

Recent research and development of PLGA/PLA microspheres/nanoparticles: A review in scientific and industrial aspects

Feng Qi (✉)^{1,2}, Jie Wu³, Hao Li², Guanghui Ma (✉)³

¹ Beijing Nuokangda Pharmaceutical Technology Co., Ltd, Beijing 100176, China

² Department of Mechanical and Aerospace Engineering, University of Missouri, MO 65211, USA

³ National Key Laboratory of Biochemical Engineering, Key Laboratory of Biopharmaceutical Production and Formulation Engineering, PLA, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

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Abstract Poly(D,L-lactic-co-glycolic acid) (PLGA)/poly(lactic acid) (PLA) microspheres/nanoparticles are one of the most successful drug delivery systems (DDS) in lab and clinic. Because of good biocompatibility and biodegradability, they can be used in various areas, such as long-term release system, vaccine adjuvant, tissue engineering, etc. There have been 15 products available on the US market, but the system still has many problems during development and manufacturing, such as wide size distribution, drug stability issues, and so on. Recently, many new and modified methods have been developed to overcome the above problems. Some of the methods are easy to scale up, and have been available on the market to achieve pilot scale or even industrial production scale. Furthermore, the relevant FDA guidance on the DDS is still incomplete, especially for abbreviated new drug application. In this review, we present some recent achievement of the PLGA/PLA microspheres/nanoparticles, and discuss some promising manufacturing methods. Finally, we focus on the current FDA guidance on the DDS. The review provides an overview on the development of the system in pharmaceutical industry.

Keywords PLGA, microspheres, drug delivery system, stability, manufacturing

1 Introduction

The discovery, development and application of therapeutic peptides/proteins or chemical drugs are rapidly increasing.

Most of their administration depends on standard parenteral injection in clinic, attributed to poor stability or permeability in gastro-intestinal tract [1]. However, the inconvenient and high-frequency injection often causes pain, and reduces patient compliance. To overcome these problems, microspheres/nanoparticles based on biocompatible and biodegradable polymers (such as natural and synthetic polymers) have been successfully studied and applied [2]. They can sustainedly release the encapsulated drugs for weeks or even for months, or modify drug release in a controlled manner [3]. Among the various polymers, polyester is the most extensively investigated synthetic one [2]. Especially, poly(D,L-lactic-co-glycolic acid) (PLGA) and poly(lactic acid) (PLA), approved by the Food and Drug Administration (FDA) and the European Medicines Agency, have been successfully applied in form of microspheres/nanoparticles in lab and in clinic for decades [3,4]. Currently, there have been 15 FDA-approved PLGA/PLA-based products available on the US market [5]. Compared with their corresponding traditional formulations (for example, Bydureon[®] vs. exenatide injection (Byetta[®]), and Risperdal[®] Consta[®] vs. risperidone tablets (Risperdal[®])), the advantages of PLGA/PLA system are numerous: (1) tuneable control of drug release and minimized fluctuation of drug concentration *in vivo*; (2) decreased side effects caused by high drug exposure; (3) good patient compliance due to reduced injection frequency [6].

Although the DDS has gained great achievement in clinic, there are still some drawbacks and limitations. Generally, most of preparation methods only meet the requirements for laboratory scale, but those that are scalable and low-cost are very valuable, and are in urgent need in industry. Stability of the encapsulated macromolecules (peptides/proteins) is another research hotspot,

attributed to unstable structure and vulnerable amino-acid side chains [7]. Moreover, the related FDA guidance for the microspheres is still incomplete compared with other traditional formulations.

The primary objective of this review is to summarize recent applications of PLGA/PLA microspheres/nanoparticles and the novel methods of development, especially the scalable methods. Then, we discuss about the stability issue of the DSS and current solutions. Finally, we focus on the current relevant FDA guidance, which can provide an overview on the development of the microspheres/nanoparticles in pharmaceutical industry.

2 Applications

Because of the biodegradable and biocompatible properties, PLGA/PLA microspheres/nanoparticles can be used in various areas. The main purpose of using PLGA/PLA is to achieve longer and better efficacy, and reduce administration frequency [8–10] by many administration routes, including subcutaneous injection, intramuscular injection, oral administration, pulmonary administration [11,12], ocular administration [13], and so on.

2.1 Long-term release system

Over the past decade, long-term release system has become one of the main applications for PLGA/PLA microspheres, and it is also the most successful application on the market. PLGA/PLA microspheres can encapsulate and deliver both hydrophobic and hydrophilic therapeutic drugs, and reduce dosing frequency and potential drug toxicity [14–16]. However, there are still some problems that may occur during development and use. For example, the current PLGA/PLA-based long-term release system often has large size. Nearly millimeter-sized OZURDEX[®] (dexamethasone-loaded PLGA intravitreal implant), indicated for treatment of macular edema, non-infectious uveitis, and diabetic macular edema, has to be injected using 22 G needles [17,18]. The large-sized needle may terrify the patients, and greatly reduce the patient compliance. Thus, Villanueva et al. optimized the preparation for small-sized PLGA microspheres encapsulating dexamethasone, and the release behavior of dexamethasone was improved by human serum albumin to achieve low burst and sustained release [19]. The size of the obtained microspheres was much smaller (20–40 μm), and was feasible for the intravitreal injection with 30 G needles. Therefore, the patient compliance was greatly improved.

On the other hand, many scientists have been dedicating efforts to improve efficacy of the system. Parumasivam et al. applied spray drying to prepare rifampine-loaded PLGA microspheres for inhaled delivery. The microspheres maintain a stable drug concentration in lung, and

encourage phagocytosis by alveolar macrophages to harbor *Mycobacterium tuberculosis* [15]. They found that PLGA microspheres with high lactic acid (LA) ratio had a superior tendency for macrophage uptake, greatly improving the efficacy. Alternatively, synergistic co-delivery of two different drugs is also feasible using microspheres. Feng et al. encapsulated doxorubicin (DOX) and paclitaxel (PTX) together into porous PLGA microspheres, due to their excellent anti-tumor efficiency against various tumors [12]. The microspheres administered by inhalation exhibited high synergism at a molar ratio of 2/1 (DOX/PTX), due to the different drug release rates, and greatly decreased the number of lesions in the tumor-bearing mice.

2.2 Vaccine adjuvant

PLGA/PLA microspheres/nanoparticles-based vaccine vehicles/adjuvants have received considerable attention over the past decades. Compared with traditional aluminum adjuvant, the particles-based antigen delivery system can sustainedly deliver antigens for long time as a depot, reduce the number of booster injections, and passively or actively target antigen-presenting cells (APCs) via non-specific or receptor-mediated phagocytosis [20].

Particles-based adjuvants take effects through combined mechanisms. The particles with comparable size to pathogens facilitate recognition and phagocytosis by APCs. It further activates NALP3 inflammasome of APCs, and regulates the pathway of antigen presentation. Moreover, the particles antigen delivery system can also induce cellular immune responses [21,22]. The PLGA/PLA-based delivery system has been proved to be a kind of promising adjuvant [23]. For example, PLA nanoparticles can induce systemic Ab titers, compared with traditional aluminum salts adjuvant.

Recently, more and more investigations are focusing on building novel delivery systems to improve immune effects. Zhang et al. intelligently designed a combined system composed of antigen-loaded PLGA nanoparticles and antigen freely mixed with blank PLGA nanoparticles [20]. The mechanism is illustrated in Fig. 1. They found that more powerful antigen-specific immune responses were induced by the system than each single component. The possible reasons are antigen-depot effect at the injection site, effective provision of both adequate initial antigen exposure and long-term antigen persistence, and efficient induction of dendritic cells (DC) activation and follicular helper T cell differentiation in draining lymph nodes. Liu et al. prepared pH-responsive PLGA nanoparticles with thin shells and larger inner space that could realize rapid intracellular release of antigen in APCs [24]. As shown in Fig. 2, the nanoparticles encapsulating ammonium bicarbonate (NH_4HCO_3) and antigen can be broken up in DC endosomes and lysosomes, because of H^+ . Afterward, the antigens are released, then escape from

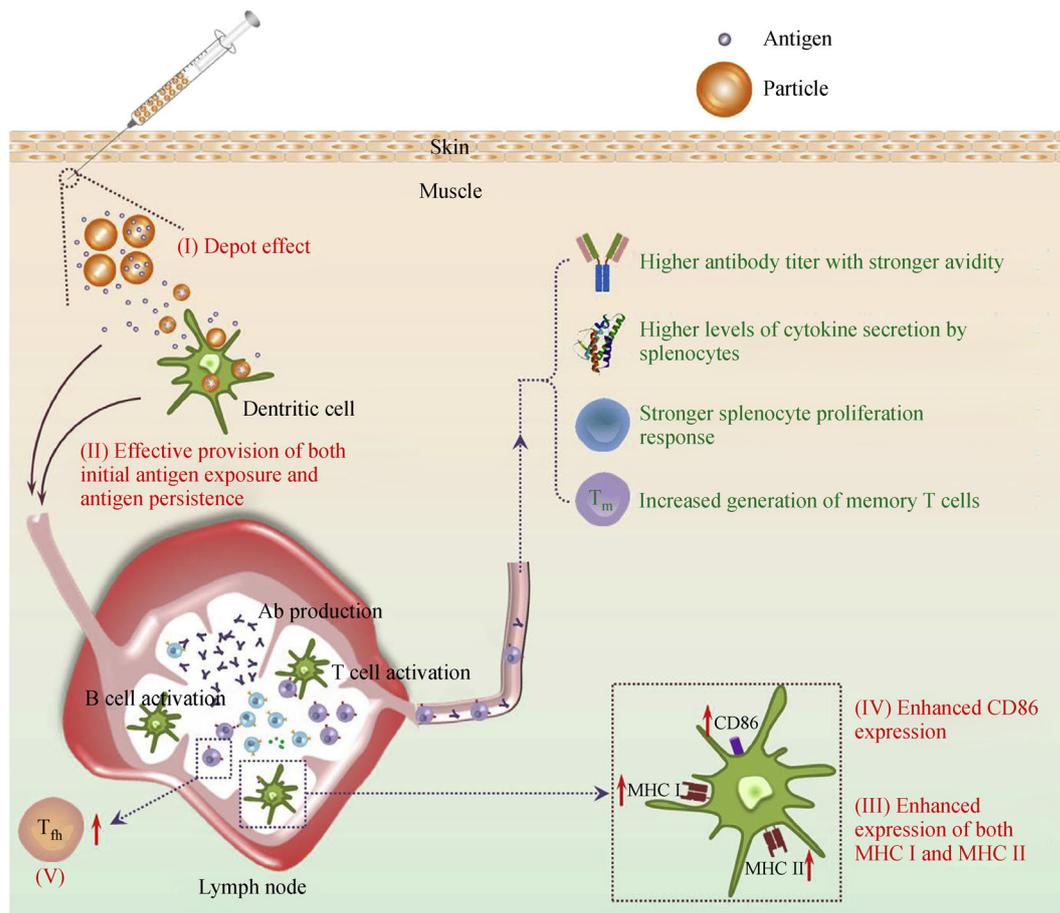


Fig. 1 Schematic illustration of the proposed action mode of the combined vaccine formulation composed of PLGA nanoparticles-encapsulated antigen and soluble antigen (reprinted from [20], copyright (2014), with permission from Elsevier)

lysosomes into cytoplasm, and further are cross-presented. Besides, the nanoparticles can induce up-regulation of costimulatory molecules, stimulate cytokine production, and improve generation of memory T cells. Thus, it is a very promising vaccine delivery and adjuvant system.

2.3 Bone tissue engineering

Biomaterials-based scaffolds are a kind of promising alternative to allografts, autografts, and xenografts [25]. In terms of biodegradability and biocompatibility, PLGA and PLA are very suitable for tissue engineering. Besides, the polymers also possess excellent reproducible mechanical and physical properties, such as tensile strength, elastic modulus and degradation rate, as well as low toxicity and immunogenicity [26]. However, the application of pure polymer for bone regeneration is limited because of poor osteoconductivity and suboptimal mechanical properties. Thus, combination of PLGA/PLA and other materials is often needed to overcome the above disadvantages.

For example, negatively charged inorganic hydroxyapatite nanoparticles and positively charged PLGA nanoparticles coated with chitosan can be combined to create a

cohesive colloidal gel via electrostatic interaction and van der Waals attraction [27]. The gel system can be used as injectable bone filling, attributed to the unique response to external shear force and recoverable properties and negligible toxicity. Wang et al. used porous nanohydroxyapatite/collagen (nHAC) scaffolds to absorb PLGA microspheres that release insulin for bone regeneration [28]. The favorable mechanical and structural properties of nHAC/PLGA composite scaffolds facilitate cell adhesion, proliferation, and differentiation into osteoblasts. Furthermore, the combination system possesses tissue compatibility and higher bone restoration capacity, compared with those without PLGA microspheres.

2.4 Applications of blank microspheres/nanoparticles

Most of the studies focus on the PLGA/PLA microspheres/nanoparticles encapsulating therapeutic drugs. However, the blank microspheres/nanoparticles also have wide applications.

Taking advantages of the controllable degradation and high biocompatibility, blank PLA microspheres have been applied for correcting shallow to deep nasolabial fold

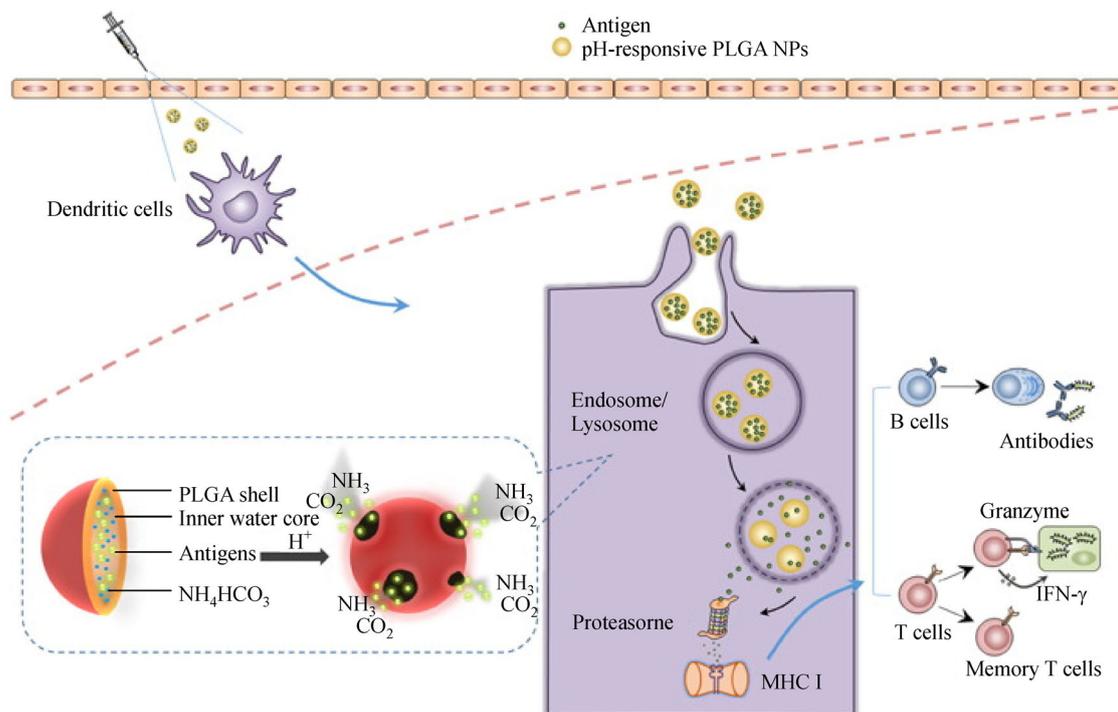


Fig. 2 Schematic illustration of the proposed mode of pH-responsive PLGA NPs (reprinted from [24], copyright (2015), with permission from ACS)

contour deficiencies and other facial wrinkles for many years. In 1999, the first injectable PLA microspheres product (NewFill, Medifill, London, UK) for treatment of scars and rhytides was approved in Europe [29]. In 2004, another similar product (Sculptra, Dermik Laboratories, USA) was approved by FDA, indicated for treatment of HIV-associated facial lipoatrophy. The product has been proved to have high safety and good efficacy in multiple clinical trials [30–33]. The particle size is in the range of 40–63 μm , and the molecular weight is extremely high (about 140000 Da). This is the reason why the filling effect lasts very long (about 2 years). PLA microspheres will cause a tissue response within the first few weeks to months (such as foreign body reaction and new collagen production), followed by metabolism in body [34]. Although the patients still need retreatment to maintain the correction, the filler greatly decreases the injection frequency and improves patient compliance.

Due to large geometric diameters but low density and small aerodynamic diameters, porous microspheres are commonly used for drug delivery through pulmonary administration [35]. Besides, the porous structure is also very accommodative to cell culture, because the 3D structure closely resembles the *in vivo* condition. As shown in Fig. 3, Zhang et al. used the blank porous PLGA microspheres to culture ovarian cancer cells. They found that the porous PLGA microspheres better supported the adhesion and proliferation of ovarian cancer cells, especially after coating with collagen I [36]. Therefore, it

is a promising carrier for pathological research and high-throughput antitumor drug screening.

Interestingly, Qi et al. found that the blank PLGA nanoparticles could be used as a kind of surfactant to stabilize Pickering emulsions [37]. Furthermore, they also investigated the stabilization mechanism, and found that molecular weight (influencing hydrophobicity), particle size, particle concentration and oil-water volume ratio could affect the stability of Pickering emulsions. Based on this study, the mechanism of Pickering emulsions applied in drug delivery, tissue engineering, and vaccination could be well interpreted [38,39].

3 Critical issues during development

3.1 Development for preparation methods

3.1.1 Lab-scale preparation methods

The commonly used methods for PLGA/PLA microspheres/nanoparticles preparation include solvent evaporation, solvent extraction, spray drying, and so on [16,40], based on which many commercial products are developed and manufactured. However, these methods still have various problems, such as wide size distribution, poor repeatability, and violent preparation conditions. For example, Risperdal[®] Consta[®], indicated for treatment of schizophrenia and bipolar I disorder, has wide size

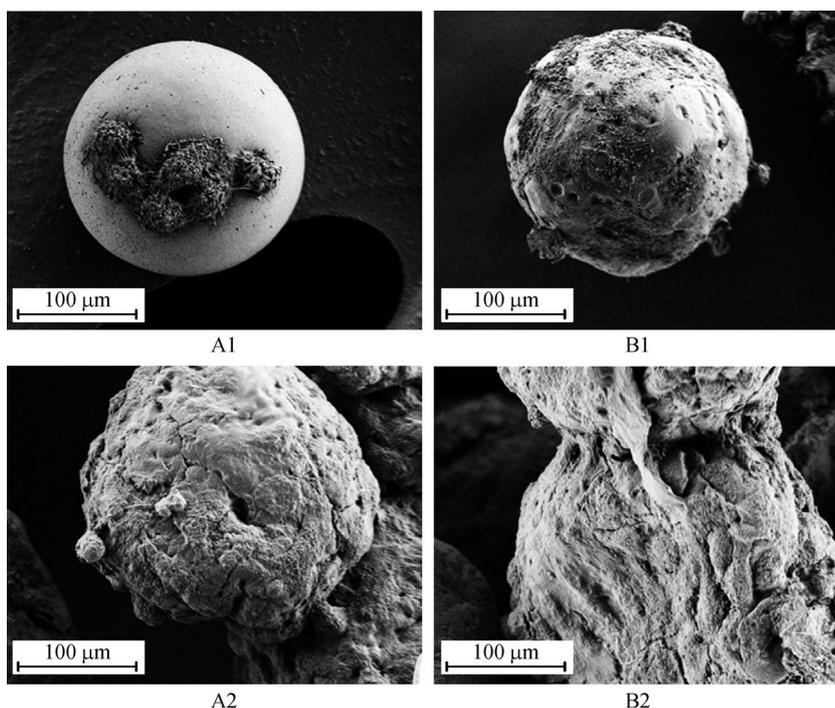


Fig. 3 SEM images of PLGA microspheres covered with HO8910 cells. The culture period is 7 d. (A1) Original nonporous PLGA microsphere; (B1) original porous PLGA microspheres; (A2) collagen I coated nonporous PLGA microspheres; (B2) collagen I coated porous PLGA microspheres (reprinted from [36], copyright (2014), with permission from Elsevier)

distribution from 25 to 150 μm [41]. In recent years, new methods or modified traditional methods have been developed and investigated to overcome these issues.

Electrospraying with a strong electric field is used to break up the organic phase containing polymer into a continuous stream of monodispersed particles [42]. Nath et al. used this method to prepare simvastatin-loaded PLGA microspheres [16]. The method can significantly improve some disadvantages of traditional methods, such as wide size distribution, and low encapsulation efficacy. However, optimization of PLGA concentration is especially needed to keep the polymer in a spherical shape, in case it will form into fibers. Alternatively, Zhang et al. used coaxial electrospraying to prepare recombinant human bone morphogenetic protein-2 (rhBMP-2)-loaded PLGA microspheres with narrow size distribution. The major advantage of the method is no direct contact between drug and organic solvent during electrospraying process, which protects unstable drugs (such as proteins, peptides, and genes) from loss of bioactivity. Moreover, the uneven surface of the obtained microspheres may enhance cell adhesion and cell proliferation, improving the bone formation effect of rhBMP-2 [43].

In most of the preparation methods, PLGA/PLA is dissolved in organic solvents (such as methylene dichloride, and ethyl acetate) as oil phase. Thus, residual solvent content is a critical index of quality control for products, in case that the high residual solvent will cause safety issues.

Supercritical emulsions extraction (SEE) that is developed from supercritical fluid technologies has been investigated using super critical-carbon dioxide as an extracting agent of the oil phase [44,45]. More recently, a new SEE has been proposed to prepare near solvent-free PLGA microspheres in a continuous process layout (SEE-C) [46,47]. As shown in Fig. 4, a counter-current packed tower is set up to reduce the emulsion processing time (solidification time), and improve the encapsulation efficiency and reproducibility [44]. Because of the mild operative temperature condition, the technology is capable of loading drugs sensitive to temperature into the microspheres [48,49], and magnetic iron oxide or gold nanoparticles into magnetic nanoparticles or light-sensitive micro/nano-devices [50].

Among the traditional preparation methods, mechanical stirring is widely used to agitate two immiscible phases to form emulsions, because of its simple operation, low cost and easy scale-up. However, sieving is often the last procedure to remove the particles out of normal size range [51], reducing the yield and increasing the cost. Thus, Liu et al. put some glass beads (4–6 mm diameter) as an adjuvant in the continuous phase to improve homogeneous distribution of the mechanical dispersion produced by stirring (Fig. 5) [52]. Homogeneity of the obtained microspheres is greatly improved (about 60% of the microspheres with uniform and specific size after selective centrifugation), compared with those prepared by the

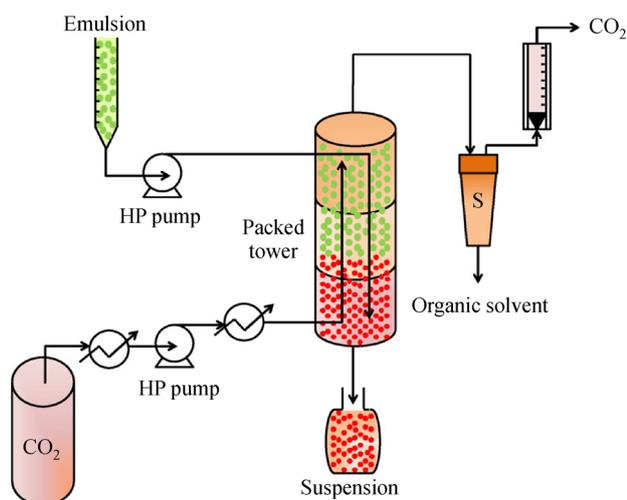


Fig. 4 Schematic representation of SEE-C process for continuous emulsion processing using a counter-current packed tower (reprinted from [45], copyright (2013), with permission from Elsevier)

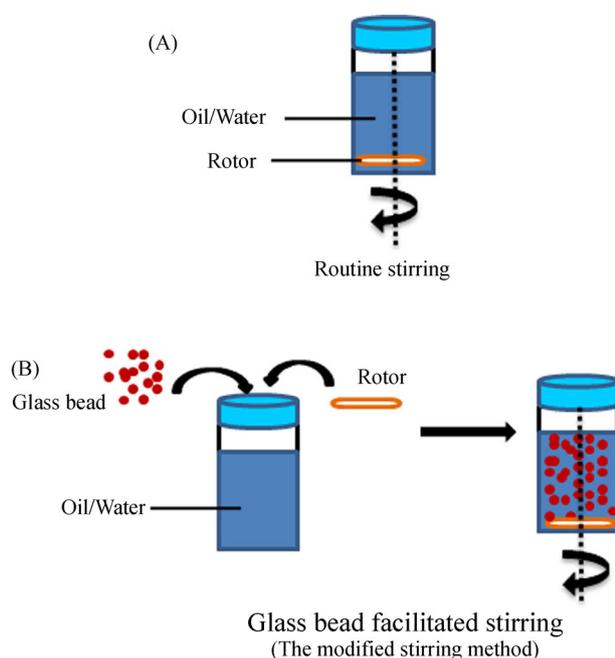


Fig. 5 Scheme of glass bead-facilitated stirring emulsification. (A) Routine mechanical stirring, and (B) glass bead-facilitated stirring (reprinted from [52], copyright (2016), with permission from Elsevier)

traditional method. This method is much easier and lower-cost, and is very promising in industrial scale-up.

Except for improvement in size distribution, application ranges of some methods have been extended. Microfluidic technique, characterized by capability of preparing mono-dispersed emulsion droplets, is widely used to prepare

microspheres with micron size range. Using this method, Hung et al. obtained nano-sized PLGA particles (down to 70 nm) by optimizing polymer concentration in solvent and relative flow rates of oil and aqueous phases in the system [53]. Premix membrane emulsification is another method to prepare uniform-sized particles with high yield and flux. Generally, the particles size prepared using this method is smaller than 8 μm [54]. Through optimizing membrane pore size and trans-membrane pressure, as well as other process parameters, Qi et al. successfully prepared PLGA microspheres with uniform size of 20 μm [14], which greatly increased the upper size limit of the technology.

3.1.2 Scale-up methods

As discussed above, the commonly used method to manufacture PLGA/PLA microspheres are mechanical stirring. However, a few new technologies have been developed in recent years, and some of them have already applied in pilot or production scale.

Hot-melt extrusion is a commonly used method in preparation of granules, pellets, sustained release tablets and other traditional dosage forms [55]. Briefly, drug is mixed with polymer, and then, the mixture is extruded under optimized conditions. For PLGA/PLA microspheres, no organic solvents are needed in this method, so the toxicity caused by organic solvents is avoided. Besides, no pores will be formed during solidification, therefore, the encapsulation efficiency of drug is almost 100% in theory [56]. Based on this method, Allergan, Inc. developed a PLGA system in form of implant encapsulating dexamethasone for intravitreal injection [17]. The product, named as OZURDEX[®] treating macular edema, was approved by FDA in 2009 [57]. However, the disadvantages of the method still limit its wide application in preparation of PLGA/PLA microspheres. Since high temperature is necessary during preparation, it is not applicable for peptide, protein or other temperature-sensitive drugs. Furthermore, the quality control for active pharmaceutical ingredient (API) is stricter, because it directly influences the physicochemical properties of microspheres or even efficacy. Thus, pre-process (such as wet milling) of API may be essential before mixing with polymers.

Recently, a new method named as FormEZE[®] has been developed by Evonik[®] to prepare PLGA microspheres in pilot scale. The method possesses many advantages, such as high yield, less post-process handling, short operation time, and so on. The mechanism is based on emulsification in a packed-bed column. Discontinuous phase in a continuous phase flows through the gaps between beads (50–1000 μm) filled inside the column, and forms into emulsions. The emulsions pass through the gaps repeatedly, which narrows down the size distribution [58]. The

size of the obtained microspheres is in the range of 10–100 μm , and the Span value (an index reflecting size distribution) calculated from the provided data is about 0.6 (indicating narrower size distribution). The flow rate of the equipment is claimed to be from 0.0001 to 100 L/min. Furthermore, the production scale of each batch can be up to at least dozens of kilograms [59]. Using this method, Edge Therapeutics has developed PLGA microspheres encapsulating nimodipine for aneurysmal subarachnoid hemorrhage therapy. Now, the product has passed Phase 1/2 study (evaluation of safety, tolerability and pharmacokinetics of the PLGA microspheres compared to oral nimodipine), and now is under Phase 3 that investigates the efficacy and monitor adverse reactions.

Membrane emulsification, characterized by mild preparation condition, low cost, fast flux and high repeatability, has been widely applied to prepare monodispersed PLGA/PLA microspheres/nanoparticles [60,61]. Generally, membrane emulsification is divided into two types: direct membrane emulsification (Fig. 6(A)) and premix membrane emulsification (Fig. 6(B)) [60].

In the direct membrane emulsification, oil phase (O) is directly pressed through the uniform pores of the membrane into aqueous phase (W) to form uniform-sized droplets on the membrane. Then, the droplets are detached by shear force to form uniform O/W emulsion. Microspheres are obtained after organic solvent is removed. Moreover, Liu et al. [62] developed the membrane emulsification to prepare W/O/W double emulsion. They optimized the size of microspheres by choosing a

membrane with proper pore size, and proved that release behavior could be modified by changing the diameters of microspheres [63]. Besides, higher encapsulation efficiency can be achieved compared with traditional method [64]. Rotating membrane emulsification, a modified direct membrane emulsification, has also been applied to prepare uniform-sized emulsions and polymer microspheres (Fig. 6(C)). The emulsions are detached from membrane by membrane rotation [65]. Liang et al. prepared PLGA-magnetic microspheres with well-controlled sizes (100–1000 μm) by this method. The obtained microspheres exhibited extremely low hemolysis ratio and excellent biocompatibility with HepG2 cells and L02 cells. Besides, the biochemical examination and the gene expression of relative HCC markers indicated that the microspheres had good biosafety and excellent therapeutic efficacy, which were promising for liver cancer therapy [66]. For premix membrane emulsification, coarse emulsions with larger droplets and broad size distribution are prepared by mechanical stirring method or homogenization, and then they are pressed through the membrane. The droplets are broken into smaller uniform ones via shear force of membrane pores [14].

The two types of membrane emulsification can prepare the PLGA/PLA microspheres with uniform size from submicron level to micron level [37]. There has been commercial equipment that can realize both lab scale and pilot scale production [60]. Using lab-scale premix membrane emulsification, Qi [14] developed exenatide-loaded PLGA microspheres. The obtained microspheres

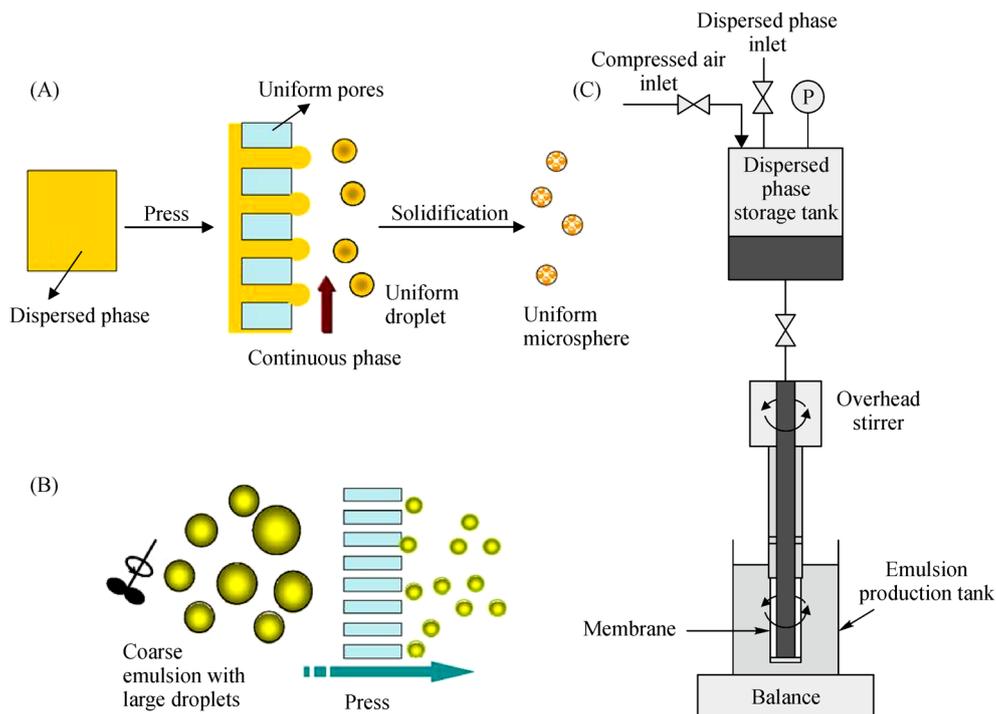


Fig. 6 Schematic diagrams of preparation principle for (A) direct membrane emulsification, (B) premix membrane emulsification, and (C) rotating membrane emulsification (reprinted from [60,65], copyright (2014), with permission from Elsevier)

have narrow size distribution and high loading efficiency. For the large-scale equipment, the production rate of direct membrane emulsification is up to 40 L/h, and that of premix membrane emulsification can be 60 L/h or even higher [60].

Precision particle fabrication (PPF) was firstly developed by Berkland et al. combined with microfluidic technology and solvent extraction, PPF can precisely control the size of microspheres and narrow down the size distribution [67]. A schematic of PPF is illustrated in Fig. 7. The equipment consists of a dual nozzle and an acoustic excitation device. The oil phase containing PLGA is pumped through a small inner nozzle to form a smooth cylindrical jet. The jet is acoustically excited to break the oil phase into uniform droplets under preset conditions. To control the microsphere size, an external phase flows concentrically around the inner jet through an outer nozzle. The external phase is pumped at a rate higher than that of the oil phase. Therefore, frictional contact between the two phases generates an additional downward force controlling the size of the droplets [68]. Orbis Biosciences, Inc. has already developed the technology for large-scale production, and the method offers high efficiency and nearly limitless scalability with virtually zero waste. The size range of the particles is from 10 μm up to 1 mm, and the production rate is from kg/h to kg/min.

3.2 Sterility

The microspheres/nanoparticles should be manufactured in an aseptic environment, using sterile starting materials. Besides, sterility testing should be conducted at every processing step to ensure the safety of products [69]. It is because that the commonly used terminal sterilization methods (such as gamma irradiation or heat sterilization) easily cause polymer degradation or protein/peptide inactivation, which badly affects performance of the products [70]. Filtration that is widely used in sterilization

of injectable formulation is not applicable for the microspheres, because the pore size of filter membrane is smaller than that of microspheres. The tests for the sterility are conducted through external and internal aspects. The external sterility testing is performed following United States Pharmacopoeia (USP) <71>. The internal one is to assess the absence of microorganisms inside the microspheres, and is often performed by dissolving microspheres in a mixed solution containing culture medium and miscible organic solvent, followed by pharmacopoeia sterility test [71]. Another important safety index is bacterial endotoxins, which can be tested following USP <85>. Moreover, validation of the manufacturing process is critical to ensure that the endotoxin limit is within the safe range [72]. Thus, to meet the above requirements for the safety of products, easy cleaning, sterilizability and simple operation are extremely important for the manufacturing equipment.

3.3 Drug stability

3.3.1 Physical and chemical instability of peptide/protein

Stability of the encapsulated therapeutic drugs, especially peptides and proteins, is one of the critical problems often met during DDS development. For instance, the existence of oil-water surface during microspheres preparation may cause aggregation of proteins [73]. Besides, the solvent type also affects the aggregation [69]. One of the most widely studied solutions is addition of additives into the internal aqueous phase (W_1), such as BSA [74], sugars (e.g., trehalose, sorbitol) [75] and PEG [76]. However, all the additives are released with the encapsulated drug together, weakening the stabilization effects with microspheres degradation. Thus, Kang et al. developed a new method: recombinant human growth hormone (rhGH) was firstly formed into microspheres with dextran, and then was encapsulated into PLGA microspheres using S/O/W

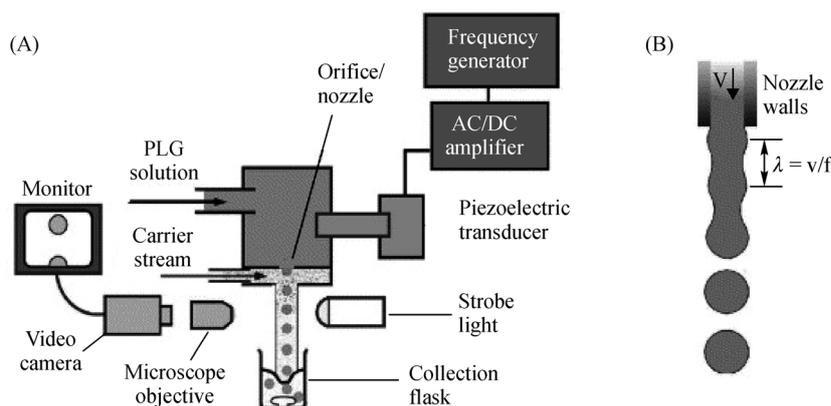


Fig. 7 (A) Schematic of the microsphere generator portraying acoustic excitation with carrier stream for microsphere production; (B) schematic indicating the variables used for acoustic excitation theory development (reprinted from [68], copyright (2001), with permission from Elsevier)

method [77]. The dextran help the protein resist interface tensions and protect it from aggregation, so the bioactivity is greatly preserved (Fig. 8(A)). Moreover, the method decreases the initial burst release and improves the release rate compared with W/O/W method (Fig. 8(B)). Except for addition of additives, another method based on double emulsion has been developed [78]. A combination of hydrophilic oils (ethylene glycol and glycerol) is used between the oil phase and outer water phase (W/O/O_h/W), which avoids protein contact with oil-water surface and escape from inner aqueous phase. Thus, the activity of the protein encapsulated is highly preserved during preparation, and encapsulation efficiency is also greatly improved.

Moreover, the acidic microenvironment formed during drug release period because of PLGA/PLA hydrolysis/degradation is another critical issue affecting drug stability. Many investigations have used counter alkaline excipients in the formulations, such as carbonate salts [7,79], and magnesium hydroxide [7], to prevent pH drop inside the PLGA/PLA microspheres. Alternatively, new types of polymers have been developed to overcome this problem. Wei et al. [73] used poly-(monomethoxypolyethylene glycol-co-D,L-lactide) (mPEGPLA, PELA) to prepare microspheres. The relatively high hydrophilicity of PELA facilitates water adsorption and polymer degrada-

tion. The degradation products of PELA are easy to diffuse out into medium, leading to less acidic microenvironment than in PLGA and PLA. Thus, proteins or peptides encapsulated in the PELA microspheres would experience a friendlier microenvironment.

3.3.2 Drug-polymer interactions

Generally, drug-polymer interaction often influences the stability and release of the drug. There are various methods to reduce or eliminate the interaction, such as addition of additives [7], modification of polymer [73], and so on.

Acylation is one of the major interactions between peptide/protein and PLGA/PLA. Nucleophilic primary amines (such as *N*-terminus and lysine sidechain), interact with the carboxylic acid end-groups of PLGA or the degradation products to form acylated derivatives, leading to activity loss, immunogenicity and toxicity. Electrostatically driven adsorption is the precursor to the acylation, followed by water existence [80]. Many solutions for acylation inhibition, such as pH modification and dications addition [81,82], have been proposed. Among these methods, addition of dication in the formulation attracts extensive attention due to high feasibility. Sophocleous et al. have proved that Ca²⁺ and

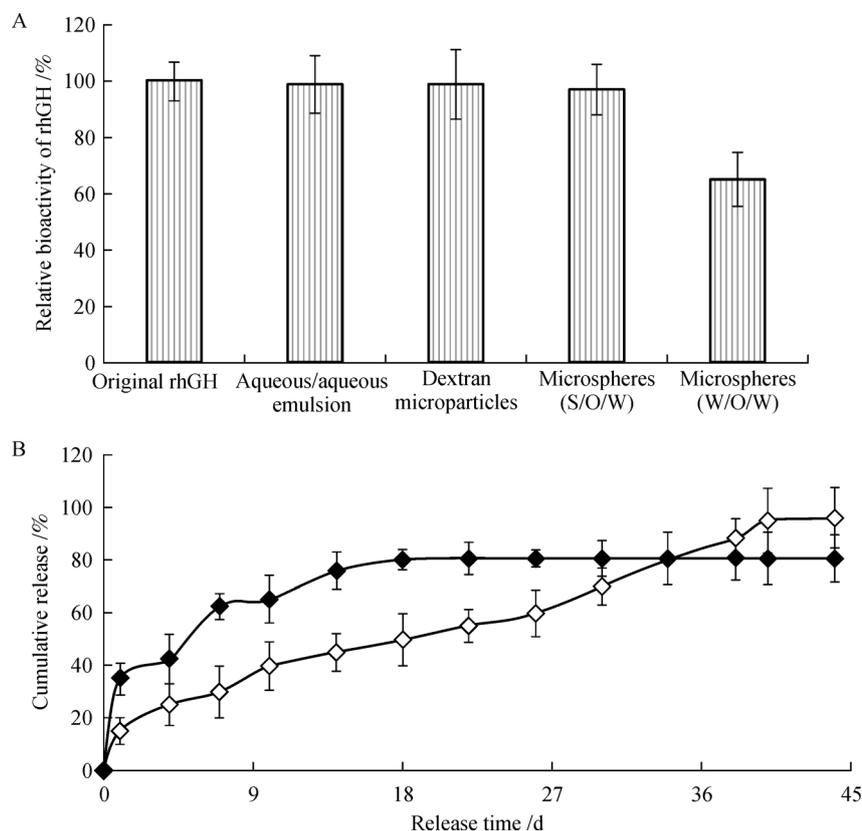


Fig. 8 (A) Recovery bioactivity from a hemostatic gauze scaffold containing rhGH-loaded dextran microspheres ($n = 5$); (B) *in vitro* release profiles of rhGH-loaded dextran microspheres ($n = 5$), ◇: microspheres prepared by S/O/W method, ◆: microspheres prepared by W/O/W method (reprinted from [77], copyright (2014), with permission from Elsevier)

Mn^{2+} have better acylation inhibition effect on octreotide (basic peptide, pI 8.3) [82]. In addition, Qi et al. used quartz crystal microbalance with dissipation to investigate the absorption mechanism of exenatide (acidic peptide, pI 4.96) on PLGA in microcosmic scale, and analyze the effects of dications (Ca^{2+} , Mn^{2+} and Zn^{2+}) on the stability of the peptide [61]. They proved that the peptide adsorbed on the PLGA via both hydrophobic effect and electrostatic interaction. Moreover, the dications have different inhibition effects on acidic peptide and basic peptide: Ca^{2+} and Mn^{2+} take effects for basic peptide, but Zn^{2+} and Mn^{2+} for acidic peptide.

However, not all the interactions are unfavorable for the DDS. Contrarily, Pakulska et al. just took advantages of the adsorption of protein to PLGA to achieve good release behavior and high stability [83]. The positively charged protein firstly adsorbs on the negatively charged surface of PLGA nanoparticles by electrostatic interactions, and the nanoparticles are embedded inside a hydrogel. With the polymer degradation, acidic degradation products are generated in the nanoparticles, decreasing local pH and neutralizing the negative charge of the nanoparticles. Then, the drug is released when the surface charge becomes neutral (Fig. 9). By adjusting nanoparticle concentration, particle size and environment pH, the release behavior can be modified. This method also protects proteins from contacting with organic solvents, O-W interface and high pressure that can badly influence the stability of proteins.

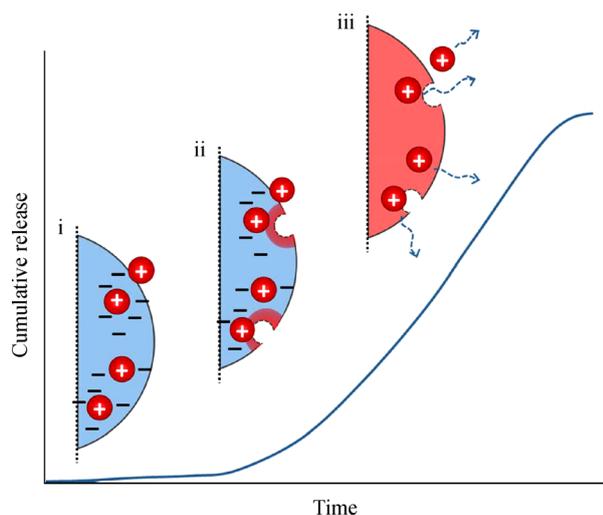


Fig. 9 Adsorption may be rate-limiting for the release of positively charged proteins from PLGA nanoparticles (reprinted from [83], copyright (2016), with permission from Science)

4 FDA Guidance for microspheres

Although there have been 15 FDA-approved PLGA/PLA-based drug products on the US market [5], the relevant FDA guidelines are much fewer than the traditional dosage

forms (such as tablets, cream, injection, etc.). Here, we summarized some studies and guidance that were appropriate for the PLGA/PLA microspheres development, manufacturing and investigational new drug (IND)/new drug application (NDA)/abbreviated new drug application (ANDA) submission to FDA.

4.1 Quality controls

As a product that will be filed for an IND, NDA or ANDA, chemical manufacture and control (CMC) is an important part in the filing. CMC includes various characterization studies on the microspheres, such as particle size, size distribution, encapsulation efficacy, stability, residual solvent content, and so on.

Moreover, as one of the major parts in CMC, release testing is critical for optimizing manufacturing process, which should be discriminatory, sensitive and reproducible. It is also a biorelevant method to investigate *in vitro-in vivo* correlation, avoid time-consuming and costly animal tests, and accelerate product development [84]. Generally, among the compendial methods for drug dissolution (release), USP dissolution apparatus 1, 2 and 3 are widely used for oral formulations. However, the standard compendial method for release testing of microspheres is lacking. To date, some noncompendial methods have been used in the testing, such as dialysis sac, reverse dialysis sac, and sample-and-separate [85], but reproducibility and inter-lab or intra-lab comparisons cannot be guaranteed. Thus, USP apparatus 4 (flow-through cell) is developed based on continuous flow method. Compared with other apparatuses or noncompendial methods, USP apparatus 4 can remain in a good sink condition, minimize microsphere aggregation, and better simulate *in vivo* environment during the testing. One of its features is the use of beads that can prevent microsphere aggregation, reduce cell's dead volume, and increase laminar flow. Tomic et al. compared USP apparatus 4 and 2 in drug release [6]. They found that USP 4 was superior to 2, because of its easy handling, and limited evaporation at high temperature during a long-time period. Rawat et al. modified USP 4, and proved that it was applicable for the compendial adaptation for drug release testing of microspheres [41].

Long-term release system often needs very long time (from days to months) to release most of the encapsulated drug, so accelerated testing is always necessary to shorten the testing time. Basically, the accelerated release testing should not change the drug release mechanism. Thus, various parameters (such as temperature, pH, ionic strength, surfactant, stirring speed) should be optimized [6]. Generally, elevated temperature is the commonly used method in the testing. Nevertheless, the temperature should be not higher than glass transition temperature (T_g) of the polymer, in case of alteration in the drug release mechanism, due to increased polymer molecular mobility [86]. Accelerated testing can be used as a tool for quality

control, if it can tell the difference between formulations. It can be also used to validate the robustness and reproducibility of the release method. Rawat et al. validated the method through comparing release rate between accelerated and real-time tests, taking Risperdal® Consta® microspheres as the model product [41]. As shown in Fig. 10, the accelerated release profile had a good correlation with the real-time one. In addition, initial burst is another important index for drug release. Thus, a real-time release testing is necessary, if the accelerated one fails to indicate the difference in the initial burst [85]. Another important part of CMC is the stability testing of the microspheres. The testing can be conducted according to ICH Q1A [87] under different temperatures and humidity to assess product quality. At each time point, indices of quality controls, such as T_g , drug loading, particle size and so on, are detected. Finally, based on the stability data, the storage condition and shelf life of the product can be determined.

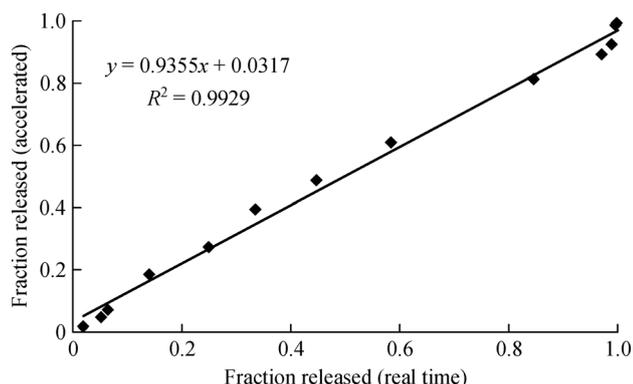


Fig. 10 Correlation between real time (37 °C) and accelerated (45 °C) fraction released (reprinted from [41], copyright (2011), with permission from Elsevier)

4.1.1 FDA regulations for ANDA

For the product filed for ANDA, pharmaceutical equivalence and bioequivalence to the reference listed drug (RLD) must be demonstrated, which means the test product must have the same API as RLD in identical strength, administration route and, dosage form. Besides, no significant difference in the rate and extent to the available drug at the action site is required for bioequivalence. In short, the test product should be qualitatively (Q1) and quantitatively (Q2) the same as the RLD [88].

Since the physiochemical properties of PLGA/PLA (such as molecular weight, molar ratio of glycolide and lactide) affect drug release behavior, the characteristics of the polymers are critical for determining Q1 sameness between the test product and RLD. There are various methods used to prepare PLGA/PLA microspheres, which can cause different release behaviors and bioavailability.

Even under high Q1/Q2, the bioequivalence among them is still not necessarily same. Unfortunately, compendial or biorelevant *in vitro* drug release testing and related characterization standards for the microspheres are still lacking. Therefore, no PLGA/PLA microspheres generic drug products have been approved by FDA so far. Besides, clinical study (such as clinical endpoint or pharmacokinetic bioequivalence study) is another challenge. Even so, the corresponding work has been carried out for long time, and it is promising to be completed soon.

5 Conclusions

PLGA/PLA microspheres/nanoparticles have been studied in lab, and applied in clinic for decades. Although there are some products available on the market, new microspheres products and preparation methods are still developed to overcome current problems or achieve better efficacy. In this review, we focus on the recent applications of the PLGA/PLA microspheres/nanoparticles, as well as some new methods to prepare them. The development in lab has been increasingly mature, and large-scale manufacturing techniques with low cost and high output have been launched into the market. It is promising for the technique transfer from lab scale to pilot or even industrial scale. Finally, although the FDA guidance is not complete, the experiences and information from the existing products are very informative and instructive. Thus, there will be more PLGA/PLA microspheres/nanoparticles products on the market in the near future.

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