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Formulation Composition, Manufacturing Process, and Characterization of Poly(lactide-co-glycolide) Microparticles

Kinam Park[#1,2], **Andrew Otte**[#1], **Farrokh Sharifi**[#1], **John Garner**[#2], **Sarah Skidmore**[#2], **Haesun Park**[#2], **Young Kuk Jhon**[#3], **Bin Qin**[#4], **Yan Wang**[#4]

[1]Purdue University, Biomedical Engineering and Pharmaceutics, 206 S. Martin Jischke Drive, West Lafayette, IN 47907, USA.

[2]Akina, Inc., 3495 Kent Avenue, Suite A200, West Lafayette, IN 47906, USA.

[3]Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Quality, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA.

[4]Food and Drug Administration, Center for Drug Evaluation and Research, Office of Generic Drugs, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA.

[#] These authors contributed equally to this work.

Abstract

Injectable long-acting formulations, specifically poly(lactide-co-glycolide) (PLGA) based systems, have been used to deliver drugs systematically for up to 6 months. Despite the benefits of using long-acting formulations, the development of clinical products and the generic versions of existing formulations has been slow. Only about two dozen formulations have been approved by the U.S. Food and Drug Administration during the last 30 years. Furthermore, less than a dozen small molecules have been incorporated and approved for clinical use in PLGA-based formulations. The limited number of clinically used products is mainly due to the incomplete understanding of PLGA polymers and the various variables involved in the composition and manufacturing process. Numerous process parameters affect the formulation properties, and their intricate interactions have been difficult to decipher. Thus, it is necessary to identify all the factors affecting the final formulation properties and determine the main contributors to enable control of each factor independently.

The composition of the formulation and the manufacturing processes determine the essential property of each formulation, i.e., *in vivo* drug release kinetics leading to their respective pharmacokinetic profiles. Since the pharmacokinetic profiles can be correlated with *in vitro* release kinetics, proper *in vitro* characterization is critical as a batch-to-batch quality control test and scale-up production. In addition to *in vitro* release kinetics, other *in vitro* characterization is essential for ensuring that the desired formulation is produced, resulting in an expected pharmacokinetic profile. This article reviews the effects of a selected number of parameters in the

Address correspondence: Kinam Park, kpark@purdue.edu.

formulation composition, manufacturing process, and characterization of microparticle systems. In particular, the emphasis is focused on the characterization of surface morphology of PLGA microparticles, as it is a manifestation of the formulation composition and the manufacturing process. Also, the implication of the surface morphology on the drug release kinetics is examined. The information described here can also be applied to *in situ* forming implants and solid implants.

Keywords

PLGA; manufacturing parameters; surface morphology; solvent/water exchange; interfacial instability; capillary pressure; drug release kinetics

1. PLGA-based injectable long-acting formulations

For injectable long-acting formulations, poly(lactide-co-glycolide) (PLGA) has been the polymer of choice for more than 30 years. Currently, three different types of injectable long-acting formulations (microparticle, *in situ* forming implant, and solid implant) have been approved by the United States Food and Drug Administration (FDA) [1]. They deliver active pharmaceutical ingredients (i.e., drugs) for durations ranging from 1 week to 6 months. The microparticle formulations deliver small molecules (minocycline, naltrexone, risperidone, and triamcinolone acetonide) and peptides (exenatide, leuprolide acetate, octreotide, pasireotide pamoate, and triptorelin pamoate). One microparticle formulation delivering a protein, somatotropin, was discontinued due to the difficult and costly manufacturing process [2]. *In situ* forming implants have been approved for delivery of doxycycline hyclate, leuprolide acetate, buprenorphine, and risperidone. Solid implants have been approved for delivering goserelin acetate, dexamethasone, mometasone furoate, afamelanotide, and bimatoprost.

PLGAs are usually characterized by their molecular weight, lactide:glycolide (L:G) ratio, end-group, and molecular shape (linear or branched). Some injectable long-acting formulations use a mixture of different PLGAs with different molecular weights, L:G ratios, and/or molecular structures. PLGA polymers commonly used are available in L:G ratios ranging from 50:50 to 100:0 (i.e., polylactide) in 5% molar ratio increments, such as 55:45, 60:40, 65:35, etc. Recent studies found that PLGAs of different L:G ratios may not dissolve in the same solvent. For example, PLGA 75:25 dissolves well in benzyl alcohol, whereas PLGA 50:50 does not [3]. Thus, a manufacturing procedure used with PLGA 75:25 may not produce the same microparticle properties if PLGAs of different L:G ratios are used. Also, PLGAs with the same L:G ratio and molecular weight may result in different microparticle properties, if the PLGA molecular structure is different, e.g., linear vs. branched [4].

In addition to the composition (i.e., drug, PLGA, solvents, and their respective ratios), variation in the manufacturing methods (e.g., extraction volume, composition, time, and temperature, etc.) may produce microparticles having dissimilar properties due to the variable 3-dimensional (3D) network structures, i.e., the macrostructure of the microparticle. The 3D network structure here means the main supporting matrices forming the overall shape of the microparticle. The composition and manufacturing process of each formulation determines the main configuration of the final microparticles. Simply put, the network

structure is analogous to the beams and columns of a building, which are surrounded by the roofs, walls, and slabs. The network structure dictates the overall architecture of the microparticle, but the appearance, i.e., surface morphology, is largely influenced by the roofs and walls.

The effects of the formulation composition and processing parameters on the properties of the microparticles have been characterized extensively over the past three decades, but exact mechanistic relationships have not been clearly understood or established. This problem has been compounded not only by the complex processes but also by the absence of adequate standard procedures [5]. Unfortunately, the PLGA microparticle preparation process is usually not described in comprehensive detail in the literature for successful replication of the manufacturing method. Therefore, difficulties arise when correlating each processing parameter and the respective microparticle properties. The cost of making PLGA-based products is usually high, and sometimes extreme to the level of discontinuing approved products from the market, e.g., Nutropin Depot® by Genentech [2].

The ultimate property of PLGA microparticle formulations that matters most is the drug release kinetics. It depends on the 3D network structure of PLGA and the respective drug loading and drug distribution throughout the microparticles. The 3D network structure is affected by the type and concentration of PLGA used (i.e., physicochemical properties of PLGA) and the manufacturing processes used (such as temperature, solvent extraction rate, etc.). Proper characterization of the final PLGA microparticle formulations is necessary for batch-to-batch quality control, reproducibility, evaluation and approval for Scale-Up and Post-Approval Changes (SUPAC), and developing future formulations with predetermined drug release profiles.

2. Factors affecting the properties of PLGA microparticles

Making PLGA-based injectable long-acting formulations is not simple, especially for microparticles and solid implants. Each and every formulation requires a specific composition and manufacturing process, followed by appropriate characterization. Here, a microparticle formulation prepared via an emulsification-type process is used as an example to illustrate the complexity. Fig. 1 describes the parameters that are known to affect formulation properties. The composition includes a drug, PLGA polymer(s), and solvent(s). Mainly hydrophobic drugs are considered in this example. If hydrophilic drugs are used, they can be dissolved in water first to form a water/oil/water (W/O/W) emulsion. When PLGA(s) and a drug are dissolved in a selected solvent, they will undergo a series of manufacturing steps to obtain the final PLGA microparticles.

As shown in Fig. 1, numerous factors contribute to forming the properties of microparticles. Inadequate control of the microparticle precursor solution(s) or suspension(s), process parameters, and storage conditions may result in significant variability in the properties of the final formulation [6, 7]. For example, the concentration of poly(vinyl alcohol) (PVA), commonly used in the aqueous continuous phase, has a significant effect on the microparticle properties [8–10]. A higher PVA concentration reduces the interfacial tension between organic and aqueous phases to produce smaller microparticles, and it also enables

faster removal of the solvent to the aqueous phase. PVA can also account for the deformation of microparticles [11]. The extraction phase temperature during processing can also cause plasticization and annealing of the solid matrix [5]. Since the glass transition temperature (T_g) of a PLGA varies depending on the quantity of solvent(s) used during manufacturing [12], controlling the temperature during the entire process is essential. The temperature during storage may also alter the drug release properties over time [13]. The impacts of many factors are self-evident. For example, solvent extraction becomes more efficient with a larger volume of water and/or higher stirring rate. What is not known, however, is how they exactly affect the properties of the final formulation, and thus, how to optimize the process and to control the properties. It is not yet clearly understood which parameters are more significant than others.

It is far too impractical to try and extensively examine all the parameters at once. Here, several parameters that are known to have significant impacts on the final properties of microparticles are highlighted and discussed. The composition and the manufacturing process are manifested in the properties of the final formulation. Thus, proper characterization of the resultant formulation can reveal information on the manufacturing process. In particular, the surface morphology can reveal a great deal of information about the formulation composition and manufacturing process.

3. Composition

The three essential components of the formulation are the drug, PLGA(s), and solvent(s). The components affect the drug release kinetics from microparticles, but how each influences the network structure and ultimately the respective release kinetics is relatively unknown. Even if the same components are used, the final formulation will likely have different physico-chemical properties based on the manufacturing process used. As shown in Fig. 1, many processing parameters potentially alter the formulation properties. Nevertheless, proper characterization of PLGA polymers is the first step toward understanding and controlling the properties of the final formulation.

PLGA is traditionally characterized by its molecular weight, L:G ratio, end-group, and molecular structure. The molecular weights of commercially available PLGAs are usually described by the inherent viscosity (IV) in hexafluoroisopropanol (for polymers with low L:G, such as 50:50 and 65:35), or chloroform (for polymers with high L:G ratios). Each lot of manufactured polymers is specified by an IV range, which needs to be converted to the molecular weight using a standard curve established for the polymer and solvent pair. The molecular weights of the polymers are also measured by gel-permeation chromatography (GPC) using polystyrene external standards. Since the molecular dimensions of PLGA and polystyrene dissolved in the same solvent are different, the calculated molecular weight of PLGA is not an accurate molecular weight [1]. The use of polystyrene as an external standard is understandable, as no PLGA standards have been available until recently. It is highly recommended to use PLGAs of known absolute molecular weights measured by multiangle dynamic light scattering. Even for PLGAs, different standards need to be used depending on their respective L:G ratio. The L:G ratio, molecuar weight, and solvent dictate the extent of molecular swelling and ultimately the molecular size; thus, appropriate PLGA

standards need to be used based on the L:G ratio. PLGA standards provide a more accurate calculation of the molecular weights than polystyrene standards.

Characterization of the L:G ratio and end-group chemistry by ^{1}H-NMR and ^{13}C-NMR has been well established [14, 15]. Recent understanding of the semi-solvent effect of dissolving PLGAs in different solvents allows fractionation and identification of PLGAs of different L:G ratios, if more than one type of PLGA is used in a formulation [3]. The non-linear molecular structure of PLGA, e.g., glucose-PLGA branched polymer, can be determined by using the Mark-Houwink equation in comparison with standard branched PLGAs with known branch numbers and molecular weights [4]. If a formulation has complex excipients, e.g., PLGAs of different molecular weights, L:G ratios, end-groups, and molecular structures, separation and characterization of individual PLGAs are still a challenge, but tools are available for such studies.

The solvents used in manufacturing have multiple effects on the formulation properties. First, solvents affect not only the PLGA solubility but also the resultant drug distribution and final physical form in the microparticle. The solvent-dependent PLGA solubility affects the molecular dimensions and ultimately the solution viscosity. The solution viscosity in turn significantly impacts the droplet breakage and resultant emulsion microdroplet size distribution [16]. The solvent miscibility with water, along with other process parameters illustrated in Fig. 1, is a further critical factor influencing the surface morphology of the final microparticles by controlling the precipitation rate of the polymer layers [17]. The solvent type also affects the formation of the microstructure, as it contributes to the change in the T_g during solvent extraction [12, 18]. The solvent, especially residual solvent, can also alter the drug release kinetics [19].

A drug may have an affinity to PLGA polymers, potentially altering the drug release kinetics. Typically, drugs are not expected to interact with the PLGA polymer chains, but some drugs are known to cause degradation. Many nucleophilic drugs (e.g., naltrexone, oxybutynin, and risperidone) are known to cleave ester bonds of PLGA polymers in organic solvents [16, 20–23]. Thus, in those situations, the contact time between the drug and the polymer dissolved in organic solvent(s) has to be minimized or tightly-controlled to obtain the desired molecular weight [16].

Multiple characterization tools and techniques have been developed to extensively characterize the initial PLGA components and the resultant formulation. A bridge needs to be developed on how to best initially choose the correct PLGA(s) for the required drug loading and desired release kinetics. Additionally, chemical changes, specifically molecular weight, that may occur during processing and/or terminal sterilization, need to be considered. Very few, if any reports on microparticle formulation consider the implications of terminal sterilization. While many formulations are likely produced aseptically, building terminal sterilization into the dosage form early in development can be a means to significantly reduce costs and ease the manufacturing burdens incurred during aseptic processing.

It is recommended to describe the components used in making PLGA microparticles in detail. It will allow a comparison of drug release properties of different formulations to understand the impacts of each component on the formulation. The recommended information on formulation components includes PLGA (molecular weight, L:G ratio, end-group, molecular structure, and the amount, or the concentration in solvent(s) for making seed emulsion), solvent(s) (the amount used to dissolve PLGA), and the drug (amount, or the weight ratio with PLGA).

4. Manufacturing process: The influence of process parameters on the properties of PLGA-microparticles

4.1. Formation of seed emulsion

Due to multiple processing variables, the manufacturing procedure needs to be tightly controlled to increase the batch-to-batch reproducibility of the microparticle properties. The difficulty in controlling them stems mainly from the lack of understanding of the exact mechanisms of microparticle formation. For the emulsion extraction method, the manufacturing process begins with the creation of an O/W seed emulsion. The conditions of forming the initial seed emulsion (e.g., mixing point of oil- and water-phases, viscosity of the oil-phase, interfacial tension between two phases, and stirring rate and time) and subsequent solvent extraction (such as the water volume, temperature, and stirring rate) have a significant influence on the final properties [24–26]. Formulations have been traditionally characterized by the drug loading, drug encapsulation efficiency, size distribution, porosity, residual solvent content, surface morphology, and drug release kinetics. The question is whether such characterization can lead to the understanding of the contribution of each process parameter. Currently, no clear relationships have been identified, and thus, the development of injectable, long-acting formulations still relies mostly on a trial-and-error approach. It is necessary to find proper characterization methods and physico-chemical properties that can correlate the drug release kinetics to the composition and manufacturing process parameters.

The drug release kinetics are expected to depend on the network structure of microparticles. As shown in Fig. 1, the network structure of microparticles can be examined by studying the surface and inner morphologies, the PLGA density distribution throughout the microparticle (including the network structure), structural relaxation during storage, and structural reconfiguration during drug release. The network structure of microparticles may be formed in the very early stage of the seed emulsion process. The solvent exchange occurring at the water-oil interface starts immediately, in the milliseconds (msec) to seconds (sec) range depending on the size of microparticles and the solvent-water miscibility [27, 28]. To date, however, no experimental studies have examined mixing and precipitation kinetics at that time scale. Computational fluid dynamics simulations have been used to gain insight into finding the optimized microparticle formation process, including scale-up manufacturing [24]. The formed seed emulsion is then transferred to the extraction solution for removing the remaining solvent(s) from the seed emulsion. This two-step solvent extraction technique is known to produce microparticles of improved quality and higher drug loading [29]. As shown in Fig. 1, numerous parameters affect the microparticle properties. Thus, describing

all process parameters in detail will improve our understanding of microparticle formation mechanisms. This, in turn, will enable design of future PLGA formulations having specific drug release properties.

4.2. Solvent extraction

The microparticle formation process may involve three simultaneous processes: solvent exchange and extraction, phase separation (via nucleation and growth or spinodal decomposition) and coarsening, and eventual phase inversion and solidification [30]. Ingress of non-solvent into the droplet initiates the solvent exchange process across the phase boundaries. To gain an understanding of this, studies have been performed using droplets of 1% sodium poly(styrene sulfonate) (NaPSS, 70 kDa) and 22 nm diameter silica SiO_2 suspension in water that were exposed to a series of external non-solvents (toluene, butyl acetate, ethyl acetate and methyl ethyl ketone) with the water solubility ranging from 0.04 to 11% v/v [30]. The same composition resulted in different microparticle structures depending on the non-solvent used. The Peclet number (*Pe*) is a dimensionless mass transport number that can be used to compare the ratio of advection of the organic solvent by the flow of non-solvent (extraction solution) to the rate of diffusion of the organic solvent through the oil-phase droplet. Rapid extraction ($Pe >> 1$) tends to result in rapid skin formation and precipitation, and thus, it is likely that the spinodal pathway is circumvented to form a hollow morphology, such as dimpled, hollow, or crumpled capsules (if the PLGA concentration is low). In contrast, comparatively slow extraction ($Pe << 1$), or slow shrinking, produces dense and compact structures [30–34].

The initial mixing process between water and solvent(s) and the solvent-water miscibility determine the solvent removal rate and the formation of the initial skin, also called a shell, crust, or envelope [35]. The formed skin may affect the subsequent solvent removal rate and the quality (i.e., mechanical strength, porosity, PLGA density, etc.) of the skin layer, which will affect the drug loading and encapsulation efficiency [16]. If freely water-miscible solvents, such as acetone or dimethyl sulfoxide, are used, they are extracted fast, resulting in the formation of filaments, strands, or thin layers, instead of spherical microgranulates. The difference in surface tension between the aqueous and oil phases causes interfacial turbulence and thermal inequalities, leading to interfacial convective flows [36]. Such non-spherical precipitation is known to occur when the solubility of a solvent in water is larger than 15% (w/w) [37]. Thus, formation of spherical microparticles indicates gradual, controlled extraction of the solvent (with the solubility in water less than ~15%) from the oil-phase to the extraction medium. Even for such solvents, the extraction rate can be slowed down by saturating the water with the solvent, extracting under positive pressure, or decreasing the extraction medium volume. Ethyl acetate, with a water solubility of ~8.3%, has also been shown to cause irregular PLGA precipitation, but it can be circumvented in part via partial saturation in extraction media or extraction volume modifications [38, 39]. Furthermore, dissolution of ionic components, such as sodium chloride, in the non-solvent water phase can affect the osmotic properties of the water phase and affect the extraction behavior [40]. Fast or slow solvent extraction here is used only in a relative sense, under a given condition for partially water-miscible solvents. It is generally understood that fast removal of partially water-miscible solvents tends to cause rapid solidification or formation

of a rough, porous shell leading to higher drug encapsulation efficiency, but also fast drug release. If the PLGA concentration is low, a thin shell is formed, and the droplets shrink or deform. If the solvent removal is too slow, however, weak, unstable skin formation occurs, and the solvent trapped inside the microparticles may increase the solvent vapor pressure, ending up with volcano-like rupture of the skin. Finding the optimum balance between the formulation variables and the manufacturing process is essential, and it is still not clearly understood or predictable a priori. A few processing parameters alone can significantly affect the properties of the final microparticles. For this reason, describing the processing conditions in depth is critical to maintaining quality and reproducibility. The details of the processing parameters have been overlooked, maybe simply because many steps seem inconsequential. The processing parameters could change as the process is scaled-up. This means that the formulation properties may change by scaling-up production. A clear understanding of the impacts of each processing parameter will contribute to the successful translation of microparticle preparation in the laboratory scale to industrial manufacturing processes [24].

4.3. Skin formation

For partially water-miscible solvents, such as ethyl acetate, with an aqueous solubility of 8.3 g/100 mL at 20 °C [41], non-solvent induced phase separation (NIPS) at the interface starts immediately after mixing [24], on the order of a few milliseconds contact in some cases [27, 28]. The solvent near the surface region is depleted into the extraction medium to form a skin layer, and this process is similar to the evaporation of solvent into the air, e.g., drying paint [42, 43]. NIPS has been used widely for making microstructured polymer materials and membranes [43]. The process of NIPS is analogous to solvent extraction by aqueous-based solutions, or even oil-based such as silicone oil that may be used in a w/o/o emulsification technique. While the mechanisms of NIPS are still debated due to their complexity, phase separation kinetics appears to be a critical step for fabricating polymeric membranes with desired properties.

Determination of the solvent removal rate is not straightforward, but the description of the relative volumes of the organic and water phases, the stirring speed, and the extraction time may be sufficient for reproducibility. The impact of the extraction phase temperature on the resulting microparticles has not been studied in detail. Only a few studies examined the structure formation mechanism of PLGA microspheres corresponding to the solvent extraction kinetics through monitoring the T_g during solvent removal [12]. As the solvent is removed, the interfacial PLGA molecules transition from a solution-state to a rubbery-state forming the initial skin layer. As additional solvent is extracted from the interfacial layer and subsequent layers beneath, the surface morphology begins to form as the *local* T_g (i.e., T_g of skin layer) rises above the processing temperature inhibiting further molecular mobility.

When water-miscible solvents are used to dissolve PLGA polymers, such as N-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide, or acetone, the phase separation of the polymer occurs immediately, and the diffusion of water into the oil emulsion droplets results in finger-like structures [27]. A simulation study on the precipitation of Nomex® (20%) (a synthetic aromatic polyamide polymer) at the water-NMP interface showed the formation of

a finger-like structure under a thin skin in about 20 msec, similar to the structures observed experimentally. The large molecular repulsive forces between Nomex and water further aided the precipitation rate and fingering, as precipitation was too fast for any segregation of the polymer to occur into rich and poor regions. The fingering eventually ceases due likely to layers of precipitated polymer behind the finger growth being too dense. As solvent extraction continues, the process results in the further shrinking of the droplets. This shrinking may result in a surface morphology unique to the process. In general, a thin skin layer may be formed, if a polymer precipitates immediately after exposure to water because there is not enough time to form coacervates [27]. In this situation, as well as when the polymer concentration is low, the skin becomes inhomogeneous, resulting in quick formation of defects, some of which initiate pores that may grow inside the polymer solution to form fingers [27].

A study to determine the time for extracting ethyl acetate from nano-emulsion droplets (500 nm radius) showed that diffusion of the solvent from the emulsion was faster than the stopped-flow apparatus could measure, i.e., 10 msec [28]. An order of magnitude of the solvent diffusion time was estimated using the mean-square displacement, $<R^2>/D$, where R is the radius of a droplet, and D is the diffusion coefficient of ethyl acetate, 2×10^{-5} cm^2/sec. For microdroplets of 50 μm radius, it takes only about 1 sec. The actual extraction time of a solvent into the water phase may vary significantly depending on the experimental conditions, but it will be in the range of seconds, not hours. The water-solubility of ethyl acetate is 8.3% at 20 °C. Even with dichloromethane, that has a water-solubility of approximately 2% at 20 °C and a diffusion coefficient of 2×10^{-7} cm^2/sec, it will take only a few minutes [19, 44]. Thus, one can easily expect that a skin layer (or a polymer shell) can be formed in a matter of seconds after an emulsion is formed. The initial skin layer may also become a barrier slowing down subsequent extraction [28]. The solvent evaporation rate from the water was examined to obtain the overall permeability coefficients of ethyl acetate, dichloromethane, and acetonitrile [45]. Depending on the experimental condition, i.e., process parameters in Fig. 1, the solvent extraction rate will vary, and will ultimately affect the surface morphology of the drug-loaded microparticles.

The surface of PLGA emulsion droplets undergoes a rapid increase in viscosity as solvent is removed, resulting in coacervation. As the concentration of PLGA in the polymer-rich phase increases, the network structure starts to form. The study of making porous polymer membranes indicates that polymers tend to form microporous structures at lower than the critical viscosity, and an asymmetric structure with a dense skin is usually formed at above the critical viscosity [46]. The critical viscosity depends on each system, and this is another reason to control the solvent extraction kinetics.

Fig. 2 shows the formation of a skin layer by the solvent extraction process, i.e., the solvent/water exchange process. As the solvent is removed, the PLGA concentration increases to form coacervates or precipitates. During this process, many water pockets are formed (which become void spaces after drying), and water may penetrate deep into the PLGA matrix, forming finger-like structures.

As the hardened microparticles undergo a drying process and water evaporation nears completion, polymer precipitates merge, and the void spaces between them are covered by the precipitates which are deformed by the water surface tension, γ, in the interstitial capillary system between precipitates [47–50]. The coalescence process occurs primarily at the surface-air interface in the dry-inversion process due to the capillary pressure acting on the spinodally phase-separated structure on the surface [48]. The capillary pressure is the major driving force for film formation, and is exerted normally to the water-coacervate interface, resulting in the deformation of the coacervates to the center, as shown in Fig. 3 [47]. The pores initially formed on the surface become closed by the lateral merging of neighboring coacervates [27]. If the PLGA shell formed is rigid to overcome the capillary force, the pore will remain. It was also suggested that at the end of the solvent extraction process, when the polymer is in a rigid state, water acts as a porogen leaving sub-nanometer voids in the places from which water molecules are removed [51]. An apparent pore-free skin layer on the microparticle surface can be explained by the observation that the surface appears to be non-porous when analyzed with scanning electron microscopy (SEM). The pores may simply be too small for detection by SEM. The pores are connected to the linked network of channels found throughout the microparticles [52]. Therefore, the solvent removal rate, with specific emphasis on the instantaneous extraction, affects the kinetics of skin formation and the resultant microparticle properties. In this process, the PLGA properties (the molecular weight and L:G ratio, and the interaction with solvent) and concentration affect the solvent extraction rate. These factors, in turn, affect the microparticle morphology, and thus, may alter the drug release profile.

The skin formation is also an important step for *in situ* forming implants. Solvents with high water miscibility will cause coacervation of PLGA quickly, forming a thicker skin layer with finger-like pores [53]. In general, fast removal of solvents (i.e., freely water-miscible or solvents with high water solubility) tend to result in finger-like morphology, while slow solvent-water exchange results in sponge-like morphology [54]. The fingers continue to grow until the layer of the precipitated polymer behind the growing fingertip becomes dense enough to prevent further extension [27]. The final structure may be an intermediate between the two extremes. The finger-like structure is known to be formed when a more viscous fluid is displaced by a less viscous fluid, known as viscous fingering [55]. As the solvent is extracted faster, the drug release becomes faster, too. The release of metoclopramide monohydrochloride (metosalt) from PLGA 50:50 *in situ* depot was decreased as triacetin, a hydrophobic co-solvent, was added to a water-miscible solvent (e.g., DMSO or NMP), resulting in a decreased rate of solvent extraction and a minimization of the subsequent initial burst peak [56].

5. Morphology of PLGA microparticle surfaces

The formed microparticles are usually characterized for their drug loading, drug encapsulation efficiency, *in vitro* release kinetics, size distribution, porosity, and surface morphology. The most important characterization is their respective *in vivo* pharmacokinetic profiles. The main goal of characterization is to ensure the development of a formulation having desired properties, such as drug release kinetics, initial release, and duration of release, ultimately providing specifications for product release. The initial characterization

allows further modification of the microparticle properties to obtain the final formulation with the desired pharmacokinetic profile. The surface morphology of the microparticles varies depending on the process parameters, even if the same composition is used. Thus, understanding the surface morphology, i.e., how and why a specific morphology is formed, allows reverse engineering of the manufacturing process.

The surface and inner morphologies of PLGA microparticles have been routinely examined by SEM [57–62] or fluorescence microscopy [63, 64]. It is still not clearly understood, however, what information the particle morphology presents, especially concerning the drug release kinetics or what quantifiable parameters from these techniques can be utilized to successfully discriminate between batches. The study becomes even more difficult if other factors are considered, such as continuous changes in the PLGA structure during drug release *in vitro* and *in vivo*. Fig. 4 shows examples of the vast number of surface morphologies that may be observed depending on the formulation and/or processing parameters.

5.1. Smooth surface

The surface of the microparticles may appear smooth overall under SEM, but it depends on the magnification used. If the magnification is sufficiently high, no surface may be considered perfectly smooth. As compared to other distinct morphologies in Fig. 4, the smooth surface lacks visible holes, pores, or other patterns. The smooth surface is formed when the skin is fully formed, or strong enough, to withstand further deformation. Alternatively, the solvent can be continuously extracted to make microparticles shrink uniformly until the whole particle solidifies. Finally, smooth surfaces may exist if there is incomplete solvent extraction or residual moisture that may inhibit any deformation that may occur through *complete* drying.

5.2. Porous and volcanic surface

Quite often, a porous surface is observed on various microparticles. One of the factors that contribute to the formation of pores is the PLGA molecular weight. The pores on the microparticle surface were formed when the molecular weight was higher than 100 kDa (Resomer RG756S, PLGA 75:25, IV=0.71–1.0 dL/g). In contrast, microparticles prepared with molecular weights lower than 40 kDa (PLGA 50:50, Resomer R502, IV=0.16–0.24 dL/g and R504, IV=0.45–0.60 dL/g) showed a smooth surface free of pores [11]. This may be due to the higher viscosity of the polymers hindering the dispersion of the oil-phase into the external aqueous-phase [11].

The pore formation also depends on the temperature used during the solvent extraction step. When microparticles of PLGA 75:25 (Resomer 755S, 64.7 kDa) were prepared at the extraction temperature of 10 °C (which is below the T_g of PLGA), they resulted in large coarse pores [51]. The open macroporous structure may result from shrinkage and rupture of the drying shell. Since the polymer is not rubbery at that temperature, larger channels and pockets initially filled with sequestered water maintain their structures [51, 67, 68]. At temperatures above T_g, the PLGA chains remain flexible to form a dense PLGA matrix with a thin skin layer [12, 51].

A non-disrupted porous surface forms when the evaporated solvent leaves a permeable skin without increasing the internal pressure. If the internal pressure is cumulated after the surface is solidified to form a thin and flexible skin, a solvent bubble can undergo local expansion and erupt to form volcanic type pores [34]. Lidocaine-loaded PLGA microparticles were prepared by a water/oil/water (W/O/W) double emulsion technique using 10% PLGA (50:50, IV= 0.45 – 0.60 dL/g, Resomer RG 504H) in dichloromethane [65]. Other polymers, such as poly(methyl methacrylate) (3.5% in dichloromethane), also showed volcanic-like pores due to the eruption of liquid bubbles through the thicker shell [69]. When the shell thickness is heterogeneous, the weakest part of the skin becomes broken to form large holes [70]. This happens when the residual solvent in the core reaches the surface facing the aqueous phase [70, 71].

5.3. Cracked surface

Recently, capillary stresses during drying have been identified as a cause for cracking of films of colloidal dispersions [72]. An elastic skin can deform to maintain the intact layer and/or close small pores on the surface. On the other hand, a rigid skin cannot deform as the maximum capillary pressure is reached, causing partial deformation or cracking. Cracks on microparticles were observed when a PLGA 50:50 of around 7~17 kDa (Resomer RG 502, IV=0.16–0.24 dL/g) was used. On the other hand, the microparticles made of higher molecular weight PLGAs did not show such cracks [11]. The shell may be cracked if the stress caused by the osmotic pressure gradient is larger than the tensile strength of the shell, as shown by the cracks on the surface of poly(ɛ-caprolactone) microparticles [70].

5.4. Buckled surface

The buckling of the microparticle surface is a common phenomenon. Buckling occurs in different forms, and it is useful to distinguish them. In a recent study, three different types of buckling were observed on PLGA microparticles of the same batch [16]. The surface was smoother at lower PLGA concentration, e.g., 10% vs. 16.85%. Formulations with 16.85% PLGA showed three different types of buckling, as shown in Fig. 5. Type I describes shallow, round buckles (similar to sags) that become smaller (transition from I to I') and level with the surrounding area, as the solvent is removed. Type II inward buckles are observed frequently [35, 47, 48, 70, 73, 74]. The inward buckles are formed by inward folding of the unstable, elastic skin, while the remaining solvent is removed. During solvent removal, PLGA polymer chains and drug molecules accumulate near the skin. As the solid region grows, mechanical stress builds up due to capillary pressure (Fig 3). As the stress is released, instability in the skin occurs, causing it to buckle, or a structure at the drop edge can fracture [35]. In some cases, due to reduced inner volume caused by the removal of the solvent, local buckling of the skin may occur provided it has sufficient elasticity. The resulting depression continues to grow to form invaginated or deflated buckles [35]. As the volume is reduced, the shell is deflated to form an axisymmetric buckle. If the shell is sufficiently thin, the buckle loses its axisymmetry upon further volume reduction, resulting in a fully buckled shape [75], such as Type III in Fig. 5. Bowl-shaped microparticles can also be formed by adding a gas-forming agent inside PLGA microparticles and removing the formed gas from the microparticles during the solvent evaporation process [76].

The addition of a diblock copolymer of methoxy(polyethylene glycol) (MePEG, 750 Da) and poly(ε-caprolactone) (PCL, 1,000, 1,250, and 1,875 Da) to PLGA (85:15, IV=0.61 dL/g) resulted in a surface morphology with Type I buckles, when the MePEG-b-PCL was 20% and the PCL block was 1,250 Da or higher [77]. It is noted that the article described the morphology as dimpled, instead of buckled. Here, however, the sags are classified as Type I buckles, as the large indentations are not homogeneously distributed throughout the surface. A dimpled morphology is used to describe the homogeneous distribution of smaller indentations, as on the surface of golf balls (see below).

5.5. Dimpled surface

Dimples are different from shallow buckles (such as Type I buckles in Fig. 5) in that the dimples occur throughout the surface homogeneously in a spherical shape. Dimples are formed by different reasons from forming buckles, and the following describes three different mechanisms.

5.5.1. Presence of drug aggregates on the surface—Some drugs are incompatible with PLGA, and thus, they phase separate from PLGA after a significant volume of solvent is removed, and the solution viscosity is relatively high. In this case, the phase separation often results in drug-rich microdomains. However, the drug microdroplets on the surface can be solubilized in water during the final stage of solvent extraction, resulting in dimples on the surface. A good example of such morphology is rifampicin-loaded microparticles made of PLGA 50:50, M_n=49,100 Da [66]. The water-solubility of rifampicin is reported to be 0.041 mg/mL [66], 0.41 mg/mL [78], and 1.31 mg/mL [79], high enough for dissolution in the continuous aqueous phase.

5.5.2. Presence of polymeric surfactants on the surface—The dimpled surface morphology was also observed with indomethacin-loaded microparticles made of PLGA 50:50 (Resomer® RG 503, 34 kDa) and Labrafil®M 1944 CS (oleoyl polyoxyl-6 glycerides) [80]. The Pluronic® triblock copolymers also generated dimpled surfaces for microparticles made of PLGA 50:50 (IV=0.88 dL/g). The dimples became more pronounced as the molecular weight of Pluronics increased. Pluronics (or poloxamers) are triblock copolymers consisting of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) segments, arranged in a PEO-PPO-PEO structure. The surface appeared irregular with low molecular weight PPO (950–1,200 Da, Pluronics L31 and L44), shallow and dimpled with mid molecular weight PPO (1,750 Da, Pluronics L62 and L64), and distinctly dimpled with a high molecular weight PPO (2,750 Da, Pluronic L92) [81]. The microparticles made of PLGAs (50:50, 153 kDa and 75:25, 114 kDa) and Pluronic F127 (PPO of 4 kDa) also showed dimpled surfaces, and the dimples were more pronounced with the PLGA 75:25 microparticles [82]. It appears that certain polymeric surfactants with higher hydrophobic segments (i.e., appropriate HLB values) reside on the surface longer to be more efficacious in stabilizing the emulsion droplet [83].

5.5.3. Presence of oils on the surface—Surface morphologies of PLGA microparticles loaded with either etoposide alone or with tricaprin (glycerol tricaprate or glycerol tridecanoate) were studied [84]. The concentration of PLGA (50:50, IV=0.61 dL/g)

in dichloromethane for making an emulsion was either 4% or 8%. The etoposide concentration ranged from 5% to 15% of the microparticle, while the tricaprin concentration varied from 0% to 50%. The etoposide-loaded microspheres had a smooth surface, while the tricaprin microspheres had a dimpled surface. The dimple size increased with the increasing tricaprin percentage. The tricaprin droplets located at the surface are removed from the surface, resulting in regularly arranged dimples [84]. Dimpled surface morphology was also observed when canola oil was added to the ciprofloxacin/PLGA solution (PLGA 50:50, 31 kDa). The PLGA concentration in dichloromethane ranged from 0.5% to 5% (w/v). The oil pockets formed on the microparticle surface were washed away [85]. The dimple size changed depending on the oil type, where the size reduced substantially as silicon oil was used instead of canola oil [85]. In another study, 2-methylpentane was added to 10% PLGA (65:35) in dichloromethane [44]. As dichloromethane was removed, 2-methylpentane (which is a nonsolvent for PLGA) leached out onto the surface to form microdroplets, resulting in a dimpled surface resembling a golf ball. The dimpled surface was formed when the weight ratio of 2-methylpentane:PLGA was 1:4 or higher. The increasing amount of 2-methylpentane resulted in larger dimples. When perfluorooctyl bromide (PFOB) was added to an ethyl acetate solution with 2.5% poly(D,L-lactide) (PLA, 13.6 or 52 kDa), the surface of microparticles made of 52 kDa PLA was covered with many small droplets of PFOB (which is a nonsolvent for PLA), as in a Pickering emulsion. During the rapid solvent extraction process, PFOB droplets near the surface were left on the surface due to increased viscosity, resulting in a dimpled surface when PFOB was removed during freeze drying [86].

5.6. Islandy surface

The islandy morphology was observed with microparticles made of 2.5~5.0% PLGA (50:50. 40–70 kDa) and cyclosporin A dissolved in dichloromethane [87]. The water-solubility of cyclosporin A is known to be 0.04 μg/mL [88]. The islands surrounded by a continuous matrix were identified as drug precipitates, as the blank PLGA microparticles showed a smooth and homogenous surface, and the dimensions of the islands increased as the drug loading was increased. The formation of drug islands was due to the absence of interactions between the drug and PLGA [87]. PLGA microparticles loaded with fucidic acid also showed islandy morphology. The water-solubility of fucidic acid is 0.0052 mg/mL. Fucidic acid-loaded microparticles were made using PLGA (50:50, 49.1 kDa) [66]. As dichloromethane was removed, fusidic acid precipitated at a relative microdroplet volume of around 30% of the total microparticle. The solution viscosity was still relatively low for the fusidic acid-rich coacervates to move from the interior of the microparticles to the surface by convective solvent transfer. As more solvent was removed, drug precipitates remained on the surface as distinctive islandy domains. In the same study described above in Section 5.5.1, rifampicin-loaded PLGA microparticles resulted in dimpled morphology, as the drug has a high enough water-solubility enabling removal from the surface. Thus, it appears that, when drug precipitates are present on the surface, the water-solubility of the drug determined whether dimpled or islandy morphology is formed.

5.7. Wrinkled and rugged surfaces

Microparticles made by a solvent extraction method using 1% PLGA (50:50, IV=0.16–0.24 dL/g, Resomer RG 502H) in propylene carbonate showed a highly wrinkled surface [89].

The water-solubility of propylene carbonate is 236 mg/mL [90]. Thus, the solvent diffuses out of the microparticle very fast by convective flow, leaving solidified particles with a wrinkled surface. The valleys of the wrinkled surface look like pores, and thus, they are often described as porous, rough, and with an irregular surface [89, 91]. The wrinkled surface was also observed when bisdemethoxycurcumin, with a theoretical drug loading of 10%, was added to 10% PLGA (50:50, 50 kDa) in an 8:2 mixture of dichloromethane and ethyl acetate [91]. The solvent mixture was used considering the drug solubility and boiling point of the volatile solvent. In a study comparing the effect of minor manufacturing changes on PLGA microparticles, two different formulations were prepared using PLGA (75:25, DLG 6E from Evonik) and risperidone, but different solvents [13]. In the first formulation, both PLGA and risperidone were dissolved in dichloromethane. The seed emulsion was formed in the 1% PVA water saturated with the solvent, followed by solvent extraction in water for 3 hours at room temperature. The resulting microparticles showed a smooth surface. In the second formulation, the PLGA and the drug were dissolved in ethyl acetate (16.7% w/w) and benzyl alcohol (24%, w/w), respectively, and the seed emulsion was prepared in 1% PVA water saturated with benzyl alcohol, followed by solvents extraction in water (2.5% v/v ethyl acetate) overnight at 4 °C. The whole surface of the microparticles was wrinkled.

Wrinkles occur as a result of stress relaxation due to various types of interfacial instability caused by mechanical stress, thermal expansion, and/or swelling-shrinking [92]. As the solvent is removed during the extraction process, the network structure starts to form. As more solvent is extracted, the network structure may contract (or shrink), increasing internal stress, which subsequently induces the bending of the skin without causing the skin fracture to form a wrinkled surface structure. Thus, the formation of wrinkled morphologies depends on the factors affecting the interfacial instability, including the surfactant concentration, curing temperature, drying method [93], and PLGA molecular weight [94]. As the interfacial instability increases, more labyrinth-like patterns are formed.

Wrinkles may be thin and compact if the skin is “softer” during the drying process [95]. For “harder” skins, the skin may shrink to form thicker and less wavy morphology, i.e., rugged morphology. As a network structure is formed under certain conditions (e.g., high PLGA concentrations, high molecular weight PLGAs, fast solvent extraction, temperatures below glass transition temperature, etc.), it becomes thicker and more rigid, making subsequent contraction more difficult. Thus, the size of microparticles with rugged structures is expected to be larger than the microparticles with a wrinkled surface, or buckled microparticles, for that matter (assuming similar starting oil droplet sizes). The wrinkle wavelength (λ) is a function of the skin stiffness and thickness, and thus, rugged morphology has a larger wavelength [96].

5.8. Irregular surface

Microparticles with an irregular shape, instead of spherical form, are observed when the solvent is removed fast, as in using a water-miscible solvent or in spray drying [97, 98]. Microparticles of an irregular shape can be observed with other polymers such as silk fibroin [99]. Ivermectin is practically insoluble in water, with a water-solubility of about 0.005

mg/mL [100]. It was formulated into microparticles using PLGA (85:15, 136 kDa) and ethyl acetate [101]. The microparticles appeared not spherical and irregularly shaped with wrinkles and buckles when the drug loading was 50%. Although the concentration of PLGA in the solvent was not disclosed in the study, the presence of wrinkles and sagging buckles indicates that the concentration was not high, and the thin skin was formed fast. As more solvent is removed from the interior core, the shell collapsed as there is an insufficient amount of PLGA to support the shell.

5.9. Surface morphology and the processing conditions

A variety of surface morphologies can be formed based on the components and process parameters. Solvent extraction kinetics affect whether the surface becomes smooth, wrinkled/rugged, or irregular, depending on the PLGA molecular weight and concentration. The molecular weight and concentration are manifested into the solution's viscosity, which influences whether the surface becomes porous, cracked, buckled, or wrinkled/rugged. The drug-PLGA interactions and the presence of surfactants can cause dimpled or islandy surfaces. Because of the intimate relationships among all the parameters that are not fully understood yet, it is essential to describe the experimental conditions, particularly the solvent extraction conditions in as much detailed as possible.

6. Drug release profiles

Morphological characterization is essential, as it presents clues on the formulation composition and manufacturing process, and means to alter the formulation to obtained more desirable properties if necessary. It is vital to compare whether different formulations have been prepared similarly or not. The surface morphology can also be related to the drug release kinetics. While *in vitro* drug release can be routinely measured using various methods, the pharmacokinetic profile is vital to a formulation's potential success. Since the pharmacokinetic study is not as readily acessible as *in vitro* release studies, establishing parameters that may influence *in vitro* - *in vivo* correlations are essential in further development of PLGA formulations. To this end, it is beneficial to examine factors known to be related to the PK profiles.

6.1. The initial burst release

Many PLGA microparticle formulations show an initial burst release followed by a duration of steady-state drug release. The extent and magnitude of the initial burst release depends on the formulation. One of the explanations for the initial burst is attributed to the drug present on the surface and the drug dissolved throughout pre-existing pores and channels, which are most likely formed during the solvent extraction process [102]. After the initial burst release that typically occurs during the first day or in the first few days, the steady-state release may be due to the reconfiguration and degradation of PLGA molecules and morphological changes throughout the microparticles. During drug release, water penetrates into the microparticles and acts as a plasticizer [103]. This leads to a reduced glass transition temperature of PLGA, and the polymer matrix becomes softened and swellable [16, 104, 105]. This process can transform the porous surface to non-porous, and make the skin layer denser, resulting in slower drug release [102, 106–108].

6.2. In vitro-in vivo correlation (IVIVC)

Predicting the pharmacokinetic profile from the *in vitro* drug release data alone is still problematic. While, correlations can often be found once both data are available, often times these correlations require various magnitudes of scaling factors or the correlations only exist for a subset of formulations in a study. The characterization and subsequent explanation becomes even more difficult if other factors are considered, such as continuous changes in the PLGA structure during drug release *in vitro* and *in vivo*. Two examples described below present data showing faster *in vivo* drug release than *in vitro*.

Two PLGA microparticle formulations encapsulating triamcinolone acetonide were implanted subcutaneously in rats using a cage for retrieval of the microsphere during release [64]. This study clearly showed that the drug release from both formulations was greatly accelerated *in vivo* compared to *in vitro*, including water uptake, rate of PLGA hydrolysis, and mass loss. A formulation made of PLGA 50:50 with an acid-endcapped low molecular weight (IV=0.19 dL/g, 7~17 kDa) exhibited erosion-controlled release *in vitro*. Another formulation made of ester-endcapped PLGA with a higher molecular weight (IV=0.61 dL/g, ~40 kDa) displayed an osmotically induced/pore diffusion mechanism [64]. This study emphasized the need for a full understanding of the *in vivo* environment and development of better *in vitro* release tests that genuinely mimic the *in vivo* environment. This study has stimulated the field to examine the differences between the *in vitro* and *in vivo* drug release kinetics, further illustrating the need for a more comprehensive *in vitro* release method that better mimics the *in vivo* conditions.

Donepezil release from PLGA microparticles was also studied *in vitro* and *in vivo*. Fig. 6 shows the cumulative drug release and swelling of 75:25 (47 kDa) PLGA microspheres *in vitro* and *in vivo*. It is striking that the cumulative drug release profiles match the swelling of microspheres [109]. The *in vitro* release showed a triphasic release profile consisting of the initial burst release of about 4%, several days of lag time, and zero-order release for the rest of the 42-day release [109]. On the other hand, *in vivo* release was much faster with a higher initial release of about 10%, followed by zero-order release for 21 days, significantly shorter than the duration of *in vitro* release. This study also showed accelerated drug release *in vivo* relative to *in vitro*, indicating that the *in vivo* drug release mechanism is different by some unknown factors, or the *in vitro* methodology used may not be a suitable method to evaluate or discriminate the formulation in question. Characterization of the glass transition temperature, mass loss, water uptake, and swelling of microspheres *in vivo* indicated that faster release of donepezil than incubation *in vitro* is due to rapid PLGA degradation from the beginning *in vivo*. Thus, the question is what differences in the subcutaneous space can account for the faster rate of degradation *in vivo* relative to *in vitro*.

It was speculated that the faster degradation was due to the presence of enzymes as well as other *in vivo* factors, including interstitial fluid volume and local pH [110]. This process may result in the outside-in degradation of the PLGA microspheres proceeding from the surface inward *in vivo*. *In vitro*, on the other hand, degradation may occur inside-out due to the autocatalytic degradation process by the accumulation of acidic oligomeric units within the microspheres [110]. This explanation, however, fails to explain a few observations. First, it is not clear why acid accumulation by degrading PLGA chains does not occur *in vivo* as

much as *in vitro*. Second, the drug release *in vivo* is faster from the first few days when the acid accumulation may not be high enough for autocatalytic degradation. Third, the concentration of surfactants used *in vitro* release may not be as high as that *in vivo*. Finally, the particles were centrifuged at 4,000 RPM for 5 min each time during collection. The centrifugal force may be high enough to result in densification of the PLGA microstructure and water expulsion from the matrix resulting in an apparent slowing of release. Without a compendial method to accurately assess release, these are questions that can be invoked in nearly every report of PLGA microparticles.

It is understandable if *in vivo* drug absorption is the same or slower than the *in vitro* drug release. It is difficult to understand, however, if the *in vivo* drug absorption is faster than the *in vitro* drug release. Various enzymes and surfactants present in the body may adsorb to the microparticle surface, altering the drug release kinetics. This aspect, however, has not been studied comprehensively yet. Understanding the exact reasons for the faster *in vivo* drug release, and thus, drug absorption, is necessary for establishing meaningful *IVIVC*. This will ultimately enable the prediction of the *in vivo* response from the available *in vitro* characterization techniques.

7. Summary and outlook

The U.S. FDA has approved about two dozen PLGA formulations during the last three decades. Considering the various advantages of injectable, long-acting formulations, one would have expected a much larger number of formulations in clinical use. This low number of clinically approaved products may indicate that developing injectable formulations that deliver over clinically relevant durations requires much more than just trial-and-error approaches. Developing safe and effective, long-acting formulations requires a clear understanding of the mechanisms of microparticle formulation. In turn, this allows controlling the properties of the formulations, in particular the drug release kinetics.

As shown in Fig. 1, many composition and manufacturing process parameters can affect the properties of PLGA microparticles. Understanding each parameter's impacts on the resultant formulation's properties may be necessary, but this is also far too resource-intensive and impractical. Thus, it is critical to identify the key parameters that may play significant roles in determining the formulation properties. Many of the parameters have already been identified and controlled in formulation studies. They include characterizing PLGA (molecular weight, L:G ratio, end-group, molecular structure, and amount), solvent (type, mixture, and amount), and drug (type and amount). Most of these parameters have been reported in all studies. On the other hand, other parameters that appear to impact the formulation properties have usually not been described in detail. For example, the seed emulsion conditions, solvent evaporation or extraction kinetics, and drying conditions are usually indicated only briefly. Also, the residual solvent in the PLGA formulation and the formulation T_g can significantly affect drug release kinetics, but often they have not been reported.

As researchers begin to elucidate the mechanisms of PLGA microparticle formation and the importance of the individual composition and process parameters, we should start to report

as much detailed information as possible. Such collective efforts will provide the field with the information necessary to decipher the mechanisms and identify the key parameters to obtain the final formulation with the desired drug loading and release kinetics. Eventually, the complexity of PLGA microparticle formulations will be deconstructed, and the task at hand is to make it as thorough and expedited as possible.

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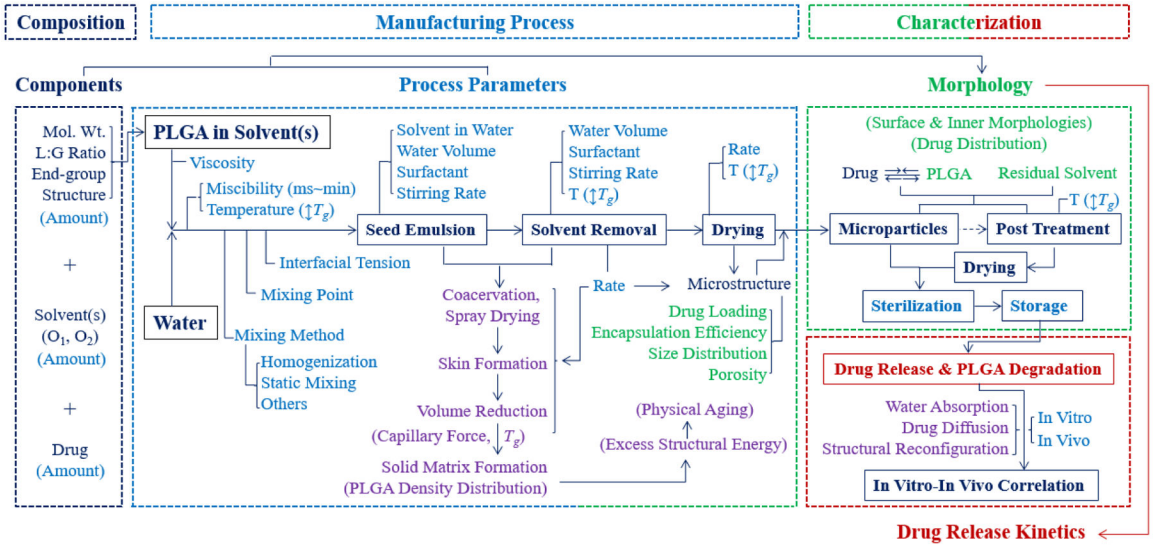

Fig. 1.

A flow chart of manufacturing PLGA microparticles by emulsion methods and the parameters affecting the properties of the formulation. Each color represents specific parameters or processes: Dark blue for components, dark blue in a box for main processes, blue for process parameters, purple for physicochemical processes, green for microparticle properties for characterization, and red for drug release characterization.

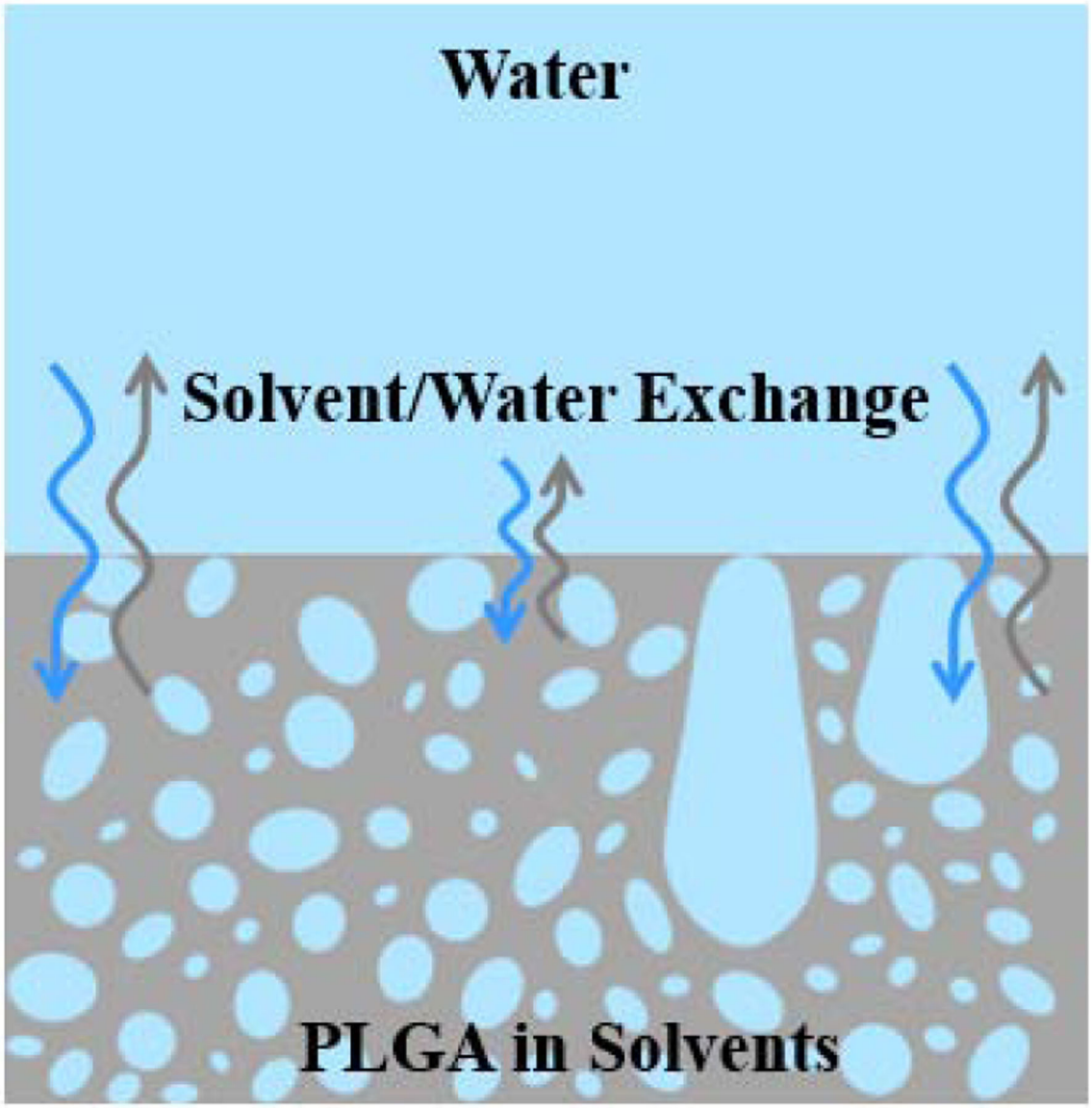

Fig. 2.
Schematic of the immersion precipitation process, where the exchange between water (blue) and solvent (grey) drives a phase separation in a PLGA microparticle surface. (Modified from [43]).

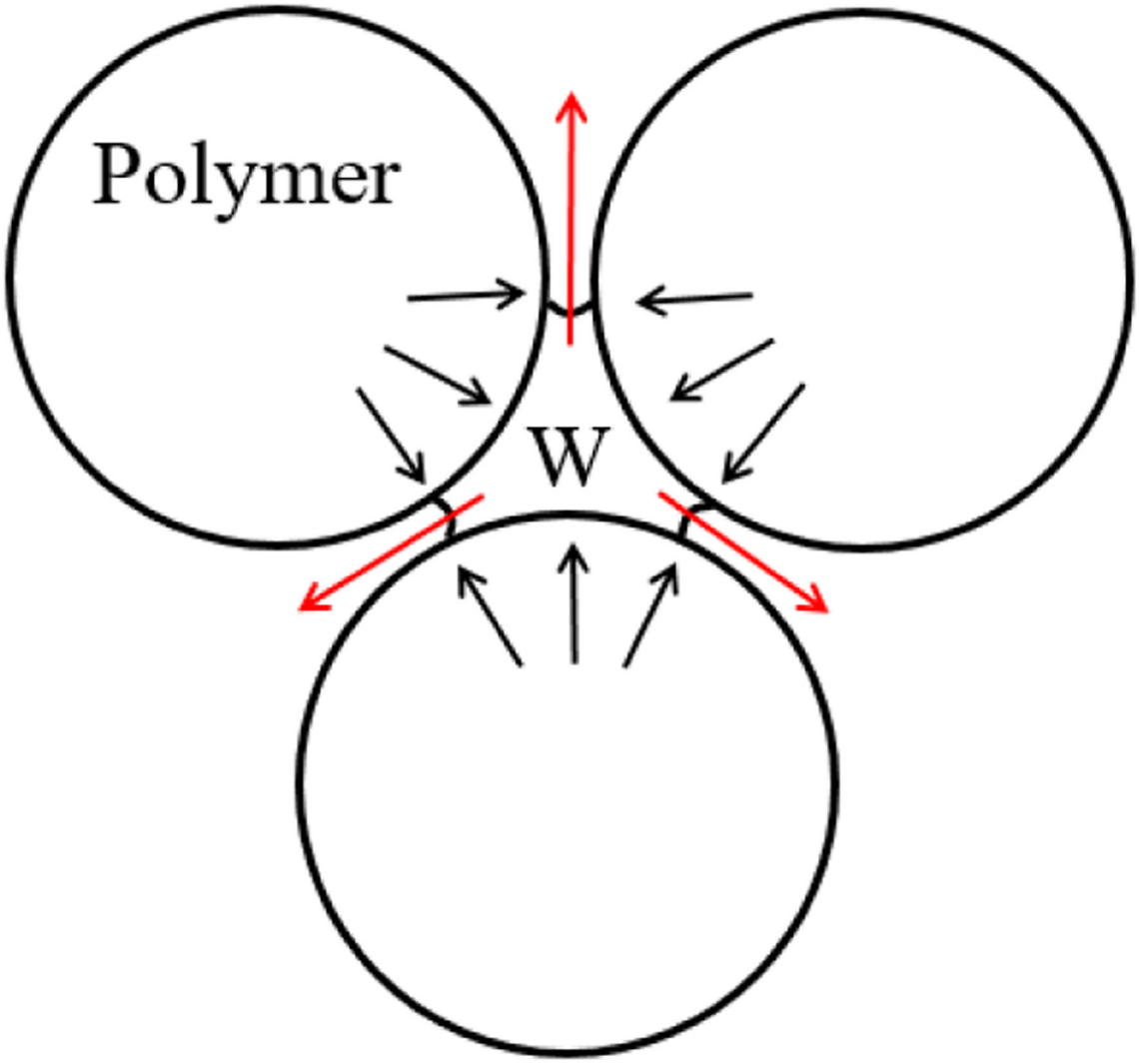

Fig. 3.

PLGA coacervates form capillary water (W) in interstices, resulting in contracting forces (black arrows) and negative pressure (red arrows). The contracting forces deform the PLGA coacervates to cover the interstices on the surface. (Modified from [47].)

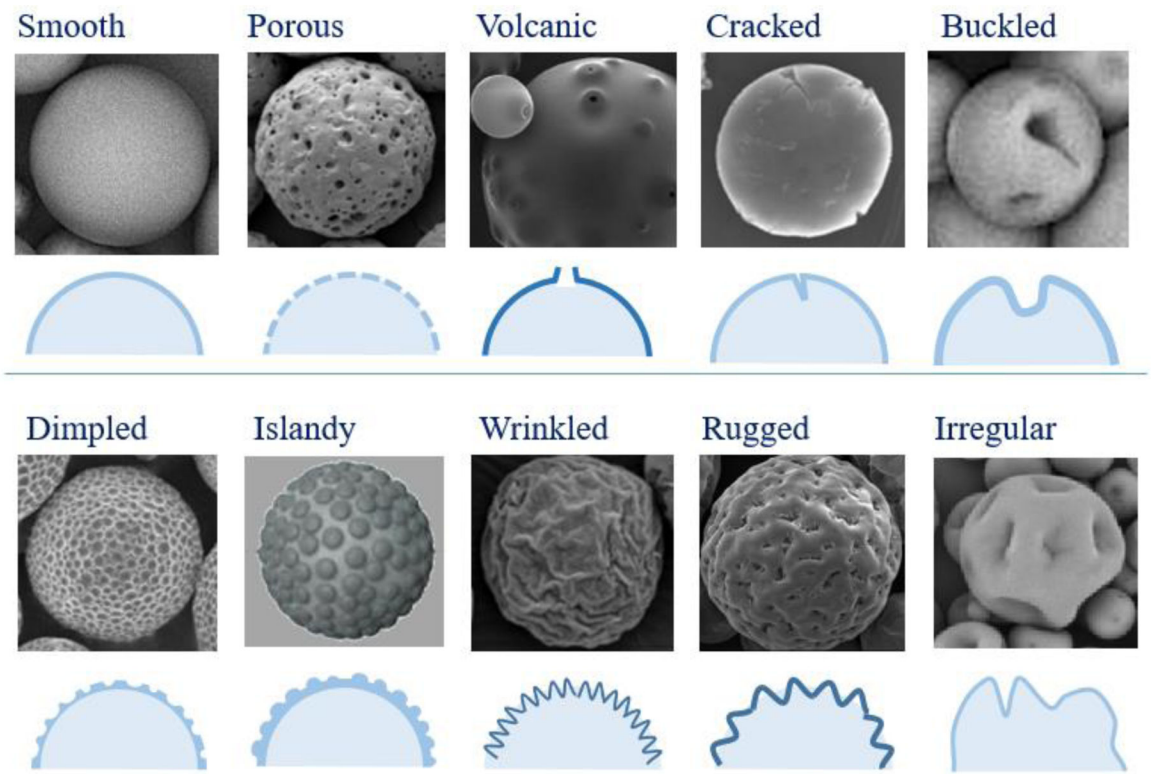

Fig. 4.

Examples of different types of surface morphologies observed on microparticles. Images of volcanic, cracked, dimpled, and islandy morphologies were obtained from references [65], [11], [45], and [66], respectively.

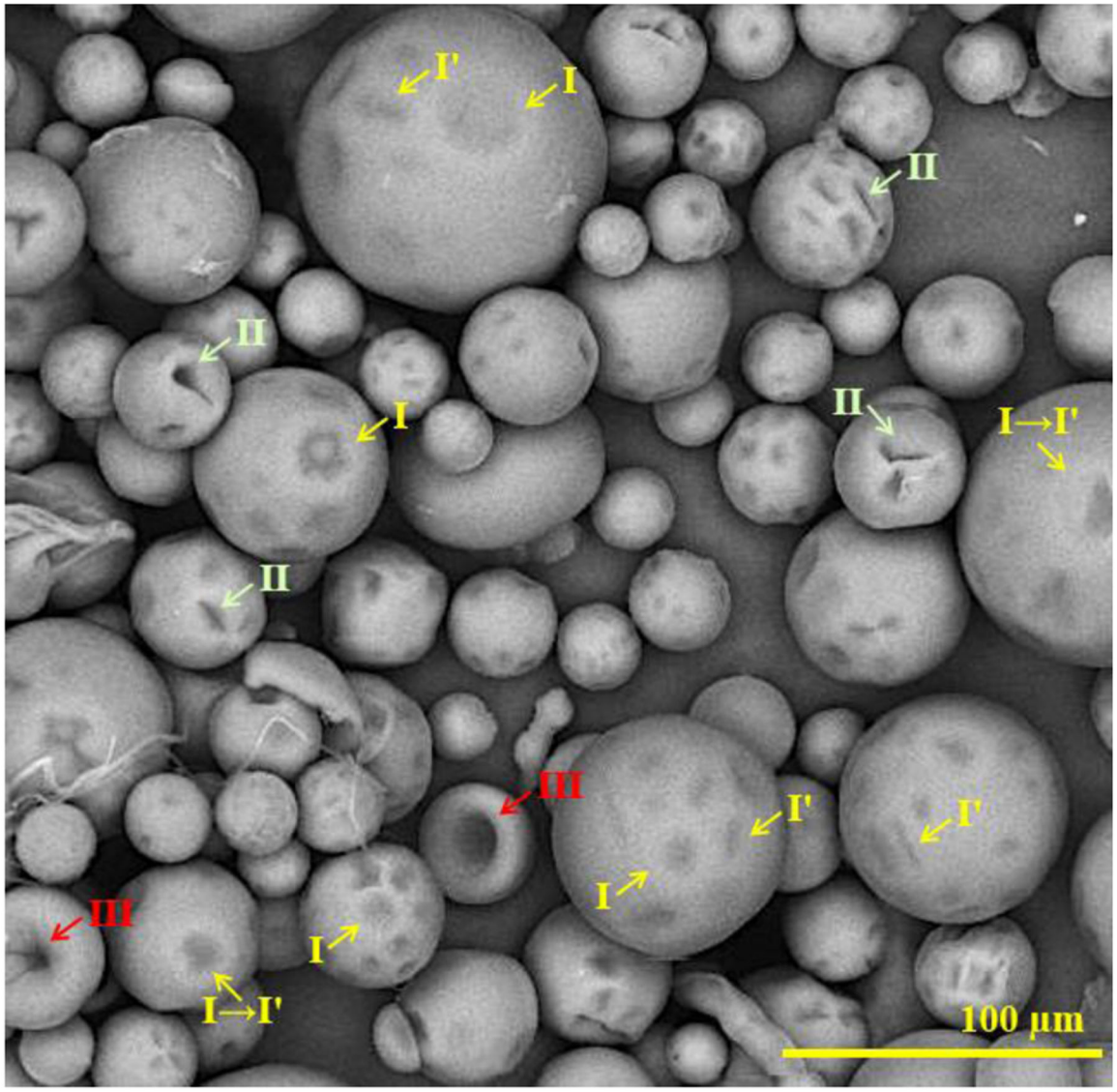

Fig. 5.

Three different types of buckling. (I) round concave dark patch area, (II) creased invagination, and (III) deflated buckles.

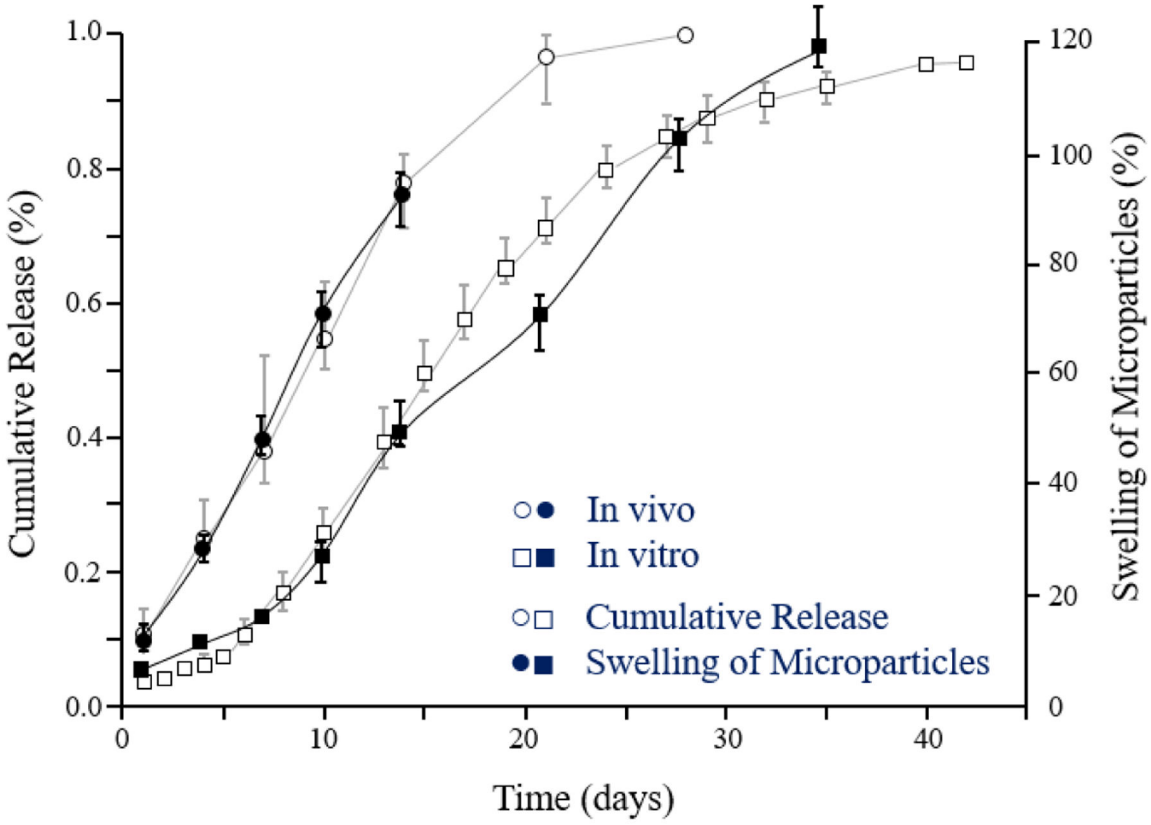

Fig. 6.

Cumulative donepezil release from PLGA microparticles and their swelling *in vitro* and *in vivo*. (Modified from [109]).