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Microspheres for Drug Delivery

Kyekyoon “Kevin” Kim and Daniel W. Pack

University of Illinois at Urbana-Champaign

2.1. INTRODUCTION

With advances in biotechnology, genomics, and combinatorial chemistry, a wide variety of new, more potent and specific therapeutics are being created. Because of common problems such as low solubility, high potency, and/or poor stability of many of these new drugs, the means of drug delivery can impact efficacy and potential for commercialization as much as the nature of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery. Indeed, drug delivery systems—designed to provide a therapeutic agent in the needed amount, at the right time, to the proper location in the body, in a manner that optimizes efficacy, increases compliance and minimizes side effects—were responsible for $47 billion in sales in 2002, and the drug delivery market is expected to grow to $67 billion by 2006.

Controlled release drug delivery systems are being developed to address many of the difficulties associated with traditional methods of administration. Controlled release drug delivery employs devices—such as polymer-based disks, rods, pellets, or microparticles—that encapsulate drug and release it at controlled rates for relatively long periods of time. Such systems offer several potential advantages over traditional methods of administration. First, drug release rates can be tailored to the needs of a specific application; for example, providing a constant rate of delivery or pulsatile release. Second, controlled release systems provide protection of drugs, especially proteins, that are otherwise rapidly destroyed by the body. Finally, controlled release systems can increase patient comfort and compliance by replacing frequent (e.g., daily) doses with infrequent (once per month or less) injection.

While a variety of devices have been used for controlled release drug delivery, biodegradable polymer microspheres are one of the most common types and hold several advantages. Microspheres can encapsulate many types of drugs including small molecules,
proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time. Several commercial products are based on polymer microspheres including Lupron Depot® and Nutropin Depot®. Disadvantages of microspheres include difficulty of large-scale manufacturing, inactivation of drug during fabrication, and poor control of drug release rates. For example, Nutropin Depot, comprising Genentech’s recombinant human growth hormone (rhGH) encapsulated within poly(d,l-lactide-co-glycolide) (PLG) microspheres using Alkermes’ proprietary ProLease® encapsulation technology, was recently pulled from the market because manufacturing and production costs were too high.

In this chapter, the factors controlling drug release rates are reviewed, followed by several examples of controlled-release microsphere applications. More importantly, methods for microparticle fabrication will be described. In particular, the chapter will focus on a precision particle fabrication (PPF) method that has been reported recently. PPF provides unprecedented control of particle size, size distribution and morphology that may translate into enhanced control of drug release rates and improved understanding of the mechanisms controlling release.

2.2. BACKGROUND

2.2.1. Factors Affecting Release Rates

The microsphere fabrication method is a governing factor in the encapsulation and release of therapeutics. In addition, a complicated array of factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microsphere formulation (e.g., for stabilization of the therapeutics), and the microsphere size can have a strong impact on the delivery rates.

First, the type of polymer used in microsphere fabrication and the way in which the polymer degrades obviously affect drug release rates. Depending on the rate of hydrolysis of their functional groups, polymers can be broadly categorized into two types: surface-eroding and bulk-eroding [29, 91, 169]. Bulk-eroding polymers, such as PLG, readily allow permeation of water into the polymer matrix and degrade throughout the microsphere matrix. In contrast, surface-eroding polymers, such as polyanhydrides, are composed of relatively hydrophobic monomers linked by labile bonds. In this way, they are able to resist the penetration of water into the polymer bulk, while degrading quickly into oligomers and monomers at the polymer/water interface via hydrolysis [148].

Bulk-eroding polymer microspheres are often characterized by a “burst” of drug—as much as 50% of the total drug load [131]—released during the first few hours of incubation, followed by a slow, diffusion-controlled release and sometimes a third phase in which the remaining drug is released quickly as a result of severe degradation of the polymer matrix. In microspheres composed of surface-eroding polymers, drug is released primarily at the surface as the polymer breaks down around it. Erosion of such polymers usually proceeds at a constant velocity [66]. If the drug of interest is homogeneously dispersed throughout a microsphere, the largest rate of release will occur at the beginning. As time proceeds, the surface area of the sphere and the release rate decrease asymptotically.
Polymer molecular weight can affect polymer degradation and drug release rates. As one might expect, an increase in molecular weight decreases diffusivity and therefore drug release rate [2, 95, 109, 118, 191]. In addition, a major mechanism for release of many drugs is diffusion through water-filled pores, formed as polymer degradation generates soluble monomers and oligomers that can diffuse out of the particle. These small products are formed more quickly upon degradation of lower molecular weight polymers. The decrease in release rates with increasing polymer molecular weight appears to hold for small molecules, peptides, and proteins [25, 123]. However, molecular weight typically has little effect on release rates from surface-eroding polyanhydride microspheres [69, 167].

The co-monomer ratios in many copolymers can also affect release rates. Most often, increasing the content of the more rapidly degrading monomer increases the release rate [112, 156, 161]. Similarly, when drug release is controlled by polymer erosion, release rate typically increases with higher concentration of the smaller and/or more soluble monomer [167]. However, the effect of the copolymer composition can be complicated by differences in the polymer phase behavior or the thermodynamics of the encapsulated drug [89].

A variety of excipients may be added to microsphere formulations to stabilize the drug during fabrication and/or release and may impact drug release through several different mechanisms. For example, to improve the encapsulation of bovine serum albumin (BSA) in microspheres of poly(ε-caprolactone) (PCL) and 65:35 PLG, Yang et al. included poly(vinyl alcohol) (PVA) in the BSA solution to stabilize the primary emulsion resulting in a more uniform BSA distribution in the microspheres [192]. Increasing concentrations of PVA decreased the initial burst of protein and the overall release rates. Jain et al. encapsulated myoglobin in PLG microspheres in the presence of a stabilizer, mannitol [77]. They report that mannitol increased the release rate and the final amount of drug released by increasing the initial porosity of the PLG matrix, leading to faster formation of the pore network within the sphere due to polymer erosion.

Clearly, microsphere size will strongly affect the rate of drug release. As size decreases, the surface area-to-volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microsphere, the rate of flux of drug out of the microsphere, per mass of formulation, will increase with decreasing particle size. In addition, water penetration into smaller particles may be quicker due to the shorter distance from the surface to the center of the particle. Also, while the decrease in surface area with particle size may lead to decreased rate of erosion of poorly water-permeable polymers like polyanhydrides, because surface area-to-volume ratio increases with decreasing particle size, drug release rates (per mass of polymer) will be faster for smaller polyanhydride microspheres.

2.2.2. Recent Applications of Controlled Release Microspheres

Controlled-release microspheres are in development for a number of interesting and important applications, especially for delivery of large, fragile drugs like proteins and nucleic acids. Several recent examples are described below.

2.2.2.1. Controlled-Release Vaccines

Vaccination has been highly successful for controlling or even eradicating many important types of infectious diseases, and new or improved vaccines are being heavily investigated for AIDS [196], hepatitis B [170], anthrax [59], and SARS [197]. A frequent problem is the need for repeated administrations—usually
injections—to ensure long-lasting immunity. For example, the current anthrax vaccine requires a series of boosters at 2 and 4 weeks, and at 6, 12, and 18 months following the first inoculation; and the Recombivax HB® vaccine for hepatitis B—required for most healthcare workers in the U.S.—is administered in three injections at 0, 1, and 6 months. The need for multiple injections poses a serious problem for patients in developing countries with limited access to medical care, where awareness is lacking, or for transient populations. One promising alternative is a single-shot vaccine in which a drug delivery device provides the necessary boosters at specified times after administration [83]. Further, the ability to more precisely control the time course of vaccine delivery may lead to more effective vaccination with current antigens and may allow utilization of antigens that were previously ineffective [114].

Single-shot Vaccine delivery systems should provide the antigen(s) and adjuvant on a prescribed schedule and maintain the bioactivity of the antigen, both during fabrication of the delivery device and during the often prolonged residence time of the device in the body. In recent years, much work has focused on developing microsphere-based, single-administration, vaccine delivery vehicles [44, 45, 149, 158] using a variety of materials including hydroxypropyl cellulose/PLG [101], poly(ε-caprolactone) [160], PLA [141], chitosan [31], and collagen [74], though the majority have been fabricated with PLG [7, 44, 47, 158, 162]. Maintenance of antigen bioactivity has been problematic due to contact of the proteins with organic solvents and the hydrophobic polymer, and the use of strong physical forces to produce the microspheres [2, 36, 81]. To enhance vaccine stability, researchers have been focusing on several approaches, including the use of adjuvants to protect the protein antigens or by choosing different microsphere materials [125, 138, 139].

A major advantage of microspheres for vaccination is that they can be passively targeted to antigen-presenting cells (APCs) such as macrophages (MΦ) and dendritic cells. The ability of APCs to phagocytose particulates is dependent on the particle size. In particular, 1- to 10-µm diameter microspheres are optimally taken up by APCs in a number of tissues [9] and have been shown to enhance antigen-specific T-helper lymphocyte (Th) responses [142] (thus leading to an enhancement in antigen-specific antibody responses) and elicit a cytotoxic T lymphocyte (CTL) response (Nixon et al. 1996). T-cell activation in response to antigen-encapsulating microspheres has been shown to be 100-1000 fold better than antigen alone [179].

2.2.2.2. Stabilization of Encapsulated Protein Therapeutics  A major problem with protein encapsulation in polymer particles is loss of protein bioactivity. Damage to proteins can occur during fabrication of the particles—via shear stresses or other physical forces, through contact with organic solvents, and by loss of water (e.g., upon lyophilization) [34, 76]—as well as during incubation and release in the warm, moist, in vivo environment. Two types of damage occur most often: (i) covalent or non-covalent intermolecular aggregation [34] and (ii) denaturation [51, 60]. Several studies have investigated the mechanisms of damage [153]. Protein stability can be enhanced by the addition of excipients to prevent aggregation and stabilize the folded protein structure or through judicious choice of polymer employed for fabrication of the devices.

Although a number of types of stabilizing excipients have been studied, poly(ethylene glycol) (PEG) and sugars have been the most common [46, 81, 88, 93, 163, 187]. For example, Perez-Rodriguez et al. very recently reported that co-dissolving PEG (M_w 5,000) and
maltose with $\alpha$-chymotrypsin in the primary emulsion greatly reduced protein aggregation and inactivation [163]. In a similar study, Castellanos et al. used PEG to enhance the stability of $\gamma$-chymotrypsin encapsulated in PLG microspheres formed by a solid-in-oil-in-water (s/o/w) emulsion process [35]. They reported that normal encapsulation procedures with protein lyophilized from buffer or a trehalose solution caused 30% protein aggregation and 50% loss in activity. Co-lyophilizing the protein with PEG prior to encapsulation reduced the activity loss to only 8%, but the protein still aggregated during encapsulation. By using PEG as an emulsifier in the secondary o/w emulsion, the resulting microspheres were free of protein aggregation and the enzyme completely maintained its activity upon extraction from the particles. Similar stabilization of horseradish peroxidase (HRP) [35] and BSA [1] was achieved with the same technique, suggesting that this may be a general approach for protein stabilization. Finally, Jiang and Schwendeman reported that upon blending of 20–30 wt% PEG in PLA microspheres encapsulating BSA, protein aggregation was avoided and in vitro protein release was sustained for 29 days [79].

Other types of excipients also are effective in the stabilization of proteins. Lysozyme inactivation, precipitation, and aggregation were largely prevented upon formation of the primary emulsion by the addition of 50 mM potassium phosphate, and the activity of the protein was largely unaffected [135]. Further, the acidic microclimate resulting from accumulation of polymer degradation products [63, 157] is a major factor causing protein instability in degrading microspheres [154]. Schwendeman and co-workers [84, 200] showed that addition of buffering salts can stabilize encapsulated proteins. Zhu et al. demonstrated that co-encapsulation of magnesium hydroxide ($\text{Mg(OH)}_2$), an antacid, increased the microclimate pH in PLG millicylinders and microspheres containing BSA [200]. The devices with Mg(OH)$_2$ showed minimal BSA aggregation and exhibited a smaller burst effect of non-aggregated BSA compared to cylinders without the antacid.

The type of polymer used for microsphere fabrication, its degradation rate, acidity of the degradation products, hydrophobicity, etc., can also impact stability of encapsulated proteins. Although PLG is the most common polymer used for polymer microspheres, PEG can be combined with polyesters to form poly(\(d,\,l\)-lactide)-co-poly(ethylene glycol) (PELA) diblock copolymers [113, 198], PLA-PEG-PLA triblock copolymers [24, 38, 111], and PLG-PEG-PLG triblocks [124]. It is thought that the more hydrophilic PEG may improve the affinity of protein for the matrix polymer and lead to better entrapment efficiency [103, 104, 199].

2.2.2.3. DNA Encapsulation Gene therapy holds tremendous potential for treating genetic diseases and acquired diseases including cancer, and as vaccines [8, 52, 53, 71, 176]. A major barrier to development of gene-based pharmaceuticals is safe and efficient DNA delivery. Much research has focused on development of gene delivery vectors including viruses [177], liposomes [55], and polymers [127]. However, parenteral administration of naked plasmid DNA (pDNA) leads to gene expression [28, 188], and controlled release of pDNA from polymeric matrices [26, 54, 116, 130, 155], microparticles [73, 116, 121, 171], and nanoparticles [48, 75, 119] has been reported recently. In particular, encapsulation and controlled release of pDNA from biodegradable microspheres may provide a number of advantages including protection from nuclease degradation, access to alternative routes of administration (e.g., nasal, pulmonary, oral, and mucosal), passive targeting to professional antigen-presenting cells [73, 115], and prolonged gene expression [116].
Problems currently facing the design of new DNA-loaded microparticle formulations include loss of bioactivity, poor encapsulation efficiency and low loading due to the difficulty of encapsulating such large hydrophilic macromolecules in the hydrophobic polymer matrix. Plasmid DNA is particularly susceptible to damage by physical forces, especially the shear forces employed in most microsphere fabrication processes [106]. In addition, loss of structural integrity can result from exposure to acidic environments [171, 180, 182]. Ando et al. devised a novel emulsion-solvent extraction/evaporation method for DNA-loaded microsphere encapsulation that prevents shear-induced damage of the plasmid [6]. In this method, the primary w/o emulsion, comprising the aqueous DNA solution and the polymer/solvent continuous phase, is formed by sonication and then quickly frozen in liquid nitrogen. The emulsion is allowed to warm slowly until reaching a temperature at which the organic phase melts, but the aqueous phase remains frozen. The primary emulsion—actually a suspension of ice particles—is then homogenized in water to form the microspheres. Because the DNA is entrapped in the frozen droplets, the plasmid does not experience the shear forces employed in homogenization. In microspheres prepared by the optimal procedure, 89% of the DNA retained its native conformation compared to only 39% using the conventional process.

2.3. FABRICATION OF POLYMER MICRO- AND NANOPARTICLES

Microsphere drug delivery systems have been fabricated by a variety of techniques including combinations of phase separation or precipitation (Young, 1999), emulsion/solvent evaporation [11, 43, 102, 117, 164, 186, 192], and/or spraying methods [58, 72, 126, 140, 183]. Variations of the fabrication parameters generally allow control of the particle size and size distribution. Drugs may be incorporated into the particles in several different ways depending on the properties of the drug. Hydrophobic therapeutics may be co-dissolved with the polymer in a solvent such as methylene chloride or ethyl acetate. Hydrophilic therapeutics, including proteins, may be suspended in the organic phase as a finely ground dry powder. Alternatively, an aqueous solution of a hydrophilic therapeutic may be mixed with the organic polymer solution to form a water-in-oil emulsion.

The emulsion-solvent extraction/evaporation methods are most commonly used, especially at the lab scale. In these processes, a solution containing the polymer (and possibly the drug to be encapsulated) is emulsified in a non-solvent phase (the continuous phase) containing a stabilizer. The emulsion can be prepared with any of a variety of physical methods including homogenization and sonication. The components are chosen such that the solvent is slightly soluble in the non-solvent. For example, to produce microspheres of PLG or polyanhydrides, common solvents are methylene chloride and ethyl acetate used in conjunction with an aqueous continuous phase containing poly(vinyl alcohol) (PVA) as a stabilizer [49]. After emulsification, the solvent is extracted into the continuous phase and allowed to evaporate. At the same time, the non-solvent may penetrate into the polymer-rich droplets. Due to loss of solvent, the dispersed phase is enriched in polymer until the droplets “harden” to become particles. The microspheres may then be filtered, washed, and lyophilized.

There are several disadvantages of the emulsion solvent-extraction/evaporation techniques that have limited their application. Because these methods are inherently batch
operations, scale up of the processes is difficult and large-scale production can be costly. Another critical problem is that size distributions of particles are generally reproducible but non-uniform. Standard deviations of the distribution equal to 50% of the average size are not uncommon. Since the size of the spheres directly affects the drug release rate and syringability, it is important that size distributions be relatively narrow. In addition, as described above the presence of organic solvents and aqueous-organic interfaces may have adverse effects on encapsulated drugs [62, 147]—decreasing or even eliminating bioactivity. Organic solvents also may be very difficult to remove completely. Since many of the commonly used organic solvents (e.g., methylene chloride) are toxic, the concentration of residual solvent in the microsphere must be tightly regulated.

2.3.1. Techniques for Fabricating Uniform Microspheres

As pointed out in the preceding sections, an important limitation in the development of biodegradable polymer microspheres for controlled-release drug delivery applications has been the difficulty of specifically designing systems exhibiting precisely controlled release rates. Because microparticle size is a primary determinant of drug release [15, 17], it is worthwhile to develop a methodology for controlling release kinetics employing monodisperse microspheres.

There have been several reports of fabrication of uniform biodegradable polymer microspheres [4–5, 105, 152, 159]. Amsden and Goosen have used electrostatics to form EVAc spheres with diameters smaller than the nozzle from which they were sprayed [5]. The minimum size obtained, however, was only ~500 µm, and they concluded that “it is not possible to obtain small microbeads having a narrow size distribution.” Amsden later reported production of uniform microspheres by extruding a polymer solution through a nozzle and into a stabilizing solution flowing perpendicular to the point of injection [3]. The PLG solution droplets exiting the nozzle are drawn into the PVA solution in a periodic fashion. The rate at which the droplets are removed from the nozzle, and therefore—under constant polymer flow rate—the size of the microspheres, are determined by properties of the stabilizing solution (density and viscosity), the diameter of the nozzle, and the interfacial tension between the polymer solution and the needle tip. Amsden reported PLG microspheres with diameters ranging from 67.7 to 295 µm. However, the size distributions varied widely, with standard deviations typically 10–30% of the average size (the minimum standard deviation was 5% of the average for 295-µm microspheres). Furthermore, formation of small particles may not be possible with this technique, as any further reduction of the particle size would apparently require smaller nozzles; as the nozzle size is decreased, shear forces at the orifice and the potential for clogging increase.

In summary, there have been several reports of the fabrication of biodegradable polymer microspheres with controlled, uniform size. However, none of these methods was successful in generating particles in the size range appropriate for drug delivery (~1–100 µm) while maintaining narrow size distributions. In addition, these conventional methods appear to be difficult to scale-up for commercial applications.

To remedy this situation, we have developed a novel microsphere fabrication technology which combines two techniques for generating monodisperse microspheres with precisely controlled sizes. This precision particle fabrication (PPF) technology also allows fabrication of predefined particle size distributions via continuous variation of the process parameters.
The fundamental physical processes underlying the PPF technology are schematically described in Figure 2.1. The method basically comprises spraying a polymer-containing solution through a nozzle with (i) acoustic excitation to produce uniform droplets (Figure 2.1A), and (ii) an annular, non-solvent carrier stream allowing further control of the droplet size (Figure 2.1B). Using the PPF technology we have fabricated uniform solid microspheres of a variety of polymers including poly(ε-caprolactone-co-glycolide) (PLG) [14, 17], polyanhydrides [18], ethylcellulose [39], chitosan [40], hetastarch [41], and gelatin hydrogel [42].

In general, the PPF apparatus (Figure 2.2) is designed to pass a solution containing the sphere material, and any drug to be encapsulated, through a small nozzle or other orifice (20-μm to a few millimeters in diameter) to form a smooth, cylindrical jet. The nozzle is
vibrated by a piezoelectric transducer driven by a wave generator at a frequency tuned to match the flow rate and the desired drop size. The mechanical excitation launches an acoustic wave along the liquid jet generating periodic instabilities that, in turn, break the stream into a train of uniform droplets (Figure 2.3A). With this apparatus alone, the minimum particle size achievable is approximately twice the nozzle opening. This approach represents an improvement over conventional ultrasonic nozzles as the acoustic wave intensity is lower and we can tightly control the match between the frequency and solution flow rate.

We can further control sphere size, and in fact form droplets much smaller than the nozzle opening, by employing an annular flow of a non-solvent phase around the polymer jet (Figure 2.1B). The annular stream is pumped at a linear velocity greater than that of the polymer stream. Thus, frictional contact between the two streams generates an additional downward force that effectively “pulls” the polymer solution away from the tip of the nozzle. The polymer stream is accelerated by this force and, therefore, thinned to a degree depending on the difference in the linear velocities of the two streams. The carrier stream allows production of microspheres as much as 100-fold smaller than the orifice size. Thus, addition of the carrier stream accommodates high-viscosity materials and reduces the risk of clogging by allowing use of large nozzles.
In order to predict the process parameters (flow rates and acoustic frequency) required to generate particles of a desired size or size distribution it is necessary to understand the theory of droplet formation as applied to the PPF system.

### 2.3.1.1. Theory of the Precision Particle Fabrication Technology

Lord Rayleigh first investigated the instabilities of a cylindrical inviscid jet subject to disturbance [145]. He found that the most unstable wavelength ($\lambda_{\text{max}}$) of a disturbance imposed on a jet surface, which will give rise to maximum growth rate and consequently result in the break-up of the jet into uniform droplets (Figure 2.1), is

$$\lambda_{\text{max}} = 9.016r_j \quad (2.1)$$

where $r_j$ is the radius of the undisturbed jet (approximately equal to, but typically slightly larger than, the diameter of the nozzle orifice). The practical range of acoustic wavelengths that give rise to the breakup of a liquid jet into uniform droplets was experimentally determined to be [146]

$$7r_j < \lambda < 36r_j. \quad (2.2)$$

Both upper and lower wavelength limits may vary somewhat depending on the noise level of the system and the amplitude of the acoustic wave.

The microsphere generator developed by us allows for control of the acoustic wave frequency and amplitude. The wavelength produced by a set frequency ($f$) is given by

$$f = \frac{v_j}{\lambda} \quad (2.3)$$

where $v_j$ is the linear velocity of the liquid jet. Knowing that the volume of the resulting sphere should be equal to the volume of a cylindrical element of the jet, the length of which is defined by the acoustic wavelength, we find that the drop radius, $r_d$, is predicted to be

$$r_d = \left(\frac{3r_j^2v_j}{4f}\right)^{1/3}. \quad (2.4)$$

At the optimum wavelength, $r_{d,\text{max}} = 1.891r_j$. Thus, by imposing a uniform, high-amplitude oscillation on the nozzle, which will dominate the random, natural instability, we can control the breakup of the stream into droplets and predict the orifice size ($\sim r_j$), solution flow rate ($v_j$), and acoustic frequency ($f$) needed to generate a desired sphere size or size distribution.

### 2.3.1.2. Uniform Polymer Microspheres Produced by PPF Technology

Uniform microspheres of controlled sizes, both solid and hollow, were previously fabricated from a variety of non-polymeric materials using acoustic excitation [56, 64, 68, 78, 85, 87, 90]. More recently, we have demonstrated the PPF technology for fabricating monodisperse microspheres of various polymers such as PLG [14, 17], polyanhydrides [18], EC [39], chitosan [40], gelatin hydrogel [41], and hetastarch [42], with average diameters from $\sim 4 \mu m$ to $>500 \mu m$ (Figure 2.3B–F). These micrographs clearly demonstrate that the PPF technology: (a) can produce uniform polymeric microspheres with precisely controlled sizes,
(b) can be used to produce particles much smaller than the nozzle opening, and (c) is insensitive to the choice of materials.

We have also demonstrated encapsulation and in vitro release of model drug compounds such as rhodamine B, piroxicam, nifedipine, and felodipine [15, 17, 39–40]. The release kinetics of these compounds depended strongly on the microsphere size, as expected. Details of in vitro release profiles and mechanisms will be discussed below.

2.3.2. Techniques for Fabricating Uniform Core-Shell Microparticles

The ability to form microcapsules exhibiting a predefined diameter and shell thickness may offer several additional advantages in drug delivery. Higher drug loads may be realized by utilizing core materials offering increased drug solubility. In addition, fragile therapeutics such as proteins and DNA may be stabilized by generating a favorable environment in the core. Advanced drug release schedules such as delayed or pulsatile release, with the removal of drug “burst,” may be possible by selectively varying the shell material or especially thickness [103–104]. For example, a surface-eroding polymer (e.g. polyanhydride) shell may be expected to protect a polyester core, and the encapsulated drug, for a prolonged time, the duration of which is governed by the shell thickness. However, efficient fabrication of such particles has not been previously reported to our knowledge. Finally, drugs could be released in tandem by selectively loading them into the core or shell phase thereby potentially enhancing drug efficacy [193–194].

Core-shell microparticles are significantly more difficult to manufacture than solid microspheres. A variety of techniques for fabricating microcapsules of varying sizes have been reported [32, 50, 65, 85, 87, 94, 120, 133, 134, 150, 172]. For example, variations of the common double-emulsion approach have also been used to prepare microcapsules by allowing the discontinuous phase of the primary emulsion to coalesce and form the core of the particle [50, 150, 172]. Such particles have shown an interesting pulsatile release profile that may be advantageous for vaccine delivery [150]. Important limitations of these approaches, however, are that the core and shell material must be immiscible and the microcapsule architecture is difficult to control; the core size and shell thickness depend strongly on the properties of the primary emulsion and the time over which the emulsion is allowed to coalesce.

It is also possible to generate microcapsules with solid cores by coating pre-formed microparticles with a second material. For example, Göpferich et al. [65] developed an ingenious method for coating microspheres with a second layer of polymer by resuspending preformed microspheres in a concentrated solution of a second (or the same) polymer followed by re-emulsifying the suspension in an aqueous continuous phase to form “microencapsulated microspheres.” Presumably, the shell thickness can be controlled by varying the polymer concentrations and mass ratios, and the two materials can be miscible with one another. However, the thinnest shell reported was >50% of the overall particle diameter.

“Double-wall” particles comprising polymer cores and shells can be formed by controlling phase separation of the two sphere-forming materials [133–134]. One of the first studies consisted of adding two separate polymers dropwise to an aqueous phase and controlling precipitation rates such that one polymer has ample time to spread on the other [96, 97, 99, 122, 132]. Another technique utilized the phase separation of PLG and PLL at certain concentrations [103–104]. The radiosensitizer etanidazole was suspended as fine
filaments (<20 µm) into the PLG phase and the two polymers were emulsified into each other forming a milky solution. Again, during the relatively prolonged extraction of solvent from these large, ~450-µm particles, the two polymers phase-separated, but some of each polymer still remained in both the core and shell phase. Etanidazole was entrapped primarily in the PLG core due to a higher affinity to the PLG phase. Most recently, a polyorthoester (POE) was encapsulated in a PLG shell [193–194]. In these studies, the overall polymer concentration remained constant at 5% (wt polymer/vol DCM) but the POE:PLG polymer ratio was adjusted to produce double-wall microspheres. When POE accounted for 50%, 60%, and 70% of the polymer weight, core-shell particles with PLG shells were formed while microspheres with intermingled polymer phases were formed at all other ratios.

These are simple and scalable processes, but several factors seem to influence the formation of complete microcapsules. First, the interfacial tension between the three phases (polymer 1, polymer 2, aqueous non-solvent) indicates which polymer will tend to spread on the other [133–134, 173]. The spreading coefficient in these cases, therefore, is expected to be a function of the polymer chemistry, solvent type, and polymer concentration [193–194]. Relying on these thermodynamically driven techniques to form microcapsules may necessitate extended solvent extraction times to allow the two polymer phases to come into intimate contact [96–100, 122, 132, 133] or phase separate [103–104] and then form a core/shell arrangement before the polymer solutions precipitate. An extended solvent extraction time may have been achieved in the reviewed work by creating large particles, >100 µm (and thus large nascent polymer droplets), lowering polymer concentration, lowering the temperature of the non-solvent bath, or adding solvent to the extraction phase. Thus, the achievable architectures, types of drugs that can be encapsulated, shell thicknesses obtainable, and release kinetics achievable may be limited.

2.3.2.1. Precision Core-Shell Microparticle Fabrication Following previous work reporting production of core/shell particles made from a variety of inorganic materials [68, 85, 87], the uniform solid microsphere fabrication methodology described in the preceding sections (Figures 2.1 and 2.2) has been further extended to produce uniform double-walled polymeric microspheres with controllable size and shell thickness. The method, as illustrated in Figure 2.4, employs three coaxial nozzles to produce a smooth coaxial jet of controllable size, comprising a carrier, annular shell and core streams, which is acoustically excited to break up into uniform core-shell droplets. As before, the non-solvent carrier stream surrounding the coaxial jet accelerates and makes it thinner before its breakup. The orientation of the jets, material flow rates, and rate of solvent extraction are controlled to vary the shell thickness.

Microcapsules have been fabricated with different arrangements of bulk-eroding poly(d,l-lactide-co-glycolide) (PLG) and surface-eroding poly[(1,6-bis-carboxyphenoxy) hexane] (PCPH) [20]. Variation of the fabrication parameters allowed complete encapsulation by the shell phase including the efficient formation of a PCPH shell encapsulating a PLG core. Utilizing this technology, microcapsule shell thickness can be varied from <2 µm to tens of microns while maintaining complete and well-centered core encapsulation for microcapsules near 50 µm in overall diameter. Scanning electron micrographs of microcapsules originally containing an oil or aqueous core are shown in Figure 2.5, and PCPH shell/PLG core and PLG core/PCPH shell microcapsules are shown in Figure 2.6. These micrographs are proof that the modified PPF technology is an effective single-step
FIGURE 2.4. Schematic description of the triple-nozzle PPF methodology of producing uniform core-shell droplets using acoustic and carrier stream schemes.

FIGURE 2.5. Scanning electron micrographs showing fractured (A) canola oil core/PLG shell microcapsule, and (b) microcapsule with an aqueous core containing 100 mg/mL dextran, 10 mg/mL BSA and a PLG shell.
method for producing uniform polymeric microcapsules of controllable size and shell thickness. Monodisperse or precisely defined particle size distributions can be achieved while maintaining the desired polymeric shell thickness.

2.3.2.2. Further Discussion of Microcapsule Fabrication Technique  In comparison with the conventional core-shell particle methods described above, precision particle fabrication (PPF) technology sprays polymer solutions that are immediately brought into intimate contact as distinct and separate phases specifically arranged in the desired orientation. Utilizing this method for creating uniform microcapsules of controllable shell thickness provides a mechanical driving force to aid this thermodynamically driven process. Additionally, the present method is not limited to using concentrations where polymers are immiscible or specific conditions that slow the extraction of solvent but rather allows the exact control of polymer concentrations and flow rates thus controlling the mass ratios of the two polymers in each nascent droplet, resulting in precision control of microcapsule diameter and shell thickness. This flexibility can also be utilized to create conditions in the nascent droplet that facilitate spreading of PCPH on a PLG core until subsequent precipitation of the polymers kinetically traps the microcapsule arrangement that otherwise would be difficult to achieve.

In summary, the modified PPF technology is a single-step method for producing uniform polymeric microcapsules of controllable size and shell thickness. Monodisperse or precisely defined particle size distributions can be achieved while maintaining the desired polymeric shell thickness. Exact control of the volumetric flow-rates of the core and shell materials also allows the formation of particle populations exhibiting discretely or incrementally increasing shell thickness. In addition to advancing control of particle architecture, methods have been developed for modulating the locale of materials and compounds of interest. Specifically, polymer, oil, and aqueous cores have been encapsulated within uniform
polymeric shells potentially enabling advancements in the areas of drug delivery, photonics, cell encapsulation, and catalysis to name a few. Monodisperse microcapsule advantages in reproducibility, control of transport phenomena, and consistency of physically derived properties may provide valuable assistance in a variety of research areas.

2.3.3. Use of Electrohydrodynamic Spraying for Fabrication of Uniform Micro and Nanospheres

A process known as electrohydrodynamic (EHD) or electrostatic (ESS) spraying can be used to fabricate both micro- and nanospheres of polymeric materials. In this method charge is injected at a controlled rate (i.e., at a controlled current) into a polymer solution contained in a nozzle such that the surface of the solution meniscus at the nozzle opening becomes highly charged. The charged meniscus surface is thus under the electrical tension forces that push the meniscus surface away from the nozzle, opposing the surface tension force that tries to minimize the meniscus surface area (Figure 2.7). The electrical forces increase with increasing charge injection, finally overcoming the surface tension force and ejecting the charged solution from the meniscus surface. The net result is that with an increase in the charge injection, there will be gradual reduction in the size of the drops that leave the nozzle. This so-called “drip mode” develops into a “single-jet mode” with further increase in the charge injection. The jet mode is invoked when the electrical tension forces at the charged meniscus surface are such that the charged solution is literally pulled away from the nozzle orifice as a thin jet, which in turn naturally breaks up into charged droplets due to the jet instability. As the charge injection is further increased, the “single-jet mode” develops into a “multijet mode” where more than one jet emanates from the charged meniscus surface at the nozzle opening. Since the electrical tension forces acting on the meniscus surface are a function of the surface charge density, the effect of increasing flow rate is similar to that of decreasing charge injection. Figure 2.8 shows this sequence of
events that take place as the charging voltage (i.e., the charge injection) is increased, for a PLG solution. In the phenomena just described, generally known as EHD spraying, the typical flow rates used are necessarily very small. When fibers are produced as the final product, which is possible with polymeric solutions or material in molten state, the process is often referred to as electrospinning.

An alternative electrohydrodynamic method called flow-limited field-injection electrostatic spraying (FFESS) [86, 190] (Kim et al. 1994; Kim et al. 1995; Berkland et al. 2004) has been developed. FFESS can be used by itself alone or in tandem with the acoustic methods described in the preceding sections. In FFESS, charge injection is achieved by using a nano-sharpened tungsten needle connected to a high voltage in a process called field injection that is field emission or ionization depending on whether the polarity of the
needle is negative or positive. Thus, the FFESS process allows one to produce sprays that are finer and more precisely controlled than those produced by conventional EHD techniques, which employ conducting hypodermic needles as the spray nozzle (Figure 2.7). The main reason is that the sharp charge-injection electrode used in FFESS renders more control and stability, and higher charge injection (Kim, 1994 & 1995). For applications involving advanced drug delivery, the effects of the key parameters controlling the FFESS process, such as applied voltage, polymer solution flow-rate, and solvent properties (surface tension, viscosity, vapor pressure) have been qualitatively evaluated [19]. For example, using the FFESS system a wide variety of uniform micro- and nanoparticles of PLG have been created. (Figure 2.9). By discretely varying production parameters, subtle changes in deposited polymer morphology are realized potentially resulting in enhanced performance of a medical or biological device. FFESS technology thus provides a simple and robust technique for fabricating devices with a precisely defined nano-structure from a broad range of biocompatible polymeric materials.

2.4. CONTROLLED RELEASE FROM PRECISION MICROSPHERES

Presented in this section are a brief review of the controlled-release literature and the results from several controlled release studies that have been carried out with PPF-produced microspheres. The data clearly show that release of model small-molecule drugs can be varied from typical diffusion-controlled profiles to slower, sigmoidal profiles as microsphere diameter is increased in the range of 10–100 µm. The data also show that drug release from mixtures of uniform microspheres corresponds to a weighted average of the release from individual uniform microspheres. As a result, it has been possible to choose appropriate mixtures to generate desired release rate profiles, in particular constant (i.e., zero-order) release. It is, therefore, concluded that microsphere mixtures with well-defined size distributions may provide a general methodology for controlling drug release rates [15, 17–18].
2.4.1. In-vitro Release from Uniform Microspheres

Long-term zero-order release of small-molecule therapeutics from biodegradable microspheres has been difficult to achieve. Release of small compounds is often rapid and diffusion controlled [23, 82]. Often, release of small molecules encapsulated in polymeric particles typically is dominated by a large initial rate of release (or “burst”) in the first 24 h, offering little advantage over conventional oral dosage forms [27, 67, 92, 137, 175, 178].

To examine the effect of microsphere size and size uniformity on drug release kinetics, we measured release profiles for two model drugs, rhodamine and piroxicam, from PLG spheres. Rhodamine release profiles are shown in Figure 2.10A. As expected, 20-µm microspheres exhibited a faster initial release than 65-µm microspheres, likely due to the increased surface-to-volume ratio of the smaller particles. Further, as drug loading increased, the initial rate of drug release increased. An interesting concave-upward (i.e., sigmoidal) profile was observed with the 65-µm particles and to a lesser extent with the 45-µm particles, wherein drug release was initially slow, then progressed to a more rapid release phase before leveling off [37, 165].
Piroxicam release profiles show similar trends (Figure 2.10B). Samples of 10-, 50- and 100-µm microspheres were studied. The microspheres span a broader size range than the rhodamine-loaded particles, resulting in a more pronounced difference in drug release profiles. The smallest microspheres (10-µm diameter) exhibited a rapid initial rate of release, with 40–60% of encapsulated piroxicam released within the first 24 hours. Initial release rates decreased with increasing microsphere diameter for all drug loadings examined. Further, the initial release rate decreased with increasing drug loading. Interestingly, the 50- and 100-µm particles exhibited sigmoidal release profiles similar to rhodamine release from 65-µm microspheres.

Modeling of release has elucidated the mechanisms controlling the varying shapes of the release profiles \[16, 143\]. The model assumes a simple diffusion-controlled release but incorporates initially non-uniform drug distribution in the microsphere and a time-dependent diffusivity. We showed that the effective drug diffusivity increased with decreasing polymer molecular weight caused by degradation. In this way, we are able to accurately predict the shapes and kinetics of the small-molecule release profiles. This model will facilitate design of microsphere systems to provide desired release rates.

2.4.2. In-vitro Release from Mixtures of Uniform Microspheres

Constant release is highly desirable for many drug delivery applications. Because there is a transition from the concave downward to sigmoidal release profiles as sphere size increases, it appears that nearly linear release may be achieved at a certain size. For example, between 10- and 50-µm, a microsphere size may exist that would provide zero-order piroxicam release over a 4- to 8-day duration (c.f. Figure 2.10). Others have reported linear or near-linear release profiles achieved with microspheres of similar size, ∼30–50 µm in diameter \[21, 110, 189\]. For example, Woo et al. formulated a leuprolide delivery system using PLA microspheres with an average diameter of 51.7 µm achieving near-linear peptide release for 135 days following a 15-day period of “diffusion-controlled release” \[189\]. This early phase of release may result from the portion of the microspheres in this formulation under ∼35-µm, which would be expected to release drug more rapidly. Further, Bezemer et al. used a poly(ethylene glycol)-poly(butylene terephthalate) (PEG-PBT) block copolymer to test the effects of microsphere size on drug release \[21\]. They also discovered that decreasing the average microsphere size from 108 µm to 29 µm causes the release kinetics to change gradually from zero-order release to release controlled by Fickian diffusion.

Other researchers have suggested that drug delivery rates may be controlled by mixing microspheres of varying sizes or characteristics. For example, Ravivarapu et al. mixed microspheres comprising 8.6-kD or 28.3-kD PLG encapsulating leuprolide acetate \[144\]. The low-molecular-weight polymer resulted in porous, quickly releasing microspheres while the high-molecular-weight formulation resulted in dense microspheres and produced a sigmoidal release profile. By mixing microspheres comprising the two polymers, release rates could be tailored, and the resulting profiles were linear combinations of those resulting from individual microspheres. Bezemer et al. produced linear lysozyme release over 25 days from PEG-PBT microspheres having a bimodal size distribution dominated by 50-µm and 110-µm particles (in essence a combination of two sizes) \[21\]. Finally, Narayani et al. combined gelatin microspheres of various size ranges producing zero-order release of methotrexate \[128\]. In general, however, zero-order release has been difficult to achieve...
Based on the different shapes of the uniform PPF microsphere release profiles, and given the reproducibility of the PPF methodology for uniform microsphere fabrication, it was reasoned that it may be possible to modulate release kinetics in a desired fashion by mixing appropriate proportions of two or more uniform microsphere preparations (Figure 2.11) [21–22, 128]. To investigate the generality of this hypothesis, we mixed uniform microspheres of different sizes to generate zero-order release of rhodamine and the clinically relevant NSAID, piroxicam. For example, multiple linear combinations of 10-, 50- and 100-µm piroxicam-containing microspheres at various drug loadings were examined computationally to identify a combination resulting in linear drug release. Two possible formulations were found. The first formulation combined 10-µm/15% and 50-µm/15% microspheres in ratios of 3:1, 1:1, and 1:3 wt:wt. This formulation resulted in slightly concave

![Figure 2.11](image-url)
downward release profiles for the 3:1 and 1:1 ratios and a linear drug release profile for the 1:3 wt:wt mixture (Figure 2.11B).

In summary, because release kinetics from uniform spheres are very predictable and reproducible, our ability to fabricate uniform microspheres enhances this technique. We found that upon mixing uniform microsphere preparations, the resulting release profile is a mass-weighted average of the release profiles of the individual microspheres. This demonstrates that the microspheres release their payload independently; there is no interaction between the particles. In these experiments, the shapes of the rhodamine and piroxicam release profiles were such that it was possible to choose appropriate microsphere mixtures that provided zero-order release kinetics (Figure 2.11). However, it may not always be possible to generate a desired release profile from mixtures of only two microsphere sizes. Depending on the desired profile and the shape of the individual release curves, one may need to mix multiple microsphere samples or to fabricate complex microsphere size distributions. Because the PPF method provides a unique ability to generate predefined microsphere sizes [147], this technology may lead to enhanced control of release rates.

2.4.3. In vitro Release with Double-Wall Microspheres

Double-walled microspheres represent an increasingly important class of drug delivery devices that provide enhanced control of drug delivery schedules. Clearly, the overall particle size and shell thickness are important parameters in modulating the drug release rates. In one study, PPF was used to fabricate double-walled microspheres of predefined uniform diameters of 40–60 µm exhibiting a PLG core and poly(l-lactide) (PL) shell of controllable thickness from approximately 2 to 10 µm [13]. In vitro release of piroxicam from uniform microspheres of pure PLG and PL is compared to release from double-walled microspheres exhibiting different PL shell thicknesses in Figure 2.12. The benefit of utilizing a PL shell around the PLG core is threefold. First, the presence of the PL shell minimizes the initial drug “burst” so often associated with microparticle-based drug delivery systems. Secondly, by

![Figure 2.12](image-url)

**Figure 2.12.** In vitro release of piroxicam from PPF particles with drug-free PLL shells and PLG cores containing piroxicam. The legend shows the weight ratios of PLL:PLG.
specifically controlling the mass ratio of PL:PLG and thus the resulting PL shell thickness, the release of piroxicam can be progressively modulated from biphasic (no shell) to zero-order (thickest shell in Figure 2.12). Lastly, the duration of piroxicam release was extended from one month to three months utilizing the same molecular weight PLG. The ability to specifically control double-walled microsphere shell thickness may provide a novel and precise approach to modulate drug release profiles.

2.4.4. Release of Macromolecules from Monodisperse Microspheres

Macromolecular therapeutics such as peptides, proteins and DNA are advancing rapidly toward the clinic. Because of typically low oral bioavailability, invasive delivery methods such as frequently repeated injections are required. Parenteral depots including biodegradable polymer microspheres offer the possibility of reduced dosing frequency but are limited by the inability to adequately control delivery rates.

The release of macromolecules typically exhibits an initial “burst” of drug, which can be as much as 10–50% of the drug load, followed by a “lag” phase exhibiting slow release and finally a period of steady release [10, 151, 184]. The initial burst of protein therapeutics has been attributed to their tendency to partition to the microsphere surface during the encapsulation process [61]. Multiple approaches have attempted to alleviate the burst by adding excipients to the polymer phase [80, 185], utilizing novel polymers [70, 166, 168, 181, 195], encapsulating particulate forms of the drugs [33], or exchanging the non-solvent aqueous phase used in the fabrication process with non-polar oils or alcohols to reduce the affinity of the encapsulated drugs for the bulk phase [57, 174]. The burst is usually followed by a lag period where diffusion is limited and little release of the macromolecule occurs. The duration and flatness of this phase is determined by the polymer degradation kinetics, particle size, and microsphere porosity [10]. In addition, the drug size, charge, and any potential interactions of the drug with the polymer can influence the lag phase [30]. Finally, the lag phase is followed by a period of steady release typically controlled by the polymer degradation rate.

To control release and investigate release mechanisms, model macromolecules were encapsulated in uniform poly(ε-caprolactone-coglycolide) (PLG) microspheres using PPF in combination with a double-emulsion method. The precision particle fabrication (PPF) technology described in Section 3 allowed the production of these monodisperse microspheres [12, 14]. Fluorescein-dextran (F-Dex) and sulforhodamine-labeled bovine serum albumin (R-BSA) were encapsulated in PLG microspheres of three different sizes; 31, 44 and 80 µm and 34, 47 and 85 µm diameter for F-Dex and R-BSA, respectively (Figure 2.13). Notably, the initial burst of drug often observed for release of hydrophilic macromolecules was not observed. At 28 hours, F-Dex-loaded microspheres had only released 1.2% of the total encapsulated drug for all three microsphere sizes while R-BSA-loaded microspheres released 1.9–4.8% depending on microsphere diameter. With the uniform microspheres, the duration of the slow release or “lag” phase varied with the microsphere diameter. In addition, the slow release phase for these formulations appears to be a diffusion-controlled process with rates that decrease with increasing sphere diameter. Plots of amount released versus the square root of time show that the diffusion-controlled phase lasts for 25–200 days, depending on the microsphere size.
Release from uniform microspheres showed only two phases: a lag and more rapid release. F-Dex release profiles were very distinctive for the three different sizes. The diffusion-controlled lag period was followed by a sharp increase in release rate (Figure 2.13). The time at which the release rate increased was strongly dependent upon but inversely proportional to microsphere size. Again, after the lag periods, release rates of molecules increased dramatically. Thus, uniform microspheres represent a new delivery system for therapeutic proteins and DNA and provide unprecedented control of delivery rates using simple injectable depot formulations.

2.5. CONCLUSIONS

The development of PPF technology has allowed the production of uniform microspheres and double-wall microspheres capable of efficiently encapsulating model drugs. Of primary importance was the ability of monodisperse microsphere formulations to eliminate
initial drug burst while modulating the onset of steady drug release. Modified PPF technology has also been established as a single-step method for producing uniform polymeric microcapsules of controllable size and shell thickness. Monodisperse or precisely defined particle size distributions can be achieved while maintaining the desired polymeric shell thickness. Exact control of the volumetric flow-rates of the core and shell materials also allows the formation of particle populations exhibiting discretely or incrementally increasing shell thickness. Controlled release systems, especially those comprising biodegradable polymer microparticles, have been heavily studied and have even reached the clinic in several cases. However, notable limitations remain, especially in controlling delivery rates. Monodisperse PPF microspheres and core-shell microparticles offer advantages in reproducibility, control, and consistency that may provide valuable assistance in designing advanced drug delivery systems. The FFESS technique is capable of producing nanometer-scale solid particles as small as 10 nm or even smaller, and may be applicable to fabrication of nanocapsules. However, to achieve precise control of the particle size and reproducibly fabricate nanocapsules the technology needs to be further refined and developed.

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