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An overview of the production methods for core–shell microspheres for parenteral controlled drug delivery

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ABSTRACT

Core-shell microspheres hold great promise as a drug delivery system because they offer several benefits over monolithic microspheres in terms of release kinetics, for instance a reduced initial burst release, the possibility of delayed (pulsatile) release, and the possibility of dual-drug release. Also, the encapsulation efficiency can significantly be improved. Various methods have proven to be successful in producing these core–shell microspheres, both the conventional bulk emulsion solvent evaporation method and methods in which the microspheres are produced drop by drop. The latter have become increasingly popular because they provide improved control over the particle characteristics. This review assesses various production methods for core–shell microspheres and summarizes the characteristics of formulations prepared by the different methods, with a focus on their release kinetics.

1. Introduction

During the last few decades, parenteral controlled release of both small-molecule drugs and biopharmaceuticals, such as peptides and proteins, has gained increasing attention. Controlled release delivery systems offer many advantages over the traditional administration of drugs because the release kinetics can be adjusted to the needs of a particular application [1,2]. The main advantage is the possibility to maintain drug levels within the therapeutic window for an extended duration which lowers the risks of side effects and systemic toxicity and allows for less frequent administrations [2]. This improves patient compliance and reduces discomfort. Furthermore, controlled release delivery systems can protect the drug in the body from most environmental influences which is especially beneficial for biopharmaceuticals that often have poor stability and a short biological half-life [1]. Therefore, the market for these drug delivery systems is growing, specifically the one for polymer-based long-acting injectables, such as microspheres [1,3]. Examples of clinically approved injectable microsphere formulations are Risperdal Consta and Vivitrol but also peptide-loaded microsphere products, such as Sandostatin LAR and Lupron Depot [4]. Most of these products consist of microspheres smaller than 200 µm [3,5,6] and can thus be administered through a rather thin, high-gauge needle of 19 to 23G [3,4]. In comparison, other controlled release delivery systems such as solid implants usually require a larger-diameter needle of e.g. 14G, which might be more painful for the patient [4]. On the other hand, the low loading capacity of specifically monolithic microspheres can sometimes be a limiting factor for their clinical application [7]. Moreover, obtaining the desired particle size, release profile, and stability of the drug remains a challenge, especially for biopharmaceuticals.

So far, most of the research has focused on traditional monolithic microspheres in which the drug is dispersed throughout the whole polymer matrix [8]. These monolithic systems have proven their suitability for sustained release drug delivery [9], but they do have some limitations with regard to the control over the release kinetics. Although methods to reduce the initial burst exist, complete elimination of burst release is often difficult or even impossible to achieve. This can be explained by the fact that drug molecules tend to preferentially accumulate at or near the surface of the microsphere, especially for water-soluble drugs [10]. Yet, for many drug products, the absence of an initial burst release is desired or even crucial as it can lead to unwanted side effects, it reduces the duration of drug release, and it compromises the efficiency of the drug delivery system [11]. Moreover, pulsatile release, zero-order release, and co-encapsulation of multiple drugs with different chemical characteristics and/or release profiles are often hard to achieve. Finally, achieving a high encapsulation efficiency (EE) can be challenging when a monolithic system is used, especially when highly water-soluble drugs with a low molecular weight are encapsulated.

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Another subcategory of microspheres, the core–shell microspheres, might offer a solution for these limitations. Core-shell microspheres are compartmentalized particles consisting of a single core or multiple cores surrounded by a polymer shell [12]. Drugs can be loaded either in the inner core or in the shell layer, or in both. There are many potential advantages to the use of core–shell microspheres over monolithic microspheres but the main one is the improved control over the release kinetics of the encapsulated drugs, as the composition and dimensions of both the core and the shell can independently be tuned. Both the initial burst release and the release duration could, respectively, be reduced and extended even further, thereby enabling sustained release over time [13,14,15,16]. This reduced initial burst release is for instance crucial for drugs with a narrow therapeutic index, such as many cytotoxic anticancer drugs [17]. Moreover, choosing the right (polymeric) excipients and fabrication process and settings also allowed for a pulsatile release profile which could be useful for the delivery of for instance vaccines [18,19]. Also, EE could significantly be improved by the addition of a shell layer and sometimes drug loading as well [14,20,21,22,23]. Therefore, core–shell microspheres offer great versatility and functionality as a controlled release drug delivery system, and have many potential pharmaceutical applications that could improve therapeutic efficiency, such as the co-delivery of two or more drugs with different functions and properties. These drugs, for instance a hydrophilic and a hydrophobic one, could be encapsulated separately in the core and shell, resulting in sequential or sometimes parallel release of the drugs [24,25,26]. Yet, despite these many advantages, to our best knowledge, no core–shell microsphere products have reached the market yet.

There are several methods for the production of core–shell microspheres. The choice of the method greatly influences the particle characteristics and thereby the release profile of the drug. Due to rapid advances in the field, the advantages and disadvantages of each system are not always clear. In this review, the different types of core–shell microspheres are discussed and the advantages over monolithic microspheres are assessed. Different conventional bulk emulsion methods and drop-by-drop methods for the production of core–shell microspheres are compared, and the influence of various process and formulation parameters on the particle characteristics, especially the release kinetics, is investigated.

2. General features and types of core–shell microspheres

In literature, the definition of what a core–shell microsphere is, varies. Both particles with a single core (mono-nuclear) and particles with multi-cores (poly-nuclear) are described as core–shell microspheres. In this review, both mono-nuclear and poly-nuclear core–shell microspheres are taken into account, as long as there is a distinct shell surrounding the core(s) and the core–shell structure is demonstrated well. Moreover, all particles with a size of approximately 1–1000 μm and a shell thickness of at least a few micrometers are considered as core–shell microspheres. As the focus of this review is on parenterally injectable controlled-release formulations, potential routes of administration are mainly the subcutaneous and the intramuscular route. However, particles with sizes of hundreds of micrometers might give injectability issues due to potential needle blockage, and they require larger needle diameters which can cause a more painful injection [3,4,27]. Core-shell microspheres smaller than 100 μm are therefore preferred as they can be administered using a 21G needle or higher [4].

Although a broad range of particles can be considered as core–shell microspheres, there are some examples in the literature where the description core–shell microsphere is unjustified. This is usually due to only partial engulfment, which means that the cores of the microspheres were not completely surrounded by a shell, or due to incomplete phase separation. There are also some examples where it is unclear why a core–shell structure was obtained. Furthermore, the core–shell structure is often not convincingly proven. As an example, a scanning electron microscopy (SEM) photo of the cross-section of only one particle is generally inconclusive. There are several methods to confirm the existence of a core–shell structure and a combination of these methods should preferably be used. The most common method is microscopy, including light microscopy, transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM) if the drug or another distinct component of the core or shell is fluorescent or fluorescently labeled. SEM is very informative as well but requires cross-sectional cutting of the microspheres which is often fairly difficult. In the case of core–shell microspheres with a solid core, selective dissolution of the core or shell using an organic solvent can give additional information regarding the polymeric distribution (Fig. 1) [15,28,29]. Other less frequently used techniques to characterize the particle structure are differential scanning calorimetry (DSC) which indicates whether phase separation completely occurred, X-ray photoelectron spectroscopy (XPS) which can give information about the chemical composition of the particle surface, and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) which can confirm the presence or absence of a certain polymer in the shell layer.

Because our definition of a core–shell microsphere is broad, there are many different types possible. First of all, a wide variety of polymers can be used for the fabrication of the shell, as long as they are biocompatible and biodegradable. In general, the same polymers are employed as for monolithic microspheres. The most frequently used polymers are poly (DL-lactide-*co*-glycolide) (PLGA), poly(DL-lactide) (PDLLA), and poly(L-lactide) (PLLA) because they have been studied extensively and have an easily adjustable degradation time. This degradation time can be adjusted by varying the lactide:glycolide monomer ratio and/or the molecular weight of the polymer[30]. Some alternatives for the shell are glucose-initiated PLGA (Glu-PLGA)[31] which is a branched polymer of PLGA chains attached to a D-glucose core, poly(ε-caprolactone) (PCL) [32,33], the polyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [34], and the polyanhydride poly[(1,6-bis-carboxyphenoxy) hexane] (PCPH) [19,28,35]. Natural polymers can also be used as shell material, for example the water-soluble sodium alginate which forms a gel after addition of calcium ions, and chitosan which is soluble at low pH but solidifies at neutral pH [24,36,23,37,38,39]. Secondly, the core contains either a gas or liquid or is composed of a polymer. Gas- or liquid-filled microspheres are also called microcapsules. In the case of liquid-filled core–shell microspheres, the core can be made of an aqueous solution or an oil. Particles with an oil-based core can have the advantage of improved solubility of hydrophobic drugs, which potentially allows an increased drug load [32]. Also, these oil-based microspheres can offer improved physical and chemical stability compared to

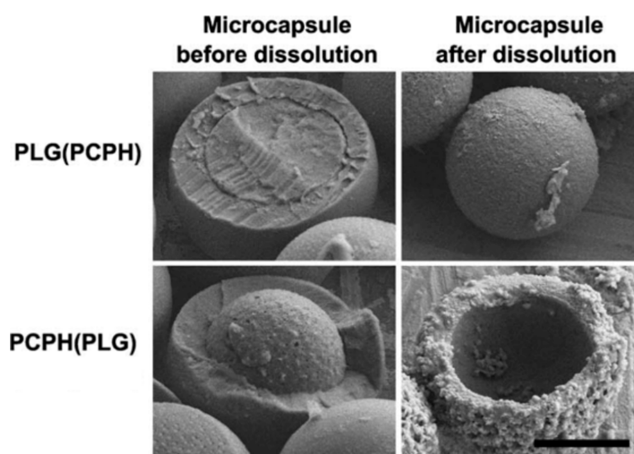


Fig. 1. SEM images of PLGA/PCPH microspheres (top row) and PCPH/PLGA microspheres (bottom row). Images illustrate the effect of selectively dissolving the PLGA phase using tetrahydrofuran. Scale bar = 25 μm. Modified from [28] with permission from Elsevier.

particles with an aqueous core because many polymer degradation products are not able to reach the drug, which was for instance the case with the protein bovine serum albumin (BSA) [18]. Silicone oil was encapsulated into PLGA-based and PCL-based core–shell microspheres with injectable size, thus offering the potential for parenteral drug delivery [19,32]. Canola oil can be used as well, if needed emulsified with the aqueous drug solution into a W/O emulsion [18]. In this way, both hydrophilic and hydrophobic drugs can be encapsulated into an oily core. In the case of an aqueous core, the water can be removed by lyophilization which turns the liquid-filled microspheres into hollow (i. e. gas-filled) core–shell microspheres loaded with a drug [40]. Gas contents can also directly be encapsulated into core–shell microspheres and these gas-filled microspheres are sometimes called microbubbles, though these microbubbles are mainly used for diagnostics and imaging instead of drug delivery [41]. The last possibility for the core composition of core–shell microspheres is a biodegradable polymer. Just like the shell, PLGA, PDLLA, and PLLA are regularly used as core material but an alternative is poly(ortho ester) (POE) [42,43]. Core-shell microspheres with a solid core and a solid shell are often called double-walled microspheres, in contrast to monolithic microspheres which are sometimes called single-walled microspheres. Finally, drugs can be incorporated into the core, shell, or both, thereby enabling the production of dual-drug release products [24,25,44].

3. Methods for the production of core–shell microspheres

Several methods can be employed for the production of core–shell microspheres, and the type of method, as well as the process and formulation parameters, determine the formulation's physico-chemical characteristics and performance. Conventional emulsion solvent evaporation is the standard method for the bulk fabrication of microspheres and is often combined with phase separation to obtain microspheres with a core–shell structure. This method, however, involves high shear stresses and often generates particles with a broad size distribution and low EE, although modified methods have been developed that generate microspheres with improved characteristics [15,45]. Therefore, other production methods, such as microfluidics, electrospraying or coaxial electrohydrodynamic atomization (CEHDA), and precision particle fabrication (PPF) technology are becoming more widely used, because of their capability of generating highly monodisperse particles with high EEs (Table 1). These methods can make use of emulsification processes as well but in contrast to the conventional emulsion solvent evaporation method, the microspheres are produced drop by drop instead of in bulk. This results in increased monodispersity of the particles. There are more methods that enable the production of core–shell microspheres, such as polymerization methods and self-assembly [46], but because the focus of this review is on widely used and commercially available polymers, these methods are not addressed here. The characteristics of the different methods can be found in Table 1.

3.1. Conventional bulk emulsion methods

Conventional bulk emulsion solvent evaporation is the most frequently used method for the production of polymeric microspheres as it is a very straightforward method, and a wide range of particle sizes can be produced from approximately 1 to 1000 µm (Table 1, Table 2). Moreover, various types of drugs can be incorporated, such as hydrophilic or hydrophobic drugs, small molecules or proteins and peptides, and a single drug or multiple drugs. This production method usually leads to the formation of monolithic microspheres but with some modifications, for instance the combination with phase separation, core–-shell microspheres can be produced as well. In this process, a double

Table 1
Overview of most common production methods for core–shell microspheres and the process characteristics.

Production method	Subcategories	Applied polymers	Particle size	Dispersity	EE	Advantages	Disadvantages
Conventional bulk emulsion solvent evaporation (combined with phase separation)	W/O/O/W W/O/W S/O/O/W O/O/W O/W Acetone-W/O S/Acetone-W/O	PLGA Glu-PLGA PDLLA PLLA Chitosan Alginate POE PDME PHBV	1–880 µm	Usually polydisperse (COV = 5–75%)	Variable (15–100%)	Simplicity of setup Variability of materials Broad particle size range possible	High shear stress Presence of aqueous-organic interface Difficult to scale-up Low monodispersity
Microfluidics (combined with phase separation)	W/O/W O/W/O O/W	PLGA PLLA PCL Alginate	45–350 µm	Monodisperse (COV < 10%)	Usually not measured, claimed to be high	Small volumes needed Little waste High monodispersity High EE	Low production speed Difficult to scale-up Narrow working window, low flexibility Risk of channel contamination/ blockage
Electrospraying/ CEHDA	Dual-capillary Tri-capillary Coaxial Emulsion Electrodropping Electrospinning	PLGA PDLLA PLLA PCL Chitosan Alginate PCL-PPE-EA	0.2–65 µm	Variable (COV = 5–40%)	Usually high (65–100%, shell materials: 40–85%)	Simplicity of setup Single-step, continuous process Cost-effectiveness Variability of materials High EE	Limited particle size range Low throughput Stable cone-jet mode required
PPF	–	PLGA PDLLA PLLA PCPH	40–115 µm	Monodisperse (COV < 10%)	Variable (5–100%)	Continuous process High reproducibility High production speed High monodispersity	Complexity

Table 2
Representative polymeric core–shell microspheres produced via conventional bulk emulsion solvent evaporation. Presented polymer molecular weights and viscosities are the weight averaged molecular weights and the inherent viscosities, respectively, unless stated otherwise.

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
[31]	W/O/O/W emulsion solvent evaporation	Core: lysozyme + PLGA (50:50, intrinsic viscosity = 0.4 dL/g), shell: Glu-PLGA (50:50, Mn = 15/50 kDa)	Burst = 5–15%, slow release up to at least 70 days	2–8 µm, COV = 13–35%	71–84%	No	
[29]	W/O/O/W emulsion solvent evaporation	Core: BSA + PLGA (50:50, 0.55–0.75 dL/g), shell: PLLA (40–70 kDa)	Short solvent evaporation: 15–22% burst, a lag phase up to day 4–26, and sustained or fast release up to day 45 or almost no further release; long solvent evaporation: ≤ 15% burst, a lag phase up to day 18–30, and sustained release up to day 58	Short solvent evaporation: 33–73 µm, COV = 24–53%; long solvent evaporation: 62–80 µm, COV = 40–64%	No data	No	
[45]	W/O/O/W emulsion solvent evaporation	Core: BSA + Glu-PLGA (50:50, Mn = 15/50 kDa), shell: PLGA (50:50, intrinsic viscosity = 0.4 dL/g)	Burst = 9–16%, a lag phase up to day 8–23, and sustained release up to (at least) 90 days	4–8 µm, COV = 6–21%	95–100%	No	
[16]	W/O/O/W emulsion solvent evaporation	Core: insulin + Glu-PLGA (50:50, Mn = 15 kDa), shell: PLGA (50:50, intrinsic viscosity = 0.4 dL/g)	Burst = 13–19%, a lag phase up to day 3–14, and sustained release up to (at least) 42 days	3–9 µm, COV = 13–23%	67–78%	No	
[14]	W/O/O/W and S/O/O/W emulsion solvent evaporation	Core: meglumine antimoniate + PLGA (50:50, 48–78 kDa), shell: PLGA (75:25, 48–78 kDa)	Burst = 17–22%, sustained release up to day 30	S/O/O/W: 31 µm, COV = 42%; W/O/O/W: 52 µm, COV = 46%	S/O/O/W: 87%, W/O/O/W: 81%	No	
[13]	W/O/O/W emulsion solvent evaporation	Core: 5-FU/BSA + PLGA (50:50, 15 kDa/53:47, 137 kDa/75:25, 118 kDa), shell: PLGA (80:20, 201 kDa)/PLLA	5-FU: burst = 4–20%, a lag phase up to day 9–19, and sustained release up to 48–70 days	515 µm and 775 µm, COV = 20% and 25%	5-FU: 81–87%	No	
[110]	W/O/W and S/O/W emulsion solvent evaporation	Core: losartan potassium, shell: PLGA (75:25, 20 kDa)	Sustained release up to 18–30 days or biphasic release up to 26–30 days (i.e. slow release up to day 10–14, followed by fast release)	19–31 µm, COV = 27–52%	EE = 57–79%	Yes, *in vivo* pharmacodynamics study	Microspheres with a gelatine or Pluronic® F-127 core were also produced
[39]	W/O/W emulsion solvent evaporation	Core: BSA/bFGF + PLGA (75:25, 40–75 kDa), shell: CHA + glycol chitosan (28 kDa)	BSA and CHA: sustained release up to day 18, CHA faster release rate than BSA	6 µm, COV = 14–21%	BSA: 75–76%	Yes, antibacterial and cell proliferation assay	
[34]	W/O/W emulsion solvent evaporation	Core: BSA (+HGF) + PLGA (50:50, 62 kDa), shell: PHBV (576 kDa)	BSA: burst = 18%, fast release up to day 7, hardly any release up to day 30, and fast release up to day 70	185 µm, COV = 12%	BSA: 91–92%, HGF: 89%	Yes, bioactivity assays of released proteins on cell lines and cell proliferation study with hepatocytes	Shell contained PLGA as well due to incomplete phase separation
[42]	W/O/W emulsion solvent evaporation	Core: CyA + POE (24 kDa), shell: BSA + PLGA (50:50, 43 kDa)	CyA: sustained release up to day 30 or 42, BSA: nearly complete release within 5 days	51–60 µm, COV = 24–41%	BSA = 60–61%, CyA = 79–83%	No	Shell contained POE as well due to incomplete phase separation
[43]	W/O/W emulsion solvent evaporation	Core: POE (24 kDa), shell: PLGA (50:50, 43 kDa)	No data	~100 µm	No data	No	
[49]	S/O/O/W emulsion solvent evaporation	Core: dexamethasone + PLGA (75:25), shell: PLLA	Lag phase up to day 70, sustained release up to at least 264 days	250 µm, COV = 3%	43%	Yes, histological and image analysis of adipose tissue	
[103]	S/O/O/W emulsion solvent evaporation	Core: BT + PLGA (50:50, 7/13/22/24 kDa), shell: PLLA (100 kDa)	Biphasic (slow release followed by faster release) or sustained release up to (at least) 40 days	100–600 µm	No data	Yes, preparation and implantation of carrier system for microspheres and *in vivo* release study	

(*continued on next page*)

Table 2 (*continued*)

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
[50]	S/O/O/W emulsion solvent evaporation	Core: bupivacaine/ chlorophenol red + PLGA (50:50, 7/24/ 33 kDa), shell: PLLA (100 kDa)	Bupivacaine: 7 kDa: fast release up to day 18; 24 kDa: fast release up to day 17, slow release up to day 33; 33 kDa: < 10% release up to day 15, fast release up to day 33	~150–200 µm	No data	Yes, implantation of microspheres into goat joint and collection of blood and synovial fluid samples + histological staining	
[54]	S/O/O/W emulsion solvent evaporation	Core: lysozyme + PLGA (50:50, 40–75 kDa), shell: PLLA (0.9–1.2 dL/g)	Small burst, slow release up to day 36, and faster release up to day 140	81 µm, COV = 42%	No data	No	
[55]	S/O/O/W emulsion solvent evaporation	Core: etanidazole + PLGA (50:50, 40–75 kDa), shell: PLLA (85–160 kDa)	Burst < 5%, a lag phase up to day 27, and linear sustained release up to day 50	422 and 432 µm, COV = 41%	55–57%	No	
[15]	O/O/W emulsion solvent evaporation	Core: aspirin + PLLA (39 kDa), shell: PLGA (50:50, 30 kDa)	Burst = 2–3%, a lag phase of 3 days, and sustained release up to day 23–31	154–179 µm, COV = 0.3–1.5%	74–81%	No	
[52]	O/O/W emulsion solvent evaporation	Core: dox + PLGA (50:50, 40–75 kDa), shell: PLLA (85–160 kDa) and the inverse	PLGA:PLLA (1:1): burst = 4%, a lag phase of 32 days, and a linear sustained release up to day 73; PLGA:PLLA (2:1 and 3:1): burst = 1 and 13%, sustained release up to day 45	167–172 µm, COV = 62–73%	60–87%	No	
[22]	O/W emulsion solvent evaporation	Core: aripiprazole, shell: PDLLA (95 kDa)	Slow release up to at least 49 days	Without homogenization: 206 µm, with homogenization: 65 µm	91–100%	Yes, *in vivo* release study after subcutaneous injection in rabbits	
[36]	O/W emulsion solvent evaporation	Core: paracetamol + PLGA (75:25, 10 kDa), shell: HSA + alginate	Paracetamol: lag phase up to 15 h, near zero-order release up to 3 days, and slower near zero-order release up to at least 12 days; HSA: complete release within 3 days	9–13 µm, COV = 11–36%	HSA: 17–62%, paracetamol: 68–90%	No	
[67]	O/W emulsion solvent evaporation	Core: ABT627, shell: PLGA (50:50)	Burst = 14%, slow release up to day 13, fast release up to day 21, and slow release up to day 25	18 µm, COV ~ 7%	~45%	No	ABT627: hydrophobic model drug
[56]	Acetone-W/O emulsion solvent evaporation	Core: risedronate, shell: PLGA (50:50, 7–17 kDa)	Sustained release up to 6 h	1 µm, COV = 35%	32%	No	
[57]	S/Acetone-W/ O emulsion solvent evaporation	Core: theophylline, shell: PDME	Sustained or zero-order release up to (at least) 8 h	630–878 µm	No data	No	

Abbreviations: 5-FU, 5-fluorouracil; bFGF, basic fibroblast growth factor; BT, brimonidine tartrate; CHA, chlorhexidine acetate; CyA, cyclosporin A; dox, doxorubicin; HGF, hepatocyte growth factor; HSA, human serum albumin; Mn, number averaged molecular weight.

emulsion (i.e. water-in-oil-in-water, W/O/W; solid-in-oil-in-water, S/O/W; oil-in-oil-in-water, O/O/W) or triple emulsion (i.e. water-in-oil-in-oil-in-water, W/O/O/W; solid-in-oil-in-oil-in-water, S/O/O/W) containing two polymers is employed. Upon solvent removal, the polymers separate into different phases to achieve the most thermodynamically stable configuration in the concentrating polymer solution(s) [47,48]. Subsequently, the combination with phase separation always leads to the formation of core–shell microspheres with a solid polymeric core. The polymers are usually dissolved in separate solutions and subsequently added together after which they phase separate. In this case, a W/O/O/W [29,31], S/O/O/W [49,50], or O/O/W [15] emulsification method is employed. Phase separation, however, can also occur when the polymers are dissolved together in one solvent, for instance with a W/O/W [42,43] or S/O/W emulsification process. This means that for double emulsions, the drug can be incorporated into an organic polymer solution as: (i) an aqueous solution (water-in-oil, W/O), (ii) a solution of the drug and another polymer in an organic solvent (oil-in-oil, O/O), or (iii) solid particles (solid-in-oil, S/O). This is the primary dispersion step. The secondary dispersion step is the emulsification of the primary dispersion, called the dispersed phase, with the external aqueous phase, called the continuous phase. After emulsification, the microspheres solidify due to the extraction of the organic solvent by the continuous phase, accompanied by solvent evaporation. In the final step, the particles are collected by centrifugation or filtration, washed, and subsequently lyophilized or bulk (vacuum) dried to remove residual solvent [51]. Fig. 2 illustrates the different emulsification configurations that can be used for the conventional bulk emulsion solvent evaporation method combined with phase separation to obtain core–shell microspheres. For triple emulsions, an extra dispersion step is needed as a primary emulsion (for W/O/O/W) or a solid dispersion (for S/O/O/W) is generated, and subsequently emulsified with another polymer solution. A hydrophobic drug can be dissolved in one or both of the polymer phases, and then preferentially localizes within one polymer over the other, ultimately yielding an oil-in-water (O/W) or O/O/W emulsion. If

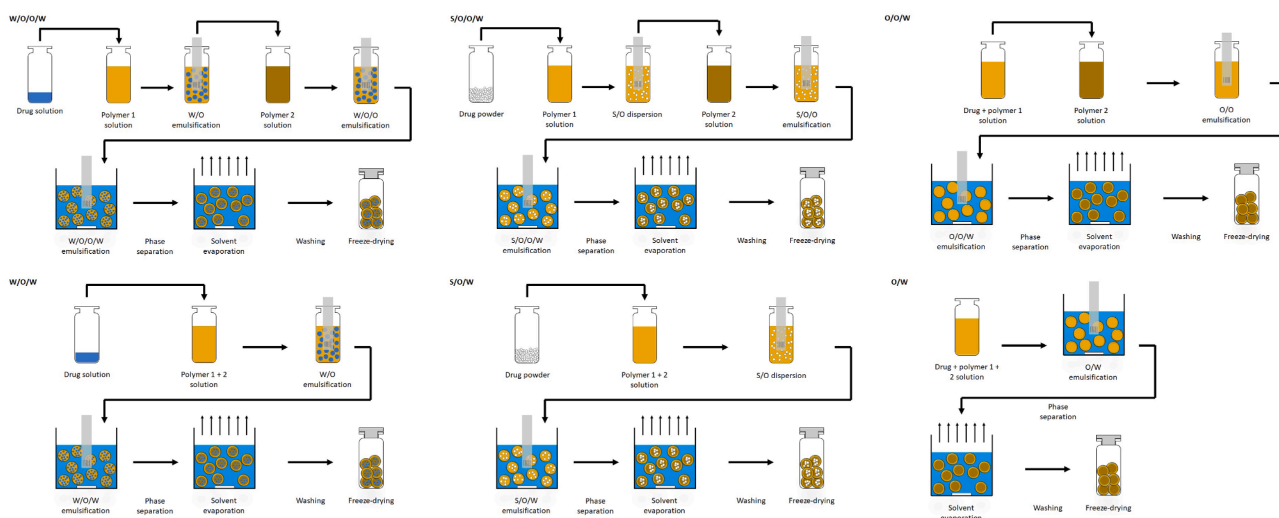


Fig. 2. Schematic illustration of the different configurations that can be used for the conventional bulk emulsion solvent evaporation method combined with phase separation for the production of polymeric core–shell microspheres.

the drug is hydrophilic, an aqueous solution of the drug is prepared and emulsified with either one or both polymer phases, thus yielding a W/O/W or W/O/O/W emulsion. The polymer phase that differs the least from the drug in terms of solubility parameters will contain the highest drug concentration [52]. The phenomenon of phase separation can be attributed to differences in hydrophilicity and crystallinity, for instance when PLGA and PLLA are used, or incompatibility of the two polymers which is reflected in for example differences in solubility. The three possible configurations that can be obtained through phase separation are complete engulfment, partial engulfment, and no engulfment as presented in Fig. 3. With a correct choice of the evaporation rate and the interfacial tensions between the liquid phases, complete engulfment of one polymer by the other can be achieved and thus a core–shell structure [47,53]. When the solvent evaporates too fast, complete phase separation may not occur, thereby causing only partial engulfment as shown by Zhu et al. for PLGA/PHBV composite microspheres [34]. In the case of PLGA and PLLA in equal amounts, PLGA usually forms the core and PLLA the shell [29,54,55] according to the spreading coefficient theory which is based on the surface tension of both polymer phases and the interfacial tension between the phases [47,53]. However, with increasing PLGA:PLLA mass ratio, core–shell inversion takes place as the polymer phase with the higher mass often forms the engulfing phase.

Core-shell microspheres with a non-polymeric core can be produced using conventional emulsion solvent evaporation as well, although this is less common. A few examples exist where an O/W or acetone-W/O emulsification method combined with phase separation was employed for this purpose. Production of aripiprazole-loaded core–shell microspheres with a high drug loading of up to 80% was achieved with a conventional O/W emulsion solvent evaporation method [22]. High molecular weight PDLLA and aripiprazole were dissolved in dichloromethane (DCM), i.e. the dispersed oil phase, and the obtained solution was added drop-by-drop to an external water phase cooled to 10 °C. After addition of the oil phase, the temperature of the water phase was gradually increased to 20 °C, which resulted in precipitation of aripiprazole in the core and eventually evaporation of the organic solvent. This caused the polymer to slowly precipitate on the outer surface of the core resulting in microspheres with a core–shell structure. Abulateefeh and Alkilany prepared aqueous core-PLGA shell microspheres with an acetone-W/O emulsification method combined with internal phase separation [56]. An aqueous risedronate solution was added to a solution of the polymer in acetone, after which the internal acetone–water phase was emulsified with the external oil phase. Subsequently, the evaporation of acetone caused the solubility of PLGA to decrease and a part of the polymer to migrate to the surface of the droplets where it precipitated. This phase separation resulted in the formation of a polynuclear core–shell structure with large aqueous cores embedded in the polymeric matrix. Core-shell microspheres containing theophylline could be produced with a similar emulsion solvent evaporation method [57]. The hydrophobic dextran derivative PDME was used as polymer. After dissolving the polymer in an acetone–water mixture, the drug was suspended in the solution and the resulting suspension was emulsified with liquid paraffin to obtain an S/acetone-W/O emulsion. At a

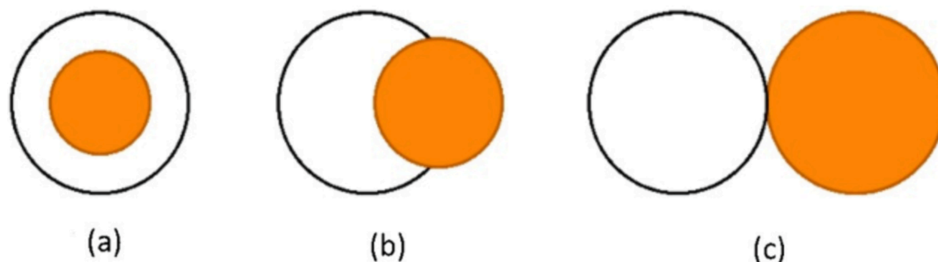


Fig. 3. The three possible configurations that can be achieved through phase separation in a two-polymer system: complete engulfing (a), partial engulfing (b), and non-engulfing (c). Reproduced from [68] with permission from MDPI.

sufficiently low acetone/water ratio, a core–shell structure was obtained as the polymer rapidly deposited on the surface of the droplets.

As already mentioned above, the conventional bulk emulsion solvent evaporation process does have some disadvantages, such as broad particle size distribution and exposure of the drug to organic solvents and high shear stresses due to high-speed homogenization. This creates hazards for the integrity of sensitive biopharmaceuticals [58,27]. Besides, the solvent evaporation rate is difficult to control, which often results in variability in particle characteristics such as size, internal structure, and EE, both within a batch and between different batches [15,59]. Another disadvantage is the difficulty of obtaining a high EE, especially with moderately or highly hydrophilic drugs with a low molecular weight [56]. There are many parameters that affect the EE, such as particle structure, stirring speed, lipophilicity of the drugs, drug loading, and polymer concentration [51,60]. In general, for all production methods that involve emulsification, the EE can be improved by decreasing the solubility of the drug in the continuous phase or by increasing the solidification rate of the microspheres but other methods are also possible [61,62]. For the conventional bulk emulsion solvent evaporation method in specific, reduction of the stirring speed during emulsification will result in lower shear forces by which a larger portion of the drug molecules will stay in the particles [63]. This might also improve the stability of drugs that are sensitive to shear stress, such as therapeutic proteins. On the other hand, a reduced stirring speed might also result in a lower solidification rate of the microspheres, and thus in a lower EE [61]. In some exceptional cases, EEs as high as 90–100% could be reached, as seen with the aripiprazole-loaded microspheres [22]. An EE of 95–100%, depending on the formulation settings, was also obtained for double-walled microspheres with a Glu-PLGA core and a PLGA shell prepared by conventional W/O/O/W emulsion solvent evaporation combined with phase separation [45]. Single-walled microspheres consisting of PLGA and Glu-PLGA were prepared as a comparison, and these particles had an EE of only 60–70%. Similar findings were obtained for PLGA-based double-walled microspheres with the hydrophilic small-molecule drug meglumine antimoniate loaded in the inner core [14]. This difference in EE between double-walled and single-walled microspheres can be attributed to the outer shell layer that acts as a barrier to the diffusion of the hydrophilic drug into the external aqueous environment during solidification of the microspheres. This also explains why core–shell microspheres generally have a higher EE than monolithic microspheres. A high EE is especially advantageous when expensive or scarcely available drugs are incorporated and a high EE can be helpful when a high drug loading is desired [22]. Higher loading may also be realized by making use of core materials that enable increased solubility of the drug. Especially for hydrophilic drugs, the target loading can significantly be increased by incorporating them into an aqueous core. When utilizing monolithic microspheres for controlled release, the maximum drug loading is usually about 30% [22]. Loadings above this value will cause the drug to also exist on the surface of the microspheres, thereby disabling slow release. Increased EE and possibly drug loading are thus a great advantage of core–shell microspheres in comparison with monolithic microspheres. The last drawback of the conventional bulk emulsion solvent evaporation method is that industrial scale-up while preserving the particle properties is often difficult and costly. This difficulty arises from the fact that the production method involves batch operation and that it requires removal of the organic solvent, though this is a problem for all production methods that involve emulsification processes [59,64]. An organic solvent is usually needed to dissolve the polymers, but most organic solvents are toxic. Solvent removal, therefore, is a key step in the production process. The most commonly used solvent is dichloromethane as it is highly volatile and poorly soluble in water. Due to its toxicity, the residual solvent level in the final microspheres must be reduced to a minimum. Solvent removal can be promoted by stirring and using elevated temperatures for the continuous phase, though residual levels may still be present after drying [65]. Therefore, alternative solvents that are less toxic could be used, such as dimethyl carbonate [66] and ethyl acetate [15,25], though their physical properties are inferior to those of dichloromethane. For example, they are less volatile and thus harder to remove, and they are a poorer solvent for some polymers such as PLGA [65]. Mao et al. produced core–shell microspheres with an O/W emulsion solvent evaporation method using DCM, and determined the glass transition temperature of the polymer and the blank microspheres with DSC, but no significant difference was found [67]. As DCM acts as a plasticizer, this indicated that the residual solvent level was very low.

3.2. *Drop-by-drop methods – Microfluidics*

Another approach for the production of core–shell microspheres is the application of microfluidics, which offers precise control over the size of the microspheres by making use of shear forces to create new interfaces between immiscible fluids [69,70]. A schematic illustration of an example of a microfluidic device for the production of core–shell microspheres is shown in Fig. 4. Droplet microfluidics, a subcategory of microfluidics, is also an emulsification method but in contrast to the conventional bulk emulsion solvent evaporation method, the emulsion is produced drop by drop and in a continuous fashion [70]. This usually results in highly monodisperse particles with a high EE after extraction and evaporation of the organic solvent. The droplets are produced by injecting two immiscible liquid phases (an oil and a water phase) via separate inlets into the microchannels of a microfluidic device. Monodisperse W/O or O/W droplets are then generated in a highly repeatable manner at the junction where the streams meet due to the shear stresses, although these stresses are substantially lower than with the conventional bulk emulsion solvent evaporation method. By making use of a third immiscible liquid phase that is injected via another inlet, the droplets are re-emulsified into this phase and a double emulsion is obtained [71]. W/O/W or oil-in-water-in-oil (O/W/O) double emulsions usually form the basis for the production of core–shell microspheres. In addition, microfluidics enables the formation of higher-order emulsions, such as triple (W/O/O/W, S/O/O/W) [72] or even quadruple emulsions, although extra channels are then required. The number and size of the inner droplets can be precisely controlled [70,71]. Moreover, microfluidics can be used for the incorporation of all kinds of molecules, such as hydrophilic and hydrophobic molecules, small molecules and macromolecules but the incorporation of two or more different types of drugs is also possible [73]. Hydrophilic drugs or dyes for visualization of the internal structure are often encapsulated into the core of core–shell microspheres by producing a W/O/W emulsion. When a hydrophobic drug is to be incorporated in the core, an O/W/O emulsification method is usually preferred. The hydrophobic core is then composed of polymer solution or oil and the hydrophilic shell is composed of e.g. alginate. In that particular case, an extra liquid phase consisting of calcium chloride solution is required to cross-link the alginate. In a study by Wu et al., the hydrophobic model drug rifampicin was encapsulated into PLGA-alginate core–shell microspheres in order to control its release [23]. Drug loading and EE could also be increased by applying the alginate shell around the PLGA core. Table 3 summarizes the representative core–shell microspheres that were produced using microfluidics.

Not only the conventional bulk emulsion solvent evaporation method can be combined with phase separation but the microfluidic method as well. Li et al. prepared core–shell microspheres from a single O/W emulsion using microfluidics [66]. The oil phase consisted of both PLGA and PCL in an organic solvent. By choosing the right solvent, the polymers underwent phase separation when the O/W emulsion droplets were collected in polyvinyl alcohol (PVA) solution, resulting in core–-shell microspheres with a PLGA shell and a PCL core which also contained tiny PLGA beads. This was caused by an increase in polymer concentration upon extraction of the organic solvent, causing an inversion of both polymers. Acetone treatment and ATR-FTIR demonstrated the localization of PLGA and PCL in the shell and the core, respectively. Rhodamine B was added as a hydrophilic fluorescent dye

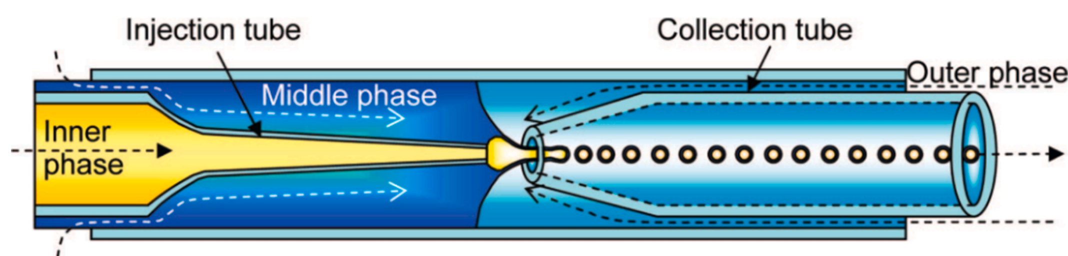


Fig. 4. Schematic illustration of the generation of a double emulsion in a microfluidic device for the production of core–shell microspheres. Reprinted with permission from [83]. Copyright 2021 American Chemical Society.

Table 3
Representative polymeric core–shell microspheres produced via microfluidics. Presented polymer molecular weights and viscosities are the weight averaged molecular weights and the inherent viscosities, respectively, unless stated otherwise.

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
[111]	Microfluidics (W/O/W)	Core: DOX-ADA + indocyanine green, shell: PLGA (50:50, 7–17 kDa)	Doxorubicin: sustained release up to 20 days	~100 µm, COV = 2%	Doxorubicin: 47%, indocyanine green: 63%	No	
[112]	Microfluidics (W/O/W)	Shell: PDLLA (89 kDa)	No data	~250 µm, monodisperse	No data	No	Microspheres with a Eudragit® S 100 core were also produced
[76]	Microfluidics (W/O/W)	Shell: PLGA (50:50, intrinsic viscosity = 0.41 dL/g / 65:35, intrinsic viscosity = 0.55–0.75 dL/g / 85:15, intrinsic viscosity = 0.66 dL/g)	No data	75–290 µm, monodisperse	No data	No	Salts (NaCl and Na_2CO_3) were added to inner water phase for osmotic annealing
[40]	Microfluidics (W/O/W)	Shell: PLGA (75:25, 66–107 kDa)	No data	Droplets: 446–921 µm, COV = 2%	No data	No	
[75]	Microfluidics (W/O/W and O/W/O)	Core: alginate, shell: PLGA (50:50, 7–17 kDa) (W/O/W) and the inverse (O/W/O)	No data	O/W/O: 69 µm, monodisperse	No data	No	
[23]	Microfluidics (O/W/O)	Core: rifampicin + PLGA (50:50, 7–17 kDa), shell: alginate	Hardly any release up to day 10, near zero-order release up to day 31	Core: 15–55 µm, whole particle: no data; COV = 8%	70%	Yes, viability studies on cell lines to confirm the biocompatibility of the microspheres	
[37]	Microfluidics (O/W/O)	Shell: alginate	No data	256 and 337 µm, COV < 2%	No data	No	
[66]	Microfluidics (O/W)	Core: PCL (130 kDa), shell: PLGA (50:50, 30 kDa)	No data	47 µm, COV = 3%	No data	No	Core contained PLGA as well due to incomplete phase separation
[33]	Microfluidics (O/W)	Core: PCL (43 kDa), shell: PLGA (65:35, 0.55–0.75 dL/g) and the inverse	No data	187–218 µm, COV = 1–4%	No data	No	
[74]	Microfluidics (O/W)	Core: Oil-Red-O, shell: PLLA (42 kDa)	Fast release up to 60 min, slow release up to 220 min	50 µm, COV = 16%	No data	No	Shell might have contained some Oil-Red-O as well

Abbreviations: DOX-ADA, doxorubicin-conjugated alginate dialdehyde.

that selectively distributes in the more hydrophilic PLGA which enabled the confirmation of the core–shell structure by CLSM. A similar study was carried out by Kim et al., in which the particle morphology could be controlled by varying the blend ratio of both polymers [33]. Complete phase separation could be induced by choosing the right blend ratio and by employing slow solvent evaporation. Furthermore, liquid-filled microspheres could be prepared using microfluidics combined with phase separation by adding dodecane to the organic polymer solution and subsequently generating an O/W emulsion [74]. Dodecane is a hydrophobic non-volatile non-solvent for PLLA, the polymer used in this study. PLLA precipitated at the droplet interface upon evaporation of the organic solvent, causing phase separation between the polymer and the non-solvent. Eventually, microspheres with a dodecane-filled core containing the hydrophobic dye Oil-Red-O were formed. The dodecane core could be removed by lyophilization, resulting in hollow microspheres with the dye in the core.

Several factors influence the size and size distribution of the inner and outer droplets, such as the geometry of the device, channel diameter, concentrations and flow rates of the different fluid phases, and ratio of the flow rates. However, when using a microfluidic junction, the geometry of the device and the channel diameter are often fixed and thus difficult to vary. Therefore, the flow rates of the different fluid phases are the most important factor in controlling the droplet characteristics but the ratio of the different flow rates influences the particle and core size as well. Ren et al. made use of this dependency to tune the dimensions of an O/W/O double emulsion. Soybean oil solution was used as the inner phase, an aqueous alginate solution as the middle phase, and another oil solution as the outer phase [37]. In the last emulsification capillary, a calcium chloride aqueous solution was injected. Due to the density difference between the O/W/O emulsion droplets and the outer

oil solution, the droplets sunk to the bottom of the emulsification capillary where the aqueous alginate layer in the droplets came into contact with the calcium chloride solution, thereby allowing the alginate to gel. By increasing the ratio between the inner phase flow rate and the middle phase flow rate, the inner oil droplet size increased linearly. The outer microsphere diameter could also be varied. When the sum of the inner phase flow rate and the middle phase flow rate increased with respect to the outer phase flow rate, the outer microsphere diameter increased. Highly monodisperse particles were obtained, with coefficient of variation (COV) values of $< 2\%$ for both the core and the whole particle (Fig. 5). Inner and outer droplet size could also be tuned by osmotic annealing [75,76]. By varying the solute concentration ratio between the inner phase and outer phase, the inner droplet volume could be altered by more than three orders of magnitude due to the osmotic pressure difference of the inner and outer phase [76]. This osmotic annealing method circumvents the need for the fabrication of a new microfluidic device with different channel dimensions.

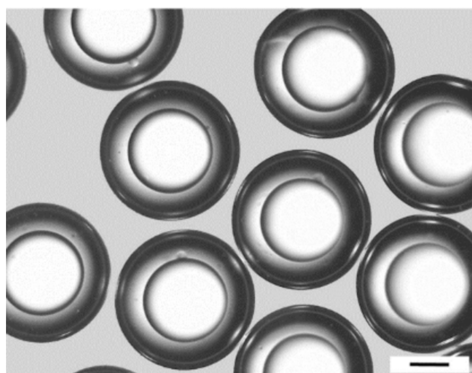
Microfluidic fabrication of microspheres also has some drawbacks, one of which is the need for pulseless flow and high responsiveness of the system [77]. Syringe pumps are the most frequently used devices for controlling the flow within the system, but even the most advanced pumps have some fluctuations in flow over time, which results in a broader particle size distribution. Furthermore, the pumps often have low responsiveness, which means that it takes some time for the flow to stabilize after adjustment of the flow rate. By making use of pressure-controlled pumps, these fluctuations in flow can be minimized and response times can be decreased [78]. A second drawback is the low throughput and the difficulty of scale-up. With a single microfluidic junction, microspheres can usually be produced at a throughput of approximately 50–300 mg/h [79], depending on the viscosity of the dispersed phase and the channel diameter. Higher throughput can be achieved by increasing the polymer concentration in combination with a decreased molecular weight of the polymer or by using a larger channel diameter. However, to achieve a significant scale-up, parallelization of microfluidic devices that operate with a minimum number of pumps is needed [73,79]. Romanowsky et al. achieved a production rate of 1 kg/day of a water/octanol/water double emulsion by using a three-dimensional array of fifteen droplet-making units in parallel [80]. Additionally, large-scale production of solid lipid nanoparticles by microfluidic mixing has proven to be possible, which can for instance be used for the mRNA-based COVID-19 vaccines [81,82]. Although these vaccines do not concern core–shell particles, it does showcase the potential of microfluidics for industrial scale-up. The last shortcoming is the limited range of suitable flow rates that can be used and droplet sizes that can be generated.

3.3. Drop-by-drop methods – Electrospraying

Coaxial electrospraying, also called CEHDA, is a single-step continuous method for the production of core–shell microspheres. Two or three liquids are separately injected via coaxial capillaries into a nozzle or spray head, called dual-capillary and tri-capillary electrospraying, respectively. An electric field is applied to the nozzle tip and at a certain voltage, the solution interface at the tip changes shape, forming a Taylor cone jet. When the critical voltage is reached, the surface tension of the drop is overcome which causes the drop to break up into very fine highly charged droplets. These charged droplets are accelerated towards the grounded collector during which the solvent is evaporated, thereby resulting in solidified particles [84,85]. Because of this rapid drying, immiscibility of the injected solutions is not necessary for a core–shell structure. The particles are usually collected on aluminum foil but they can also be collected in ethanol, water, or another aqueous solution, although the process then demands an additional washing and/or drying step. Because a coaxial nozzle is used, the core fluid is surrounded by an annular fluid which enables the production of core–shell particles. A schematic representation of this production method is shown in Fig. 6. Table 4 provides an overview of representative core–shell microspheres produced with this method.

The terms electrospraying and electrospinning are sometimes used interchangeably but in general, electrospraying refers to the production of microspheres while electrospinning refers to the production of fibers [86,87]. Both are electro-hydrodynamic techniques that use a similar setup but the methods differ in terms of applied voltage and the properties of the polymer solution, such as the molecular weight and concentration of the polymer and the solvent properties [86]. Low viscosity solutions usually cause electrospraying as stream breakup is more likely to occur, in contrast to high viscosity solutions that will rather cause electrospinning. Hiep et al. produced core–shell microspheres composed of a PLGA core and a chitosan shell with electrospinning to determine the influence of the polymer concentration and the applied voltage on the particle morphology [38]. Increasing the PLGA concentration from 7 to 10% w/w indeed caused the morphology of the particles to change from spheres into fibers. Furthermore, a voltage of 25 kV was required to obtain core–shell microspheres. In another study, core–shell microspheres with a PLGA (lactide:glycolide ratio 75:25) core and a PLGA (lactide:glycolide ratio 85:15) shell also showed a change in shape from microspheres to more fiber-like structures when the core polymer concentration was increased from 6 to 7.5% w/w and the shell polymer concentration from 4 to 5% w/w [86].

Some modifications have been made to the coaxial electrospraying setup, of which coaxial electro-dropping is one. Microspheres with varying sizes and a core–shell structure could be produced using this method. Similar to electrospraying, an electric field is applied to electrically charge the injected solutions but in the case of electro-dropping,



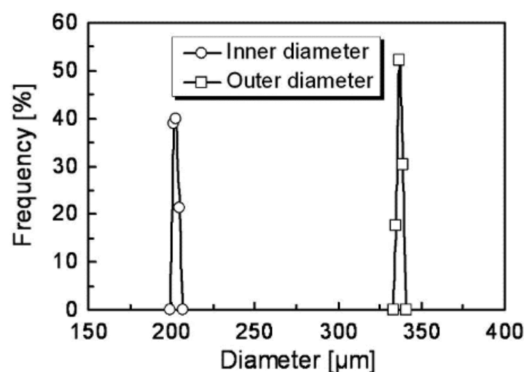


Fig. 5. Optical light micrograph (left) and the size distribution (right) of highly monodisperse O/W/O core–shell microspheres with an alginate shell and soybean oil solution in the core. Scale bar = 100 μm. Modified from [37] with permission from Elsevier.

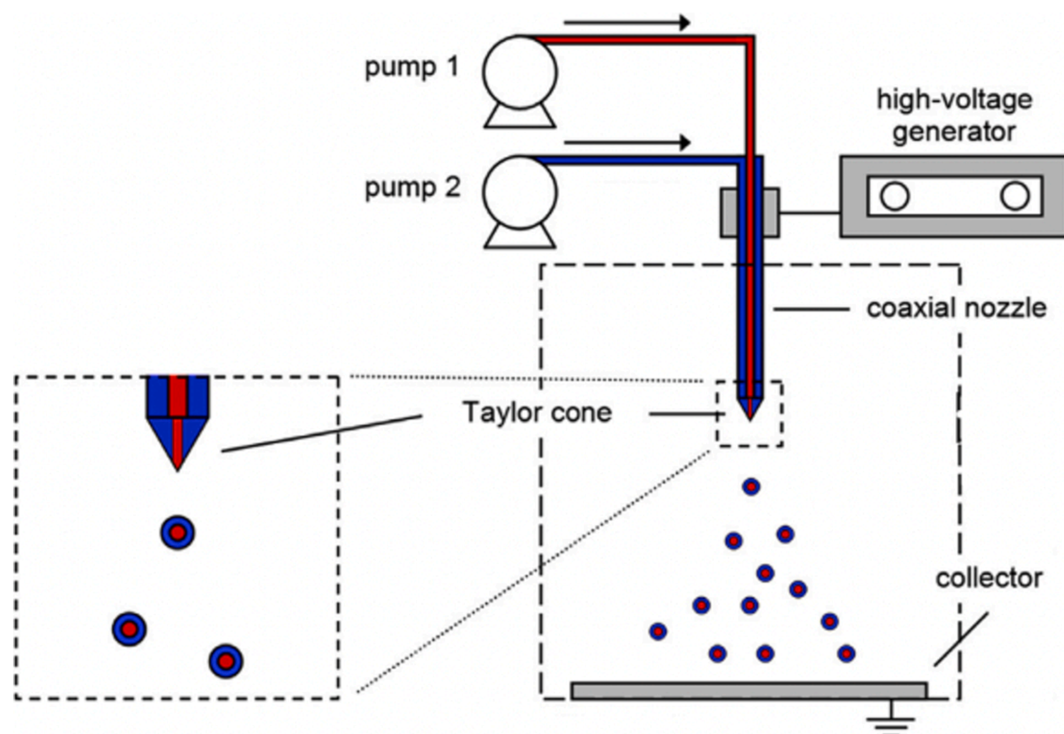


Fig. 6. CEHDA setup for the production of core–shell microspheres. The inner liquid is delivered using pump 1, the outer liquid is delivered using pump 2. Modified from [85] with permission from Elsevier.

the liquids are slowly pumped through coaxial needles. Core-shell microspheres of several hundred micrometers were prepared by loading a PLGA solution and an alginate solution separately in a syringe, and slowly pumping the liquids out through the inner and outer needle, respectively [24]. The two immiscible viscous liquids met at the tip of the coaxial nozzle which resulted in the formation of a droplet, and eventually in the formation of a semisolid particle upon collection in crosslinking calcium chloride solution. The collected particles were then washed and filtered. Single or multiple PLGA cores were observed in the microspheres, and the osteogenic induction factors bone morphogenetic protein 2 (BMP-2) and dexamethasone could be encapsulated separately in the core and shell, respectively, and vice versa.

Also without the coaxial setup, it is possible to obtain core–shell structured microspheres by means of electrospraying. In a study by Wu et al., core–shell microspheres were fabricated using a single-step emulsion electrospraying method with BSA encapsulated in the core and the amphiphilic biodegradable polymer poly(ε-caprolactone)-poly-amino-ethyl ethylene phosphate (PCL-PPE-EA) as shell material [88]. A W/O emulsion was prepared by adding an aqueous solution of BSA drop by drop to DCM solution containing the polymer, after which this emulsion was electrosprayed. Due to the amphiphilic properties of the polymer, core–shell structured particles instead of monolithic particles were formed, as agglomeration of the small water droplets in the W/O emulsion resulted in a monolithic protein core. This core–shell structure was verified by both TEM and CLSM photos and by an SEM photo of a freeze-fractured particle.

A great advantage of electrospraying is the variety in the combination of polymers and drugs that can be used, even materials that are sensitive to high shear stresses and elevated temperatures, such as proteins [87,89]. The ability to operate at ambient temperature and pressure makes it a very versatile and convenient production method. For the shell, PLGAs of different monomer ratios are the most commonly used polymers, but also PDLLA, PLLA, PCL, chitosan, and alginate have been used. Gao et al. produced monodisperse core–shell microspheres with a PCL shell and a silicon oil core containing the hydrophobic model drug Sudan Red G [32]. Oil-based cores have gained increased attention as problems related to drug solubility can be reduced and the chemical or physical stability of moisture-sensitive drugs can be improved in comparison with aqueous cores. This shows the potential of coaxial electrospraying for hydrophobic drug encapsulation without the need for a polymeric core. Furthermore, particle size and shell thickness can easily be controlled by varying the polymer concentration, inner and outer flow rate, applied voltage, and collection distance [32]. Another advantage of CEHDA over the conventional bulk emulsion solvent evaporation method is the fact that there is no need for stirring to create emulsions so high shear rates are circumvented. Also, the formulation does not require contact with an outer aqueous environment, which enhances the ability to load hydrophilic drugs in the core. This makes CEHDA very suitable for producing core–shell microspheres with hydrophilic drugs loaded in the core and hydrophobic drugs loaded in the shell but also vice versa in a single step. In a study by Nie et al., core–shell microspheres were fabricated as a multi-drug release system of which the core consisted of PLLA and the shell of PLGA [25]. The hydrophobic small molecule paclitaxel was incorporated into the shell whereas the hydrophilic small molecule suramin was incorporated into the core but the reverse was also constructed for comparison. Although the EE of the core material was high, i.e. 81–91%, the EE of the shell material was compromised. Paclitaxel was encapsulated in the shell at an EE of 54–59% but a significantly lower EE of 39–46% was obtained for suramin in the shell. The low EE of suramin in the shell was ascribed to its hydrophilicity and to jet instability caused by the mixture of ethyl acetate (EtAc), ethanol, and water which was used as solvent for the drug. Also, the microspheres were collected in anhydrous ethanol instead of on aluminum foil so the drug on the surface might have washed off. The EE of the core materials for CEHDA in general is relatively high (approximately 65–100%), although this is also dependent on the formulation parameters, such as polymer concentration and drug loading. The reason for these high EE values is that the core materials are

Table 4
Representative polymeric core–shell microspheres produced via electrospraying/CEHDA. Presented polymer molecular weights and viscosities are the weight averaged molecular weights and the inherent viscosities, respectively, unless stated otherwise.

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
[113]	CEHDA	Core: simvastatin + PLLA (85–160 kDa), shell: PDGF + alginate	Simvastatin: sustained release up to day 40; PDGF: fast release up to day 6, slow release up to day 9	~500 µm	Core materials: 73–78%, shell materials: 65%	Yes, micro-CT, histological, and immunohistochemical assessments on rats	
[114]	CEHDA	Core: BSA/PDGF/ simvastatin + PDLLA (24–75 kDa), shell: BSA/simvastatin/ PDGF + PLGA (50:50, 31–44 kDa)	PDGF and simvastatin: no burst, sustained release up to day 14	18–21 µm, COV = 4–10%	Core materials: 85–96%, shell materials: 54–83%	Yes, micro-CT, histological, and histomorphometric assessments on rats	
[85]	CEHDA	Core: dox + PLGA (50:50, 0.61 dL/g), shell: PDLLA (0.37 and 0.70 dL/g)	Hardly any release up to day 19, fast release up to day 43, and slower release up to day 152	28–32 µm, COV = 14–19%	71–91%	No	
[115]	CEHDA	Core: BSA/ simvastatin + PDLLA (24–75 kDa), shell: BSA/PDGF + PLGA (31–44 kDa)	Sustained release up to day 14	14–17 µm, COV = 22–29%	Core materials: 81–90%, shell materials: 65–73%	Yes, placement of microspheres in rats, micro-CT assessments and histological examination	
[106]	CEHDA	Core: simvastatin/ BSA/PDGF + PDLLA (24–75 kDa), shell: BSA/simvastatin/ PDGF + PLGA (50:50, 31–44 kDa)	Sustained release up to (at least) 14 days	15–22 µm, COV = 13–21%	Core materials: 83–92%, shell materials: 51–71%	Yes, *in vivo* biocompatibility assay	
[109]	CEHDA	Core: BSA/ simvastatin/PDGF + PDLLA, shell: BSA/ PDGF/simvastatin + PLGA	Sustained release up to (at least) 14 days	15–20 µm	Core materials: 65–92%, shell materials: 41–71%	Yes, micro-CT, histological, inflammation, cell viability, and bone resorption studies	
[25]	CEHDA	Core: paclitaxel/ suramin + PLLA (85–160 kDa), shell: suramin/paclitaxel + PLGA (50:50, 40–75 kDa)	Sustained release up to day 30; resp. sequential and parallel release of both drugs	~20 µm	Core materials: 81–91%, shell materials: 39–59%	Yes, cytotoxicity assay and cellular apoptosis study	
[44]	CEHDA	Core: paclitaxel/ suramin + PLLA (85–160 kDa), shell: suramin/paclitaxel + PLGA (50:50, 40–75 kDa)	Sustained release up to 30 days; resp. sequential and parallel release of both drugs	~10–20 µm	No data	Yes, cytotoxicity and cellular apoptosis assays; tumor inhibition and imaging study, histological and immunohistochemical analysis	
[104]	Coaxial electrospraying	Core: VEGF, shell: PLGA (50:50, 120 kDa)	Burst = 20–35%, fast release up to day 6	6 µm, COV = 28–29%	65–70%	Yes, cell culture and staining	
[116]	Coaxial electrospraying	Core: reGFP, shell: PLGA (50:50)	No data	2–6 µm, COV = 13–26%	No data	No	
[117]	Dual-capillary electrospraying	Core and shell: budesonide/ theophylline + PLGA (50:50, 7–17 kDa/ 50:50, 24–38 kDa/ 85:15, 50–75 kDa)	Both drugs: sustained release up to at least 50 h	0.4 and 1.1 µm, geometric standard deviation = 1.4	Both drugs: 88–97%	No	
[32]	Coaxial electrospraying	Core: Sudan Red G, shell: PCL (45 kDa)	Fast release up to day 1, slow release up to day 5	30–62 µm, monodisperse	No data	Yes, biological evaluation/*in vitro* cell studies: cytotoxicity and cell growth behavior	
[26]	Coaxial electrospraying	Core: BMP-2 + PLGA (75:25, 10 kDa), shell: VEGF + PDLLA (10 kDa)	Sustained release up to day 28; VEGF faster release than BMP-2	0.7 µm, COV = 37–40%	BMP-2: 80–85%, VEGF: 73–80%	Yes, cell tests and implantation of microspheres into rat calvarium	
[86]	Coaxial electrospraying	Core: lacosamide + PLGA (72:25, 60 kDa), shell: PLGA (85:15, 60 kDa)	Sustained release up to day 18, ~50% release within the 1st day	4 µm, COV = 24%	94%	No	
[20]	Coaxial electrospraying	Core: BSA, shell: PLGA (50:50, 31–58 kDa and 58–92 kDa)	PLGA 58–92 kDa: fast release up to day 3, a lag phase up to at least 43 days; PLGA 31–58 kDa: fast release up to day 3, a lag phase up to day 29, and near zero-order	3–6 µm, COV = 17–38%	48–75%	No	

(*continued on next page*)

Table 4 (*continued*)

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
			release up to at least 43 days				
[107]	Dual-capillary electrospraying	Core: budesonide/ EGCG, shell: PLGA (50:50, 5–15 kDa)	Budesonide: burst = 5–60%, two-stage release up to 25–225 h	0.2–1.2 µm, COV = 3–11%	Budesonide: 90–95%, EGCG: 88–92%	No	
[90]	Coaxial tri-capillary electrospraying	Core: EGCG (+PLGA), middle phase: budesonide (+PLGA), shell: PLGA (50:50, 40–75 kDa)	EGCG: biphasic release (slow release followed by faster release) up to 18–24 days, budesonide: sustained release up to 18–24 days	3 µm, COV = 7–18%	EGCG: 90–93%, budesonide: 87–92%	No	
[88]	Emulsion electrospraying	Core: BSA, shell: PCL-PPE-EA	Sustained release up to day 20	3 µm	90%	No	
[24]	Coaxial electro-dropping	Core: BMP-2 + BSA + PLGA (50:50, 40 kDa), shell: dexamethasone + alginate	Sustained release up to day 30; faster release of shell drug than of core drug	200–1000 µm	No data	No	BSA added as stabilizer. Position of BMP-2 and dexamethasone could be switched
[38]	Electrospinning	Core: PLGA (85:15), shell: chitosan	No data	0.2–20 µm	No data	Yes, cytotoxicity and cell proliferation test on cells	

Abbreviations: EGCG, epigallocatechin gallate; PDGF, platelet-derived growth factor; reGFP, recombinant enhanced green fluorescent protein; VEGF, vascular endothelial growth factor.

loaded through the inner needle which reduces the chance of diffusion of the core drugs into the outer phase. This in turn reduces the possibility that the materials get wasted in the atomization process. This is a great advantage in comparison with some of the other production methods. The EE, for instance, significantly improved when BSA-loaded microspheres were produced with coaxial electrospraying instead of emulsion electrospraying. The core–shell microspheres prepared with coaxial electrospraying had an EE of 69–72%, in contrast to the monolithic microspheres prepared with emulsion electrospraying that had an EE of only 47–54% while using the same theoretical BSA loading and polymer concentration [20]. This again shows the benefit of a core–shell structure.

Coaxial electrospraying also allows for the production of small particles in the nanometer to micrometer range but the production of particles larger than 100 µm does not seem to be possible (Table 1, Table 4). Moreover, precise control over size and shape, i.e. microspheres or fibers, of the product is complicated. COV values of < 10% could be achieved, especially when operated in cone-jet mode but the formation of a stable cone-jet is much more difficult with dual-capillary electrospraying than with single-capillary electrospraying. This is caused by the differences in the electrical properties of both phases [20]. Lee et al., however, did succeed in producing monodisperse tri-layered microspheres with COV values of 7–18% by using acetonitrile as the solvent for both the inner, middle, and outer phase. The miscibility of the three liquids resulted in a stable cone-jet and, thus, a narrow particle size distribution [90].

3.4. Drop-by-drop methods – PPF

A less common method for the fabrication of core–shell microspheres is PPF, which uses multiple concentric nozzles to coaxially spray a jet that is composed of the core and annular shell material. The jet is acoustically excited via an ultrasonic transducer and subsequently broken up into uniform core–shell droplets by piezoelectric vibration. The frequency of the vibrations and the concentrations and flow rates of the solutions control the droplet size. An additional coaxial nozzle generates a co-flowing non-solvent carrier stream that surrounds the polymer jet. This carrier stream can reduce the jet diameter and thus allows for the production of droplets smaller than the nozzle diameter [28,91]. The reported diameter of the obtained microspheres is 40–115 µm and thus within a size range that is suitable for parenteral administration. After collecting the droplets in PVA solution, the organic solvent is extracted and evaporated, the particles are washed, and eventually freeze-dried. Table 5 gives an overview of representative core–shell microspheres produced with PPF. Because this technique offers great control over the particle size and shell thickness due to both the carrier stream and the use of acoustic excitation, a narrow size distribution is often achieved (Table 5). Also, microspheres can be produced with a high production speed and reproducibility and in a continuous fashion which makes it a very profitable method, and there is no need for high-speed homogenization. Fig. 7 provides a schematic representation of the production method.

In most of the studies, a PLGA or a P(D)LLA solution was used for the core jet so that core–shell microspheres with a solid core were produced. For the shell phase, the same polymers were primarily employed. The PPF method is not confined to the use of immiscible polymers or polymers solutions. In some studies, however, the use of miscible polymers, for instance PLGA and PCPH, resulted in the presence of some domains of the core polymer in the shell layer [19,35]. Furthermore, both a hydrophobic and a hydrophilic model small-molecule drug, i.e. piroxicam [92,93] and doxorubicin [94,95,96], respectively, could be loaded in the core phase in order to control their release rate. In the case of doxorubicin, the drug was first dissolved in water and subsequently emulsified with the polymeric core phase. Chi-p53 (gene delivery vectors comprising chitosan and a plasmid DNA encoding p53) nanoparticles were added to the shell phase to obtain a dual-drug delivery system for anticancer therapy by combining both chemotherapy and gene therapy [94,96]. Proteins can also be incorporated into microspheres using PPF. BSA was successfully encapsulated in a PLGA core surrounded by a PLLA or PDLLA shell [21,97]. An exception to the use of PLGA, PDLLA, and PLLA is the surface-eroding polyanhydride polymer PCPH. Berkland et al. prepared double-walled microspheres with a PCPH core and a PLGA shell [28]. However, reversing the arrangement of the two polymers while keeping all other production conditions unchanged, resulted in incomplete encapsulation of the PLGA core by PCPH. By adjusting the polymer concentrations and flow rates, full engulfment could eventually be achieved. This shows that there are no standard settings for microsphere production with PPF, and that the production conditions have to be optimized when a different polymer is used or a different arrangement is desired.

A great advantage of PPF is the possibility of easily achieving a non-solid core that only contains the drug. For example, Berkland et al. demonstrated that liquid-filled core–shell microspheres with either an oil or aqueous core could be produced using PPF technology [19]. Three

Table 5
Representative polymeric core–shell microspheres produced via PPF. Presented polymer molecular weights and viscosities are the weight averaged molecular weights and the inherent viscosities, respectively, unless stated otherwise.

Ref.	Production method	Materials	Release profile	Particle size and dispersity	EE	*In vivo* or *ex vivo* data?	Comments
[18]	PPF	Core: BSA, shell: PLGA (50:50, 15/38/88 kDa)	15 kDa: fast release up to day 5; 38 kDa: sustained release up to day 40; 88 kDa: slow release (up to 10–30%) followed by a pulse over ~ 7 days from day 22, 32, or 35	73–85 µm, monodisperse	15 kDa: 5–10%, 38 kDa: 15–30%, 88 kDa: 55–65%	No	
[21]	PPF	Core: BSA + PLGA (50:50, 4 kDa), shell: PLLA (43/106/192 kDa)	Fast release up to day 10, sustained release up to 70 days	55 µm, COV = 3–5%	Only DCM: 20–35%, EtAc + DCM: 40–55%	No	
[96]	PPF	Core: dox + PLGA (50:50, 0.61 dL/g), shell: Chi-p53 nanoparticles + PLLA (1.05 dL/g)	Chi-p53: burst = 15%, near zero-order release up to at least 125 days; dox: burst = 10–30%, lag phase up to day 20, sustained release up to at least 125 days	50–75 µm	No data	Yes, cytotoxicity and cellular expression study, and immuno-fluorescence staining	
[95]	PPF	Core: dox + PLGA (50:50, 0.61 dL/g), shell: PDLLA (0.37 and 0.70 dL/g)/PLLA (1.05 dL/g)	Burst = 2–10%, lag phase up to day 26, sustained release up to at least 125 days	50–75 µm	79–80%	No	
[97]	PPF	Core: BSA + PLGA (50:50, 4 kDa), shell: PDLLA (43 kDa)	EtAc + DCM: fast release of 35% up to day 10, sustained or near zero-order release up to day 140	60–77 µm, COV = 2–4%	Only DCM: 25–30%, EtAc + DCM: 45–50%	No	
[94]	PPF	Core: dox + PLGA (50:50, 0.61 dL/g), shell: Chi-p53 nanoparticles + PDLLA (0.37 and 0.70 dL/g)/PLLA (1.05 dL/g)	Dox: burst = 2–30%, lag phase up to day 26, sustained release up to at least 125 days; chi-p53: burst = 2–15%, slow release up to at least 125 days	63–75 µm, COV = 4–7%	Dox: 79–83%, Chi-p53: 25–37%, Dox + Chi-p53: 32–47% and 27–37%, resp.	No	
[19]	PPF	Core: dextran/BSA, silicone/canola oil, and PLGA; shell: resp. PLGA, PLGA, and PCPH (n.b. PLGA 50:50, 10–65 kDa)	BSA and dextran: burst < 5%, lag phase up to day 20, pulse over ~ 5 days from day 30, slow release up to day 60	Aqueous core: 115 µm, oil core: 110 µm, solid core: 60 µm; monodisperse	No data	No	
[93]	PPF	Core: piroxicam + PLGA (50:50, 0.39 dL/g), shell: PDLLA (0.24 dL/g)	Burst = 4–12%, biphasic release (slow release followed by faster release) up to 40–50 days	47–86 µm, COV = 5–16%	74–97%	No	
[35]	PPF	Core: PCPH, shell: PLGA (50:50, 0.82 dL/g) and the inverse	No data	44 µm, COV = 4–6%	No data	No	
[92]	PPF	Core: piroxicam + PLGA (50:50, 35 kDa), shell: PLLA (100 kDa)	Burst ~ 10%, sustained release up to day 90	40–60 µm, monodisperse	3–8%	No	
[28]	PPF	Core: PLGA (50:50, 85/130 kDa), shell: PCPH and the inverse	No data	58 µm, monodisperse	No data	No	

different core types were tested: a solid PLGA core with a PCPH shell, a silicone or canola oil core with a PLGA shell, and an aqueous dextran or BSA core with a PLGA shell. For all arrangements, a distinct core–shell structure was visible and a narrow size distribution was obtained. However, for the solid and oil core formulations, some mixing of phases did take place at the interface of the materials as portrayed by SEM photos of cross-sectioned particles. Microspheres with a canola oil core displayed small unconnected pores at the particle surface, indicating the breaching of canola oil into the PLGA shell. Aqueous core microspheres did not display this minimal intrusion of the core phase into the shell phase as these phases are less miscible.

A drawback of this production method is the fact that the obtained EE is variable from as low as 3% to up to 97%, though the low EE values are often the consequence of the chosen production settings instead of being inherent to the production method. Xia et al. prepared both BSA-loaded single-walled and double-walled microspheres, from PLGA and PLGA/PLLA [21] or PLGA/PDLLA [97], respectively. Double-walled microspheres had an EE of only 20–35% when DCM was used as the solvent. However, the EE increased to 40–55% when both DCM and EtAc were used for the core and the shell phase, respectively, which was ascribed to differences in the particle hardening time. The EE also appeared to increase with increasing molecular weight of PLLA, i.e. the shell polymer, due to an increase in the solution viscosity which prevented the protein from diffusing out of the core [21]. These EE values are relatively low in comparison with microfluidics and CEHDA. The single-walled microspheres had an EE of only 20% or 30%, depending on the production settings. The improved EE values for the double-walled microspheres can again be attributed to the presence of a drug-free shell layer. However, the opposite was observed in a study by Berkland et al. [92], where the EE drastically decreased from 49% (PLGA microspheres) or 85% (PLLA microspheres) to only 3–8% (PLGA/PLLA core–shell microspheres). It is said that the large volume of solvent in the shell phase of each droplet is a driving force for the diffusion of the drug towards the droplet surface. When doxorubicin was incorporated into the core of the previously described PLGA/PLLA double-walled microspheres, the EE increased from 61% for single-walled PLGA-

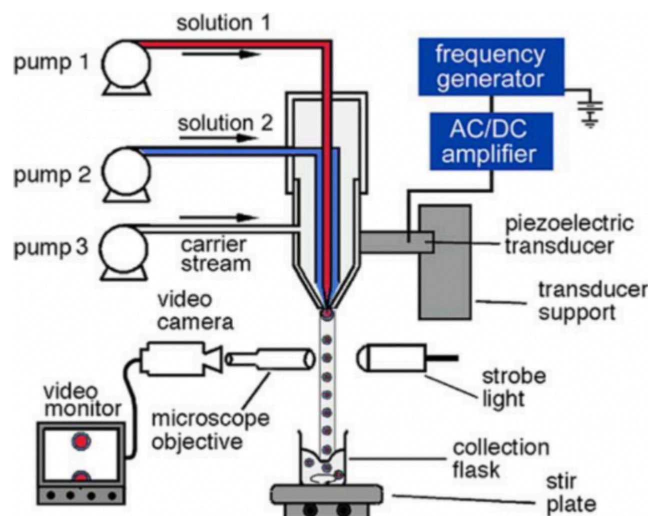


Fig. 7. Schematic PPF setup for the production of uniform core–shell microspheres. The inner liquid is delivered using pump 1, the outer liquid is delivered using pump 2, and the carrier stream is delivered using pump 3. Modified from [28] with permission from Elsevier.

based microspheres to 79–83% [94]. Yet, when chi-p53 nanoparticles were added to the shell, the EE of doxorubicin decreased from 79 to 83% to 32–47% as the nanoparticle dispersion was emulsified with the shell phase, thereby facilitating the diffusion of doxorubicin out of the particles during microsphere solidification. The EE of the nanoparticles was only 25–37%. It has to be noted that the EE of drugs in the shell phase is generally 40–80% and thus much lower than the EE of drugs in the core, which can be explained by the shorter diffusion distance from the shell. Another disadvantage of PPF is the complexity of the production method and so far, only a few studies have used the technique for the production of core–shell microspheres for pharmaceutical use. Therefore, the information on the possible applications and the optimal production conditions is limited, and further research is required.

4. Drug release profiles from core-shell microspheres

One of the major advantages of core–shell microspheres over monolithic microspheres is the increased control over the release kinetics of the encapsulated drugs because the properties of the shell, such as shell material and shell thickness, can be tailored. Examples of improved release kinetics are a reduced initial burst release [14,31,45], a prolonged total release [14,21,23,92,97], and a delayed (pulsatile) release [18,19,29,55,50,85] as the shell layer presents a diffusion barrier to the drugs in the core. A prolonged release is especially advantageous for drugs that frequently have to be administered via parenteral injection which is very uncomfortable and unpractical for the patient. Another example of improved release kinetics is the dual-drug release of, for example, a hydrophobic and a hydrophilic drug with different release patterns [24,26]. Dual-drug release is especially beneficial in the therapy for tissue regeneration and cancer, as these are multistage processes that can be influenced by several growth factors or inhibition factors and other proteins and drugs that can regulate the tissue or tumor growth [44,98]. These therapies often require sequential or parallel delivery of the different drugs, which can be achieved by loading these drugs separately in the core and shell. In order to mimic the natural bone healing process, Wang et al. produced PLGA/PDLLA core–shell microspheres using coaxial electrospraying, with vascular endothelial growth factor (VEGF) incorporated in the shell layer and BMP-2 in the core (Fig. 8) [26]. Both drugs exhibited a sustained release profile up to 28 days, although they were delivered at different release rates and thus in a sequential manner. As VEGF was loaded in the shell, this growth factor exhibited an initial burst release of nearly 40% and a total release of approximately 70% within the first ten days, while only 3% of BMP-2 was released from the core within the first 24 h. The released VEGF can promote angiogenesis, followed by the release of BMP-2 inducing osteoblast differentiation. Choi et al. prepared core–shell microspheres with two osteogenic induction factors, BMP-2 and dexamethasone, loaded separately in the core and shell, thereby establishing a dual-drug delivery system [24]. In this way, both drugs could be released simultaneously at different release rates, which means that stem cell differentiation could be regulated in a coordinated fashion. Additionally, the respective drugs could be switched from core to shell position

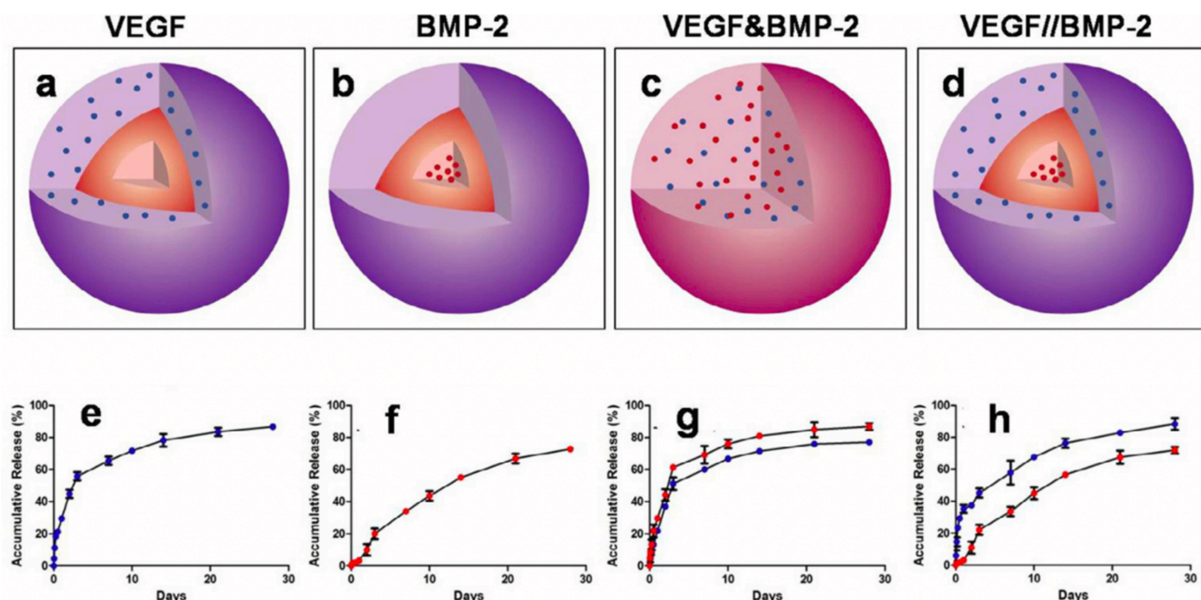


Fig. 8. Schematic illustration of VEGF and BMP-2 releasing monolithic and core–shell microspheres (a-d): core–shell microsphere with only VEGF in the shell (a), core–shell microsphere with only BMP-2 in the core (b), monolithic microsphere with both VEGF and BMP-2 (c), core–shell microsphere with both VEGF and BMP-2 in the shell and core, respectively. In vitro release profiles of VEGF and BMP-2 from microspheres a-d (e-h). Blue dots represent VEGF, red dots represent BMP-2. Modified from [26] with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and vice versa, while maintaining the physical separation. The drugs displayed a sustained release profile for at least thirty days, with the drug incorporated in the shell displaying a higher burst release and a faster overall release than the drug incorporated in the core. Although the incorporation of two drugs into a single microsphere has some advantages, such as the need for only one production line, it is also possible to incorporate two drugs into two different batches of microspheres. By adding these batches together into one syringe, a dual-drug release formulation can be obtained.

The desired release profile depends on the intended application of the drug(s) in the microspheres. A pulsatile release profile, such as a delayed pulsatile release (i.e. a pulse after a certain lag time) or a triphasic release profile, is often aimed for when core–shell microspheres are employed. There are various indications for which continuous drug delivery is not optimal and where a pulsatile release profile might be preferred [99]. Examples are drugs with a high first-pass effect or with specific chronopharmacological demands, for example hormones. Hormones regulate many internal functions in the body, often following the circadian rhythm, which means that pulsatile release is required to mimic certain endogenous patterns and thus improve therapeutic efficiency. Moreover, a triphasic-release formulation might be beneficial for the delivery of vaccines that generally demand a second and sometimes third booster dose to confer protective immunity against the targeted pathogen [100]. A single injection of a vaccine delivery system with such a triphasic release covers both the primer and the booster dose, and thus circumvents the need for multiple injections [65]. This improves vaccinee's convenience and compliance and reduces the costs. A sustained-release formulation can also be applied for vaccine delivery but a pulsatile release profile gives a better imitation of the current multiple injection regimen used for conventional vaccines, and a sustained release profile might induce immune tolerance [65,101]. Sanchez et al. developed a single-shot tetanus vaccine formulation using PLGA-based microspheres with an oily core containing the model antigen tetanus toxoid surrounded by a vaccine-free polymer shell [102]. Two formulations with different grades of PLGA were tested and both exhibited a delayed pulsatile release of tetanus toxoid as seen in Fig. 9. Delayed release of the highly water-soluble radiosensitizer etanidazole was achieved by incorporating the drug as solid crystals into core–shell microspheres with a PLLA shell and a PLGA core, although the lag phase was not followed by a pulsatile release but by a sustained release (Fig. 10) [55]. A low initial burst of<5% was observed, followed by a lag phase of four weeks and a nearly linear release for two weeks. Such a release profile might greatly improve the treatment of tumors. The initial release was diffusion controlled while the subsequent release was controlled by the degradation of the polymeric shell layer as the formation of pores and channels, caused by the presence of PLGA domains in the shell, predominated. Similar microspheres were prepared with a conventional W/O/O/W emulsion solvent evaporation method combined with phase separation with BSA as model protein [29]. The release profile could be altered by saturating the aqueous continuous phase with DCM, thereby changing the solvent evaporation kinetics, and by adding ethanol to the PLLA solution, i.e. the shell phase. The first reduced the solvent efflux from the dispersed oil phase into the aqueous continuous phase and the last caused an increased solubility of DCM in the aqueous continuous phase. Both methods ultimately influenced the protein distribution within the microspheres. For all formulations, an initial burst release of<20% was obtained that was followed by a lag time in which hardly any protein was released. The lag time duration could be varied from 4 to 30 days and the total release period from 30 to at least 58 days by altering the solvent evaporation kinetics and/or the ethanol content. For PLGA-based core–shell microspheres with an oily core, a delayed pulsatile release of BSA was obtained as well when the molecular weight of the polymer was high enough, i.e. 88 kDa [18]. For lower molecular weight PLGA, i.e. 15 and 38 kDa, fast or sustained release was obtained without a lag phase as the liquid-core engulfment efficiencies were significantly lower for these formulations, which indicates that a high percentage of the microspheres did actually not have a core–shell structure. Pek et al. also demonstrated this dependency of the *in vitro* release profile on the molecular weight of the polymer for PLGA/PLLA core–shell microspheres loaded with bupivacaine powder in the inner core [50]. In a study by Berkland et al., PLGA-based core–shell microspheres with an aqueous core containing BSA and dextran were produced [19]. Both compounds generated a pulsatile release after a lag time of approximately twenty days with minimal initial burst release. In this case, however, a low molecular weight of PLGA (15 kDa) was enough to obtain a high core engulfment efficiency, and thus, such a pulsatile release profile. In summary, polymeric core–shell microspheres can provide a delayed pulsatile release profile as long as they meet certain structural criteria. The core should be completely surrounded by a drug-free shell layer with minimal porosity, and distinct phase separation of the core and shell phase is essential, with the drug being spatially localized in the core.

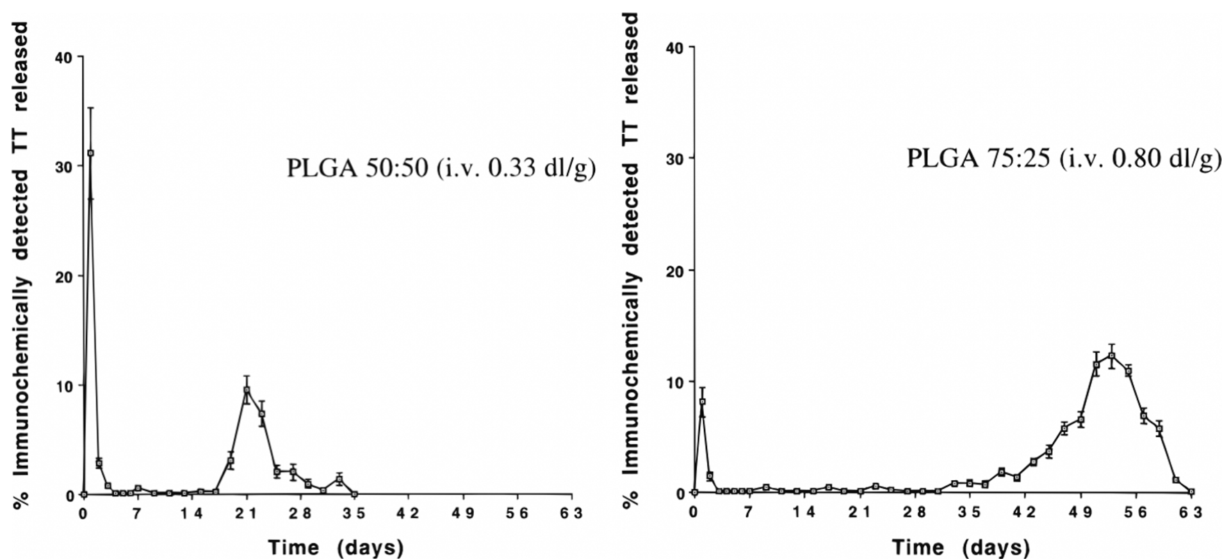


Fig. 9. In vitro release profiles of immunochemically detected TT from oil-based core–shell microspheres with a PLGA shell. PLGA with a lactide:glycolide ratio of 75:25 and an inherent viscosity (i.v.) of 0.33 dL/g (left) and PLGA with a lactide:glycolide ratio of 75:25 and an i.v. of 0.80 dL/g (right) were compared. Modified from [102] with permission from Elsevier.

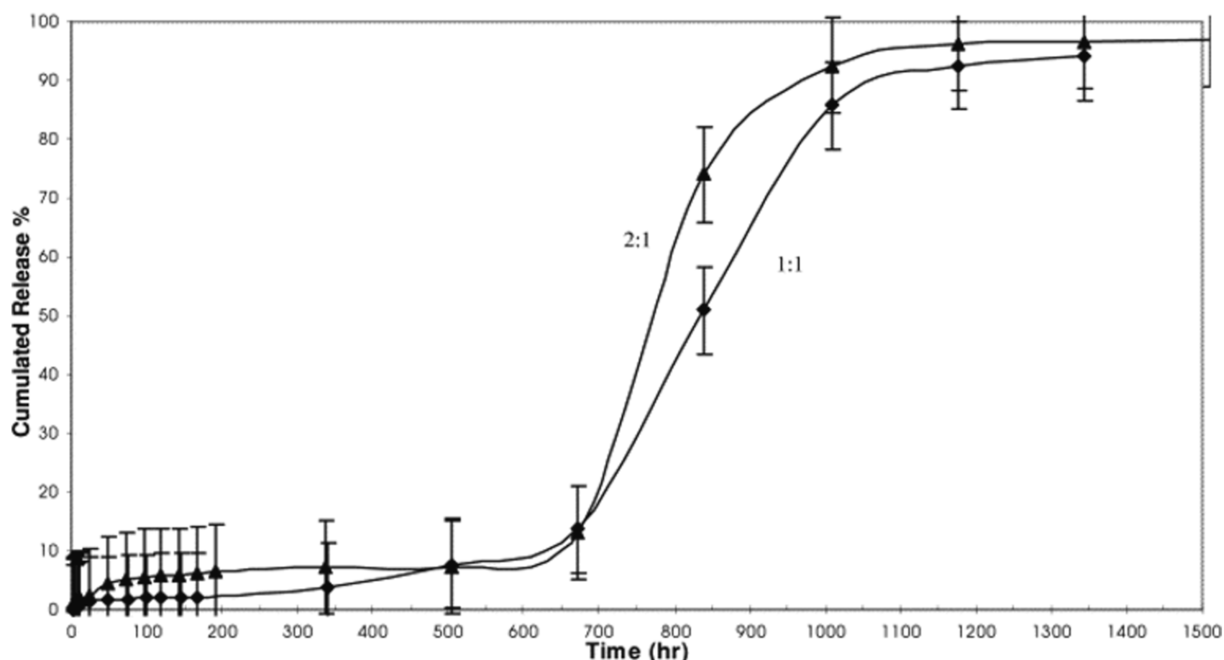


Fig. 10. In vitro release profiles of etanidazole from PLGA-PLLA core–shell microspheres with different polymer mass ratios (w/w) of PLLA to PLGA (2:1 (▲) and 1:1 (◆)). Reproduced from [55] with permission from Elsevier.

One of the aims that is often pursued with delayed (pulsatile) release core–shell microspheres, is the ability to modulate the lag time by tuning the properties of the core and/or shell. In this way, the microspheres are suitable for numerous applications. The lag time could be modulated by adding ethanol to the polymer solution which altered the protein distribution and the microsphere structure but many more mechanisms are possible [29]. PLGA/PLLA core–shell microspheres that were γ-irradiated with a sterilization dosage of 25 kGy displayed a decrease in lag time of two weeks compared to the nonirradiated microspheres [55]. This was explained by a reduction in molecular weight of the shell polymers as a result of irradiation, which caused a decrease in degradation time. The duration of the lag time was, on the other hand, independent of the polymer mass ratio of PLLA and PLGA, and thus independent of the shell thickness of the microspheres as shown in Fig. 10. This might be explained by the fact that PLLA and PLGA are bulk-degrading polymers and not surface-eroding which means that the lag time is only determined by the polymer characteristics. The influence of the polymer molecular weight on the release characteristics has been demonstrated multiple times [18,20,50], although the polymer composition and primarily the monomer ratio in the case of PLGA, have a greater influence, mainly on the onset of the pulse. An increase in lactide content results in a more hydrophobic and thus a slower degrading polymer which eventually could lead to a longer lag time. In the case of the single-shot tetanus vaccine formulation, PLGAs with two different monomer ratios and molecular weights were used to vary the lag time [102]. For both formulations, the antigen was released in a pulsatile manner after a certain lag time. The lag time was three weeks for PLGA with a lactide:glycolide ratio of 50:50 and a relatively low molecular weight, and seven weeks for PLGA with a lactide:glycolide ratio of 75:25 and a relatively high molecular weight. Both the monomer ratio and the molecular weight might have caused the difference in lag time. An initial burst release of 30 and 10%, respectively, was observed which is presumably due to the migration of some of the antigen-loaded droplets towards the particle surface. However, no other studies could be found in which the influence of the lactide:glycolide ratio of the polymer used as shell material on the lag time of core–shell microspheres was investigated. Zheng determined the influence of the polymer composition of the core material on the release profile [13]. Core-shell microspheres with 5-fluorouracil loaded in a PLGA core surrounded by a PLLA shell were produced and the monomer ratio and molecular weight of PLGA in the core were varied. The lag phase was the shortest for microspheres made from PLGA with a relatively low lactide content and molecular weight and the longest for microspheres made from PLGA with a relatively high lactide content and molecular weight, although the differences were marginal. The *in vitro* release rate after the lag phase decreased as well with increasing lactide content and molecular weight. Both observations could be explained by the occurrence of autocatalytic degradation of the shell polymer. PLGA with the lowest lactide content and molecular weight will degrade the fastest once water has reached the core and thus generate more carboxylic acids that cause faster autocatalytic degradation of the shell. The lag time also increased with increasing shell thickness, which relationship was also shown in some other studies [18,93]. On the other hand, BSA release studies of different core–shell microsphere formulations with a shell made from PLGA with a lactide:glycolide ratio of 50:50 all demonstrated similar lag times of approximately three to four weeks [18,19,20]. These results indicate that the lag time is indeed solely dependent on the polymer composition and not on the particle size and/or shell thickness which was also demonstrated by Xu et al. [85]. It is unclear why different results were obtained. More research should be conducted on the dependence of the lag time on various particle characteristics such as size and hydrophilicity of the drug, polymer composition, polymer and drug localization, and shell thickness.

Another interesting finding is the fact that core–shell microspheres often do not exhibit a delayed (pulsatile) release profile. In the majority of the studies, a sustained release profile was obtained, whether or not preceded by an initial burst release. In some cases, (near) zero-order [24,103] or even immediate [104] release was obtained. Drug release from polymeric core–shell microspheres is influenced by a combination of water penetration, drug diffusion, and polymer degradation [105]. There are several causes for a high initial burst release and/or the absence of a lag phase, one of which is incomplete phase separation. This can occur during the solvent evaporation and microsphere hardening process, for instance when the solvent evaporation is too fast [16]. As a result, two discontinuous layers of polymer are formed with tiny beads of the core polymer embedded in the shell layer, which causes some of

the drug molecules to be present in the shell layer as well. These drug molecules in the vicinity of the surface can cause an initial burst release or release during the lag phase [29,54,52,56]. Moreover, a completely non-porous shell is necessary to prevent any drug from being released during the lag phase and to prevent an initial burst release. Many examples can be found of core–shell microspheres with small or large pores in the shell through which the drug can diffuse out [14,21,42,44,88,94,106]. Furthermore, drug diffusion can sometimes occur through the polymer matrix. This is mainly the case for small hydrophobic drugs [32,92,107] but whether the shell layer can act as a diffusive barrier depends on both the properties of the shell material and the properties of the drug. Large hydrophilic proteins, for example, can sometimes diffuse through the polymer shell as well if this shell is made of for example glycol chitosan [39]. In some cases, however, core–shell microspheres with a non-porous, non-permeable shell are formed in which the drug molecules are solely encapsulated in the core but still no delayed pulsatile release is obtained [97]. This shows that release mechanisms are often still unclear and that research into the release mechanisms of especially core–shell microspheres is desired.

Lastly, *in vitro* release data are often lacking, especially for microfluidically produced core–shell microspheres (Table 3). These studies often focus on the technical part of the production process and on the influence of the production settings on the particle characteristics. Incorporation of a drug and measurement of the *in vitro* release of this drug, however, would definitely be of added value. *In vivo* data are even more scarce and because for many drugs, the release is difficult to measure *in vivo*, the therapeutic effect of the administered drug is often measured instead, for instance tumor weight and volume [108] or bone resorption [109]. Because *in vitro* release data are often not an accurate predictor of the *in vivo* performance, the acquisition of *in vivo* data should be prioritized in the future. Additionally, *in vitro* release studies are often terminated after a few weeks, even when drug release still seems to continue. In order to get a complete picture of the release profile and to determine the underlying release mechanisms, continuation of the release studies over a longer period is warranted.

5. Conclusion

Core-shell microspheres seem to have multiple advantages over monolithic microspheres, and the addition of a shell might offer improved functionality and versatility for parenteral drug delivery. In the first place, core–shell microspheres can provide increased control over the release kinetics of the incorporated drugs. Examples are reduction of the initial burst, increased circulation time of the drug in the body, and the ability to obtain a pulsatile or dual-drug release. Secondly, core–shell microspheres generally have a higher EE than monolithic microspheres. Many different types of core–shell microspheres are possible, both with a solid polymeric, gas-filled, or liquid-filled core, and various polymers can be employed although PLGA, PDLLA, and PLLA are used in the majority of the studies. Unfortunately, data that prove the existence of a core–shell structure are frequently lacking, and a combination of confirmation methods is desired. Various methods can be applied for the production of core–shell microspheres but drop-by-drop methods, such as microfluidics, CEHDA, and PPF are the most attractive because they allow for better control over the particle structure and size and because there is no high-speed homogenization involved. PPF is a very promising method but rather complex and CEHDA is only feasible for the production of small particles. Therefore, microfluidics is the preferred method although large-scale production is still a challenge. Yet, conventional bulk emulsion solvent evaporation (combined with phase separation) is still the most common production method but the obtained particles usually have a broad size distribution and it makes use of harsh production conditions. Various release profiles can be obtained with core–shell microspheres but the release mechanisms are often unclear and many studies lack *in vivo* or even *in vitro* release data. Hence, future research should focus on elucidating the mechanism behind the different release profiles and release profiles should be determined more often, especially *in vivo* and for a longer period of time. Overall, core–shell microspheres have many potential implications on clinical practice, for instance the incorporation of drugs with a narrow therapeutic index that, therefore, require complete absence of burst release. Another example is the use of core–shell microspheres with a pulsatile release profile as a single-injection vaccine formulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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