International Journal of Pharmaceutics 407 (2011) 1–11

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy?

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 1 January 2011
Accepted 12 January 2011
Available online 21 January 2011

Keywords:
Monodispersed microparticles
Biodegradable polymer
Controlled delivery
Pharmaceutical field

ABSTRACT

Drug delivery *via* biodegradable microparticles benefits from both the protection of the encapsulated drug from hazardous conditions and the controlled release of the encapsulated drug, thereby reducing the administration frequency and improving patient compliance. Microsphere-size particle distribution is considered as being an important factor that affects the choice of the administration route and the drug-release rate. Significant research efforts have been directed towards the production of monodispersed "designer" particles. Amongst various techniques, some have been examined from lab-scale to industrial-scale. This review provides a global overview of monodispersed microparticle production methods and then focuses on recent processes being used to produce biodegradable microparticles applied in the pharmaceutical field. Further discussion about the choice of process according to the microparticle objectives of use is suggested.

Contents

1. Introduction

Since microencapsulation technology was first studied in the 1930s, a great deal of research has focused on drug encapsulation and drug delivery (Freiberg and Zhu, 2004). To be effective, microspheres must fulfil certain criteria: (i) high encapsulation efficiency, (ii) preservation of drug activity during encapsulation and storage, (iii) easy administration to the target site and (iv) controlled release rate to achieve a therapeutic effect while minimising side-effects. The administration of drugs *via* microspheres benefits from both the protection of the encapsulated drug from hazardous conditions and a release profile for a desired period. The administration frequency is thus reduced and patient comfort and compliance are

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doi:10.1016/j.ijpharm.2011.01.027

improved. Various microsphere materials have been used for drug-controlled delivery (Haider et al., 2004; Kim, 1994; Passerini et al., 2003). As the lack of biodegradability in some systems implies the requirement of eventual surgical removal, major research has been focused on biodegradable polymers for drug delivery. Biodegradable and biocompatible microspheres can be achieved by using natural polymers such as alginate, chitosan, collagen or synthetic polymers made from naturally occurring monomers such as lactic and glycolic acids (Boontheekul et al., 2005; Lee et al., 2001; Ma et al., 2001; Tatard et al., 2005b). The use of natural polymers, especially alginate, must be considered carefully since they can be widely contaminated by endotoxins or immunogenic proteins (Jork et al., 2000). From its discovery in the 1970s, the synthetic, biodegradable and biocompatible poly (lactic acid) (PLA) and its copolymers poly (lactic-co-glycolic) acid (PLGA) have become the most popular polymers used due to their lack of contamination sources and their controllable degradation rate *via* their molecular weight and the modification of the ratio of lactic acid/glycolic acid (Edlund and Albertsson, 2002; Stevanović and Uskoković, 2009). Recently, there has been a trend of using copolymers of PLGA and poly (ethylene glycol) (PEG), PLGA–PEG–PLGA, in drug delivery, especially for protein delivery, due to their highly hydrophilic characteristics that facilitate drug release by diffusion (Kissel et al., 2002; Paillard-Giteau et al., 2010).

The use of microparticles must initially take into account the chosen administration route. For oral administration, although there is no upper limit of microsphere size for administration, it has been found that decreasing the microsphere size from 7.2 μm to 2.1 μm doubled gastrointestinal adsorption (Gaumet et al., 2009; Lamprecht et al., 2001; Wei et al., 2008a,b). For the pulmonary route, microspheres should be around 3 μm to achieve good results (Mohamed and Van Der Walle, 2008; Rawat et al., 2008). For subcutaneous, intramuscular or intravitreal administration routes, microspheres should be in the range of 10–250 μm in order to avoid particle uptake by macrophage phagocytosis and to minimise inflammatory reaction. (Anderson and Shive, 1997; Gasparini et al., 2007; Herrero-Vanrell and Molina-Martinez, 2007; Katare et al., 2005; Senuma et al., 1999; Thomas et al., 2010; Yamamoto et al., 2002). In the case of specific organs such as the brain, microsphere size should not exceed 100 μm so as not to disturb the 3D structure of the brain (Tatard et al., 2005a). Microspheres should therefore be sufficiently large to contain a reasonable amount of active ingredient but not so large as to cause discomfort upon administration (Mitragotri and Lahann, 2009; Wang et al., 1997).

The encapsulation yield and drug distribution must be carefully considered in order to avoid side effects, especially for narrow, therapeutic index drugs such as anticancer drugs, antibiotics or proteins. By sieving the 5-FU-loaded PLGA microspheres of the same formulation into various fractions of size from under 35 μm to 125 μm, Siepmann et al. (2004) found that initial drug loading increased with increasing microsphere size because large microspheres could contain both large and small drug crystals whereas only small ones could be encapsulated into small microspheres. On the contrary, by encapsulating piroxicam and rhodamine B into PLGA microspheres, Berkland et al. (2003a, 2004b) found higher encapsulation efficiency in small microspheres of 10–20 μm and large microspheres of 100–120 μm than with medium microspheres of 40–50 μm when the drug was soluble in polymer solvent. The practical encapsulation yield in this case was thus the result of competition between drug diffusion and the polymer precipitation rate (Dawes et al., 2009). Furthermore, drug distribution in microspheres is influenced by microsphere size. For small microspheres (10–20 μm), the drug was distributed homogeneously throughout the microspheres (Berkland et al., 2003a, 2004b). For microspheres larger than 40 μm, hydrophilic drugs had a high tendency to be distributed near the surface whereas hydrophobic drugs shifted towards the microsphere core (Berkland et al., 2003a). Microsphere size influenced drug distribution and thus affected the drug-release profile.

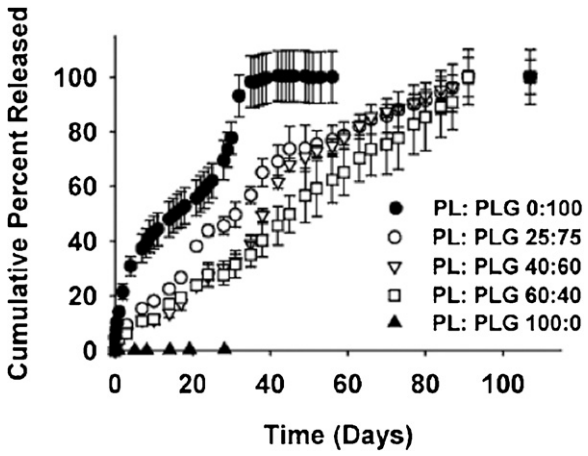

Fig. 1. *In vitro* piroxicam release from uniform PLG and PL microspheres and PL-PLG double-wall microspheres.
Reprinted from Berkland et al. (2004a) with permission from John Wiley and Sons.

At the initial stage of the release profile, the larger surface area of the smaller particles led to faster drug release (Berkland et al., 2003a; Klose et al., 2006; Leach et al., 2005); whereas larger particles with larger cores increased the length of the diffusion pathways and decreased drug concentration gradients; drugs thus took longer to be released (Bezemer et al., 2000; Dunne et al., 2000; Redhead et al., 2001; Siepmann and Göpferich, 2001). At the latter stage, microsphere degradation interfered with the release rate. In the case of PLA and PLGA microspheres, due to the autocatalytic effect, the limited diffusion of oligomers in large microspheres increased the degradation rate and transformed the slow-release phase into a faster one (Berchane et al., 2007; Dunne et al., 2000). The onset-time of this transition phase decreased with increasing microsphere diameter (45 and 20 days for 34 and 85 μm microspheres, respectively) (Berkland et al., 2007a). A constant (i.e., zero-order) release rate is often preferred to maintain an effective drug concentration in the host tissue (Chen and Lu, 1999). The release profile can be adjusted by varying the ratio of microspheres of various sizes to obtain a zero-order release rate (Berkland et al., 2007a; Pollauf et al., 2005).

Core–shell microspheres provide an alternative to control the drug-release rate. When a drug is localised in the core matrices, the shell prolongs the diffusion path of water-in and drug-out, and hence the initial burst release can be limited (Lee et al., 2002). A polymeric shell (e.g. PLA) could delay the degradation of a polyester core, and increasing the PLA shell thickness shifts the release profile from a biphasic shape for pure PLGA microspheres to zero-order piroxicam release over 3 months for the thickest (10 μm) PLA shells (Berkland et al., 2004a) (Fig. 1). Furthermore, by switching the position of bone morphogenic protein (BMP) and dexamethasone in PLGA core and alginate shells, Choi et al. (2010) altered their release profiles. When these microspheres were then incubated with rat, bone-marrow stromal cells, a different production of osteogenic material was observed. However, conventional core–shell microparticles could be obtained by encapsulating a preformed core into the shell matrix or by using the phase-separation phenomenon of two polymers in solution when a critical concentration is reached (Choi et al., 2010; Lee et al., 2002; Wang et al., 2010). For the latter process, the inherent thermodynamic properties of the system such as polymer chemistry, core–shell mass ratio, and drug–polymer affinity, limited drug distribution and particle architecture (Lee et al., 2002; Mathiowitz and Langer, 1999;

Table 1
Conventional microencapsulation process.

From monomer	From polymer
	Coacervation (complex or simple)
Polycondensation	Solvent evaporation/extraction emulsion (rotor-stator, homogenisation, ultrasound)
Polymerisation	Spraying
	Extrusion
	Jet break-up

Shi et al., 2003). These conventional core–shell microparticles were often large (>150 μm) and polydispersed (Lee et al., 2002). It was therefore desirable to find a technique that allowed the production of multi core–shells with selective polymer and drug localisation as well as controlling the particle size.

Conventional microencapsulation processes can be classified as shown in Table 1 according to the initial material (from monomer or polymer). Microencapsulation processes from polymers are not classified because some techniques can be hybrids of two or more methods or can use different mechanisms simultaneously (e.g. Jet break-up process can be used with solvent extraction/evaporation or thermal gelification). Depending on the polymer type, additive, solvent and process parameters, the microsphere size and size distribution can be modified (Berchane et al., 2007; Dawes et al., 2009; Giteau et al., 2008; Klose et al., 2006; Senuma et al., 1999). However, this factor can vary from batch to batch and from lab to lab (Gong et al., 2007; Johnson and Tracy, 1999). A process for monodispersed microsphere production could be an answer to the problem of variability. A microencapsulation process from monomers could lead to monodispersed microspheres. However, their size is limited to several micrometres and, due to the potential toxicity of monomers, this process is not widely employed for pharmaceutical application (Arshady, 1989; Song et al., 2009). For all processes with preformed polymers, the initial step is droplet formation that in turn defines the size and size distribution of the resulting microparticles. The droplet is most commonly formed with conventional turbulence-based methods, such as homogenisation, rotor-stator systems, ultrasound or spraying (Freitas et al., 2005; Li et al., 2008). Although these processes are relatively easy to do, droplets are formed randomly and size distribution is wide, finally resulting in broad microsphere size distribution. By applying an electric charge to the droplets, Hong et al. (2008) managed to separate the satellite droplets and main droplets in the spraying method due to their different mass, and quasi monodispersed microspheres were obtained. However, the loss of initial materials could not be avoided. To develop monodispersed microsphere encapsulation processes, the control of droplet size is required (Charcosset and Fessi, 2005; Charcosset et al., 2004; Serra and Chang, 2008). This review provides a global overview on monodispersed mono or multi core–shell microparticle production methods, and then focuses on recent processes that are being used for the preparation of biodegradable microparticles applied in the pharmaceutical field.

2. Preparation of monodispersed microparticles

Processes used to prepare monodispersed particles can be classified into two types: microfluidic devices and jet break-up. In microfluidic devices, the dispersed phase is pushed through a micro-channel and broken into droplets at the end of the channel. In the jet break-up technique, the dispersed phase is pushed through a nozzle to form a jet and then broken up into a chain of monodispersed droplets.

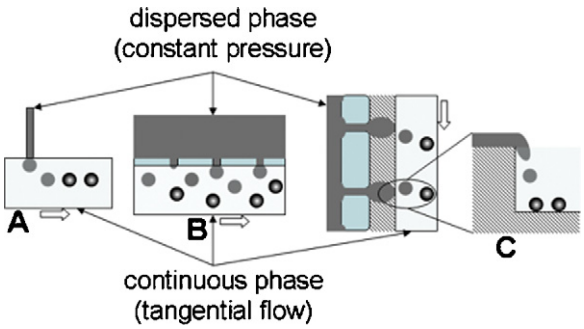

Fig. 2. (A) A microfluid device process syringe infusion pump, (B) microporous membrane and (C) microchannel device.

2.1. Microfluidic devices

The simplest lab-scale process for uniform microsphere formulation was developed by Amsden et al. (Amsden, 1999; Amsden, 2001) and was named the 'syringe infusion pump' (Fig. 2A). This method was developed from the gravity drip casting method, where the polymer phase was dripping from the nozzle to create monodispersed droplets under the force of gravity. However, the production rate of the gravity casting method is ultimately low. A syringe was applied to push the polymer solution more quickly. Briefly, a continuous phase of PLGA in dichloromethane (DCM) is continuously injected *via* a stainless-steel, blunt-ended needle into a perpendicular flow of an aqueous polyvinyl alcohol (PVA) solution used as the continuous phase. Mean microsphere size varied between 68 and 295 μm (CV = 5–35%). In the same way, by injecting a suspension of BSA in PLGA/acetonitrile solution into a flowing continuous phase of cotton seed oil, through a 120 μm needle, Leach et al. produced monodispersed microspheres of 123 μm (CV = 6%) (Leach et al., 2005). A reduction of the injection diameter and an increase of the continuous phase velocity decreased microsphere size (Amsden, 1999; Leelarasamee et al., 1988; Xu et al., 2009a); this process is unsuitable for the production of small microspheres (<30 μm) and their throughput was quite low (<2 mL/min) (Amsden, 2001). Scaling-up may be feasible through the parallel use of a quantity of needles. However, no information about the retention of particle distribution was made. More widely used, microporous membrane (Fig. 2B) and microchannel devices (Fig. 2C) are discussed below.

2.1.1. Microporous membrane emulsification

The Shirasu Porous Glass (SPG) membrane emulsification technique was first proposed by Nakashima and Shimizu (Kandori et al., 1991) and later developed by Omi et al. (Omi, 1995; Omi et al., 1995) and Ma et al. (1999). Dispersed drops are generated at the membrane surface by pushing the dispersed phase through the membrane pores into the continuous phase under applied pressure of about 0.5–5 bars from nitrogen gas (Fig. 2B). The membrane pore size exerted the most dominant influence on droplet size distribution (Lambrich and Schubert, 2005). Droplet size ranged from 2 to 10 times the membrane pore diameters, depending upon the interfacial tension between the dispersed phase, the continuous phase and the membrane surface with the flow shear stress level (Joscelyne and Tragardh, 2000; Makino et al., 2004). Given the rapid progress in micro engineering, membranes with pore sizes ranging from 0.49 to 40 μm have been achieved (Gasparini et al., 2007; Omi et al., 1995; Sugiura et al., 2002a). Microspheres produced with this process ranged from 100 μm to around 10 μm (Gasparini et al., 2007; Shiga et al., 1996; Wei et al., 2008a,b). Recently, another alternative named the 'premix-membrane' was developed to increase the production rate and to obtain microspheres of 1–2 μm (Doan

and Olivier, 2009). In this alternative, a coarse emulsion O/W was prepared classically by stirring and then pushed to pass through the membrane for many cycles in order to obtain a fairly narrow degree of distribution.

The process should avoid wetting the membrane so as to avoid blocking the membrane pores. The most commonly used membrane for the preparation of emulsions is the SPG (Vladisavljević and Schubert, 2003; Williams et al., 1998). This membrane contains monosized pores in a cylindrical shell tube consisting of hydrophilicsillimanite (Al_2O_3–SiO_2) (Omi, 1995). Due to its hydrophilicity, this membrane is an appropriate choice for oily-dispersed phase emulsions (O/W emulsion). The hydrophilicity of the membrane can be altered by changing the surface chemistry; this needs to be repeated at the beginning of each formulation (Lau et al., 2009). Silicone-based membranes have been used recently due to their anti-adherence properties, which makes them suitable for the production of various emulsion types (O/W, W/O, W/O/W, S/W/O, and S/O/W) (Lau et al., 2009; Veldhuis et al., 2009).

Although the membrane process offers the opportunity of producing emulsions with narrow droplet size distribution, without high mechanical stress, and with low energy input (10^4–10^6 J/m^3) compared to conventional mechanical methods (10^6–10^8 J/m^3), the process is limited due to their low production rates of between 0.01 and 10 mL/h as well as the coalescence of microparticles (Sugiura et al., 2005). These limits are mainly due to the porosity of the membrane. Firstly, the porosity of the membrane determines the distance between two adjacent pores. This distance is critical to ensure that two adjacent droplets do not come sufficiently close to each other to make contact, which may lead to coalescence (Abrahamse et al., 2002; Charcosset et al., 2004; Williams et al., 1998). Secondly, all the pores do not become active at the same critical pressure, even though they have the same diameter. Indeed, the pressure drops under the membrane as soon as the disperse phase flows through some pores, and hence prevents other pores from becoming active (Vladisavljević and Schubert, 2003; Wang et al., 2005).

2.1.2. Terrace-like microchannel system

Nakajima et al. first applied this system in emulsification technology (Kawakatsu et al., 1997; Sugiura et al., 2002c). The microchannels open up to a terrace that descends to a well through which the continuous phase slowly passes. The dispersed phase is pressed through a channel and spread over the terrace until it reaches the rim of the well. When flowing over the rim into the well, interfacial forces contract the fluid to form a droplet (Fig. 2C) (Kawakatsu et al., 1999; Kobayashi et al., 2003; Sugiura et al., 2002b). Droplet formation is only governed by interfacial forces, leading to monodispersed droplets (Kawakatsu et al., 1997). Droplets from a few micrometres up to 100 μm, with a relative standard deviation lower than 5% were produced (Ikkai et al., 2005; Iwamoto et al., 2002; Sugiura et al., 2002a). Although the interfacial force was the main factor creating droplet formation, there was no direct link between the static interfacial tension and the resulting droplet size (van Dijke et al., 2010). The geometry of the microchannel plate, however, had a dominant effect on the microparticle size (Lambrich and Schubert, 2005). Larger microspheres were prepared using a microchannel with deeper and longer terraces. However, it is difficult to fabricate microchannels deeper than 16 μm and terraces exceeding 240 μm by the wet-etching process (Sugiura et al., 2002a). The miroparticle size was thus limited to less than 100 μm.

Since no additional forces for droplet detachment are necessary, the process conditions for microchannel emulsification are relatively easy (Lambrich and Schubert, 2005). However, the production rate is still limited by the dispersed phase flow rate. This process has been used for the production of monodispersed microparticles of gelatin and alginate (Chuah et al., 2010; Iwamoto et al., 2002); the drug encapsulated into these microparticles was not reported. Another limitation of membranes and microchannels is the need for low viscosity solutions. Other processes reported below can use viscous solutions.

2.2. Jet break-up process

In the jet break-up processes, a polymeric solution is pushed through a nozzle at a constant flow rate, forming a laminar jet. The jet is then broken into a chain of monodispersed droplets. Depending on the mechanism of jet break-up used, various processes exist as shown in Fig. 2.

2.2.1. Electrostatic droplet generation

In this process, an electric current is applied between the nozzle and the hardening solution (Fig. 3A). Under the electrostatic force, the liquid breaks up into droplets. By selecting the appropriate conditions, the generated droplets can have a narrow size distribution (Amsden and Goosen, 1997; Bugarski, 1994a,b). There are two working methods in electrostatically assisted processes: the dripping mode and the jet mode.

In the dripping mode, the polymer solution is pushed gently through the nozzle where a low electric current is applied (up to about 4 kV). The liquid is broken up at the out-put of the nozzle due to the electrostatic force (Fig. 3A$_1$) (Moghadam et al., 2008). Microspheres of 500–1500 μm are obtained and the production rate is lower than 30 mL/h. Smaller microbeads can be attained by increasing the electric current and using a smaller needle (Moghadam et al., 2008; Poncelet et al., 1994; Xie and Wang, 2007). However, increasing the electric current induces a much broader diameter distribution (Amsden and Goosen, 1997; Bugarski et al., 1994a,b); the microsphere size is reduced to less than 100 μm but their CV increases to over 15%. The dripping mode is thus limited by low productivity and large microparticle size (Bugarski et al., 1994a,b; Heinzen et al., 2004; Lewińska et al., 2008; Xue et al., 2006).

At a higher velocity, a smooth and stable jet is formed, but a higher electric current is necessary to break the jet into small droplets (Fig. 3A$_2$). In this mode, the production of mono, small microparticles within 1–15 μm with high productivity can be achieved (Ding et al., 2005; Lee et al., 2010). However, in their work, the formulation of microspheres over 20 μm was not mentioned. This could be due to the high electric repulsion causing the break-up of the droplets.

The first advantage of this process is that the equipment is very simple and easy to operate. Furthermore, in the cone-jet mode, the microsphere size can be controlled by the electric current independently of the nozzle diameter, so a nozzle in the millimetre range can be used and the nozzle-blocking problem can thus be limited (Xie et al., 2008). Interestingly, the electrical charge on the droplets prevents their coalescence and the need for a surfactant is thus limited. It has been shown that proteins (BSA, lysozyme, bone morphogenetic protein) and cells can maintain their active forms and viability under electrostatic forces up to 10 kV (Lewińska et al., 2008; Strand et al., 2002; Wang et al., 2010; Xie and Wang, 2007; Xie et al., 2008). Although this process can work with non-Newtonian viscous liquids (Moghadam et al., 2008), highly viscous polymer solutions (15% PLGA/DCM) are usually elongated in the given condition, yielding micro- or nano-fibres (Choi et al., 2010; Fantini et al., 2006; Xie et al., 2008).

2.2.2. Jet-cutter technology

In this process, a dispersed jet is cut into uniform segments by means of a cutting tool consisting of several wires (Fig. 3B) (Prüße et al., 1998a,b). The height of these cylinders and, therefore, the diameter of the resulting droplet, is determined by the number of cutting wires, the number of rotations of the cutting tool, and the

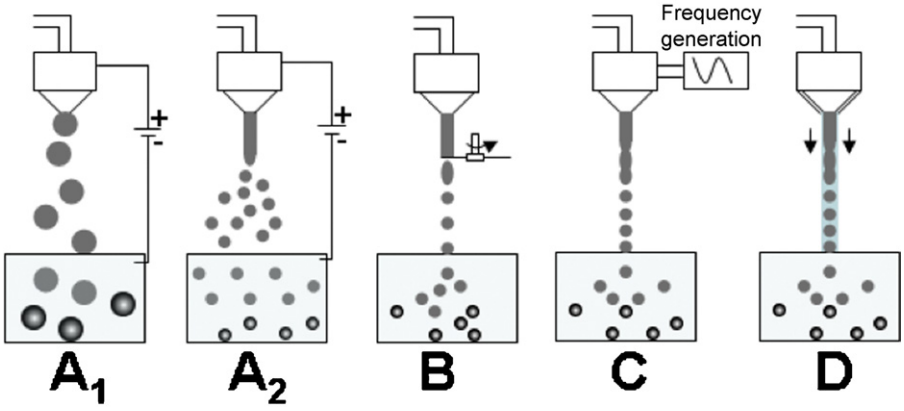

Fig. 3. Jet break-up processes: (A_1), (A_2) electrostatic generation, (B) jet cutter, (C) acoustic jet excitation, and (D) flow focusing.

mass flow through the nozzle (Prüße et al., 1998b). This encapsulation method is simple, efficient for the production of microparticles, even with highly viscous fluids (greater than 500 mPa s), and has a high flow rate (10–30 m/s) with correspondingly high production rates (Prüße et al., 2000, 1998a,b). However, microparticle size is large (from 200 μm to millimetre size range). Moreover, cutting through the liquid jet causes a cutting loss which is assumed to be in proportion to the size of the cutting wire diameter (Prüße et al., 2000). The occurrence of a cutting loss is inherent in this method and, therefore, cannot be completely avoided. Nevertheless, the reduction of cutting losses to a satisfying level (below 5%) is feasible (Prüsse et al., 1998; Prüße et al., 1998b). Although this process has been reported for the production of biodegradable, monodispersed microparticles such as alginate, pectin, chitosan and gelatine (Prüße et al., 2000), no information about drug encapsulation was found.

Other variations of jet cutters are rotary disc atomising and centrifugal disc atomising (Teunou and Poncelet, 2005). In these processes, the polymer solution is fed onto a rotary disc, and the polymer solution is projected at the edge of the disc under centrifugal force. In rotary disc atomising, a flat tray was divided into different parts and used to collect the particles of different sizes. By carefully controlling the process parameters, the centrifugal atomising disc can fabricate monodispersed microparticles. However, no report about drug encapsulation by using these methods has been found.

2.2.3. Jet excitation

In this method, the jet is broken into uniform segments by means of vibration excitation (Fig. 3C). The vibration of a liquid jet for its disruption into droplets was originally studied by Lord Rayleigh in the late 19th century (Rayley, 1879). A longitudinal oscillation imposed on a liquid stream causes periodic surface instability, which breaks up the liquid into a chain of uniform droplets. Weber (1931) extended the analysis by including the effect of viscosity (μ) into the analysis (Koch et al., 2003). The optimum wavelength (λ) for break-up is thus given by (Weber, 1931):

$$\lambda = 4.443 d_j \sqrt{\frac{3\mu}{\sqrt{\rho\sigma d_j}} + 1}$$

where d_j, ρ, σ indicate the diameter of the jet, the density, and the interfacial tension of polymer solution. One droplet was generated by each hertz of vibration (each sinus wave led to the formation of one droplet), the drop diameter, d_d, can be calculated as follows (Seifert and Phillips, 1997):

$$d_d = \sqrt[3]{\frac{3.d_j^2.\lambda}{2}}$$

Increasing polymer concentration and flow rate increased droplet size (Berkland et al., 2001; Mazzitelli et al., 2008; Seifert and Phillips, 1997). The longer the wavelength of the jet break-up, and the shorter the distance to the impact plane of the hardening bath, the less likely is drop coalescence (Brandenberger and Widmer, 1999; Seifert and Phillips, 1997). Thus, smaller nozzle diameters and higher frequencies increase the possibility of coalescence. The frequency is usually kept as low as possible in order to avoid the formation of satellite droplets leading to a broader size range (Del Gaudio et al., 2009).

This process has been used for the production of particles from nanometre to millimetre range (Dumas et al., 1992; Seifert and Phillips, 1997). The production of microparticles by the vibrating nozzle device is highly reproducible, time saving, can be performed under aseptic and scaled-up conditions (Zvonar et al., 2009).

2.2.4. Flow focusing

Flow focusing was first developed by Gañán-Calvo and Gordillo (2001). This process is based on the principle of hydrodynamic focusing. The dispersed phase flows into a central nozzle while an immiscible phase (aqueous or gaseous) is delivered through two side channels (Fig. 3D) (Gañán-Calvo, 1998). The outer phase has a flow rate several orders of magnitude higher than the inner dispersal phase (Anna et al., 2003; Schneider et al., 2008). Thus, the central stream is forced into a thin, jet-like stream and broken up into droplets smaller than the nozzle, with a narrow size distribution (Schneider et al., 2008).

The composition of the focusing fluid defines the respective method, either aerodynamic (i.e., using gas) or hydrodynamic flow focusing (i.e., using fluids). When gas is used as a focusing fluid, the monodispersity of droplets is characterised by the Weber formula:

$$\mathrm{We} = \frac{P_g v_g^2 d_j}{g}$$

where P_g, v_g, d_j and g are respectively gas pressure, gas velocity at the nozzle, diameter of the jet and surface tension of the jet. With an experimental value below 40, the resulting droplet stream is monodispersed. Increasing the gas pressure increases the We number. The resulting spray shows significant polydispersity in this case (Gañán-Calvo, 1998).

In the case of monodispersed microparticles, the diameter of microparticles (d_p) could be predicted by taking into account the polymer concentration (C), the polymer solution density (rp) the nozzle diameter (D), the focused and focusing fluid flow rate (Q_d

and Q_t):

$$d_p = \left(\frac{3pC}{2k\rho_p}\right)^{1/3} \cdot \left(\frac{Q_d}{Q_t}\right)^{1/2} .D$$

k is the wave number of the fastest growing perturbation on the jet (approximately $k \approx 0.5$ for most liquid–liquid combinations) (Martín-Banderas et al., 2010). The particle diameter is generally 1/10 to 1/30 the diameter of the orifice (Martín-Banderas et al., 2006).

Increasing the carrier stream flow rate and decreasing the polymer flow rate reduces the droplet diameter (Schneider et al., 2008; Gañán-Calvo et al., 2006). Although microparticle size obtained with this method is usually from 2–3 μm up to 50 μm, this method is mainly used for the production of microspheres under 10 μm (Xu et al., 2009b; Martín-Banderas et al., 2010). Holgado et al. (2009) compared lidocaine-loaded PLGA microspheres produced by the conventional solvent evaporation method, and the flow-focusing method. Microspheres obtained from the flow-focusing method were uniform and presented an acceptable CV of 15% smaller than ones obtained from the conventional stirring method (>23%) (Anna et al., 2003; Schneider et al., 2008; Xu et al., 2009b).

The method of flow focusing has many general advantages: (1) it is generally a simple one-step approach, (2) particle size can be adjusted by changing the fluid flow velocity of the two phases, (3) droplet size is not limited by the injector and orifice size (i.e., droplets can be much smaller than the orifice size), (4) the flow focusing process is scalable, and (5) offers the generation of droplets and microspheres at low cost (Hunik and Tramper, 1993; Martín-Banderas et al., 2005; Schneider et al., 2008). This method has mainly been reported for PLGA monodispersed microsphere production (Holgado et al., 2008, 2009; Schneider et al., 2008).

2.2.5. Precision particles fabrication

Berkland et al. (2001) also used an annular or 'carrier' stream of PVA 1% to decrease jet diameter of PLGA/DCM but jet break-up is always due to jet excitation. This method for uniform microparticle generation was named precision particle fabrication (PPF) (Fig. 4A). The 'carrier' stream help to reduce the diameter of the droplets to be smaller than the nozzle. The size of microspheres obtained from this method could be reduced to 6 μm (Fig. 4B–D). For one droplets diameter, the precision particle fabrication process can work with a larger nozzle than simple jet excitation process. The problems of nozzle clogging and high shear force were thus avoided. Furthermore, by simple modify the process parameters, from a 100 μm nozzle, a range of monodispersed microspheres from 40 to 60 could be obtained. In latter experiences, uniform microspheres from 1 to over 500 μm in diameter were obtained (Berkland et al., 2007a; Raman et al., 2005). Choy et al. (2007) also applied this process for the fabrication of a series of hydrogel microspheres of chitosan and alginate in a size range of 50–200 μm from the nozzle of 250 μm. The only limit of this method is the complexity of the apparatus.

2.3. Multi core–shell microspheres

The simplest multi core–shell microspheres were prepared with two different steps: a W/O emulsion was firstly prepared with a simple stirring method and then this emulsion was subsequently pressed through a nozzle and divided into monodispersed droplets using the flow flocusing method (He, 2008). Although the diameter of microcapsules was controlled, the number of cores in each microcapsules could not be mastered. Another alternative to control the core number in each microcapsules is to inject separately the core and shell solutions into two, co-concentric nozzles and then broken out into monodispersed droplets. The mechanism of droplet splitting can be electrostatic (Choi et al., 2010; Jaworek, 2008), by jet-cutter (Prüße et al., 2000) or by jet excitation (Berkland et al., 2004a). When the core solution has a high tendency to spread out into the shell, which results in an uncompleted core–shell structure, the shell solution concentration should be reduced to increase the fluidity which may in aid the spreading of the shell phase onto the core polymer. Moreover, dichloromethane was added into outer phase to extend the extraction time, allowing more time for complete capsule formation, and may also alter the interfacial tension between the polymer solution and the aqueous phase. (Berkland et al., 2004c). The thickness of the shell wall increases by increasing the inner space between the outer diameter of inner capillary and the inner diameter of the outer capillary or by increasing the flow rate of shell to core solutions (Berkland et al., 2007b; Lee et al., 2010). Furthermore, by controlling the droplet formation frequency between the inner phase and outer phase, the multi-inner core or unique core encapsulated in the unique shell wall can be achieved (Berkland et al., 2004a; Bocanegra et al., 2005). The liquid core can be essential oils, plant extracts (coffee, menthol, citrus oil, etc.), drugs or cells in a culture medium (Berkland et al., 2007b; Bocanegra et al., 2005; Martín-Banderas et al., 2005; Nedovic and Willaert, 2004). When the partition coefficients of the polymer in the solvent of the two phases are close to each other, the diffusion of the wall material into the capsule core can be observed (Nedovic and Willaert, 2004). When the viscosities between the core and shell solution are nearly similar, the break-up frequency of the core solution is less than that of the shell solution, and thus multi-core microcapsules are formed (Bocanegra et al., 2005). Bocanegra et al. (2005) studied the evolution of a concentric stream of three immiscible liquids forced through a small orifice to reduce core–shell microsphere sizes. The interfacial tension of the core-to-focusing liquid should be larger than that of the shell-to-focusing liquid to ensure that the shell liquid encase the core liquid (Bocanegra et al., 2005).

Another alternative is that the aqueous core and the polymeric shell solutions be separately injected into two different nozzles. The liquid streams are broken into a series of uniform droplets and the location of the nozzles is manipulated to cause collisions between the drops. The polymer covers the aqueous droplets forming the microcapsules (Langer and Yamate, 1969; Yeo et al., 2004). This process is limited by the complexity of the experimental set-up and the difficulty of maintaining monodispersity of the microcapsules.

2.4. Consolidation procedure

Once the droplets are formed, the consolidation procedure must take place as soon as possible to prevent either the aggregation of polymer droplets or the undesired leakage of medical drugs (Huang et al., 1999; Yang et al., 2007). The chemical nature of the droplet phase (dispersed phase) determines the next step, a consolidation procedure, in which the droplets are transformed into solid microparticles: this entails temperature modification, such as cooling for collagen or heating with an appropriate inlet temperature to evaporate the solvent for PLGA (Holgado et al., 2009), chemical reactions or ionic cross-linking for water-soluble polymers such as alginate (Seifert and Phillips, 1997), gelatine (Huang et al., 1999), chitosan, collagen (Dumas et al., 1992; Yang et al., 2007) or k-carrageenan (Hunik and Tramper, 1993) or solvent evaporation/extraction for oil-soluble polymers such as PLA, PLGA, PLGA–PEG–PLGA (Berkland et al., 2001; Leach et al., 2005). The solvent evaporation/extraction method can be carried out either in air or in liquid. The freezing method implies recovering the droplets in a frozen solvent (MeOH at −70 °C) to instantly precipitate droplets, and then the solvent gradually evaporates when the temperature increases, leading to solid microspheres. Although this process can be applied to various solvents, it is complex, expensive, and usually takes more than one day per

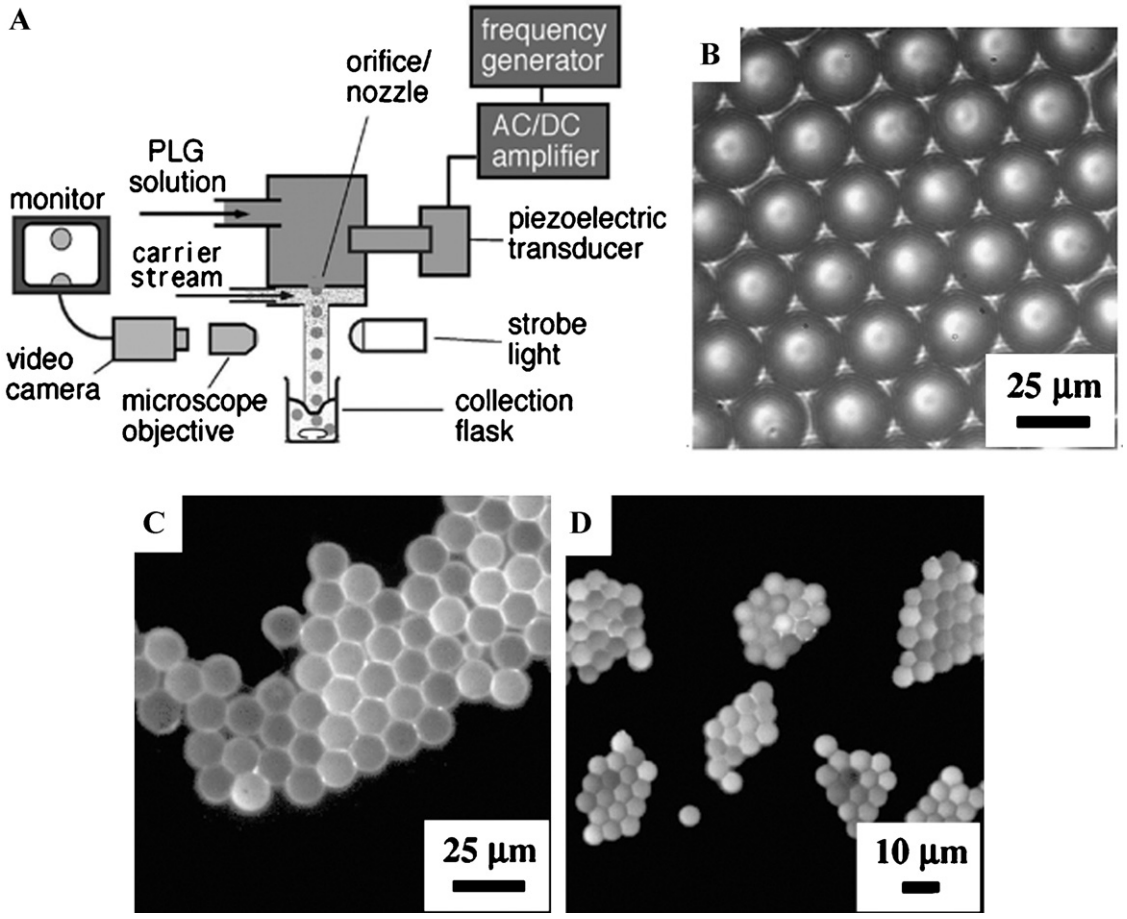

Fig. 4. Diagram of the microsphere generator with the precision particle fabrication process (A). Light and fluorescence micrographs of uniform PLG microspheres demonstrate the lower achievable limit of the size range. Sizes are approximately (B) 24 μm, (C) 11 μm, and (D) 6 μm.
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batch (Amsden and Goosen, 1997; Leach et al., 2005). For droplets smaller than 30 μm, the volatile solvent can be eliminated during the fall process under ambient temperature (for DCM) or in a thermostatic chamber (55 °C for ethyl acetate) to obtain the hardened microspheres (Gañán-Calvo and Gordillo, 2001; Xie et al., 2008). However, the solvent evaporation rate should be taken into consideration in order to avoid rough microsphere surface morphology (Xie et al., 2008). The conventional evaporation/extraction of DCM in a solution of 1% PVA can also be used (Berkland et al., 2001).

During the hardening process, the droplet sizes can shrink. This shrinkage is influenced by the type of polymer, polymer concentration, the hardening medium, and the ratio of polymeric phase/hardening medium (Hunik and Tramper, 1993; Yang et al., 2007). Depending on the polymer concentration within the droplet, the size of the final polymer particle is usually smaller than that of original droplet by 2–30%, due to the higher density of the polymer (Serra and Chang, 2008; Berkland et al., 2001).

3. Monodispersed microparticle production scale-up

It is well known that a number of problems may be associated with the conventional, existing methods of production. The energy needs for the large-scale production of emulsions using traditional methods (rotor-stator) is high, and gets worse as the vessel size increases. This can lead to increasing the complexity of scaling-up. The electrostatic method has not been reported, most probably for safety reasons. Furthermore, due to the cutting loss of the jet-cutter, this method has not been considered as being appropriate for scaling-up. Nevertheless, pilot scale-ups for microporous membranes and acoustic excitation have been performed. Using the membrane emulsion process, Veldhuis et al. (2009) achieved the production of a large number of biodegradable microspheres of PLGA, PCL, etc. The production rate was successfully scaled up to 1 kg/day. Williams et al. (1998) reported the pilot scale-up using continuous membrane production; the uniformity of the droplets was constant between seven batches. Furthermore, it was stated that by using this process, the original structure of fragile drugs and the viability of encapsulated cells were preserved (Sugiura et al., 2005). A commercial membrane emulsification system for uniform microspheres named Nanomi is now on the market.

The jet-excitation process was patented for the production of uniform microspheres of alginate (Brandau, 1995), collagen (Dumas, 1992) and PLGA (Berkland et al., 2003b; Kim et al., 2008). The scale-up of the vibration process is easily done by using a multi-nozzle system (e.g. 240 nozzles (Brandau, 2002)) without changing other parameters such as the flow rate and the vibration frequency (Brandenberger and Widmer, 1998; Hunik and Tramper, 1993; Seifert and Phillips, 1997). The most important element is about the arrangement of the nozzles. The geometry must ensure equal jet formation and equal pressure drops between the nozzles (Brandenberger and Widmer, 1998). The pilot apparatus using this technique is now being sold by Brace GmbH (Germany), Nisco Inc. (Switzerland) and Inotech AG (Switzerland) (Brandau, 2002; Brandenberger et al., 1999; Magyar et al., 2001). Furthermore, Orbis

Table 2
Characteristics of monodispersed microsphere processes.

Process	Polymer phase viscosity	Particle diameter (μm)	Multi-wall microspheres	Polymer types	Advantages	Disadvantages
Microporous membrane emulsification	Low	3–100	No	PLA, PLGA, PCL, PLGA–PEG–PLGA	Low mechanical stress	Low production rate
						Possibility of coalescence
Terrace-like microchannel system	Low	3–100	No	Gelatin, alginate, chitosan	Low mechanical stress	Low production rate
						No drug encapsulation reported
Flow focusing	High	30–100	Yes	All types	Particle size adjusted by changing the fluid flow of both phases. Droplet size is not limited by the orifice size	Only works at a specific flow rate ratio
Jet cutter	High	>200	Yes	Gelatin, alginate, chitosan	Simple and easy to operate	Cutting loss
						No drug encapsulation reported
Electrostatic dripping	Medium	>500	No	All types	Simple and easy to operate	Low production rate
Electrostatic cone-jet mode	Medium	2–20	Yes	All types	Prevents droplet coalescence	Large diameter
						Small diameter Viscous polymer solution yielding microfibres
Acoustic jet excitation	Low	>40	Yes	All types	Highly reproducible	Polymer solution must be less viscous than other processes
					Time saving Scaleable	
Precision particle fabrication	High	>10	Yes	All types	Nozzle clogging and shearforce avoided Monodispersed microparticles of different size from one nozzle diameter	More complex apparatus

Biosciences is successfully scaling up the precision particle fabrication process by using a multi-nozzle set-up.

4. Discussion and conclusion

Although the monodispersed microsphere production processes discussed are different, some common factors that affect microsphere size can be found. For the intrinsic factors, the microsphere size increases with increasing polymer concentration (Lee et al., 2010). For the extrinsic factors, the diameter depends firstly on the nozzle through which the dispersed phase passes (Dumas et al., 1992). Secondly, the diameter depends upon the droplet formation frequency (extrusion through membrane pores, vibration frequency, disk rotation speed, electrical field, etc.). Increasing the droplet formation rate decreases the volume of the droplets and thus decreases the microsphere size (Lee et al., 2010; Senuma et al., 1999). Furthermore, the final microsphere size and size distribution are also determined by the degree of coalescence and the stability of the droplets before and during the hardening process (Joscelyne and Tragardh, 2000; Vladisavljević and Schubert, 2003). The coalescence of droplets during the fall or impact at the hardening solution interface increases the standard deviation of the particle-size distribution. By charging the droplet with a high voltage of 400–1400 V, Brandenberger et al. (1999) eliminated the coalescence of alginate microparticles. Furthermore, the electrostatic force allowed the use of surfactant-free processes for non-toxic particles (Choy et al., 2007).

The characterisation of various reported processes is summarised in Table 2. Considering the administration route and syringeability, the microsphere size used for drug delivery should be smaller than 250 μm. The jet cutter and electrostatic dripping methods are thus not the best choices in this case. Furthermore, the microparticle sizes formed by the electrostatic cone-jet mode are limited to 20 μm or less. The microporous membrane and terrace-like microchannel methods are suitable for microspheres up to 100 μm, but these processes are limited for the production of multi-wall microspheres. Multi-wall microspheres can be achieved using the flow-focusing or acoustic-jet excitation process. The precision particle fabrication covers the principle characteristics required: viscosity of polymer solution, size range, and multi-wall microsphere production. However, this combination implies the use of equipment whose scaling-up is difficult.

For parenteral clinical assays, microsphere production must be carried out under aseptic conditions. This step must be considered, especially for protein formulations: temperature or γ-ray irradiation induces protein degradation (Igartua et al., 2007), sterilisation by ethylene oxide gas induces toxicity (Athanasiou et al., 1996) and autoclaving induces the hydrolysis of the polymer (Seifert and Phillips, 1997). Microparticle formation under aseptic conditions using sterile starting materials and sterility tests at each processing step could provide an answer (Brandenberger and Widmer, 1998; Toguchi, 1999). The jet excitation process was reported for production under sterile conditions (Brandenberger et al., 1999). Indeed, a sterile polymer solution was pushed into a nozzle placed in a

sealed, sterile compartment where the microencapsulation process was carried out. Upon the hardening of the microspheres, the suspension is flushed out through a valve and collected on a filter. A washing step is performed in a sterile compartment and finally the microspheres are collected and removed aseptically.

Furthermore, limits concerning production equipment can eliminate certain methods. The most common challenge for all narrow-dispersed microsphere production methods consists of avoiding nozzle/pore clogging, especially for nozzles smaller than 30 μm (Kim et al., 2008). Even one large particle (aggregate polymer or drug or foreign dust particle) could be sufficient to clog the nozzle, therefore filtration is essential and careful washing of all equipment is required (Anna et al., 2003; Doan and Olivier, 2009). The use of the in-line filter is helpful in this regard (Leach et al., 2005). Furthermore, in the conventional preparation of microspheres, it is possible to increase polymer concentration up to saturation level; polymer precipitation occurs immediately after its dispersion into the continuous phase, thereby preventing the drug from diffusing out of the desolvated polymer matrix. However, a high level of viscosity and the fast agglomeration of a dense polymer solution may result in clogging the nozzle, so the polymer concentration must be taken into consideration when choosing the nozzle diameter (15% PCL/DCM for 700 μm nozzle or 5% of PLGA/DCM for 1.1 μm pore diameter in porous membrane process (Ding et al., 2005; Shiga et al., 1996)). The jet-cutter or electrostatic process can be useful when working with highly viscous polymer solutions, but the microspheres obtained by these processes are often too large (from over 200 μm to a millimetre) or too small (under 20 μm). The precision particle fabrication not only helps to produce monodispersed microparticles with all size ranges, but also works with all types of microparticles (mono or multi core–shell microspheres).

An optimal process should allow the production of microparticles with various size ranges and particle types. Moreover, it should not be too sophisticated, thereby allowing easy scaling-up, and should be capable of production under sterile conditions. Taking into account these considerations, some of the processes discussed are highly relevant, such as the porous membrane, jet excitation or the precision particle fabrication. Obviously, the selection of these processes depends on the objective of using microparticles.

Acknowledgment

The authors thank the French '*Ministère de l'Education Nationale et de la Recherche*' for its financial support.

References

Abrahamse, A.J., Van Lierop, R., Van der Sman, R.G.M., Van der Padt, A., Boom, R.M., 2002. Analysis of droplet formation and interactions during cross-flow membrane emulsification. J. Membr. Sci. 204, 125–137.
Amsden, B., 1999. The production of uniformly sized polymer microspheres. Pharm. Res. 16, 1140–1143.
Amsden, B.G., 2001. US Patent no 6224794.
Amsden, B.G., Goosen, M.F.A., 1997. An examination of factors affecting the size, distribution and release characteristics of polymer microbeads made using electrostatics. J. Control. Rel. 43, 183–196.
Anderson, J.M., Shive, M.S., 1997. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Deliv. Rev. 28, 5–24.
Anna, S.L., Bontoux, N., Stone, H.A., 2003. Formation of dispersions using "flow focusing" in microchannels. Appl. Phys. Lett. 82, 364–366.
Arshady, R., 1989. Preparation of microspheres and microcapsules by interfacial polycondensation techniques. J. Microencapsul. 6, 13–28.
Athanasiou, K.A., Niederauer, G.G., Agrawal, C.M., 1996. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 17, 93–102.
Berchane, N.S., Carson, K.H., Rice-Ficht, A.C., Andrews, M.J., 2007. Effect of mean diameter and polydispersity of PLG microspheres on drug release: experiment and theory. Int. J. Pharm. 337, 118–126.
Berkland, C., Cox, A., Kim, K.K., Pack, D.W., 2004a. Three-month, zero-order piroxicam release from monodispersed double-walled microspheres of controlled shell thickness. J. Biomed. Mater. Res. 70A, 576–584.
Berkland, C., Kim, K., Pack, D.W., 2001. Fabrication of PLG microspheres with precisely controlled and monodisperse size distributions. J. Control. Rel. 73, 59–74.
Berkland, C., Kim, K., Pack, D.W., 2003a. PLG microsphere size controls drug release rate through several competing factors. Pharm. Res. 20, 1055–1062.
Berkland, C., Kipper, M.J., Narasimhan, B., Kim, K., Pack, D.W., 2004b. Microsphere size, precipitation kinetics and drug distribution control drug release from biodegradable polyanhydride microspheres. J. Control. Rel. 94, 129–141.
Berkland, C., Pack, D.W., Kim, K., 2003b. Microparticles, US Patent 6,669,961 B2.
Berkland, C., Pollauf, E., Pack, D.W., Kim, K., 2004c. Uniform double-walled polymer microspheres of controllable shell thickness. J. Control. Rel. 96, 101–111.
Berkland, C., Pollauf, E., Raman, C., Silverman, R., Kim, K., Pack, D.W., 2007a. Macromolecule release from monodisperse PLG microspheres: control of release rates and investigation of release mechanism. J. Pharm. Sci. 96, 1176–1191.
Berkland, C., Pollauf, E., Varde, N., Pack, D.W., Kim, K., 2007b. Monodisperse liquid-filled biodegradable microcapsules. Pharm. Res. 24, 1007–1013.
Bezemer, J.M., Radersma, R., Grijpma, D.W., Dijkstra, P.J., Van Blitterswijk, C.A., Feijen, J., 2000. Microspheres for protein delivery prepared from amphiphilic multiblock copolymers. 1. Influence of preparation techniques on particle characteristics and protein delivery. J. Control. Rel. 67, 233–248.
Bocanegra, R., Sampedro, J.L., Gañán-Calvo, A., Marquez, M., 2005. Monodisperse structured multi-vesicle microencapsulation using flow-focusing and controlled disturbance. J. Microencapsul. 22, 745–759.
Boontheekul, T., Kong, H.-J., Mooney, D.J., 2005. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials 26, 2455–2465.
Brandau, E., 1995. US Patent no 54726485.
Brandau, T., 2002. Preparation of monodisperse controlled release microcapsules. Int. J. Pharm. 242, 179–184.
Brandenberger, H., Nüssli, D., Piëch, V., Widmer, F., 1999. Monodisperse particle production: a method to prevent drop coalescence using electrostatic forces. J. Electrostat. 45, 227–238.
Brandenberger, H., Widmer, F., 1998. A new multinozzle encapsulation/immobilisation system to produce uniform beads of alginate. J. Biotechnol. 63, 73–80.
Brandenberger, H.R., Widmer, F., 1999. Immobilization of highly concentrated cell suspensions using the laminar jet breakup technique. Biotechnol. Prog. 15, 366–372.
Bugarski, B., Amsden, B., Neufeld, R.J., Poncelet, D., Goosen, M.F.A., 1994a. Effect of electrode geometry and charge on the production of polymer microbeads by electrostatics. Can. J. Chem. Eng. 72, 517–521.
Bugarski, B., Li, Q., Goosen, M.F.A., Poncelet, D., Neufeld, R., Vunjak, G., 1994b. Electrostatic droplet generator-mechanism of polymer droplet formation. AICHE J. 40, 1026–1031.
Charcosset, C., Fessi, H., 2005. Membrane emulsification and microchannel emulsification processes. Rev. Chem. Eng. 21, 1–32.
Charcosset, C., Limayem, I., Fessi, H., 2004. The membrane emulsification process—a review. J. Chem. Technol. Biotechnol. 79, 209–218.
Chen, W., Lu, D.R., 1999. Carboplatin-loaded PLGA microspheres for intracerebral injection: formulation and characterization. J. Microencapsul. 16, 551–563.
Choi, D.H., Park, C.H., Kim, I.H., Chun, H.J., Park, K., Han, D.K., 2010. Fabrication of core–shell microcapsules using PLGA and alginate for dual growth factor delivery system. J. Control. Rel. 147, 193–201.
Choy, Y.B., Choi, H., Kim, K., 2007. Uniform biodegradable hydrogel microspheres fabricated by a surfactant-free electric-field-assisted method. Macromol. Biosci. 7, 423–428.
Chuah, A.M., Kuroiwa, T., Kobayashi, I., Zhang, X., Nakajima, M., 2010. Preparation of uniformly sized alginate microspheres using the novel combined methods of microchannel emulsification and external gelation. Colloids Surf. A 351, 9–17.
Dawes, G.J.S., Fratila-Apachitei, L.E., Mulia, K., Apachitei, I., Witkamp, G.-J., Duszczyk, J., 2009. Size effect of PLGA spheres on drug loading efficiency and release profiles. J. Mater. Sci: Mater. Med. 20, 1089–1094.
Del Gaudio, P., Russo, P., Rosaria Lauro, M., Colombo, P., Aquino, R.P., 2009. Encapsulation of ketoprofen and ketoprofen lysinate by prilling for controlled drug release. AAPS PharmSciTech 10, 1178–1185.
Ding, L., Lee, T., Wang, C.H., 2005. Fabrication of monodispersed Taxol-loaded particles using electrohydrodynamic atomization. J. Control. Rel. 102, 395–413.
Doan, T.V.P., Olivier, J.C., 2009. Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization. Int. J. Pharm..
Dumas, H., Tardy, M., Rochar, M.H., Tayot, J.L., 1992. Prilling process applied to collagen solutions. Drug Dev. Ind. Pharm. 18, 1395–1409.
Dumas, H., 1992. FR Patent no EP0351296B1.
Dunne, M., Corrigan, O.I., Ramtoola, Z., 2000. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. Biomaterials 21, 1659–1668.
Edlund, U., Albertsson, A.-C., 2002. Degradable polymer microspheres for controlled drug delivery. Adv. Pol. Sci., 67–112.
Fantini, D., Zanetti, M., Costa, L., 2006. Polystyrene microspheres and nanospheres produced by electrospray. Macromol. Com. 27, 2038–2042.
Freiberg, S., Zhu, X.X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.
Freitas, S., Merkle, H.P., Gander, B., 2005. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. J. Control. Rel. 102, 313–332.
Gañán-Calvo, A.M., 1998. Generation of steady liquid microthreads and micron-sized monodisperse sprays in gas streams. Phys. Rev. Lett. 80, 285–288.

Gañán-Calvo, A.M., Gordillo, J.M., 2001. Perfectly monodisperse microbubbling by capillary flow focusing. Phys. Rev. Lett. 87, 2745011–2745014.
Gasparini, G., Kosvintsev, S.R., Stillwell, M.T., Holdich, R.G., 2007. Preparation and characterization of PLGA particles for subcutaneous controlled drug release by membrane emulsification. Colloids Surf. B 61, 199–207.
Gañán-Calvo, A.M., Martín-Banderas, L., González-Prieto, R., Rodríguez-Gil, A., Berdún-Álvarez, T., Cebolla, A., Chávez, S., Flores-Mosquera, M., 2006. Straightforward production of encoded microbeads by flow focusing: potential applications for biomolecule detection. Int. J. Pharm. 324, 19–26.
Gaumet, M., Gurny, R., Delie, F., 2009. Localization and quantification of biodegradable particles in an intestinal cell model: the influence of particle size. Eur. J. Pharm. Sci. 36, 465–473.
Giteau, A., Venier-Julienne, M.C., Aubert-Pouëssel, A., Benoit, J.P., 2008. How to achieve sustained and complete protein release from PLGA-based microparticles? Int. J. Pharm. 350, 14–26.
Gong, X., Lu, Y., Xiang, Z., Zhang, Y., Luo, G., 2007. Preparation of uniform microcapsules with silicone oil as continuous phase in a micro-dispersion process. J. Microencapsul. 24, 767–776.
Haider, M., Megeed, Z., Ghandehari, H., 2004. Genetically engineered polymers: status and prospects for controlled release. J. Control. Rel. 95, 1–26.
He, Y., 2008. Application of flow-focusing to the break-up of an emulsion jet for the production of matrix-structured microparticles. Chem. Eng. Sci. 63, 2500–2507.
Heinzen, C., Berger, A., Marison, I., 2004. Use of vibration technology for jet breakup for encapsulation of cells and liquids in monodisperse microcapsules. In: Nedovic, V., Willaert, R. (Eds.), Fundamentals of Cell Immobilisation Biotechnology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 257–275.
Herrero-Vanrell, R., Molina-Martinez, I.T., 2007. PLA and PLGA microparticles for intravitreal drug delivery: an overview. J. Drug Deliv. Sci. Technol. 17, 11–17.
Holgado, M.A., Arias, J.L., Cózar, M.J., Alvarez-Fuentes, J., Gañán-Calvo, A.M., Fernández-Arévalo, M., 2008. Synthesis of lidocaine-loaded PLGA microparticles by flow focusing: effects on drug loading and release properties. Int. J. Pharm. 358, 27–35.
Holgado, M.A., Cózar-Bernal, M.J., Salas, S., Arias, J.L., Álvarez-Fuentes, J., Fernández-Arévalo, M., 2009. Protein-loaded PLGA microparticles engineered by flow focusing: Physicochemical characterization and protein detection by reversed-phase HPLC. Int. J. Pharm. 380, 147–154.
Hong, Y., Li, Y., Yin, Y., Li, D., Zou, G., 2008. Electrohydrodynamic atomization of quasi-monodisperse drug-loaded spherical/wrinkled microparticles. J. Aero. Sci. 39, 525–536.
Huang, K.S., Lu, K., Yeh, C.S., Chung, S.R., Lin, C.H., Yang, C.H., Dong, Y.S., 1999. Microfluidic controlling monodisperse microdroplet for 5-fluorouracil loaded genipin-gelatin microcapsules. J. Control. Rel. 137, 15–19.
Hunik, J.H., Tramper, J., 1993. Large-scale production of kappa-carrageenan droplets for gel-bead production: theoretical and practical limitations of size and production rate. Biotechnol. Prog. 9, 186–192.
Igartua, M., Hernández, R.M., Rosas, J.E., Patarroyo, M.E., Pedraz, J.L., 2007. γ-Irradiation effects on biopharmaceutical properties of PLGA microspheres loaded with SPf66 synthetic vaccine. Eur. J. Pharm. Biopharm. 69, 519–526.
Ikkai, F., Iwamoto, S., Adachi, E., Nakajima, M., 2005. New method of producing mono-sized polymer gel particles using microchannel emulsification and UV irradiation. Colloid Polym. Sci. 283, 1149–1153.
Iwamoto, S., Nakagawa, K., Sugiura, S., Nakajima, M., 2002. Preparation of gelatin microbeads with a narrow size distribution using microchannel emulsification. AAPS PharmSciTech [electronic resource] 3, 25.
Jaworek, A., 2008. Electrostatic micro- and nanoencapsulation and electroemulsification: a brief review. J. Microencapsul. 25, 443–468.
Johnson, O.L., Tracy, M.A., 1999. Peptide and protein drug delivery. In: Mathiowitz, E. (Ed.), Encyclopedia of Controlled Drug Delivery. John Wiley & Sons, pp. 816–832.
Jork, A., Thürmer, F., Cramer, H., Zimmermann, G., Gessner, P., Hämel, K., Hofmann, G., Kuttler, B., Hahn, H.-J., Josimovic-Alasevic, O., Fritsch, K.-G., Zimmermann, U., 2000. Biocompatible alginate from freshly collected *Laminaria pallida* for implantation. Appl. Microbiol. Biotechnol. 53, 224–229.
Joscelyne, S.M., Trägårdh, G., 2000. Membrane emulsification—a literature review. J. Membr. Sci. 169, 107–117.
Kandori, K., Kishi, K., Ishikawa, T., 1991. Preparation of monodispersed W/O emulsions by Shirasu-porous-glass filter emulsification technique. Colloid Surf. 55, 73–78.
Katare, Y.K., Muthukumaran, T., Panda, A.K., 2005. Influence of particle size, antigen load, dose and additional adjuvant on the immune response from antigen loaded PLA microparticles. Int. J. Pharm. 301, 149–160.
Kawakatsu, T., Kikuchi, Y., Nakajima, M., 1997. Regular-sized cell creation in microchannel emulsification by visual microprocessing method. JAOCS, J. Am. Oil. Chem. Soc. 74, 317–321.
Kawakatsu, T., Nakajima, K.H., Kikuchi, M., Yonemoto, Y.T., 1999. Production of monodispersed oil-in-water emulsion using crossflow-type silicon microchannel plate. J. Chem. Eng. Jpn. 32, 241–244.
Kim, K., 1994. Fabrication of glass micro- and nanospheres from liquid precursors using droplet generation and sol–gel processing. In: Materials Research Society Symposium—Proceedings. Materials Research Society , Boston, MA, USA, pp. 25–32.
Kim, K., Park, D. W., Berkland, C., 2008. US Patent no 7368130.
Kissel, T., Li, Y., Unger, F., 2002. ABA-triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly(ethylene oxide) B-blocks as a candidate for in situ forming hydrogel delivery systems for proteins. Adv. Drug Deliv. Rev. 54, 99–134.
Klose, D., Siepmann, F., Elkharraz, K., Krenzlin, S., Siepmann, J., 2006. How porosity and size affect the drug release mechanisms from PLGA-based microparticles. Int. J. Pharm. 314, 198–206.
Kobayashi, I.I.Y., Iwamoto, S., Kimura, S., Nakajima, M., 2003. Preparation characteristics of lipid microspheres using microchannel emulsification and solvent evaporation methods. J. Chem. Eng. Jpn. 36, 996–1000.
Koch, S., Schwinger, C., Kressler, J., Heinzen, C., Rainov, N.G., 2003. Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. J. Microencapsul. 20, 303–316.
Lambrich, U., Schubert, H., 2005. Emulsification using microporous systems. J. Membr. Sci. 257, 76–84.
Lamprecht, A., Schäfer, U., Lehr, C.-M., 2001. Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm. Res. 18, 788–793.
Langer, G., Yamate, G., 1969. Encapsulation of liquid and solid aerosol particles to form dry powders. J. Colloid Interface Sci. 29, 450–455.
Lau, A.N.K., Cassel, J. M., Bridgham, J. A., 2009. WO Patent no WO/2008/144288.
Leach, W.T., Simpson, D.T., Val, T.N., Yu, Z., Lim, K.T., Park, E.J., Williams Iii, R.O., Johnston, K.P., 2005. Encapsulation of protein nanoparticles into uniform-sized microspheres formed in a spinning oil film. AAPS PharmSciTech 6, E605–E617.
Lee, C.H., Singla, A., Lee, Y., 2001. Biomedical applications of collagen. Int. J. Pharm. 221, 1–22.
Lee, T.H., Wang, J., Wang, C.H., 2002. Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies. J. Control. Rel. 83, 437–452.
Lee, Y.H., Mei, F., Bai, M.Y., Zhao, S., Chen, D.R., 2010. Release profile characteristics of biodegradable-polymer-coated drug particles fabricated by dual-capillary electrospray. J. Control. Rel. 145, 58–65.
Leelarasamee, N., Howard, S.A., Malanga, C.J., Ma, J.K.H., 1988. A method for the preparation of polylactic acid microcapsules of controlled particle size and drug loading. J. Microencapsul. 5, 147–157.
Lewińska, D., Bukowski, J., Kożuchowski, M., Kinasiewicz, A., Weryński, A., 2008. Electrostatic microencapsulation of living cells. Biocybern. Biomed. Eng. 28, 69–84.
Li, M., Rouaud, O., Poncelet, D., 2008. Microencapsulation by solvent evaporation: State of the art for process engineering approaches. Int. J. Pharm. 363, 26–39.
Ma, G.H., Nagai, M., Omi, S., 1999. Study on preparation and morphology of uniform artificial polystyrene–poly(methyl methacrylate) composite microspheres by employing the SPG (Shirasu Porous Glass) membrane emulsification technique. J. Colloid Interface Sci. 214, 264–282.
Ma, J., Wang, H., He, B., Chen, J., 2001. A preliminary in vitro study on the fabrication and tissue engineering applications of a novel chitosan bilayer material as a scaffold of human neofetal dermal fibroblasts. Biomaterials 22, 331–336.
Magyar, J.P., Nemir, M., Ehler, E., Suter, N., Perriard, J.C., Eppenberger, H.M., 2001. Mass production of embryoid bodies in microbeads. Ann. N.Y. Acad. Sci. 944, 135–143.
Makino, K., Nakajima, T., Shikamura, M., Ito, F., Ando, S., Kochi, C., Inagawa, H., Soma, G.-I., Terada, H., 2004. Efficient intracellular delivery of rifampicin to alveolar macrophages using rifampicin-loaded PLGA microspheres: Effects of molecular weight and composition of PLGA on release of rifampicin. Colloid Surf. B 36, 35–42.
Martín-Banderas, L., Rodriguez-Gil, A., Cebolla, A., Chavez, S., Berdun-Alverez, T., Fernandez Garcia, J.M., Flores-Mosquera, M., Gañán-Calvo, A.M., 2006. Towards high-throughput production of uniformly encoded microparticles. Adv. Materials 18, 559–564.
Martín-Banderas, L., Flores-Masquera, M., Riesco-Chueca, P., Rodríguez-Gil, A., Cebolla, Á., Chávez, S., Gañán-Calvo, A.M., 2005. Flow focusing: a versatile technology to produce size-controlled and specific-morphology microparticles. Small 1, 688–692.
Martín-Banderas, L., Gonzalez-Prieto, R., Rodríguez-Gil, A., Fernandez-Arevalo, M., Flores-Masquera, M., Chavez, S., Alfonso, M., 2010. Application of flow focusing to the break-up of a magnetite suspension jet for the production of paramagnetic microparticles. J. Nanomater., doi:10.1155/2011/527437.
Mathiowitz, E., Langer, R.S., 1999. US Patent no 5912017.
Mazzitelli, S., Tosi, A., Balestra, C., Nastruzzi, C., Luca, G., Mancuso, F., Calafiore, R., Calvitti, M., 2008. Production and characterization of alginate microcapsules produced by a vibrational encapsulation device. J. Biomater. Appl. 23, 123–145.
Mitragotri, S., Lahann, J., 2009. Physical approaches to biomaterial design. Nat. Mater. 8, 15–23.
Moghadam, H., Samimi, M., Samimi, A., Khorram, M., 2008. Electro-spray of high viscous liquids for producing mono-sized spherical alginate beads. Particuology 6, 271–275.
Mohamed, F., Van Der Walle, C.F., 2008. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. J. Pharm. Sci. 97, 71–87.
Nedovic, V., Willaert, R., 2004. Fundamental of Cell Immobilisation Biotechnology, Focus on Biotechnology. Kluwer Academic Publisher, Dordrecht.
Omi, S., 1995. Preparation of monodisperse microspheres using the Shirasu porous glass emulsification technique. Colloids Surf. A 109, 97–107.
Omi, S., Katami, K., Taguchi, T., Kaneko, K., Iso, M., 1995. Synthesis of uniform PMMA microspheres employing modifiec SPG (Shirasu Porous Glass) Emulsification Technique. J. Appl. Polym. Sci. 57, 1013–1024.
Paillard-Giteau, A., Tran, V.T., Thomas, O., Garric, X., Coudane, J., Marchal, S., Chourpa, I., Benoit, J.P., Montero-Menei, C.N., Venier-Julienne, M.C., 2010. Effect of various additives and polymers on lysozyme release from PLGA micro-

spheres prepared by an s/o/w emulsion technique. Eur. J. Pharm. Biopharm. 75, 128–136.
Passerini, N., Perissutti, B., Albertini, B., Voinovich, D., Moneghini, M., Rodriguez, L., 2003. Controlled release of verapamil hydrochloride from waxy microparticles prepared by spray congealing. J. Control. Rel. 88, 263–275.
Pollauf, E.J., Kim, K.K., Pack, D.W., 2005. Small-molecule release from poly(D,L-lactide)/poly(D,L-lactide-co-glycolide) composite microparticles. J. Pharm. Sci. 94, 2013–2022.
Poncelet, D., Bugarski, B., Amsden, B.G., Zhu, J., Neufeld, R., MF, G., 1994. A parallel plate electrostatic droplet generator: parameters affecting microbead size. Appl. Microbiol. Biotechnol. 42, 251–255.
Prüsse, U., Bruske, F., Breford, J., Vorlop, K.-D., 1998. Improvement of the jet cutting method for the preparation of spherical particles from viscous polymer solutions. Chem. Eng. Technol. 21, 153–157.
Prüße, U., Dalluhn, J., Breford, J., Vorlop, K.-D., 2000. Production of spherical beads by jet cutting. Chem. Eng. Technol. 23, 1105–1110.
Prüße, U., Fox, B., Kirchhoff, M., Bruske, F., Breford, J., Vorlop, K.-D., 1998a. The jet cutting method as a new immobilization technique. Biotechnol. Technol. 12, 105–108.
Prüße, U., Fox, B., Kirchhoff, M., Bruske, F., Breford, J., Vorlop, K.-D., 1998b. New process (jet cutting method) for the production of spherical beads from highly viscous polymer solutions. Chem. Eng. Technol. 21, 29–33.
Raman, C., Berkland, C., Kim, K., Pack, D.W., 2005. Modeling small-molecule release from PLG microspheres: Effects of polymer degradation and nonuniform drug distribution. J. Control. Rel. 103, 149–158.
Rawat, A., Majumder, Q.H., Ahsan, F., 2008. Inhalable large porous microspheres of low molecular weight heparin: in vitro and in vivo evaluation. J. Control. Rel. 128, 224–232.
Rayley, L., 1879. On the stability of jets. Proc. Lond. Math. Soc. 10, 4–13.
Redhead, H.M., Davis, S.S., Illum, L., 2001. Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: In vitro characterisation and in vivo evaluation. J. Control. Rel. 70, 353–363.
Schneider, T., Zhao, H., Jackson, J.K., Chapman, G.H., Dykes, J., Häfeli, U.O., 2008. Use of hydrodynamic flow focusing for the generation of biodegradable camptothecin-loaded polymer microspheres. J. Pharm. Sci. 97, 4943–4954.
Seifert, D.B., Phillips, J.A., 1997. Production of small, monodispersed alginate beads for cell immobilization. Biotechnol. Prog. 13, 562–568.
Senuma, Y., Franceschin, S., Hilborn, J.G., Tissières, P., Bisson, I., Frey, P., 1999. Bioresorbable microspheres by spinning disk atomization as injectable cell carrier: From preparation to in vitro evaluation. Biomaterials 21, 1135–1144.
Serra, C.A., Chang, Z., 2008. Microfluidic-assisted synthesis of polymer particles. Chem. Eng. Technol. 31, 1099–1115.
Shi, M., Yang, Y.Y., Chaw, C.S., Goh, S.H., Moochhala, S.M., Ng, S., Heller, J., 2003. Double walled POE/PLGA microspheres: encapsulation of water-soluble and water-insoluble proteins and their release properties. J. Control. Rel. 89, 167–177.
Shiga, K., Muramatsu, N., Kondo, T., 1996. Preparation of poly(D,L-lactide) and copoly(lactide-glycolide) microspheres of uniform size. J. Pharm. Pharmacol. 48, 891–895.
Siepmann, J., Faisant, N., Akiki, J., Richard, J., Benoit, J.P., 2004. Effect of the size of biodegradable microparticles on drug release: experiment and theory. J. Control. Rel. 96, 123–134.
Siepmann, J., Göpferich, A., 2001. Mathematical modeling of bioerodible, polymeric drug delivery systems. Adv. Drug Deliv. Rev. 48, 229–247.
Song, S., Zhang, W., Hu, Z., Zhang, Z., 2009. Monodisperse micrometer-size carboxyl-functionalized polystyrene particles obtained by two-stage radiation-induced dispersion polymerization. Colloids Surf. A 348, 1–8.
Stevanović, M., Uskoković, D., 2009. Poly(lactide-co-glycolide)-based micro and nanoparticles for the controlled drug delivery of vitamins. Curr. Nanosci. 5, 1–14.
Strand, B.L., Gåserød, O., Kulseng, B., Espevik, T., Skjåk-Bræk, G., 2002. Alginate-polylysine-alginate microcapsules: effect of size reduction on capsule properties. J. Microencapsul. 19, 615–630.
Sugiura, S., Nakajima, M., Seki, M., 2002a. Preparation of monodispersed polymeric microspheres over 50 μm employing microchannel emulsification. Ind. Eng. Chem. Res. 41, 4043–4047.
Sugiura, S., Oda, T., Izumida, Y., Aoyagi, Y., Satake, M., Ochiai, A., Ohkohchi, N., Nakajima, M., 2005. Size control of calcium alginate beads containing living cells using micro-nozzle array. Biomaterials 26, 3327–3331.
Sugiura, S., Nakajima, M., Seki, M., 2002b. Preparation of monodispersed emulsion with large droplets using microchannel emulsification. J. Am. Oil Chem. Soc. 79, 515–519.
Sugiura, S., Nakajima, M., Seki, M., 2002c. Preparation of monodispersed emulsion with large droplets using microchannel emulsification. J. Am. Oil. Chem. Soc. 79, 515–519.
Tatard, V.M., Menei, P., Benoit, J.P., Montero-Menei, C.N., 2005a. Combining polymeric devices and stem cells for the treatment of neurological disorders: a promising therapeutic approach. Curr. Drug Targets 6, 81–96.
Tatard, V.M., Venier-Julienne, M.C., Saulnier, P., Prechter, E., Benoit, J.P., Menei, P., Montero-Menei, C.N., 2005b. Pharmacologically active microcarriers: a tool for cell therapy. Biomaterials 26, 3727–3737.
Teunou, E., Poncelet, D., 2005. Rotary disc atomisation for microencapsulation applications-prediction of the particle trajectories. J. Food Eng. 71, 345–353.
Thomas, C., Gupta, V., Ahsan, F., 2010. Particle size influences the immune response produced by hepatitis B vaccine formulated in inhalable particles. Pharm. Res. 27, 905–919.
Toguchi, H., 1999. Sterility assurance of microspheres. J. Control. Rel. 62, 51–55.
van Dijke, K., Kobayashi, I., Schroën, K., Uemura, K., Nakajima, M., Boom, R., 2010. Effect of viscosities of dispersed and continuous phases in microchannel oil-in-water emulsification. Microfluid. Nanofluid. 9, 77–85.
Veldhuis, G., Gironês, M., Bingham, D., 2009. Monodisperse microspheres for parenteral drug delivery. Drug Deliv. Technol. 9, 24–31.
Vladisavljević, G.T., Schubert, H., 2003. Influence of process parameters on droplet size distribution in SPG membrane emulsification and stability of prepared emulsion droplets. J. Membr. Sci. 225, 15–23.
Wang, L.-Y., Ma, G.-H., Su, Z.-G., 2005. Preparation of uniform sized chitosan microspheres by membrane emulsification technique and application as a carrier of protein drug. J. Control. Rel. 106, 62–75.
Wang, W., Zhou, S., Sun, L., Huang, C., 2010. Controlled delivery of paracetamol and protein at different stages from core–shell biodegradable microspheres. Carbohydr. Polym. 79, 437–444.
Wang, Y.M., Sato, H., Horikoshi, I., 1997. In vitro and in vivo evaluation of taxol release from poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate and degradation of the microspheres. J. Control. Rel. 49, 157–166.
Weber, C., 1931. Zum Zerfall eines Flüssigkeitstahles. Z. Angew. Math. Mech. 11, 136–155.
Wei, Q., Wei, W., Tian, R., Wang, L.Y., Su, Z.G., Ma, G.H., 2008a. Preparation of uniform-sized PELA microspheres with high encapsulation efficiency of antigen by premix membrane emulsification. J. Colloid Interface Sci. 323, 267–273.
Wei, W., Wang, L.-Y., Yuan, L., Yang, X.-D., Su, Z.-G., Ma, G.-H., 2008b. Bioprocess of uniform-sized crosslinked chitosan microspheres in rats following oral administration. Eur. J. Pharm. Biopharm. 69, 878–886.
Williams, R.A., Peng, S.J., Wheeler, D.A., Morley, N.C., Taylor, D., Whalley, M., Houldsworth, D.W., 1998. Controlled production of emulsions using a cross-flow membrane. Part II: Industrial scale manufacture. Chem. Eng. Res. Des. 76, 902–910.
Xie, J., Ng, W.J., Lee, L.Y., Wang, C.-H., 2008. Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray. J. Colloid Interface Sci. 317, 469–476.
Xie, J., Wang, C.-H., 2007. Encapsulation of proteins in biodegradable polymeric microparticles using electrospray in the Taylor cone-jet mode. Biotechnol. Bioeng. 97, 1278–1290.
Xu, J.H., Li, S.W., Tostado, C., Lan, W.J., Luo, G.S., 2009a. Preparation of monodispersed chitosan microspheres and in situ encapsulation of BSA in a co-axial microfluidic device. Biomed. Microdevices 11, 243–249.
Xu, Q., Hashimoto, M., Dang, T.T., Hoare, T., Kohane, D.S., Whitesides, G.M., Langer, R., Anderson, D.G., 2009b. Preparation of monodisperse biodegradable polymer microparticles using a microfluidic flow-focusing device for controlled drug delivery. Small 5, 1575–1581.
Xue, W., Liu, X., Yu, W., Ma, X., 2006. Preparation of protein-loaded microspheres with size <10 μm by electrostatic droplet generation technology. Chin. Sci. Bull. 51, 279–286.
Yamamoto, N., Fukai, F., Ohshima, H., Terada, H., Makino, K., 2002. Dependence of the phagocytic uptake of polystyrene microspheres by differentiated HL60 upon the size and surface properties of the microspheres. Colloids Surf. B 25, 157–162.
Yang, C.C., Huang, K.S., Chang, J.Y., 2007. Manufacturing monodisperse chitosan microparticles containing ampicillin using a microchannel chip. Biomed. Microdevices 9, 253–259.
Yeo, Y., Chen, A.U., Basaran, O.A., Park, K., 2004. Solvent exchange method: a novel microencapsulation technique using dual microdispensers. Pharm. Res. 21, 1419–1427.
Zvonar, A., Kristl, J., Kerf ç, J., Grabnar, P.A., 2009. High celecoxib-loaded nanoparticles prepared by a vibrating nozzle device. J. Microencapsul. 26, 748–759.