi-solvent insoluble fractions were dissolved in DCM for further characterization.

the insoluble fraction. PLGAs with the initial L:G ratios of 64:36, 68:32, 75:25, and 88:12 show this trend clearly. PLGA 78:22 does not show such a trend, and the values are too close to distinguish. It may be that 2-pentanone cannot distinguish PLGA fractions if the L:G ratio is 78:22 and above. PLGA 95:5 is unique because it was fractionated by toluene into portions with the same lactide content but higher and lower R_{cms} values for soluble and insoluble portions, respectively. The data indicates a relationship between the semi-solvent and R_{cms}. In general, the R_{cms} increases as the lactide content increases (Fig. 2).

3.2. Semi-solvent evaluation

To date, more than eighty solvents or solvent mixtures have been evaluated to obtain the solubility properties for PLGA based on the L:G ratio under a standardized condition of overnight incubation at 30 °C. The solvents have been categorized into three classes: Good solvents (or full solvents) that dissolve PLGAs of all lactide contents (larger than L50%) to ≥25 mg/mL (e.g., dichloromethane and hexafluoroisopropanol); poor solvents (or non-solvents) that dissolve PLGAs at <5 mg/mL (e.g., water and hexane); and semi-solvents that exhibit a lactide-dependent solubility and display a discrete transition point at a given lactide content above which they dissolve ≥ 10 mg/mL PLGA (e.g., 2-pentanone and toluene). The criterion of 10 mg/mL was chosen arbitrarily, as it allowed separation of PLGAs having different L:G ratios in our studies. All other semi-solvents characterized to date have exhibited an increasing solubility with higher lactide content. Only PEG 400 showed a reverse trend that PLGAs with lower lactide appeared to dissolve better (Hadar et al., 2017).

In search of more semi-solvents for PLGA 50:50, various solvents were tested, and the results are summarized in Table 2. Acetone:2-pentanone (1:3), 4-phenyl-2-butanone, cyclohexanone, and methyl cyclobutane-carboxylate exhibited the semi-solvent properties, and other solvents tested all showed good solvent properties. One of the good solvents is ethyl lactate acetate, which was prepared by attaching an additional ester through an acetate bond to ethyl lactate. Ethyl lactate is a semi-solvent that displays a transition at L52% as shown in Fig. 1. It appears that the molecular structure of ethyl lactate acetate allows easier penetration to the G-G blocks than ethyl lactate can, or the additional ester moiety may increase the solvent-PLGA attraction in general. For PLGA with 100:0 (i.e., polylactide or PLA), 2-octanone was previously found to be a poor solvent with 4.3 mg/mL solubility. Additional poor solvents with the PLA solubility of only 1 mg/mL or less were identified, including 2-methyl-3-hexanone, *tert*-butyl propionate, 2,4-dimethyl-3-pentanone, mesitylene, 1,2,4 trimethylbenzene, and cyclopentanol.

Table 3 shows semi-solvents with their lactide-content transition point and the measured slope of this transition. As shown in the table, PLGAs with the lactide content (L%) ranging from 51% to 94% have

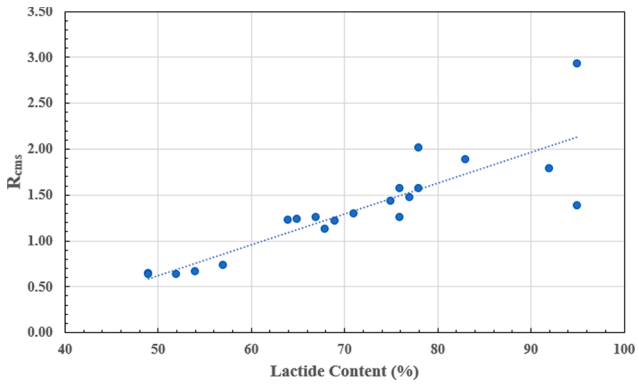


Fig. 2. Relationship between lactide content and R_{cms} in fractionated PLGAs. The dotted line represents the line of best fit.

Table 2
Semi-solvents and good solvents for PLGA 50:50.

PLGA Dissolution	Solvents
10.0 mg/mL	Acetone:2-pentanone (1:3)
15.3 mg/mL	4-Phenyl-2-butanone
16.5 mg/mL	Cyclohexanone, methyl cyclobutane-carboxylate
>24.0 mg/mL (Good solvents)	Acetone, acetone:2-pentanone (1:1 & 1:2), acetonitrile, acetonitrile: trichloroethylene (1:2), anisole, caprolactone, dimethyl carbonate, ethyl acetate, ethyl lactate acetate, 1-ethyl-2-pyrrolidinone, formic acid, methyl cyclopropane-carboxylate, methyl lactate, propylene carbonate, valerolactone

their corresponding semi-solvents. The presence of diverse semi-solvents for all L:G ratios allows a more accurate separation of PLGAs even if they have close L:G ratios. The table also lists the slope at the transition L%, which provides information on how steep a transition occurs. The higher slope, i.e., the steeper transition, makes the separation of PLGAs easier. Fig. 3 plots the transition slopes at the transition L%. The plot shows no particular relationship between the L% and the slope, indicating that the transition slope depends on the semi-solvent itself and not on the transition L%. Table 3

3.3. Solvent shape effect on PLGA dissolution

A trend noticed earlier for semi-solvents is that certain classes of solvents (notably esters and ketones) tend to behave as semi-solvents. The solvents with increasing length require higher L% to achieve solubility. For example, reviewing simple ketones on the second carbon displays the trend from the strongest solvent to the weakest solvent: acetone (good solvent) > butanone (L51%) > 2-pentanone (L69%) > 2-hexanone (L82%) > 2-heptanone (L91%) > 2-octanone (poor solvent). The hydrophobic contributions of the extended chain play some role in the solvent's behavior. However, it was not clear whether the solvent's molar volume partially contributed to this trend. For further investigation, a series of isomeric solvents with the same number of carbons but different configurations were tested, and relationships between molar volume and solubility were established.

The effects of molar volume on the semi-solvent effect were examined using a series of semi-solvents in the same class (esters and ketones) and the same overall number of carbons. PLGA polymers start dissolution in semi-solvent as their L% increases. The lactide content (L%) of a PLGA that dissolved in each solvent at 10 mg/mL concentration was identified as the transition point from a poor solvent to a semi-solvent. Table 4 lists the transition L% of tested esters and ketones and their corresponding molecular volumes and chemical structures. One trend observed is that the semi-solvent molecules with cyclic groups can dissolve PLGAs with the lactide contents < 60%, i.e., lower L:G ratios. This indicates that the cyclic compounds can penetrate glycolide blocks more readily than those without cyclic structures. The correlation between molar volume and solubility transition for each class of semi-solvent is shown in Fig. 4. At the same molar volume, esters are more prone to dissolve PLGAs with lower L% (i.e., higher G%), indicating better ability to dissolve PLGAs with higher G-G blockiness. For both esters or ketones, semi-solvents with lower carbon numbers have lower molar volumes and are better at dissolving PLGAs with higher G-G blocks. Hexafluoroisopropanol, which dissolves polyglycolide, has a molar volume of 109 cm^3/mol. The data collectively supports the hypothesis that one of the driving forces for the semi-solvent effects is the solvent's ability to penetrate crystalline glycolide-rich regions within lactide-poor PLGA segments.

Table 3
Transition lactide% of semi-solvents and their measured slopes at transition.

Transition Lactide %	Slope*	Solvent	Transition Lactide %	Slope	Solvent
51	1.1	Butanone (MEK)	80	1.3	2-Methyl tetrahydrofuran: xylene (1:1)
52	0.6	Acetonitrile:trichloroethylene (1:4)		1.3	n-Butyl acetate:xylene (1:1)
52	2.3	Ethyl lactate		2.7	Propyl propionate
53	3.1	Ethylcyclopropane carboxylate^		1.3	Isobutyrophenone
56	0.8	Acetone:2-pentanone (1:4)	82	1.2	Toluene:xylene (1:1)
	1.4	Benzyl alcohol		2.0	Isobutyl acetate
57	0.8	Propiophenone		1.8	2-Hexanone
59	3.0	Cycloheptanone		1.8	Toluene:xylene (4:1)
	0.9	Methyl ethyl ketone:xylene (1:1)		1.6	3-Methyl-2-pentanone
61	0.9	Ethyl benzoate	83	1.2	Trichloroethylene:xylene (1:1)
63	0.6	Propyl acetate		1.6	2,2-Dimethyl-propiophenone
64	11.5	Methyl cyclopentanecarboxylate^^		1.4	3,3-Dimethyl-2-butonone
67	2.6	Trichloroethylene		2.0	Toluene:xylene (3:1)
68	2.0	Ethyl propionate		1.9	Toluene:xylene (2:1)
69	1.5	Chlorobenzene	84	0.9	3-Hexanone
	1.8	3-Pentanone		1.2	Butane-2,3-dipropionate^^
	2.5	2-Pentanone		2.0	4-Methyl-2-pentanone
70	2.8	2-methyl tetrahydrofuran		2.0	Pentyl acetate
71	0.8	N-Butyl acetate		2.0	Isopentyl acetate
73	2.6	Propyl lactate^	85	2.4	Ethyl isobutyrate
74	0.4	2-Pentanone:xylene (1:1)	86	2.5	Isobutyl benzoate
	2.9	Propyl benzoate	87	2.3	Butyl benzoate
78	1.2	Toluene		1.7	2-Methyl-3-pentanone^
79	4.1	Butyrophenone	91	1.3	2-Heptanone
			92	1.7	Xylenes
				0.5	4,4-Dimethyl-2-pentanone
			93	0.5	Isobutyl propionate
			94	1.0	5-Methyl-2-hexanone

$* \ Slope = \frac{\Delta Dissolved}{\Delta Lactide\%}$
n = 3, except n = 2^ and n = 1^^

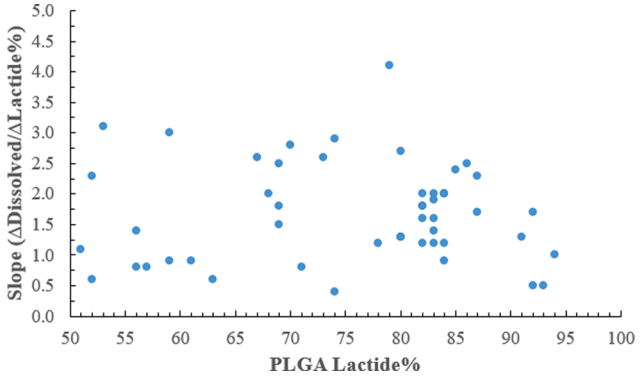


Fig. 3. The transition lactide% of semi-solvents and their measured slopes at transition.

4. Discussion

4.1. Uniqueness of PLGA polymers

PLGA polymers were synthesized around the turn of 1970 (Blasi, 2019), and the first PLGA-based injectable, long-acting formulation was approved by the Food and Drug Administration (FDA) in 1989 (Park et al., 2019). The history of PLGA is relatively short compared to other commodity polymers that the public enjoys nowadays, such as polyethylenes, nylons, and polyacrylates. The applications of PLGA polymers are still mainly limited to pharmaceutical formulations and biomedical devices. The lack of comprehensive characterization methods for PLGA polymers has been a bottleneck in developing more PLGA products. This is mainly because the drug release kinetics have not been controlled by the design of formulations. Formulation developments have relied mainly on the trial-and-error approach. As more techniques are developed for better characterization of PLGAs, more understanding of the polymers follows. The formulation properties can be controlled by tuning the PLGA properties and manufacturing processes.

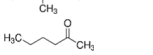
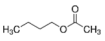
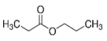
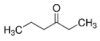
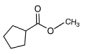
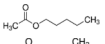
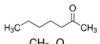
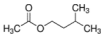
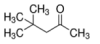
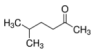
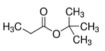
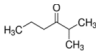
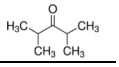
The ring-opening polymerization of lactide and glycolide monomers involves monomer solubilization into polymer melt, polymer chain growth, and transesterification reactions between polymer chains (Qian et al., 2011). Thus, a synthesized PLGA batch is a polydisperse product in terms of chain MW, monomer composition (L:G ratio), and comonomer sequencing (G-L or G-G) described by R_{cms} values. GPC cannot separate PLGA polymers on their L:G ratios or comonomer sequencing. The inability of GPC in separating PLGA polymers based on their L:G ratios and R_{cms} made it challenging to identify individual components from multi-PLGA products, such as Trelstar®. The semi-solvent method has provided a solution to separate PLGA polymers based on their L:G ratios and R_{cms} for further analysis (Skidmore et al., 2019).

4.2. Usefulness of routine fractionation using semi-solvents

The ability to separate PLGAs based on their L:G ratios and R_{cms} is useful for analyzing complex PLGA formulations not only for quality control but also for making generic products of PLGA-based reference listed drugs (RLDs). As shown in Table 1, PLGAs 64:36 and 68:32 can be treated with 2-P to remove 2P-soluble portions, which have the L:G ratio of 67:33 and 69:31, respectively. The 2P-soluble fractions also have lower MWs and slightly higher R_{cms} values. For PLGA 75:25 and 78:22, however, 2P-soluble fractions are closer to the original L:G ratios. This is expected because 2P's transition L% is 69%. For highly heterogeneous PLGAs, such as PLGA 88:12 in Table 1, simple treatment with toluene can separate two distinct fractions having L:G ratios of 76:24 and 92:8 with different MWs. If a PLGA is homogeneous, e.g., 95:5 in Table 1, toluene treatment will result in the fractions which are almost the same. The general trend observed is that the R_{cms} (i.e., the L-G sequences) increases as the L% increases. This is intuitive, as the G-G blockiness decreases as the glycolide fractions decrease.

The understanding of the semi-solvent effects has just begun. In this study, more semi-solvents were identified with their transition L%s ranging from 50:50 to 90:10 (Tables 2 and 3). The usefulness of semi-solvents increases as more of them are identified. A wide array of semi-solvents can be used to obtain PLGAs with narrow L:G ratio, MW distribution, and R_{cms}. Such well-defined PLGAs can be used to make PLGA formulations to find an answer to how the PLGA properties affect

Table 4
Solvents with the lactide content (L%) for 10 mg/mL dissolution transition, molar volume (V_m in cm^3/mol, ACDLabs predicted), boiling point (°C), and structure.

Solvent	Solvent Name	L%	V_m, B_p	Structure	Solvent	Solvent Name	L%	V_m, B_p	Structure
Ester (6 carbon)	Methyl cyclo- butanecarboxylate	50	109, 139		Ketone (6 carbon)	Cyclohexanone	50	103, 156	
	Caprolactone	50	112, 466			3-Methyl-2-pentanone	82	125, 118	
	Ethyl *cyclo*-propanecarboxylate	53	107, 134			2-Hexanone	82	125, 128	
	Butyl acetate	71	131, 259			3,3-Dimethyl-2-butanone	83	125, 106	
	Propyl propionate	80	131, 252			4-Methyl-2-pentanone	84	125, 116	
						3-Hexanone	86	125, 123	
						2-Methyl-3-pentanone	87	125, 113	
Ester (7 carbon)	Methyl *cyclo*-pentanecarboxylate	64	126, 158		Ketone (7 carbon)	Cycloheptanone	59	121, 179	
	Pentyl acetate	84	148, 149			2-Heptanone	91	141, 151	
	Isopentyl acetate	84	148, 142			4,4-Dimethyl-2-pentanone	92	141, 126	
	Isobutyl propionate	93	148, 137			5-methyl-2-hexanone	94	142, 144	
	tert-Butyl propionate	100	148, 118			2-Methyl-3-hexanone	100	142, 135	
						2,4-Dimethyl-3-pentanone	100	142, 124	

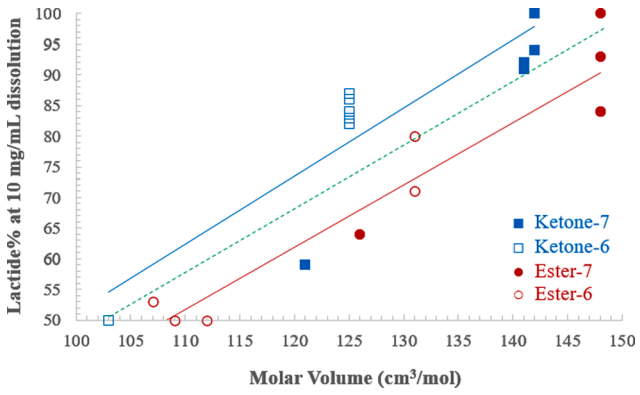


Fig. 4. Correlation between the transition lactide % at 10 mg/mL dissolution and semi-solvent molar volume. The number after ester and ketone represents the number of carbons. The solid blue, solid red, and green dotted lines are the best fits for ketones, esters, and ketones and esters, respectively.

the formulation's drug release kinetics, assuming all other manufacturing conditions remain the same. The extent of such impacts may also provide further information for controlling the formulation properties.

4.3. The mechanisms of semi-solvent effects

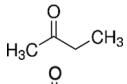
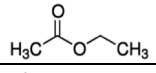
The semi-solvent effect mechanisms were examined using the molar volume and boiling point of semi-solvents (Table 4). As shown in Fig. 4, the molar volume appears to be the main factor for determining the transition L%. As the molar volume becomes smaller, the transition L% becomes closer to 50%. This is also understandable, as the G-G block segment can be more easily dissociated with the smaller semi-solvent molecules. One primary property appears to be the molar volume of a solvent. At the same molar volume, esters seem to be more efficient than ketones. The difference in performance between esters and ketones of comparable molar volumes and even (aside from the main moiety) similar chemical compositions indicates that the chemical parameters of the solvent are also a driving factor. As an illustrative example, methyl ethyl ketone (2-butanone) and its corollary ester version, ethyl acetate, are compared. By testing ethyl acetate is a full solvent (>24 mg/ml for L % down to 50%) while methyl ethyl ketone is a semi-solvent starting from L51% to have a transition > 10 mg/ml solubility (Table 3).

Despite the minor increase in the molar volume of ethyl acetate relative to butanone, the presence of the oxygen atom in ethyl acetate (Table 5) decreases the polar contribution while increasing the hydrogen contribution of the Hansen parameters. This indicates that these two parameters play an essential role in dissociating G-G segments. Similarly, ethyl lactate acetate, although being a larger molecule, demonstrated a higher solubility for PLGA than ethyl lactate. This indicates that similar structural properties of solvents to those of the PLGA can help drive its solubility. Due to the propensity of G-G rich regions to self-crystalize, those solvents with both smaller molar volume and stronger attraction to the G-G block are expected to solubilize PLGAs with lower L%, i.e., PLGAs with higher G-G segments.

4.4. Semi-solvent effect in studying properties of PLGA formulations

The information on the L:G ratio and R_{cms}, in addition to MW, is critical to understanding the properties of PLGA microparticles or other forms of PLGA formulations. These factors affect how PLGA polymers precipitate during the formulation manufacturing steps, which subsequently affects the polymer density, morphology, and, most importantly, the drug release kinetics (Park et al., 2021a). For example, the

Table 5
Comparative properties of ethyl acetate and butanone.

Solvent Properties			Hansen Parameters (Hansen, 2018)		
Solvent	Structure	V_m*	δD	δP	δH
Butanone (MEK)		91.6	16.00	9.00	5.10
Ethyl acetate		98.0	15.80	5.30	7.20

*Molar volume in cm^3/mol (ACDLabs predicted).

precipitation of PLGA polymers during the manufacturing process depends on various parameters (Park et al., 2021a,b), including but are not limited to the type of solvent(s) used and the solvent extraction kinetics. For the PLGAs having the same MW but different L:G ratios and R_{cms}, the drug release from the final formulations may vary significantly. The ability of semi-solvents to separate different PLGAs is expected to increase our ability to characterize PLGA polymers in more detail than before, allowing a clear understanding of the reasons for different drug release kinetics. PLGAs obtained from different manufacturers may have similar properties in the L:G ratio and molecular weight, but the two PLGAs may be quite different in the G-G sequences (or blockiness), affecting the solubility in different solvents, PLGA compactness during drying, and the drug release kinetics from the final formulation. This will increase the reproducibility of the formulation properties and enhance meeting the Q1/Q2 requirements in developing generic versions of RLD products. Continued efforts of identifying more semi-solvents and finding new ways of utilizing them will hopefully lead to making PLGA formulations with desirable properties, such as reducing the initial burst release and extending the drug release duration for various drugs.

5. Conclusions

A semi-solvent is a liquid that selectively dissolves PLGA polymers having the lactide content above a certain threshold. Thus, it exhibits a selective solubility for PLGAs based on their L:G ratio. All semi-solvents, except PEG 400, have higher solubility as the L% increases. The solvents tested to date provide an array of L% thresholds above which PLGAs dissolve at $>$ 10 mg/ml. For ketones and esters, semi-solvents can dissolve PLGAs with lower L% (or higher G%, increasing the blockiness) as the solvent molar volume is smaller for easier penetration into the semi-crystalline glycolide-rich segments. By leveraging the semi-solvent effect, formulations containing two or more PLGA polymers with different molecular properties (such as L:G ratios, blockiness, and molecular weights) can be analyzed for their PLGA compositions.

CRediT authorship contribution statement

John Garner: Conceptualization, Methodology, Experiments, Writing - original draft, Writing the response to reviewers. **Sarah Skidmore:** Conceptualization, Methodology, Experiments, Data curation and analysis. **Justin Hadar:** Methodology, Experiments, Data analysis and validation. **Haesun Park:** Project administration. Funding acquisition. Conceptualization, Supervision. **Kinam Park:** Conceptualization, Investigation, Writing. **Young Kuk Jhon:** Investigation, Validation, Writing – review and editing, Writing the response to reviewers. **Bin Qin:** Validation, Writing – review and editing, Writing the response to reviewers. **Yan Wang:** Conceptualization, Writing – review and editing, Writing the response to reviewers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by BAA Contract # 75F40119C10096 from the Center for Drug Evaluation Research of the Food and Drug Administration (FDA) and in part by the Showalter Research Trust Fund. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the FDA.

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