



Review

Strategies for encapsulation of small hydrophilic and amphiphilic drugs in PLGA microspheres: State-of-the-art and challenges



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ABSTRACT

Poly(lactide-co-glycolide) (PLGA) microspheres are efficient delivery systems for controlled release of low molecular weight drugs as well as therapeutic macromolecules. The most common microencapsulation methods are based on emulsification procedures, in which emulsified droplets of polymer and drug solidify into microspheres when the solvent is extracted from the polymeric phase. Although high encapsulation efficiencies have been reported for hydrophobic small molecules, encapsulation of hydrophilic and/or amphiphilic small molecules is challenging due to the partitioning of drug from the polymeric phase into the external phase before solidification of the particles. This review addresses formulation-related aspects for efficient encapsulation of small hydrophilic/amphiphilic molecules into PLGA microspheres using conventional emulsification methods (e.g., oil/water, water/oil/water, solid/oil/water, water/oil/oil) and highlights novel emulsification technologies such as microfluidics, membrane emulsification and other techniques including spray drying and inkjet printing. Collectively, these novel microencapsulation technologies afford production of this type of drug loaded microspheres in a robust and well controlled manner.

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Abbreviations: PLGA, poly(D,L-lactide-co-glycolide); logP, partition coefficient; M_w , molecular weight; $W_1/O/W_2$, water/oil/water; O/W, oil/water; W_2 , outer water phase; W_1 , inner water phase; S/O₁/O₂, solid/oil/oil; S/O/W, solid/oil/water; W/O₁/O₂, water/oil/oil; ME, membrane emulsification; IJP, inkjet printing; EE, encapsulation efficiency; LC, loading capacity; FTIR, fourier transform infrared spectroscopy; SEM, scanning electron microscopy; DCM, dichloromethane; ACN, acetonitrile; DMSO, dimethyl sulfoxide; DMAc, N,N-dimethylacetamide; PVA, polyvinylalcohol; BCNU, carmustine; PBS, phosphate buffered saline; SPG, shirasu porous glass; USP, United States Pharmacopeia.

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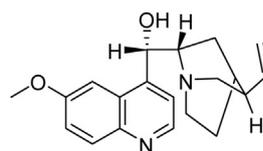
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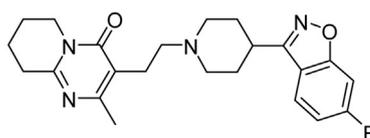
1. Introduction

Small drug molecules of biopharmaceutical classification type I and III (hydrophilic or amphiphilic drugs) have good water

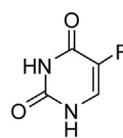
solubility and are extensively used for the treatment of various diseases. Their clinical application can however be restricted due to fast clearance or unfavorable biodistribution necessitating frequent dosing (Chen, 2010; Vrignaud et al., 2011). The



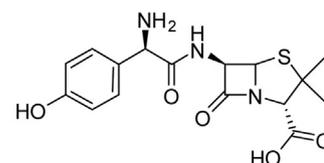
Quinidine
Mw: 324; logP: 2.51



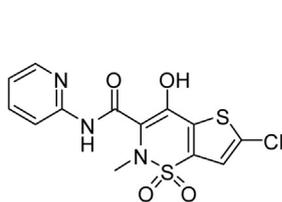
Risperidone
Mw: 410; logP: 2.63



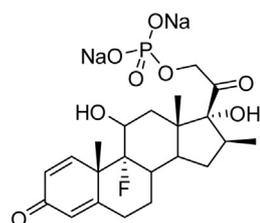
5-fluorouracil
Mw:130; logP: - 0.66



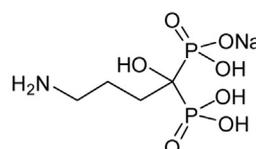
Amoxicillin
Mw: 365; logP: - 2.31



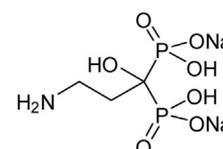
Lornoxicam
Mw: 371; logP: 0.60



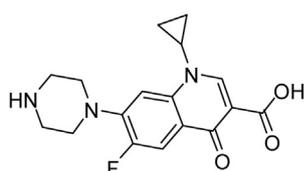
Betamethasonephosphate disodium
Mw: 504; logD at pH 7.4: -3.19



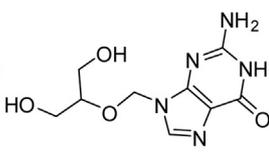
Alendronate sodium
Mw: 325; logD at pH 7.4: - 6.6



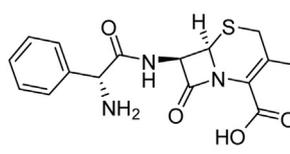
Pamidronate disodium
Mw:235; logD at pH 7.4: -6.9



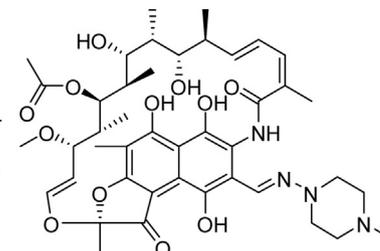
Ciprofloxacin
Mw: 331; logP: - 0.81



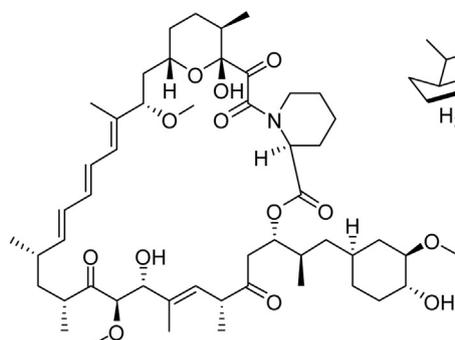
Ganciclovir
Mw:225; logP: - 1.66



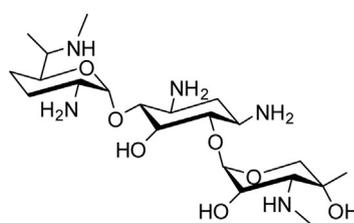
Cephalexin
Mw: 347; logP: - 2.51



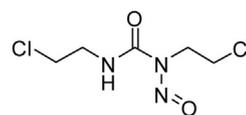
Rifampicin
Mw: 823; logP: 3.2



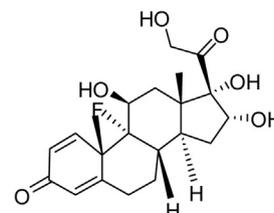
Rapamycin
Mw: 914; LogP:7.45



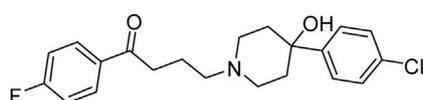
Gentamicin
Mw:477; logP: - 4.0



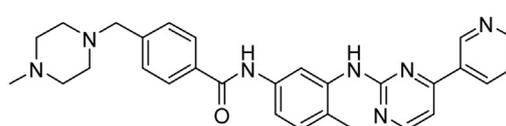
Carmustine
Mw: 214; logP:1.02



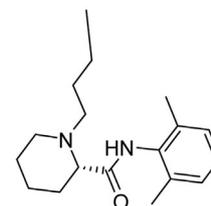
Triamcinolone
Mw: 394; logP: 1.1



Haloperidol
Mw: 375; logP: 3.5



Imatinib
Mw: 493; logP: 4.38



Bupivacaine
Mw: 288; logP: 3.4

Fig. 1. Chemical structure of amphiphilic and hydrophilic small molecules used in this review. The images are arranged based on the order of mentioning in the text.

therapeutic value of such compounds can be improved by encapsulating these drugs into delivery systems, for example polymeric microspheres, which allow sustained release and hence a more patient-friendly dosing regimen. Biodegradable polymeric microspheres possess several attractive features that contribute to their widespread use in controlled drug delivery for a variety of drugs, ranging from small molecules to proteins and plasmid DNA. Microspheres can be administered relatively easily via subcutaneous or intramuscular injection, without the necessity of surgery. Such locally injected depots provide long-term sustained drug release, thus enabling the safe dosing of drugs with pharmacokinetics issues like rapid systemic clearance or with a narrow therapeutic window (Chen, 2010; Vrignaud et al., 2011). Drug loaded microspheres have also been administered with the intention to overcome poor local distribution of drugs, for instance by intracranial local injection or via administration in the eye (Benny et al., 2005, 2009; Ford Versypt et al., 2013; Herrero-Vanrell et al., 2014).

PLGA is a widely used biodegradable polymer for preparation of drug-loaded microspheres (D'Aurizio et al., 2011a; Ozeki et al., 2012; Shive and Anderson, 1997). It is commercially available in different copolymer compositions, molecular weights and capping groups which offers possibilities to tune degradation and drug release kinetics (Casalini et al., 2014; Gasparini et al., 2010). The common technology for the fabrication of drug-loaded PLGA microspheres is emulsification e.g., oil/water (O/W), water/oil/

water ($W_1/O/W_2$), water/oil/oil ($W/O_1/O_2$), or solid/oil/water (S/O/W). Apart from these conventional methods, novel emulsification technologies such as microfluidics and membrane emulsification have also been applied for the manufacturing of PLGA microspheres (Falke et al., 2015; Kazazi-Hyseni et al., 2014; Ramazani et al., 2015a,b; Xu et al., 2009b; Ye et al., 2010b; Zhang et al., 2011). Other techniques of microparticle preparation, including spray drying and inkjet printing are also discussed in this review (Kolakovc et al., 2013; Marquette et al., 2014). These novel technologies allow the production of drug loaded microspheres in a robust and well controlled manner. Most of the examples discussed in this review are PLGA-based microspheres loaded with small hydrophilic and amphiphilic molecules (Fig. 1). Due to the novelty of their processing methods, some hydrophobic small molecules loaded microspheres or microspheres based on polymers other than PLGA have also been exemplified. Encapsulation of small hydrophilic and amphiphilic molecules in PLGA microspheres using emulsification is challenging due to the partitioning of the drug from the organic droplets to the larger volume of the external water phase (Chaisri et al., 2009; Ng et al., 2010b; Ramazani et al., 2015a). High encapsulation efficiency (EE) and drug loading capacity (LC) are critical particularly for expensive drugs and depots that provide prolonged drug release. EE is expressed as the amount of encapsulated drug divided by the amount of drug used for encapsulation. LC is the encapsulated amount of drug divided by the total weight of drug and polymer

Table 1
Overview of encapsulation methods for preparation of PLGA microspheres loaded with small hydrophilic and amphiphilic drugs.

Drug	Preparation method of microspheres	Solvents for organic phase	Comment	Reference
Quinidine sulfate	O/W emulsification	Ethanol/DCM	EE was improved using ethanol as cosolvent	Al-Maaieh and Flanagan (2001)
Risperidone	O/W emulsification	Methyl dichloroacetate	Improved solvent extraction by aminolysis of organic solvent	Sah and Lee (2006)
5-fluorouracil	$W_1/O/W_2$ emulsification	DCM	Improved encapsulation by reduction of inner water phase	Parikh et al. (2003)
Amoxicillin	$W_1/O/W_2$ and S/O/W emulsification	DCM	Solid dispersion of the drug using S/O/W method resulted in higher EE	Xu et al. (2009a)
Lornoxicam	S/O/W emulsification	DCM	Solid dispersion resulted in high EE and LC	Zhang et al. (2011)
Betamethasone disodium phosphate	$W_1/O_1/O_2$ emulsification	Acetone, ethyl acetate	Spray drying and double emulsion with external organic phase showed better EE than $W_1/O/W_2$	Chaw et al. (2003)
Alendronate sodium	Spray drying $W_1/O/W_2$ $W/O_1/O_2$ $S/O_1/O_2$ emulsification	DCM; DCM/ACN	EE was improved by emulsification in liquid paraffin as non-aqueous external phase	Nafea et al. (2007)
Pamidronate sodium	$S/O_1/O_2$ emulsification	DCM/ACN	EE was improved by emulsification in non-aqueous external phase	Weidenauer et al. (2003)
Ciprofloxacin	$S/O_1/O_2$ emulsification	Acetone	high EE obtained was obtained with sorbitan monooleate (Span 80) as non-aqueous external phase	Jeong et al. (2009)
Ganciclovir	$S/O_1/O_2$ emulsification	Acetone	high EE was obtained with silicon oil as non-aqueous external phase	Herrero-Vanrell et al. (2000)
Rifampicin	Membrane emulsification	DCM	Relatively monodisperse microspheres were obtained, the EE was independent of particle size	Ito and Makino (2004)
Rapamycin	Membrane emulsification	DCM	microspheres with narrow polydispersity were obtained by membrane emulsification	Falke et al. (2015)
Bupivacaine	Co-flow capillary device	DCM	Homogenous distribution of the drug in the microspheres	Xu et al. (2009b)
Paclitaxel	Ink jet printing	<i>N,N</i> -dimethylacetamide	Uniform shape and size for each of the chosen geometries	Lee et al. (2012)
Triamcinolone	Spray drying	Acetone	Spray dried microspheres with up to 50% drug content were obtained	da Silva et al. (2009)
Carmustine	Spray drying	DCM	BCNU loaded microspheres were prepared by spray drying and subsequently compression molded into wafers	Seong et al. (2003)
Gentamicin	$W_1/O/W_2$ emulsification	DCM	High EE was achieved by increasing the osmotic value of the external water phase	Chaisri et al. (2011)
Haloperidol	O/W emulsification	DCM	Acid terminated PLGA showed higher EE compared to the ester capped PLGA.	Budhian et al. (2005)
5-fluorouracil	$S/O_1/O_2$ emulsification	DCM	high EE were achieved with paraffin oil containing lecithin as external phase	Yeh et al. (2001)
Imatinib mesylate	$W_1/O/W_2$ emulsification	DCM	high EE was achieved by lowering the solubility of the drug in external water phase with high pH	Ramazani et al. (2015)

used for preparation of microspheres. In the first part of this study, state-of-the-art technologies in microencapsulation are reviewed. Table 1 summarizes the discussed drugs and encapsulation methods. In the second part, process-related parameters are discussed that are critical for obtaining high encapsulation efficiency of small hydrophilic and amphiphilic molecules into PLGA microspheres with a focus on emulsion solvent extraction/evaporation-based methodologies.

2. Methods and strategies to encapsulate small hydrophilic and amphiphilic drugs in PLGA microspheres

2.1. Aqueous emulsion solvent evaporation based methods

Hydrophobic drug molecules are often encapsulated in PLGA microspheres by O/W solvent evaporation/extraction method (D'Aurizio et al., 2011b; Wischke and Schwendeman, 2008). Both the polymer and the drug are dissolved in a volatile organic solvent, commonly dichloromethane (DCM), and the resulting organic phase is emulsified under mechanical force in a continuous aqueous phase which contains an emulsifier for instance poly (vinyl alcohol) (PVA). Subsequently, as a result of solvent extraction and evaporation, the primary droplets shrink and transform into finally solid polymeric particles. To facilitate the encapsulation of drugs that are poorly soluble in DCM, a variety of co-solvents (e.g., dimethyl sulfoxide, acetone, acetonitrile, dimethylformamide or (m) ethanol) have been added to the polymer-DCM solution (Al-Maaieh and Flanagan, 2001; Jaraswekin et al., 2007; Kazazi-Hyseni et al., 2014). Utilizing water miscible cosolvents along with DCM results in a relatively fast mass-transfer from the organic solvents into the water phase and in a quick solidification of the particles. In a study of Al-Maaieh and Flanagan (2001) it was shown that the EE of quinidine sulfate, an antiarrhythmic drug, increased from 31% to 61%, resulting in a LC of 11% by using ethanol/DCM (1:5) instead of DCM which only achieved a LC of 5%. The microspheres prepared using ethanol/DCM or DCM as organic solvent were spherical with a non-porous surface which released the drug in a sustained manner for about one month. Additionally, the burst was higher for microspheres prepared with only DCM instead of ethanol/DCM (about 60% and 30% of the loading, respectively). Confocal microscopy images of the fluorescent microspheres (quinidine is fluorescent) showed that the drug was homogeneously distributed in the polymer matrix when ethanol was used as a cosolvent, while quinidine was heterogeneously distributed within and close to the surface of the microspheres when only DCM was used. This resulted in a higher burst release for microspheres prepared by only DCM compared to DCM/ethanol. Sah et al. prepared PLGA microspheres loaded with risperidone, an antipsychotic amphiphilic drug, using methyl dichloroacetate (water solubility of 4.6 g/l) instead of DCM (water solubility of 13 g/l) as solvent for the dispersed phase. Methyl dichloroacetate will be hydrolyzed rapidly into methanol and dichloroacetamide in diluted ammonia solutions, which will enhance the speed of solvent extraction from the formed emulsified droplets. This approach resulted in microspheres with nearly 100% EE and 40% LC (Sah and Lee, 2006). ¹HNMR, TGA and mass spectroscopy analysis showed that the microspheres did not contain any significant amount of residual methyl dichloroacetate demonstrating completely aminolysis of the organic solvent. The cross-sectional view of the SEM images displayed that the microspheres had a number of small inner cavities. The authors did not investigate the *in vitro* drug release from the microspheres, nor was it shown whether adding ammonia to the aqueous phase causes hydrolysis of PLGA or not. This is a concern since it has been suggested that aliphatic polyesters are more susceptible to hydrolysis at alkaline pH (de Jong et al., 2001; Göpferich 1996).

W₁/O/W₂ double emulsification is a practical approach to encapsulate water-soluble drugs (Gaignaux et al., 2012; Ramazani et al., 2015a; Ye et al., 2010b). First, an aqueous solution of the drug is dispersed in the organic phase which contains the polymer. The resulting primary W/O emulsion is subsequently dispersed into a large volume of an emulsifier-containing external water phase. Solidified microspheres are formed upon extraction and evaporation of the organic solvent. The initial W₁/O emulsion's stability is of primary importance for obtaining high EE of the resulting microspheres and is mostly governed by the volume ratio of the inner water droplets (W₁) to the volume of the organic phase. When the inner water volume approaches the volume of the organic phase the primary emulsion will break during subsequent double emulsification and step solvent extraction steps, resulting in polymeric particles with a low drug entrapment and a high burst release (Rosca et al., 2004). Keeping the outer water phase's volume (W₂) constant, the EE of 5-fluorouracil, a nucleoside metabolic inhibitor, increased from 28% to 35% when the W₁ volume decreased from 1.25 ml to 0.75 ml per 10 ml of organic phase (Parikh et al., 2003).

As an alternative to double emulsification, solid drug particles can be mixed into a polymer solution forming the initial drug-containing dispersed phase. The S/O suspension can subsequently be emulsified into polymeric droplets using similar procedures as reported for the earlier discussed methods for preparing microspheres. It is worth to mention that the drug particles have to be in the lower micrometer range to enable their encapsulation in PLGA microspheres, for instance by grinding, spray drying, spray freeze-drying, lyophilization and/or utilizing supercritical fluids technology (Martin and Cocero, 2008). Microspheres loaded with amoxicillin, a β-lactam antibiotic, were prepared with either W₁/O/W₂ emulsification or by S/O/W technique (Xu et al., 2009a). EE of amoxicillin improved using the S/O/W technique from 35% to 61% as compared W₁/O/W₂ emulsification method with an increase in LC from 7% to about 12% (Xu et al., 2009a). Moreover, this method has been applied to encapsulate lornoxicam, a nonsteroidal anti-inflammatory drug, into PLGA microspheres with high EE (82%) and LC (12%) aimed for intra-articular drug delivery (Zhang et al., 2011).

2.2. Non-aqueous emulsification methods

Aqueous emulsification of hydrophilic and amphiphilic molecules in polymeric particles can be compromised by relatively high drug flux into the continuous phase during the encapsulation process. To circumvent this, emulsion systems with non-aqueous external phases have been explored. In so-called W/O₁/O₂ emulsification methods a primary emulsion, prepared by an aqueous solution of drug mixed with the polymer solution, is dispersed into a large volume of a non-mixable organic oil which contains a suitable emulsifier (e.g., cotton oil/lecithin). Using this method, betamethasone phosphate disodium, a corticosteroid drug, reached an EE of 78% in PLGA microspheres, while its encapsulation was less than 15% using an aqueous W₁/O/W₂ emulsion method (Chaw et al., 2003). The EE of sodium alendronate, an inhibitor of bone resorption, improved from 1% for W₁/O/W₂ versus nearly 100% for W/O₁/O₂ and 86% for S/O₁/O₂ emulsification with liquid paraffin as external organic phase and 4% Span 85 as emulsifier (Nafea et al., 2007). The poor encapsulation of alendronate using W₁/O/W₂ was attributed to the high water solubility of this hydrophilic drug which favored its partitioning into the external water phase before hardening of the polymeric particles.

S/O₁/O₂ method employs micronized drug particles, similar as described above for S/O/W preparation of polymeric microspheres. While double emulsion methods with external water phases (W₁/

O/W₂, S/O/W) failed to encapsulate sufficient amounts of pamidronate disodium, a hydrophilic bisphosphonate drug for preventing osteoporosis, the S/O₁/O₂ method resulted in a high EE (~87%) and LC (~30%) of the drug (Weidenauer et al., 2003). Similar approaches, using emulsion systems with external organic phases, have been applied to encapsulate ciprofloxacin, a quinolone antibiotic, (Jeong et al., 2009) and ganciclovir, an antiviral nucleoside analogue, (Herrero-Vanrell et al., 2000) into PLGA microspheres. The S/O₁/O₂ method resulted in a high EE (98% and 95%) and LC (9.8% and 8.5%) of ciprofloxacin and ganciclovir loaded microspheres, respectively.

2.3. Membrane emulsification procedures for preparation of monodisperse microspheres

The particle size and size distribution are important for controlling the microparticle degradation as well as release kinetics of an encapsulated drug (Fredenberg et al., 2011; Tran et al., 2011). The particle size distribution is also an important factor in the *in vivo* fate of the particles by influencing the foreign body reaction. Small microspheres (~5 μm) are phagocytosed, while large microspheres (~30 μm) are not (Carr et al., 2012; Schmidt et al., 2013; Zandstra et al., 2014). Therefore, controlling the average particle size and size distribution can result in a better batch-to-batch reproducibility and optimized therapeutic efficacy. Recent advancements in microspheres manufacturing technology

permit the preparation of monodisperse microspheres with controlled particle size distribution (Acharya et al., 2010; Ito et al., 2010; Kazazi Hyseni et al., 2015; Tran et al., 2011).

One of the novel approaches for the fabrication of monodisperse microspheres is membrane emulsification (Makino et al., 2004). The basic principle of microencapsulation using membrane emulsification (ME) is similar to the previously described emulsification methods. In ME, small droplets of the organic phase are formed at the membrane pore openings and detach from the membrane surface by the shear stress of continuous phase. Depending on the process, microspheres can be formed by either O/W or W₁/O/W₂ methods (Fig. 2). In the O/W membrane emulsification approach, a continuous phase (emulsifier containing water) is flowing over the membrane through which the dispersed phase (drug dissolved in polymer solution) is pumped, resulting in the formation of emulsified droplets of uniform size. In double W₁/O/W₂ membrane emulsification method, the primary emulsion (W₁/O) is formed by mixing the aqueous drug solution into an organic solution (e.g., PLGA in DCM) using a homogenizer. The W₁/O emulsion is subsequently processed over the membrane to generate monodisperse droplets (Qi et al., 2014; Vladislavjević and Schubert, 2003).

Shirasu Porous Glass (SPG) is the most commonly used membrane for the preparation of monodisperse microspheres (Vladislavjević and Williams, 2005). The membranes are designed by a phase separation process of calcium aluminum borosilicate

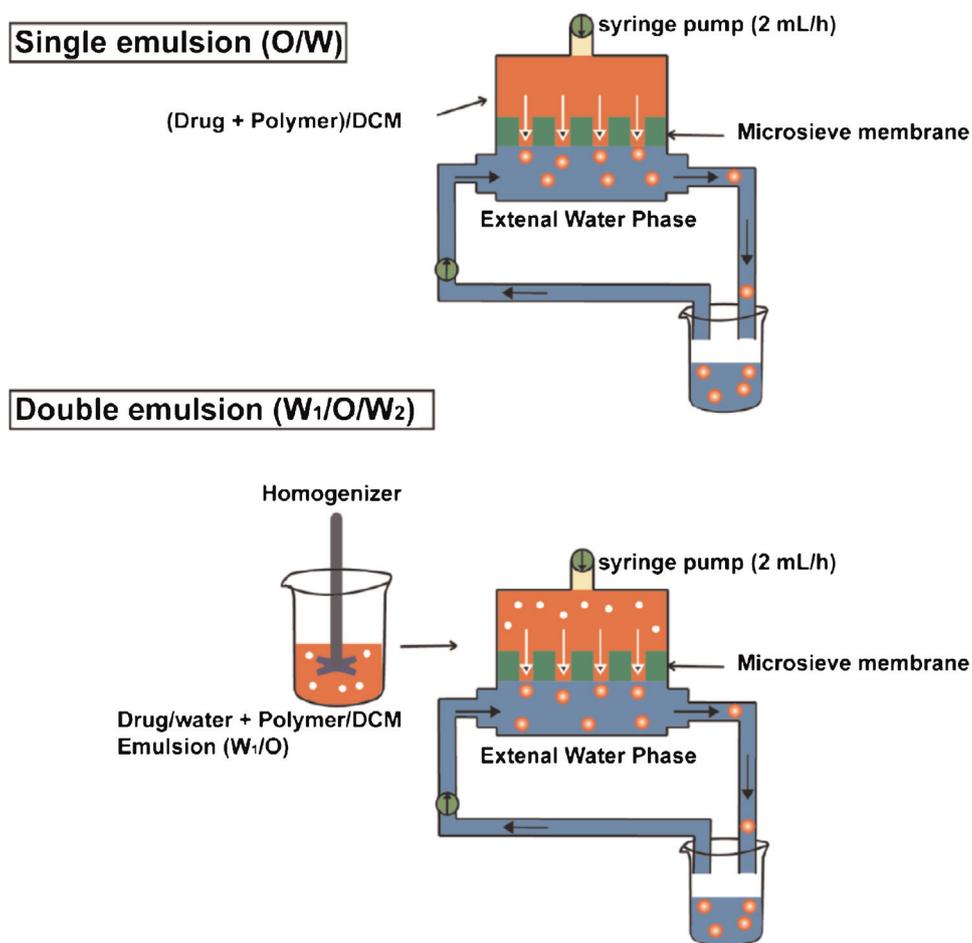


Fig. 2. Schematic representation of membrane emulsification techniques for fabricating monodisperse microparticles. Upper panel: O/W membrane emulsification approach. An organic phase containing drug and polymer is dispersed over the membrane into a continuous water phase. Bottom panel: double W₁/O/W₂ membrane emulsification approach. A primary W/O emulsion is formed by mixing the aqueous drug solution into an organic solution using conventional homogenizer. The W₁/O emulsion is subsequently processed over the membrane to generate monodisperse droplets. Modified from Kazazi-Hyseni et al., (2014) Pharm. Res. 31 (2014) 2844–2856 with permission from Elsevier.

glass heated at 1350 °C and subsequent acid leakage to form the membrane (Tran et al., 2011). The membranes are compatible with different emulsions (O/W, W₁/O/W₂, S/O/W, and S/O₁/O₂) by changing the surface chemistry to tune the hydrophilicity/hydrophobicity balance of the membrane surface (Tran et al., 2011). Using SPG membrane technology rifampicin loaded PLGA microspheres with a narrow size distribution was obtained (Ito and Makino, 2004). The EE of rifampicin ranged from 50–67% and it was independent of the microsphere size. However, the release of rifampicin was faster for microspheres of about 2 μm compared to particles with average size of 9 μm. Nanomi company has developed novel silicone-based membranes (Microsieves™) fabricated by photolithographic technique (Mao et al., 2008). The surface properties of this type of membranes can similarly be altered as reported for SPG membranes. In a recent study of Falke et al. using (2015) Microsieves™ technology, rapamycin-loaded monodisperse microspheres were fabricated by membrane sieving technology. The resulting rapamycin-loaded microspheres had an average particle size of (35 ± 5 μm) and 14% LC of rapamycin. The microspheres released rapamycin for about 12 days in an *in vitro* release study.

2.4. Microfluidics for preparation of monodisperse microspheres

Monodisperse polymeric microspheres have also been produced by microfluidic technologies (Kang et al., 2008; Perez et al., 2015). The microfluidic devices can be classified into co-flow capillary devices, flow-focusing capillary devices and the combination of these two principles as depicted in Fig. 3 (Shah et al.,

2008). This figure shows a schematic drawing of microfluidic devices for preparation of monodisperse particles using either single or double emulsion techniques. In co-flow capillary devices (Fig. 3A), the aqueous phase (e.g., PVA in water) is introduced into the two side channels and the organic phase that contains drug and polymer is directed into the central channel of the device, using syringe pumps with constant flow rates. Monodisperse emulsion droplets are continuously formed at the junction points of the combined microfluidic channels. (Perez et al., 2015; Shah et al., 2008).

Xu et al. (2009b) used a co-flow capillary device for preparation of microspheres loaded bupivacaine, an amphiphilic local anesthetic drug. In their study, microspheres with defined sizes, ranging from 10–50 μm depending on the applied flow rates were engineered by co-flow microfluidics emulsification, with very low polydispersity, while conventional emulsification produced particles with a broad size range. More importantly, striking differences were observed in the bupivacaine release profile of the microspheres produced by these techniques, with lower burst release and also overall lower release rates for the fluidics-based microspheres. The authors suggested that a more homogenous distribution of the drug in the microspheres prepared by microfluidics contributed to the lower burst release, as well as the absence of very small particles that are normally present in microspheres prepared by conventional emulsification.

In flow-focusing capillary devices, the two fluid phases (i.e., organic phase containing drug and polymer and continuous water phase) are introduced from opposite directions into the microfluidic mixing cell (Fig. 3B). The internal organic phase is flow

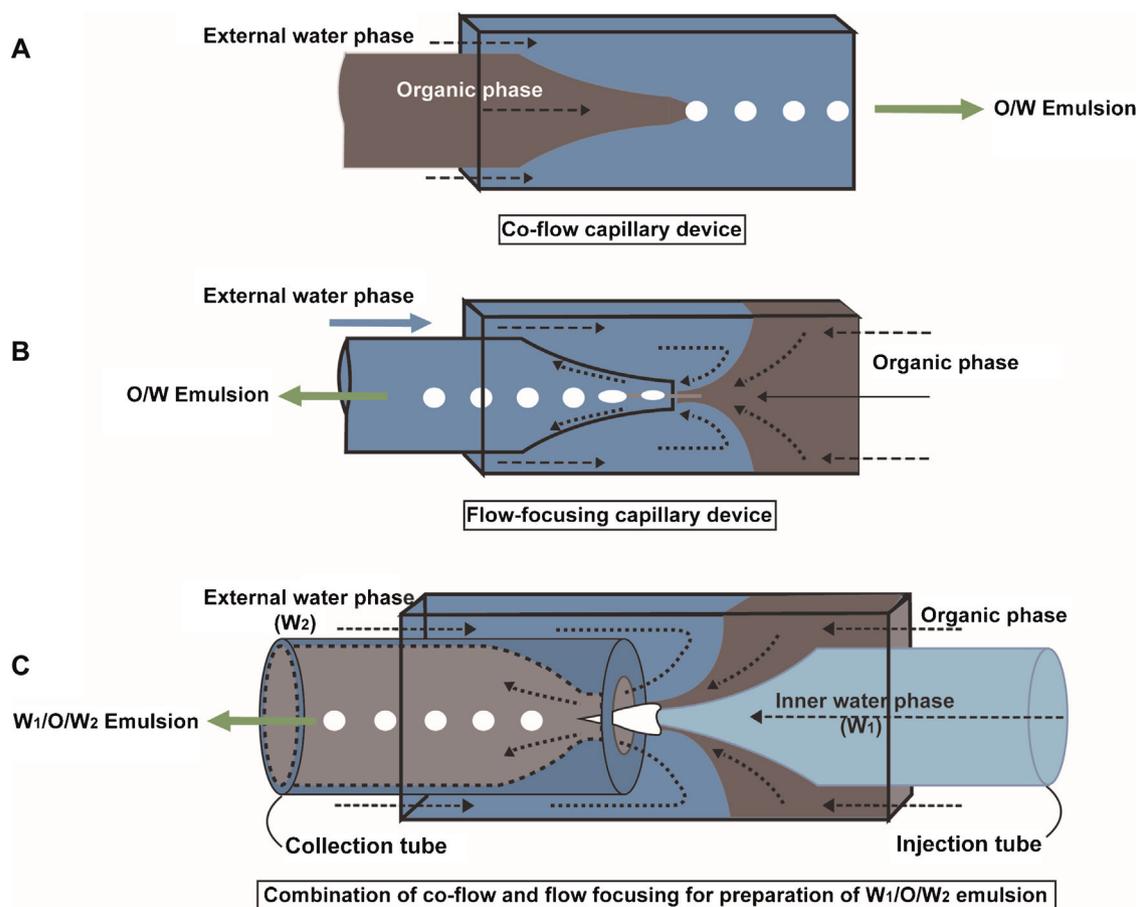


Fig. 3. Schematic demonstration of microfluidic devices for fabricating monodisperse microparticles. A: co-flow capillary device, B: flow-focusing capillary device and C: combination of co-flow and flow focusing for fabricating particles with W₁/O/W₂ emulsion method. Modified with permission from Shah et al., (2008) Materials Today, 11 (2008) 18–27.

focused hydrodynamically by the external aqueous fluid through the orifice. As the organic phase enters the orifice, it breaks up (by dripping or under jetting conditions) to generate monodisperse emulsion droplets (Tran et al., 2011). Compared to the conventional O/W emulsion method, blank PLGA microspheres that fabricated by flow focusing technique were narrower in particle size distribution (Perez et al., 2015).

Double emulsion based microspheres have also been prepared by microfluidic flow focusing techniques with the combination of a co-flow and flow focusing apparatus (Fig. 3C). Briefly, the inner water phase (*i.e.*, the phase that contains the drug dissolved in water) is pushed through a narrowing circular tube delivering small water droplets into the organic phase that is processed in a flow-focusing device, thus forming $W_1/O/W_2$ emulsion droplets (Marre and Jensen, 2010; Shah et al., 2008; Tu and Lee, 2012). Utada et al. (2005) used such a microfluidic device for preparing monodisperse microspheres that contained a single inner droplet in a core-shell geometry. Despite the limited production scale of a single microfluidic device (~ 50 – 300 mg/h), scaling up of this technology is certainly possible by operating multiple microfluidic devices in parallel simultaneously.

2.5. Ink jet printing for manufacturing microspheres with desired shape

Inkjet printing (IJP) is a method for manufacturing differently shaped devices (Agarwal et al., 2012; Kolakovic et al., 2013; Lee et al., 2012) and can be classified into continuous and drop-on-demand approaches. In the continuous approach, using a high-pressure pump, a liquid ink (drug and polymer solution dissolved in an organic solvent) is released through an orifice with defined diameter (typically 50 – 80 μm) creating a constant current of ink. The stream of ink breaks down into micro-droplets using a piezoelectric crystal or by heating. In thermal IJP, small volumes of the ink solvent are vaporized by a micro-heater creating the pulse that ejects droplets from the printer head. In piezoelectric inkjet printing, an electric current is applied to the ink in the nozzle by a piezoelectric actuator causing a shock wave that pushes out the ink through the nozzle (Fig. 4A). Ink viscosity and surface tension will affect the geometry and size of the final particles. The viscosity of the ink should be low (< 20 cP) as the piezoelectric printer used for preparation of such particles has only low jetting power (Lee et al., 2012; Sumerel et al., 2006). Moderate surface tension (30 – 70 mN/m) of the ink is required for obtaining well defined printed microspheres (Shah et al., 2008). Low surface tension causes dripping of the ink from the outlet and high surface tension is not good for proper distribution of the ink over the substrate. In drop-

on-demand method, drops of ink are only ejected as required while in continuous mode printers, droplets are propelled in constant manner. In the drop-on-demand method, thermal elements or piezoelectric crystals are also used to break down the ink to particles.

Printing technology allows the production of well-defined structures with unique control over the three-dimensional properties of the obtained products. Although best known for production of larger constructs, 3D printing has also been applied for the preparation of drug-loaded microspheres. Lee et al. (2012) printed paclitaxel loaded with PLGA microspheres with diverse shapes as shown in Fig. 4 using a continuous mode piezoelectric printer. The ink used for printing of these microspheres consisted of paclitaxel and PLGA dissolved in *N,N*-dimethylacetamide (DMAc), a non-volatile solvent. The resulting microspheres with 10% LC showed a fairly uniform shape and size for each of the chosen geometries (Fig. 4B). It is worth noting that the authors did not check the residual content of organic solvent in the formed microspheres. According to the United States Pharmacopeia (USP), DMAc is classified as class 2 solvent and its content in formulations should be limited to 1090 ppm. One of the attractive findings of the above study is that the release rate of paclitaxel correlated nicely to the total surface area of the printed particles, as determined using a surface profilometer. It was found that the drug release was highest for particles with a relatively larger surface area, with lowest release for spherical particles (circle) and highest release for relative open microstructures like grid and honeycomb printed microspheres. (Fig. 4C) (Lee et al., 2012).

Regarding the 'ink' formulation, it is worth to mention that the viscosity and surface tension plays a crucial role in the printing process. As mentioned above, ink viscosity and surface tension will affect the geometry and size of the final particles. There is no optimum value for the viscosity and surface tension in inkjet printing as they are dependent on the printer devices. Compared to volatile solvents, organic solvents with high boiling points such as DMSO (Scoutaris et al., 2011) and isobutanol (Tarcha et al., 2007) are shown to prevent nozzle clogging during printing.

2.6. Spray drying techniques for preparation of microspheres

Spray-drying has intensively been studied for the production of protein and plasmid DNA loaded microspheres (Daugherty et al., 2011; Gavini et al., 2004; Marquette et al., 2014; Ye et al., 2010a). This technology has also been applied for the encapsulation of small molecules (Beck-Broichsitter et al., 2015; Chaw et al., 2003; da Silva et al., 2009; Seong et al., 2003). Microdroplets of W/O emulsions (*i.e.*, emulsions containing the drug in the inner water

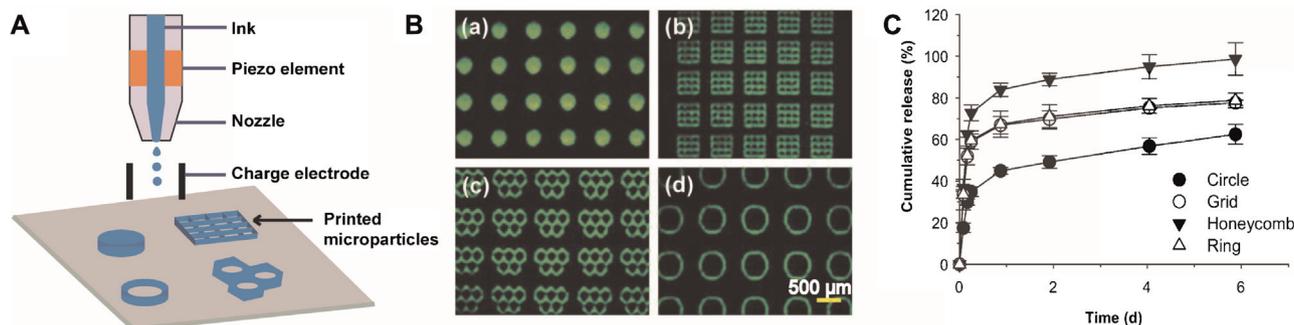


Fig. 4. Production of drug-loaded PLGA microstructures by inkjet printing technology. A: schematic representation of the inkjet system which contains a Piezo element for controlling ink flow. B: Fluorescence microscopy images of paclitaxel-loaded PLGA microparticles with different shapes. The scale bar is 500 μm . C: *in vitro* release profiles of printed microparticles. Modified from Lee et al., (2012) Int. J. Pharm. 427 (2012) 305–310 with permission from Elsevier.

phase and the polymer in the organic phase) can be spray dried using an appropriate nozzle, with a proper inlet/outlet temperature, to form microspheres. Such an approach is also feasible for or any of the other types polymeric formulations discussed, like dispersions of micronized drug particles in the polymer-containing organic phase. Since no outer solvent phase is used in such a spray drying process, high EE can be reached for hydrophilic and amphiphilic drugs, as was demonstrated for betamethasone phosphate (EE >90%, as compared to only 15% when emulsified by conventional $W_1/O/W_2$ method) (Chaw et al., 2003). Similarly high EE (90–98%) and LC (~50%) were achieved for spray-dried PLGA microspheres of triamcinolone, a corticosteroid drug, (da Silva et al., 2009). Moreover, spray drying technique was utilized to encapsulate carmustine (BCNU), an anti-cancer drug, into PLGA microspheres with about 90% encapsulation efficiency (Seong et al., 2003). BCNU was released *in vitro* (pH 7.4 phosphate buffered saline) from these microspheres up to 8 weeks.

Spray drying is a relatively simple and versatile method for large-scale production of microspheres. However, it has its drawbacks for temperature-sensitive drugs and for small batch production (*i.e.*, lab scale) as in this case the yield is usually low.

3. Optimization of formulation parameters to improve drug encapsulation efficiency using emulsification methods

The solidification time is one of the mostly discussed factors regarding encapsulation efficiency of drugs in PLGA microspheres prepared using emulsion solvent evaporation methods (Katou et al., 2008; Ng et al., 2010a; Yeo and Park, 2004). The solidification of microspheres occurs in two steps, namely extraction and evaporation. Since drug partitioning in the continuous phase substantially retards when the polymer droplets have solidified, process and formulation parameters which accelerate the solidification of the particles will improve the EE of amphiphilic and hydrophilic drugs (Ng et al., 2010a; Yeo and Park, 2004). Important factors that contribute to the rate of solvent extraction/evaporation are the solubility of the organic solvent in the external water phase, its boiling point and the volume ratios of the dispersed phase and continuous phase. The effect of these parameters and several other factors that play a role in microsphere solidification and consequently in drug encapsulation efficiency are addressed in the following sections.

3.1. The influence of polymer concentration, molecular weight and composition

Polymer molecular weight and concentration are positively correlated to the organic phase's viscosity that subsequently correlates to the drug diffusion constant (Katou et al., 2008). A high viscosity of the organic phase, either by using polymers of relatively high molecular weight or by using high concentrations of the polymer will result in high EE. Chaisri et al. (2011) showed that increasing the PLGA concentration in DCM from 10% to 15% resulted in an increase in gentamicin, an aminoglycoside antibiotic, EE from 17% to 68%. A higher polymer concentration does not only increase the viscosity of the organic phase but also result (at the same solvent evaporation kinetics) in a faster solidification of the PLGA droplets.

It has been shown that for PLGA, an increase in lactide/glycolide ratio and a decrease in polymer molecular weight increases its solubility in DCM (Yeo and Park, 2004). Furthermore, PLGA is commercially available with either a terminal free carboxylic group or an ester terminated ('end-capped' PLGA) group (Tracy et al., 1999). End-capped PLGA usually has a methyl, ethyl or lauryl ester and has a better solubility in DCM as compared to acid terminated polymers (Yeo and Park, 2004). Budhian et al. (2005) loaded

haloperidol, an amphiphilic antipsychotic drug, into PLGA using O/W emulsion solvent evaporation. The EE was 30% using acid terminated PLGA as compared to 10% in particles based on capped PLGA. The authors proposed that the interaction of haloperidol with the carboxylic acid end groups of PLGA results in slower drug diffusion from the polymeric particles prepared with acid terminated PLGA. Based on the molar ratio of acid terminated PLGA to haloperidol (1/3), about 30% of the loaded drug had the ability to interact by hydrogen bonding with PLGA. However, the authors did not verify the presence of hydrogen bonding between the drug and the polymer by means of analytical techniques such as FTIR spectroscopy (Blasi et al., 2007).

3.2. Solubility of drug in the external phase

Different approaches have been applied to decrease the efflux of amphiphilic drugs into the external water phase. The encapsulation of quinidine sulfate increased by adding different salts (NaCl, NaBr, NaSCN, NaClO₄ and Na₂SO₄) to the external medium (Al-Maaieh and Flanagan, 2001). The EE of quinidine sulfate was 31% without adding salt in the outer phase, whereas it increased up to 100% by adding 0.5 M NaSCN to the external water phase. Adding Ca²⁺ as counter ion of alendronate to the external phase improved the EE of alendronate from 4.5% (without using Ca²⁺) to 83.4% (by using 1/2 alendronate/calcium ratio) (Cohen-Sela et al., 2009). The High EE can be explained by the reduced alendronate solubility in the organic/aqueous interphase. Ca²⁺ most likely neutralizes the drug charge by coupling to the phosphate groups of alendronate. The bis-alendronate calcium salt is more hydrophobic than sodium alendronate, thus explaining its efficient partitioning into the organic phase.

The water solubility of ionizable drugs is highly dependent on the pH (Ramazani et al., 2015b). For efficient encapsulating of such drugs, one should use an external buffer with pH that favors a high logD of the drug, *i.e.*, a pH at which the drug carries no net charge and thus has the lowest aqueous solubility. On the other hand, the pH of inner water phase has to be adjusted to a pH at which the drug is charged. A charged drug has a higher water solubility and this results in microspheres with high LC (Al-Maaieh and Flanagan, 2001; Labouta et al., 2009; Ramazani et al., 2015a). Imatinib mesylate, a bcr-abl kinase inhibitor, was formulated in PLGA microspheres with high EE and LC by optimizing the pH of the internal (W_1) and external (W_2) water phases (Ramazani et al., 2015a). The W_2 consisted of buffers with pH values ranging from 5.0–9.0 which contained 1% PVA as emulsifier. It was found that the EE of imatinib was significantly pH dependent: the EE was 10% at pH 5.0 which increased to 90% for a pH 9.0 of W_2 phase while the pH of W_1 phase was rather acidic (pH 5.0). Interestingly, only 4% of mesylate, its counter ion, was encapsulated in the microspheres at the same situation (W_2 of pH 9.0). It was concluded that since mesylate is extremely water soluble, it will not partition into the organic phase. Conversely, imatinib, with a pK_a of about 8, is uncharged at pH 9.0 which can explain its high EE when the particles were prepared using an external water phase of pH 9.0. To avoid possible hydrolysis of the ester bonds of PLGA during preparation of microspheres at high pH (Göpferich, 1996; Makadia and Siegel, 2011), the external buffer was replaced by a buffer of pH 7.0 after solidification of the microspheres. In conclusion, adjusting the pH of water phase is a key in improving the encapsulation efficiency of ionizable drugs.

3.3. The effect of emulsifiers

In the $W_1/O/W_2$ emulsion method, the stability of the initial W_1/O emulsion is an important factor in achieving high EE of water soluble drugs. The stability of the inner W_1/O emulsion can be

enhanced by adding emulsifiers such as PVA to the primary emulsion. Such emulsifiers can however also increase the solubility of drugs in the external W_2 phase, thus leading to low encapsulation efficiencies especially when concentrations above their critical micelle concentration (CMC) are used (Giunchedi et al., 1998). For example, increasing lecithin concentrations from 0.05% to 2% in the W_2 phase resulted in a decrease in EE of 5-fluorouracil from 79% to 35% (Yeh et al., 2001).

4. Conclusion

The present article provides an overview of methodologies for the encapsulation of small hydrophilic and amphiphilic drugs into PLGA microspheres. Furthermore, formulation-related parameters which are important for efficient encapsulation of small hydrophilic and amphiphilic molecules into PLGA microspheres are discussed. Hydrophobic small drug molecules, due to their inherently high logP, typically have a high EE in emulsion solvent extraction/evaporation methods. On the other hand, hydrophilic and amphiphilic compounds, with low to intermediate logP, have a tendency to distribute into the excess of the external water phase, which often results in low encapsulation efficiency. Therefore, the development of new methods or improving the existing methods for improving the encapsulation of small hydrophilic and amphiphilic drugs in PLGA microspheres is of great interest. The main factors involved in the preparation of PLGA microspheres with high encapsulation efficiency are the PLGA concentration in the organic phase, PLGA molecular weight and composition, the water miscibility of the organic solvent and/or cosolvents that have been utilized to dissolve the polymer or the drug, the type and concentration of emulsifier and the pH of internal/external water phases in case of ionizable drugs. In addition, water-free methodologies such as emulsion with external organic phases, spray drying and inkjet printing can produce microspheres with high encapsulation efficiency. Novel double emulsion procedures using microfluidics devices that can produce microspheres in a robust and well controlled manner with controlled inner droplets size are promising for efficient encapsulation of water soluble drugs. These technological advancements in the field of particle production can increase the number of long-acting small molecules formulations entering the market in the near future.

Conflict of interest

The authors declare that there is no conflict of interest in this work.

References

- Acharya, G., Shin, C.S., McDermott, M., Mishra, H., Park, H., Kwon, I.C., Park, K., 2010. The hydrogel template method for fabrication of homogeneous nano/microparticles. *J. Control. Release* 141, 314–319.
- Agarwal, R., Singh, V., Journey, P., Shi, L., Sreenivasan, S.V., Roy, K., 2012. Scalable imprinting of shape-specific polymeric nanocarriers using a release layer of switchable water solubility. *ACS Nano* 6, 2524–2531.
- Al-Maaieh, A., Flanagan, D.R., 2001. Salt and cosolvent effects on ionic drug loading into microspheres using an O/W method. *J. Control. Release* 70, 169–181.
- Beck-Broichsitter, M., Paulus, I.E., Greiner, A., Kissel, T., 2015. Modified vibrating-mesh nozzles for advanced spray-drying applications. *Eur. J. Pharm. Biopharm.* 92, 96–101.
- Benny, O., Duvshani-Eshet, M., Cargioli, T., Bello, L., Bikfalvi, A., Carroll, R.S., Machluf, M., 2005. Continuous delivery of endogenous inhibitors from poly(lactide-co-glycolic acid) polymeric microspheres inhibits glioma tumor growth. *Clin. Cancer Res.* 11, 768–776.
- Benny, O., Menon, L.G., Ariel, G., Goren, E., Kim, S.K., Stewman, C., Black, P.M., Carroll, R.S., Machluf, M., 2009. Local delivery of poly(lactide-co-glycolic acid) microspheres containing imatinib mesylate inhibits intracranial xenograft glioma growth. *Clin. Cancer Res.* 15, 1222–1231.
- Blasi, P., Schoubben, A., Giovagnoli, S., Perioli, L., Ricci, M., Rossi, C., 2007. Ketoprofen poly(lactide-co-glycolide) physical interaction. *AAPS PharmSciTech* 8, 1530–9932.
- Budhian, A., Siegel, S.J., Winey, K.I., 2005. Production of haloperidol-loaded PLGA nanoparticles for extended controlled drug release of haloperidol. *J. Microencapsul.* 22, 773–785.
- Carr, K.E., Smyth, S.H., McCullough, M.T., Morris, J.F., Moyes, S.M., 2012. Morphological aspects of interactions between microparticles and mammalian cells: intestinal uptake and onward movement. *Prog. Histochem. Cytochem.* 46, 185–252.
- Casalini, T., Rossi, F., Lazzari, S., Perale, G., Masi, M., 2014. Mathematical modeling of PLGA microparticles: from polymer degradation to drug release. *Mol. Pharm.* 11, 4036–4048.
- Chaisri, W., Ghassemi, A.H., Hennink, W.E., Okonogi, S., 2011. Enhanced gentamicin loading and release of PLGA and PLHMGA microspheres by varying the formulation parameters. *Colloids Surf. B Biointerfaces* 84, 508–514.
- Chaisri, W., Hennink, W.E., Okonogi, S., 2009. Preparation and characterization of cephalixin loaded PLGA microspheres. *Curr. Drug Deliv.* 6, 69–75.
- Chaw, C.S., Yang, Y.Y., Lim, I.J., Phan, T.T., 2003. Water-soluble betamethasone-loaded poly(lactide-co-glycolide) hollow microparticles as a sustained release dosage form. *J. Microencapsul.* 20, 349–359.
- Chen, Z.G., 2010. Small-molecule delivery by nanoparticles for anticancer therapy. *Trends Mol. Med.* 16, 594–602.
- Cohen-Sela, E., Chorny, M., Koroukhov, N., Danenberg, H.D., Golomb, G., 2009. A new double emulsion solvent diffusion technique for encapsulating hydrophilic molecules in PLGA nanoparticles. *J. Control. Release* 133, 90–95.
- da Silva Jr., A.A., de Matos, J.R., Formariz, T.P., Rossanezi, G., Scarpa, M.V., do Egito, E. S., de Oliveira, A.G., 2009. Thermal behavior and stability of biodegradable spray-dried microparticles containing triamcinolone. *Int. J. Pharm.* 368, 45–55.
- Daugherty, A.L., Rangell, L.K., Eckert, R., Zavala-Solorio, J., Peale, F., Mrsny, R.J., 2011. Sustained release formulations of rhVEGF(1)(6)(5) produce a durable response in a murine model of peripheral angiogenesis. *Eur. J. Pharm. Biopharm.* 78, 289–297.
- D'Aurizio, E., Sozio, P., Cerasa, L.S., Vacca, M., Brunetti, L., Orlando, G., Chiavaroli, A., Kok, R.J., Hennink, W.E., Di Stefano, A., 2011a. Biodegradable microspheres loaded with an anti-Parkinson prodrug: an in vivo pharmacokinetic study. *Mol. Pharm.* 8, 2408–2415.
- D'Aurizio, E., van Nostrum, C.F., van Steenberghe, M.J., Sozio, P., Siepmann, F., Siepmann, J., Hennink, W.E., Di Stefano, A., 2011b. Preparation and characterization of poly(lactide-co-glycolic acid) microspheres loaded with a labile antiparkinson prodrug. *Int. J. Pharm.* 409, 289–296.
- de Jong, S.J., van Eerdenbrugh, B., van Nostrum, C.F., Kettenes-van den Bosch, J.J., Hennink, W.E., 2001. Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: degradation and protein release behavior. *J. Control. Release* 71, 261–275.
- Falke, L.L., van Vuuren, S.H., Kazazi-Hyseni, F., Ramazani, F., Nguyen, T.Q., Veldhuis, G.J., Maarseveen, E.M., Zandstra, J., Zuidema, J., Duque, L.F., Steendam, R., Popa, E. R., Kok, R.J., Goldschmeding, R., 2015. Local therapeutic efficacy with reduced systemic side effects by rapamycin-loaded subcapsular microspheres. *Biomaterials* 42, 151–160.
- Ford Versypt, A.N., Pack, D.W., Braatz, R.D., 2013. Mathematical modeling of drug delivery from autocatalytically degradable PLGA microspheres—a review. *J. Control. Release* 165, 29–37.
- Fredenberg, S., Wahlgren, M., Reslow, M., Axelsson, A., 2011. The mechanisms of drug release in poly(lactide-co-glycolic acid)-based drug delivery systems—a review. *Int. J. Pharm.* 415, 34–52.
- Gaignaux, A., Reeff, J., Siepmann, F., Siepmann, J., De Vriese, C., Goole, J., Amighi, K., 2012. Development and evaluation of sustained-release clonidine-loaded PLGA microparticles. *Int. J. Pharm.* 437, 20–28.
- Gasparini, G., Holdich, R.G., Kosvintsev, S.R., 2010. PLGA particle production for water-soluble drug encapsulation: degradation and release behaviour. *Colloids Surf. B Biointerfaces* 75, 557–564.
- Gavini, E., Chetoni, P., Cossu, M., Alvarez, M.G., Saettone, M.F., Giunchedi, P., 2004. PLGA microspheres for the ocular delivery of a peptide drug, vancomycin using emulsification/spray-drying as the preparation method: in vitro/in vivo studies. *Eur. J. Pharm. Biopharm.* 57, 207–212.
- Giunchedi, P., Alpar, H.O., Conte, U., 1998. PDLLA microspheres containing steroids: spray-drying, o/w and w/o/w emulsifications as preparation methods. *J. Microencapsul.* 15, 185–195.
- Göpferich, A., 1996. Mechanisms of polymer degradation and erosion. *Biomate* 17, 103–114.
- Herrero-Vanrell, R., Bravo-Osuna, I., Andres-Guerrero, V., Vicario-de-la-Torre, M., Molina-Martinez, I.T., 2014. The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Prog. Retinal Eye Res.* 42, 27–43.
- Herrero-Vanrell, R., Ramirez, L., Fernandez-Carballido, A., Refojo, M.F., 2000. Biodegradable PLGA microspheres loaded with ganciclovir for intraocular administration. Encapsulation technique, in vitro release profiles, and sterilization process. *Pharm. Res.* 17, 1323–1328.
- Ito, F., Fujimori, H., Honnami, H., Kawakami, H., Kanamura, K., Makino, K., 2010. Control of drug loading efficiency and drug release behavior in preparation of hydrophilic-drug-containing monodisperse PLGA microspheres. *J. Mater. Sci. Mater. Med.* 21, 1563–1571.
- Ito, F., Makino, K., 2004. Preparation and properties of monodispersed rifampicin-loaded poly(lactide-co-glycolide) microspheres. *Colloids Surf. B Biointerfaces* 39, 17–21.
- Jaraswekin, S., Prakongpan, S., Bodmeier, R., 2007. Effect of poly(lactide-co-glycolide) molecular weight on the release of dexamethasone sodium phosphate from microparticles. *J. Microencapsul.* 24, 117–128.

- Jeong, Y.I., Na, H.S., Nah, J.W., Lee, H.C., 2009. Preparation of ciprofloxacin-encapsulated poly(D,L-lactide-co-glycolide) microspheres and its antibacterial activity. *J. Pharm. Sci.* 98, 3659–3665.
- Kang, L., Chung, B.G., Langer, R., Khademhosseini, A., 2008. Microfluidics for drug discovery and development: from target selection to product lifecycle management. *Drug Discov. Today* 13, 1–13.
- Katou, H., Wandrey, A.J., Gander, B., 2008. Kinetics of solvent extraction/evaporation process for PLGA microparticle fabrication. *Int. J. Pharm.* 364, 45–53.
- Kazazi Hyseni, F., van Vuuren, S.H., van der Giezen, D.M., Pieters, E., Ramazani, F., Rodriguez, S., Veldhuis, G.J., Goldschmeding, R., van Nostrum, C.F., Hennink, W.E., Kok, R.J., 2015. Release and pharmacokinetics of near-infrared labeled albumin from monodisperse poly(D,L-lactide-co-hydroxymethyl glycolic acid) microspheres after subcapsular renal injection. *Acta Biomater.* 22, 141–154.
- Kazazi-Hyseni, F., Landin, M., Lathuile, A., Veldhuis, G.J., Rahimian, Z., Hennink, W.E., Kok, R.J., van Nostrum, C.F., 2014. Computer modeling assisted design of monodisperse PLGA microspheres with controlled porosity affords zero order release of an encapsulated macromolecule for 3 months. *Pharm. Res.* 31, 2844–2856.
- Kolakovic, R., Viitala, T., Ihalainen, P., Genina, N., Peltonen, J., Sandler, N., 2013. Printing technologies in fabrication of drug delivery systems. *Expert Opin. Drug Deliv.* 10, 1711–1723.
- Labouta, H.I., el-Khordagui, L.K., Molokhia, A.M., Ghaly, G.M., 2009. Multivariate modeling of encapsulation and release of an ionizable drug from polymer microspheres. *J. Pharm. Sci.* 98, 4603–4615.
- Lee, B.K., Yun, Y.H., Choi, J.S., Choi, Y.C., Kim, J.D., Cho, Y.W., 2012. Fabrication of drug-loaded polymer microparticles with arbitrary geometries using a piezoelectric inkjet printing system. *Int. J. Pharm.* 427, 305–310.
- Makadia, H.K., Siegel, S.J., 2011. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 3, 1377–1397.
- Makino, K., Nakajima, T., Shikamura, M., Ito, F., Ando, S., Kochi, C., Inagawa, H., Soma, G., 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. *Colloids Surf. B Biointerfaces* 36, 35–42.
- Mao, S., Shi, Y., Li, L., Xu, J., Schaper, A., Kissel, T., 2008. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. *Eur. J. Pharm.* 68, 214–223.
- Marquette, S., Peerboom, C., Yates, A., Denis, L., Langer, I., Amighi, K., Goole, J., 2014. Stability study of full-length antibody (anti-TNF alpha) loaded PLGA microspheres. *Int. J. Pharm.* 470, 41–50.
- Marre, S., Jensen, K.F., 2010. Synthesis of micro and nanostructures in microfluidic systems. *Chem. Soc. Rev.* 39, 1183–1202.
- Martin, A., Cocero, M.J., 2008. Micronization processes with supercritical fluids: fundamentals and mechanisms. *Adv. Drug Deliv. Rev.* 60, 339–350.
- Nafea, E.H., El-Massik, M.A., El-Khordagui, L.K., Marei, M.K., Khalafallah, N.M., 2007. Alendronate PLGA microspheres with high loading efficiency for dental applications. *J. Microencapsul.* 24, 525–538.
- Ng, S., Choi, J., Han, H., Huh, J., Lim, J.O., 2010a. Novel microencapsulation of potential drugs with low molecular weight and high hydrophilicity: hydrogen peroxide as a candidate compound. *Int. J. Pharm.* 384, 120–127.
- Ng, S., Choi, J., Han, H., Huh, J., Lim, J.O., 2010b. Novel microencapsulation of potential drugs with low molecular weight and high hydrophilicity: hydrogen peroxide as a candidate compound. *Int. J. Pharm.* 384, 120–127.
- Ozeki, T., Kaneko, D., Hashizawa, K., Imai, Y., Tagami, T., Okada, H., 2012. Improvement of survival in C6 rat glioma model by a sustained drug release from localized PLGA microspheres in a thermoreversible hydrogel. *Int. J. Pharm.* 427, 299–304.
- Parikh, R.H., Parikh, J.R., Dubey, R.R., Soni, H.N., Kapadia, K.N., 2003. Poly(D,L-lactide-co-glycolide) microspheres containing 5-fluorouracil: optimization of process parameters. *AAPS PharmSciTech* 4, 1530–9932.
- Perez, A., Hernandez, R., Velasco, D., Voicu, D., Mijangos, C., 2015. Poly (lactic-co-glycolic acid) particles prepared by microfluidics and conventional methods: modulated particle size and rheology. *J. Colloid Interface Sci.* 441, 90–97.
- Qi, F., Wu, J., Hao, D., Yang, T., Ren, Y., Ma, G., Su, Z., 2014. Comparative studies on the influences of primary emulsion preparation on properties of uniform-sized exenatide-loaded PLGA microspheres. *Pharm. Res.* 31, 1566–1574.
- Ramazani, F., Chen, W., Van Nostrum, C.F., Storm, G., Kiessling, F., Lammers, T., Hennink, W.E., Kok, R.J., 2015a. Formulation and characterization of microspheres loaded with imatinib for sustained delivery. *Int. J. Pharm.* 482, 123–130.
- Ramazani, F., Hiemstra, C., Steendam, R., Kazazi-Hyseni, F., Van Nostrum, C.F., Storm, G., Kiessling, F., Lammers, T., Hennink, W.E., Kok, R.J., 2015b. Sunitinib microspheres based on [PDLA-PEG-PDLA]-b-PLLA multi-block copolymers for ocular drug delivery. *Eur. J. Pharm. Biopharm.* 95 (Pt B), 368–377.
- Rosca, I.D., Watari, F., Uo, M., 2004. Microparticle formation and its mechanism in single and double emulsion solvent evaporation. *J. Control. Release* 99, 271–280.
- Sah, H., Lee, B., 2006. Development of new microencapsulation techniques useful for the preparation of PLGA microspheres. *Macromol. Rapid Commun.* 27, 1845–1851.
- Schmidt, C., Lautenschlaeger, C., Collnot, E.M., Schumann, M., Bojarski, C., Schulzke, J.D., Lehr, C.M., Stallmach, A., 2013. Nano- and microscaled particles for drug targeting to inflamed intestinal mucosa: a first in vivo study in human patients. *J. Control. Release* 165, 139–145.
- Scoutaris, N., Alexander, M.R., Gellert, P.R., Roberts, C.J., 2011. Inkjet printing as a novel medicine formulation technique. *J. Control. Release* 156, 179–185.
- Seong, H., An, T.K., Khang, G., Choi, S.U., Lee, C.O., Lee, H.B., 2003. BCNU-loaded poly(D,L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells in vitro. *Int. J. Pharm.* 251, 1–12.
- Shah, R.K., Shum, H.C., Rowat, A.C., Lee, D., Agresti, J.J., Utada, A.S., Chu, L., Kim, J., Fernandez-Nieves, A., Martinez, C.J., Weitz, D.A., 2008. Designer emulsions using microfluidics. *Materials* 11, 18–27.
- Shive, M.S., Anderson, J.M., 1997. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Deliv. Rev.* 28, 5–24.
- Sumerel, J., Lewis, J., Doraiswamy, A., Deravi, L.F., Sewell, S.L., Gerdon, A.E., Wright, D. W., Narayan, R.J., 2006. Piezoelectric ink jet processing of materials for medical and biological applications. *Biotechnol. J.* 1, 976–987.
- Tarcha, P.J., Verlee, D., Hui, H.W., Setesak, J., Antohe, B., Radulescu, D., Wallace, D., 2007. The application of ink-jet technology for the coating and loading of drug-eluting stents. *Ann. Biomed. Eng.* 35, 1791–1799.
- Tracy, M.A., Ward, K.L., Firouzabadian, L., Wang, Y., Dong, N., Qian, R., Zhang, Y., 1999. Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro. *Biomaterials* 20, 1057–1062.
- Tran, V.T., Benoit, J.P., Venier-Julienne, M.C., 2011. Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy? *Int. J. Pharm.* 407, 1–11.
- Tu, F., Lee, D., 2012. Controlling the stability and size of double-emulsion-templated poly(lactic-co-glycolic) acid microcapsules. *Langmuir* 28, 9944–9952.
- Utada, A.S., Lorenceau, E., Link, D.R., Kaplan, P.D., Stone, H.A., Weitz, D.A., 2005. Monodisperse double emulsions generated from a microcapillary device. *Science* 308, 537–541.
- 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.
- Vladisavljević, G.T., Williams, R.A., 2005. Recent developments in manufacturing emulsions and particulate products using membranes. *Adv. Colloid Interface Sci.* 113, 1–20.
- Vrignaud, S., Benoit, J.P., Saulnier, P., 2011. Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles. *Biomaterials* 32, 8593–8604.
- Weidenauer, U., Bodmer, D., Kissel, T., 2003. Microencapsulation of hydrophilic drug substances using biodegradable polyesters: part I: evaluation of different techniques for the encapsulation of pamidronate di-sodium salt. *J. Microencapsul.* 20, 509–524.
- Wischke, C., Schwendeman, S.P., 2008. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *Int. J. Pharm.* 364, 298–327.
- Xu, Q., Crossley, A., Czernuszka, J., 2009a. Preparation and characterization of negatively charged poly(lactic-co-glycolic acid) microspheres. *J. Pharm. Sci.* 98, 2377–2389.
- 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.
- Ye, M., Kim, S., Park, K., 2010a. Issues in long-term protein delivery using biodegradable microparticles. *J. Control. Release* 146, 241–260.
- Ye, M., Kim, S., Park, K., 2010b. Issues in long-term protein delivery using biodegradable microparticles. *J. Control. Release* 146, 241–260.
- Yeh, M.K., Tung, S.M., Lu, D.W., Chen, J.L., Chiang, C.H., 2001. Formulation factors for preparing ocular biodegradable delivery system of 5-fluorouracil microparticles. *J. Microencapsul.* 18, 507–519.
- Yeo, Y., Park, K., 2004. Control of encapsulation efficiency and initial burst in polymeric microparticle systems. *Arch. Pharm. Res.* 27, 1–12.
- Zandstra, J., Hiemstra, C., Petersen, A.H., Zuidema, J., van Beuge, M.M., Rodriguez, S., Lathuile, A.A., Veldhuis, G.J., Steendam, R., Bank, R.A., Poppa, E.R., 2014. Microsphere size influences the foreign body reaction. *Eur. Cell. Mater.* 28, 335–347.
- Zhang, Z., Bi, X., Li, H., Huang, G., 2011. Enhanced targeting efficiency of PLGA microspheres loaded with Lornoxicam for intra-articular administration. *Drug Deliv.* 18, 536–544.