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Long-acting PLGA microspheres: Advances in excipient and product analysis toward improved product understanding

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ABSTRACT

Poly(lactic-co-glycolic acid) (PLGA) microspheres are a sustained-release drug delivery system with several successful commercial products used for the treatment of a variety of diseases. By utilizing PLGA polymers with different compositions, therapeutic agents can be released over durations varying from several weeks to several months. However, precise quality control of PLGA polymers and a fundamental understanding of all the factors associated with the performance of PLGA microsphere formulations remains challenging. This knowledge gap can hinder product development of both innovator and generic products.

In this review, variability of the key release controlling excipient (PLGA), as well as advanced physicochemical characterization techniques for the PLGA polymer and PLGA microspheres are discussed. The relative merits and challenges of different *in vitro* release testing methods, *in vivo* pharmacokinetic studies, and *in vitro-in vivo* correlation development are also summarized. This review is intended to provide an in-depth understanding of long-acting microsphere products and consequently facilitate the development of these complex products.

Contents

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1. Introduction

Poly(lactic-co-glycolic acid) microspheres have been developed to deliver different active pharmaceutical ingredients (*e.g.* small molecules, peptides, and proteins) with sustained release durations (varying from a couple of weeks to several months) [123] and/or controllable pulsatile drug delivery [67,76]. There are twelve PLGA-based microsphere drug products that have been approved by the U.S. Food and Drug Administration (FDA) (Table 1). In addition, there are many studies listed in the *clinicaltrials.gov* online database associated with PLGA-based products, including PLGA microspheres (Table 2). These include both new active ingredients and previously approved active ingredients that are being formulated into extended-release microsphere products, as well as current microsphere products that are being tested for new indications. Accordingly, it is evident that PLGA-based microspheres are considered reliable sustained-release drug delivery systems. However, the development of generic versions of existing products is challenging and despite the expiry of relevant patents, no generic versions of microspheres are available on the market. Moreover, these drug products are considered to be high risk, since the drug loading is typically high since they are intended for long-term drug release. Unintended alterations in product performance (for example as a result of dose dumping) can lead to severe safety issues for patients. Therefore, it is critical to understand the key process and formulation related parameters (such as release-controlling excipients) to develop appropriate quality control standards to ensure microsphere performance and safety, as well as to assist in the product development and regulatory approval processes.

As a polymer used in FDA and EMA approved products, PLGA has great potential in research and for future clinical usage. This polymer has been extensively investigated in a variety of drug delivery systems for numerous diseases, in scaffolds for tissue regeneration, and in a range of diagnostic tools [60,61,70,74]. Moreover, due to its tunable biodegradation rate, PLGA is the predominant release-controlling excipient used in long-acting parenterals such as microspheres [123]. By selecting different types and properties (such as molecular weight, monomer (L/G) ratio, and terminal groups) of PLGAs, release profiles can be tailored and specific pharmacokinetic requirements can be met based on the indications of the active pharmaceutical ingredients [38,46]. However, this ease of excipient modification also brings the challenge of standardization of excipient characterization. Some researchers have reported that minor variations in PLGA characteristics can alter the properties and performance of PLGA formulations [118,119]. The difficulty in regulating PLGA characteristics has been recognized as a critical variable limiting PLGA microsphere drug product development [123]. Thus, a comprehensive investigation of excipient and formulation characteristics is extremely relevant and beneficial to the development of generic and brand microsphere products.

In addition, development of *in vitro-in vivo* correlations (IVIVCs) has been a long-term pursuit for the pharmaceutical science community since the 1950s [79]. IVIVCs can be used to facilitate formulation development, set dissolution specifications, and serve as a surrogate for bioequivalence studies. However, the success rate of IVIVCs is low in regulatory submissions [55,80]. This indicates that IVIVC development remains challenging, particularly for long-acting parenterals such as PLGA microspheres. This may be attributed to: 1) the complexity of formulation characteristics and release mechanisms; 2) the lack of compendial, bio-relevant *in vitro* release testing methods; and 3) inadequate consideration of the *in vivo* release/absorption process under physiological conditions. Accordingly, more effort is required into the development of appropriate release testing methods and into achieving a mechanistic understanding of the release process.

This review highlights the current advances in physicochemical characterization techniques for polymers and PLGA microspheres. Moreover, recent investigations in *in vitro* release testing methods and *in vivo* pharmacokinetic studies as well as IVIVC developments are summarized.

2. Poly(lactic-co-glycolic acid) variability

To ensure consistent product manufacturing and to guarantee market demand, pharmaceutical companies tend to have multiple excipient suppliers [28]. However, changes in excipient suppliers can lead to excipient variability [28]. As reported, PLGAs from different manufacturers can exhibit different physicochemical properties such as inherent viscosity, molecular weight, L/G ratio, blockiness, and amount of residual solvent [118,119]. Moreover, differences in PLGA L/G ratio, blockiness, and block length among various commercially available PLGA polymer products have been reported [111]. Likewise, intra-source (batch-to-batch) variations have been reported [28,77]. These variations may come from several different aspects: 1) lack of detailed regulatory guidance and specifications; 2) differences in manufacturing processes among different manufacturers; 3) unintended alterations during manufacturing processes; and 4) different quality control methods and non-unified certificate of analysis of polymer products.

2.1. Regulatory background

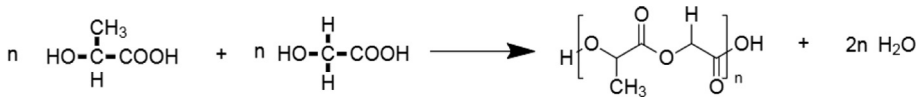
Excipients or inactive ingredients have been defined by different regulatory agencies and organizations. The 21 CFR 210.3(b)(8) states "*inactive ingredient means any component other than an active ingredient.*" The International Pharmaceutical Excipients

Table 1
List of commercially available brand-name PLGA microsphere drug products approved by the U.S. FDA.

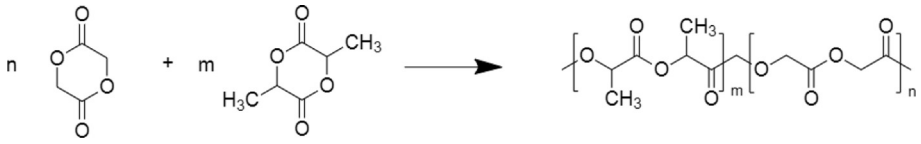
Brand Name	API	Indication	Administration Route	Dose	Encapsulation method	FDA's recommendation on *in vitro* release testing	Company
Bydureon™	Exenatide	Type 2 diabetes mellitus	Subcutaneous	2 mg per week	Coacervation	NA	AstraZeneca
Sandostatin LAR® Depot	Octreotide	Acromegaly, Carcinoid Tumors, Vasoactive Intestinal Peptide Tumors	Intramuscular	10/20/30 mg per month	Coacervation	Using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method	Novartis
Trelstar®	Triptorelin pamoate	Palliative treatment of advanced prostate cancer	Intramuscular	3.75 mg per month 11.25 mg per 3 months 22.5 mg per 6 months	Coacervation/Hot-melt-extrusion/ Emulsion solvent extraction	Apparatu II (Paddle)/Water-Methanol (95:5); Reconstitute vial in 2 mL Water for Injection, add to 500 mL medium at 37 °C	Verity Pharmaceuticals
Arestin®	Minocycline HCI	Periodontitis	Periodontal	1 mg per 2 weeks	Coacervation	NA	OraPharma Inc
Lupron Depot®	Leuprolide acetate	Palliative treatment of advanced prostatic cancer	Intramuscular	7.5 mg per month 22.5 mg per 3 months 30 mg per 4 months 45 mg per 6 months	W/O/W emulsion solvent evaporation	Same as the recommendation for Sandostatin LAR® Depot	AbbVie
Risperdal® Consta®	Risperidone	Schizophrenia, Bipolar I Disorder	Intramuscular	12.5/25/37.5/50 mg per 2 weeks	O/W emulsion solvent extraction	Same as the recommendation for Sandostatin LAR® Depot	Janssen Pharmaceuticals
Vivitrol®	Naltrexone	Alcohol dependence, prevention of relapse to opioid dependence	Intramuscular	380 mg per month	O/W emulsion solvent extraction	Using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method. Phosphate buffered saline with 0.02% Tween 20 and 0.02% Sodium azide, pH 7.4 (final osmolality should be 270 ± 20 mOsm), or any other appropriate medium, at 37 °C.	Alkermes
Signifor® LAR™	Pasireotide	Acromegaly	Intramuscular	20/40/60 mg per month	O/W emulsion solvent evaporation	NA	Novartis
Zilretta®	Triamcinolone acetoamide	Osteoarthritis	Intra-articular	32 mg per 3 months	S/O/W emulsion solvent evaporation/ Spray drying	NA	Flexion Therapeutics
Nutropin depot®	Somatotropin	Growth failure/adult growth hormone deficiency	Subcutaneous	13.5/18/22.5 mg per month	Cryogenic spray-drying	NA	Genentech
Lupaneta pack™	Leuprolide acetate	Endometriosis	Intramuscular	3.75 mg per month 11.25 mg per 3 months	W/O/W emulsion solvent evaporation	Same as the recommendation for Sandostatin LAR® Depot	Abbott

Table 2
List of PLGA–based products undergoing clinical trials.

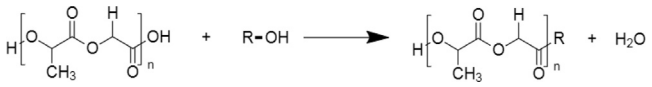
	Study title	Conditions	Interventions	Responsible party	Phase	NCT number
1	Dendritic Cell Activating Scaffold in Melanoma	Melanoma	Biological: WDVAX	Dana-Farber Cancer Institute	I	NCT01753089
2	Study of Probable Benefit of the Neuro-Spinal Scaffold™ in Subjects With Complete Thoracic AIS A Spinal Cord Injury as Compared to Standard of Care	Injury, Spinal Cord	Device: Neuro-Spinal Scaffold	InVivo Therapeutics	NA	NCT03762655
3	Ahmed Valve Implantation Coated With Poly Lactic –Co-glycolic Acid (PLGA) Saturated With Mitomycin-C in the Management of Adult Onset Glaucoma in Sturge Weber Syndrome	Glaucoma	Procedure: Ahmed Valve	Sohag University	III	NCT04735601
4	First-in-man Clinical Trial of CEB-01 PLGA Membrane in Recurrent or Locally Advanced Retroperitoneal Soft Tissue Sarcoma	• Locally Advanced Soft Tissue Sarcoma • Recurrent Soft Tissue Sarcoma	Combination Product: CEB-01 membrane loaded with SN-38	CEBIOTEX	I	NCT04619056
5	Comparative Trial of Operative Treatment of Distal Pediatric Forearm Fractures With Biodegradable Nails and K-wires	Fracture Wrist	Procedure: Distal radial and/or ulnar metaphyseal fracture fixation with bidegradable PLGA-based (Activa Im-Nail) implants	Péterfy Sándor Hospital	NA	NCT04848818
6	Autologous Transplantation of Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium for Geographic Atrophy Associated With Age-Related Macular Degeneration	Age-Related Macular Degeneration	Drug: iPSC-derived RPE/ PLGA transplantation	National Eye Institute	I&II	NCT04339764
7	Dose Escalation Study of Immunomodulatory Nanoparticles	Advanced Solid Tumor	Drug: PRECIOUS-01	Radboud University Medical Center	I	NCT04751786
8	Use of the Bioabsorbable Activa IM-Nail™ in Pediatric Diaphyseal Forearm Fractures	• Fracture Fixation, Intramedullary • Forearm Fracture • Fracture Healing	Device: Activa IM-Nail	Children's Fractures Interest Group	NA	NCT04941612
9	Genoss DES in Patients With a High Risk of Ischemic Events	• Drug-eluting Stent • Coronary Artery Disease	Device: Genoss DES	Yonsei University	NA	NCT05448625
10	Dexamethasone Implant for Retinal Detachment in Uveal Melanoma	Exudative Retinal Detachment and Uveal Melanoma	Drug: Dexamethasone intravitreal implant	Massachusetts Eye and Ear Infirmary	I	NCT04082962
11	Use of Extended Release Triamcinolone in the Treatment of Rotator Cuff Disease	• Rotator Cuff Tears • Rotator Cuff Tendinitis • Rotator Cuff Impingement	Drug: FX006 Injection	Northwell Health	III	NCT04094298
12	Study to Evaluate the Safety, Tolerability, PDs, and Efficacy of CNP-104 in Subjects With Primary Biliary Cholangitis	Primary Biliary Cholangitis	• Drug: CNP-104 • Drug: Placebo	COUR Pharmaceutical Development Company, Inc.	I&II	NCT05104853
13	CNP-201 in Subjects With Peanut Allergy	• Peanut Allergy	• Drug: CNP-201 • Drug: Placebo	COUR Pharmaceutical Development Company, Inc.	I&II	NCT05250856
14	Safety and Effectiveness of BIOSURE RG in Cruciate Ligaments Reconstruction in Chinese	• Cruciate Ligament Reconstruction • Knee	• Device: Investigational device: Biosure Regenesorb Interference Screw • Device: Control device: BIOSURE HA Interference Screw	Smith & Nephew, Inc.	NA	NCT04012567
15	Alveolar Ridge Preservation by Socket Seal Techniques	• Alveolar Bone Resorption	• Other: Routine treatment of the extraction socket • Procedure: Socket seal technique by a free gingival graft • Procedure: Socket seal technique using a synthetic resorbable membrane	Aristotle University Of Thessaloniki	NA	NCT05577663



Scheme 1. Direct condensation using lactic and glycolic acid.



Scheme 2. Ring opening polymerization using lactide and glycolide.



Scheme 3. Ester end-capping for acid-terminated PLGA.

Council defines pharmaceutical excipients as "*substances other than the pharmacologically active drug or pro-drug which are included in the manufacturing process or are contained in a finished pharmaceutical product dosage form.*" The United States Pharmacopeia (USP 35. General information/⟨1080⟩ Bulk Pharmaceutical Excipients) has one definition stating that "*the excipient is often a natural substance, mixture, or polymer whose chemical and physical properties are difficult to quantify and that is often used with a broad range of active pharmaceutical ingredients and in a diverse range of finished dosage forms.*" It is reported that excipients with different chemical and physical properties can affect the solubility, permeability, drug absorption, and pre-system metabolism of active pharmaceutical ingredients [31]. Moreover, more and more researches are reporting on the significance of excipients in the formulation development and bioavailability of drug products.

Although the U.S. pharmacopeia provides specifications (such as identity, purity and quality) for excipients, there is still a lack of understanding of excipient functionality [77,129]. Without a thorough understanding of excipient functionality and characteristics, variability may compromise drug product quality even though current monograph specifications are met. Considering the substantial amount of drug released over a long duration, it is of the utmost important to have a comprehensive understanding and clear specifications for key release controlling excipients (such as PLGA) used in long-acting parenteral formulations.

According to the 21 CFR 314.94(a)(9)(iii), parenteral generic drug products (such as PLGA microspheres) must contain the same inactive ingredients and in the same amount as the RLDs. However, due to the inherent heterogeneity of PLGA, the determination of PLGA sameness is extremely challenging with limited scientific reports. Moreover, there is no guidance from the regulatory agencies and the specifications are inadequate. In order to determine the sameness of PLGA as well as to control the quality and performance of drug products, it is essential to characterize the raw PLGAs as well as the PLGAs as presented in the final drug products.

2.2. Manufacturing process

There are two major synthesis techniques for PLGA: 1) direct condensation (Scheme 1), and 2) ring opening polymerization (Scheme 2).

Direct condensation is a process using lactic acid and glycolic acid for PLGA synthesis directly. Two equilibria: the dehydration/hydration process and the cyclic dimer/polymer chain equilibrium occur in this method. For ring opening polymerization, cyclic dimers (lactide and glycolide) are used as the reactants. As a consequence, the polymer chains synthesized by ring opening polymerization are poly-dimer chains (poly(lactide-co-glycolide)), which can be very different from polymer synthesized *via* direct condensation. The optimization and maintenance of synthesis conditions (temperature, monomer ratio and catalyst, *etc.*) are critical to the degree of polymerization, and hence product quality [32]. For example, the molecular weight of PLGA can undergo a sharp increase due to high polymerization rates with increase of organometallic catalysts (such as triethyl aluminum and stannous octoate) levels [11,48]. However, further increase in catalyst levels can lead to decreased polymer molecular weight due to the formation of a higher number of initiating species [59]. Moreover, owning to different reactivity, glycolide requires more severe conditions for polymerization compared to lactide. Accordingly, under the same conditions, lower molecular weight PLGA can be synthesized with a higher glycolide/lactide feeding ratio [11]. Moreover, the reaction time and temperature can influence the reaction rate and the final polymer quality. It has been reported that long reaction times may cause significant de-polymerization at high temperatures, which should be avoided. In addition, it is known that dehydration is critical for polymer chain growth and the prevention of hydrolysis. Therefore, high vacuum/inert gas conditions are preferred to control polymer quality [10]. Normally, PLGA polymers have acid end groups. The acid end group has been reported to improve the drug loading for some ionizable peptide drugs [82] due to ionic interaction. However, ester end-capping can also be performed *via* esterification using alkyl alcohol (with different alkyl chain length) (Scheme 3). Ester end capping increases the hydrophobicity of the polymer and allows higher drug loading of hydrophobic drugs, it also minimizes the autocatalysis effect [19,62]. Moreover, some novel end-capping methods have been reported by different researchers. For example, Zhao et al., reported a synthesis method to achieve tetra-amino-terminated PLGA using lysine [131]. Wang et al., reported a direct modification technique to prepare lipoyl ester-capped star PLGA for controlled drug delivery using lipoic acid [122].

2.3. Non-unified certificate of analysis

Typically, polymer manufacturers report certain physicochemical properties and specifications on the certificate of analysis based on the available USP requirements as well as their own methods. As summarized in Table 3, it is clear that different techniques and methods are used for quality control by different vendors. For example, different testing temperatures and polymer concentration are used for inherent viscosity analysis among different polymer manufacturers. This can hinder direct comparison of polymer size based on the reported inherent viscosity data. Moreover,

Table 3
List of properties and testing methods of PLGA polymer products reported on certificate of analyses from different manufacturers.

Brand	Tests											
	Appearance	Identity	Inherent viscosity	Molecular weight distribution	Monomer ratio	Specific rotation	Water	Tin	Residual solvent	Residual monomer	Elemental impurities	Bioburden	Bacterial endotoxins
Evonik	Visual test	NMR spectroscopy	Viscometry (Chloroform, 25 °C, 0.1%)	NA	NMR spectroscopy	NA	Coulometric titration	Inductively coupled plasma - mass	Gas chromatography	Gas chromatography	NA	Ph.Eur./ USP	Ph.Eur./ USP
Lactel	Visual test	NMR and FTIR spectroscopy	Viscometry (Chloroform, 30 °C, Conc ~ 0.5 g/dL	GPC (THF)	NMR spectroscopy	NA	NA	√	√	√	NA	√	√
Corbion	Visual test	FTIR spectroscopy	Viscometry (Chloroform, 20 °C, Conc = 1 g/dL)	NA	NMR spectroscopy	Polarimetry (Chloroform, 20 °C)	Coulometric titration	Inductively coupled plasma	Gas chromatography	Gas chromatography	USP method	NA	NA
Wako Pure Chemical Industries, Ltd	Visual test	√	√	√	NA	NA	√	NA	NA	√	√	NA	NA
Merck KGaA	Visual test	√	Viscometry (Chloroform, 25 °C, 0.5%)	GPC (THF)	NMR spectroscopy	NA	Volumetric titration	Inductively coupled plasma - optical emission spectrometry	NMR spectroscopy	NMR spectroscopy	√	NA	NA
PolySciTech	NA	FTIR spectroscopy	NA	GPC (THF)	NMR spectroscopy	NA	NA	NA	NA	NA	NA	NA	NA

NA: not reported on the certificate of analysis; √: test reported without characterization method.

not every vendor provides detailed molecular weight distribution results. In addition, the typical reported molecular weight determination methods are based on conventional gel permeation chromatography and polystyrene standards, which cannot provide absolute molecular weights. It is recommended to analyze the accurate molecular weight of PLGA *via* multi angle static light scattering technique (and not rely on external standards) [86]. Different quality control methods for amount of residual solvent, bioburden, and specific rotation can also be noticed among the certificate of analyses from different manufacturers.

3. Current advances in formulation preparation

The currently available commercial PLGA microsphere products, utilize three manufacturing techniques: *1)* emulsion-solvent extraction/evaporation, *2)* phase separation (coacervation), and *3)* spray drying (Table 1). These methods have successfully produced drug products at large scaling and with adequate control of particle size. However, owning to the complexity of PLGA microsphere formulations, it remains very challenging to produce products with high yield and high consistency (*e.g.* comparable physicochemical properties between batches) *via* these classical manufacturing techniques. Moreover, to facilitate the development of both brand and generic microsphere products, it is critical to understand the relationship between particle microstructure and product performance. Additionally, new techniques for real-time monitoring of the manufacturing process are needed.

Microfluidics is an emerging tool for microsphere preparation owning to its ability to achieve precise control over particle size and morphology [2,88,124]. In microfluidics, immiscible phases from different micro-sized channels are injected at certain intersection channels [44]. The droplets are formed one at a time, *via* shearing forces and this process results in mono-dispersed particles [63]. Based on their geometry, microfluidic channels can be classified as T-shaped channel, co-flow channel, and flow-focusing channel. Using these different channel setups and different flow rates, single and multiple-emulsions with different sizes can be generated [21,99]. However, researchers have reported low production efficiency and an inability to achieve mass production *via* microfluidic techniques [93,110]. Attempts (such as using a multi-layer geometry structure generator) have been made to improve product yield [78].

Another technique that is being investigated to achieve high production efficiency and scale up of microspheres is spray drying [120]. Spray drying is a rapid, scalable, one-step technique that has been shown to be a great option for microsphere manufacturing. One problem in spray drying is the need for elevated temperatures during the particle formation process, which limits applications to heat stable drugs. To overcome this limitation, spray freeze drying can be an alternative strategy [25]. This is a hybrid technique combining spray-drying (atomization of droplets) and freeze drying (freezing of droplets and sublimation process) [100]. Using this technique, the stability of thermo-sensitive therapeutics (such as proteins, peptides, and nucleic acids) can be improved without compromising the production efficiency of the final products [49,115].

Process analytical technology (PAT) has started to be implemented in both research and manufacturing of other complex parenteral products (such as micelles and liposomes) [24,37,42]. Implementing PAT into microsphere manufacturing (such as microfluidic) will ensure product quality and minimize waste. It is anticipated that new microsphere drug products will utilize advanced manufacturing methods with online PAT.

4. Current advances in polymer and formulation characterization

4.1. Polymer molecular weight

The polymer molecular weight is a key parameter that influences both the microsphere properties and performance [57,81]. It is noted that the PLGA molecular weight can change during formulation processing (high speed sheering, aqueous media incubation and drug-catalyzed degradation) [22,92,98], sterilization (gamma irradiation) [14] and storage (high temperature and humidity) [9]. Accordingly, monitoring the PLGA molecular weight is critical.

Conventionally, the molecular weight is determined *via* gel permeation chromatography (GPC) equipped with different kinds of detectors (differential refractive index detector, UV or evaporative light scattering detector) [96]. This separation technique is based on the size of the polymer coils in the mobile phase, not on the actual molecular weight. Therefore, this technique is method dependent and the results obtained can be affected by the different detectors used). Moreover, the most widely used standards for calibration are polystyrene standards. These external standards can exhibit different molecular dimensions compared to the testing samples (PLGAs) due to differences in polymer interactions with different solvents [87]. Currently, PLGA/PLA standards are available on the market. These specific polymer standards may provide more accurate molecular weight results compared to the conventional standards.

However, due to the inherent heterogeneity of PLGA, the PLGA/PLA standards may not perfectly match the chemistry (L/G ratio and blockiness) of all testing samples. Advanced techniques such as multi angle static light scattering and multi-detector GPC which do not rely on external standards for the molecular weight determination are suitable to acquire accurate molecular weight.

4.2. Polymer L/G ratio

PLGA is a copolymer composed of lactic and glycolic units. Differences in L/G ratios of PLGAs can alter the polymer properties (*e.g.* solubility in different solvents), formulation processes and ultimately drug release characteristics. Currently, the most widely used method to determine the L/G ratio is nuclear magnetic resonance (NMR). Samples are dissolved in deuterated organic solvents such as chloroform with appropriate concentration for analysis. Due to the different chemical environments, the hydrogens on the methyl group (CH_3) and methylene group (CH_2) exhibit different signals with specific chemical shifts. The experimental L/G ratio can be calculated using the integrated peak area of the different monomers *via* the formula below:

$$\%\,\mathrm{D,\,L-Lactic\,unit} = \frac{(CH_3)\mathrm{integral\,area}/3}{(CH_3)\mathrm{integral\,area}/3 + (CH_2)\mathrm{integral\,area}/2} \times 100\%$$

$$\%\,\mathrm{Glycolic\,unit} = \frac{(CH_2)\mathrm{integral\,area}/2}{(CH_3)\mathrm{integral\,area}/3 + (CH_2)\mathrm{integral\,area}/2} \times 100\%$$

4.3. Polymer blockiness

PLGA blockiness indicates the occurrence or incidence of the glycolic-glycolic linkages (G-G) over the glycolic-lactic linkages (G-L) in the PLGA sequence [43]. This property can be affected by different synthetic methods and may vary among different batches as well as among different manufacturers. As reported by researches, polymers with different blockiness can show different solubilities in organic solvents [108]. This can lead to alterations in formulation attributes. Moreover, higher blockiness normally facilitates water uptake and hydrolytic degradation, which can increase the drug release rate [114].

Normally, PLGA blockiness is determined using ^{13}C NMR. By comparing the peak area of the G-G linkage (166.3 ppm) to the peak area of the G-L linkage (166.4 ppm), the blockiness can be calculated [119].

$$R_c = \frac{\mathrm{G-G\,integral}}{\mathrm{G-L\,integral}}$$

Wan et al., has reported a strong linear correlation between polymer blockiness and microspheres release performance and degradation rates (see Figs. 1 and 2) [119]. This phenomenon indicates the importance of blockiness as a critical material attribute for PLGA polymer based formulations.

4.4. Residual solvent content

Various solvents are involved in polymer synthesis/purification as well as in the formulation development processes. It is always a concern whether the stability and quality of a drug product may be affected by residual solvent, and whether the daily exposure of solvent residues *in vivo* is important.

Different techniques can be applied to determine the presence and amount of residual solvent, such as loss on drying (LOD), thermo-gravimetric analysis (TGA), Karl Fischer titration and Fourier transform infrared (FTIR) spectroscopy. However, there may be more than one kind of residual solvent in samples. To identify the structure and amount of individual residual solvent with high specificity, gas chromatography–mass spectrometry (GC–MS) is the most widely use analytical tool.

4.5. Crystallinity

Polymer mechanical strength, water uptake, and hydrolysis rate are closely related to the crystallinity of PLGA. PLGA can vary from fully crystalline to fully amorphous based on the L/G ratio and types of monomer (L-lactide or D-lactide). The glass transition

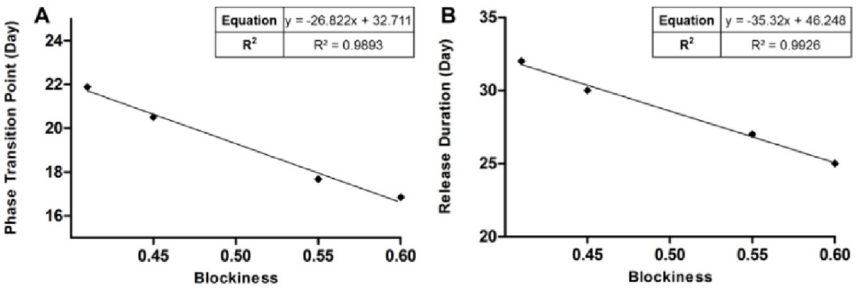


Fig. 1. (A) Linear regression of the phase transition point against the blockiness of the prepared microsphere formulations. (B) Linear regression of the release duration against the blockiness of the prepared microsphere formulations [119].

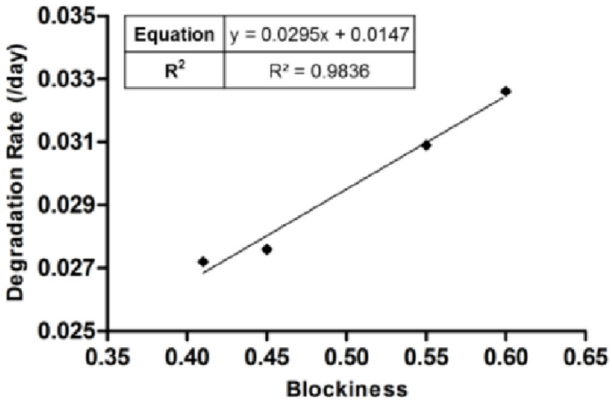


Fig. 2. Linear regression of the degradation rates against the blockiness of the prepared microsphere formulations [119].

temperature of PLGA can vary from 40 to 60 °C which is above the human body temperature. The crystallinity of PLGA can be investigated using X-ray crystallography.

4.6. Drug content

Drug loading is the percentage amount of drug in the final product (w/w). To evaluate drug loading, samples are dissolved in organic solvent, such as alcohol or dimethyl sulfoxide (depending on the solubility of the drug and excipients), to release the drug entrapped within the formulation. Then, the drug content is analyzed by high-performance liquid chromatography (HPLC) or other analytical instruments according to the pharmacopeia monograph.

4.7. Particle size and surface morphology

Particle size and surface morphology are critical factors influencing microsphere performance, resulting from porosity, surface area and the diffusional path length. Different techniques including light microscopy, scanning electron microscopy, laser light scattering, multi-size coulter counter, morphologically-directed raman spectroscopy, and X-ray micro-computed tomography can be used to analyze particle morphology and size [51].

Light microscopy is a conventional method for the observation of particles. The sample preparation process is relatively simple. This method can be used to observe not only the particle size and shape, but also the phase separation phenomena during formulation preparation [95]. However, the resolution of light microscopy is low and evaluating the detailed surface properties of small size particles is not feasible *via* light microscopy. By contrast, scanning electron microscopy provides higher resolution (one nanometer) and 3-D images of the surface topography of samples are possible due to the application of a focused electron beam. However, complex sample preparation, such as drying and coating (gold or platinum), are required. Apart from direct visualization of surface morphology, microscopy techniques can provide particle size distribution data *via* application of data analysis software such as ImageJ.

Laser diffraction and light obstruction are other popular methods for particle size measurement. Comprehensive data (population/area/volume weight mean particle size, D10, D50, D90 and span value) can be obtained and the sample preparation process is simple. However, precipitation and agglomerates may occur and this can affect the results and decrease the accuracy of this method.

4.8. Internal microstructure

Microspheres can exhibit different internal microstructure owing to changes in components (surfactant, polymer molecular weight) and processing parameters (solvent system, solidification kinetics). It is reported that the porosity and pore size greatly impact formulation performance. Hence, a thorough evaluation of the internal porous structure is essential.

Mercury intrusion porosimetry is a widely used technique to measure the porosity and pore size of porous samples [1,75]. This method can analyze different samples over a wide range (meso- and macro-pore range) based on compressibility [85]. It should be noted that this method relies on the capillary effect. Therefore, if the samples have an ink well structure (internal cavities with no surface pores) the accuracy of this method can be compromised.

To better understand the complex structure of microparticles, application of 3D image technology (X-ray computed tomography (CT) and focused ion beam scanning electron microscopy (FIB-SEM)) has recently attracted attention [23,68]. X-ray CT is a non-invasive technique to evaluate the internal structure of samples. Test samples can be scanned to generate image slides corresponding to individual cross-sections. By stacking all image slides, the 3-D structure of the samples can be acquired. For example, Gajjar et al., determined powder microstructure and the spatial correlation between particles, precisely size, shape, and density using X-ray micro-computed tomography [39]. FIB-SEM utilizes focused ion beam to mill the test samples, the milled cross sections can then be imaged using SEM. Artificial intelligence-based image processing allows different phases (drug phase, polymer matrix, and internal cavity) to be clearly identified [130]. For example, Clark et al., determined different microstructure between nine samples by FIB-SEM (Fig. 3) [23]. Sample D1153 had a uniformly distributed pore network throughout the cross section of the microspheres, whereas sample D1406 showed fewer pores which appeared to be mostly clustered near the center of the microspheres [23]. However, the formation of damaged amorphous layers, and voids at the sample surface may occur [58,94]. In order to achieve a complete picture of the sample microstructure, a combination of different techniques is recommended for internal microstructure analysis.

4.9. Drug distribution

Different drugs can exhibit different distributions in the polymer matrix due to variations in their physicochemical properties. It is reported that highly hydrophilic drugs such as proteins and peptides tend to distribute towards the exterior of microspheres due to their higher affinity for aqueous continuous phase [126]. Moreover, different processing methods (freeze-drying and air-drying) can change the drug distribution due to different extents of convection induced migration [47,121].

Many techniques have been reported to understand how drugs are distributed in microspheres. For example, SEM conjugated with X-ray spectroscopy can provide elemental information, indicating the drug distribution within the microspheres (Figs. 4, 5) [130]. Moreover, confocal Raman spectroscopy, stimulated Raman scattering (SRS) and laser scanning confocal imaging techniques have been used to examine peptide distribution in the polymer phase (Fig. 6) [109].

4.10. Drug polymer compatibility

Compatibility between the polymer and encapsulated drugs can influence the drug content, release mechanism and the therapeutic effect [69,82,83,108,109]. Theoretically, the Flory–Huggins interaction parameter χ is normally used to estimate drug polymer compatibility, as shown below.

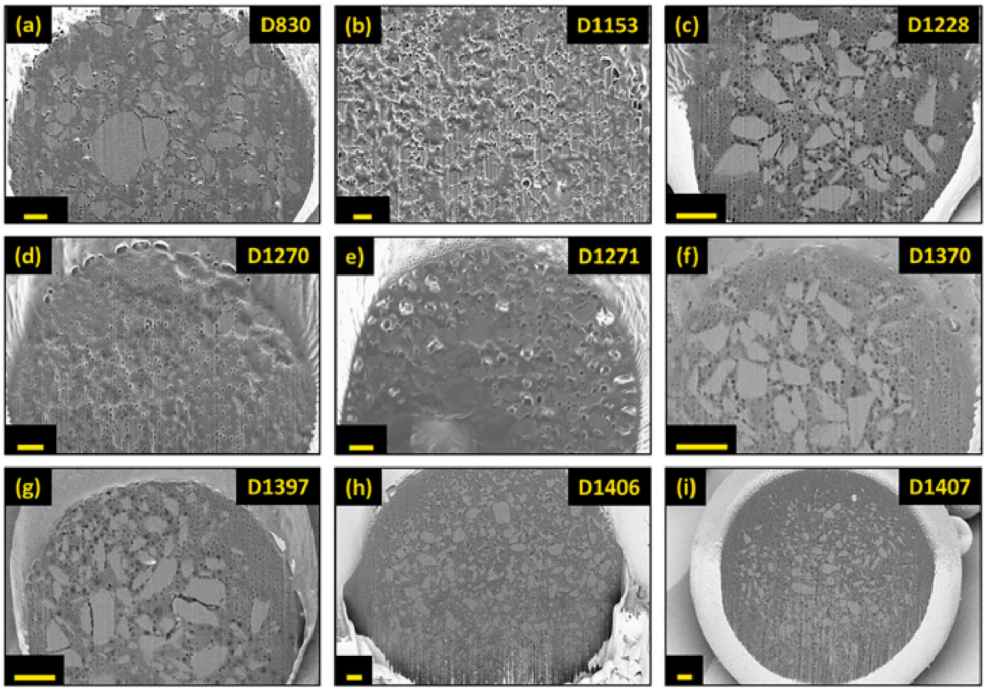


Fig. 3. 2D FIB-SEM cross sectional images of: (a) D830, (b) D1153, (c) D1228, (d) D1270, (e) D1271, (f) D1370, (g) D1397, (h) D1406, and (i) D1407. All scale bars are 3 μm [23].

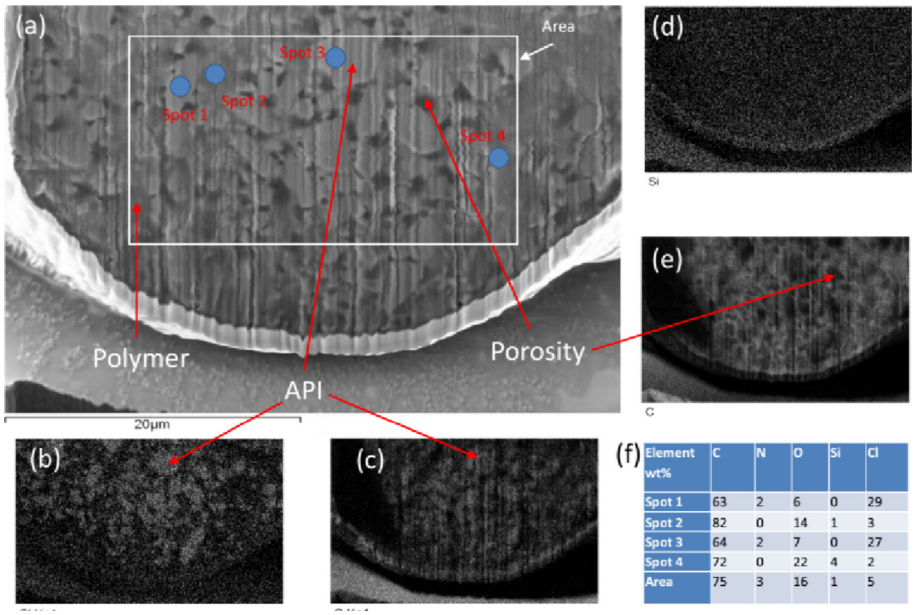


Fig. 4. Confirmation of material phases with EDS map. (a) Cross-section of a bottom half of a microsphere sample. (b) Chlorine distribution map on the same area as (a). (d) Oxygen map. (c) Silicon map. (e) Carbon map. (f) Spot and area EDS corresponding to the annotations in (a) [130].

$$\chi = (\delta_a - \delta_b)^2 \frac{V}{RT}$$

In this formula,δ(Hildebrand solubility parameter) represents the total solubility parameter of each component of the system. Contrary to the one-dimensional Hildebrand solubility parameter, the Hansen solubility parameter comprises the contributions from van der Waals forces, dipole–dipole interactions, and hydrogen bonding, and can be calculated by the group contribution method and/or Yamamoto Molecule Break method. Lübtow et al., empirically determined the compatibility of 18 different amphiphilic polymers for five different hydrophobic drugs, and compared these to the theoretical values [69]. The results showed that Hansen solubility parameter gave a better estimation compared to the Flory–Huggins interaction parameter. This may facilitate formulation development for future products [69]. In addition, different techniques have been used to study drug polymer interactions. NMR spectroscopy has been used to understand ionic interactions between acid end groups of PLGA and basic amino acid residues of peptides. For example, Okada reported a chemical shift in the arginyl and histidyl protons of leuprolide to a lower magnetic field

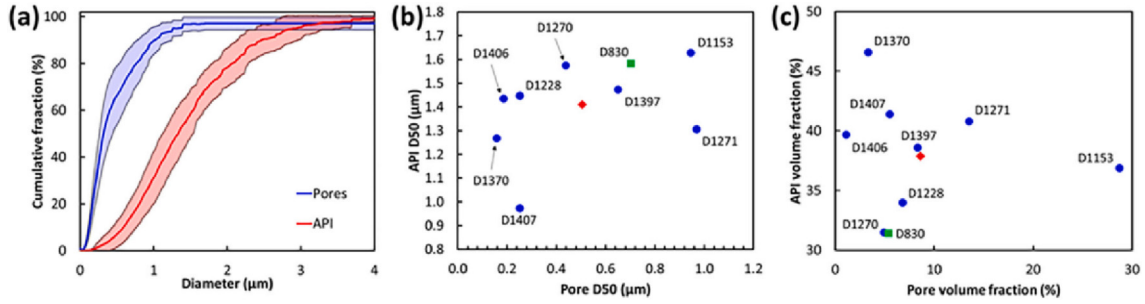


Fig. 5. (a) Average size distributions for all nine microspheres of the API (red) and porosity (blue) as determined from the 2D FIB-SEM images. Shaded regions indicate one standard deviation from the average. (b) D50 for the API distributions vs. the porous D50 for the nine samples (blue circles and a green square for the commercial product) and the average of the nine samples (red diamond), with the individual microsphere samples indicated. (c) API volume fraction vs. pore volume fraction for the nine microsphere samples [23].

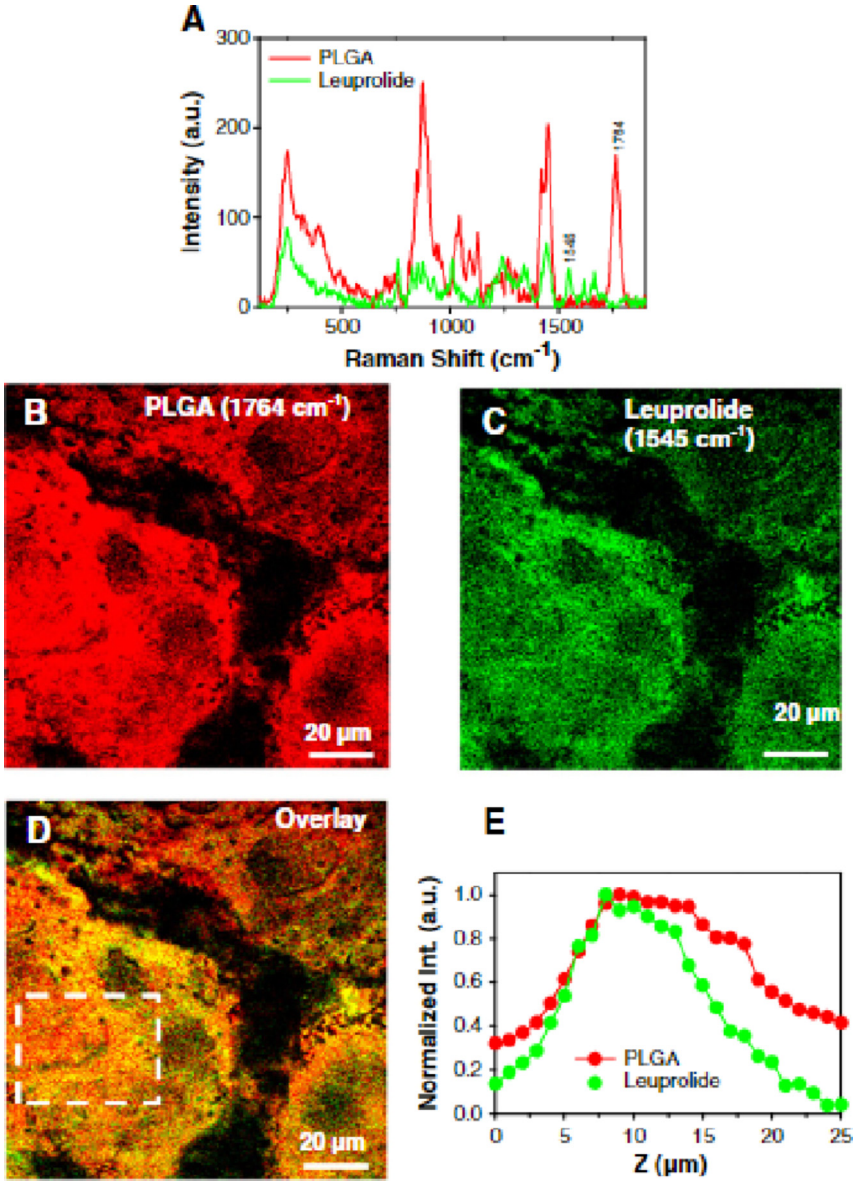


Fig. 6. Direct visualization of peptide penetration into PLGA films by SRS imaging. (A) Raman spectra from pure PLGA film (red) and leuprolide (green). (B) SRS image of PLGA distribution (red) in the film at 1764 cm^{-1}. (C) SRS image of leuprolide distribution (green) at the same location and depth in the film at 1545 cm^{-1}. (D) Overlay image of (B) and (C). (E) Average intensity of PLGA and leuprolide in the area indicated by the white box in D as a function of depth along the film [109].

in the NMR spectra of the prepared emulsion, confirming the ionic interaction between cationic peptides and PLGAs [84]. Moreover, FTIR spectroscopy has been used to detect drug polymer interactions with the help of reference standard spectra [6].

4.11. Water uptake

Microspheres may undergo swelling due to water uptake. This is a critical process which can affect drug diffusion and the initial of hydrolytic degradation of PLGA. The percent water uptake is investigated by measuring the weight value changes of hydrated and dried microsphere samples, as shown in the formula below:

$$\%\ \text{water uptake} = \frac{\text{weight of hydrated microspheres} - \text{weight of dry microspheres}}{\text{weight of dry microspheres}}$$

4.12. Microclimate

Microclimate is normally referred to as pH value within the PLGA matrix. Due to the properties of the encapsulated drug and the PLGA, as well as different degradation mechanisms (bulk/surface erosion), the microclimate in formulations can vary from acidic to neutral. This uncontrolled internal pH can be problematic for drug stability and product performance, particularly for proteins and peptides. Different techniques can be applied to determine the average pH value within PLGA matrices including ^{31}P NMR, electron paramagnetic resonance, and potentiometry [16,17,107]. Moreover, more detailed pH mapping of PLGA microspheres can be visualized *via* fluorescent pH-sensitive probes (SNARF-1 dextran and Lysosensor yellow/blue dextran) and confocal microscopy [64,65,66]. As reported by Liu, *et al.*, accurate pixel-by-pixel microclimate distribution maps in both the neutral (pH 5.8–8.0) and acidic ranges (pH 2.8–5.8) can be created with/without protein co-encapsulation [66].

5. *In vitro* and *in vivo* drug release

In vivo biopharmaceutical studies such as bioavailability and bioequivalence are critical for drug product development and ensuring optimal therapeutic efficacy and safety for patients [68]. However, *in vivo* studies (animal and human) are expensive and time consuming, specifically for long-acting parenterals such as microspheres. A robust, discriminatory and clinically relevant *in vitro* release method can reduce the burden of *in vivo* studies and bridge the formulation development challenges between *in vitro* and *in vivo* product performance [68]. However, method development remains challenging with limited regulatory guidance provided (Table 1).

Currently, many *in vitro* release methods have been developed for different microsphere formulations. In order to make the methods bio-relevant, different factors such as local pH, body temperature, metabolism, and buffer capacity should be taken into consideration [5].

5.1. Current in vitro release testing methods

5.1.1. Non-compendial sample-and-separation methods

Owing to simple device set-up, flexibility and practicability, non-compendial sample-and-separation methods are popular for the *in vitro* release testing for different microsphere formulations. In general, the formulations are dispersed in the release media and agitated by a motor driven paddle with a constant stirring rate. Sampling time points are determined and the withdrawn release samples are centrifuged or filtered for separation of the particles and supernatant. The separated microspheres are dispersed in fresh release media and transferred back to the apparatus, and the drug amount in the supernatant is quantified by chromatography assays.

Different parameters including vessel size (500 μL–500 mL), agitation (magnetic stirring, water shaker bath and rotation), and separation method (filtration or centrifugation) can be optimized to match specific needs based on the properties of the testing formulations (sample amount, drug loading, density, drug solubility, *etc.*) [6,54,105]. However, this method has some inevitable limitations such as sample loss and particle aggregation. Moreover, due to the non-standardized devices/equipment (with different dimensions and materials), the reproducibility of the results may be compromised, and can limit inter-laboratory comparisons and regulatory approval.

5.1.2. USP apparatus II with paddle method

USP apparatus II is a most commonly used standardized method with good robustness. It is widely used for solid oral dosage forms such as tablets and capsules and has also been applied to parenteral dosage forms such as microspheres. It is noted that USP II has the same disadvantage of sample loss, as non-compendial sample-and-separation methods. In addition, a large volume of release medium is required for this method. This method may not be practical if the sample is in limited amount. Moreover, it is reported that peak vessels are recommended in order to prevent cone formation due to the hydrodynamic issues of conventional USP vessels/paddles (static water under paddle) [12]. Additionally, microspheres may aggregate and in some cases float on top of the media causing variability in the collected data [102,103,106,132,133].

5.1.3. Dialysis methods

Dialysis methods are widely used for the *in vitro* release testing of different kinds of parenteral formulations. Using dialysis membranes, formulations (kept in dialysis sacs or tubes) can be separated from the outer bulk medium. Samples can then be collected from the outer medium for analysis at the chosen sampling time points. This reduces sample loss that normally occurs in the sample-and-separation methods. However, regular dialysis methods have some limitations including particle aggregation (due to lack of agitation) and obstruction of the porous dialysis membrane. In addition, sink conditions are often violated within the dialysis sacs causing irreproducible and often incomplete drug release. These problems can change the release performance of microsphere formulations and lead to unsatisfactory correlation between *in vitro* and *in vivo* profiles. Reverse dialysis can be applied to overcome these limitations, in particular violation of sink conditions is not a problem. In reverse dialysis, formulations are dispersed in the bulk media. Release samples are then collected from the dialysis sacs [18]. This method has much improved agitation, preventing particle aggregation and facilitating drug diffusion [54].

5.1.4. USP apparatus IV method

USP IV is a method based on the continuous flow-through principle, which is designed to simulate the *in vivo* conditions [13,50,91]. This method was originally developed for sustained release oral dosage forms such as tablets, capsules, and is suitable for special dosage forms such as granules, powders, suppositories and implants with different cell/device designs. Moreover, the application has been extended to parenteral formulations such as microspheres [72,73,132,135]. Different reports have noted that the USP IV is a preferred method for *in vitro* release testing of microspheres [72].

This method has various advantages including: (1) prevents sample loss and particle aggregation; (2) prevents media evapora-

tion; (3) provides better hydrodynamic (laminar flow, no dead volume); and (4) increases flexibility of media type, volume and flow rate. However, back-pressure, leaking and blockage may occur during testing due to: (1) generation of nano-scaled degradation products especially for long term (month or longer) testing of microspheres; (2) aging of rubber rings (V/O-ring) and inappropriate filter membrane. These issues can be prevented by routine checking and solvent change [5]. Moreover, drug adsorption may occur for macromolecules such as proteins due to the high surface area of the glass beads and the tubes used in the USP IV system. This can be overcome by selecting appropriate device materials and by the addition of surface active agents such as sodium dodecyl sulfate [54].

5.2. In vivo pharmacokinetic study

In vivo release/absorption studies can provide direct and comprehensive pharmacokinetic information. Different animal models (rat, rabbit, and dog) have been reported for use in *in vivo* drug release studies [7,53,56]. Interspecies differences need to be considered especially when extrapolation of animal data to human clinical studies is required.

In general, for systemic drug delivery, microsphere formulations are administered to the animals and blood/urinal samples are then collected at predetermined time points. Drug or target drug metabolites are extracted from the collected biological samples *via* different extraction methods (such as protein precipitation, liquid–liquid extraction) based on the properties of the target molecules and biological samples. The structure and concentration of the target molecules can be determined using liquid chromatography-tandem mass spectroscopy. For local drug delivery systems (*e.g.*, periodontal cavity or knee joint space), the local drug concentration is more relevant and critical to determine the therapeutic effect and the detection of systemic drug concentrations may not be feasible [104]. End-point studies (quantify drug amount in certain tissues) with different animal models have been used. However, it is not ethically desirable and high variations between different subjects are inevitable [54]. Microdialysis is an alternative method to understanding drug concentration at the local site with minimal invasion [54].

Different factors including delivery system independent (such as interstitial fluid component and viscosity, temperature, foreign body reaction and motion at site) and delivery system dependent (such as properties of the drug and polymer, enzymatic degradation and protein adsorption) may change the drug release/absorption behavior and result in discrepancies between *in vitro* and *in vivo* performance [18]. It has been reported that triamcinolone acetonide microspheres displayed an osmotically induced/pore diffusion mechanism which was not observed *in vitro* [29]. Researchers found that the rate of hydrolysis, mass loss, and water uptake all increased in *in vivo* compared to *in vitro*. Similar phenomenon have been reported for donepezil-loaded microspheres, which undergo an increased rate of water uptake, and hence faster drug release/absorption *in vivo* compared to *in vitro* [33]. In addition, other researchers have reported that increased *in vivo* drug release/absorption can be attributed to changes in local pH [52,133]. As reported by Zolnik, due to the limited interstitial tissue volume, the accumulation of acidic oligomeric polymer degradation material may lead to the release mechanism changing from bulk erosion to surface erosion with an increase in the release/absorption rate *in vivo*. More investigations are necessary to identify all the features leading to release mechanism changes between *in vitro* and *in vivo* conditions.

Moreover, tissue response (acute inflammatory response, chronic inflammatory response, granulation tissue development, and the foreign body reaction) have been reported after administration of microspheres. The intensity and duration of these processes are related to the size, physicochemical properties (L/G ratio) and the morphological properties (shape, pore) of the microspheres, as well as to the injection site and volume, and the syringe needle gauge used [3,116,117]. The formation of fibrosis can isolate microspheres from the surrounding environment. Hence, the drug absorption process can be hindered, and consequently the drug release/absorption rate is decreased *in vivo*. This tissue response may be one of the reasons for slower drug release/absorption in human studies [90]. In addition, the properties of the loaded drug should be taken into consideration, especially if the drug has cytotoxic, inflammatory or anti-inflammatory effects [4,45,125].

6. *In vitro-in vivo* correlation

Considering the high cost and long duration of *in vivo* pharmacokinetic studies, using *in vitro* dissolution/release testing to accurately and precisely predict the *in vivo* behavior of a drug product is a long-held goal of the scientific community [35,71]. The U.S. FDA has defined IVIVC as a predictive mathematical model describing the relationship between *in vitro* properties (such as drug release rate or extent) and relevant *in vivo* responses (such as the drug plasma concentration or the amount of drug absorbed) [35].

The establishment of an IVIVC has various benefits: (1) serve as surrogate for bioequivalence studies; (2) set dissolution specifications; and (3) support formulation development and provide a mechanistic understanding drug release. Various U.S. FDA guidelines have been provided for IVIVC on oral dosage forms [34,36]. Currently, there is no regulatory guidance for parenteral dosage forms such as PLGA microspheres. The principle of model development and validation has been extrapolated from oral dosage forms to non-oral dosage forms (parenteral, transdermal and ocular) based on the currently available guidance [41,104,127].

6.1. Different levels of IVIVC

There are four levels of IVIVC (A, B, C, D). Level A is a point-to-point model that describes the relationship between the entire *in vitro* release profile and the entire *in vivo* absorption profile. Therefore, a level A IVIVC is the most informative model and can be used as a surrogate for bioequivalence studies for the initial approval processes as well as for pre-/post-approval changes (such as formulation, equipment, process, and manufacturing site changes) [35]. Consequently, human studies can be minimized, and the regulatory burden can be reduced without sacrificing product quality. Level B is a statistical moment analysis instead of a point-to-point correlation. It compares the mean *in vitro* dissolution time with the mean *in vivo* residence time. It is not intended for regulatory approval considerations due to the lack of sufficient predictability. Level C can be subdivided into single point and multi point IVIVC. For single point, it attempts to set up a correlation between an *in vitro* dissolution parameter such as a particular time for a certain amount of drug to be released (such as $T_{50\%}$) or a percent amount of drug dissolved in a certain time to an *in vivo* pharmacokinetic parameter such as the AUC or Cmax. Although it cannot provide a whole picture of an *in vivo* plasma curve due to its single point analysis nature, a single point level C correlation can be used in early-stage formulation development. For a multi-level C IVIVC, multiple time points on an *in vitro* release profile are used to correlate with pharmacokinetic parameters. Level D is a qualitative rather than a quantitative correlation, which provides a rank order comparison between *in vitro* and *in vivo* profiles. It is not adopted in the regulatory guidance.

6.2. Development and validation of IVIVC

To develop an IVIVC, firstly, at least two preferably three or more formulations with different release rates should be selected to acquire *in vitro* release profiles using the same testing method. One formulation is acceptable if the release characteristics are independent of the *in vitro* release test conditions (such as pH, release media and flow rate, *etc.*). Secondly, drug plasma concentration–time profiles of the selected formulation should be acquired *via* pharmacokinetic studies using appropriate animal models or human clinical studies. Thirdly, convolution and/or deconvolution studies are required. Different methods are available including model-dependent such as the Wagner-Nelson method (for one compartment model), and the Loo-Riegelman method (for two compartment model), as well as model-independent numerical methods and, mechanistic methods (physiologically-based pharmacokinetic/biopharmaceutic modeling) as shown below:

$$\text{Wagner}-\text{Nelson}: \ F = \frac{X_t}{X_\infty} = \frac{C_t + k_e \int_0^t C\,dt}{k_e \int_0^\infty C\,dt}$$

$$\text{Loo}-\text{Riegelman}: \ F = \frac{X_t}{X_\infty} = \frac{C_t + k_{10} \int_0^t C\,dt + \frac{X_{p,t}}{V_c}}{k_{10} \int_0^\infty C\,dt}$$

$$\text{Numerical}: \ C(t) = \int_0^t C(t-u) r_{ab}(u) du$$

The F represents the fraction absorbed *in vivo*, k_e and k_{10} represent the elimination constant in the one and two compartment models, respectively. X_p represents the amount of drug in the peripheral compartment. V_c represents the apparent volume of the central compartment. r_{ab} represents absorption rate time course. C represents the plasma-drug concentration time profile resulting from instantaneous absorption of a unit amount of drug. C(t) is the plasma-drug concentration time profile of the formulations.

Correlations can be established *via* either a linear (preferably) or non-linear model (such as Sigmoid, Hixon–Crowell, Weibull, and Logistic) using the *in vivo* absorption profiles and the corresponding *in vitro* release profiles [30]. Due to the differences between the *in vitro* and *in vivo* conditions as well as interspecies differences, scaling and shifting factors can be introduced to compensate for the differences between the release/absorption profiles *in vitro* and *in vivo*, as shown in the equation below:

$$X_{vivo}(t) = a_1 + a_2 X_{vitro}(b_1 + b_2 t)$$

In this formula, a_1 and b_1 represent the shifting factors for absorption and time, respectively. a_2 and b_2 represent the scaling factors for absorption and time, respectively.

It is noted that these factors should be kept the same for all formulations involved in model development. If the same shifting or scaling factors cannot be applied to all formulations, this indicates an absence of a reliable IVIVC [112].

The predictability of the developed IVIVC models is validated using the percent prediction error (%PE) of the critical pharmacokinetic parameters (AUC and C_{max}) internally and externally. According to the IVIVC guidance from U.S. FDA, in order to demonstrate that the developed models are conclusive, the %PE of both the AUC and the Cmax should be less than 15% for each formulation and the average %PE of both the AUC and the Cmax for all internal formulations should be less than 10%. If these criteria cannot be achieved or the drug has a narrow therapeutic index, external validation is required, and the %PE of both the AUC and Cmax of the external formulation should be less than 10%.

6.3. Challenges for IVIVC development for parenteral PLGA microspheres

Effects have been made to develop IVIVC models for PLGA microspheres using different methods [15,20,27,133]. However, most of these studies are"proof-of-concept" based. These studies discuss the probability of developing point to point correlations or Level B correlations based on one formulation [97,113]. Although some recent research has reported Level A IVIVCs using multiple qualitative and quantitative equivalent PLGA microsphere formulations, the development of universal and reliable IVIVC models for these dosage forms remains challenging [6,8,20,26].

As discussed in the previous section, appropriate *in vitro* release testing methods are critical for IVIVC development. However, currently there is no compendial *in vitro* release method for PLGA microspheres. Moreover, PLGA microspheres normally exhibit multi-phasic release characteristics such as burst release and a lag phase, followed by a fast release phase. Accurately and precisely describing these detailed release characteristics in a discriminative, robust and bio-relevant *in vitro* release test method is a prerequisite for IVIVC development. Although sample-and-separation as well as dialysis methods have been reported for IVIVC model development, USP apparatus IV is the most highly recommended method for microsphere IVIVC development [105]. According to published results, developed IVIVC models showed improved model predictability, particularly the initial burst phase using *in vitro* release profiles acquired from USP IV compared to the sample-and-separation methods [105]. In addition, many literature reports describe changed mechanisms and release/absorption profiles between *in vitro* and *in vivo* conditions. For example, dexamethasone microspheres show triphasic *in vitro* release profiles, and yet biphasic *in vivo* profiles with shortened overall duration [134]. Accordingly, IVIVCs have been developed using normalized, post-burst release phase *in vitro* release data [5]. Moreover, another research report describes differences in burst release of peptide loaded microspheres between *in vitro* and *in vivo* conditions. IVIVCs were developed using formulations with similar release characteristics to improve predictability [7]. Accordingly, more investigations should be conducted to elucidate the factors resulting in the mechanism change between *in vitro* and *in vivo* conditions. Different methods incorporating physiological properties and formulation characteristics may be developed for improved correlation and better data explanation.

With the motivation for better prediction of *in vivo* performance, mechanistic PBPK models have been investigated to identify critical quality attributes and to understand the impact on *in vitro* and *in vivo* performance of different long-acting products. For example, Rajoli et al., developed PBPK models (using SimBiology®) for eight anti-retroviral products using clinical PK data. The whole-body PBPK model was established to predict the PK profiles and to identify the optimal dose for the anti-retroviral products [89]. Another example was reported by Lukacova et al. The GastroPlus PBPK model was used to predict the performance of PLGA-based products [101]. Different formulation factors are considered including autocatalysis of polymer, monomer ratio, and pH-dependent solubility of the drug. In addition, physiological factors are considered. For example, a model describing drug diffusion through an immune cell layer with a time-varying thickness and nonspecific tissue binding was developed and incorporated in the GastroPlus PBPK model [101]. Most recently, Gao et al., reported a novel bio-relevant medium mimicking the physiological ion and protein background [40]. They quantitatively investigated the contributions of *in vitro* drug release, drug degradation, diffusion process, and lymphatic transport to the drug absorption *via* a mechanistic modeling approach (using Stella® Architect), which

facilitated the establishment of IVIVCs (using Phoenix®) for PLGA microsphere formulations [40].

In addition, considering the long duration (several weeks to several months) and the high cost of these long-acting parenterals, a complete crossover study design may not be practical [128]. Considering the high cost of clinical trials, different animal models have also been used to investigate the feasibility of IVIVC model development. However, it is essential to consider any interspecies differences, particularly when extrapolating animal data to future human clinical trials. It is noted that there is still a long way ahead to achieve clinical IVIVCs for microsphere products. Non-clinical IVIVC research may pave the way for understanding model establishment.

7. Conclusions

In conclusion, although significant advances have been reported in microsphere development, there is still a lack of in-depth understanding and clear guidance for quality control (excipients and formulations), as well as for product performance. Advanced understanding of both the release controlling excipients and complex formulations can be achieved with current cutting-edge technologies, including: 1) multi angle static light scattering for molecular weight analysis; 2) liquid/solid state nuclear magnetic resonance for blockiness analysis; 3) gas chromatography–mass spectrometry for residual solvent quantification; 4) X-ray computed tomography and focused ion beam scanning electron microscopy for microstructure analysis, *etc*. These methods are essential for regulatory consideration and future product development for both innovator and generic companies.

Moreover, different release methods were introduced and compared, suggesting USP apparatus IV is the most preferred method of *in vitro* release testing of parenteral microsphere products. It can be used as a reliable quality control tool and most importantly, as a bio-relevant method to predict the *in vivo* performance of these complex long-acting products. Understanding of IVIVC for long-acting parenterals remains at an early stage. Although, some IVIVCs for long-acting parenterals such as microspheres have been developed with different conventional methods, extensive effort is still required to fill knowledge gaps to achieve accurate and reliable prediction for various products. What is critical for clinically applicable IVIVC models for these products is not only bio-relevant, standardized *in vitro* testing methods, but also an in-depth understanding of the physiological environment.

Data availability

The data that has been used is confidential.

Declaration of Competing Interest

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

References

[1] W.I. Abdel-Fattah, T. Jiang, G.-E.-T. El-Bassyouni, C.T. Laurencin, Synthesis, characterization of chitosans and fabrication of sintered chitosan microsphere matrices for bone tissue engineering, Acta Biomater. 3 (2007) 503–514.

[2] L.M.C. Aguilar, S. Duchi, C. Onofrillo, C.D. O'Connell, C. Di Bella, S.E. Moulton, Formation of alginate microspheres prepared by optimized microfluidics parameters for high encapsulation of bioactive molecules, J. Colloid Interface Sci. 587 (2021) 240–251.

[3] J.M. Anderson, In vivo biocompatibility of implantable delivery systems and biomaterials, Eur. J. Pharm. Biopharm. 40 (1994) 1–8.

[4] J.M. Anderson, R. Marchant, M. McClurken, Tissue response to drug delivery systems: the cage implant system, Long-acting contraceptive delivery systems, harper and row, 1984, 248–255.

[5] J.V. Andhariya, D.J. Burgess, Recent advances in testing of microsphere drug delivery systems, Expert Opin. Drug Deliv. 13 (2016) 593–608.

[6] J.V. Andhariya, R. Jog, J. Shen, S. Choi, Y. Wang, Y. Zou, D.J. Burgess, Development of Level A in vitro-in vivo correlations for peptide loaded PLGA microspheres, J. Control. Release 308 (2019) 1–13.

[7] J.V. Andhariya, R. Jog, J. Shen, S. Choi, Y. Wang, Y. Zou, D.J. Burgess, In vitro-in vivo correlation of parenteral PLGA microspheres: effect of variable burst release, J. Control. Release 314 (2019) 25–37.

[8] J.V. Andhariya, J. Shen, S. Choi, Y. Wang, Y. Zou, D.J. Burgess, Development of in vitro-in vivo correlation of parenteral naltrexone loaded polymeric microspheres, J. Control. Release 255 (2017) 27–35.

[9] J.V. Andhariya, J. Shen, Y. Wang, S. Choi, D.J. Burgess, Effect of minor manufacturing changes on stability of compositionally equivalent PLGA microspheres, Int. J. Pharm. 566 (2019) 532–540.

[10] K. Avgoustakis, Polylactic-co-glycolic acid (PLGA), Encycl. Biomater. Biomed. Eng. 1 (2005) 1–11.

[11] K. Avgoustakis, J. Nixon, Biodegradable controlled release tablets 1: preparative variables affecting the properties of poly (lactide-co-glycolide) copolymers as matrix forming material, Int. J. Pharm. 70 (1991) 77–85.

[12] J.L. Baxter, J. Kukura, F.J. Muzzio, Shear-induced variability in the United States Pharmacopeia Apparatus 2: modifications to the existing system, AAPS J. 7 (2005) E857–E864.

[13] U. Bhardwaj, D.J. Burgess, A novel USP apparatus 4 based release testing method for dispersed systems, Int. J. Pharm. 388 (2010) 287–294.

[14] B. Bittner, K. Mäder, C. Kroll, H.-H. Borchert, T. Kissel, Tetracycline-HCl-loaded poly (DL-lactide-co-glycolide) microspheres prepared by a spray drying technique: influence of γ-irradiation on radical formation and polymer degradation, J. Control. Release 59 (1999) 23–32.

[15] M.A.J. Blanco-Prɐto, M.A. Campanero, K. Besseghir, F. Heimgatner, B. Gander, Importance of single or blended polymer types for controlled in vitro release and plasma levels of a somatostatin analogue entrapped in PLA/PLGA microspheres, J. Control. Release 96 (2004) 437–448.

[16] A. Brunner, K. Mäder, A. Göpferich, pH and osmotic pressure inside biodegradable microspheres during erosion1, Pharm. Res. 16 (1999) 847–853.

[17] P. Burke, Determination of internal pH in PLGA microspheres using 31P NMR spectroscopy, in: Proc Int Symp Control Release Bioact Mater, 1996, p. e134.

[18] N. Chidambaram, D. Burgess, A novel in vitro release method for submicron-sized dispersed systems, AAPS PharmSci 1 (1999) 32–40.

[19] S.-W. Choi, J.-H. Kim, Design of surface-modified poly (D, L-lactide-co-glycolide) nanoparticles for targeted drug delivery to bone, J. Control. Release 122 (2007) 24–30.

[20] D.-F. Chu, X.-Q. Fu, W.-H. Liu, K. Liu, Y.-X. Li, Pharmacokinetics and in vitro and in vivo correlation of huperzine A loaded poly (lactic-co-glycolic acid) microspheres in dogs, Int. J. Pharm. 325 (2006) 116–123.

[21] L.Y. Chu, A.S. Utada, R.K. Shah, J.W. Kim, D.A. Weitz, Controllable monodisperse multiple emulsions, Angew. Chem. 119 (2007) 9128–9132.

[22] Z. Chu, Q. Zheng, M. Guo, J. Yao, P. Xu, W. Feng, Y. Hou, G. Zhou, L. Wang, X. Li, The effect of fluid shear stress on the in vitro degradation of poly (lactide-co-glycolide) acid membranes, J. Biomed. Mater. Res. A 104 (2016) 2315–2324.

[23] A.G. Clark, R. Wang, Y. Qin, Y. Wang, A. Zhu, J. Lomeo, Q. Bao, D.J. Burgess, J. Chen, B. Qin, Assessing microstructural critical quality attributes in PLGA microspheres by FIB-SEM analytics, J. Control. Release 349 (2022) 580–591.

[24] A.P. Costa, Continuous Processing of Liposomes to Control and Predict Physical Properties, 2016.

[25] H.R. Costantino, L. Firouzabadian, C. Wu, K.G. Carrasquillo, K. Griebenow, S.E. Zale, M.A. Tracy, Protein spray freeze drying. 2. Effect of formulation variables on particle size and stability, J. Pharm. Sci. 91 (2002) 388–395.

[26] S. D'Souza, J.A. Faraj, S. Giovagnoli, P.P. DeLuca, IVIVC from long acting olanzapine microspheres, Int. J. Biomater. (2014).

[27] S. D'Souza, J.A. Faraj, S. Giovagnoli, P.P. DeLuca, In vitro–in vivo correlation from lactide-co-glycolide polymeric dosage forms, Prog. Biomater. 3 (2014) 131–142.

[28] V.S. Dave, S.D. Saoji, N.A. Raut, R.V. Haware, Excipient variability and its impact on dosage form functionality, J. Pharm. Sci. 104 (2015) 906–915.

[29] A.C. Doty, D.G. Weinstein, K. Hirota, K.F. Olsen, R. Ackermann, Y. Wang, S. Choi, S.P. Schwendeman, Mechanisms of in vivo release of triamcinolone acetonide from PLGA microspheres, J. Control. Release 256 (2017) 19–25.

[30] A. Dunne, Approaches to developing in vitro-in vivo correlation models, Drugs Pharm. Sci. 165 (2007) 47.

[31] D.P. Elder, M. Kuentz, R. Holm, Pharmaceutical excipients—quality, regulatory and biopharmaceutical considerations, Eur. J. Pharm. Sci. 87 (2016) 88–99.

[32] C.D.A.C. Erbetta, R.J. Alves, J. Magalh, R.F. de Souza Freitas, R.G. de Sousa, Synthesis and characterization of poly (D, L-lactide-co-glycolide) copolymer, 2012.

[33] Y. Fang, N. Zhang, Q. Li, J. Chen, S. Xiong, W. Pan, Characterizing the release mechanism of donepezil-loaded PLGA microspheres in vitro and in vivo, J. Drug Delivery Sci. Technol. 51 (2019) 430–437.

[34] FDA, Guidance for industry SUPAC-MR: Modified release solid oral dosage forms scale-up and postapproval changes: Chemistry, manufacturing, and controls. Vitro Dissolution Testing and In Vivo Bioequivalence Documentation, 1997.

[35] FDA, Guidance for Industry: Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro. Vivo Correlations, 1997b.
[36] FDA, Bioavailability and bioequivalence studies for orally administered drug products, FDA, Center for Drug Evaluation and Research, Rockville, 2003 [Google Scholar].
[37] Food, Administration, D., Guidance for industry, PAT-A framework for innovative pharmaceutical development, manufacturing and quality assurance, 2004, http://www.fda.gov/cder/guidance/published.html.
[38] S. Fredenberg, M. Wahlgren, M. Reslow, A. Axelsson, The mechanisms of drug release in poly (lactic-co-glycolic acid)-based drug delivery systems—a review, Int. J. Pharm. 415 (2011) 34–52.
[39] P. Gajjar, I.D. Styliari, T. Nguyen, J. Carr, X. Chen, J. Elliott, R. Hammond, T. Burnett, K. Roberts, P. Withers, 3D characterisation of dry powder inhaler formulations: Developing X-ray micro computed tomography approaches, Eur. J. Pharm. Biopharm. 151 (2020) 32–44.
[40] G.F. Gao, M. Ashtikar, R. Kojima, T. Yoshida, M. Kaihara, T. Tajiri, S. Shanehsazzadeh, H. Modh, M.G. Wacker, Predicting drug release and degradation kinetics of long-acting microsphere formulations of tacrolimus for subcutaneous injection, J. Control. Release 329 (2021) 372–384.
[41] A.P. Gorle, S.G. Gattani, Design and evaluation of polymeric ocular drug delivery system, Chem. Pharm. Bull. 57 (2009) 914–919.
[42] A. Gupta, A.P. Costa, X. Xu, D.J. Burgess, Continuous processing of paclitaxel polymeric micelles, Int. J. Pharm. 607 (2021).
[43] A.G. Hausberger, P.P. DeLuca, Characterization of biodegradable poly (D, L-lactide-co-glycolide) polymers and microspheres, J. Pharm. Biomed. Anal. 13 (1995) 747–760.
[44] C. He, W. Zeng, Y. Su, R. Sun, Y. Xiao, B. Zhang, W. Liu, R. Wang, X. Zhang, C. Chen, Microfluidic-based fabrication and characterization of drug-loaded PLGA magnetic microspheres with tunable shell thickness, Drug Deliv. 28 (2021) 692–699.
[45] T. Hickey, D. Kreutzer, D. Burgess, F. Moussy, Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices, Biomaterials 23 (2002) 1649–1656.
[46] A.S. Hoffman, The origins and evolution of "controlled" drug delivery systems, J. Control. Release 132 (2008) 153–163.
[47] X. Huang, C.S. Brazel, On the importance and mechanisms of burst release in matrix-controlled drug delivery systems, J. Control. Release 73 (2001) 121–136.
[48] S.-H. Hyon, K. Jamshidi, Y. Ikada, Synthesis of polylactides with different molecular weights, Biomaterials 18 (1997) 1503–1508.
[49] S.P. Ishwarya, C. Anandharamakrishnan, A.G. Stapley, Spray-freeze-drying: a novel process for the drying of foods and bioproducts, Trends Food Sci. Technol. 41 (2015) 161–181.
[50] S.S. Iyer, W.H. Barr, M.E. Dance, P.R. Coleman, H.T. Karnes, A 'biorelevant' system to investigate in vitro drug released from a naltrexone implant, Int. J. Pharm. 340 (2007) 104–118.
[51] P. Jadhav, P. Patil, D. Bhagwat, V. Gaikwad, P.P. Mehta, Recent advances in orthogonal analytical techniques for microstructural understanding of inhalable particles: present status and future perspective, J. Drug Delivery Sci. Technol. (2022).
[52] G. Jiang, B.H. Woo, F. Kang, J. Singh, P.P. DeLuca, Assessment of protein release kinetics, stability and protein polymer interaction of lysozyme encapsulated poly (D, L-lactide-co-glycolide) microspheres, J. Control. Release 79 (2002) 137–145.
[53] M.K.D. Jonge, G. Van den Bos, G. Elzinga, Changes of microsphere density with time in myocardial infarcts in dogs, Cardiovasc. Res. 14 (1980) 741–744.
[54] M. Kastellorizios, D.J. Burgess, In vitro drug release testing and in vivo/in vitro correlation for long acting implants and injections, in: Long Acting Injections and Implants, Springer, 2012, pp. 475–503.
[55] P. Kaur, X. Jiang, J. Duan, E. Stier, Applications of in vitro–in vivo correlations in generic drug development: case studies, AAPS J. 17 (2015) 1035–1039.
[56] A.S. Kennedy, C. Nutting, D. Coldwell, J. Gaiser, C. Drachenberg, Pathologic response and microdosimetry of 90Y microspheres in man: review of four explanted whole livers, Int. J. Radiat. Oncol.* Biol.* Phys. 60 (2004) 1552–1563.
[57] M. Kohno, J.V. Andhariya, B. Wan, Q. Bao, S. Rothstein, M. Hezel, Y. Wang, D.J. Burgess, The effect of PLGA molecular weight differences on risperidone release from microspheres, Int. J. Pharm. 582 (2020).
[58] M. Kolíbal, T. Matlocha, T. Vystavěl, T. Šikola, Low energy focused ion beam milling of silicon and germanium nanostructures, Nanotechnology 22 (2011).
[59] H.R. Kricheldorf, I. Kreiser-Saunders, A. Stricker, Polylactones 48. SnOct2-initiated polymerizations of lactide: a mechanistic study, Macromolecules 33 (2000) 702–709.
[60] J.-M. Lü, X. Wang, C. Marin-Muller, H. Wang, P.H. Lin, Q. Yao, C. Chen, Current advances in research and clinical applications of PLGA-based nanotechnology, Expert Rev. Mol. Diagn. 9 (2009) 325–341.
[61] R.P.F. Lanao, A.M. Jonker, J.G. Wolke, J.A. Jansen, J.C. van Hest, S.C. Leeuwenburgh, Physicochemical properties and applications of poly (lactic-co-glycolic acid) for use in bone regeneration, Tissue Eng. B Rev. 19 (2013) 380–390.
[62] S.Y. Lee, H. Hyun, J.Y. Youn, B.S. Kim, I.B. Song, M.S. Kim, B. Lee, G. Khang, H.B. Lee, Preparation of nano-emulsified paclitaxel using MPEG–PLGA diblock copolymers, Colloids Surf. A: Physicochem. Eng. Asp. 313 (2008) 126–130.
[63] G. Li, L. Yao, J. Li, X. Qin, Z. Qiu, W. Chen, Preparation of poly (lactide-co-glycolide) microspheres and evaluation of pharmacokinetics and tissue distribution of BDMC-PLGA-MS in rats, Asian J. Pharm. Sci. 13 (2018) 82–90.
[64] L. Li, S.P. Schwendeman, Mapping neutral microclimate pH in PLGA microspheres, J. Control. Release 101 (2005) 163–173.
[65] Y. Liu, A.H. Ghassemi, W.E. Hennink, S.P. Schwendeman, The microclimate pH in poly (D, L-lactide-co-hydroxymethyl glycolide) microspheres during biodegradation, Biomaterials 33 (2012) 7584–7593.
[66] Y. Liu, S.P. Schwendeman, Mapping microclimate pH distribution inside protein-encapsulated PLGA microspheres using confocal laser scanning microscopy, Mol. Pharm. 9 (2012) 1342–1350.
[67] X. Lu, L. Miao, W. Gao, Z. Chen, K.J. McHugh, Y. Sun, Z. Tochka, S. Tomasic, K. Sadtler, A. Hyacinthe, Engineered PLGA microparticles for long-term, pulsatile release of STING agonist for cancer immunotherapy, Sci. Transl. Med. 12 (2020).
[68] X. Lu, B. Ning, N. Fotaki, S.S. Sharp, D. Burgess, S. Haddouchi, American Association of Pharmaceutical Scientists (AAPS) and Chinese National Institutes for Food and Drug Control (NIFDC) Joint Workshop on Dissolution, Bioequivalence, Product Performance, and Quality, Dissolut. Technol. 27 (2020) 42–51.
[69] M.M. Lübtow, M.S. Haider, M. Kirsch, S. Klisch, R. Luxenhofer, Like dissolves like? A comprehensive evaluation of partial solubility parameters to predict polymer–drug compatibility in ultrahigh drug-loaded polymer micelles, Biomacromolecules 20 (2019) 3041–3056.
[70] H.K. Makadia, S.J. Siegel, Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier, Polymers 3 (2011) 1377–1397.
[71] H. Malinowski, P. Marroum, V.R. Uppoor, W. Gillespie, H.-Y. Ahn, P. Lockwood, J. Henderson, R. Baweja, M. Hossain, N. Fleischer, Draft guidance for industry extended-release solid oral dosage forms, in: In Vitro-in Vivo Correlations, Springer, 1997, pp. 269–288.
[72] M. Martinez, M. Rathbone, D. Burgess, M. Huynh, In vitro and in vivo considerations associated with parenteral sustained release products: a review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting, J. Control. Release 129 (2008) 79–87.
[73] M.N. Martinez, M.J. Rathbone, D. Burgess, M. Huynh, Breakout session summary from AAPS/CRS joint workshop on critical variables in the in vitro and in vivo performance of parenteral sustained release products, J. Control. Release 142 (2010) 2–7.
[74] C. Martins, F. Sousa, F. Araujo, B. Sarmento, Functionalizing PLGA and PLGA derivatives for drug delivery and tissue regeneration applications, Adv. Healthc. Mater. 7 (2018).
[75] A.Y.P. Mateus, C.C. Barrias, C. Ribeiro, M.P. Ferraz, F.J. Monteiro, Comparative study of nanohydroxyapatite microspheres for medical applications, J. Biomed. Mater. Res. Part A: Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Kor. Soc. Biomater. 86 (2008) 483–493.
[76] K.J. McHugh, T.D. Nguyen, A.R. Linehan, D. Yang, A.M. Behrens, S. Rose, Z.L. Tochka, S.Y. Tzeng, J.J. Norman, A.C. Anselmo, Fabrication of fillable microparticles and other complex 3D microstructures, Science 357 (2017) 1138–1142.
[77] C. Moreton, EXCIPIENTS-functionality and performance of excipients in a quality-by-design world part 2: excipient variability, QbD and robust formulations1, Am. Pharm. Rev. 12 (2009) 40.
[78] S. Nawar, J.K. Stolaroff, C. Ye, H. Wu, F. Xin, D.A. Weitz, Parallelizable microfluidic dropmakers with multilayer geometry for the generation of double emulsions, Lab Chip 20 (2020) 147–154.
[79] E. Nelson, Solution rate of theophylline salts and effects from oral administration, J. Am. Pharm. Assoc. 46 (1957) 607–614.
[80] M. Nguyen, T. Flanagan, M. Brewster, F. Kesisoglou, S. Beato, J. Biewenga, J. Crison, R. Holm, R. Li, E. Mannaert, A survey on IVIVC/IVIVR development in the pharmaceutical industry–past experience and current perspectives, Eur. J. Pharm. Sci. 102 (2017) 1–13.
[81] M. Ochi, B. Wan, Q. Bao, D.J. Burgess, Influence of PLGA molecular weight distribution on leuprolide release from microspheres, Int. J. Pharm. 599 (2021).
[82] Y. Ogawa, M. Yamamoto, H. Okada, T. Yashiki, T. Shimamoto, A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly (lactic/glycolic) acid, Chem. Pharm. Bull. 36 (1988) 1095–1103.
[83] H. Okada, Y. Doken, Y. Ogawa, H. Toguchi, Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers, Pharm. Res. 11 (1994) 1143–1147.
[84] H. Okada, M. Yamamoto, T. Heya, Y. Inoue, S. Kamei, Y. Ogawa, H. Toguchi, Drug delivery using biodegradable microspheres, in: Advances in Drug Delivery Systems, Elsevier, 1994, pp. 121–129.
[85] G.N. Okolo, R.C. Everson, H.W. Neomagus, M.J. Roberts, R. Sakurovs, Comparing the porosity and surface areas of coal as measured by gas adsorption, mercury intrusion and SAXS techniques, Fuel 141 (2015) 293–304.
[86] K. Park, A. Otte, F. Sharifi, J. Garner, S. Skidmore, H. Park, Y.K. Jhon, B. Qin, Y. Wang, Formulation composition, manufacturing process, and characterization of poly (lactide-co-glycolide) microparticles, J. Control. Release 329 (2021) 1150–1161.
[87] K. Park, S. Skidmore, J. Hadar, J. Garner, H. Park, A. Otte, B.K. Soh, G. Yoon, D. Yu, Y. Yun, Injectable, long-acting PLGA formulations: analyzing PLGA and understanding microparticle formation, J. Control. Release 304 (2019) 125–134.

[88] H.-Y. Peng, W. Wang, R. Xie, X.-J. Ju, Z. Liu, Y. Faraj, L.-Y. Chu, Mesoscale regulation of droplet templates to tailor microparticle structures and functions, Particuology 48 (2020) 74–87.
[89] R.K. Rajoli, D.J. Back, S. Rannard, C.L. Freel Meyers, C. Flexner, A. Owen, M. Siccardi, Physiologically based pharmacokinetic modelling to inform development of intramuscular long-acting nanoformulations for HIV, Clin. Pharmacokinet. 54 (2015) 639–650.
[90] A. Rawat, U. Bhardwaj, D.J. Burgess, Comparison of in vitro–in vivo release of Risperdal® Consta® microspheres, Int. J. Pharm. 434 (2012) 115–121.
[91] A. Rawat, D.J. Burgess, USP apparatus 4 method for in vitro release testing of protein loaded microspheres, Int. J. Pharm. 409 (2011) 178–184.
[92] A. Rawat, E. Stippler, V.P. Shah, D.J. Burgess, Validation of USP apparatus 4 method for microsphere in vitro release testing using Risperdal® Consta®, Int. J. Pharm. 420 (2011) 198–205.
[93] S. Rezvantalab, M.K. Moraveji, Microfluidic assisted synthesis of PLGA drug delivery systems, RSC Adv. 9 (2019) 2055–2072.
[94] L. Romano, G. Impellizzeri, M. Tomasello, F. Giannazzo, C. Spinella, M. Grimaldi, Nanostructuring in Ge by self-ion implantation, J. Appl. Phys. 107 (2010).
[95] J. Ruiz, B. Tissier, J. Benoit, Microencapsulation of peptide: a study of the phase separation of poly (D, L-lactic acid-co-glycolic acid) copolymers 50/50 by silicone oil, Int. J. Pharm. 49 (1989) 69–77.
[96] G.Saunders, B. Maccreath, Guide to multi-detector gel permeation chromatography, Agilent Technologies, Inc., 2012, Available online: https://www.agilent.com/cs/library/primers/Public/5990-7196EN.pdf (accessed on 19 July 2019).
[97] G. Schliecker, C. Schmidt, S. Fuchs, A. Ehinger, J. Sandow, T. Kissel, In vitro and in vivo correlation of Buserelin release from biodegradable implants using statistical moment analysis, J. Control. Release 94 (2004) 25–37.
[98] F. Selmin, P. Blasi, P.P. DeLuca, Accelerated polymer biodegradation of risperidone poly (D, L-lactide-co-glycolide) microspheres, AAPS PharmSciTech 13 (2012) 1465–1472.
[99] R.K. Shah, H.C. Shum, A.C. Rowat, D. Lee, J.J. Agresti, A.S. Utada, L.-Y. Chu, J.-W. Kim, A. Fernandez-Nieves, C.J. Martinez, Designer emulsions using microfluidics, Mater. Today 11 (2008) 18–27.
[100] H. Shahin, B.P. Vinjamuri, A.A. Mahmoud, S.M. Mansour, M.B. Chougule, L. Chablani, Formulation and optimization of sildenafil citrate-loaded PLGA large porous microparticles using spray freeze-drying technique: a factorial design and in-vivo pharmacokinetic study, Int. J. Pharm. 597 (2021).
[101] S. Sharan, L. Fang, V. Lukacova, X. Chen, A.C. Hooker, M.O. Karlsson, Model-informed drug development for long-acting injectable products: summary of American College of Clinical Pharmacology Symposium, Clin. Pharmacol. Drug Dev. 10 (2021) 220–228.
[102] J. Shen, D.J. Burgess, Accelerated in-vitro release testing methods for extended-release parenteral dosage forms, J. Pharm. Pharmacol. 64 (2012) 986–996.
[103] J. Shen, D.J. Burgess, Accelerated in vitro release testing of implantable PLGA microsphere/PVA hydrogel composite coatings, Int. J. Pharm. 422 (2012) 341–348.
[104] J. Shen, D.J. Burgess, In vitro–in vivo correlation for complex non-oral drug products: where do we stand?, J Control. Release 219 (2015) 644–651.
[105] J. Shen, S. Choi, W. Qu, Y. Wang, D.J. Burgess, In vitro-in vivo correlation of parenteral risperidone polymeric microspheres, J. Control. Release 218 (2015) 2–12.
[106] J. Shen, K. Lee, S. Choi, W. Qu, Y. Wang, D.J. Burgess, A reproducible accelerated in vitro release testing method for PLGA microspheres, Int. J. Pharm. 498 (2016) 274–282.
[107] A. Shenderova, A.G. Ding, S.P. Schwendeman, Potentiometric method for determination of microclimate pH in poly (lactic-co-glycolic acid) films, Macromolecules 37 (2004) 10052–10058.
[108] S. Skidmore, J. Hadar, J. Garner, H. Park, K. Park, Y. Wang, X.J. Jiang, Complex sameness: separation of mixed poly (lactide-co-glycolide) s based on the lactide: glycolide ratio, J. Control. Release 300 (2019) 174–184.
[109] A.M. Sophocleous, K.-G.-H. Desai, J.M. Mazzara, L. Tong, J.-X. Cheng, K.F. Olsen, S.P. Schwendeman, The nature of peptide interactions with acid end-group PLGAs and facile aqueous-based microencapsulation of therapeutic peptides, J. Control. Release 172 (2013) 662–670.
[110] Y. Su, B. Zhang, R. Sun, W. Liu, Q. Zhu, X. Zhang, R. Wang, C. Chen, PLGA-based biodegradable microspheres in drug delivery: recent advances in research and application, Drug Deliv. 28 (2021) 1397–1418.
[111] J. Sun, J. Walker, M. Beck-Broichsitter, S. Schwendeman, Characterization of Commercial PLGAs by NMR Spectroscopy, 2021.
[112] V.R.S. Uppoor, Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations, J. Control. Release 72 (2001) 127–132.
[113] G.A. Van Buskirk, D. Arsulowicz, P. Basu, L. Block, B. Cai, G.W. Cleary, T. Ghosh, M.A. González, D. Kanios, M. Marques, Passive transdermal systems whitepaper incorporating current chemistry, manufacturing and controls (CMC) development principles, AAPS PharmSciTech 13 (2012) 218–230.
[114] E. Vey, C. Rodger, J. Booth, M. Claybourn, A.F. Miller, A. Saiani, Degradation kinetics of poly (lactic-co-glycolic) acid block copolymer cast films in phosphate buffer solution as revealed by infrared and Raman spectroscopies, Polym. Degrad. Stab. 96 (2011) 1882–1889.
[115] D. Vishali, J. Monisha, S. Sivakamasundari, J. Moses, C. Anandharamakrishnan, Spray freeze drying: emerging applications in drug delivery, J. Control. Release 300 (2019) 93–101.
[116] G. Visscher, J. Pearson, J. Fong, G. Argentieri, R. Robison, H. Maulding, Effect of particle size on the in vitro and in vivo degradation rates of poly (DL-lactide-co-glycolide) microcapsules, J. Biomed. Mater. Res. 22 (1988) 733–746.
[117] G. Visscher, R. Robison, H. Maulding, J. Fong, J. Pearson, G. Argentieri, Biodegradation of and tissue reaction to 50: 50 poly (DL-lactide-co-glycolide) microcapsules, J. Biomed. Mater. Res. 19 (1985) 349–365.
[118] B. Wan, J.V. Andhariya, Q. Bao, Y. Wang, Y. Zou, D.J. Burgess, Effect of polymer source on in vitro drug release from PLGA microspheres, Int. J. Pharm. 607 (2021).
[119] B. Wan, Q. Bao, Y. Zou, Y. Wang, D.J. Burgess, Effect of polymer source variation on the properties and performance of risperidone microspheres, Int. J. Pharm. (2021).
[120] F. Wan, M. Yang, Design of PLGA-based depot delivery systems for biopharmaceuticals prepared by spray drying, Int. J. Pharm. 498 (2016) 82–95.
[121] H. Wang, E. Schmitt, D. Flanagan, R. Linhardt, Influence of formulation methods on the in vitro controlled release of protein from poly (ester) microspheres, J. Control. Release 17 (1991) 23–31.
[122] X. Wang, R. Cheng, L. Cheng, Z. Zhong, Lipoyl ester terminated star PLGA as a simple and smart material for controlled drug delivery application, Biomacromolecules 19 (2018) 1368–1373.
[123] Y. Wang, W. Qu, S. Choi, FDA's regulatory science program for generic PLA/PLGA-based drug products, Am. Pharm. Rev. (2016).
[124] Q. Xu, M. Hashimoto, T.T. Dang, T. Hoare, D.S. Kohane, G.M. Whitesides, R. Langer, D.G. Anderson, Preparation of monodisperse biodegradable polymer microparticles using a microfluidic flow-focusing device for controlled drug delivery, Small 5 (2009) 1575–1581.
[125] K. Yamaguchi, J.M. Anderson, Biocompatibility studies of naltrexone sustained release formulations, J. Control. Release 19 (1992) 299–314.
[126] Y. Yamaguchi, M. Takenaga, A. Kitagawa, Y. Ogawa, Y. Mizushima, R. Igarashi, Insulin-loaded biodegradable PLGA microcapsules: initial burst release controlled by hydrophilic additives, J. Control. Release 81 (2002) 235–249.
[127] Y. Yang, P. Manda, N. Pavurala, M.A. Khan, Y.S. Krishnaiah, Development and validation of in vitro–in vivo correlation (IVIVC) for estradiol transdermal drug delivery systems, J. Control. Release 210 (2015) 58–66.
[128] D. Young, In vitro–in vivo correlation for modified release parenteral drug delivery systems, in: Pharmaceutical Product Development, CRC Press, 2007, pp. 141–151.
[129] P. Zarmpi, T. Flanagan, E. Meehan, J. Mann, N. Fotaki, Biopharmaceutical aspects and implications of excipient variability in drug product performance, Eur. J. Pharm. Biopharm. 111 (2017) 1–15.
[130] S. Zhang, D. Wu, L. Zhou, Characterization of controlled release microspheres using FIB-SEM and image-based release prediction, AAPS PharmSciTech 21 (2020) 1–14.
[131] X.-Y. Zhao, J. Xie, M.-Z. Wang, A. Xing, Synthesis and characterization of novel biodegradable tetra-amino-terminated PLGA telechelic copolymer, J. Mater. Sci. 48 (2013) 659–664.
[132] B. Zolnik, J. Raton, D. Burgess, Application of USP apparatus 4 and in situ fiber optic analysis to microsphere release testing, Dissolution Technol. 12 (2005) 11–14.
[133] B.S. Zolnik, D.J. Burgess, Evaluation of in vivo–in vitro release of dexamethasone from PLGA microspheres, J. Control. Release 127 (2008) 137–145.
[134] B.S. Zolnik, D.J. Burgess, In vitro–in vivo correlation on parenteral dosage forms, in: Biopharmaceutics Applications in Drug Development, Springer, 2008, pp. 336–358.
[135] B.S. Zolnik, P.E. Leary, D.J. Burgess, Elevated temperature accelerated release testing of PLGA microspheres, J. Control. Release 112 (2006) 293–300.