EXPERT OPINION

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Recent advances in polymeric microspheres for parenteral drug delivery – part 1

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Introduction: Polymeric microspheres have been established as a valuable parenteral drug delivery system for sustained release of therapeutic agents via subcutaneous or intramuscular injection.

Areas covered: Biodegradable polymers which are either synthetic or from natural sources are reviewed with respect to recent advances in exploring their applications for microsphere fabrications. New information on the impact of formulation variables on the properties of microspheres formed by an emulsion method was also presented. The characterization of microspheres using advanced physical analytical techniques was also reviewed and the utilization of the information in assessing *in vivo* performance of the product was also highlighted.

Expert opinion: The broad clinical use of microspheres for delivery of therapeutic agents in particular biologics such as proteins has not been realized commercially. The limited availability of biodegradable polymers with a long history of regulatory approval and the challenges in gaining regulatory approval of a new polymer have hindered the development of microspheres for parenteral drug delivery.

Keywords: microsphere characterization, microsphere formulations, parenteral drug delivery, polymeric microspheres

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1. Introduction

Polymeric microspheres as a parenteral drug delivery system are primarily developed for sustained release of drugs for prolonged systemic therapeutic effects after subcutaneous (SC) or intramuscular (IM) administration. The key microsphere injectable products marketed in the United States are listed in **Table 1**. Polymers used for formulation of microspheres are biodegradable and biocompatible. Microspheres in the finished product are in a dry powder form which is produced either as the end product of the manufacturing process or by the removal of the solvent/dispersion liquid medium after formation of microspheres by lyophilization or filtration with final drying. Prior to administration, a microsphere product is reconstituted in a liquid diluent which can be supplied in a separate container or in the liquid compartment of a dual-chamber prefilled syringe. While the size and size distribution of a microsphere product are two key factors that control the drug release rate and the resultant duration of sustained release, these two factors also affect the syringeability and injectability of the product with respect to a specific gauge size of the syringe used. The ease of reconstitution and injectability via SC and IM routes of administration of microspheres are the main advantages over implants and viscous injectable polymeric gels as a parenteral drug delivery system. The small particle size and the dispersibility of microspheres upon injection have also made microspheres more acceptable by patients as compared with implants which can cause discomfort during and after implantation.

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The total dose of drug delivered in a microsphere product is determined by the total drug loading, the concentration of microspheres in the reconstituted suspension for injection, and the volume for SC and IM administration. Depending on the hydrophilicity of the drug and the hydrophobic nature/biodegradability of the polymer, the total drug loading can vary remarkably. In general, a drug loading of 20% can be the upper limit for a water-soluble drug such as a peptide in a microsphere product designed for sustained release longer than a week. Because of the viscosity-related problems in syringeability and injectability, the final microsphere concentration in the reconstituted suspension usually does not exceed 20% w/v. When taking into the account the volume limitation (not more than 2 mL) for SC and IM administration, microspheres are more suitable for the delivery of more potent drugs with a lower total drug dose requirement.

Microspheres for parenteral administration are sterile and pyrogen-free, thereby the finished product is produced either by aseptic processing or terminal sterilization usually by irradiation. Due to the complexity and challenges of the manufacturing process in terms of scale-up and process validation, the cost and time for process development are significantly higher than those for implants and injectable gels. In addition to the complex manufacturing process, the use of a new biodegradable polymer also faces a highly scrutinized regulatory path for final product approval. Although the potential for microspheres as a parenteral drug delivery system has been broadly recognized, these high development hurdles have contributed to the slow market introduction of new microsphere products (**Table 1**) [1].

2. Polymers and formulation variables

When selecting a polymer for fabrication of microspheres designed for parenteral drug delivery, the properties of the polymer to be considered are the chemical composition, physical and mechanical properties, mechanism and rate of biodegradation, toxicological profile, and regulatory history. In addition, the physicochemical properties of the drug such as its aqueous solubility and compatibility with the polymer during manufacturing, storage, and *in vivo* degradation are key factors to be evaluated during development. The desired drug loading in the microspheres as related to the dose of drug in the final product is also a critical formulation requirement which in some cases can be quite challenging to achieve. Different types of polymers are reviewed below with recent references.

2.1 Synthetic polymers

2.1.1 *Polylactide, polyglycolide, and copolymers*

Poly(D,L-lactide) (PLA), Poly(glycolide) (PGA), and their copolymer Poly(D,L-lactide-co-glycolide) (PLGA) are the most commonly used polymers in the preparation of microspheres for parenteral delivery of drugs because of their long history of safety and FDA approval for human uses (**Table 1**). The two-stage degradation of PLGA is characterized by initial hydrolytic depolymerization resulting in a decrease in molecular weight of the polymer followed by continued polymer chain scission as a result of autocatalysis by the acidic degradation products [2], leading to the subsequent loss in mass.

The pH gradient in a microsphere as caused by the formation of lactic and glycolic acids was determined during the course of polymer degradation [3]. It was shown that the pH gradient profile was significantly influenced by the diameter of the microsphere. Microspheres with a larger diameter tended to maintain a lower internal pH for a longer time simply because of a greater diffusion distance for the acid byproducts to leach out of the microsphere [3]. Microspheres with a higher porosity showed a less drastic drop in internal pH, which can be explained by the faster diffusion of the acid byproducts out of the microspheres via the porous structures [4].

The low pH microenvironment within a degrading polymer matrix can cause degradation of entrapped drugs that are susceptible to acid catalytic degradation such as proteins [5]. The addition of alkalinizing excipients in the formulation has been shown to be effective in suppressing the negative impact of the low pH and enhancing the stability of entrapped proteins [6]. It was reported that PLGA polymers could be blended with biocompatible hydrophilic polymers including polyvinyl alcohol (PVA), poly(ethylene glycol) (PEG), and chitin to enhance the hydration of the polymer matrix and increase polymer degradation rate to effect faster release of proteins [7].

2.1.2 *Poly(caprolactone)*

Poly(caprolactone) (PCL) is a semi-crystalline polymer with good solubility in organic solvents commonly used in pharmaceutical manufacturing such as dichloromethane and cyclohexanone. PCL and its copolymers degrade via hydrolysis in two phases: decrease in molecular weight due to chain scission followed by weight loss. ε-Hydroxycaproic acid formed by complete hydrolysis of the polymer and water were the only metabolites detected *in vivo* [8]. Unlike PLGA copolymers, degradation of PCL polymers will not result in an acidic microenvironment. In general, PCL homopolymers degrade relatively slower than PLGA copolymers, making PCL more suitable for sustained release beyond 1 year. PCL blends can be produced with other polymers and biodegradation of the blended polymer can be enhanced by incorporating polylactic acid and polyglycolic acid [8-10]. A recent review has provided a list of drugs which were incorporated in PCL-based microspheres intended for drug delivery [11].

2.1.3 *Polyanhydrides*

Polyanhydrides are biodegradable hydrophobic polymers with the backbone formed by the linkages of repeating units via water-labile anhydride bonds (**Table 2**). In recent years, polyanhydrides have attracted increasing research interest in the drug delivery field; their methods of synthesis, structural characterization, and pharmaceutical applications have been extensively reviewed [12,13]. Since the hydrolytic scission of

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Table 1. Key microsphere products marketed in the United States.

Company	Product	Drug	Polymer	Indication	Year approval
Abbott	Lupron Depot, 7.5 mg, 1-month	Leuprolide acetate	PLGA copolymer	Palliative treatment of advanced prostatic cancer	1989
	Lupron Depot, 3.75 mg, 1-month			Endometriosis	1990
	Lupron Depot, 11.25 mg, 3-month		Polylactic acid		1997
	Lupron Depot, 22.5 mg, 3-month			Palliative treatment of advanced	1996
	Lupron Depot, 30 mg, 4-month			prostatic cancer	1997
	Lupron Depot, 45 mg, 6-month				2011
	Lupron Depot PED, 7.5 mg, 1-month		PLGA copolymer	Children with precocious puberty	1993
	Lupron Depot PED, 11.25 mg & 15 mg, 3-month		Polylactic acids		2011
Novartis	Santostatin LAR Depot, 1-month	Octreotide acetate	PLGA copolymer	Acromegaly Carcinoid tumors VIPomas	1998
Waston Pharma/Debiopharm*	Trelstar LA, 3.75 mg, 1-month	Triptorelin pamoate	PLGA copolymer	Palliative treatment of advanced prostatic cancer	2000
	Trelstar LA, 11.25 mg, 3-month				2001
	Trelstar LA, 22.5 mg, 6-month				2010
J&J/Alkermes*	Resperidal Consta, biweekly	Resperidone	PLGA copolymer	Schizophrenia bipolar disorders	2003 2009‡
Alkermes	Vitrol, 1-month	Naltraxone	PLGA copolymer	Alcohol dependence Opioid dependence	2006 2010‡
Amylin/Alkermes*	Bydureon weekly	Exenatide	PLGA copolymer	Type 2 diabetes	2012

*Manufacturer.
‡Approval for additional indication.

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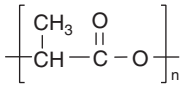
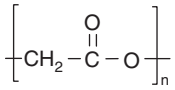
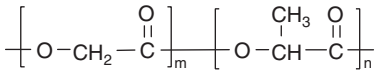
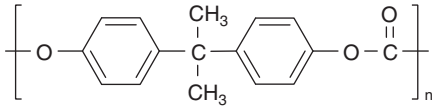
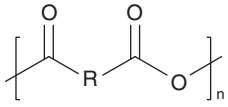
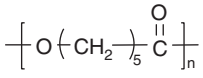
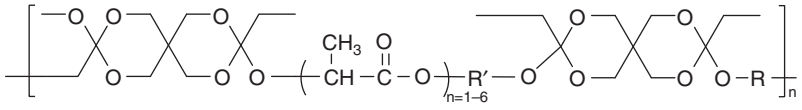
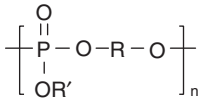
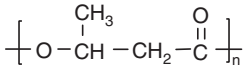
Table 2. Chemical structure of representative synthesized biodegradable polymers.

Name of the polymer	Representative structure
Poly(lactide acid) (PLA)	
Poly(glycolic acid) (PGA)	
Poly(D,L-lactide-co-glycolide)(PLGA)	
Polycarbonate	
Polyanhydrides	
Poly(caprolactone) (PCL)	
Poly(ortho esters) IV	
Poly(phosphoesters) (PPE)	
Polyhydroxybutyrate	

the anhydride bonds occurs at a rate much faster than water penetration into the polymer matrix, a surface erosion of the polymer matrix is exhibited [14]. Polyanhydrides degrade *in vivo* giving rise to nontoxic diacid byproducts which are subsequently eliminated from the body as metabolites. The degradation rate of polyanhydrides can be varied from days to years by changing monomer compositions and co-monomer ratios [14]. Polyanhydrides and their degradation products have been found to be nontoxic and biocompatible and they have been approved by FDA for human use [12].

2.1.4 *Polycarbonate*

Polycarbonates are long-chain linear polyesters of carbonic acid and dihydric phenols. Aliphatic polycarbonates such as poly(trimethylene carbonate) (PTMC) and poly(5,5-dimethyl trimethylene carbonate) (PDTC) represent a family of biodegradable materials used as drug carriers and implant materials because of their good biodegradability, high biocompatibility, low toxicity, low immunogenicity, superior mechanical properties, and mild inflammatory response caused by the degraded products [15]. Aliphatic polycarbonates can be modified with other functional groups such as ester and carboxyl to improve their thermal properties and degradability [16]. Some copolymers and modified polycarbonates of PTMC have been synthesized with various functional groups and are being evaluated as drug carrier because of their low glass transition temperatures and amorphous nature under physiological conditions [17,18].

2.1.5 *Poly(ortho esters)*

Poly(ortho esters) (POEs) are a family of hydrophobic, biocompatible, and bioerodible polymers with many desirable key features as a parenteral drug delivery system. The synthesis and degradation of POEs as well as fabrication of POE-based

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drug delivery systems have been extensively reviewed [19]. Amongst the various POE classes, Class IV POEs are the most hydrophobic and formed with the backbone consisting of lactides or glycolides, which are incorporated to catalyze polymer hydrolysis allowing better control of polymer erosion and drug release [20]. The polymer matrix undergoes erosion confined predominantly to the surface layers (surface erosion) and a neutral pH in the interior of the matrix can be maintained as acidic hydrolytic products diffuse away from the surface immediately after they are formed. Also, POEs are excellent thermoplastic materials by which drug delivery systems can be easily fabricated by extrusion, injection molding or compression molding. POE-based microspheres for controlled release of a local anesthetic agent [21], proteins, and genes have been reported [22].

2.1.6 *Poly(phosphoesters) (PPEs)*

Poly(phosphoesters) (PPEs) is a class of biodegradable inorganic polymers with a representative structure shown in **Table 2**, where R, R' are usually alkoxy group, amino group or halogens. Since their chemical structure can be tailored by varying R and R' during synthesis, it is possible to obtain PPEs with a wide range of physicochemical properties. These polymers were found to be biocompatible and to degrade forming nontoxic low molecular weight products such as ethanol, glycine, phosphate, and ammonia at a rate dependent on the physicochemical characteristics of the substituent groups. PPE polymers degrade via a combined mechanism of surface erosion and bulk degradation [23]. The mode of degradation and drug delivery applications of biodegradable PPE have been well reported [24]. PPEs have been used in drug and gene delivery [25]. PPE microspheres containing nerve growth factor have been prepared and evaluated [26].

2.1.7 *Polyhydroxybutyrate*

Polyhydroxybutyrate (PHB) is a polyester that is highly hydrophobic in nature and relatively resistant to hydrolytic degradation. Chemically, it belongs to the class of polyhydroxyalkanoates (PHAs), which are biodegradable and biocompatible with animal tissues [27]. PHB has been evaluated as a material for tissue engineering scaffolds and controlled drug-release carriers owing to its biodegradability, optical activity, and isotacticity. Controlled release of tramadol from PHB microspheres has been reported [28]. PHB was used to fabricate microspheres using PEG 40kDa as a large molecular weight hydrophilic model drug for sustained release via intramuscular injection [27].

2.2 Natural polymers

2.2.1 *Chitosan*

Chitosan is a nontoxic material in animals as well as humans with an LD_{50} of 16 g/kg in rats [29]. Every deacetylated subunit of chitosan contains a primary amine group with a pKa value of about 6.5, making it soluble in acidic media but insoluble at neutral and alkaline pH. In addition to pH, the solubility of chitosan is greatly influenced by the degree of deacetylation, molecular weight, and ionic strength of the medium. In the physiological environment, chitosan can be readily digested either by lysozymes or by chitinases which can be produced by the normal flora in the human intestine and exist in the blood [29]. It has a unique chemical structure as a linear polyelectrolyte with a high charge density as well as reactive hydroxyl and amino groups, allowing chemical modification. Chitosan has great potential for pharmaceutical applications due to its biocompatibility, high charge density, safety, mucoadhesion, and permeation enhancing effect across the biological surfaces. Chitosan-based microspheres have been evaluated as a carrier for controlled release of many drugs, improving the bioavailability of unstable substances such as protein and peptides, enhancing the uptake of hydrophilic substances across the epithelial layers [30]. Chitosan microspheres are being investigated for parenteral drug delivery [31,32].

2.2.2 *Alginate*

Alginates are natural polysaccharide isolated from seaweed and algae and are derived from bacterial sources. They are linear block copolymers composed of D-mannuronic acid (M) and L-guluronic acid (G) via 1,4 linkage. As part of the polymer structure, there are homopolymeric regions of M or G and alternated MG blocks with highly variable composition and monomer sequences. The physical and chemical properties of alginate are determined by the M/G ratio and the ratios and segment lengths of the MM, GG, and MG blocks. The ability of hydrogel formation between alginate and multivalent cations such as calcium is a property that distinguishes alginate from other polysaccharides. Specific intermolecular interactions between calcium and the carboxylic groups of the guluronate moieties in GG sequences lead to a particular structure called the "egg-box," while the M and G/M blocks provide flexibility to the resulting networks [33].

Alginate is stable against breakdown by mammalian enzymes but dissolves and is eliminated through the kidneys *in vivo*; alternatively, partial oxidation of the guluronic units of sodium alginate can render alginate susceptible to hydrolytic breakdown *in vivo* [34]. Recently, alginates have attracted much attention as a drug delivery system because they are nontoxic, biodegradable, low immunogenic, and are capable of forming gel under mild conditions with multivalent cations [35]. Alginate microspheres can be easily prepared by coacervation and the size of the particles is dependent on the alginate concentration and the preparation method. A number of bioactive proteins have been incorporated into alginate-based microspheres under mild preparative conditions (room temperature and physiological pH) without the use of organic solvents [34,36].

2.2.3 *Hyaluronic acid*

Hyaluronic acid (HA) is a natural mucopolysaccharide consisting of repeating units of d-glucuronic acid and

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N-acetyl-d-glucosamine. It is known to be an ideal biomaterial for pharmaceutical applications due to its biocompatibility, nonimmunogenecity, biodegradability, and viscoelasticity [37]. HA is capable of eliciting specific cell interaction via the CD44 receptor, which promotes wound healing and induces chondrogenesis [38]. HA-based microspheres have been successfully utilized for drug and gene delivery applications by using a nontoxic and aqueous-based adipic dihydrazide-mediated cross-linking chemistry [39]. Adipose tissue-derived mesenchymal stem cell aggregates prepared by using porous HA microspheres could be delivered in an injectable manner into the body and could have great therapeutic potential for soft tissue augmentation and reconstruction [40].

2.2.4 Gelatin

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the bones, connective tissues, organs, and some intestines of animals mainly cattle. Gelatin-based drug delivery systems have been extensively studied because of its long history of safety. Since gelatin microspheres can be prepared by thermal cross-linking at a temperature lower than that needed for albumin microsphere cross-linking, there is a lower probability of thermal degradation of the encapsulated drugs. Gelatin microspheres have shown potential to be used as an embolic material for transcatheter arterial embolization for several organs [41]. Also, it was suggested that gelatin microsphere-mediated delivery of therapeutic peptides is a promising means to provide efficient neuroprotection in the postischemic brain [42]. Because of the potential cause of bovine spongiform encephalopathy (BSE) by gelatin from infected cattle, most national and international authorities have established statutory guidelines or legislation ensuring that the gelatin raw materials are subject to continuous rigorous checks on their safety and origin [43].

2.2.5 Starch

Starch consists of two types of molecules: linear and helical amylose and branched amylopectin. Because of its biodegradability and biocompatibility, starch-based drug delivery systems have been studied extensively. Injection of degradable starch microspheres together with an adenovirus vector through the hepatic artery can result in efficient and cancer-selective transfer of genes to hepatocellular carcinomas [44]. It was reported that following intravenous administration of terbutaline sulfate (TBS)-loaded degradable starch microspheres, initial uptake of microspheres by the lung was higher than those of other organs [45]. Degradable starch microspheres provide transient occlusion of small arteries and are thought to improve the therapeutic effect of anticancer drugs for liver metastases therapy [46].

2.2.6 Dextran

Dextran is a complex, branched glucan composed of chains of varying lengths; the straight chain consists of α-1,6 glycosidic linkages between glucose molecules and branches are formed via α-1,3 linkages. Dextran and its derivatives, such as methacrylated-dextran, have been used as controlled release vehicles for drugs and bioactive proteins. Diwan *et al.* [47]. attached tetanus toxoid covalently to dextran microspheres and administrated parenterally to rats resulting in considerable immune responses. Intratumoral injections of doxorubicin-loaded sulfopropyl dextran microspheres were tolerated much better than systemic administration of equivalent drug concentrations. There was a modest (up to 34%) delay of tumor growth compared with groups receiving no treatment or blank microspheres [48]. The *in vitro* to *in vivo* correlation of a sustained release formulation for human growth hormone (hGH) based on hydroxyethyl methacrylated dextran (dex-HEMA) microspheres was investigated in Pit-1-deficient Snell dwarf mice and in healthy human volunteers. Single subcutaneous administration of the microspheres in mice resulted in a good correlation between hGH released *in vitro* and *in vivo* effects for the hGH-loaded microsphere formulation similar to daily injected hGH indicating a retained bioactivity. Good *in vitro* to *in vivo* correlations were obtained for hGH-loaded dex-HEMA microspheres [49]. These microspheres have also been investigated for encapsulation and sustained release of liposomes [50].

2.3 Formulation variables

The characteristics of microspheres such as particle size, burst and sustained release profile, drug loading, and morphology are influenced by the formulation variables with respect to microsphere composition. However, the impact of these formulation variables on the final quality of the microspheres is highly dependent on the method of preparation and the materials (type and quantity) used during the manufacturing process, which are generally called processing excipients or aids. Although most of the processing aids are not intended to be part of the final composition of the microspheres and are mostly removed during manufacturing, their impact on the final microsphere quality can be significant. Since emulsion-solvent evaporation is the most commonly used industrial process for microsphere fabrication, some recent findings on the highly complex interaction effects of formulation variables including processing aids on the final quality of the microspheres are included in the discussion below.

2.3.1 Polymer properties

Polymer type is one of the most predominant factors affecting the final properties of microspheres. Its selection should be based on physicochemical characteristics of the drug, drug loading requirements, and desirable rate and duration of release. In the previous sections, the properties of different polymers were discussed with respect to their applications as the microsphere matrix. By using the single emulsion method, Mao *et al.* investigated the effect of PLGA polymers with the same molecular weight but different end groups on the properties of microspheres. It was found that the particle size and encapsulation efficiency decreased slightly for microspheres prepared with the polymer containing free carboxyl

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groups (uncapped) compared to those formed with the polymer containing ester end groups (capped) [51]. The porosity and the resultant drug burst release were also shown to be different for microspheres formed with PLGA polymers with free or capped end groups. Larger pores were found on the surface of microspheres formed with the uncapped polymers leading to a higher burst drug release [51]. By using a double emulsion method, it was shown that an increase in molecular weight of the polymers with same chemical composition resulted in microspheres with a larger size [52].

The rate of polymer degradation is also dependent on the polymer type and this can influence the *in vitro* and *in vivo* release of the drug. Significantly faster release rates were observed for microspheres prepared with uncapped PLGA polymer after 18 days, which can be explained by the more rapid degradation of uncapped polymer because of increased hydrophilicity [51]. Also, the auto-catalytic degradation by the free carboxylic acid groups may be suppressed by blocking the end groups in the capped polymer leading to slower degradation [53]. Physicochemical properties and degradation rates of specific polymers can also be fine-tuned by blending with different polymer types [54-56].

Polymer molecular weight could affect not only particle size, encapsulation efficiency but also *in vitro/in vivo* drug release profiles of microspheres. It was reported that using PLGA with higher molecular weight led to a higher encapsulation efficiency with low burst effect and desirable release pattern [57,58]. Another study showed that the encapsulation of GDNF (Glial cell-line derived neurotrophic factor) was higher when using hydrophilic polymers with high molecular weight such as RG 503H [59]. It has also been demonstrated that with the increase of PLGA molecular weight, the *in vitro* and *in vivo* release periods of huperzine A were prolonged [60].

2.3.2 Drug loading

The determination of drug loading in a microsphere formulation is primarily dependent on the total drug dose desired in the final product with the additional consideration of the duration of release. When a hydrophobic drug is encapsulated, drug loading in the range of 20 – 45% does not influence either the mean particle size or size distribution significantly. Internal morphology examination revealed that increasing drug loading did not change the microstructures of the microspheres [61]. However, *in vitro* drug release can be influenced by drug loading; with a hydrophobic drug, it was found that a higher drug loading resulted in a slower release rate [61]. Similar results were also reported by others [62]. This can be explained by the fact that at high drug loading, upon recrystallization of the drug, phase separation between the drug and polymer occurs leading to the formation of large hydrophobic drug aggregates that exhibit slower drug dissolution and release. On the contrary, at a low drug loading level, drug crystallizes within the polymer matrix giving rise to a molecular dispersion showing enhanced drug dissolution and release. However, with a slightly water-soluble crystalline drug (fentanyl), Choi *et al.* reported that a faster drug release was found with PLGA microspheres with a higher drug loading [63]. This result was explained by the fact that with increasing concentration of a water-soluble drug, the dissolution of the drug in the dissolution medium penetrating into the polymer matrix leading to the formation of channels filled with drug solution, where enhanced drug diffusion and release took place.

The loading of a hydrophilic drug in PLGA microspheres at a level lower than 10% did not influence either the mean particle size or size distribution significantly [51,64]. No remarkable difference in drug encapsulation efficiency was found between a drug loading of 1 and 5%. However, the encapsulation efficiency decreased considerably when the drug loading increased to 10%. A similar phenomenon was observed for rhodamine-loaded microspheres [65]. It was reported that at a loading levels of 0.5 – 1.6%, the protein molecular weight can influence the release kinetics of microspheres as lager protein molecules are released through pores formed during polymer degradation but smaller protein molecules can diffuse through pores initially present in the polymer matrix. At a higher drug loading (4.8 – 6.9%), the release of the entrapped protein will not be dependent on its molecular weight [66].

2.3.3 Stabilizers

For efficient encapsulation of a drug dissolved in an aqueous phase which is subsequently dispersed in an organic phase or vice verse, stabilization of the W/O or O/W emulsion is critical. Surfactants or viscosity enhancers are the most commonly used stabilizers including nonionic surfactants, anionic surfactants, and hydrophilic polymers [2,67-69]. The addition of a surfactant results in lowering of the surface tension of the continuous phase which facilitates reduction of globule size, but this effect can level off when the surfactant concentration reaches the critical micelle concentration (CMC). Hydrogel-forming hydrophilic polymers can stabilize emulsions through increased solution viscosity [2].

When antigens are being encapsulated in microspheres, surfactants provide additional protection against irreversible aggregation [2]. Poloxamer 188 lowered BSA encapsulation in PLGA microspheres by up to 20%; however, the percentage of BSA aggregates was reduced from 31% to 5% [2]. The bioactivity of urease in PLGA microspheres was enhanced with poloxamer 407 added [70]. Drug release rate, biodistribution, mucoadhesion, and cellular uptake of the microspheres have been shown to be influenced by the type and concentration of the stabilizers used [69].

Polyvinyl alcohol (PVA) is the most commonly used stabilizer in the preparation of PLGA microspheres. Jeong *et al.* reported that particle size of microspheres decreased with increasing concentration of PVA in the aqueous phase but loading efficiency of all-trans retinoic acid decreased when PVA concentration was reduced [62]. Mao *et al.* reported

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that a ten-time increase of PVA concentration (0.1 – 1%) caused insignificant decrease in particle size [51]. The same change in PVA concentration also reduced the burst release of the drug [51]. Similar observations were also reported by others [61,64]. Jeong *et al.* reported that the size of microspheres decreased with the increase of PVA molecular weight, which also caused a small decrease in loading efficiency [62].

Poloxamer and carbopol are considered to be valuable alternatives for PVA [69]. Pradeesh *et al.* investigated the effect of three stabilizers, Tween-40, sodium laurate and PVA, on the size and geometry of hydroxyapatite microspheres. Their results show that only the microspheres formed with PVA were spherical in shape and with no agglomeration. The particle size decreased with the increase of PVA concentration. In the case of Tween-40 and sodium laurate, the increase of stabilizer concentration up to 1% has no effect on microspheres formed with irregular shape [71]. Instead of using a surfactant as a stabilizer, recent studies show that it is possible to prepare microspheres with an amphiphilic biodegradable polymer with poly(L-lactide) sequences grafted on a water-soluble poly-α,β-[N-(2-hydroxyethyl)-1-aspartamide] backbone [72].

2.3.4 Osmolytes

Osmolytes such as polyols, carbohydrates, and amino acids are frequently used as protein stabilizers in parenteral microsphere formulations [2]. One of their stabilizing effects is the maintenance of structural integrity of the water molecules surrounding the protein, which favors the compact native form of proteins and inhibits protein unfolding. Osmolytes can replace water during drying and provide additional stabilization effect. In PLGA microsphere formulations of rhGH, trehalose and mannitol were added as stabilizer during emulsification with methylene chloride. The stability of rhIFN-g was found to be fully preserved with trehalose but only slight improvement was shown with mannitol (63%) [2]. The stabilizing effect of osmolytes on antigens being encapsulated in microspheres appears to be controlled by two counteracting factors: the destabilization effect due to binding of the osmolyte to the antigen on the surface of the microspheres and the stabilization effect when the osmolyte was not in contact with the antigen [2]. It was suggested that interaction between the protein backbone and osmolyte polar groups is more favorable than the corresponding interaction with nonpolar groups [73].

2.3.5 Pore-forming agents

Some ingredients such as co-solvent and pore-forming agents are added in the dispersed phase to modify the drug release of microspheres. Co-solvents are added to dissolve the drug that is not totally soluble in the solvent used as the disperse phase. Organic solvents miscible with water such as methanol and ethanol are the commonly used co-solvents. A pore-forming agent is incorporated to generate pores inside the microspheres, which consequently accelerates polymer degradation and enhances drug release. Organic solvents such as hexane, which do not dissolve poly(lactic acid) and poly(lactic-co-glycol acid) can be incorporated into microspheres to form pores [74]. When an appropriate amount of n-heptane was added in the ethylcellulose/methylene chloride emulsion for encapsulation of aspirin, an increase in microsphere porosity was shown. However, if an excess of n-heptane is added, microspheres were formed with high porosity but low drug encapsulation efficiency [75]. Other substances added as a pore-forming agent include sodium chloride [76], gelatin [77], polyethylene glycol [78], ammonium bicarbonate [79], and pluronic F127 [80]. The initial burst release of a highly water-soluble model peptide, octreotide acetate from PLGA microspheres was significantly reduced by the coencapsulation of 0.2% of glucose, which is attributed to the decreased permeability of the microspheres at low glucose concentration [81].

2.3.6 Polymer concentration

The drug loading and particle size of microspheres are both influenced by the polymer concentration in the organic solvent phase. Mao *et al.* reported that a higher PLGA concentration resulted in an increase in drug loading, encapsulation efficiency, and size of microspheres. This was attributed to the increased viscosity of the organic phase, reducing the outflow of drug during the hardening phase [51,61]. When developing fentanyl-loaded PLGA microspheres, it was reported that the slowest drug release rate was observed with microspheres prepared with the lowest polymer concentration [63]. This phenomenon was thought to be caused by the density difference between the organic phase with different polymer concentrations.

The polymer concentration was also found to affect the external and internal morphology of microspheres as well as the burst release. At a low PLGA concentration (8%), microspheres with wrinkled surface and less surface porosity but large internal porosity were formed. When the PLGA concentration was increased to 32%, microspheres were formed with a smoother but a more porous surface. The increase in polymer concentration also resulted in a significant decrease in the initial drug burst release [51,61,82].

2.3.7 Solvent type

In general, the desirable properties of a solvent used in fabrication of microspheres are high polymer solubility, poor solubility in the continuous phase, high volatility, low boiling point, and low toxicity [74]. Commonly used solvents are methylene chloride, ethyl acetate, and ethyl formate. Methylene chloride is the most commonly used solvent for PLGA microsphere preparation by solvent evaporation because of its high volatility, low boiling point, and low miscibility with water. Its relatively high saturated vapor pressure compared to other solvents allows a high solvent evaporation rate, which significantly shortens the duration of fabrication. However, methylene chloride is confirmed to be carcinogenic according to EPA (Environmental Protection Agency) data

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and researchers have been trying to find less toxic solvents as a replacement [74].

Ethyl acetate has shown promise as a substitute of methylene chloride because of its lower toxicity, ease of removal, and excellent polymer solubility. It was reported that ethyl acetate resulted in microspheres with a larger particle size than those prepared with methylene chloride but with no significant difference in drug loading [62]. However, PLA microspheres prepared using ethyl acetate were found to be immunogenic and to elicit similar total antibody responses as the alum formulation of beta-galactosidase [83]. PLGA microspheres were successfully prepared with ethyl formate, showing that the evaporation rate of ethyl formate in water was 2.1 times faster than that of methylene chloride in spite of its lower vapor pressure and higher boiling point [84]. The author attributed the fast evaporation rate to the fact that more molecules of ethyl formate are exposed to the air–liquid interface because of its higher water solubility. This work also demonstrated that water immiscibility of a solvent is not an absolute prerequisite for its use in microsphere fabrication by an emulsion method. When microspheres containing proteins or peptides are prepared, the solvent used not only determines the encapsulation efficiency but also affects protein stability [2,67,74].

2.3.8 *Continuous phase/dispersed phase ratio*

When preparing microspheres using an emulsification method, the continuous phase to dispersed phase ratio (CP/DP) can influence the drug loading, encapsulation efficiency, porosity, and thereby burst drug release. By using a single emulsion method, Mao *et al.* reported that the drug loading and encapsulation efficiency of PLGA microspheres increased remarkably with increasing CP/DP ratio even though there was no change in particle size [61]. A similar phenomenon was previously reported for the encapsulation of progesterone [85]. It was also found that the porosity of microspheres was CP/DP ratio-dependent. A core-shell structure was observed in the microspheres prepared at CP/DP ratio of 20 and the porosity decreased with increasing CP/DP ratio [61]. PLGA microspheres were formed with a smoother surface and low porosity at higher CP/DP ratio, probably due to faster polymer precipitation in the presence of a larger amount of water. With a less porous structure, microspheres formed at a high CP/DP ratio showed significantly lower burst drug release [61].

The influence of CP/DP ratio was also dependent on the drug property and method of preparation. By using the double emulsion ($W_1/O/W_2$) method and FITC dextran (FD4) as the model drug, Mao *et al.* investigated the influence of the continuous phase volume on the properties of microspheres including particle size, drug encapsulation efficiency, and porosity [51]. They concluded that an increase in the volume of the continuous water phase (W_2) tended to result in larger particle size and lower encapsulation efficiency, but some leveling effect was shown when the volume reach a specific level. In addition to the CP/DP ratio, the ratio of the internal aqueous phase (W_1) to the continuous aqueous phase (W_2) volume used in a double emulsion method ($W_1/O/W_2$) can also influence the properties of the microspheres including drug loading, encapsulation efficiency and initial burst drug release, drug loading, and encapsulation efficiency [51].

2.3.9 *pH*

The pH of the continuous phase is another key variable influencing drug encapsulation efficiency. If the solubility of the drug in the continuous phase is much higher than that in the dispersed phase, the drug will readily partition and diffuse into the continuous phase resulting in low drug encapsulation [61]. A higher encapsulation efficiency of gentamicin was achieved with an internal aqueous phase at pH 6 where the drug was more soluble [86]. Therefore, when the solubility of the drug is pH-dependent, controlling the pH of the aqueous phase is essential to achieve high drug loading in microspheres.

3. Characterization techniques

Characterization of microspheres usually involves the measurement and determination of particle size and size distribution, surface morphology and internal structures (porosity), drug loading, encapsulation efficiency, zeta potential, burst drug release, *in vitro* release profile, syringeability/injectability, content uniformity, water contents, residual solvent level, polymer molecular weight, and drug polymer interaction. Some of these properties are determined during the development phase of a microsphere product with the aim to guide formulation and process development and some are tested for quality control purposes. The recent advances in some of the characterization techniques are reviewed below.

3.1 Particle size and size distribution

Microspheres prepared for parenteral administration generally have a particle size less than 50 μm in diameter. In recent years, microspheres also called microparticles, with a size range less than 10 μm have been developed for delivery of biomolecules such as recombinant proteins, genes, and vaccines in research and clinical trials. The size of microspheres can affect the rate of drug release, drug encapsulation efficiency, product syringeability/injectability, *in vivo* fate in terms of uptake by phagocytic cells, biodistribution of the particles after subcutaneous injection [87,88] as well as efficacy and side effect [89].

Optical microscopy is suitable for measuring the size of microspheres larger than 10 μm. With imaging analysis capability, determining the size distribution of microspheres using optical microscopy is less labor-intensive and more accurate than visual examination alone. The major advantage of microscopy is the direct visual examination of microspheres so that the shape and state of aggregation can be

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determined [77]. In recent years, particle sizing using automated laser diffraction has become the method of choice because of the speed of analysis and the capability of handling a large number of particles [90]. Microsphere samples can be measured as a dry powder or after being dispersed in a suitable liquid medium. A mean particle size, frequency distribution profile (number- or volume-based), and polydispersibility index are usually obtained as the output from the instrument [91].

3.2 Surface morphology and internal microstructures

Scanning electron microscopy (SEM) is the most widely used technique for examining external particle morphology such as size, shape and surface texture and internal microstructures including wall thickness and porosity [61]. Although some of the results generated using SEM are qualitative in nature, the information obtained is valuable in particular for comparison purposes. The use of SEM allows visualization of the internal structure of samples prepared from microspheres using microtome cutting [51] or freeze-fracture [92]; however, a quantitative measurement of porosity can only be achieved using other techniques such as mercury intrusion porosimetry [81,93].

Confocal laser scanning microscopy (CLSM) is a technique for obtaining high-resolution optical images with depth selectivity. CLSM can be used to characterize the structure of multiple-walled microspheres [94], to visualize the uniformity of drug distribution throughout microspheres prepared under different conditions with three-dimensional images. Lysozyme distribution in PLGA microspheres were analyzed by CLSM showing a homogeneous distribution of the protein throughout the microspheres and internal cavities formed by the water-in-oil emulsion method [95]. As revealed by results obtained by confocal microscopic analysis, the freeze–drying process was shown to generate water-escaping microchannels, through which the encapsulated molecules were presumably dumped out [96]. CLSM was used to determine the impact of method of preparation and drug loading on the distribution of fluorescein isothiocyanate in dextran microspheres [51,78].

3.3 Zeta potential

The surface charge of microspheres can have an impact on the degree of aggregation upon reconstitution with diluent and the resultant syringeability/injectability. The state of dispersion upon injection into tissues can also be affected by the surface charge of the microspheres [69,97]. Zeta potential instead of surface charge of the microspheres is usually measured to determine the electrostatic interactions between microspheres as influenced by their surface charge. Zeta potential of microspheres can be measured by microelectrophoretic procedures in which the movements of individual particles under the influence of a known electric field are followed microscopically. Electrophoretic light scattering can be used for determining the zeta potential of microspheres with a smaller particle size (< 5 μm) based on the analysis of the Doppler shift and is called laser Doppler velocimetry (LDV) [98].

3.4 Polymer and polymer–drug interaction

The molecular weight of the polymer is a critical formulation variable as this affects not only the release rate but also duration of release of the encapsulated drug. Polymer degradation (depolymerization) during manufacturing in particular irradiation sterilization can lead to significant decrease in molecular weight [99]. Gel permeation chromatography (GCP) is the standard method for determining polymer molecular weight for in-process control, product release, and stability testing.

The thermal properties of a polymer such as the glass transition temperature can be assessed by using differential scanning calorimetry (DSC). This is also a valuable tool for examining the impact of the entrapped drug on the thermal properties of the polymer as an indication of potential polymer–drug interaction [77,100]. DSC is also often used to analyze the physical state of drugs encapsulated in polymeric microspheres. The disappearance of its melting peak indicates that in the microspheres the drug existed as an amorphous form rather than a crystalline state [97]. When FITC (fluorescein isothiocyanate)-dextran 40KDa was encapsulated in PLGA microspheres at a relatively low drug loading (< 10%), there was no obvious change of PLGA glass transition temperature and no drug melting peak found. These results indicate that there was no chemical interaction between these two components and FITC was molecularly dispersed in PLGA matrix. At a higher drug loading (theoretical loading 20%), a drug melting peak was observed, which was a good evidence that some of the drug was distributed in the microspheres in a crystalline state [78].

X-ray diffraction (XRD) is a technique usually employed to investigate the physical state properties of a drug entrapped in microsphere matrix. Data from an X-ray diffraction experiment provide information on the drug–polymer interaction [63,77]. Polymer–drug interaction in microspheres can also be evaluated by using FT-IR [77,101].

Raman spectroscopy was used to investigate and confirm the possible interactions between drug and copolymer [102]. The possible interactions between a model drug diclofenac sodium (DS) and the water-insoluble ammonio methacrylate copolymer (AMC) was assessed using Raman spectroscopy. Films with different drug/polymer ratios were prepared by the solvent casting method and investigated as a preformulation study toward sustained release microparticles. The Raman spectra confirmed that none of the major structural changes revealed any significant difference, which can indicate a strong ionic interaction between the DS and the AMC. The investigations provided good information for the selection of a DS/AMC ratio in the development phase of the microsphere preparation process, in conformity with the therapeutic aim [103].

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3.5 *In vitro* drug release

During the formulation and process development phase, the influence of different process and formulation variables on drug release is determined by conducting *in vitro* release experiments. Due to the simplicity of experimental setups and the low operation cost, *in vitro* drug release tests are particularly attractive as a tool for formulation and process parameter screening. In general, drug-loaded microspheres are suspended in PBS medium or surfactants containing medium (to achieve sink conditions) in screw-capped tubes and placed in an orbital shaker maintained at 37°C. At predetermined time intervals, the tubes were taken out of the shaker and centrifuged. The supernatant is removed for drug assay and the precipitated microspheres are resuspended in fresh buffer and placed back in the shaker. Since microspheres are developed for sustained release of drug over periods of weeks or months, it becomes impractical to use an *in vitro* release test at 37°C as a quality control tool. Therefore, accelerated methods are developed, in which a higher temperature (i.e., 45°C) is usually applied. The mechanism for drug release should be characterized and confirmed that it will not be altered at the elevated temperature so that the relevance of the *in vitro* release to *in vivo* product performance can be established [104].

3.6 *In vivo* drug release and *in vitro* to *in vivo* correlation

In general, *in vivo* release kinetics can be estimated either from residual drug contents in microspheres retrieved from the excised tissue at the injection site or from drug input rates calculated from plasma concentrations and clearance [105]. *In vivo* pharmacokinetic studies by Kim *et al.* demonstrated that PEGylated TNF-related apoptosis-inducing ligand (PEG-TRAIL) microspheres demonstrated a sustained release profile (18 days), and that the steady-state concentration of PEG-TRAIL in rat plasma was reached at day 3 and maintained until day 15 [106]. IFN-2b microspheres provided sustained and steady plasma levels of active IFN-2b for about 13 days with a single subcutaneous injection, which was faster as predicted by the *in vitro* results since the degradation of PLGA *in vivo* proceeded faster than *in vitro* due to the foreign body response [107].

It is desirable to establish *in vitro* to *in vivo* correlation (IVIVC) for microspheres, which will allow more accurate prediction of *in vivo* performance based on *in vitro* drug release data. IVIVC can be achieved by mathematical modeling and is the basis for the quality control of microspheres by using *in vitro* release method. This approach has been attempted to be a surrogate for bioequivalence study [108]. To investigate whether an *in vitro* and *in vivo* relationship could be established for dexamethasone release, Zolnik *et al.* prepared two PLGA microsphere formulations with different polymer molecular weights [108]. The *in vitro* drug release from both formulations followed the typical triphasic profile, with initial burst release, followed by a lag phase and a secondary zero-order phase. The *in vivo* release profiles differed in that the lag phase was not observed and drug release rates were faster compared to the *in vitro* studies. The absence of lag phase *in vivo* was explained by different PLGA degradation mechanisms *in vivo* owing to the presence of enzymes as well as other *in vivo* factors such as interstitial fluid volume and local pH. A linear *in vitro–in vivo* relationship was established after normalization of the time required to reach plateau for the *in vitro* and *in vivo* data and the *in vitro* release data were demonstrated to be predictive of *in vivo* release. Established IVIVC was also reported for gestodene, ethinyl estradiol, and hydroxyapatite-loaded PLGA microspheres [58,109].

With several vapreotide-loaded PLGA microsphere formulations, IVIVC analysis showed a linear correlation between the mean residence time *in vivo* and the mean *in vitro* dissolution time. There was also a correlation between the amount released between 6 h and 14 days and the $AUC_{6h\text{-}14d}$. For several other parameters or time periods, no IVIVC was found [105]. IVIVC was influenced by administration route and particle size. It was reported that after intramuscular administration, the linear relationship between the *in vitro* and the *in vivo* release data was better than that after subcutaneous administration [60]. When the particle size of the microspheres was smaller, the values of correlation coefficient were higher [60]. The *in vivo* sustained release of a drug entrapped in microspheres can also be assessed by monitoring the therapeutic effect. Pharmacodynamic studies on microspheres containing proteins or peptides can be conducted to provide evidence that the biological activity of the encapsulated agent is preserved during the sustained release period of the microspheres. A significant elevation of red blood cell counts and hemoglobin level was shown in male rats starting the fourth day to the 37th day after receiving a single injection of rhEPO-loaded microspheres. This prolonged therapeutic effect as a result of sustained *in vivo* release was also predicted by the *in vitro* release data [82].

4. Expert opinion

Existing marketed microsphere products are composed of homo and copolymers of lactides and glycolides (PLGA). These polymers have a long history of human safety and regulatory approval. However, they share a few shortcomings for their use as a biodegradable matrix of microspheres such as a less controllable drug release pattern governed by bulk erosion, an acidic microenvironment in a degrading matrix, and less adaptability to manufacturing processes due to high glass transition temperature. Recently, significant research effort has been reported on the use of new biodegradable polymers which have exhibited different, in some cases superior, drug delivery and processing properties compared with PLGA in microsphere formulations. While preclinical studies and clinical trials of microspheres formulated with these new polymers have been reported, the regulatory

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approval and subsequent commercialization of these products can be challenging to realize in the near future simply because of the lengthy trials for safety and efficacy of the polymer with the encapsulated therapeutic agent. In addition to extensive chemical and physical characterization, costly biological tests including assessment of biocompatibility and determination of metabolic pathways for *in vivo* breakdown by-products are required for a new polymer with the results submitted for regulatory review as part of a new drug application. Furthermore, the regulatory approval of a new biodegradable polymer can face additional hurdles if the product fails either safety or efficacy associated with the therapeutic agent since there is not a regulatory pathway for the approval of a new polymer alone.

The properties of microspheres are highly dependent on the composition of the formulation (drug, polymers, and other excipients) and the processing aids used during the manufacturing process. The complex interplay of formulation and process variables has made process development for microspheres very challenging and time consuming. Furthermore, the cause–effect relationship between these variables are likely to be different when the manufacturing method changes. A systematic investigation on the impact of these variables on the final product properties can be very valuable for manufacturing process optimization with the aim of achieving the desirable end product quality. The availability of new advanced physical characterization techniques has allowed a better understanding of the influence of formulation and processing variables as well as their interactive effects on the final properties of the microspheres. Information from the more in-depth characterization of microspheres further facilitates formulation and process development allowing *in vitro* evaluation better reflective of *in vivo* performance of the microspheres.

Reasonable IVIVC results have been reported recently for microspheres, but the challenges should not be overlooked. The *in vivo* environments surrounding microspheres after subcutaneous or intramuscular injection are difficult to simulate *in vitro* primarily because of the existence of enzymes and other cellular and tissue responses such inflammation, foreign body response, and fibrous capsule formation. All of these *in vivo* events can significantly alter the degradation of the polymer and release kinetics of the encapsulated drugs as compared to those shown by *in vitro* drug release testing. These are the major factors contributing to poor or lack of IVIVC. *In vivo* drug release studies are commonly performed for polymeric implants and injectable gels in animals by determining the amount of drug remaining in the partially degraded samples retrieved from the injection site. While conducting a similar *in vivo* drug release study with microspheres, it becomes difficult to attain reliable results simply because complete retrieval of the degrading microspheres from the injection site is very challenging due to the much smaller particle size of microspheres and their tendency to disperse upon injection. Therefore, a pharmacokinetic (PK) or pharmacodynamic (PD) study using appropriate animal models is usually performed for characterization of the *in vivo* sustained drug profile of microspheres. PK and PD characterization of microspheres in animal models are valuable studies during the preclinical development phase of the product. However, the translation of animal PK or PD results to clinical performance in humans may not be straightforward, in particular when the *in vivo* fate of the drug in animals and humans are different.

Declaration of interest

LC Li, C Guo, and Y Shi are employees of Abbott.

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