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PLGA-based long-acting injectable (LAI) formulations

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ABSTRACT

Long-acting injectable (LAI) formulations, which deliver drugs over weeks or months, have been in use for more than three decades. Most clinically approved LAI products are formulated using poly(lactide-*co*-glycolide) (PLGA) polymers. Historically, the development of PLGA-based LAI formulations has relied predominantly on trial-and-error methods, primarily due to a limited understanding of the complex factors involved in LAI formulations and insufficient analytical techniques available for characterizing individual PLGA polymers of the prepared formulations. This article offers a personal perspective on recent advancements in characterization methods for PLGA polymers within final formulations, i.e., products, as well as enhanced insights into the drug release mechanisms associated with LAI products. With a deeper understanding of PLGA polymer properties and drug release mechanisms, the formulation development process can transition from traditional trial-and-error practices to a more systematic Quality by Design (QbD) approach. Additionally, this article explores the emerging role of artificial intelligence (AI) in formulation science and its potential, when applied carefully, to enhance the future development of PLGA-based LAI formulations.

1. Long-acting injectable (LAI) formulations

Since the introduction of the first controlled-release drug delivery system, known as Spansule, numerous controlled-release formulations have been developed through the smart use of various polymers, ranging from natural to synthetic, water-soluble to water-insoluble, and nondegradable to biodegradable polymers [1]. Biodegradable polymers are preferred for any formulation designed to be implanted inside the body, as retrieval of nondegradable formulations after use is highly undesirable. Of the many biodegradable polymers available for drug delivery and biomaterial applications, poly(lactide-*co*-glycolide) polymers have been widely used. Poly(lactide-*co*-glycolide), also known as poly(lactic-*co*-glycolic acid), is usually abbreviated as PLGA. In developing clinically used LAI formulations, PLGA polymers have been used exclusively, even though other biodegradable polymers have also been available [2,3]. This is largely due to the fact that the U.S. Food and Drug Administration (FDA) has approved many LAI formulations based on PLGA polymers, for which general safety has been established. There is no reason for pharmaceutical companies to explore new biodegradable polymers if their safety profiles have not been established. Using new polymers adds a new burden for companies to undergo tedious and costly safety studies. Thus, PLGA polymers have become the polymers of choice for developing LAI formulations.

One of the primary goals of formulation research is to optimize drug delivery systems, enabling the formulation to be reproducible and further improved based on scientific data. To achieve this goal, the formulation approach should not be empirical but rather based on a fundamental understanding of the formulations and their ingredients. Since one of the critical components of LAI formulations is PLGA, full characterization of PLGA polymers is essential in developing an ideal formulation for each drug. PLGA polymers, however, are highly diverse in molecular structures and compositions, and thus, their physicochemical properties and variability between vendors.

This article is focused on LAI formulations based on PLGA polymers. There are 27 LAI products approved by the FDA, all of which are based on PLGA polymers. The LAI formulations have been administered via intramuscular (IM), subcutaneous (SQ), intraarticular, and intravitreal routes, using microparticles, solid implants, and in situ gel-forming implants. While the information in this article applies to all three PLGA formulations, the discussion focuses on microparticle formulations, where specific examples and explanations are necessary. For example, the debate on drug release kinetics primarily focuses on

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microparticle formulations, which may not necessarily be applicable to in situ forming implants.

The overall outline of this article is described in Fig. 1. The numbers attached to the square boxes in Fig. 1 refer to the section numbers. Section 1 describes the benefits and difficulties of developing LAI formulations. Section 2 provides an in-depth discussion of PLGA polymers, enabling the selection and characterization of suitable PLGAs for intended formulations. Section 3 deals with drug release mechanisms and the initial burst release, which occurs in all LAI formulations. Section 4 deals with the formulation development approach based on Quality by Design (QbD). While the discussion on QbD is universal, PLGA microparticle formulations are used as a representative formulation when specific examples are necessary. The quality target product profile (QTPP) is first determined and then translated into the critical quality attributes (CQAs). The CQAs depend on critical material attributes (CMAs) and critical process parameters (CPPs). CMAs are clear, as formulation components are well-defined. However, CPPs are more complex due to numerous known and unknown variables that can impact the properties of LAI formulations and, consequently, critical quality attributes (CQAs), which in turn affect the quality target product profile (QTPP). Furthermore, a slight variation in each critical processing parameter can have a significant impact on CQAs. Each parameter may work independently, but it is often related to the other parameters. Such interactions between parameters are difficult to recognize. This is why it is essential to develop simpler methods for producing microparticles, allowing for better control of the parameters and, consequently, improved reproducibility. Examples include efficient solvent-free remote encapsulation based on electrostatic interactions between anionic PLGA and cationic peptide [4] and the preparation of leuprolide microparticles using a single emulsion (with methylene chloride and methanol) instead of a double emulsion [5]. Another practical reason for developing a simpler process is to decrease the cost and time needed to produce the microparticles under current Good Manufacturing Practice (cGMP) conditions. Section 5 provides a brief description of the process for translating laboratory formulations into FDA-approved products. Most formulation scientists in academia do not pay enough attention to translating laboratory formulations into products. Years of research and subsequent innovation in formulation development can be justified mainly with laboratory-to-clinic translations [6]. It is FDA-approved products administered to patients that treat diseases, not innovations for the sake of innovation, limited to the laboratory. One of the reasons for the lightning-speed development of the COVID-19 mRNA vaccine was the availability of lipid nanoparticle formulation used for the delivery of siRNA (Onpattro®). The final section examines the potential application of artificial intelligence/machine learning (AI/ML) in the development of LAI formulations.

1.1. The benefits of LAI formulations

The ultimate goal of formulation studies is to produce products that patients can use to treat diseases [7]. For use by patients, the formulation has to be approved by the FDA for its safety and efficacy in the drug's proposed use [8]. FDA approval means that the drug formulation is determined to provide benefits that outweigh its known and potential risks for the intended population [9].

LAI formulations have been utilized to treat chronic conditions, offering several advantages [10]. LAI formulations offer patients convenience and compliance, providing a more effective treatment of the medication [11,12]. There appear to be more than 200 long-acting injectable formulations in clinical use [13]. The first LAI formulation is known to be fluphenazine enanthate, which was developed by G.R. Daniels at E.R. Squibb & Sons in 1966 [14]. Since then, numerous LAI formulations have been developed. LAI formulations include oily solutions, aqueous drug suspensions, microparticles, solid implants, and in situ forming implants [12,15,16]. In situ forming implants include PLGA polymers dissolved in organic solvents, thermosensitive copolymers containing poly(ethylene glycol) (PEG), such as PEG-PLGA copolymers [17,18], and lyotropic liquid crystals (LLCs) [19]. The drugs with poor

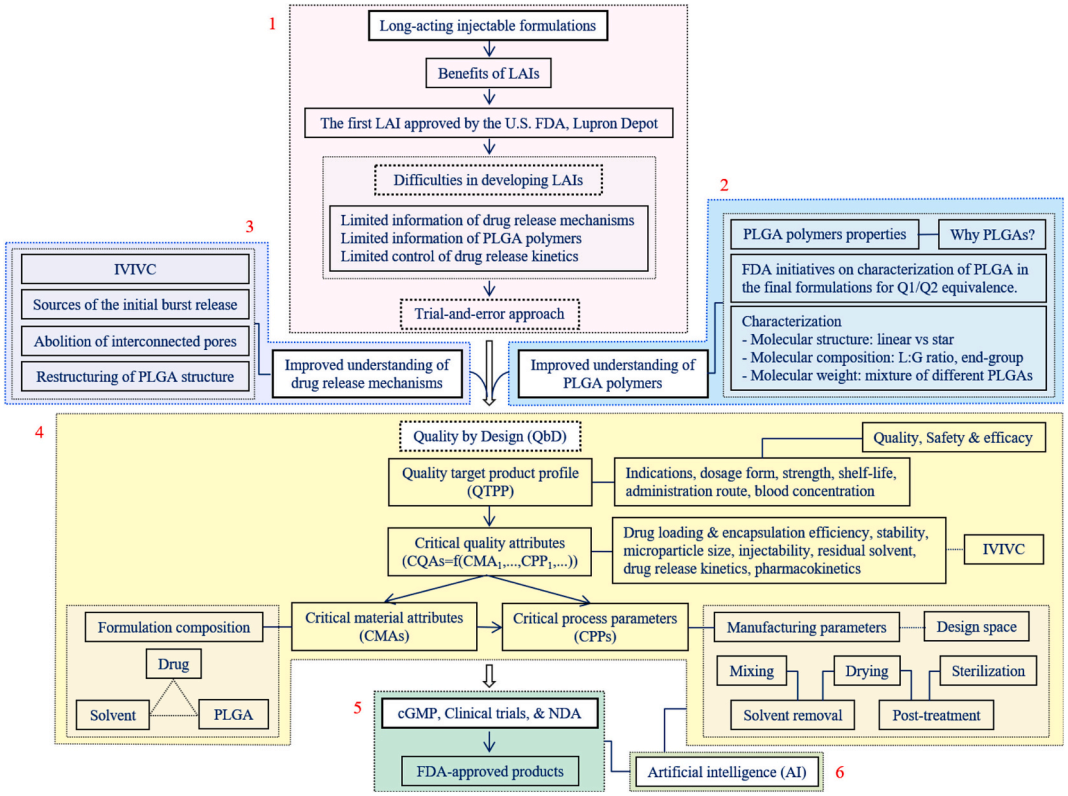

Fig. 1. Overall outline of the contents. The numbers beside the square boxes refer to the section numbers.

water solubility can be administered as drug suspensions or oil solutions [12,16]. Drug suspensions are often in the form of drug nanocrystals stabilized by surfactants, and they are useful for drugs that are practically insoluble in water and/or require high doses [11,20,21]. For example, Cabenuva is a 2-drug co-packaged product consisting of cabotegravir (400 mg/2 mL or 600 mg/3 mL, Apretude) and rilpivirine (600 mg/2 mL or 900 mg/2 mL) developed for the treatment of HIV-1 infection and administered intramuscularly every month [22]. For such large amounts of drugs to be delivered, it would not be practical to use any polymeric vehicles to control the drug release kinetics. Most drugs, however, do not require such large amounts, and it has been common to use polymers to control the drug release kinetics for up to 6 months.

1.2. The first long-acting injectable formulation, Lupron depot

The first PLGA-based long-acting injectable formulation was a 1-month release Lupron Depot® approved by the U.S. FDA in 1989 for the treatment of prostate cancer. In Europe, Decapeptyl® SR, which delivers triptorelin, was first approved in 1986 [2]. Lupron Depot® consisted of PLGA microparticles delivering leuprolide acetate for 1 month after IM injection. The advances made in producing Lupron Depot using the water/oil/water (W/O/W) double emulsion method include improving the drug loading to 12 % (*w*/w) by increasing the viscosity of the internal W/O emulsion. It was achieved by adding gelatin to the internal water phase or by increasing the drug concentration, decreasing the PLGA concentration in an organic solvent, and cooling the W/O phase before emulsifying it into the external aqueous phase [23,24]. The increased viscosity in the internal W/O phase reduced the extraction of the internal water, resulting in improved loading capacity [24,25]. The polymers used were poly(D,L-lactic acid) (PLA) and PLGA (L:G ratio of 90:10) with a molecular weight of around 18–22 kDa [24,26]. The lessons learned from preparing Lupron Depot were: (i) the leuprolide loading could be increased to 20 % by increasing the viscosity of the W/O internal phase and partly due to the ionic interaction between the negatively charged carboxylates of PLGA/PLA polymers and the positively charged amino acid groups of the drug, (ii) 1-month sustained release was obtained by optimizing the drug loading, e.g., 12 % instead of 20 % as the higher drug loading resulted in the formation of more internal water channels resulting in immature fast release profiles, (iii) a higher drug loading was achieved by increasing the particle size, (iv) PLA and PLGA 90:10 released only around 20 % of the drug in 1 month, (v) PLGAs with lower molecular weight (14 kDa) and lower L:G ratio (75:25) resulted in nearly a zero-order release over 1 month, and (vi) PLA of 15 kDa resulted in the drug release over 3 months [23,24,26–28]. The study paved the way for optimizing the desired drug release kinetics through the judicious selection of the L:G ratio and molecular weight of PLGAs. Following the 1-month Lupron Depot, microparticle formulations delivering leuprolide (7.5 mg/month) for 3, 4, and 6 months were introduced in 1996, 1997, and 2011, respectively.

1.3. Difficulties in developing LAI formulations

The PLGA-based LAI formulations are listed in Table 1. In Table 1, three drugs (leuprolide, triptorelin, and risperidone) have been approved by different companies over the years. Since the first approval of the leuprolide LAI formulation (Lupron Depot) in 1989, two additional microparticle formulations (Lupaneta in 2012 and Lutrate in 2018) and two in situ gel-forming formulations (Eligard in 2002 and Camcevi in 2021) were introduced to the market. For risperidone, three additional long-acting injectable (LAI) formulations (Rykindo in 2023, Perseris in 2018, and Uzedy in 2023) have been introduced since the initial approval of Risperdal in 2003.

The availability of multiple LAI formulations for leuprolide, triptorelin, and risperidone highlights the importance of these drugs, their market sizes, and the relatively straightforward development of LAI formulations. On the other hand, only a limited number of drugs (a total of 17) have been developed into LAI formulations over the last 35 years, which also indicates the difficulties of developing LAI formulations for new drugs. LAI formulations require long-term (months to years) data on safety and efficacy, making the development of new LAI formulations challenging. As shown in Table 1, the technologies used in LAI

Table 1
PLGA-based long-acting injectable formulations approved by the FDA.

Microparticles				
Lupron, 1989–2011 Leuprolide acetate 1, 3, 4, 6 months 7.5 mg/month	Sandostatin, 1989 Octreotide acetate 1 month 20 mg/month	Nutropin, 1999 Somatropin 1 month 13.5 mg/month	Trelstar, 2000–2010 Triptorelin pamoate 1, 3, 6 months 3.75 mg/month	Arestin, 2001 Minocycline HCl 2 weeks 1 mg/2 weeks
Risperdal, 2003 Risperidone 2 weeks 25 mg/2 weeks	Vivitrol, 2006 Naltrexone 1 month 380 mg/month	Somatulin Depot, 2007 Lanreotide 1 month 60, 120 mg	Bydureon, 2017 Exenatide 1 week 2 mg/week	Lupaneta, 2012 Leuprolide acetate 3 months 3.75 mg/month
Signifor, 2014 Pasireotide 1 month 20, 40, 60 mg/month	Triptodur, 2017 Triptorelin 6 months 22.5 mg/6 months	Zilretta, 2017 Triamcinolone aetonide 3 months 32 mg/3 months	Lutrate, 2018 Leuprolide acetate 3 months 22.5 mg/month	Rykindo, 2023 Risperidone 2 weeks 25–50 mg/2 weeks
Solid implants				
Zoladex, 1989 Goserelin acetate 1, 3 months 3.6 mg/month	Ozurdex, 2009 Dexamethasone 3 months 0.7 mg/3 months	Propel, 2011 Mometasone furoate 1 month 0.37 mg/month	Scenesse, 2019 Afamelanotide 2 months 8 mg/month	Durysta, 2020 Bimatoprost 6 months 10 μg/6 months
In situ gel-forming implants				
Atridox, 1998 Doxycycline hyclate 1 week 50 mg/week	Eligard, 2002 Leuprolide acetate 1, 3, 4, 6 months 7.5 mg/month	Sublocade, 2017 Buprenorphine 1 month 100, 300 mg/month	Perseris, 2018 Risperidone 1 month 90, 120 mg/month	Fensolvi, 2020 Leuprolide acetate 6 months 45 mg/6 months
Camcevi, 2021 Leuprolide 6 months 42 mg/6 months	Uzedy, 2023 Risperidone 1–2 months 50–100 mg/month			

formulations approved by the FDA have remained relatively unchanged over the last three decades. One of the primary reasons for the difficulties in developing LAI formulations by utilizing existing technologies is the lack of a clear understanding of PLGA polymers and their properties, which makes it challenging to prepare suitable LAI formulations with the desired drug release kinetics. Other reasons include a lack of understanding of the fine control of drug loading, drug-PLGA interactions, and the long-term degradation behavior in vivo. Most of the LAI formulations have been based on the trial-and-error approach [29].

1.4. Trial-and-error approach

Since the basic requirements for FDA approval of new drug applications are safety and efficacy, the applied formulations will be approved as long as these requirements are met, regardless of the drug's release kinetics. All drug release kinetics show that there is, almost always, an initial burst release, causing the drug concentration in the blood to reach up to 500 times higher than the steady-state concentration [30,31]. Those are safe, or the benefit is significantly larger than the potential risk. The question is why such an initial burst release could not be controlled. It was simply due to a poor understanding of the formulations. Although such formulations are still safe and effective, some patients may not be able to tolerate such high doses. More importantly, the release of unnecessarily large amounts of the drug in the beginning is a waste of the drug that could have been used for longer efficacy.

The manufacturers of brand-name products often argue that such a high initial burst release is important for the drug's long-term efficacy. This, however, is not true. The daily injection of the same drug exhibits no initial burst release, unlike LAI formulations, but maintains the same efficacy. The initial burst release, most of the time, does not enhance the drug's effectiveness, except in unique situations where a rapid rise in the drug concentration in the blood is necessary. The daily injections are simply inconvenient. Furthermore, the release of the same drug (e.g., leuprolide) from different formulations (e.g., Lupron vs. Eligard) is orders of magnitude different, and yet all formulations have shown the same efficacy [31].

2. Understanding PLGA polymers

PLGA polymers have been used extensively since their introduction in 1989 for the development of the first LAI formulation (see Table 1). One question frequently raised is why PLGA polymers have been used exclusively when there are other biodegradable polymers, such as poly (ε-caprolactone), polyorthoesters, polypeptides, and proteins [32]. Non-PLGA polymers have been used in FDA-approved products, including tri (ethylene glycol)-poly(orthoester (Zynrelef and Sustol) and poly[bis(*p*-carboxyphenoxy)propane:sebacic acid] (Gliadel). Other non-biodegradable polymers used are poly(ethylene-*co*-vinyl acetate) (Probuphine and Implanon) and poly(2-hydroxyethyl methacrylate)/poly(2-hydroxypropyl methacrylate) (Vantas and Supprelin LA) [16]. Each non-PLGA polymer has only one or two products, and most FDA-approved LAI products are based on PLGA polymers. One of the disadvantages of using PLGA polymers is the generation of acid as they degrade, which significantly lowers the local pH and frequently causes inflammation [33]. Despite these drawbacks, PLGA polymers have been the polymer of choice by formulation scientists. This is because using PLGA polymers, especially those used in FDA-approved products, will not raise any new concerns related to the use of polymers that have not been proven safe in the human body. If new biodegradable polymers are used, their safety profiles must be examined through short-term studies to assess immediate adverse effects, tolerability, and initial pharmacokinetic profiles. The long-term study monitoring for delayed adverse effects may extend to several years. Even for formulations that did not use polymers (e.g., the 6-month paliperidone palmitate formulation), a 3-year follow-up study was conducted for safety and efficacy [34]. Even if new polymers have better properties (however they are defined) than PLGA polymers, proving the safety of new polymers is highly costly. The cost of demonstrating the safety profile and unknown potential effect on the drug efficacy may hinder pharmaceutical companies from using them. It is unlikely that other biodegradable polymers will be used for LAI formulations unless their properties are significantly better than those of the current PLGA polymers. The release of drugs, polymer degradation kinetics, and body responses are still difficult to control. New biodegradable polymers may become the choice if they can maintain those properties and substantially improve LAI formulations. Until then, PLGA polymers are expected to remain the polymer of choice for developing LAI formulations for the foreseeable future.

2.1. PLGA polymer properties

Although PLGA polymers have been used for over five decades, they are not fully understood. It is important to appreciate the true nature of PLGA polymers when selecting the appropriate PLGA for specific applications.

The first conventional assumption made by researchers new to PLGA polymers is that they are hydrophobic and, thus, dissolve in any organic solvent. This turns out to be quite untrue. Another common assumption is that the properties of PLGA polymers depend mainly on their molecular weight. This is only partly true, as other parameters of PLGA polymers, such as molecular structure and composition, also influence their properties. Furthermore, currently, there are no PLGA standards that provide known properties for scientists to use as controls.

PLGA polymers are highly diverse, and their properties depend on multiple factors, primarily molecular weight, molecular structure, and molecular composition, as illustrated in Fig. 2. For polymer properties, molecular weight is one of the key factors to consider. Sandostatin LAR® Depot is based on branched glucose-PLGA (Glu-PLGA), also known as star PLGA, synthesized by Thomas Kissel [35]. Branched PLGA polymers have a lower viscosity and molecular size than the corresponding linear polymers of the same molecular weight. PLGA polymer properties are also influenced by the molecular composition, including the lactide: glycolide (L:G) ratio and the end-cap (or end-group).

Characterization of PLGA polymers, including their structure and composition, is crucial in selecting the appropriate PLGA for each application. PLGA interacts with drugs, solvents, and other excipients. PLGA polymers are generally low-glassy polymers that can be easily plasticized by drugs, solvents, other excipients, and temperature [36]. Plasticization can benefit certain processes but also produce high variability in drug release kinetics. The type and concentration of a drug may plasticize PLGA differently, resulting in different drug release profiles. This is one of the reasons why using the same PLGA does not guarantee the same release profile of another drug, even if all other variables remain the same. As PLGA polymers are inherently diverse in their molecular weights, L:G ratios, and end-caps, it is not easy to expect the same properties from polymers obtained from different lots. Even for the same lot, if small samples are collected in other sections of the lot, their properties may not be identical.

2.2. Characterization of PLGA in the final formulations

Despite extensive use, PLGA polymers were poorly understood. For example, only recently was it known that PLGA dissolution in organic solvents depended on the type of PLGA, mainly its L:G ratio [37]. In a given solvent, PLGA with a lower molecular weight has higher solubility than PLGA with a higher molecular weight. Such differences raised awareness that the PLGA polymer initially used may not remain the same after the multi-processing steps involved in making LAI formulations. Even if the same PLGA is used for making microparticles, the properties of the PLGA constituting the final formulation that underwent complex processing may be different. This is because other processes may use different solvents, mixing methods, washing procedures, temperatures, and post-treatments. It is the properties of the PLGA of the

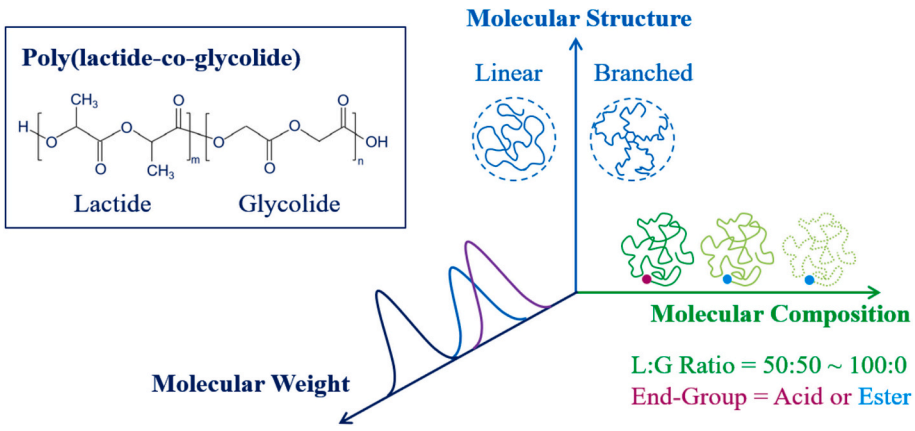

Fig. 2. The structure of PLGA and three molecular properties (molecular structure, molecular composition, and molecular weight) affecting the polymer properties.

final formulation, i.e., the product, that the FDA is interested in knowing, not the initial raw PLGA purchased from manufacturers.

The efforts to better understand PLGA polymers were initiated by the FDA Center for Drug Evaluation and Research (CDER) more than a decade ago. The goal was to understand the properties of PLGA polymers in Reference Listed Drugs (RLDs), or products, used in patients so that generic products could match the Q1/Q2 (qualitative/quantitative sameness) requirements. The three main challenges identified to be associated with developing generic PLGA-based LAI formulations were: (i) lack of full understanding of the impact of CMAs and CPPs on product performance, (ii) lack of compendial in vitro release testing (IVRT) methods that can discriminate formulations with manufacturing differences and predict in vivo performance, and (iii) complicated bioequivalence study designs because of long application durations and complex multi-phasic in vivo pharmacokinetic profiles [38,39]. Here, drug release and drug dissolution are used interchangeably, even though the two terms are technically different. Drug release is a result of multi-step processes, including drug dissolution [40].

Until the FDA's CDER initiated a systematic program to develop new characterization methods and understand PLGA properties in detail, it was challenging to compare the PLGA polymers used in generic products and RLDs. The CDER's efforts were particularly critical because characterizing the PLGA polymers in products, rather than the raw polymers used at the beginning of formulation, is not trivial. Even if only one type of PLGA is used in a product, the polymer is inherently heterogeneous and is likely to undergo product-specific changes after the manufacturing process [11]. The PLGA polymers in the products may be identified if sufficient sample quantities and necessary assay methods are available. Many formulations, however, are made of more than one type of PLGA, differing in molecular weight, molecular structure, and molecular composition. The difficulty arises when no assay method is established to assess certain PLGA properties properly, such as molecular structures, particularly those of branched PLGAs. The difficulty becomes compounded if PLGAs of different molecular structures have similar molecular weights and molecular compositions. The difficulty becomes even more complicated if there are not enough PLGAs available in a formulation. These situations are discussed using a few commercial products as examples.

2.3. Characterization of PLGA polymers

Characterization methods of raw, linear PLGA polymers are well established. However, the difficulty arises when PLGA polymers must be characterized after preparing an LAI formulation. When preparing the final formulations, fractions of polymers may be lost, which can alter the overall properties of the remaining PLGA polymers.

2.3.1. Characterization of molecular structure: Linear vs. star

Sandostatin LAR Depot (octreotide acetate for injectable suspension) is prepared using glucose-initiated PLGA polymers, usually described as glucose-PLGA (Glu-PLGA) or star PLGA (branched PLGA). Glu-PLGA has a lower viscosity and molecular size than the corresponding linear PLGA [35]. No assay method was available to accurately characterize Glu-PLGA until the paper on the characterization of branched PLGA polymers was published in 2019 [31,41,42]. Traditionally, it was assumed that Glu-PLGA would have 5 PLGA chains due to the presence of 5 alcohol groups that can initiate the polymerization of PLGA. However, at ring-opening polymerization temperatures, glucose may participate in oxidative side reactions (caramelization), and not all hydroxyl groups may be equally accessible (e.g., due to steric hindrance), so 5-arm branching may or may not be fully achieved for any given glucose molecule. Without a suitable assay method, however, it was impossible to determine the number of branches (or arms) per each glucose and polydispersity index of Glu-PLGA and arms. Naturally, determining the Q1/Q2 for generic products was not possible. The ensemble identification methods, including the measurement of intrinsic viscosity as a function of polymer molecular weight to establish Mark-Houwink plots, enabled the comparison of Glu-PLGAs used in different formulations. Fig. 3 shows an example of the Mark-Houwink plots of branched PLGAs against the branch standards of 2–6 arms. At a given molecular weight, the intrinsic viscosity allows the determination of the branch unit per molecule. The intrinsic viscosity can also be used to calculate the branch unit using the drainage factor as a function of the molecular weight [41]. Four samples of Glu-PLGAs from Sandostatin LAR exhibit heterogeneity in the branch units per molecule, with this heterogeneity increasing as the molecular weight increases. Sando 10, 28, and 66 indicate Sandostatin samples of 3 different lots. The data shows that all Sandostatin LAR Glu-PLGAs have two arms, i.e., linear PLGA, at the lower end of the molecular weight, but they mostly overlap with the 3-arm standard. Only at the higher molecular weight end ($> \sim 80{,}000$ g/mol) does the branch unit reach 4. Only <6 % of Glu-PLGA showed a branch unit of 4, and the average branching value ranges from 3.10 to 3.25 for the Sandostatin extracts [41].

2.3.2. Characterization of molecular compositions: L:G ratios

Trelstar® (triptorelin pamoate for injectable suspension) is made of more than one type of PLGA. The information on PLGA compositions is a trade secret and, therefore, not publicly available. At least it was known that linear PLGAs were used. Thus, the main difficulty was centered around how many different L:G ratios were used. To identify individual PLGAs with different L:G ratios, a new assay method, called the semi-solvent method, was developed [31,37]. The method is based on the finding that PLGAs of different L:G ratios dissolve in different organic solvents. PLGA polymers with an L:G ratio of 50:50 (PLGA 50:50), for

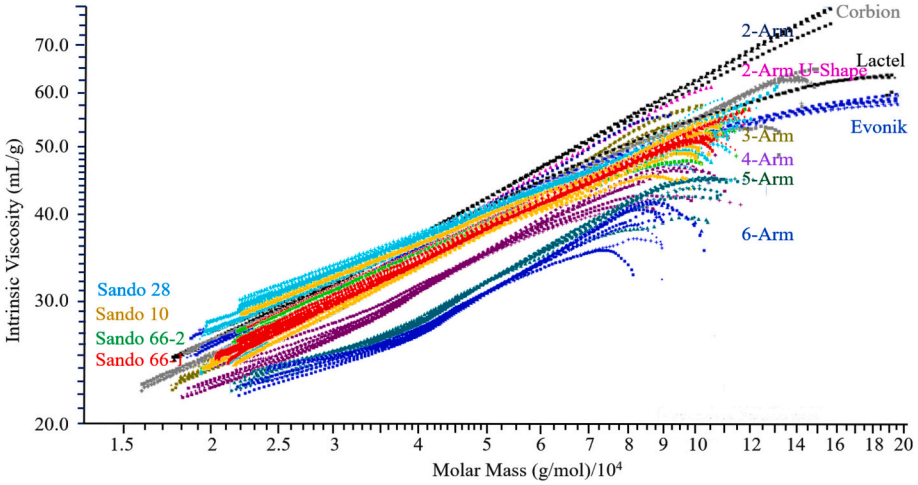

Fig. 3. Mark-Houwink plots of Glu-PLGAs of Sandostatin, Corbion, Evonik, and Lactel overlapped with branch standards of 2–6 arms. (Redrawn from reference [41]).

example, have very different solubility properties in various organic solvents as compared to PLGAs with higher lactide contents, such as 60:40, 75:25, and 90:10 [43]. Dozens of different organic solvents were tested to determine the L:G ratio-dependent solubilities in organic solvents [37,43]. The semi-solvent effect method was used to separate PLGAs having different L:G ratios. In addition, the comparison of monomer sequence distributions (Rc = (G-G)/(G-L)) was examined using ^{13}C NMR. The information was used to obtain L:G sequencing in terms of the degrees of randomness (or blockiness). This was examined in each fraction of the isolated PLGA polymer. Combining the Rc and molecular weight information with the L:G ratios of PLGA fractions separated using semi-solvents allowed the determination of 3 different PLGA types used in Trelstar [37]. This may also represent various degrees of lactide content in multiple types of PLGA in the blended product, as PLGA polymers exhibit dispersion in lactide content across chains.

2.3.3. Characterization of molecular compositions: The end-cap

The nature of the end-cap of PLGA polymers plays a significant role in drug loading and release. For example, a drug with positive charges, such as octreotide, can interact with the negatively charged acid end-caps of PLGA molecules. It was demonstrated that octreotide formed a salt with the acid end-caps of linear PLGA chains present in Sandostatin LAR, which is composed of Glu-PLGA. The linear PLGAs may exist as a byproduct of the degradation of Glu-PLGA. The PLGA-octreotide salt is thought to catalyze octreotide acylation, resulting in extended peptide release [44]. Leuprolide is also known to interact with the acid groups of PLGA [45].

Identification of the PLGA end-cap seems straightforward based on NMR measurement. However, it requires long hours of measurement time and special equipment [46]. If a product contains two types of PLGAs with two different end-caps with unknown molecular weights, quantifying the percentage of PLGA-acid and PGLA-ester becomes challenging. The PLGAs with acid end-caps can be measured by the total acid number (TAN) using potentiometric and colorimetric methods [47–49], but they require large quantities. Measuring the TAN for a product with a total weight of less than 1 mg (sub-milligram quantity) causes significant difficulties. A new analytical method for quantifying PLGAs with acid end-cap was developed [50]. The method was based on modifying the acid end-cap with pyrene methylamine, a UV dye, to enhance the signal and compare it with the total PLGA quantity measured by the refractive index (RI) after the sample was run through a gel permeation chromatography (GPC) system. This GPC-UV/RI approach was applied to quantify the acid content in an Ozurdex-similar formulation. Ozurdex implant consists of 116 μg of PLGA-ester and 350 μg of PLGA-acid in the size of 0.46 mm in diameter × 6 mm in length [51,52]. Since Ozurdex could not be purchased for research, an Ozurdex-similar formulation developed by Professor Feng Zhang at the University of Texas-Austin was used for assay [53,54]. The acid content in the Ozurdex-similar sample was 76 ± 23 %, closely matching the literature value but with a wide standard deviation. The large variation is due to several factors: (i) the small sample size tested, (ii) the extraction process from the drug-loaded sample, and (iii) the blend aspects of the sample. When the same method was applied to neat polymer controls, the variation was small and consistent with the degree of variation observed during titration-based techniques on very large sample quantities.

2.3.4. Characterization of microstructural arrangements of PLGA in microparticles

During the microparticle formation process, each microparticle experiences different conditions, from forming an emulsion through solvent extraction to the drying and post-processing steps. This variation in individual microparticles is often overlooked, as most conventional testing methods (e.g., in vitro release, encapsulation efficiency, and polymer extraction and analysis) are based on the analysis of a large number of microparticles. It has been challenging to examine an individual microparticle for its PLGA composition and microparticle structure, specifically its microstructural arrangement and morphology. The dynamic nature of microstructure, particularly the opening and healing of pores on the PLGA microparticle surface, has been demonstrated previously [55–58].

The microstructure is the manifestation of the use of different types of PLGAs and varying manufacturing conditions [43,59]. To this end, a new technique was developed, known as surface analysis after sequential semi-solvent impact (SASSI), which utilizes sequential semi-solvent vapors (SSV), also referred to as surface analysis of (semi-solvent) vapor impact (SAVI) [60,61]. The key here is to use a semi-solvent vapor, ensuring that exposure to semi-solvents does not dislodge the microparticles during the introduction and removal of the semi-solvents. The use of semi-solvent vapors also facilitated an easy transition to the next semi-solvent, eliminating the need for significant drying time when liquid semi-solvents were employed. Since there is no liquid, the PLGA polymers are gradually dissolved by semi-solvent vapors, exposing the inner parts of the microparticles. The particle morphological changes were quantified by image analysis using laser scanning confocal microscopy. This allowed for the investigation of changes in the surface and inner structural shape of the same microparticles during

degradation or drug release processes.

The SAVI analysis focuses on individual microparticles; thus, the actual deviations of particle behavior among individual microparticles become apparent. Although large standard deviations occur among microparticles prepared the same way, the changes in the microparticle properties observed by SAVI are distinct enough to compare different batches of microparticles. The SAVI is ideal for investigating the microstructural properties of PLGA microparticles. Additionally, depending on the solubility properties of the loaded drug, further features may be observed, such as the drug structure within the microparticles (e.g., naltrexone, which is observed to exhibit a cubic structure inside loaded particles). This technique also allows probing microstructural changes of the microparticles during in vitro release testing [60,61]. The approach is useful because minor differences in PLGA properties (such as L:G ratio and molecular weight) can result in drastic effects on the microstructural arrangement of formulated microparticles, affecting drug release profiles [60,61]. Thus, examining the microstructures of PLGA formulations may provide insight into whether the microparticles are composed of the same formulation compositions or produced under the same processing conditions. Such information can add assurance of the similarity to other properties, such as in vitro release and pharmacokinetic profiles.

3. Understanding drug release mechanisms

3.1. In vitro-in vivo correlation (IVIVC)

The ultimate test of PLGA microparticle formulations is to obtain a pharmacokinetic profile in humans, but it is unattainable until the Phase 1 study. Thus, animal pharmacokinetic studies are used at the beginning of formulation development. The results of animal studies indicate whether the microparticle formulations are reproducible using the same materials and processing. The use of animal pharmacokinetic profiles is not intended to predict human pharmacokinetic outcomes; rather, it aims to establish a correlation with in vitro release data. This correlation enables the effective assessment of the reproducibility of the formulation using in vitro release data. Additionally, it suggests that formulations exhibiting the same in vitro release profiles are likely to exhibit similar in vivo pharmacokinetic behaviors. Since conducting animal studies is cost-intensive and time-consuming, in vitro release studies are routinely used to represent pharmacokinetic profiles after an IVIVC is established. Even before IVIVC is established, in vitro release studies are used to examine whether the prepared microparticles have the expected properties. In the absence of compendial in vitro release testing methods [39], it is critical to understand the impact of the test conditions on the release kinetics and interpretation of test results [62,63].

In an ideal situation, in vitro drug release should be correlated to in vivo drug release [38,58]. Measuring in vivo drug release, however, is significantly more challenging than determining the pharmacokinetic profile, as it requires retrieval of the remaining microparticles, e.g., using a cage [58] or carefully removing them [64]. The retrieval of remaining microparticles is prone to experimental errors due to the degradation of PLGA polymers and the inability to collect all microparticles that remain. This is why in vivo pharmacokinetic profiles are commonly used. In vitro release testing, also known as dissolution testing, is important because it links the formulation to the in vivo pharmacokinetic profile, thereby demonstrating proven safety and efficacy. In vitro release testing also links lots of the same formulations manufactured at different times and sites to safety and efficacy. The selection of an in vitro release test is critical because it should be able to discriminate the effect of material and process variabilities in the formulation. It goes without saying that in vitro release testing should be done under the same conditions. A slight change in any parameter, such as the pH of the release medium or temperature, can significantly affect release profiles [65–67].

All the impacts of variables in LAI formulations and processing are manifested in in vitro release profiles. Likewise, in vivo release or absorption profiles reflect all variables, such as the type of animals used, the type of injection (e.g., SQ vs. IM), the injection site (e.g., deltoid vs. glute), the animal's diet, and the animal's health. This is why IVIVC can be established regardless of the details of measuring in vitro release and in vivo release or absorption. Thus, an IVIVC can be established as long as the processes obtaining in vitro release and in vivo drug absorption are consistent [68]. Since clinical performance can be linked to product quality through IVIVC, the impact of the drug product's CQAs on in vivo performance can be studied. Thus, CMAs and CPPs during product development and manufacturing with assured clinical outcomes can be examined using in vitro release testing as a surrogate [69,70]. The ability to substitute in vivo pharmacokinetic studies with in vitro release studies is critical in the early stage of product development. In general, IVIVC is also essential for quality control, regulatory compliance [71,72], and validation of scale-up production [73]. The established IVIVC for a microparticle formulation may not apply to other types of LAI formulations, even for the same drug [12,68].

IVIVC can also include physiologically-based pharmacokinetic (PBPK) models to incorporate specific mechanisms, such as the impact of physiological response at the site of injection, that might affect the drug release from the microparticles and absorption into the blood using the drug's physicochemical properties, drug release properties, and the blood flow through the injection site [12]. The PBPK model can accelerate the development of LAI formulations by reducing the burden on animal experimentation [74].

3.2. In vitro drug release studies

Drug release kinetics from LAI formulations is one of the most important parameters that must be examined carefully and understood to make desirable formulations. Thus, many scientists have dedicated their efforts to understanding and, hopefully, controlling the drug release kinetics for LAI formulations. Fig. 4 shows four typical in vitro release profiles usually observed in LAI formulations.

Although LAI formulations are designed to release the loaded drug in a controlled manner to achieve the desired therapeutic effects, the drug release profiles cannot be easily controlled. The drug release kinetics are affected by various factors depending on the formulation. Thus, no two formulations may have the same release profiles. However, most drug release profiles are variations of the four commonly observed release profiles [75,76], as described in Fig. 4. Fig. 4-A represents one of the most common release profiles. Most of the loaded drug is released in the beginning, and the subsequent release is very limited. Naturally, their duration of efficacy is short. Fig. 4-B is ideal as the initial, small burst release is followed by a steady-state release to maintain the drug efficacy over the intended period. Fig. 4-C is another common drug release profile observed with LAI formulations. After the low initial burst

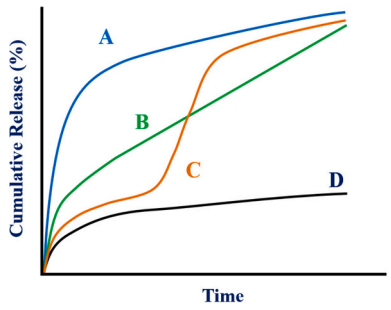

Fig. 4. Four typical drug release profiles observed in LAI formulations. (A) High initial burst release (>30 %) followed by little additional release. (B) Low initial burst release (<30 %) followed by steady-state release. (C) Low initial burst release followed by a burst/steady-state release. (D) Low initial burst release followed by negligible additional release.

release, not much drug is released for a while. Then, a new phase of drug release begins to have a steady-state release. This type of release usually indicates the degradation of PLGA, resulting in the release of the remaining drug [77,78]. Fig. 4-D shows the initial drug release with no further significant release. This is usually due to the use of unsuitable PLGA types that may degrade too slowly for the intended drug release, or the drug loading is too low. One of the reasons for the limited drug release in the beginning in Figs. 4-C and D is either a drug is extremely hydrophobic or hydrophilic drugs (e.g., positively charged peptide drugs) interacting with negatively charged PLGA electrostatically, limiting the drug release until PLGA breaks down [78,79]. One thing noticeable in Fig. 4 is that all profiles exhibit an initial burst release, and the question is how large or small it is, rather than whether it exists or not. The initial burst release can be considered the initial dose for drug efficacy, and thus, the initial burst release of a suitable amount is desirable. Controlling the initial burst release requires understanding the sources of the initial burst release.

Each model is only as good as the assumptions and parameter estimations made during model development, which are peculiar to each formulation [77,80–82]. Since each microparticle formulation is made with different components and vastly different processes, it is unlikely that one model can accurately represent all formulations. The release kinetics calculated from the model may resemble the actual release profile. Still, it simply means that some of the assumptions used in the model may apply to the actual formulation. The release profiles in Fig. 4 provide typical release patterns, offering only a general idea of what may be responsible for drug release. Two formulations made from the same PLGA in molecular weight, L:G ratio, and concentration can produce totally different release profiles due to the different processes used and the way the final formulations are treated. At the same time, two formulations made of entirely different PLGAs may show similar release profiles. Instead of attempting to develop a universal model that explains all types of drug release, it would be more practical to understand drug release kinetics based on formulation composition and process parameters, thereby improving the formulation to achieve the desired drug release profiles.

3.3. Sources of initial burst release

The initial burst release is generally defined by the initial release of the drug in substantially higher amounts at the beginning than that observed at the steady state. The initial burst release was typically attributed to the drug available on the surface and dissolution through pre-existing pores and channels, which formed during the solvent extraction process [83,84]. This assumption is reasonable because the drug molecules can migrate from the center of the microparticles to the surface during the solvent removal and drying processes. When solvents used to prepare PLGA microparticles are removed by extraction in water and the drying process, hydrophobic drugs can migrate with the solvents to the surface. Hydrophilic drugs, including peptides and proteins, can migrate to the surface during solvent extraction in water. The enriched presence of drug molecules on the surface is most pronounced in spray-dried PLGA microparticles [85]. While this explanation is reasonable and most prevalent in the literature, it may not fully account for the substantial burst release where most loaded drugs are released, such as Fig. 4-A. Thus, alternative and supplementary explanations are necessary.

3.3.1. Presence of interconnected open pores

When dried microparticles are introduced into an aqueous environment, the first thing that happens is the fast diffusion of water molecules into the core of PLGA microparticles and the release of the dissolved drug molecules. In vivo, the peak drug concentrations resulting from the initial burst release occur in a matter of several hours for the formulations delivering drugs for up to 6 months [25,31,86–88]. There are reasons for such extremely fast drug release from PLGA microparticles. First, the seemingly smooth microparticle surface is full of interconnected pores throughout the entire volume. The pores are formed due to the initial presence of oil (i.e., solvent) that has to be extracted into water and replaced with water, which is removed during the drying process. The study on the distribution of ovalbumin in PLGA microparticles using transmission electron microscopy showed that more than 60 % of all ovalbumin was located on the microparticle surface or distributed in the large pores connected to the surface [89]. Another study using spray-dried PLGA microparticles showed that albumin signals were detected approximately 20 nm from the topmost surface [85]. Lysozyme distributional analysis in PLGA microparticles using confocal laser light microscopy indicated that burst release is not caused by the protein located at the surface of the microparticles but most likely caused by diffusion of the lysozyme through water-filled pores in the PLGA matrix [90]. The common observation for the initial burst release is the presence of interconnected open pores. The surface of the microparticles appears smooth without visible pores, even when examined by scanning electron microscopy (SEM). This is likely due to the pores that are too small to be detected by SEM [91]. Microparticles in the dry state, in particular, have smooth surfaces due to the formation of a film-like surface layer during the drying process. The pores may open as the surface is introduced to water due to the absorption of water, resulting in the swelling and rearrangement of polymer molecules [58]. During this process, new pores may be formed.

3.3.2. Fast water absorption to open pores

The presence of interconnected pores is important for explaining the fast initial burst release. The capillary action of interconnected pores can easily explain the rapid absorption of water, resulting in rapid drug release. Edward W. Washburn studied the penetration of water into a porous body [92]. The Washburn equation can be applied to water penetration to various porous systems, such as fibrous structures, e.g., fabrics or paper [93], powders [94], compressed powder cakes [95], and moisture absorption into concrete [96].

The Washburn equation, shown below, can be used to determine the time necessary for water to diffuse to the microparticle core.

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

where h, r. γ, c, η, and t are penetration depth (cm) of water at time t, the radius of a pore (cm), the surface tension of water (72.8 dynes/cm or 72.8 g/sec^2), the contact angle of water on PLGA, the viscosity of water (1 cP or 0.01 g/cm·sec), and penetration time (sec). Thus, the penetration time of water (t) into the microparticle core (h) can be calculated using Eq. 2.

$$t = \frac{2\eta}{r\gamma cos\theta}h^2 \tag{2}$$

For example, the radius of pores, h (the radius of microparticles), and the contact angle are set to 1 nm, 100 μm, and 89.99° (thus, $cos\theta$ = 0.00017).

$$t = \frac{2x0.01\,\frac{g}{cm\bullet sec}}{(10^{-7}cm)\left(72.8\,\frac{g}{sec^2}\right)(0.00017)}0.01^2\,cm^2 = 1616\,sec \tag{3}$$

For real microparticles used in drug release studies, the pore size (r) and the contact angle will undoubtedly vary. As an example, the study on the pore size distribution of PLGA microparticles determined by helium and nitrogen pycnometry indicated the presence of two separate pore populations, one smaller than 0.36 nm (in which only helium can diffuse) and another larger than 3.9 μm (in which mercury can penetrate), with not more than 6 % of the total porosity lying in between (in which nitrogen can penetrate), all of which accounts for 20 % of the total volume [97]. As the contact angle may decrease below 90° due to the presence of various surfactants used in the emulsification process and

the body, the time (t) will decrease. The time will also decrease for the microparticles with a radius smaller than 100 μm. The only variable that may vary widely is the pore radius (r). Even assuming 100-fold fluctuations of the pore size, the penetration time ranges from 1 s to 5 h. Thus, it is not surprising that the initial peak concentration is observed with several hours of administration of LAI formulations. Even though the above calculation is performed under ideal conditions where water penetration follows straight capillary channels, the calculation indicates that water penetration to the core occurs within a matter of hours, not days. In real microparticles, the open channels are tortuous and sometimes not interconnected throughout the microparticles. Also, the drug-PLGA interactions can contribute to the drug release kinetics [98]. However, the pharmacokinetic profiles of most LAI formulations show the peak drug concentration in several hours. This indicates that fast water penetration is the first step toward the initial burst release.

3.4. Methods to prevent the initial burst release

Since fast water absorption occurs in all types of microparticles, one way of avoiding the initial burst release may be to prevent the formation of open channels inside the microparticles. It, however, turns out to be very difficult. Open channels are formed due to the removal of the oil phase from the oil/water emulsion droplets and the subsequent removal of water while obtaining dry microparticles.

The drug release kinetics is affected by almost all factors involved in microparticle production, ranging from the formulation composition, processing parameters, and interactions among components and processes [31,99]. Of those, the initial burst release is affected more by certain factors than others. As water absorption occurs in a matter of minutes and hours after the microparticles are exposed to water, the water solubility of the drug is one of the first factors to consider. The spatial distribution of hydrophilic drugs is expected to be critical for the initial burst release. If the total drug loading is, e.g., 1 % of the total weight, the initial burst release is bound to be lower than the same formulation with high drug loading, e.g., 10 % or higher. Thus, prevention of the initial burst release is necessary when the maximum drug loading is achieved. The fast water absorption and the presence of more drugs on the microparticle surface account for the initial burst release. Thus, approaches that prevent (or reduce) the initial burst release include altering the spatial distribution of drug molecules throughout the microparticles.

3.4.1. Increasing osmotic pressure in the solvent extraction phase

The initial burst release of albumin from PLGA microparticles using dichloromethane (DCM) was decreased from 80 % to 50 %, 40 %, and 30 % by adding 20 % sucrose, 1.8 % NaCl, and 3.6 % NaCl, respectively, in the extraction aqueous phase [100]. The encapsulation efficiencies were similar. Thus, the lower initial burst release was thought to be caused by a denser internal structure formed by the higher osmotic pressure, resulting in lower porosity [100]. Since the osmotic pressure of 20 % sucrose is similar to 1.8 % NaCl, the superior efficacy of NaCl in reducing the initial burst release may also be due to other effects, such as slower DCM extraction in the presence of NaCl [100]. It is also possible that the sucrose that remains inside the microparticles may function as a porogen [101] and/or as a plasticizer, lowering the T_g of the final PLGA microparticles [102], resulting in a higher initial burst release than NaCl. It is noted that the addition of sucrose still decreased the initial burst release as compared with the native PLGA microparticles. The inclusion of a small amount of glucose (0.2 % w/w) also showed a reduced initial burst release of octreotide acetate [103].

3.4.2. Lowering T_g of PLGA by pretreatment with ethanol or glycerol

Adding glycerol or ethanol to the primary dichloromethane dispersion of PLGA (6600 Da) results in a drastically suppressed initial release. As an additional effect of glycerol, the initial burst of insulin was further suppressed due to the decrease of the glass transition temperature of PLGA from 42.5 to 36.7 °C [104]. Since the annealing of PLGA molecules took place at around 37 °C, the porous structure of microspheres immediately disappeared after immersion in PBS or subcutaneous administration. The insulin diffusion through the water-filled pores would be effectively prevented [104].

3.4.3. Lowering T_g of PLGA by post-treatment with ethanol

The enriched presence of drug molecules near the surface of microparticles can be altered by the redistribution of the surface drugs throughout the entire volume of the microparticles. This redistribution of hydrophobic drugs can be done using post-treatment in a 25 % ethanol solution [60,105]. The post-treatment condition lowered the T_g, enabling the restructuring of PLGA molecules and the rearranging of drug molecules. Post-treatment has a substantial effect in preventing or reducing the initial burst release of risperidone [105].

The post-treatment with ethanol also showed a significant reduction of a high initial burst release of antisense oligonucleotides from PLGA microparticles prepared by the multiple emulsion (W/O/W) method [106]. The ethanol/water mixture can function as a plasticizer, significantly lowering the T_g of the PLGA. It allows PLGA microparticles to become soft, and the surface pores closed during treatment. As a result, microparticles with reduced surface pores resulting in a low initial burst were obtained. When a polymer solvent (e.g., acetone)/water mixture was used, however, the decrease in the initial burst release was less significant [84,106]. The effect of post-treatment with ethanol appears to be drug-specific. The post-treatment with ethanol (at 40 %) for an hour resulted in a higher initial burst release of TGF-β_3 and BMP-2 proteins, maybe due to the diffusion of the protein from the innermost layers of the microparticles to the surface [107]. Since each PLGA microparticle formulation is prepared differently for different drugs, the effect of ethanol post-treatment may result from a competition between PLGA rearrangements and the reduction of porosity, thereby reducing drug diffusion from the core to the surface. If a drug does not dissolve well in an ethanol/water mixture, it may not diffuse to the surface and vice versa.

3.4.4. Adding a hydrophobic excipient as a barrier

LAI formulations using triethyl citrate, a common plasticizer, showed lower initial burst release than those without, probably by acting as a hydrophobic barrier for peptide drugs [25,108,109]. It is also likely that triethyl citrate functions as a plasticizer, lowering T_g and allowing the restructuring of PLGA molecules to close existing pores. In case the release is limited, as shown in Fig. 4-D, due to the hydrophobic nature of the drug, then hydrophilic excipients can be added, e.g., adding PEG to PLGA for phase separation to result in water-filled channels for the release of paclitaxel [110].

3.4.5. Surface modification of PLGA microparticle surface

The prepared microparticles can be dispersed in a gelatin solution to coat their surface with a gelatin film, thereby reducing the initial burst release. The post-coating of gelatin reduced the initial burst release substantially (up to 98 %) in proportion to the coating thickness or the gelatin concentration used for coating [111].

The surface of PLGA microparticles can be crosslinked to form an additional diffusional resistance for drug release [112]. The crosslinking agent used includes ethylene glycol dimethacrylate and tri(ethylene glycol) dimethacrylate, which are not toxic to the human body. This approach may be effective in practical applications if the crosslinking agent is both degradable and safe.

3.5. Steady-state drug release following the initial burst release

Despite the fast water absorption through the open channels and subsequent burst release, the remaining drug is usually released over the lifetime of each formulation, which can vary from weeks to months. It was initially thought that the steady state release was due to the drug

release being dependent on slow polymer erosion [84]. Still, the drug release at the steady state can occur without polymer erosion. "Erosion" refers to the loss of PLGA material as oligomers and monomers that move away from the PLGA structure, while "degradation" refers to the PLGA polymer chain scission reaction [113,114]. Degradation is the first step of erosion.

The release of the drug from the surface region is governed by the water solubility of the drug and its diffusion coefficient through the PLGA matrix and channels. Water functions as a plasticizer, lowering T_g [115], and the T_g decrease occurs up to about 15 °C [116,117]. Thus, as PLGA microparticles absorb water, the microparticles swell and increase the mobility of PLGA polymers [118–120]. This allows reorientation (healing or annealing) of PLGA polymers to form a skin layer on the surface, closing the pores, resulting in a diffusion-controlled reservoir system for zero-order release [84]. Such rapid morphological changes occur at the surface of the microparticles during the first 24 h to form a skin layer (film or membrane) around the microparticles [55,84,106]. This is a similar process of active self-healing of the surface of PLGA microparticles by raising the temperature above T_g [121,122]. The skin layer becomes dense due to the coacervation of PLGA to cover the interstices on the surface, forming a dense film [59]. The newly formed surface layer functions as a diffusion-controlled barrier, resulting in zero-order drug release kinetics. The lower molecular weight may lead to localized reorientation, leading to an additional effective channel to release free volume frozen in the microparticles [123].

4. Quality by design (QbD)

4.1. What is QbD?

In the production of highly regulated drug products, even a small, seemingly trivial change has to be approved by the FDA. Even if manufacturing can be done more efficiently based on an improved understanding of the processes and product quality, the production relies on the manufacturing methods used when the product was approved. In many conventional pharmaceutical manufacturing processes, samples are collected after each batch processing step and tested offline to ensure that quality specifications are met before proceeding to the next step in the fixed process. Such a strategy relies on "quality by testing (QbT)," but quality does not improve by testing [124]. The quality here means the features and characteristics of a product with the consistent assurance of safety and efficacy, and free of defects and contamination [125,126]. Recognizing that traditional testing strategies restrain innovation and continuous improvement in manufacturing processes, the FDA issued guidance for the transition from the inefficiencies associated with QbT to a new method of "quality by design" (QbD) [127]. QbD in product development focuses on building quality into products by design to meet the intended product performance from the beginning, through understanding the impacts of different factors in the formulation and manufacturing process on the final product quality [128]. In short, QbD is designed to provide the pharmaceutical manufacturing sector with maximal efficiency, agility, and flexibility to produce high-quality drugs reliably without extensive regulatory oversight [126].

The International Conference on Harmonization (ICH) Quality guidelines Q8(R2) defines QbD as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [128,129]. In other words, QbD refers to the design and production of formulations and manufacturing techniques aimed at maintaining predefined consistency in product quality [130,131]. Thus, the implementation of QbD begins with the construction or identification of a quality target product profile (QTPP). QTPP is a prospective summary of the drug product characteristics, typically achieved to ensure the desired quality, taking safety and efficacy into account based on risk assessment [129]. Simply put, defining the QTPP is to decide the product's function, the type of dosage form, and the manufacturing method [132]. QTPP is different from the target product profile (TPP). TPP is a patient-centered product labeling goal that helps focus and guide development activities, ultimately translating into a drug package insert. QTPP is a quantitative safety and efficacy support to create and optimize a formulation and manufacturing process [133]. QTPP is a sub-branch of TPP released by the FDA that focuses on the chemical, manufacturing, and controls (CMC) stages of development [133]. QTPP describes various aspects of the product to ensure its safety and efficacy. QTPP is converted into critical quality attributes (CQAs), which, in turn, define the critical material attributes (CMAs) and critical process parameters (CPPs) [134].

4.1.1. Design of experiment, design space, and process analytical technology

The complex relationships between formulation components and processing parameters impact product quality. The QbD approach is implemented to minimize product variability and defects through controlled manufacturing, thereby enhancing product development and manufacturing efficiencies for consistent finished products [130,135,136]. The design of experiments (DoE) is used to quantitatively examine the impact of each parameter and its potential interaction with other factors of CMAs and CPPs on the CQAs. Thus, DoE is considered a systematic approach to performing experimentation that helps establish a relationship between the input factors (CMAs and CPPs) and output responses (CQAs), i.e., a cause-and-effect relationship [137,138]. The DoE subsequently determines the range (known as the design space) of the CMAs and CPPs that have been proven to ensure product quality, thereby representing safety and efficacy. The concept of design space is a key component of QbD, as working within the design space is not considered a change and would not initiate a regulatory post-approval change process [125]. Naturally, the design space proposed by the applicant is subject to regulatory assessment and approval. Essentially, DoE is used to examine the butterfly effect and determine the design space with minimal or no impact from it. The butterfly effect refers to the sensitive dependence on initial conditions, where small variations in initial conditions result in large, divergent, and dynamic transformations in output events [139,140]. Changes in parameters within the design space will not significantly affect the outcome and will enable the product to meet its specifications.

QbD is based on continuously monitoring key quality attributes during the process to enable early detection of improvements through process analytical technology (PAT). PAT is a system for designing, analyzing, and controlling the manufacturing process through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes to ensure final product quality [141]. In PAT, the term 'analytical' includes chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner [141]. Analytical method development is a part of QbD and product development [131]. Within the scope of PAT, the design and optimization of drug formulations and manufacturing processes may include (i) identification and measurement of critical component, material, and process variable factors responsible for the change in the properties of the in-process material and product, (ii) designing a process evaluation system that allows real-time monitoring of all critical variables, (iii) designing process controls that allow for corrections to ensure adequate control of all critical variable factors and process results, and (iv) developing valid mathematical correlation relationships between the product and its quality requirements and the results from the evaluation of all critical components, materials, and process components [133]. PAT has not been widely adopted due to technological barriers, including the need for increased sampling density and inspection frequency, as well as a lack of economic incentives [133].

4.1.2. Quality risk management

The increased understanding of the acceptable ranges for the CQAs

and control of the process allows some flexibility or variations within the ranges to produce products with a predefined quality consistently [130]. Thus, QbD is intended to enhance the knowledge and focus on risk-based approaches for risk management and encourage continuous improvement [142,143]. An example of risk assessment is assessing the probability of a vulnerable step going wrong and the potential results of the risk [136]. Risk assessment needs to be done for material attributes (i.e., formulation variables) and process parameters that can affect the CQAs and design space, and thus, the drug product [128,132]. In the end, QbD establishes a robust product and manufacturing process along with clinically relevant specifications [135].

The number of possible formulation and processing parameters that could influence the CQAs of a pharmaceutical formulation (e.g., PLGA-based microparticles) exceeds a dozen, making it impractical to study all of them, even without considering the interactions among the parameters [132]. Creating a design space that encompasses all the parameters that can impact the quality of the finished product is both costly and time-consuming. The situation becomes uncontrollable when the interactions between variables are considered. Thus, risk assessment is often used to determine the key experiments necessary to identify the CQAs [132]. Risk assessment aims to identify possible causes of problems based on the literature information, the theoretical model used, the formulation data, and personal experience. Low-risk parameters can be set aside, and medium-risk parameters may be considered later after the high-risk parameters have been carefully examined. For example, if a chosen solvent or a mixture of solvents dissolves both the drug and PLGA well, the solvent selection becomes a low-risk parameter. However, if a solvent dissolves the drug but not PLGA, and vice versa, the solvent selection becomes a high-risk parameter. Another example is that if a formulation is made using a solid/oil/water emulsion, the drug's particle size distribution may significantly influence the overall release rate and become a high-risk parameter. However, if formulated in oil/water emulsion, the drug particle size distribution may be a low-risk parameter. This is where the literature information and the experience/expertise of a formulator play a significant role in determining CMAs and CPPs, which are critical for CQAs. Once key parameters are specified, the application of DoE can be more effective in anticipating interactions and defining the region of interest for ideal modeling. In other words, DoE analysis is used to significantly reduce the number of experiments required to create a model design space. The correct tools for developing design space are determined by numerous aspects, including the system's complexity under consideration and scientific data. Thus, it would be fair to say that there is no ideal experimental design or tool for every situation [133].

The release of drugs from PLGA microparticles is influenced by various parameters that are both independent and dependent on others. Therefore, the interplay among multiple parameters of CMAs and CPPs must be understood to determine the key parameters. The number of data points necessary for such an understanding can be drastically reduced using various methods, such as a multiple full factorial-central composite design [144] or a two-factor fractional factorial design based on an Ishikawa diagram (also known as a fishbone diagram or a cause-and-effect diagram) [132,138,145]. However, it is better to understand the entire process of making microparticles and develop a mechanistic model, rather than an empirical one, to eliminate variables that are not critical to CQAs. Furthermore, it is not necessary to find the perfect formulation, which may not even exist, as long as an optimized formulation yields a product that possesses most of the desired features.

4.1.3. QbD for PLGA-based LAIs

Applying QbD to the development of LAI formulations is not straightforward. The materials and processing conditions involved in producing LAIs are diverse, and many variables interact with one another, resulting in a complex array of factors to consider. Thus, identifying CMAs and CPPs is not trivial. Naturally, the transition from trial-and-error approaches to QbD-based approaches will be hard. However, as our understanding of the PLGA-based LAIs improves, such transitions will be made eventually.

For LAI formulations based on PLGA polymers, QbD begins with a clear setup of QTPP that defines the desired performance of the formulation, including indications (uses for which the drug product is approved by the FDA), dosage form, strength, shelf life, packaging, administration route, and blood concentration. QTPP allows for identifying CQAs, which are properties or characteristics that ensure the desired product quality when controlled within a defined limit [143]. CQAs include drug loading and encapsulation efficiency, microparticle size, injectability, residual solvent, drug release kinetics, pharmacokinetics, and stability or shelf life. CQAs are a function of CMAs and CPPs. CQAs are identified to determine the effect of the variation of a CMA or a CPP on product quality. Thus, numerous parameters must be understood and adjusted to consistently reproduce CQAs. Therefore, selecting the parameters relevant to CMAs and CPPs of LAIs with desired properties is critical. The CQA list may be updated based on improved knowledge of CMAs and CPPs. The key approach vital in developing robust LAI formulations is understanding drug release mechanisms from PLGA microparticles, the properties of PLGA polymers, and the selected process parameters.

The complexity of PLGA microparticles and poor reproducibility in scale-up manufacturing present major obstacles to the LAI development [146]. For PLGA microparticle production, PAT may be critical if a manufacturing process involves a step with a very narrow design space. In this situation, applying PAT is critical in maintaining real-time manufacturing control within the acceptance range and quality assurance [141]. Essentially, PAT can be used to ensure consistency and reproducibility within a predetermined design space. PAT can be in-line, on-line, and at-line. For certain formulations, the size of the drug crystals inside the microparticles may be a factor in determining product quality. It can be monitored at-line via powder X-ray diffraction or in-line based on which CPP largely dictates crystal size. In other formulations, the size and shape of PLGA particles may be a factor and may be monitored in-line via laser diffraction. In some formulations, post-treatment with ethanol may be a decisive factor in controlling the drug release kinetics and, consequently, the pharmacokinetics. Therefore, an experienced formulator can establish a suitable PAT to obtain CQAs, depending on the formulations.

4.2. Quality target product profile (QTPP) of LAI formulations

The QTPP of PLGA-based LAI is a summary of the quality characteristics that the final LAI formulation (or the finished product) must possess for guaranteed safety and efficacy. QTPP is the base of design for the development of the product, i.e., "Begin with the end in mind" [147]. QTPP includes the desired performance of the formulation, such as indications, intended use (dosage form, administration route, and container closure system), quality attributes of the drug product (appearance, identity, strength, assay, uniformity, purity/impurity, stability, and shelf-life), and attributes affecting pharmacokinetics (drug release) [145]. An example of QTPP of a PLGA LAI microparticle is listed in Table 2, following the exhaustive list of QTPP for parenteral products [29,147,148].

4.3. Critical quality attributes (CQAs)

Once the QTPP is defined, CQAs need to be identified. CQAs are quality attributes that, if failed, result in severe harm to patients by compromising safety and efficacy [148]. CQAs are used to describe various aspects that influence product performance. CQAs of finished products influence product performance within the desired quality, efficacy, and safety space [133]. Thus, CQAs need to be identified before risk control is considered. CQAs are derived parameters from the QTPP, and maintaining all CQAs within the specified limits is crucial for achieving the predefined QTPPs [147]. CQAs include in vitro, animal,

Table 2
An example of the QTPP of a PLGA LAI microparticle formulation delivering naltrexone for 3 months.

Quality attributes	Target
Indication	Treatment of alcohol dependence or prevention of relapse to opioid dependence
Dosage form	Dried microparticles (suspension at the time of use)
Administration route	Injection, IM or SQ
Appearance	White to yellowish powder of individual microparticles
Dose strength	380 mg
Drug loading (assay)	90–110 % of 380 mg
% Yield (encapsulation efficiency)	≥80 %
Content Uniformity	Uniform per USP <905>
Particle size	10–150 μm (D90: <150 μm, D50: 80 μm, D10: >10 μm)
pH	7
Tonicity	Isotonic
Stability /Shelf life	Stable for more than 2 years at 4 °C.
Impurities	Single impurity ≤0.5 %, total impurity ≤1.5 %
Particulate matter	None
Bacterial endotoxins	<0.92 EU/mg per USP <85>
Sterility	Sterile per USP <71>
Residual solvents	Ethyl acetate ≤1.0 %, ethanol ≤0.1 %
Fill volume	3 mL
in vitro release	≥90 % release in 3 months with a low initial burst release
Pharmacokinetics	Maintain effective drug concentration for >3 months

and clinical data, prior knowledge, established science, and published information, which are used to assess the impact on pharmacokinetics and pharmacodynamics, potency, immunogenicity, and safety [126]. The CQAs depend on the type of dosage form, formulation, and manufacturing method chosen among many possible and clinically equivalent alternatives [132]. Identifying potential CQAs for PLGA-based LAIs (e.g., Table 3) is challenging due to the large number of attributes involved in various processes. Various key factors are intercorrelated and interact with each other quite extensively [29]. Thus, it might not be feasible to study all the attributes in detail and their impact on patient safety and efficacy [147]. Thus, instead of trying to study all attributes in detail, risk assessment can be used for ranking or prioritizing quality attributes [147].

4.3.1. Drug release kinetics

Drug release from microparticles depends on various factors, primarily the formulation composition (CMAs) and manufacturing parameters (CPPs). Both are critical in obtaining a formulation possessing CQAs. Another factor that controls the drug release kinetics is the in vitro test methods [149,150]. Thus, if the drug release kinetics are to be used for comparing different formulations, the same test method should be used to compare the CQAs. It is important to understand that CMAs and CPPs can be controlled and reproduced. However, the phenomena that occur after microparticles are introduced to the test medium are not easy to control and understand. These factors can only be considered using a model. A mechanistic model accounting for testing conditions and all known CQAs was developed [150]. When microparticles are first introduced into the release medium, water diffuses into the porous structure and dissolves the loaded drug. As the dissolved drug is released, PLGA molecules begin to reorganize their structure in the presence of water and a lowered T_g. This process forms the PLGA surface layer, which functions as a rate-controlling membrane, resulting in steady-state release kinetics. This process continues until a substantial amount of the PLGA polymers is degraded in water, causing faster drug release. The beauty of the mechanistic model by Mittapelly et al. is that it considers both intrinsic (e.g., CMAs) and extrinsic factors [150]. The extrinsic factors include the composition of the release media (e.g., the presence of surfactants), test conditions that affect fluid penetration into the microparticles, and the PLGA degradation rate. The inclusion of extrinsic factors is critically important because they substantially alter the drug release kinetics. Since a model requires dozens of parameters, some are bound to be estimated values associated with significant uncertainty. In particular, no model can account for the microstructural changes that occur after microparticles are exposed to a release medium and PLGA degradation, which may be accelerated during the release study. Thus, curve fitting is inevitable during parameter estimation, as it is in any model. While a model may not describe the exact mechanisms of drug release, it surely provides insights into the drug release kinetics and mechanisms and, more importantly, improves the formulation.

Table 3
An example of the CQAs of a PLGA LAI microparticle formulation.

Quality attributes	Justification
Appearance	Individual microparticles should not be aggregated.
Drug loading (assay)	Variability affects safety and efficacy.
% Yield	Encapsulation efficiency affects efficacy and inadequate processing.
Content Uniformity	Variability affects safety and efficacy.
Particle size	Different sizes affect the injectability and drug release kinetics.
in vitro release	The drug release kinetics is the main factor for efficacy.
Residual solvents	They significantly affect the appearance, drug release kinetics, stability, and safety.
Pharmacokinetics	Maintain effective drug concentration in the body.
Stability /Shelf life	Stable for more than 2 years at 4 °C.
Bacterial endotoxins	The safety and efficacy of the final product
Impurities	Increased impurities indicate drug degradation, affecting safety.
Sterility	Prevents degradation and assures safety

4.3.2. Particle size and size distribution

The microparticle size controls the drug release kinetics, provided that all other properties remain constant. The smaller microparticles have shorter diffusion path lengths and higher total surface area if the same amount of drug is to be delivered. Usually, the microparticle size has a distribution between certain size ranges. The microparticle size is an important parameter for the syringeability and the route of administration, as smaller microparticles are better for IM or SQ injectables [151]. To avoid such heterogeneous microparticle sizes, microparticles of homogeneous sizes have been prepared by various methods, including nano/microfabrication [75,152–156] and microfluidic approaches [36,151,157–164]. Some droplet microfluidic systems offer unprecedented control over droplet size, but at the expense of low droplet productivity. Such low productivity was overcome by step microfluidic emulsification [164]. While microfluidic devices produce monodispersed droplets, the obtained oil/water emulsion droplets must undergo subsequent processes, including solvent extraction, particle washing, solvent evaporation, and freeze drying, similar to conventional emulsion methods. It would be ideal if microparticles of homogeneous size possess the necessary CQAs. However, it is difficult for microfluidic devices to produce microparticles with the necessary CQA because many processing parameters critical to achieving CQAs are not available or achievable in microfluidic devices. The monodispersed size is just one aspect of a list of properties controlling the QTPPs.

4.3.3. Stability

Organic solvents are used in the production of PLGA microparticles, and they may not be completely removed despite solvent extraction and drying processes. Thus, the quantity of residual solvents has to be below the specific allowable limits set by the ICH Q3C [165]. DCM, one of the most widely used solvents, belongs to the Class 2 category, which is toxic with the possibility of neurotoxicity and teratogenicity [151]. Removing residual solvents from the formulation is difficult because a solvent may have an affinity to hydrophobic PLGA polymers. The solvent can also affect the microparticle quality, such as the T_g, drug dissolution

property, drug stability, and activity of the drug (especially peptides and proteins sensitive to organic solvents) [151,165]. The stability of a formulation depends on many factors, but controlling the level of residual solvents is a key parameter for maintaining CQAs.

4.4. Identification of CMAs and CPPs

Once CQAs are recognized, CMAs and CPPs need to be identified, and then the functional relationships that link CMAs/CPPs to CQAs need to be established.

4.4.1. CMAs and CPPs in PLGA microparticle formulations

PLGA microparticles are prepared by selecting formulation composition and processing parameters. The qualitative and quantitative information of drugs and excipients are considered raw material attributes [135]. Selecting proper raw excipient materials is as important as selecting parameters in CPPs. Processing parameters affect the product quality immensely, even with subtle changes, but they cannot be identified once the formulation is prepared. For this reason, the impact of each processing parameter on the properties of the final formulation is not clearly understood and can only be evaluated independently. Furthermore, numerous unknown processing parameters influence product quality.

As shown in Fig. 5, CMAs include properties of formulation components, such as drug, solvent, and PLGA. In contrast, CPPs encompass various manufacturing parameters, including the mixing of water and oil phases, solvent removal, drying, post-treatment, sterilization, and storage.

Fig. 5 illustrates the three primary components of microparticle formulation: the drug, PLGA, and solvent. A drug can vary significantly in its physicochemical properties and potency, which restricts the selection of PLGA and solvents. Some solvents can dissolve PLGA but not drugs, and vice versa. In such a specific situation, using two different solvents is not unusual. The selection of solvents is critical as it affects the solubilities of selected PLGAs and drugs, as well as the viscosity of the solution and extraction rate based on the solvent's solubility in water. Furthermore, the residual solvent can function as a plasticizer, leading to various undesirable and unexpected outcomes, such as the aggregation of microparticles and higher initial burst release. Depending on the properties, drugs can also function as a plasticizer of PLGA polymers. The selection of the right PLGA is highly critical. The importance of selecting PLGAs with the right molecular weight and composition cannot be overemphasized. The amount of PLGA used, i.e., the PLGA concentration in the solvent, is another critical factor affecting the processing steps.

Fig. 5 illustrates the essential steps in microparticle processing. The emulsification of the oil phase (drug/PLGA/solvent) in water is achieved by mixing the two phases using a homogenizer. Here, the homogenizer speed, input rates, and time affect the formation of the seed emulsion. This step is followed by solvent removal in a large quantity of water. Again, the solvent removal rate and duration determine the microparticle properties [166]. Fast solvent removal tends to result in large microparticle sizes, thin shell layers or skins, high porosity, and irregular shapes [167]. Due to the rapid exchange between the solvent and water, the shell tends to have a finger-like void space resulting from the rapid precipitation of PLGA. On the other hand, slow solvent extraction results in small microparticle size due to the continuous breakup of seed emulsion droplets. Thus, finding optimum solvent removal conditions is not a trivial matter. The microparticle morphology is a manifestation of many processing conditions [59]. Once the solvent is removed, the microparticles need to be dried, and the drying conditions also affect their properties. The presence of residual solvent, depending on the quantity, has visible impacts, such as aggregation of microparticles and/or higher initial burst release. It is not uncommon for dried microparticles to undergo post-treatment, typically involving washing in a 25 % ethanol solution. The process is known to remove residual solvent, distribute drug molecules, and rearrange the microstructure of PLGA molecules. While the whole microparticle manufacturing process can be done in aseptic conditions, it is not practical. Thus, the microparticles can be terminally sterilized through exposure to γ-ray or e-beam. PLGA polymers are known to degrade when exposed to γ-irradiation or e-beam treatment. Therefore, it is reasonable to anticipate that the PLGA polymers in the microparticle formulation will also degrade, potentially affecting the drug release kinetics. Once the microparticles are fully formed, the PLGA polymers are entangled with each other to form the overall structure. As a result, any change in drug release kinetics may be minimal and unlikely to significantly impact the overall drug release profile, particularly if the drug is released before the polymers begin to degrade substantially. The potential effects of terminal sterilization on the drug release properties of the final formulation will vary depending on the specific characteristics of each formulation. The impacts of terminal sterilization on drug release kinetics need to be examined [168].

4.4.2. Design of experiments (DoE) and design space

As shown in Fig. 5, numerous parameters must be optimized to make microparticles possess the desired drug-release properties. It is common to optimize more than a dozen parameters in formulation composition and processing. Since there are numerous material attributes and process parameters to be assessed, identifying CQAs is highly challenging. The CQA risk assessment can be used to reduce the number of attributes. Quality risk management allows risk-based development [148]. This is followed by developing an appropriate Control Strategy, including

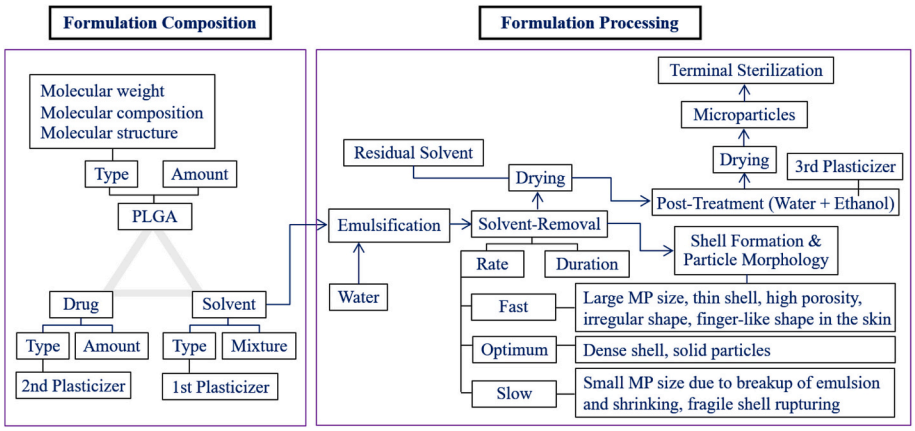

Fig. 5. Formulation components and processing parameters of microparticle production that result in CMAs and CPPs. (This is an example of microparticles prepared by an emulsion method, followed by post-treatment and terminal sterilization.)

justification based on scientific evidence, such as scientific literature, prior knowledge, and DoEs. The DoE is used to narrow down the number of parameters.

The number of experiments increases exponentially as the number of parameters in the experimental design increases. Thus, only a small number of key parameters with major impacts affecting CQAs are identified using screening designs, such as Fractional Factorial Design, Taguchi Design, and Plackett-Burman Design [138,169,170]. The key parameters identified by the screening design are further optimized using response surface designs to determine the main effects of individual factors and the impact of interactions between factors. This allows the identification of the levels of CMAs at high, medium, or low levels. The most widely used designs for response surface optimization include Full Factorial Design, Central Composite Design, Box-Behnken Design, Optimal Design, and Mixture Design [138,170]. Nowadays, machine learning (ML) has emerged as an alternative to traditional approaches due to its ability to analyze extensive and complex data patterns and find key features [171].

CQAs are achieved through a complete understanding and control of the CMAs and CPPs. Such an understanding is provided by the DoE. Those who have extensively studied PLGA LAI microparticles have a good understanding of which parameters are more critical than others. In addition, the impacts of some CMAs and CPPs on CQAs for specific microparticles are available in the literature [172]. It is noted that the CMAs and CPPs are unique for that particular drug and microparticle formulation. For example, in making 1-month leuprolide-loaded PLGA microparticles, the polymer concentration, homogenization speed, water phase volume, and stirring time were found to have little effect on the drug release [173]. Such information on specific microparticles may not apply to other microparticle formulations. However, such information and other general trends found in the literature can be a valuable source of information. This is where the experience of a formulator plays a significant role in the DoE.

Even if only a two-level full factorial design is used to estimate the main effect of CMAs and CPPs, the total number of experiments necessary for 4, 6, 8, 10, and 12 parameters becomes 16, 64, 256, 1024, and 4096, respectively. Thus, optimizing only a small number, e.g., 4–6, of parameters appears to be practical. Even in those situations, examining only two levels may not be sufficient to evaluate the true effect of each parameter. To alleviate this burden, fractional factorial design is typically used to determine the main effects of CMAs and CPPs with fewer experiments. However, this can result in misidentifying a parameter's main effect due to interactions of unrelated factors. If a three-level full factorial design is used, the total number of experiments necessary for 4, 6, 8, 10, and 12 parameters becomes 81, 729, 6561, 59,049, and 531,441. Here, optimizing only four parameters becomes highly challenging. Thus, optimization designs of three levels or higher are used only for a few input parameters. Various optimization designs have been developed to obtain the major effects of input parameters using a manageable number of experiments [172,174–176]. Regardless of which optimization design is used, the most important thing is to obtain high-quality data that are reproducible and consistent.

4.5. Critical material attributes (CMAs)

4.5.1. Drug

The physicochemical properties of drugs play important roles in drug loading and release from microparticles. As the solvent is removed from the emulsion, phase separation forms solvent-rich and polymer-rich phases. Depending on the drug's solubility in PLGA, the drug partitioning in the polymer or solvent phase will be determined. As the solvent is removed, the drug will be distributed throughout the PLGA matrix or separated as crystals [177].

Some drugs are also known to affect the PLGA degradation mechanisms and kinetics [76,178,179]. When PLGA 50:50 was used to load (at the same 20 % level) six different drugs (thiothixene, haloperidol, hydrochlorothiazide, corticosterone, ibuprofen, and aspirin) in disk-shaped pellets, different drugs resulted in significantly different polymer-degradation rates and the drug release profiles [180]. Many nucleophilic drugs, such as naltrexone, risperidone, and oxybutynin, degrade the ester bonds of PLGA polymers in organic solvents [105,181–183]. Such degradation will alter the PLGA properties, including molecular weight, which in turn affects the drug release profile from microparticles.

4.5.2. PLGA

The properties of PLGA polymers depend on their molecular characteristics, such as molecular weight, structure, and composition [31,37,43,59]. It is important to note that the drug release properties of PLGA microparticles, which are compositionally identical, can vary depending on the microparticle manufacturing process used [60,61]. The molecular structure of PLGA molecules presents a unique problem in establishing its CMA and QTPP. For example, the branch unit per molecule of Glu-PLGA is heterogeneous and varies extensively, even for the same product. Determining the sameness of two different Glu-PLGA polymers remains to be developed. The key question to be answered for branch polymers is whether linear and Glu-PLGA with the same molecular weight and composition provide comparable or different product performances [11]. It was reported that the linear and *star*-branched PLGA polymers have totally different degradation profiles [184]. Thus, mimicking the degradation profile of branched polymers, a mixture of linear PLGA polymers with different molecular weights may have to be used [184]. However, it is unclear whether such differences in degradation also affect the overall performance, particularly the drug release kinetics, of the final products. The same question also applies to formulations that utilize two or more PLGA polymers with varying molecular compositions and molecular structures. Is combining different PLGA polymers necessary to obtain the desired drug release properties that simpler formulations cannot get?

The various properties of PLGA and the manufacturing techniques affect the performance outcome of the microparticle formulations [173,185,186]. PLGA molecular weight affects viscosity and may alter molecular packing. The molecular weight distribution differences of PLGA are known to influence the in vitro release profile of leuprolide acetate microspheres when they were prepared using the same manufacturing process. The initial burst release increased with increasing amounts of low molecular weight fractions of PLGA. The molecular weight and the molecular weight distribution of PLGA must be controlled [187]. These interwoven factors (molecular weight and PLGA microstructure) that lead to differences in drug release profiles are poorly understood. Overall, there is a need for further understanding of the drug release properties of LAI microparticles and the associated polymer composition and microstructural arrangement [60,61].

The L:G ratio is an important property that can affect the properties of PLGA polymers, such as solubility in various solvents. The blockiness of glycolide segments arises from the large disparity in reactivity between lactide and glycolide during the ring-opening polymerization. The new polymerization method, known as feed rate-controlled polymerization, was developed to produce uniform PLGA polymers in terms of the spatial distribution of lactide and glycolide [188]. The usefulness of such uniform PLGA polymers for enhancing product properties remains to be seen; however, advances in controlling PLGA composition allow for better control of PLGA properties.

PLGA polymers with higher lactide contents tend to degrade more slowly than those with lower ones. This is due to the more hydrophobic properties of lactide (L) than glycolide (G), and the tendency of hydrolytic scission of ester bonds primarily targeting the G-L or G-G linkages [189]. The L:G ratio is an important factor in controlling the degradation rate and the drug delivery duration [190,191] Other factors, such as the PLGA concentration, drug loading, and residual solvents, should also be considered when adjusting the drug release duration.

4.5.3. Solvents

PLGA polymers dissolve in organic solvents. This statement is only partially correct. Not all PLGA polymers dissolve in all organic solvents. The solubility of PLGA polymers in organic solvents depends on the L:G ratio. As listed in Table 4, the solubility depends on the L:G ratio for PLGA. Table 4 was established based on the fact that the solubility of PLGA depends on the polymer concentration, molecular weight, and temperature. The solubility in Table 4 is based on a PLGA molecular weight of around 80,000 Da and a concentration of 2.5 % (*w/v*) at 30 or 37 °C. The PLGAs used were all in the DL form, and most had acid end-caps. In Table 4, the dark blue indicates that PLGA is soluble in these solvents at a concentration of 2.5 %, the lighter blue indicates that PLGA may be soluble at lower concentrations, and the white means that it is insoluble.

Solvents play a critical role in the drug release from the final microparticles in various ways. The types of solvent and cosolvent influence the solvent extraction kinetics from seed emulsion droplets into the aqueous solution and the stability of the prepared microparticles [166,192,193]. Different solvents have different abilities to dissolve PLGAs, and the residual solvents may alter the T_g differently [177]. The different water solubility of solvents may also affect skin formation during microparticle formation [194], significantly altering the initial burst release. In a study of making naltrexone microparticles, a change in solvent from dichloromethane (DCM) & benzyl alcohol (BA) to ethyl acetate (EA) & BA resulted in substantially faster drug release [195]. Microparticles made of DCM & BA resulted in round spherical particles with a porosity of 49.8 %. In comparison, those made of EA & BA resulted in irregular cylindrical or hexagonal shapes with many dimples and higher porosity (58.3 %).

Even though plasticization can be favorable to the processing and the manufacturing of scaffolds and other devices, it can be detrimental to microparticle manufacturing and storage stability [36]. The susceptibility of these polymers to boundary conditions and the interaction with other materials, drugs included, makes PLGA microparticle manufacturing prone to high variability without robust control over all process parameters [36]. The residual solvent present in the final product can lead to various undesirable effects.

4.6. Critical process parameters (CPPs)

As shown in Fig. 5, even the highly abbreviated schematic description of the PLGA microparticle manufacturing process is complicated, with multiple steps and parameters. Another complicating factor is that these individual parameters may interact with one another. Thus, altering one parameter may have an unknown influence on other factors. Nevertheless, the impacts of changing individual parameters have been extensively studied over the years. While such studies provide valuable information, each study should be considered a unique case rather than a general fact that can be applied to all PLGA formulations. The data obtained for a specific drug through specific processing steps may not apply to other types of drugs unless the drugs belong to the same category in terms of their solubility in solvent, PLGA, and water.

4.6.1. Manufacturing process complexity

The parameters of the microparticle formulation process determine the properties of the microparticles. The manufacturing of LAI formulations must consider the complexity (or simplicity) associated with production costs and reproducibility. The more complex manufacturing process increases the cost and reduces the reproducibility. One well-known example is the discontinued Genentech's Nutropin Depot®, Merck Serono's Prolease r-hFSH, and Janssen's Procrit Prolease, all employing the Alkermes' Prolease® platform [29,36,196]. Nutropin was discontinued mainly due to high manufacturing costs. The Prolease process involves freezing the microemulsion in liquid nitrogen, followed by the removal of the solvent used in methanol. Such a complex process might have been acceptable initially when no suitable manufacturing methods were available. However, unsustainably high costs cannot be justified without sustainable high profit. Creating commercially viable products requires manufacturing processes that are as simple and cost-effective as possible. The significant challenges associated with producing PLGA microparticles loaded with protein drugs are underscored by the absence of any approved protein-loaded LAI formulations since

Table 4
The L:G ratio-dependent solubility of PLGA in different solvents. (Edited from references [31, 37, 43]).

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 formamide											
Dimethyl sulfoxide											
Ethyl acetate											
Butanone											
Ethyl lactate											
Methyl ethyl ketone											
Tetrahydrofuran											
Benzyl alcohol											
Methyl n-propyl ketone											
Ethyl benzoate											
n-Propyl acetate											
Chlorobenzene											
2-Methyl tetrahydrofuran											
2-Pentanone											
Trichloroethylene											
n-Butyl acetate											
Isobutyl acetate											
Toluene											
2-Hexanone											
Butyl lactate											
1,2-Dichlorobenzene											
Methyl isobutyl ketone											
Pentyl acetate											
2-Heptanone											
5-Methyl-2-hexanone											
p-Xylene											
2-Octanone											

the introduction of Nutropin Depot. It is evident that new and innovative technologies must be developed to create LAI protein formulations.

For small molecules and peptide drugs, maintaining bioactivity may not be a significant issue. The same is true for nucleotides, such as siRNA and mRNA, which do not require maintaining their 3-dimensional structures. However, if a protein drug is to be delivered, its bioactivity must be maintained during formulation preparation and after release from PLGA microparticles. Proteins tend to denature, losing their bioactivity due to exposure to water/solvent and air/water interfaces. Furthermore, various processing parameters, including homogenization and drying, can easily damage the protein structure. Thus, protein delivery by PLGA microparticles requires processing that is highly delicate and expensive. The spray drying technologies have been advanced as a one-step continuous process for biopharmaceuticals [197,198]. However, no PLGA formulations for protein delivery prepared by spray drying have yet been approved.

The manufacturing process affects drug release kinetics through its impact on the microparticle size, size distribution, porosity, surface morphology, and spatial drug distribution [11]. Optimizing the manufacturing process requires an understanding of all potential factors involved in microparticle production. Controlling the formulation and processing parameters allows the scaleup production that has the same drug release kinetics [199].

4.6.2. Homogenization speed for emulsification

In a study on the preparation of naltrexone microparticles, a change in preparation method from magnetic stirring (600 rpm for 15 min) to homogenization (3200 rpm for 60 s) resulted in substantially faster drug release, partly due to the smaller microparticle sizes obtained through homogenization [195]. The optimum homogenization speed also depends on the viscosity of the drug/PLGA/solvent combination, as well as the feed ratio of the oil and water phases. Depending on the solvent properties, the extraction kinetics in water differ, which in turn affects the microparticle properties. The homogenization speed and time are critical factors in controlling the drug loading, e.g., by reducing the drug leakage to the outer water phase [200]. Thus, a study is needed to determine a range of homogenization settings that produce an emulsion within the targeted size distribution to be included in the design space.

4.6.3. Solvent extraction

During the PLGA microparticle preparation by the emulsion extraction method, the solvent extraction conditions, such as local shear rates and dissipation rates, and the extraction rate of the organic solvent have a substantial influence on the final properties [201,202]. These parameters have to be optimized for scale-up manufacturing. To this end, correlations need to be determined, e.g., using a computational fluid dynamics simulation, to predict the microparticle properties as functions of the process parameters [202]. The solvent extraction kinetics change as the batch size increases, and the change in the kinetics affects the microparticle morphology and drug release [203].

The solvent removal rate is critical in determining the microparticle properties. Solvent removal kinetics depend on the water solubility of the solvent [166]. Slow removal results in weak, unstable skin formation. The weak skin may be ruptured due to the increased solvent vapor inside the microparticle. This results in lower encapsulation efficiency due to the longer period for drug molecules to diffuse. On the other hand, fast solvent removal causes the rapid solidification of the porous skin, resulting in higher encapsulation efficiency but quick drug release. Optimum solvent removal provides adequate time for drug and polymer redistribution, resulting in higher encapsulation efficiency with reduced burst release [59].

4.6.4. Process temperature

The process temperature affects the microparticle properties as it influences the flexibility of the polymer chains. The process temperature below and above T_g, which changes as the solvent is extracted, affects drug distribution and the morphology of microparticles. The temperature for extracting DCM into water was varied between 10 and 35 °C. As the temperature increased, the amount of DCM in microparticles decreased from 3 % to 1 % after 4 h of extraction [177]. When the preparation temperature of 10 °C was used, the microparticles showed a sponge-like porous structure with no visible shell, and risperidone release occurred almost linearly without burst release. At higher processing temperatures, more compact structures were formed, exhibiting a lag time of more than a week before initiating drug release [177].

4.6.5. Drying

Drying at $T < T_g$ results in faster gelation with a thin, porous skin, leading to higher encapsulation efficiency and faster drug release. Drying at $T > T_g$ forms denser skin with lower encapsulation efficiency but delayed release [204]. In most cases, the drying of PLGA microparticles is performed at room temperature; however, it is worthwhile to examine the effect of varying the drying temperature on the drug release properties.

4.6.6. Post-treatment

As described above in Section 3.4.3, post-treatment with ethanol lowers the T_g of PLGA, redistributing drug and polymer molecules to reduce the initial burst release. This approach is particularly effective for hydrophobic drugs loaded into microparticles. In a study using risperidone or naltrexone, the post-treatment in 25 % ethanol resulted in different drug release profiles [205]. A typical triphasic in vitro release pattern was observed at low wash temperatures (10–30 °C) or a biphasic pattern with an elevated release rate at higher post-treatment temperatures (30–35 °C). The different temperatures affect the residual solvent levels, glass transition temperature, particle morphology, and drug loading, subsequently influencing the drug release rate. The post-treatment process is important in obtaining a formulation within the desired product profile [205].

4.6.7. Three-dimensional (3D) structures of microparticles

Another question that needs to be answered is whether different PLGA formulations can be identified based on their 3D structural arrangement. Typically, microparticle formulations are created by emulsion methods. Even minor changes in PLGA type, solvent, pH, temperature, agitation rate, and other processing parameters drastically affect the microparticle structure and resultant drug release kinetics [206,207]. In addition to the material properties of the PLGA type and drug content, the drug release rate can be significantly affected by the microstructure of the manufactured PLGA microparticles. This includes the distribution of the drug across the microparticle (interior versus surface, evenly dispersed or present as discrete crystals or in amorphous form), presence of other excipient components, residual solvents, interactions between drug and polymer, as well as a plethora of different factors which could affect the drug release and degradation properties of the microparticles.

Many process parameters affect the properties of the prepared PLGA microparticles. The same process parameters can have different effects on the final particle properties when the batch size is increased. This is why the particle properties often change when the batch size increases [202].

4.7. Continuous manufacturing

The pharmaceutical industry is increasingly adopting continuous manufacturing to enhance batch-to-batch consistency and manufacturability. Consequently, a pertinent question arises: Can continuous manufacturing be effectively implemented in the production of PLGA-based LAI formulations? Continuous manufacturing of LAI formulations offers significant advantages for scaling up production, yet it also presents several challenges.

To illustrate this, let's consider the formulation process depicted in

Fig. 5. The entire process can be streamlined into a continuous system that includes mixing the oil and water phases to create an emulsion, removing solvents while flowing in water through an extended tube, collecting microparticles in a chamber by draining excess water, proceeding to drying, post-treatment with ethanol, and finally, sieving to obtain the microparticles into a specified size range. Within this continuous framework, quality control can be maintained through careful regulation of processing parameters such as temperature, flow rate, volume, and drying rate. Achieving this quality control requires extensive research to establish correlations between CPPs and overall processing parameters, which in turn necessitates the development of a robust design space.

While continuous manufacturing may initially entail higher costs, it promises to deliver a high-throughput process that will ultimately lead to cost-effectiveness. In addition to monitoring processing parameters, the characteristics of the microparticles during production can be assessed using techniques such as particle size measurement via light scattering and solvent removal analysis through spectroscopy. Here, process analytical technology (PAT) plays a crucial role, as outlined in section *4.1.1. Design of experiment, design space, and process analytical technology*.

5. cGMP, clinical trials, and NDA

Most LAI formulations have been developed based on trial-and-error approaches to identify CMAs and CPPs. Applying the QbD approach for the future development of PLGA microparticles may allow easier translation of the laboratory technologies to scale-up production [29].

Most formulation scientists in academia do not consider bringing their research to products as their primary function. Scientists in academia have different research priorities than those in the pharmaceutical industry. It is noted, however, that the discrepancy between the two is so wide that academic research is not easily translated into products. The academic research tends to focus on new findings and subsequent publication in high-impact journals. However, product development is intensive in terms of labor, time, and finances [29]. Furthermore, product development is primarily focused on safety and efficacy, and as long as these two criteria are met, the formulations do not have to be elaborate. In fact, the simpler, the better for scale-up manufacturing and quality control. Going through the cGMP manufacturing, pre-IND meetings with the FDA, IND application, clinical studies, and NDA filing are all beyond the capability of most formulation scientists. Many experts in regulatory affairs are involved in such processes. However, suppose academic researchers understand that they provide innovation and advances for the pharmaceutical industry to create newer, better products for treating diseases in patients, as well as the difficulties in translating their research into products. If they do, their research focus may be shifted toward innovation with practical applications.

Academic researchers are strongly encouraged to become familiar with the development of LAI formulations from an industrial perspective [29]. Realizing what is required to obtain FDA approval will open a new horizon for academic researchers regarding the necessary steps to produce the products. Tens of thousands of published research articles cannot treat any disease. It is the products that can help patients. No one is rooting for the status quo. At the same time, no one is spearheading new policies that can change how academic researchers can work to be more productive in product development. Pharmaceutical research is far different from astrophysics, which studies the potential earth-like planets millions of light years away in the universe. Pharmaceutical research is for the present on earth. Maybe a radical change is just what we need. The leaders who can implement such top-down, radical changes must be cultivated to understand that innovation must be accompanied by practical applications. This is achievable, but the current academic landscape needs to change to encourage such initiatives.

6. Human intelligence or artificial intelligence (AI)?

PLGA polymers have been used for several decades. Yet, it was difficult to decide scientifically whether PLGA polymers used in one formulation can be considered the same or not as those used in other formulations. Determining the Q1/Q2 sameness of complex formulations requires highly advanced scientific testing, and such testing methods were developed only recently. The development of improved PLGA LAI formulations requires a deeper understanding of PLGA properties, process parameters, and the control of drug release kinetics. Recent advances in artificial intelligence/machine learning (AI/ML) are expected to accelerate the further development of PLGA-based LAI formulations.

The FDA has recognized the growing role of AI throughout the drug product life cycle, spanning nonclinical, clinical, postmarketing, and manufacturing phases, as well as various therapeutic areas [208]. In its guidance [209], the FDA outlines how AI can be utilized to generate information or data that supports regulatory decision-making related to the safety, efficacy, or quality of drugs. As AI increasingly becomes a vital component in drug development, the FDA is expected to provide more information on how to leverage AI to foster innovation while ensuring patient safety [208]. While AI will undoubtedly serve as an indispensable tool for formulation scientists, it is essential to fully understand both its capabilities and limitations.

6.1. The quality and quantity of data necessary for CQAs

With the advent of AI, the identification and optimization of critical parameters have become more data-driven rather than relying on the past experiences of individual scientists. It is essential to note that the effectiveness of AI, particularly machine learning (ML), relies on the quality and quantity of the available data [210]. The quantity is a crucial aspect of the successful application of ML, as there are more than a dozen parameters to optimize for creating a microparticle formulation with the desired properties. Optimization of a dozen parameters (when it is not known which parameters are critical for impacting the CQAs) will require a large number of data points, which are not easily attainable. The size of the design space increases exponentially as the number of design parameters increases. For example, testing three different values of each parameter in a 10-dimensional space equals 59,049 experiments. For a new formulation, obtaining even 100 data points is a daunting challenge. It is even more daunting when equipment at the benchtop scale is often challenging to scale to pilot or clinical scale, so additional scale-up studies need to be considered. Thus, in practice, one can optimize only a few parameters using AI, and the question arises as to whether such a limited number of optimized parameters accurately reflects the overall process. Many parameters that may have a significant influence on the CQAs can be overlooked, leading to errors in optimizing the CQAs. By ignoring other parameters, this scenario overlooks the interactions between the chosen and neglected parameters that can significantly impact the CQAs. Often, hundreds or thousands of literature data are collected for AI analysis to predict the drug release profile based on the information collected. Thousands of data points may not be large enough in an 8-dimensional space or higher. Furthermore, collecting random data overlooks the fact that each drug is unique in its physicochemical properties, and the conditions used in a drug formulation may not be optimal for other drugs. Essentially, the data obtained for each drug cannot be applied to other drugs, rendering thousands of data points less useful for generalization. Additionally, while dimensionality reduction of all design parameters may help facilitate the training of an AI/ML model using a small set of experimental data, the established model can still encounter limitations in making a reliable prediction for new sets of design conditions.

6.2. Human experience vs. machine learning in PLGA microparticle formulation development

Let's consider the following hypothetical scenarios to understand the role of AI in the development of PLGA-based LAI formulations. The microparticle properties depend on the formulation components and processing parameters [59,202]. Despite significant progress in designing PLGA-based LAI formulations, the exact drug release profiles cannot be predicted a priori. If the desired drug release profile is not achieved, the formulation compositions and processing parameters need to be adjusted. Since there are dozens of parameters to control, it is not feasible to adjust all of them. This is where the extensive experience of a formulation scientist proves to be an invaluable asset. A seasoned scientist may be able to separate parameters based on the degree of risk. Low-risk parameters can be eliminated initially, and the focus can be on high-risk parameters. Let's compare two experts.

Expert 1 has made a few hundred PLGA microparticle formulations while varying 5 parameters (such as the PLGA type and concentration, solvent type, drug concentration, solvent removal rate, and post-treatment). For each parameter, 3 variables need to be tested to obtain a statistically significant relationship. This allows 243 formulations. If 7 parameters (e.g., adding the drug type (base or salt form) and drying condition) were studied, the total number of formulations would become 2187. The question is whether the 5 or 7 parameters chosen can adequately represent the true properties of PLGA microparticles, ultimately controlling the drug release kinetics. This process cannot include all relevant parameters, which may exceed a dozen, as the required data increases exponentially. In practice, various screening designs can narrow the number of important parameters. Alternatively, an expert's intuition, based on their experiences and knowledge, becomes the primary tool, and different parameters may be selected for various applications. Expert 2, on the other hand, does not rely on any particular parameters important for controlling drug release. Rather, Expert 2 collects millions of data points from the literature, regardless of the drug used, experimental conditions, and data quality, to examine more than a dozen parameters and correlate them to the release kinetics of each formulation. From there, the expert knows which formulation to use for certain drug release kinetics.

Experts 1 and 2 represent a seasoned formulation scientist and ML-based adaptation, respectively. (It may be argued that Expert 1 is pre-trained with a lot of data, as in ML. For now, however, we distinguish experience/training-based human learning from data-driven ML.) One can reasonably expect that Expert 2 can present a formulation that satisfies the desired release kinetic profile, as it is not necessary to understand which parameters best represent the PLGA LAI formulation. However, collecting so many data points from different formulations is impractical, as the drugs used have distinct physicochemical properties and interactions with PLGAs and solvents. Even if all the release kinetics available in the literature were collected, it would be less than 10,000. Recently, numerous machine-learning models have been employed to compare predicted drug release kinetics with experimental data [211–213]. The downside of this approach is that the formulation design is not based on several key parameters or established mechanisms. Thus, if the predicted formulation does not work in practice, it would not be easy to remedy. The best that can be achieved by ML is to show that the release profile produced by AI/ML is similar to the actual release profile. It does not provide any mechanistic understanding of the formulation and processing conditions, making it difficult to improve further as necessary. As AI/ML is further improved, however, various new techniques will undoubtedly be developed to interpret the data for mechanistic understanding [214], such as identifying which design parameters are critical over others.

While ML can potentially suggest near-perfect formulations with the desired release kinetics, there is simply not enough data to feed to ML yet to correlate the CMAs and CPPs with the release kinetics. This issue may be resolved through high-throughput experiments that generate large datasets. Even then, it is not clear whether any developed model for one drug can be extended to other drugs. For this, drug chemistry may also have to be included as a design parameter, further increasing the dimensionality. Different drugs may have different tendencies to form drug crystals, and their distribution throughout the microparticle can differ significantly [76,82,178]. Different drugs may also have distinct interactions with PLGAs and solvents, and the lack of information on the interactions among parameters makes it challenging to determine the drug release kinetics. Basically, each PLGA microparticle product is unique and governed by different CMAs and CPPs. One way of confronting this conundrum is to set systematic methods for all formulation scientists to publish data using FAIR (findable, accessible, interoperable, and reproducible) practices for standardized data handling, publishing, and representation [212]. ML is a new approach that the drug delivery field is adopting. However, simply mentioning AI/ML or artificial neural networks for optimizing a few parameters has little impact on advancing the field. The selection of the critical features (i.e., CMAs and CPPs) is critical to avoid overfitting (including noise) when dealing with a limited data set [215]. Also, data scale and quality have to be improved for acceptable predictive performance and generalization ability [210,216]. For all these reasons, formulation scientists with experience and insights into PLGA LAI formulations may still be the choice for developing new formulations in the foreseeable future.

7. Epilogue

7.1. Scientists whose research focus has been on PLGA formulations

Over the last 5 decades of research on PLGA formulations, numerous scientists have contributed to the advances of the field. Many of their published papers are cited in this article. In particular, the PLGA-based LAI field owes a debt of gratitude to Patrick DeLuca, Thomas Kissel, Diane Burgess, Steven P. Schwendeman, Juergen Siepmann, and Kerstin Vay for their seminal contributions. Many more scientists have made similarly impactful contributions, and they may have been omitted due to the narrow focus of this article on microparticle formulations. The field also owes a debt of appreciation to many scientists in the pharmaceutical industry who have developed all FDA-approved PLGA LAI formulations. They are the real heroes, and without them, we would not have long-acting treatment tools for many diseases.

7.2. Future scientists who will transcend current PLGA formulations

The future is always bright, as new scientists with fresh minds continually advance the current state of science, bringing it to a new level. All we can do now is to provide an environment that will allow them to flourish quickly through a stable funding situation and a new approach to evaluating their progress. It is not easy, and so it has not been implemented yet. Any meaningful progress requires decades of steady research. Providing a stable research environment will generate new ideas and results from decades of research. As the history of the drug delivery field shows [1,217–219], it takes decades to go through any particular trendy research topic. It is anticipated that the current trend in AI-based research will persist for a few decades. Imagine a scenario where all researchers utilize AI, even when it has no place in their study. As of the end of 2024, almost all presentations at research conferences have AI in their titles. As mentioned above, utilizing AI to optimize the design space of a PLGA LAI system requires significantly more training data than what is typically collected. Using an insufficient amount of data for AI analysis is likely to result in overfitting, biases, and significant uncertainties, leading to inaccurate conclusions. Thus, the results should not be accepted as the answer simply because they were generated by AI. For the responsible use of AI, researchers must be proficient in its application or have close collaboration with AI experts who are familiar with the limitations of AI.

7.3. Personal optimistic expectations for future PLGA formulations

Scientists, like any other professionals, are typically classified as successful or not, depending on their level of productivity in terms of funding, publications, and awards. In an interview in 1989, Marlon Brando was asked whether he considered himself the greatest actor ever. His answer is so relevant today: "What's the difference? See, that's a part of the sickness in America, that you have to think in terms of who wins, who loses, who's good, who's bad, who's best, who's worst. We always think in those terms, in the extreme terms. And I don't like to think that way. Everybody has their own value in a different way. And I don't like to think who was the best at this or that. What's the point of it?" [220]. If we adopt the idea of one of the greatest actors ever, we can respect other scientists who have their own values in their own ways. Currently, research funding appears to be skewed toward high-profile researchers and organizations despite the fact that others do equally valuable or even more important work. We must have a mechanism to support those whose research topic may not be in fashion or highly visible. This approach aligns with the true ideals of America and the world, which allow all people to progress equally. Out of millions of scientists focusing on various research subjects, some will produce truly breakthrough findings over time if they all do their work with minimal interruption. I sincerely hope that a series of breakthroughs come quickly to cure cancers, Alzheimer's disease, heart attacks, diabetes, addictions, and many other diseases. For absolutely selfish reasons, I wish for a breakthrough in AI-based PLGA microparticle gene therapy to improve my golf game and become a scratch golfer. I also hope that it will enable me to overexpress alcohol dehydrogenase, allowing me to explore every single distilled spirit available in the world during my lifetime. It is quite unlikely to happen. However, dreams are the most powerful tool for scientists to remain optimistic. Even if a tiny fraction of my dream of future PLGA formulations comes true, even remotely, I shall return to report the progress with a cup filled with high spirits.

CRediT authorship contribution statement

Kinam Park: Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

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