



## Injectable, long-acting PLGA formulations: Analyzing PLGA and understanding microparticle formation

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### ABSTRACT

Injectable, long-acting depot formulations based on poly(lactide-co-glycolide) (PLGA) have been used clinically since 1989. Despite 30 years of development, however, there are only 19 different drugs in PLGA formulations approved by the U.S. Food and Drug Administration (FDA). The difficulty in developing depot formulations stems in large part from the lack of a clear molecular understanding of PLGA polymers and a mechanistic understanding of PLGA microparticles formation. The difficulty is readily apparent by the absence of approved PLGA-based generic products, limiting access to affordable medicines to all patients.

PLGA has been traditionally characterized by its molecular weight, lactide:glycolide (L:G) ratio, and end group. Characterization of non-linear PLGA, such as star-shaped glucose-PLGA, has been difficult due to the shortcomings in analytical methods typically used for PLGA. In addition, separation of a mixture of different PLGAs has not been previously identified, especially when only their L:G ratios are different while the molecular weights are the same. New analytical methods were developed to determine the branch number of star-shaped PLGAs, and to separate PLGAs based on L:G ratios regardless of the molecular weight. A deeper understanding of complex PLGA formulations can be achieved with these new characterization methods. Such methods are important for further development of not only PLGA depot formulations with controllable drug release kinetics, but also generic formulations of current brand-name products.

## 1. Introduction

### 1.1. PLGA-based injectable long-acting formulations

In 1952, Smith Kline & French introduced the first 12-h oral delivery system, known as the Spansule<sup>®</sup> formulation, marking the beginning of controlled drug delivery technology [1]. Patients used to take oral medications 3 or 4 times a day, and the new twice-a-day formulation was a game changer in patient's convenience and compliance. Since then, numerous controlled drug delivery systems have been developed [2–4]. Despite > 60 years of advances in the controlled drug delivery field, developing injectable long-acting drug delivery systems has been slow. Since the first approval of Lupron Depot based on biodegradable poly(lactide-co-glycolide) (PLGA), also known as poly(lactic-co-glycolic acid), in January 1989 by the U.S. Food and Drug Administration (FDA)

[5], only 19 drugs have been formulated into injectable, long-acting depot formulations. This number is negligible compared with the thousands of oral sustained release formulations, including both brand-name and generic products, approved by the FDA. This indicates great difficulties in the development of injectable long-acting formulations for clinical use. The duration of drug release typically ranges from 1 week to 6 months. It has been difficult to control the drug loading and drug release kinetics from PLGA formulations for all drug types. Most formulations have a huge initial burst release which often consumes a quarter of the total drug in the first day.

### 1.2. NDA (brand-name) vs. ANDA (generic) formulations

Injectable, long-acting formulations present another conundrum in developing abbreviated new drug application (ANDA, or generic)

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versions of new drug application (NDA, or brand-name) formulations. The FDA recognizes that too many patients are being priced out of the medicines they need [6], and it initiated the Drug Competition Action Plan in June 2017 to promote competition, reduce prices, and enable more patients to have access to affordable medicines [6]. The FDA has been trying to improve generic drug submissions and reduce review times to approve more generic applications on their first cycle of review. > 1000 generic drug applications were approved by the FDA in 2017 alone [7]. The FDA is also acting to deter extending a drug's monopoly beyond what Congress intended through 'gaming' of the generic drug approval process [7]. Some branded firms make it unnecessarily difficult for generic manufacturers to get access to physical doses (2000–5000 doses) of a branded drug necessary to prove their generic medicine is the same as the branded drug, and exploit the citizen petition process to add resource burdens on the generic drug review process and the FDA's regulatory decision making [7]. Generic drugs have saved about \$500 for every American each year for the last 10 years [8]. The FDA's goal is to broaden access to safe and effective generic drugs while maintaining FDA's gold standard for rigorous, science-based regulation. Under the Generic Drug User Fee Amendments (GDUFA) [8], the regulatory science program was established. One of the aims is to develop a rigorous scientific program that can facilitate approvals of generic long-acting injectable formulations. This includes thorough characterization of PLGA which is a main excipient for long-term drug release. The systematic characterization of PLGA is critical in understanding and controlling drug release kinetics from PLGA-based formulations.

## 2. PLGA formulations in clinical use

PLGA formulations have been used to deliver small molecules, peptides, and proteins for periods ranging from 1 week to 6 months [9,10]. Table 1 describes PLGA-based injectable long-acting depot formulations in the order of the FDA approval date. On average, a new drug depot formulation is introduced every year and a half. Long-acting PLGA formulations have been approved as microparticles, a solid implant, or an in situ gel-forming implant. Of these, microparticle formulations have been used most widely due to the ease of administration relative to others. For example, administration of solid implants uses a large diameter needle, e.g., 14-gauge (2.108 mm outer diameter) for Zoladex [11], and in situ gel formulations require thorough mixing of

the contents in two syringes, each containing PLGA dissolved in solvent or the drug powder, by pushing the contents back and forth for 45 s, e.g., Eligard [12]. The in situ gel forming implant method, however, has been used only for small molecule and peptide drugs which do not have the tertiary structure of proteins. The only protein-delivering PLGA microparticle formulation in clinical use was Nutropin Depot [13], but it has not been available since 2004 due to manufacturing difficulties [14]. Clearly, a need exists for PLGA-based long-acting protein formulations to maximize the benefit of various protein drugs.

### 2.1. Challenges in developing PLGA microparticle formulations

Availability of only about 20 different drug products in 30 years indicates that the development of injectable, long-acting depot formulations is challenging. The advantages of long-acting formulations include improved patient compliance and convenience, and a lower dose of drug relative to the daily oral regimen. Then, a question here is what makes it so difficult to develop long-acting PLGA formulations? First, unlike oral dosage forms, the bioavailability of injectable long-acting formulations is extremely challenging to predict based on the drug properties, making it difficult to predict in vivo drug efficacy from in vitro release studies. In addition, minor changes in the in vitro release conditions can have a dramatic effect on the resultant release profile [15]. More importantly, many long-acting PLGA formulations have a significant initial burst release. Only a few formulations, including Risperdal Consta and Bydureon, show delayed drug release [16,17]. Neither huge initial burst release nor delayed release is typically desirable.

For most long-acting depot formulations, the drug concentration in the first day of administration is frequently 100 times higher than that at the steady state drug concentration in the blood. Fig. 1 shows two examples of such huge initial burst release [18]. The NDA on Trelstar LA (triptorelin pamoate lyophilized, 11.25 mg) describes that the 11.25 mg formulation releases the drug at a rate of 3.75 mg/month over 3-month [19], and the FDA approval package on the 22.5 mg 6-month formulation indicates triptorelin is released at the rate of 3.75 mg/month over 6 months [20]. Fig. 1-A shows the pharmacokinetic profile of 15 patients administered with the 6-month formulation [20]. The huge initial burst release results in the maximum serum concentration of ~40 ng/mL only 3 h after administration, and it is followed by a relatively steady serum concentration of < 1 ng/mL. This

**Table 1**

Examples of PLGA-based injectable depot formulations approved by the U.S. FDA.

Product name	API <sup>a</sup>	Type	Duration	Dose	Approved
Lupron Depot <sup>®</sup>	Leuprolide acetate	Microparticle	1,3,4,6 months	7.5 mg/month	1989, 1996, 1997, 2011
Zoladex <sup>®</sup> Depot	Goserelin acetate	Solid implant	1,3 months	3.6 mg/month	1989
Sandostatin <sup>®</sup> LAR	Octreotide acetate	Microparticle	1 month	10–30 mg/month	1998
Atridox <sup>®</sup>	Doxycycline hyclate	In situ gel <sup>a</sup>	1 week	42.5 mg/week	1998
Nutropin Depot <sup>®</sup>	Somatotropin	Microparticle	1 month	13.5 mg/month	1999
Trelstar <sup>®</sup>	Triptorelin pamoate	Microparticle	1,3,6 months	3.75 mg/month	2000, 2001, 2010
Somatuline <sup>®</sup> Depot	Lanreotide	Microparticle	1 month	60 mg/month	2000
Arestin <sup>®</sup>	Minocycline HCl	Microparticle	2 weeks	0.5 mg/week	2001
Eligard <sup>®</sup>	Leuprolide	In situ gel <sup>b</sup>	1,3,4,6 months	7.5 mg/month	2002
Risperdal <sup>®</sup> Consta <sup>®</sup>	Risperidone	Microparticle	2 weeks	12.5 mg/week	2003
Vivitrol <sup>®</sup>	Naltrexone	Microparticle	1 month	380 mg/month	2006
Ozurdex <sup>®</sup>	Dexamethasone	Solid implant	3 months	0.23 mg/month	2009
Propel <sup>®</sup>	Mometasone furoate	Solid implant	1 month	0.37 mg/month	2011
Bydureon <sup>®</sup>	Exenatide	Microparticles	1 week	2.0 mg/week	2012
Lupaneta Pack <sup>™</sup>	Leuprolide acetate	Microparticles	3 months	3.75 mg/month	2012
Signifor <sup>®</sup> LAR	Pasireotide	Microparticles	1 month	20–60 mg/month	2014
Zilretta <sup>®</sup>	Triamcinolone acetoamide	Microparticles	3 month	32 mg/3 months	2017
Sublocade <sup>™</sup>	Buprenorphine	In situ gel <sup>a</sup>	1 month	100, 300 mg/month	2017
Perseris <sup>™</sup>	Risperidone	In situ gel <sup>a</sup>	1 month	90, 120 mg/month	2018

<sup>a</sup> Active Pharmaceutical Ingredient.

<sup>b</sup> In situ gel is also known as in situ forming implant.

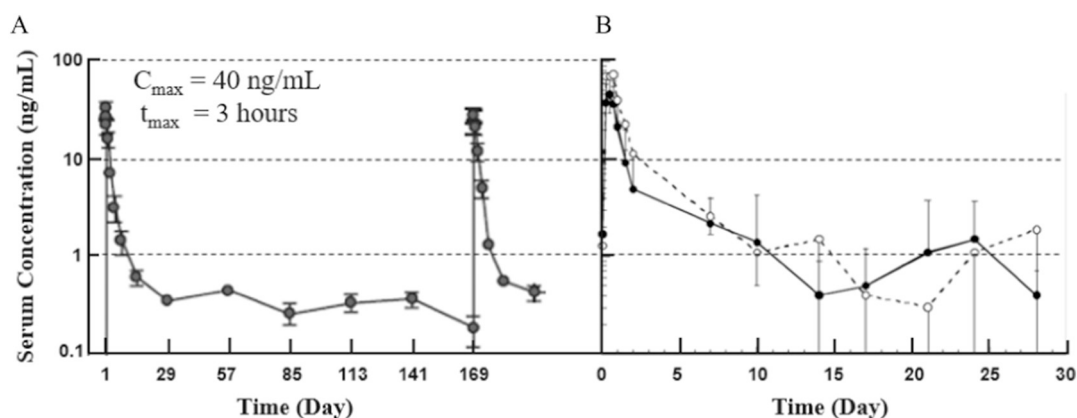


Fig. 1. (A) Mean triptorelin concentrations after the first and second triptorelin injections (Trelstar) in a subset of 15 patients. The formulation showed an initial release of triptorelin after each injection [20]. (B) Single dose mean growth hormone concentrations in pediatric growth hormone deficiency patients after subcutaneous injection of 0.75 mg/kg (●,  $n = 12$ ) and 1.5 mg/kg (○,  $n = 8$ ) (Nutropin Depot) [13].

pharmacokinetic profile in Fig. 1-A indicates that the initial triptorelin release rate is much higher than 3.75 mg/month as described in the Trelstar NDA.

Another example of a huge initial burst release from a PLGA-based product is of somatotropin shown in Fig. 1-B for Nutropin Depot. It has been the only PLGA formulation delivering a protein drug (recombinant human growth hormone somatotropin, a protein of 191 amino acid residues, with the molecular weight of 22,124 Da). Nutropin Depot was manufactured by the Alkermes' ProLease<sup>®</sup> process [21–23] and approved by the FDA in 1999. However, its production was discontinued in 2004 due to manufacturing difficulties [14]. The initial huge burst releases in Fig. 1, as well as in Fig. 2 below, may not be relevant to either drug's efficacy [18]. A daily injection of the same drug at a dose that maintains the serum concentration of 1 ng/mL should be as effective, but simply not as convenient as the long-acting depot formulations. An issue here is whether the cause of the initial huge burst release resulting in about 100 times higher serum concentration than that of the steady state can be understood, and thus, be prevented. Formulations with such high initial drug release have been approved,

indicating that the overall benefit is still larger than the potential risk. Controlling the drug release kinetics, including elimination of the initial burst release of PLGA formulations, will accelerate development of more clinically useful long-acting PLGA formulations for various drugs. More importantly, the drug consumed by the initial burst release may be used for extending the efficacy of the formulation, e.g., > 6 months using the same total dose.

### 3. Understanding drug release from PLGA depot formulations

#### 3.1. Microparticles, solid implant, and in situ forming gels

As listed in Table 1, there are three different types of PLGA formulations approved by the FDA: microparticles; solid implant; and in situ forming implant (or in situ gel). In situ forming implants are based on mixing drug powder with PLGA dissolved in solvent, usually *N*-methyl-2-pyrrolidone (NMP), just before injection. After the injection, NMP, a water-miscible solvent, is diluted with water at the injection site, leading to gel formation and solidification of the drug/PLGA mixture [24].

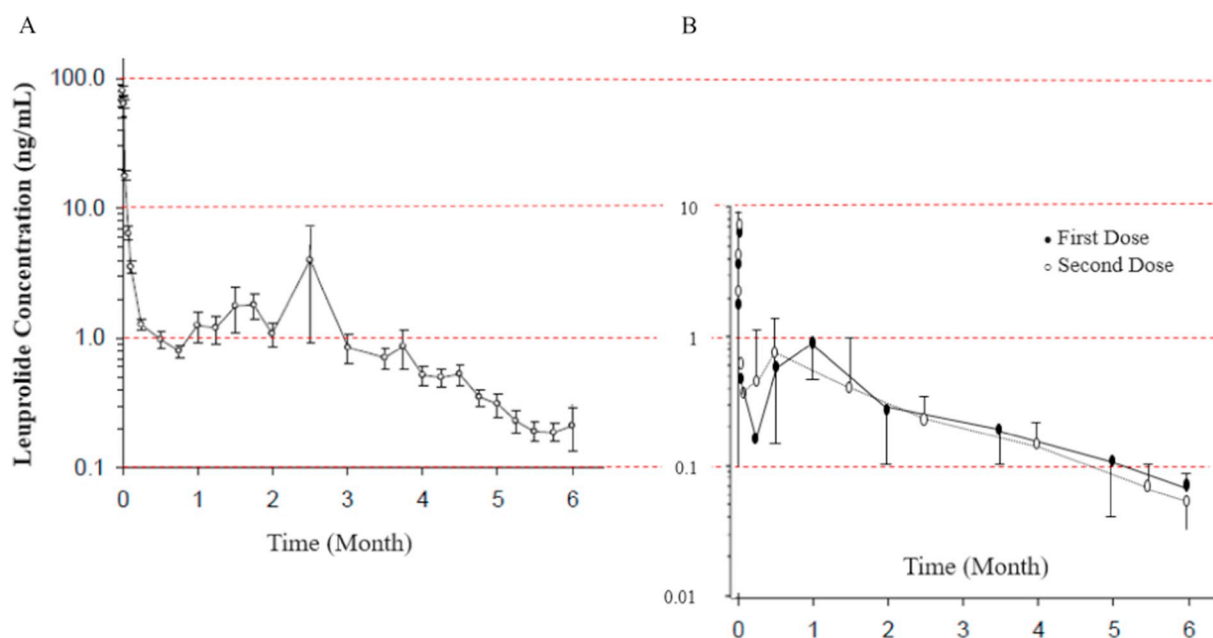


Fig. 2. The serum leuprolide concentrations in human patients after injection of Eligard (A) and Lupron (B). Both Eligard [12] and Lupron [25] deliver 45 mg leuprolide for 6-month efficacy.

Because there is no barrier to drug release before forming a gel, in situ gel formulations usually result in a huge initial burst release which is even larger than that observed in microparticle and solid implant formulations. Fig. 2 shows a comparison of leuprolide concentrations of Eligard (in situ gel formulation) and Lupron (microparticle formulation). Both formulations deliver 45 mg of leuprolide for 6 months.

The comparison of pharmacokinetic profiles of Eligard and Lupron reveals drastic differences in the serum leuprolide concentrations over the 6-month duration. In particular, the difference in the initial burst release is striking. The peak serum concentration of leuprolide for Eligard is 10 times higher than that by Lupron. Since both formulations are equally effective for 6 months, serum concentrations below 0.1 ng/mL should still be clinically effective. This means that the initial burst release results in 100 times and 1000 times higher drug concentration from Lupron and Eligard, respectively. Again, such high concentrations may still be safe as both formulations are approved by the FDA. Nevertheless, the question arises as to whether it is necessary to release large amounts. Although it is recognized that the initial burst release could be important for the onset of efficacy, the high initial burst release is probably not necessary in many cases, especially when the drug is potent and very expensive. Therefore, controlling the initial burst release could be beneficial as it can reduce the total dose without affecting product performances. The cause of the initial burst release is still not fully understood [26], and controlling the initial burst release is still difficult in many formulations. The ability to control the drug release kinetics, including the initial burst release, may be key to developing more clinically useful injectable, long-acting PLGA depot formulations.

As shown in Fig. 2, the magnitude of drug release from microparticle formulations is substantially lower than that from in situ gel formulations. The initial burst release from microparticle formulations, however, is still substantial. The same is true for PLGA solid implant formulations. It is important to understand the factors causing the initial burst release, enabling prevention and control of the drug release kinetics. Since in situ gel formulations provide no physical barrier for drug release until the solvent (e.g., NMP) is removed from the gel, they are not really designed to prevent initial burst release. The main reason for using such formulations is that the manufacturing process is less complex, relative to solid implants and microparticles. As long as a formulation's benefits outweigh the risks, it is typically approved by the FDA. This may be why the last two formulations approved by the FDA in Table 1 are in situ gel formulations both containing drugs and polymer demonstrating historically safe and effective usage. For those drugs that require strict control of the drug concentrations in the blood and cannot afford 100- or 1000-times difference, PLGA depot formulations with precise control of the initial burst release and duration of release are necessary. Thus, the following section examines the reasons for the initial burst release in microparticle and solid implant formulations.

### 3.2. Initial burst release from PLGA microparticles and solid implants

The huge initial burst release typically observed from in situ gel formulations is understandable, but the burst release from pre-

hardened microparticle and solid implant formulations is difficult to comprehend. Solid implants manufactured by a hot-melt extrusion method, such as Zoladex Depot [11] and Ozurdex [27,28], also result in an initial burst. One might assume that melt-extruded, pressurized polymeric rods would be immune to the burst, but that is not the case. Describing and proving a definitive mechanism of the initial burst from these formulations is challenging, especially when the surface area is extremely low.

A significant initial burst release from most microparticle depot formulations is often thought to result from drug on the surface or in the surface layer. Thus, it is necessary to understand why the initial burst release is often prevalent in both PLGA microparticle and solid implant formulations, even though they are manufactured under different processing conditions (emulsion methods vs. hot melt extrusion) and their final configurations are nearly opposite (a single solid rod vs. numerous microparticles). Regardless of the manufacturing method, the initial burst release is commonly observed, and thus, the reason(s) for the initial burst release may be the same. To further complicate matters, some PLGA microparticle formulations show delayed release depending on the manufacturing process, e.g., Risperidal Consta and Bydureon manufactured by Alkermes.

Another important aspect of the initial burst release is why the drug release slows down after the initial burst release. As shown in Figs. 1 and 2-B, the initial burst release is followed by a steady state release. It is easy to assume that the initial burst release is due to the release of the drug present on the surface, but this does not explain the slower release of the remaining drug. One likely explanation of the initial burst release followed by a steady state release is spontaneous external and internal morphological changes during the initial 24-h of drug release [29–31]. It has been well established that PLGA microparticles undergo continuous reorganization of the structure through pore opening and closing at the body temperature [32–36]. In addition to the pore healing, the interaction between the drug and PLGA polymers can also affect the drug release kinetics [37]. This can also explain that, in some instances, the drug release is delayed instead of the initial burst release.

## 4. Factors affecting PLGA microparticle formations

The mechanisms of PLGA microparticle formation are not fully understood. In the absence of a mechanistic understanding, development of injectable long-acting formulations has been based on a trial-and-error approach. It is necessary to delineate the factors critical to making PLGA microparticles by single or double emulsion methods, so that the microparticle properties can be controlled. Since PLGA depot formulations are developed for clinical applications, it is useful to examine the PLGA microparticle properties that are within clinically relevant limits.

### 4.1. PLGA microparticle properties important to clinical applications

The important properties of microparticles for clinical applications (or microparticle properties) include a microparticle size small enough

**Table 2**  
Properties to control for developing clinically useful PLGA microparticle formulations.

Property	Importance
Microparticle size	The largest size of microparticle needs to be less than ~100 μm for easy injection using 21–25 gauge needles
Drug loading	The higher drug loading allows injection of fewer microparticles. The desired drug loading capacity (i.e., the percentage of drug weight over the total solid contents) can be up to 40% depending on the drug type
Drug loading efficiency	The higher loading efficiency is critical for expensive drugs, such as peptide and protein drugs
Initial burst release	Currently, most PLGA formulations result in an initial burst release, where almost a quarter of the total drug is released in the first day without any enhanced therapeutic benefit
Drug release kinetics	The duration of drug release ranges from 1 week to 6 months, but it has to be accompanied with the above four parameters under control. In addition, the drug release kinetics need to match the PLGA degradation kinetics



for injection, high drug loading, high drug loading efficiency, and long duration of drug release without the huge initial burst release, as listed in Table 2. Studies in the literature have shown that each parameter can be controlled by adjusting the processing parameters, type of PLGA, and type of solvent or solvent combination. However, there has been no comprehensive study to control all five properties in Table 2. This is in part due to the absence of a mechanistic understanding of the PLGA microparticle formation.

One of the important properties to control is the microparticle size. The PLGA microparticle formulations currently in clinical use have large sizes, requiring large-diameter gauge needles for injection. For example, Trelstar and Risperidal Consta in Table 1 use 21-gauge needles (having an outer diameter (OD) of 0.813 mm and an inner diameter (ID) of 0.495 mm). Nutropin Depot, before it was withdrawn from the market, also used the 21-gauge needle. The size of Risperidal Consta microparticles ranges from 25  $\mu\text{m}$  to 180  $\mu\text{m}$  [16,38]. Delivery of the extended-release injectable formulation of naltrexone (marketed as Vivitrol) uses a 20-gauge needle (with an OD of 0.902 mm and an ID of 0.584 mm). In comparison, delivery of insulin typically uses a 28-gauge needle (with an OD of 0.356 mm and an ID of 0.165 mm). PLGA microparticles that can be administered using a 28-gauge needle or thinner needles will undoubtedly make clinical use more patient-friendly, although making smaller PLGA microparticles may come at the expense of other properties in Table 2. The control of microparticle size will be even more important for developing formulations for ocular applications treating age related macular degeneration. For intravitreal injection, 29- or 30-gauge needles are preferred to lower the patients' pain level [39].

#### 4.2. Mechanistic understanding of PLGA microparticle formation processes

The PLGA microparticle formation process can be dissected in several steps to obtain a mechanistic understanding of the process, and thus, to control the PLGA microparticle properties. Fig. 3 shows the steps critical to creating PLGA microparticles with desired properties, such as size, drug loading, and drug release kinetics. Solvent extraction from the embryonic emulsion (also known as seed emulsion) is the first step towards making PLGA microparticles. As solvent is extracted, dissolved PLGA molecules undergo coalescence leading to formation of a shell (also referred to as skin or membrane). As solvent extraction continues, the embryonic microparticles shrink to form localized, dense drug-PLGA microstructures throughout the microparticles. Simultaneously, water molecules diffuse in to replace solvent molecules. The spaces occupied by water and residual solvent become void after drying. After the desired amount of solvent has been extracted from the microparticles into the water phase, the hardened microparticles are collected and dried by freeze-drying. The dried microparticles may be treated with water (sometimes with added ethanol) again for further

control of the drug release. The sequential steps shown in Fig. 3 delineate the factors critical to producing PLGA microparticles with desirable properties. The mechanistic understanding and control of these steps is essential to preparing microparticles with preset properties to deliver a variety of drugs for treating various diseases.

Several important steps in microparticle formation in Fig. 3 are recapped. The solvent extraction kinetics is important for forming a stable shell, and it depends on the type of solvent used, temperature, and the type of PLGA. The solvent-dependent PLGA solubility is described in more detail below. The PLGA-solvent interaction also affects the PLGA coalescence and shell formation, ultimately leading to the quality of the formed microparticles. The mutual solubility of water and solvent affects the solvent extraction kinetics. More importantly, the selection of solvent dictates the drug solubility in the PLGA-drug mixture, especially for hydrophobic drugs, and thus, influencing the drug loading, drug loading efficiency, and drug release kinetics. The interactions among drug, PLGA, and solvent affect the microparticle properties in unpredictable ways, as their impacts are not linear. A small amount of residual solvent in the final PLGA microparticles can significantly influence the drug release kinetics. Due to each drug's unique physicochemical properties, each drug formulation requires an ideal combination of PLGA type, solvent type, and microparticle formation conditions. Nevertheless, the key steps described in Fig. 3 provide important parameters to control when designing an experimental approach based on quality by design.

#### 4.3. Presence of pores in PLGA microparticles

While a complete understanding of the mechanisms of PLGA microparticle formation and resultant drug release kinetics is still lacking, the presence of an initial burst release from most microparticles implies one common phenomenon. Water diffuses into the core of PLGA microparticles fast, and the dissolved drug is also released fast. As shown in Fig. 1-A, the peak triptorelin concentration occurs 3 h after injection. Other pharmacokinetic data in Figs. 1 and 2 also show a similar extremely fast drug release or initial burst. It is common to see pharmacokinetic data showing the peak drug concentration in the first day of injection. In fact, it is even typical to observe the peak in a matter of hours [40–42]. What may be the most likely reason for this extremely fast drug release from PLGA microparticles?

Zhao and Rodgers studied the transient ovalbumin distribution in PLGA microparticles using transmission electron microscopy (TEM) and coupled the TEM images to ovalbumin release [43]. Their study showed that 60.7% of all ovalbumin (i.e., 3.15% of the total 5.2% w/w ovalbumin/microparticle) was located on the microparticle surface or distributed in the large pores connected to the surface. The presence of interconnected channels leading to the surface is understandable as the PLGA molecules cannot possibly fill the whole volume that the initial oil droplet occupied. Thus, the initial high porosity seems to explain rapid release, but the surface of microparticles appears smooth without visible pores when examined by scanning electron microscopy (SEM). Thus, a large amount of drug released from PLGA microparticles in a matter of hours is still difficult to explain. This dilemma was explained by Fredenberg et al. who concluded that the pores on the surface may initially have been too small for detection by SEM [44]. Alternatively, water uptake into PLGA microspheres with a seemingly smooth, thin surface layer can directly trigger drug release by multiple processes, including polymer swelling resulting in surface pore formation or new pore networks [36]. In addition, the drug-PLGA interactions are also important in the initial burst release [45]. Either way, fast water influx, or uptake, is the first step for the drug release.

The presence of interconnected pores in PLGA microparticles essentially means that each microparticle is a porous body. The resolution limit of SEM, except recent ultra-high resolution SEM, is in the 10 nm range [46]. Thus, the pores present on the PLGA microparticle surface may not be visible, if they are much smaller than 10 nm. Penetration of

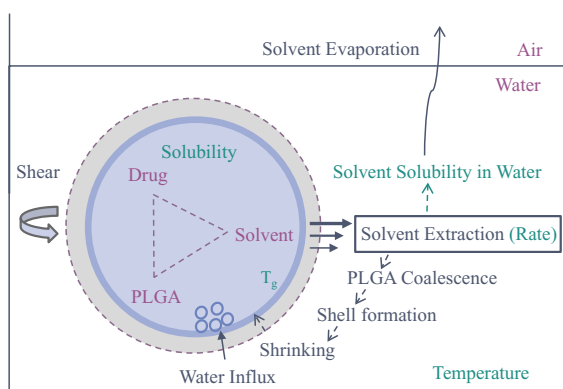


Fig. 3. Steps describing the initial process of embryonic microparticle formation.

**Table 3**  
Parameters and their values used to calculate water penetration into porous PLGA microparticles.

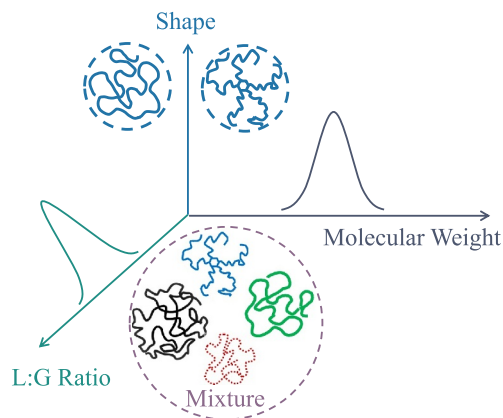
Symbol	Value and unit
$h$	Penetration depth cm or $\mu\text{m}$
$r$	Radius of a pore $1 \text{ nm} = 10^{-7} \text{ cm}$ (Any pore radius larger than 1 nm will result in increased penetration depth)
$\gamma$	Surface tension of water $72.8 \text{ dynes/cm}$ ( $= (\text{g cm/s}^2)/\text{cm} = \text{g/s}^2$ ) (Lower surface tension will reduce the penetration depth)
$\eta$	Viscosity of water $1 \text{ cP} = 0.01 \text{ g/(cm} \cdot \text{sec)}$
$\theta$	Contact angle of water $89^\circ$ ( $\cos 89 = 0.017$ ) (Any contact angle $< 89^\circ$ will result in increased penetration depth)
$t$	Penetration time 1 s

water into a porous body was first analyzed by Edward W. Washburn by treating it as an assembly of very small cylindrical capillaries [47]. This treatment of a porous body as assembled capillaries can be applied to studying liquid penetration to various systems, including powders [48], compressed powder cakes [49], moisture absorption into concrete [50], and fibrous structures such as fabrics [51].

According to the Washburn equation, the volume of a liquid that penetrates is proportional to the square root of the surface tension and viscosity of the penetrating liquid, as shown in Eq. (1), and Table 3 lists the parameters in Eq. (1) and their values.

$$h = \left( \frac{r\gamma\cos\theta}{2\eta} t \right)^{\frac{1}{2}} \quad (1)$$

For a sample calculation, the radius of pores is assumed to be 1 nm, and the contact angle of PLGA microparticle surface of  $89^\circ$  is used. The contact angle of PLGA microparticles is not above  $90^\circ$ , as there are surfactants, such as poly(vinyl alcohol) (PVA) used during the emulsification step, and Tween 20 used in the drug release medium. Entering the values from Table 3 into Eq. (1) leads to a  $25 \mu\text{m}$  penetration in 1 s. In reality, interconnected pores in PLGA microparticles are highly tortuous and some of them may not be connected. At the same time, the actual sizes of pores may be much larger than 1 nm used in the above calculation. Other factors, such as the PLGA density, hydrophobicity, porosity, and interconnectivity, all affect the actual time for water to infiltrate into the microparticles. Nevertheless, the Washburn equation suggests that it will be a matter of minutes or hours, not days, for water to infiltrate into the microparticles. This explains the observation of  $t_{max}$  in hours, rather than days or weeks, for water-soluble molecules, as seen in Fig. 1-A.



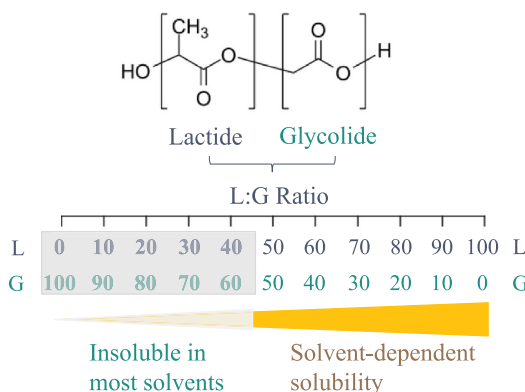
#### 4.4. Solid-state forms of the drug and polymer mixture

An often-neglected property of PLGA microparticles is the solid-state form of the drug and PLGA mixture. The drug-PLGA mixture can exist in three states: (i) molecular dispersion where the drug is at or below the equilibrium solubility in the PLGA; (ii) amorphous solid dispersion where the drug exists in an amorphous state dispersed in the PLGA matrix; and (iii) a crystalline suspension where the drug exists in a crystalline state suspended/dispersed in the polymer matrix. The drug release profile is further influenced by the physical location of the solid-state forms, e.g., uniform drug distribution or segregated drug domains throughout the PLGA matrix. The processing parameters, including the solvent type, extraction time, extraction medium, PLGA type, and drug-PLGA interactions, affects the solid-state form of the drug and drug distribution in microparticles.

### 5. Characterization of PLGA

The initial burst release, to a certain extent, can be controlled by using different PLGAs, e.g., PLGA with larger molecular weights and/or with higher lactide:glycolide (L:G) ratios. These properties affect the final structures of the microparticles, as they influence other events during microparticle formation, such as PLGA coalescence, shell formation, and solidification. This, in turn, impacts the formation of pores throughout the microparticles, ultimately determining the initial burst and subsequent drug release kinetics. Thus, it is critical to characterize a PLGA or a mixture of PLGAs present in the final formulation, as well as the raw PLGA materials.

A full characterization of PLGA typically relies on accurate measurements of molecular weight, L:G ratio, end group (ester or acid), and polymer shape (linear or branched), as shown in Fig. 4. Of these, the L:G ratio has a greater impact than others on the polymer solubility in organic solvents. As the L:G ratio increases, i.e., as the lactide portion increases, PLGA dissolves in a larger number of solvents. When the L:G ratio is smaller than 50:50 (i.e., more glycolide), these PLGAs dissolve only in highly fluorinated solvents, such as hexafluoroisopropanol. The shape of PLGA affects the physicochemical properties, but characterization of PLGA shape has been difficult. The shape of PLGA, or any polymer for that matter, cannot be determined by molecular weight alone. Characterization of the accurate molecular weight, L:G ratio, and shape of PLGA becomes critical when a formulation utilizes a mixture of different PLGAs with different molecular weights, L:G ratios, and shapes. Understanding these parameters is important not only for quality control purposes, but also for controlling the drug release



**Fig. 4.** (A) Characterization of PLGA includes molecular weight, L:G ratio, and shape (e.g., linear or branched). If a formulation consists of a mixture of PLGAs with more than one type of molecular weight, L:G ratio, and shape (as shown inside the dotted circle), identification and characterization of individual PLGA components are challenging. (B) PLGAs with different L:G ratios dissolve in different solvents, and this property allows separation of PLGAs even if their molecular weights are the same.

**Table 4**

Molecular weights of PLGA with different L:G ratios measured by GPC coupled to multi angle static light scattering (MALS) and by using polystyrene external standards (PES).

Sample	L:G ratio	M <sub>w</sub> (Da)			M <sub>n</sub> (Da)		
		MALS	PES	Difference	MALS	PES	Difference
PLGA 50 L-S	51:49	8303	12,523	51%	5834	8415	44%
PLGA 50 L-M	50:50	23,790	34,975	47%	16,280	24,121	48%
PLGA 75 L-S	78:22	17,830	28,889	62%	12,180	20,557	69%
PLGA 75 L-M	72:28	25,370	37,973	50%	16,290	24,595	51%
PLGA 75 L-H	71:29	96,330	141,117	46%	76,170	97,812	29%
PLA 100 L-S	100:0	15,890	13,829	−13%	8142	9350	15%
PLA 100 L-M	100:0	33,900	53,429	58%	24,900	34,998	41%
PLA 100 L-H	100:0	132,700	176,477	33%	103,200	104,474	1%

(The mobile phase for the DAWN HELEOS II MALS measurement was acetone. On the other hand, the mobile phase for the polystyrene external standard was tetrahydrofuran (THF). THF was not suitable for MALS measurement due to its low refractive index [57].)

properties. In addition, a thorough characterization plays an important role in developing generic versions of PLGA-based products approved by the FDA. For example, the 6-month Trelstar formulation is expected to have more than one type of PLGA [19,52,53], and thus, separation of different PLGAs is essential in meeting the Q1/Q2 sameness requirement for parenteral drug products under 21 CFR 314 [54]. Another clinical product, Sandostatin LAR delivering 20 mg octreotide acetate for 1 month, is made of branched PLGA, also called star-PLGA, based on glucose (Glu-PLGA) [55]. It is not known whether the formulation consisting of Glu-PLGA has different drug release properties as compared with the formulation consisting of linear PLGAs with the same molecular weight. Thus, new methods need to be developed for characterization of branched PLGAs and for separation and identification of individual PLGAs from a mixture of different PLGAs.

### 5.1. Molecular weight

The molecular weights of PLGAs are routinely measured by gel-permeation chromatography (GPC) using external standards or by inherent viscosity. External standards typically used are polystyrene standards. The molecular dimension of polystyrene in a given solvent is different from that of PLGA due to dissimilar polymer-solvent interactions. Thus, the molecular weight obtained by polystyrene standards may be acceptable only for a relative comparison. Molecular weights of PLGAs available from commercial sources are often calculated from measuring inherent viscosity. This again provides inaccurate information, as the molecular dimension of PLGA in a given solvent also depends on the L:G ratio, and thus, it is difficult to rely on estimating molecular weights of PLGAs with different L:G ratios using one standard curve obtained using a given solvent. Accurate molecular weights of PLGA can be obtained by multi angle static light scattering (MALS) that does not rely on external standards for the molecular weight determination. Table 4 shows a comparison of molecular weights of PLGAs determined by both MALS and polystyrene external standards (PES) [56]. The differences between the values obtained by MALS and PES are quite large, and the conventional PES method under the applied conditions overestimates the molecular weight, up to almost 70% in one example. For accurate measurements of PLGA, it is necessary to use MALS, and the limitations of the PES method stem from the differences in solvent interactions of PLGA and polystyrene.

### 5.2. L:G ratio

The properties of PLGA depend in large part on the L:G ratio, molecular weight, and end group, either acid or ester. A small difference in the L:G ratio, e.g., 50:50 vs. 65:35, results in a different solubility profile, degradation kinetics, and interactions with a drug, leading to different drug loading and drug release kinetics [9,58]. As the ratio of lactide increases (i.e., higher L:G ratio), the degradation slows down

due to the presence of a hydrophobic methyl group resulting in slower absorption and diffusion of water. Other formulation- and process-related parameters may affect the overall in vivo drug release kinetics as well. Formulation-related factors include the type of organic solvent used, the concentration of polymer used, and the drug-polymer interactions [59]. Although the organic solvent used during the processing is removed, it can influence the final formulation structure and drug release kinetics. Table 5 shows a general trend of PLGA solubility in different solvents as a function of the L:G ratio [60,61].

### 5.3. Molecular shape

According to the information available from the package insert/drug label of injectable, long-acting PLGA formulations in clinical use, it seems that almost all formulations utilize linear PLGA except Sandostatin which specifies the use of branched (or star-shape) PLGA, i.e., Glu-PLGA. To this date, no literature information has been available for determining the branch number of Glu-PLGA. Measuring the branch number of Glu-PLGAs by <sup>1</sup>H NMR has been challenging, because the glucose structure, as present in the esterified polymer form, does not provide any unique signal that is distinguishable from other signals from PLGA. It is possible to use <sup>13</sup>C-glucose to calculate the number of branch units, but the signal has been too weak thus far or obscured by the signal of long PLGA chains. Thus, the best approach to determine the branch number of Glu-PLGA is to measure the intrinsic viscosity, [η], as a function of molecular weight after running GPC [56]. The Mark-Houwink equation allows determination of the branch units per Glu-PLGA molecule when the plot is compared with those of standard branched PLGAs. To validate the branch number of Glu-PLGA, a series of star-PLGAs with known branch number ranging from 3 to 6 (e.g., trimethylolpropane for 3, pentaerythritol for 4, adonitol for 5, and dipentaerythritol for 6) were synthesized, and the branch unit per molecule was confirmed using <sup>1</sup>H NMR. Alternatively, the branch number can be calculated using a star-polymer model based on the mean square radii of gyration of branched and linear PLGAs or intrinsic viscosities of branched and linear PLGAs along with the drainage factor, e.g., Astra 7 software from Wyatt [63]. Fig. 5 shows an example of determining the branch numbers of Glu-PLGAs synthesized in the laboratory and obtained commercially. As shown in Fig. 5, the Glu-PLGAs tested in our laboratory have branch number between 2 and 3 and molecular weights between 32,000 and 64,000 Da. Several Glu-PLGA samples are currently tested to determine the number of branch units as a function of the molecular weight, effectively presenting the fingerprint characteristic unique for each Glu-PLGA.

## 6. Better characterization of PLGA

In the development of new injectable, long-acting depot formulations, the characterization of PLGA was usually limited to the molecular

**Table 5**  
Dissolution of PLGA in different solvents depending on the L:G ratio.

Solvent	PLGA L:G Ratio										
	50:50	55:45	60:40	65:35	70:30	75:25	80:20	85:15	90:10	95:5	100:0
Dichloromethane											
Dimethyl sulfoxide											
Dimethyl formamide											
Ethyl acetate											
Methyl ethyl ketone											
Tetrahydrofuran											
Ethyl benzoate											
Chlorobenzene											
Benzyl alcohol											
Methyl n-propyl ketone											
n-Butyl acetate											
Trichloroethylene											
Ethyl-L-lactate											
2-Methyl tetrahydrofuran											
1,2-Dichlorobenzene											
Toluene											
Methyl isobutyl ketone											
Butyl lactate											
p-Xylene											

(The PLGA dissolution depends on the polymer concentration, polymer molecular weight and temperature. The information is based on a PLGA molecular weight around 80,000 Da and concentration of 2.5% (w/v) at 37 °C [62]. The PLGAs used were all in the DL form, and most of them have acid end-caps. The end-cap does not appear to affect the solvent solubility. The dark blue

weight, L:G ratio, and end group. As long as PLGA formulation's benefits outweigh the risk in clinical trials, it may be approved for clinical applications. There are, however, various reasons why PLGA characterization needs to be more thorough especially for the development of corresponding generic products. PLGAs from different vendors are often supplied with specifications providing similar molecular weights, L:G ratios, and end groups. The current state of PLGA characterization, however, does not provide nearly enough discriminatory power to elucidate clear differences between varying batches and suppliers. The reality is that they are very different in their properties, as observed by the difference in drug release profiles, even though the PLGA formulations are made following exactly the same method. Currently, there are no compendial testing methods to fully characterize PLGAs.

As described above, the ultimate property that matters is the drug release, largely depending on the physicochemical properties of PLGA. Without thorough characterization methods, it is difficult to reproduce the data and even more difficult to develop better formulations having desirable drug release profiles. For PLGAs with non-linear shape, e.g., star-shape, no literature information is available yet on how to characterize the branch number of Glu-PLGA (i.e., the branched PLGA used in Sandostatin). Due to the absence of the method determining the exact number of branches and chain lengths of branches, it has been challenging to understand exactly which PLGA molecule, or molecules, are used in the product. If a product contains a mixture of PLGAs, different PLGAs need to be separated before characterization. GPC can only separate PLGAs based on the molecular weight, and thus, it cannot separate PLGAs of the same molecular weight, but different L:G ratios. This may also lead to issues during quality control testing.

As listed in Table 1, currently there are 19 PLGA-based injectable, long-acting depot formulations approved by the FDA. Many of them are

not under patent protection any longer. And yet, developing generic equivalents of PLGA formulations has been difficult, partly due to the absence of any known protocol for characterization of PLGA properties. Meeting the Q1/Q2 requirements for generic products has been challenging. Characterization of Glu-PLGA is a case in point. The absence of available methods for characterization of Glu-PLGA makes the development of generic products referencing Sandostatin difficult. This brings an interesting way of extending a product lifecycle even after its patents are expired. If a PLGA used in a product cannot be characterized and/or duplicated, it will be difficult to develop generic products. It is also hard to control post-marketing changes in the formulation for brand-name products. In this way, the brand-name product is shielded from generic competition even after the patent expiration.

While the information described above shows the characterization of non-linear PLGA, such as Glu-PLGA, and the separation of PLGAs based on the L:G ratios, additional studies are necessary to improve the techniques, so that PLGAs with even minute differences can be isolated and characterized. It is anticipated that the new long-acting formulations in the future use mixtures of PLGAs with different molecular weights, different L:G ratios, and even different molecular structures (i.e., linear or branched). Finding a formulation that can meet all the properties in Table 2 may necessitate complex PLGA systems. The ability to isolate different PLGAs and characterize them, in turn, will enhance our understanding of PLGA formulation development, e.g., making microparticles with the same properties reproducibly. The progress made on PLGA characterization over the last few years has provided a strong foundation for bigger and faster advances in the PLGA characterization methods and development of new and generic long-acting depot formulations.



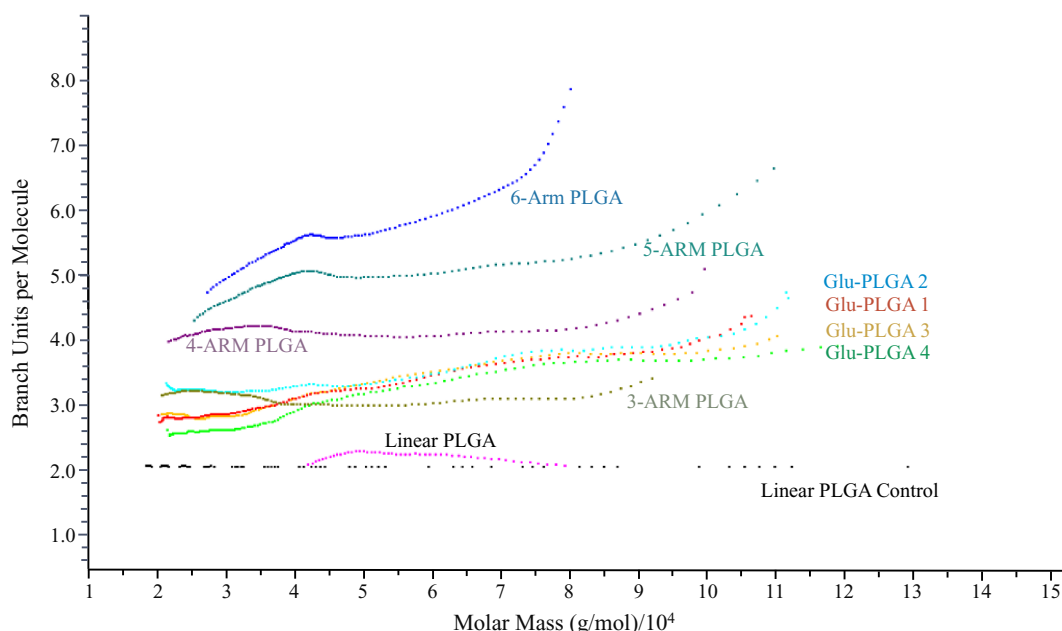


Fig. 5. Measurements of the number branch units of Glu-PLGAs and standard star-PLGAs of 3, 4, 5, and 6 arms. All Glu-PLGAs have a 55:45 L:G ratio.

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