Solvent-dependent PLGA solubility for separation of PLGAs with different L:G ratios

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Introduction

- Poly(lactide-co-glycolide) (PLGA) is a group of biodegradable polymers commonly used in various injectable long-acting depot formulations.
- There are many different types of PLGAs, resulting from different lactide:glycolide (L:G) ratios, endcaps, and molecular weights.
- Ensuring qualitative and quantitative (Q1/Q2) sameness between a reference listed drug and a proposed generic formulation in injectable depot formulations requires careful characterization of the PLGA components.

Purpose

- To develop a method to separate and characterize different PLGAs when used together in one product by leveraging their different solubilities in various solvents based on their L:G ratios.

Methods

Solubility Assay

- Initially, a series of PLGA polymers (MW of 80 ± 20 kDa) were characterized to determine their L:G ratios based on proton nuclear magnetic resonance (HNMR). For each PLGA, 100 mg was weighed into a tared glass vial and 4 ml of solvent was added. Each PLGA/solvent vial was then placed in shaking orbital incubator (100 RPM) overnight at a controlled temperature (30, 40, or 50°C).
- The solution was removed, and the remaining PLGA was vacuum dried to determine the remaining mass.

Separation Protocol

- Microparticles were prepared from PLGA 85:15 (85L) and 57:43 (57L) (Table 1), respectively, by emulsifying a dichloromethane (DCM)-polymer solution into an excess of 0.5% polyvinyl-alcohol (PVA) (Mowiol 4-88) solution and sizing/ collecting the particles by filtration. The molecular weight of the PLGAs were characterized by gel permeation chromatography (GPC) (Table 1).
- The microparticles were dry-blended along with excipients and additives listed in Table 1. This mixture was separated into 283 mg vials to create an example formulation.
- Each sample was transferred into tared glass centrifuge tubes containing a tared 3 mm glass ball.
- Each tube was washed with water (10 mL) (wash step: 4 °C/overnight), ethanol (10 mL), and hexane (10 mL) (wash step: 100 RPM/ 30 °C/ 1 hour/ centrifuge (3400 RPM/ 5 min).
- The remaining material was dried at 50°C overnight, and the mass was recorded (take NMR 'SAMPLE-TOTAL')
- The sample was dissolved in 10 mL chlorobenzene (100 RPM/ 30°C/ overnight) and centrifuged to separate away PLGAs with high lactide ratios (>75L).
- The remaining sample was dissolved in 10 mL acetone (100 RPM/ 30°C/ overnight) and centrifuged to separate away PLGAs with low lactide ratios (<75L).

Table 1. The composition of dry-blended microparticles.

Component	Mass (mg)	MW*
PLGA 57L microparticles	1,123.7	48,568
PLGA 85L microparticles	1,129.0	85,720
Mannitol, USP	917.5	NA
Sodium CMC	314.2	NA
Polysorbate 80	23.8	NA

*Molecular weights of PLGAs of prepared microparticles were measured by GPC.

Results

- The solubility results for PLGA include 'full solvents' which dissolve PLGAs regardless of L:G ratios (acetone, acetonitrile, anisole, chloroform, dichloro-methane, dimethylformamide, dimethylsulfoxide, ethyl acetate, and dioxane) and non-solvents which do not dissolve PLGAs (castor oil, ethanol, decanol, diethyl ether, hexane, lactic acid, and methanol).
- The solubility results also include semi-solvents, which have variable solubility for PLGA depending on the L:G ratio in the PLGA polymer.
- Table 2 shows select data from this study.

Table 2. Average mass dissolved (%) of 25 mg/ml solution (N=3).

	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature
L:G Ratio	50:50			75:25			88:12		
Solvent	30°C	40°C	50°C	30°C	40°C	50°C	30°C	40°C	50°C
Ethyl Acetate	99±0	98±0	97±3	99±0	98±0	97±0	98±0	98±0	98±0
Chlorobenzene	1±0	0±0	-7±1	99±0	99±0	99±0	97±1	98±0	97±1
n-Butyl Acetate	0±3	2±0	2±1	53±5	79±7	97±1	93±3	94±1	96±1

- Trends were noticed for esters and ketones in that longer alkyl chains resulted in greater selectivity for lactide content (Fig 1, 2)

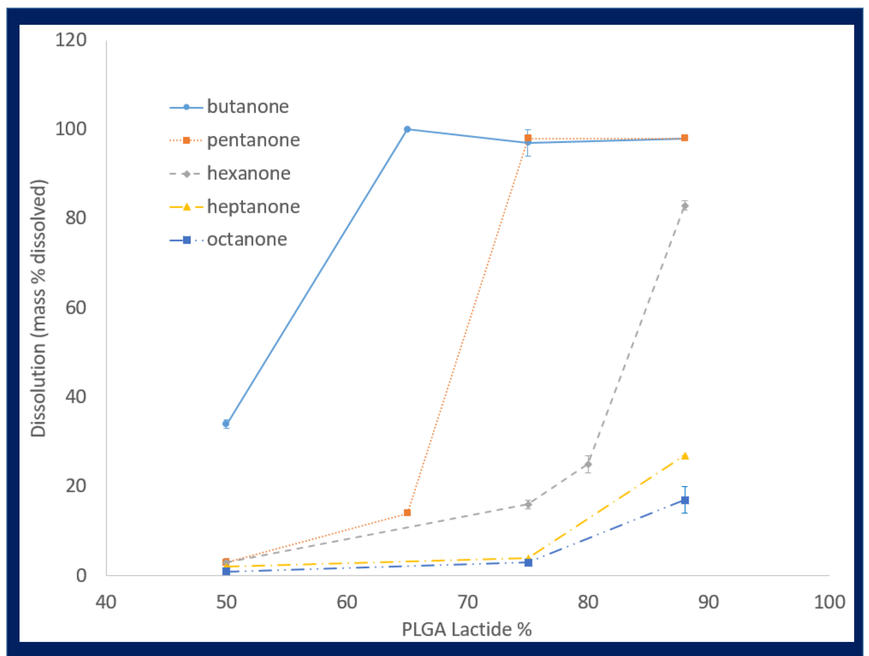

Fig. 1. PLGA solubility in ketones at 30 °C.

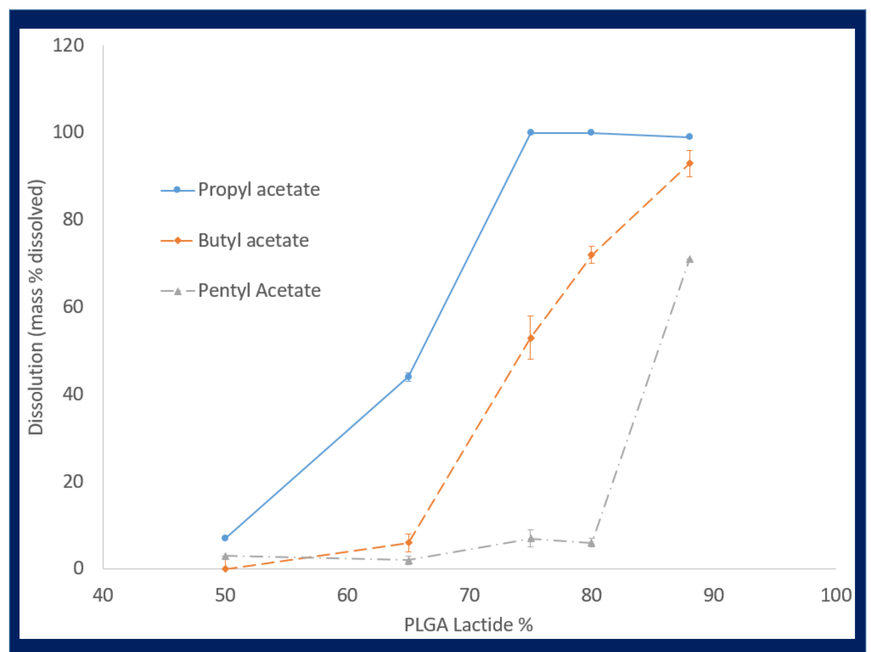

Fig. 2. PLGA solubility in esters at 30 °C.

- From this work a series of solvents were identified which showed an array of dissolution properties (Fig 3).
- Based on this capacity of solvents to variably dissolve components, a test separation assay was performed for a prepared mixture of polymer microparticles with two different PLGA types.
- Results from test separation protocol are listed in Table 3.
- The resulting NMR determined that lactide ratios were within ±4 % of the original polymer which is below the acceptable range of ±5% lactide ratio.
- The average resulting molecular weights determined were within <1,500 Da of those from the original polymer with no significant difference between the molecular weights before and after separation.

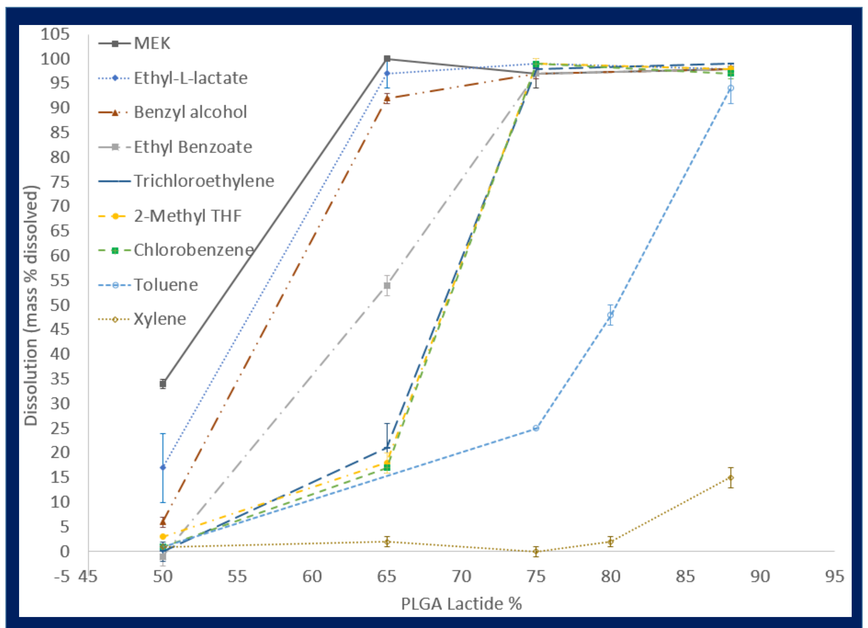

Fig. 3. PLGA solubility in various solvents at 30 °C.

Table 3. Characterization of PLGAs separated based on the protocol as compared with original polymers.

Description	Measured*	Original
Mixture (L%)[1]	69.5±0.8	NA
High-Lactide Fraction[1]	83.2±0.3%	85%
High-Lactide Fraction (MW)[2]	86,202±985	85720
Low-Lactide Fraction[1]	53.8±0.2%	57%
Low-Lactide Fraction (MW)[2]	47,269 ± 1,960	48568
Weight ratio (High Lactide/Low Lactide)[3]	1.10±0.09	1.004

*Average ± Standard deviation, n = 6.

1: HNMR, 2: GPC, 3: gravimetric measurement.

Conclusion

In the absence of semi-solvent effect, mixtures of PLGAs of varying L:G ratios remain inextractable. However, by leveraging the natural tendencies of selected solvents to preferentially dissolve PLGAs of certain L:G ratio, mixtures of the two types of PLGAs can be separated and characterized individually.

This technology enables the Q1/Q2 characterization of complex mixtures of PLGAs from a single sample to ensure the sameness between a proposed generic and an reference listed drug formulations.

Trends regarding the semi-solvent effects and PLGA L:G ratios have been identified. The mechanisms of the semi-solvent effects need to be fully elucidated with further studies. Understanding such mechanisms allows rational design of PLGA depot formulations with desirable drug release kinetics.

Acknowledgements

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