Expert Opinion





# Production methodologies of polymeric and hydrogel particles for drug delivery applications

Ana Catarina Lima, Praveen Sher & João F Mano[†]
[†]*University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, 3B's Research Group-Biomaterials, Biodegradables and Biomimetics, Guimarães, Portugal*

***Introduction:*** Polymeric particles are ideal vehicles for controlled delivery applications due to their ability to encapsulate a variety of substances, namely low- and high-molecular mass therapeutics, antigens or DNA. Micro and nano scale spherical materials have been developed as carriers for therapies, using appropriated methodologies, in order to achieve a prolonged and controlled drug administration.

***Areas covered:*** This paper reviews the methodologies used for the production of polymeric micro/nanoparticles. Emulsions, phase separation, spray drying, ionic gelation, polyelectrolyte complexation and supercritical fluids precipitation are all widely used processes for polymeric micro/nanoencapsulation. This paper also discusses the recent developments and patents reported in this field. Other less conventional methodologies are also described, such as the use of superhydrophobic substrates to produce hydrogel and polymeric particulate biomaterials.

***Expert opinion:*** Polymeric drug delivery systems have gained increased importance due to the need for improving the efficiency and versatility of existing therapies. This allows the development of innovative concepts that could create more efficient systems, which in turn may address many healthcare needs worldwide. The existing methods to produce polymeric release systems have some critical drawbacks, which compromise the efficiency of these techniques. Improvements and development of new methodologies could be achieved by using multidisciplinary approaches and tools taken from other subjects, including nanotechnologies, biomimetics, tissue engineering, polymer science or microfluidics.

**Keywords**: bioactive molecules, drug delivery systems, hydrogels, microencapsulation, nanoencapsulation, polymers

*Expert Opin. Drug Deliv. (2012) 9(2):231-248*

## 1. Introduction

Conventional drug delivery systems often require multiple administrations leading to irregular systemic drug distribution. To overcome these drawbacks, controlled delivery systems were developed allowing the release of the drug at predeterminated rates for predefined periods of time [1,2]. Controlled systems improve drug efficacy, reduce its toxicity and improve patient compliance. Other characteristics include the protection of the drug from degradation, alterations in pharmacokinetics and biodistribution, and reduction of clearance and side effects [3]. The success of a therapy involving molecule release depends mostly on the characteristics of the system developed for that purpose.

In last few decades, biodegradable and biocompatible polymeric particles have been proposed as carriers for controlled delivery of bioactive substances [4-7]. These

informa
healthcare

10.1517/17425247.2012.652614 © 2012 Informa UK, Ltd. ISSN 1742-5247

**Article highlights.**

- Micro and nano polymeric particles are ideal vehicles for many controlled delivery applications due to their ability to encapsulate a variety of substances.
- The methodologies used for the production of polymeric micro/nanoparticles to be used in drug delivery applications determine the characteristics of the obtained product and consequently the profile release of the bioactive molecules.
- Currently, the most used processes for micro/nanoencapsulation are solvent evaporation/extraction, phase separation, spray drying, ionic gelation and supercritical fluid precipitation.
- Due to the drawbacks of the existing techniques to produce polymeric systems for drug release, searches for new methods or improvements on existing techniques have been a subject of intensive study as well as for translation to the market.

This box summarizes key points contained in the article.



systems also allow an easy administration of molecules, through multiple routes, such as oral, nasal, injectable or transdermal [3,8]. Having taken this factor into account, subsequent innovations involve the study of micro/nanosphere or micro/nanocapsule applications in healthcare, namely, pharmaceuticals and nutraceuticals, cosmetics, veterinary, agriculture and food industry. The particle shape and size constitute important characteristics of the formulations. For example, different solid formulations carriers may be produced and administered through nasal route (aerosols) or oral ingestion (capsules and tablets). In addition, sub micrometer solid sized systems can be also injected into the blood stream. Bioactive compounds such as low- and high-molecular mass therapeutics, antigens or DNA may be then incorporated into such spherical supports and be released inside the body in a sustained way.

Strategies have been continuously developed in order to improve the efficiency and efficacy of the action of the bioactive compounds by targeting them specifically to the sites of action [9,10]. In some cases, particles obtained from stimuli-responsive materials have the ability to respond to external stimuli and consequently deliver the active agent in specific sites in the body and in a controlled fashion [11]; examples have been reported on magnetically targetable particles and antibodies-conjugated drugs and particles for drug delivery applications [12-15]. Such strategies may prevent unwanted systemic side effects and dose requirements are substantially reduced. Moreover, more challenging therapeutic approaches have created more demanding specifications of the dosage forms in which the particles need to exhibit other functions such as stabilization of the active molecules and modulate their release profile. Particles for drug delivery release may also be used as a diagnosis tool, especially providing imaging information [16]; the so-called theragnostic approaches have been shown to have potential to be used in cancer therapy [17]. Therefore, micro and nanoparticles in drug release applications should not be seen now as a passive carrier, but rather as an essential multifunctional element in the therapeutic or vaccination processes.

The production route used to fabricate the particles will determine their shape, size distribution, porosity, surface topography and drug entrapment capacity. The choice of the processing method will depend on factors such as the nature of the polymer and of the drug, the application (nature of the therapy and local of administration) and the duration of the therapy. The methods to encapsulate such substances must accommodate some requirements that should be followed: i) during the encapsulation process, the drug should maintain its stability and biological activity; ii) the distribution of sizes and the anisotropy of the obtained carriers should be as small as possible; iii) the encapsulation efficiency should be high; iv) the loading capability should be high; v) the drug release profile should be reproducible within specific limits; vi) the particles should exhibit low level of aggregation or adherence; and vii) the polymer composing the matrix of the particles should exhibit a degradation profile adequate with the site of administration and type of therapeutic application, and should not cause adverse or systemic responses. Langer [8] described in detail the fundamentals of release mechanisms when the molecules are entrapped in polymeric carriers. Diffusion is the primary mechanism through which the molecules are delivered from the polymer matrix or through a porous membrane. Another possibility to release encapsulated molecules is through chemical reactions in which the polymeric structure is hydrolytically or enzimatically degraded and the bonds between the drug and the polymer are cleaved. The third mechanism occurs when the polymer loaded with drug swells due the entrance of external solvents changing the diffusion properties of the molecules in the matrix. The release of the active agents is also influenced by drug molecular size, the amount entrapped, dimensions and shape of the matrix [18-20].

In this work, we provide an overview and discuss the most used polymers and processing techniques used to prepare polymeric particles for drug delivery applications; due to the importance of this topic in the pharmaceutical industry, the most recent patents are also revised. Less conventional or promising particle fabrication methods are also addressed.

## 2. Drug delivery systems

### 2.1 Biodegradable polymers

Different biodegradable polymers have been proposed in the preparation of particles for drug delivery applications [21,22]. Among them, synthetic polymers have been widely used, as they can typically be obtained with controlled molecular mass and with a tailored chemical structure. The biggest advantage of using synthetic polymers is the wide choice in the chemical nature and architecture of the macromolecular chains; besides the great variety of homopolymers that can

be synthesized, many combinations of co-polymers may provide structures with a great control of final chemical and physical properties. Poly(amides), poly(amino-acids), poly(esters), poly(orthoesters), poly(urethanes) or poly(acrylamides) are examples of synthetic polymer families that have been used in the production of drug loaded devices [23-26]. However, the aliphatic polyesters such as poly(glycolic acid), poly(lactic acid) and especially the corresponding co-polymers (poly(lactide-co-glycolide acid; PLGA) have been, by far, the most used synthetic biodegradable macromolecules in the production of particles for drug delivery applications [23-25,27,28]. These polyesters exhibit low immunogenicity and toxicity, whose physiochemical properties may be controlled by changing parameters such as molecular mass, formulation of the co-polymer and functionalization.

Natural-origin polymers usually contain domains that can send relevant signals to guide cells at various stages of their development [29-31]; this may permit eliciting the correct interfacial response between cells/tissues and the implanted device and enhancing and promoting the desired functions of the surrounding cells, tissues and organs. However, such bioactivity can cause problems with antigenicity [31] and their processing is often difficult. The most used sources of natural-derived polymers include proteins, especially from extracellular matrices (e.g., collagen), polypeptides, polysaccharides (including chitosan, starch, dextran and alginate) and poly(hydroxyalkanoates). Most of such materials have reactive chemical groups that permit their modification, being possible to tailor many properties, including water affinity, charge or degradation profile. An interesting possibility to combine natural-based polymers is using the layer-by-layer technology to assemble oppositely-charged macromolecules in nanostructured multilayers that can be used to control the permeability of drug delivery particles [32,33].

The combination of synthetic and natural-based polymers may be a way to combine advantages of both families of macromolecules. For example, PLGA and a starch-derivative have been proposed for the production of microspheres in drug delivery systems [34]; in another invention, hyaluronic acid, a non-sulfated glycosaminoglycan that is distributed widely throughout the human body in connective, epithelial and neural tissues, is processed in the form of particles and coated, for example, with PLGA, which permits controlling the degradation rate of the polysaccharide [35].

#### 2.1.1 *Hydrogels*

Hydrogels are polymeric networks able to absorb and retain large amounts of water providing good biocompatibility [36,37], while maintaining their shape. Physical or chemical crosslinking of the polymeric chains prevents the dissolution of the matrix and the maintenance of mechanical integrity. Different crosslinking agents may be used, such as epoxy compounds, glutaraldehyde, formaldehyde, dialdehyde starch, dimethyl suberimidate, carbodiimides, succinimidyls, diisocyanates, acyl azide, tris(hydroxymethyl)phosphine, ascorbate-copper, glucose-lysine or photo-oxidizers; physicochemical treatments may be also used, such as UV irradiation and dehydrothermal treatments. Natural-origin crosslinkers have been also proposed, such as genipin [38], due to their biocompatibility and environmental-friendly nature. The most common crosslinking methods for producing hydrogels were reviewed by Hennink and van Nostrum [39].

The strong hydration state in equilibrium makes many physical properties of hydrogels similar to living tissues. Proteins do not adsorb easily to hydrogels because of their low interfacial tension. Therefore, there is great interest in the use of natural, synthetic and hybrid hydrogels in areas such as tissue engineering, drug delivery systems and bionanotechnology. In particular, hydrogels have been widely used as drug delivery vehicles as they permit molecules of different sizes to diffuse into (drug loading) and out (drug release) [40,41]. Moreover, they can incorporate stimuli-responsive elements that permit delivering the bioactive agents in an 'intelligent' way [11,42,43].

Hydrogels can be processed in the shape of particles at micro and nano scale being adequate to develop biocompatible drug delivery systems. Different methods have been proposed to process hydrogel particles, using both biopolymer and synthetic polymers. We consider that crosslinking possibilities able to form networks in mild conditions, such as the ones mediated by enzymes [44,] are especially relevant in the context of encapsulation as this process may be performed together with the presence of the active agent.

Most of the hydrogels processing techniques involve wet environments; this means that liquid droplets containing the polymer and the bioactive molecule are developed in a liquid medium, either organic or aqueous [45]. As is described later, other methodologies should be used to avoid such contact between distinct liquid phases to improve encapsulation efficiency and decrease contamination.

In order to obtain a controlled release, environmentally-responsive hydrogels (or smart hydrogels) have been applied in delivery systems [40]. External stimulus such as temperature, pH, ultrasounds, electric and magnetic field, oxidation light, mechanical stress, ionic strength, enzymes and biomolecules [1,11,46] could cause swelling, collapse or degradation of the hydrogel structure switching the release profile of an entrapped drug.

### 2.2 Micro/nano sized polymeric particles

Polymeric particles including spheres and capsules with sizes at the micro or nano level constitute the kind of carriers most studied in the pharmaceutical field. Microparticles, with sizes between 1 and 1000 μm, have the ability to encapsulate high amounts of active agents and may be administered by minimally invasive procedures or intranasally (in dry powder format) or ingested [47]. The use of biodegradable polymeric microparticles avoids surgical procedures of removing them after the release of the bioactive agent [48]. Silva *et al.* [48] reviewed the most important characteristics of

microparticles to be used in biomedical applications, including size, size distribution, porosity and pore structure and surface area.

Nanoparticles, which include nanocapsules and nanospheres, present a large surface area when compared with microparticles and help in increasing drug stability [49]. Due to their small size, nanoparticles may be injected and circulated in the blood stream constituting an advantage for some therapies. The circulation of nanoparticles in the blood stream is prolonged because of their ultra small volume, which also allows the passage through capillary vessels and avoids clearance by phagocytes. However, nanoparticles may lead to adverse immunological responses when they are phagocytosed by macrophages. Strategies such as the pegylation of the particles surface may decrease such an effect [50]. Nanoparticles arrive easily to targeted organs such as liver, spleen, lung, spinal cord and lymph because they can penetrate cells and tissue gaps [51] which is advantageous for successful targeted delivery systems.

The active molecules in the core of micro/nanoparticles could be released by erosion, permeation or rupture of the shell; depending on the thickness or the material composing the shell, the rate or timing of core releasing could be controlled [3,52]. Most of shells and cores are polymeric; however, they could be also made of lipids and waxes [53].

By focusing on the micro/nanoparticle production, the concept of encapsulation has to be understood. Micro/nanoencapsulation is defined as a process of producing particles or droplets at micro/nano scale with an active substance (core) coated or embedded in a polymer (shell) [54]. The shell should be inert and has the function to isolate and protect the core against aggressive environments and control the permeability or diffusion rate of the substances. These systems constitute interesting approaches in medical, pharmaceutical and veterinary fields by acting as a controlled and sustained drug delivery, masking tastes or odors of many drugs, stabilizing and protecting the drugs from degradation, protecting the body against the toxic effects of drugs, targeting the delivery for a specific site and preventing incompatibility between drugs [3].

Different methods have been proposed to process polymeric micro and nanoparticles loaded with active agents and their final characteristics are directly related to the methodology chosen for their production. The most used techniques are emulsions, phase separation and spray drying [48,55]. More recently, technologies such as supercritical fluid (SCF) precipitation and microfluidic devices were developed and shown to be useful for producing micro/nanoparticles incorporating active agents entrapped. Such methodologies will be described and discussed in more detail later in this review; **Figure 1** summarizes the size of the particles that each technique is able to produce. Other methods or deviations of the traditional methodologies have been proposed and were reviewed by Oliveira and Mano in the context of tissue engineering applications [56].

## 3. Micro/nanoencapsulation technologies

### 3.1 Emulsions

Techniques of micro/nanoencapsulation based on the use of emulsions are the oldest and widely applied in pharmaceutical industries [57-60]. Different procedures may be used in this context, depending of the nature of the drug and the characteristics of the final particles. For example, size, porosity and surface characteristics of the obtained particles may be controlled up to certain extent, so that they can meet the necessary requirements for the applications [10]. The basis of these techniques is the formation of an emulsion of a polymeric solution inside a continuous phase. The application of mechanical forces induces the deformation of an interface between the two phases and consequently spherical drops are formed [61]. The mechanical forces applied (controlled by the mixing or stirring speed), the viscosity of the polymer solution and also the amount of stabilizers present in continuous phase to avoid coalescence of the drops determine the size of the particles obtained. For insoluble or poorly water soluble molecules, the oil-in-water method (o/w) is usually used (**Figure 2A**). The polymer that will give rise to the matrix encapsulating the drug is first dissolved in a water immiscible, volatile, organic solvent into which the bioactive molecule is also dispersed. This dispersion (polymer/solvent/drug) is then added to a large amount of an aqueous solution containing an emulsifying agent (e.g., poly(vinyl alcohol)). The solvent is then removed by either evaporation or extraction resulting in the formation of polymeric spheres loaded with drug. In the first case, the solvent evaporates under appropriate temperature, pressure and stirring conditions, during which the particles harden; an example of this application can be seen elsewhere [62]. In the latter case, the emulsion is moved to a large quantity of water that may also contain surfactant [63]; under such conditions, the solvent is diffused out and the solid spheres are finally obtained after washing, isolated using filtration, sieving or centrifugation, and dried. Moreover, inverse emulsions (water-in-oil (w/o)) also can be performed. In this case, an aqueous drug and polymeric solution or suspension is added into an oil continuous phase where stabilizers (Span 80 and Aerosol OT) and specific crosslinking agents (according with the polymer used) are present. These kind of emulsions are also used to encapsulate DNA and cells.

The major drawback of the o/w methodology is the poor encapsulation efficiencies of drugs that are water soluble or moderately water soluble, as they could diffuse towards the aqueous phase during the processing of the particles. In such cases, a double (or multiple) emulsion process would be more suitable to encapsulate water-soluble substances.

In water-in-oil-in-water (w/o/w) methodologies (**Figure 2B**), an aqueous solution of the drug is added in an organic phase containing the polymer; the first w/o emulsion is then formed. This emulsion is then added into water containing an emulsifier to form the w/o/w emulsion under stirring. The organic solvent

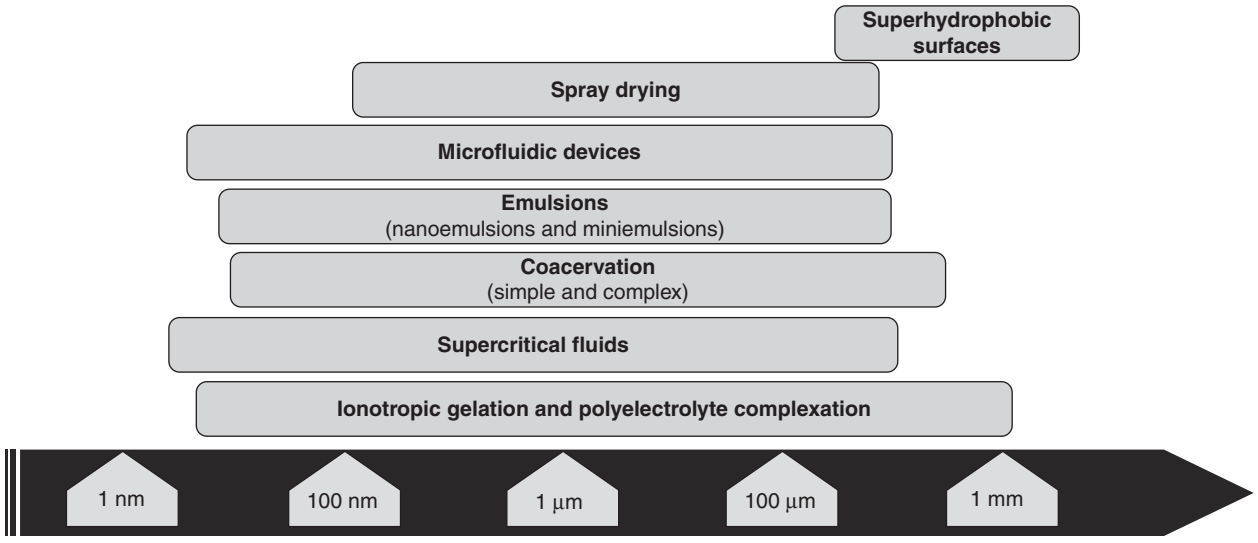


**Figure 1.** Schematic representation of the polymeric particles size range of each encapsulation methodology.



is finally removed by either evaporation or extraction as explained before. An example of application of the w/o/w, aimed to encapsulate a peptide, can be seen elsewhere [64].

Many hydrophobic and hydrophilic drugs have been encapsulated into polymeric matrices using the solvent evaporation methods mentioned above [58], including proteins [65-68]. Such macromolecules are usually very unstable and protein denaturation can easily occur during the shear and cavitation forces generated during the dispersion steps of the encapsulation processing, such as mechanical/magnetic stirring, homogenization and sonication. During the solvent evaporation step, the interfacial stress also could destroy the protein structure. The final solvent removal or drying process may also generate irreversible conformational or chemical changes in the protein structure and aggregation, due to the loss of hydrating water shell. Such issues that may lead to the loss of structural integrity of proteins, thus compromising their therapeutic efficacy, were discussed in more detail by van de Weert *et al.* [69]. Another issue is to control the release of the encapsulated drug. It was suggested that the creation of interfaces between the lipophilic and the hydrophilic/amphyphilic domains, using a lipophilic additive solubilized inside the droplets, could help in delaying the release of the active agent [70]. Another major drawback of emulsions is the environmental aspect related to the removal of the organic solvents. Sometimes high temperatures are used to remove the solvent from the particles; in these cases, temperature-sensitive polymers or bioactive molecules could be damaged. An alternative to the use of lower temperatures is the employment of a closed system which requires the increase of the pressure inside the vessel where the particles are being dried [71,72]. In any case, conventional emulsion-based methods always required multiple steps that should be desirably reduced.

There are several patents reporting small changes in emulsion-based methodologies in order to improve the process and the resultant particles characteristics. For example, patent WO/2001/035933 described a method to encapsulate vitamins, food complements oils or pharmaceuticals using an emulsion solvent removal technique with non-chlorinated solvents, which are physiologically more acceptable [73]. Moreover, special devices have been developed to obtain micro and nanoparticles in a controlled and reproducible manner using a solvent removal process. In the case of the invention presented in the patent WO/2006/082263, the apparatus contains a capillary focusing system which combines the application of hydrodynamic forces and the use of a specific geometry [74]. The system is composed of a device restrained in a pressurized chamber, which is immersed in a liquid that is a component of the external phase of the emulsion. The second liquid flows in the interior chamber. Pressure drives the flow of the second liquid to the exterior through a special nozzle. The set up permits the formation of a homogeneous emulsion with droplets with controlled size. The use of nozzles with various concentric channels results in the formation of spheres with several layers. This device may be applied in drug, cell and microorganism encapsulation, diagnosis and clinical analysis. The major advantages of this system are: i) production of very small sized particles, ii) particle size control, iii) narrow distribution size of particles, iv) reproducibility, v) no contact between internal fluid and the outlet, avoiding stress problems, vi) a large range of fluids may be used with distinct physicochemical properties (e.g., viscosity or chemical composition) and vii) continuous production of particles.

Another interesting system was described by Little *et al.* who developed a high-throughput system to produce

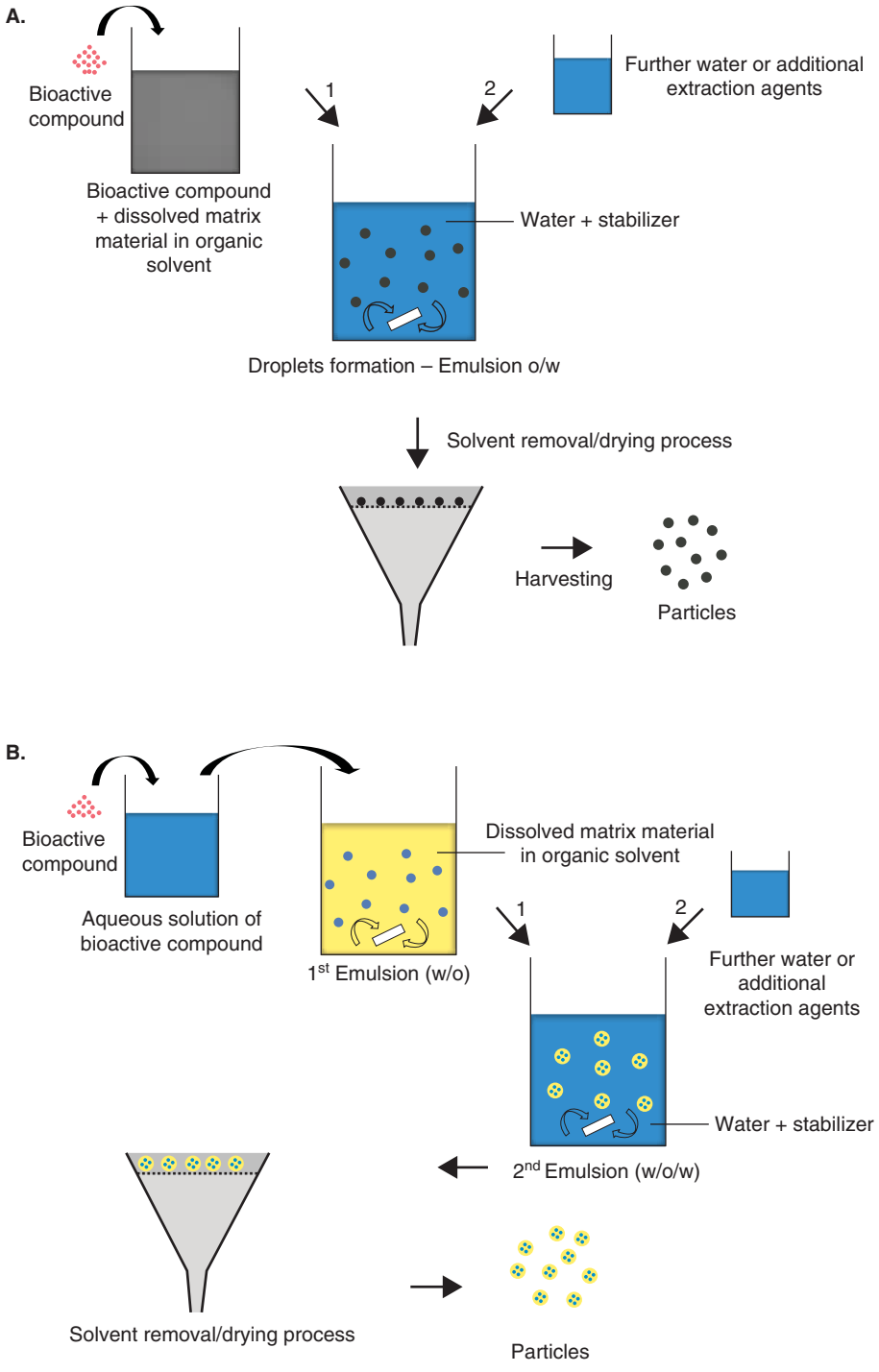


**Figure 2.** Solvent evaporation/extraction encapsulation method: single emulsion (**A**) and multiple emulsions (**B**).

different microparticles formulations simultaneously (for particle screening and optimizations) using a double emulsion/solvent evaporation method [75]. Due to the small dimensions, this system requires small amounts of reagents which allow saving costs. In addition, time is also saved because it is possible to test different material combinations.

**Table 1** presents some recent patents that use the emulsion-based methods to produce specific polymeric particles for different applications.

**Table 1. Patents that use solvent evaporation/extraction method to produce polymeric particles.**

| Patent number | Inventors | Description | Applications |
|---|---|---|---|
| WO1998032423 | Kamei *et al.* [140] | Pamoic acid microspheres for sustained release of a physiologic active peptide | Drug release |
| WO2002028371 | Jönsson *et al.* [141] | Starch microparticles with an immunologically active substance entrapped | Vaccine composition |
| WO2002092132 | Hural *et al.* [142] | Viral vector conjugated with a microparticle that increases drastically the efficacy of immune response | Vaccines |
| WO2005011741 | Hughes and Olejnik [143] | Biodegradable polymeric particles with ester prodrug entrapped to treat or prevent a disease in posterior part of a mammal eye | Drug sustained release |
| WO2008157614 | Lavik *et al.* [144] | Biodegradable polymeric microparticles (PLGA and PLA) as carriers for one or more active agents (e.g., timolol maleate) for sustained release in eye | Drug sustained release |
| WO2008075762 | Futo *et al.* [145] | Lactic acid microcapsules with physiologic active substance (peptide LHRH) entrapped. Suppression of the initial excessive release was achieved | Drug sustained release |

LHRH: Luteinizing hormone-releasing hormone; PLA: Poly(lactic acid); PLGA: Poly(lactide-co-glycolide acid.



In terms of recent research in the area of emulsions, due to the great stability of the obtained systems and the high potential in pharmaceutical applications, the physical and physicochemical fundamentals of micro and nanoemulsions have been studied [76].

Landfester [77] reviewed the ability of miniemulsions to form *in situ* complex structured polymeric nanoparticles and their capacity to encapsulate solid/liquid, inorganic/organic or hydrophobic/hydrophilic materials into polymeric shells. One example of their work is the production of degradable polyester nanoparticles (~ 200 nm) with excellent cellular uptake and drug delivery properties [78].

### 3.2 Phase separation (Coacervation)

Phase separation method is based on the decrease of the solubility of the encapsulating polymer through the addition of a non-solvent to the organic solution containing the polymer and the bioactive compound. During this process two separated liquid phases are generated: the core of the spheres and the polymer surrounding the core [24]. During coacervation processing other molecular entities may be included in the particles, such as binding components that can interact with the bioactive molecules [79]. The process happens in three basic steps [54,55] shown in **Figure 3**.

Depending of the method that induces the phase separation, coacervation techniques could be classified as: non-solvent addition, temperature change, incompatible polymer or salt addition and polymer–polymer interaction (complex coacervation when two opposite polyelectrolytes are used and simple coacervation when just a single polyelectrolyte is used) [55,80].

The coacervation technique is an aqueous method able to encapsulate water soluble and insoluble drugs with reasonable efficiency. A major drawback of this technique is the non-straightforward scale-up for the production of large quantities of particles because of the difficulty of controlling several processing parameters, such as the rate of non-solvent addition, agitation conditions and viscosity of the two organic media. Kumar *et al.* proposed an innovation to scale-up this process; the coacervation agent is added to the mixture comprising the bioactive molecule and the polymer in at least two distinct stages of the particle production [81]. In this new methodology, water soluble biologically active polypeptides may be encapsulated without loss of biological activity.

Other disadvantages of coacervation technique include: agglomeration of particles, large quantities of organic solvent required and difficulty in removing residual solvents from the final particles [24].

Some minor optimizations of phase separation methods are also described in patent literature, for example, Dardelle and Normand describe in patent WO/2007/113706 a coacervation methodology for microencapsulation by gelling warm gelatin (from fish, pork, beef and poultry) with transglutaminase at temperatures in the range of 13 – 25°C [82]. Microcapsules were obtained with an average diameter of 250 µm. The advantages of this process are that the energy required to heat or cool is minimized, and the loss of volatile reagents through evaporation and the degradation of the molecules are also reduced. Gheith *et al.* proposed a coacervation methodology using a cationic and an anionic polyelectrolyte to produce novel capsules without using crosslinking agents for encapsulation of hydrophobic molecules such as carotenoids [80]; Lee, in patent WO/2009/054841, described the process of production of thermally stable microcapsules by complex coacervation, also without crosslinking agents, for applications in food and

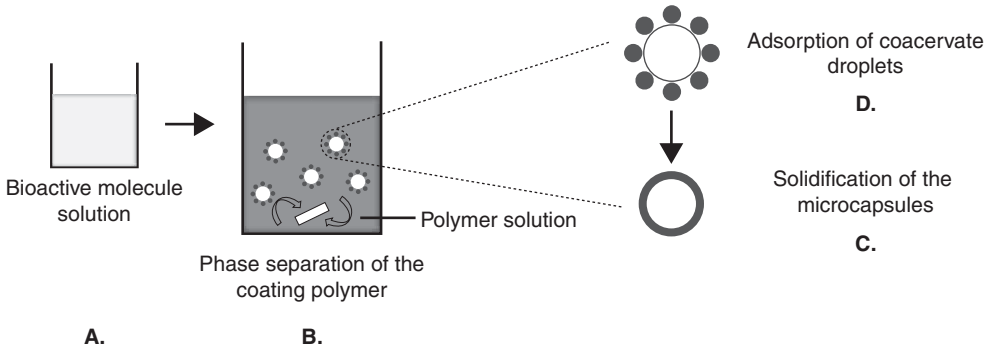


**Figure 3.** Phase separation method: a solution containing the bioactive molecule (**A**) is added to a polymeric solution (**B**) where a phase separation occurs and coats polymer form coacervate droplets onto the bioactive molecule solution (**C**). Finally, droplets solidify and a loaded particle is obtained (**D**).



pharmaceutical industry [83]; Trapani and Herbert focused their investigation in discovering a methodology to improve non-aqueous quench liquids and washing systems to reduce the amount and concentration of hardening agents used in coacervation processes [84].

**Table 2** indicates relevant patents in which the phase separation method is used to produce diverse polymeric particles.

### 3.3 Spray drying

Spray drying permits creating solid matrix spheres (using either hydrophobic or hydrophilic polymers) by spraying liquid mixtures such as solutions, emulsions or free-flowing slurries, into a hot drying medium (**Figure 4**). It is a continuous processing method that involves several operational steps, namely atomization, mixing of spray with the drying gas, evaporation and product collection [85,86].

Atomization design influences directly the particle size of the final product. Four different types of atomizers are used in most industrial applications, including rotary atomizers, pressure nozzles, two-fluid nozzles and ultrasonic atomizers [86]. More complex nozzles have been proposed. For example, Ozeki *et al.* [87] produced submicron particles of ethylcellulose and PLGA in combination with polyethylenimine using a 4-fluid nozzle. This special nozzle has two liquid and two gas passages which allow the encapsulation of two different drugs dissolved in different solvents.

Despite the easy scale-up and the high degree of reproducibility, spray drying also offers a narrow particle size distribution. However, a considerable loss of the product may occur during spray drying as the particles may adhere to the inside wall of the apparatus. Moreover, agglomeration of particles may also take place. Takada *et al.* suggested a way to overcome such problems, by using a double-nozzle spray drying technique using mannitol as an anti-adherent agent [88]; in another solution, a powdering agent is introduced during the spraying step, preventing the capsules from sticking together [89].

In this method, the particle size is difficult to control and temperature-sensitive compounds could be degraded if it is necessary for high temperatures to remove the solvents [90]. The resolution for this issue has been addressed, for example, by reducing the temperature during particle formation due to increase of the pressure inside the device where the particles are being produced [91].

The major application of the particles obtained by spray drying is pulmonary drug delivery due to the large porous particles obtained by this methodology. However, for other applications the high porosity could be a disadvantage because the release of the drugs occurs very fast. The particle morphology and drug release profile are determined by the evaporation rate and diffusion flux of the solvent and till now the drying mechanism is not completely understood [92].

In conventional spray drying systems it is necessary to consume considerable amounts of energy to dry the droplets produced. Patent WO/2010/085143 describes a new system and method for reducing the amount of energy required for spray drying [93]. The system comprises a fluid reservoir and a spraying device with at least one outflow. The droplets are projected into a determined trajectory and the energy to dry the particles is focused on the droplet trajectory.

Another innovative application of spray drying was proposed by Koch *et al.* [94] to produce a specific atmosphere inside an exposure room in order to obtain a controlled atmosphere to perform clinical tests. A liquid spray formulation comprising a solvent, a carrier and an allergen (a natural element such as plant pollen) is transformed in an aerosol and the room contained a device to generate a temporally and spatially constant allergen concentration. The effect of the amount of the allergen present in the atmosphere is then studied.

One other application of spray drying was explored by Origuchi *et al.* [95] who produced polyurethane particles coated with hydrophilic silica micropowders for cosmetic applications. Moreover, in patent WO/2010/037142, D'Souza [96] proposed a new procedure to obtain nanospheres containing bioactive material for cellular uptake. The polymer (e.g., albumin or β-cyclodextrin) is first mixed with a crosslinking agent (e.g., glutaraldehyde) and a neutralizing agent (e.g., sodium bisulfate)

**Table 2. Patents that use phase separation method to produce polymer particles.**

| Patent number | Inventors | Description | Application |
|---|---|---|---|
| WO1996032191 | Jason and Kalota [146] | Gelatin and polyaspartic acid are coacervates to create non-permeable, stable and uniformly spherical microparticles. An aldehyde (gluteraldehyde) is used as a crosslinker | Drug delivery |
| WO2005105290 | Lumsdon *et al.* [147] | Encapsulation of water insoluble oils inducing the reduction of their oxidative degradation | Agricultural and pharmaceutical applications |
| WO2006109104 | Forster [148] | Micelle production containing a new type anionic surfactant and small molecular cationic complexing agent | Isolating, stabilization and removing of substances such as proteins, lipids, dyes, radioactive substances, isolating nucleic acids and encapsulation bioactive molecules |
| WO2006072613 | Ruiz [149] | Microparticles of an insoluble polymer salt containing functional groups with ionic bonds with the active agent | Drug sustained release |
| WO2006055142 | Lai *et al.* [150] | Pharmaceutical compositions for making taste-masked microparticles and orally disintegrating tablets | Drug delivery |
| WO2006023207 | Heller [79] | Microparticles for the sustained release of therapeutic agents | Drug sustained release |
| WO2007026307 | Bouquerand *et al.* [151] | Particles with effective barriers to an oxygen-sensitive active ingredient | Oral administration systems or food products |



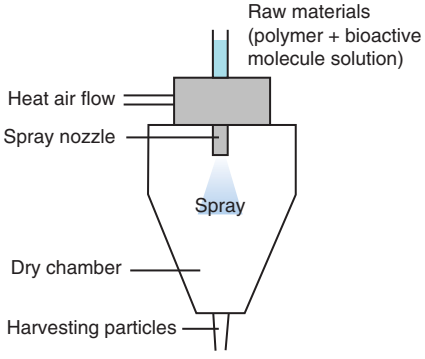


**Figure 4. Spray drying process.**

in an aqueous medium, and then complemented with the bioactive material. Finally, the mixture is sprayed and dried to create the nanospheres.

An alternative technique to spray drying is spray chilling in which the drug is dissolved or dispersed in a melted carrier without using solvent. The fluid is sprayed into a cold air stream, at a temperature lower than the melting point of the carrier, to solidify the particles [55]. Spray freeze drying is also an alternative to combat the disadvantage of the high temperatures required in spray drying process. This technology is able to maintain the biological activity of proteins and other temperature sensitive molecules. It consists of the atomization of the solution containing the polymers and the active compound directly into the freezing medium, such as cryogenic liquid nitrogen. The solvent where the polymer and the active molecule were dissolved is then removed through a sublimation process, such as liophilization. Studies [97,98] demonstrated that spray freeze drying is suitable for drug encapsulation. An application of this method – the ProLease® technology to encapsulate proteins under sterile conditions – was developed and commercialized by the company Alkermes, Inc. (USA) [99,100].

### 3.4 Ionotropic gelation and polyelectrolyte complexation

The principle of ionic gelation is the ability of polyelectrolytes to crosslink and form hydrogels in the presence of ions. This methodology has been used for cell [101] and drug encapsulation [99,102-104]. Examples of polymers with these characteristics are alginate [51,105,106], chitosan [107], dextran [108], carrageenan [109], gellan gum [110] and poly-di(carboxylatophenoxy)phosphazene [39]. The introduction of stimuli-responsive polymers in such formulations, such as poly(N-isopropylacrylamide), can lead to smart semi-interpenetrated networks beads [111,112].

In general, micro/nanospheres are produced by dropping a polymeric solution containing cells or bioactive molecules into an aqueous bath containing specific ions able to crosslink

the polymers. The ions diffuse into the drug loaded drops allowing the crosslinking and forming a 3D network hydrogel. When using syringe needles, the size of the particles generated is relatively high; however, smaller droplets can be formed using a system of air atomization (**Figure 5**). Zhao *et al.* [113] used an electrodispersion reactor, where electric fields are used to atomize the sodium alginate solution, to prepare calcium alginate microgels for pharmaceuticals encapsulation. The mild processing conditions used in ionotropic gelation (without use of organic solvents or high temperatures) could maintain cell viability and protein integrity, constituting an advantage compared with other methods such as emulsions, spray drying or coacervation. One of the major disadvantages of this technique is the loss of drug during particle production. Also, some crosslinking agents could react with specific groups establishing bonds between the polymer and the bioactive molecule inhibiting or delaying the molecule release.

Patent WO/2005/071060 proposes a novel system for cell encapsulation [114]. The device is constituted by two chambers separated by a plate with several apertures. The first chamber contains a cell suspension in a polymeric solution and the second one contains a solution with the crosslinking agent. A pressure is applied in the cell suspension forcing the passage through to the apertures of the plate and its collection in the second chamber in the form of particles. As examples related to the use of ionic gelation to obtain particles for drug release, Park *et al.* [115] developed chitosan capsules crosslinked in a phytic acid bath and Reis *et al.* [116] performed a combination between ionic gelation and emulsions methods to produce polymeric particles. The last case demonstrated that the combination of distinct established encapsulation methodologies may overcome the drawbacks of the processes when used alone.

In order to improve some characteristics of the hydrogel produced, namely the mechanical strength and the permeability of the particle, other polyelectrolytes may be added to the particles obtained by ionotropic gelation. For example, chitosan and negatively charged biopolymers, such as chondroitin sulfate, heparin, dextran sulfate or carragenan, have been used to prepare particulate systems that could be suitable for drug delivery applications [117,118].

The sequential deposition of polyelectrolytes with opposite charges using the layer-by-layer methodology allows the formation of membranes or layers surrounding the core of the particle. The core could be liquefied or removed and capsules capable of controlling the release of the entrapped molecules are obtained [55,119]. Costa *et al.* [120] proposed a liquefied LbL system (capsules with 2 mm) composed of alginate and chitosan for cell encapsulation. For applications in therapeutic delivery, Becker *et al.* [121] reviewed the most relevant LbL 3D systems able to encapsulate small molecules, DNA and proteins.

### 3.5 SCF precipitation

SCFs have been used in several biomedical technologies [122], including the development of particles for bioactive molecules delivery. The most used principle is based on the fact that rapid expansion of SCFs causes precipitation of solutes dissolved therein [123].

Depending on the materials, the molecules to be encapsulated and the final product characteristics, SCF could be used as a solvent, solute, anti-solvent and aerosolization aid [124].

The most common methods using SCF for encapsulation are the rapid expansion of supercritical solutions (RESS), gas anti-solvent (GAS) and aerosol solvent extraction system (ASES). In RESS, the drug and the polymer are dissolved in an SCF (usually $CO_2$) at high pressure and after a rapid decompression (expansion) precipitation occurs. Also, after expansion the SCF becomes a gas which means that the solid product in a pure form is recovered, without any trace of solvent. In the GAS process, the solutes (polymer and bioactive molecule) are dissolved in a suitable organic phase. The solution is pressurized and the SCF, acting as the anti-solvent fluid, enters into the solution precipitating the solutes. The pressure is then decreased (expansion) and the solid product is collected. This method is adequate when it is difficult to dissolve polymers or bioactive molecules (such as peptides or proteins) in SCFs [55,125]. Recently, Garay *et al.* [126] used GAS to obtain Eudragit EPO® (an acrylate–methacrylate co-polymer) particles with 2 – 12 μm suitable for bioactive molecule entrapment. In the ASES method, a solution containing the solutes is dispersed by a spray device and mixed with the SCF (the anti-solvent) promoting the precipitation of the solute. Kunastitchai *et al.* [127] showed that this method could be used for the preparation of liposomes for drug release applications.

The use of SCFs instead of organic solvents presents several advantages. For example, the most common SCF used is $CO_2$ which is a non-toxic, environmentally acceptable, non-flammable and inexpensive product. The process does not require high temperatures, has potential to be scaled up and it is possible to apply it in aseptic conditions. The drawbacks of these processes are the difficult dissolution of some polymers and drugs in SCF and the control of the morphology of the obtained particles may not straightforward [52].

Patent WO/1998/015348 [52] describes a microencapsulation method in mild conditions using an SCF neither requiring polymers nor bioactive molecules soluble in SCFs. In this innovation, the bioactive compound has to be mixed with an encapsulating polymer and the SCF is supplied to the resulting mixture. The SCF has to be capable of inducing the swelling of the polymer, penetrating and liquefying the polymer under temperature and pressure conditions able to maintain the fluid in a supercritical state. Finally, the rapid decreasing of the pressure induces the solidification of the polymer around the core material. In patent WO/2009/016677, Reverchon and Porta [128] described a method to produce continuously micro and nanoparticles. Supercritical $CO_2$ or other similar solvent contact with emulsions (o/w, w/o, multiple emulsions) forming a solution with the dispersed phase of

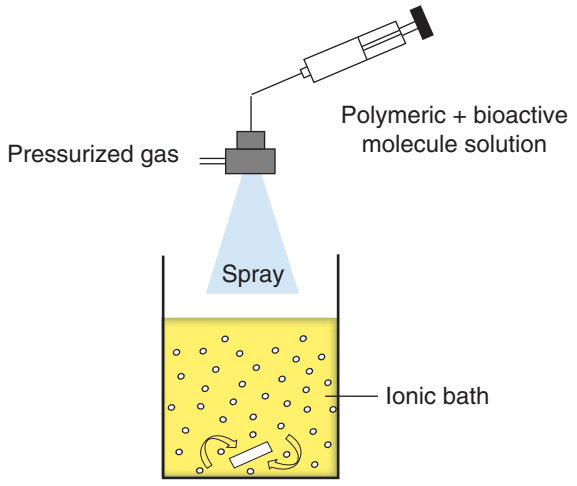


**Figure 5. Ionotropic gelation method using an air atomization system.**

the emulsion and it induces the formation of the desired particles. The process is carried out in a counter-current packed column wherein the expanded emulsion is fed from the top and the expanded liquid is fed from the bottom. The expanded liquid avoids the solid particle deposition on the apparatus walls and a suspension of structured particles is collected continuously at the bottom of the column.

Tu *et al.* [129] presented a method to produce particles with two or more pharmaceutical active compounds. The first bioactive compound was suspended in a medium miscible with SCF. After this, a solution with the second bioactive compound was prepared in a solvent also miscible with SCF. After mixing both, the precipitation of the second compound in the presence of the first is induced by combining the SCF of the suspension and the solution. The SCF acts as an antisolvent dispersing and extracting the suspension medium and the solvent.

### 3.6 Microfluidic devices

Some encapsulation methods require harmful procedures that could damage cells or cause loss of bioactivity of some molecules. The use of microfluidic devices may help in developing simple, continuous, uniform, cell-friendly and less contaminated systems capable of encapsulating cells and molecules in mild conditions. In most of the cases, these devices have distinct inlets, one for continuous liquids and other for polymeric solutions where drugs or cells are dissolved or suspended. The operating principle is based on the use of a pulsed airflow to conduct the polymeric solution through microchannels forming microbeads due to the presence of two immiscible phases (emulsions principle). The particles are delivered out of the system through a microcapillary tube [130]. The formed particles could after be solidified using UV light, solvent evaporation, chemical reactions or ionic gelation [131]. Similar to the technologies described above, a few parameters have to be controlled in order to obtain particles with desired morphologies and sizes, including the flow rates of the liquids and the reaction time [132].

Microfluidic devices with distinct designs have been developed to produce continuously beads, at micro scale, for cell encapsulation applications [130-134]. Variations of these methodologies may be used to produce more complex particles, including Janus and ternary microparticles with narrow size distribution [135]. Due to the compartmentalization of the obtained particles, this microfluidic system has great potential to innovate drug delivery systems.

Patent WO/2004/071638 [136] describes microfluidic systems able to control the viscous shear forces of the o/w imiscible liquids. This caracteristic provides optimal conditions to produce continuously amphiphilic vesicles, namely lipossomes, polymer vesicles or micelles both at micro and nano scales. Droplet formation speed, droplet size ranges, multi-lamellar amphiphilic layers and asymmetic visicles are parameters that may be controled varying the design of the device.

Patent WO/2009/015296 [137] describes a high-throughput microfluidic device with a specific design and constituted by 3-valve pump, able to control with precision the size of the droplets obtained by an emulsion process. The pulsatile flow profile of the microfabricated pumps provides the control over droplet generation. In addition, DNA/RNA molecules or cells are easily encapsulated due to the use of biological compatible oils.

### 3.7 Superhydrophobic surfaces

As seen before, in most technologies used to fabricate spherical particles the initial liquid droplets harden into a hydrogel or solid form while immersed in another insoluble liquid substrate, during which a fraction of the molecules that are initially in the liquid phase may be lost. Then, in these 'wet' methods, the encapsulation of proteins or other molecules will be never totally efficient and some molecules cannot maintain the biological activity. One other drawback of most of the presented processes is the use of organic liquids that are required in several steps, which limit the possibility of encapsulating living cells or microorganisms.

In order to overcome the disadvantages of these encapsulation methods, Song *et al.* [138] presented a method to prepare spherical hydrogel or polymeric particles with high encapsulation efficiency. In this fabrication process, using superhydrophobic substrates, spheres are produced involving basically liquid–air interfaces which mean that spheres harden in a dry environment (**Figure 6**). This environment-friendly method shows interesting characteristics because it is easy, presents high encapsulation efficiency, shows absence of mechanical forces, and has low production costs and scale-up possibility. It was shown that this methodology could be used for cell encapsulation [138] and also for encapsulation of proteins in stimuli-responsive matrices [139]. Until now, just mili and micro sized particles were produced using this

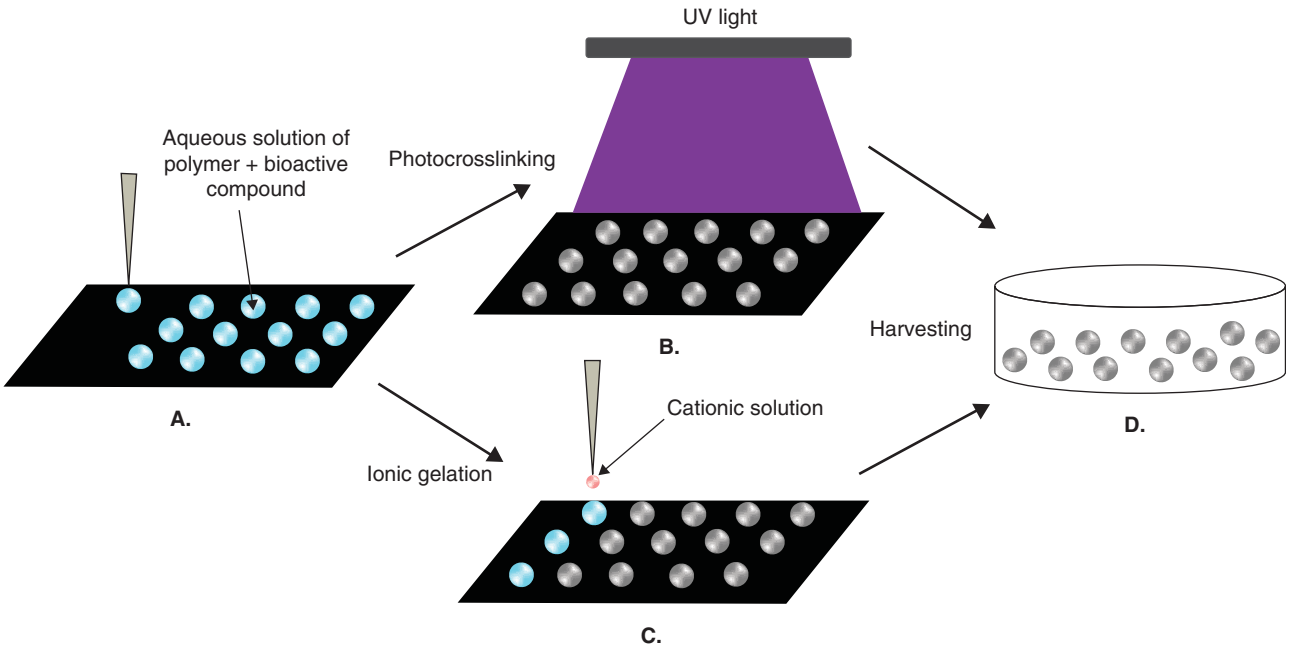


**Figure 6.** Production of polymeric particles using superhydrophobic substrates: droplets of an aqueous polymeric solution containing a bioactive compound are dropped on superhydrophobic surfaces (**A**). The particles harden in a dry environment through a photocrosslinking process (**B**) or ionic gelation (**C**). Afterwards, they are easily harvested (**D**) to be applied or dried and stored.



technology. The use of spraying devices to deposit small droplets into the superhydrophobic surfaces could allow production of particles with smaller sizes.

## 4. Conclusion

The use of polymeric and hydrogel drug delivery systems constitutes an interesting approach to improve the efficiency of the developed drugs when required for therapeutic or disease prevention. The encapsulation allows the increase of active agents' stability due to the protection against the environment as also their sustained and controlled release in a target site.

This overview made regarding the most important methodologies for encapsulating active agents and cells or microorganisms shows that the selected technique used to perform the encapsulation may influence the performance of delivery systems. Moreover, the patents reviewed permitted having a better perception of the technological developments made to improve the existing methodologies. It is clear that most of the efforts have focused on improving the existing processes in order to overcome the disadvantages of the conventional techniques making the processes more efficient and consequently saving the costs of production to obtain more efficient delivery systems. We believe that completely new strategies should also be explored such as the last example presented, in which the critical parameter should include the scalability and the economical viability in the case of industrial implementation.

## 5. Expert opinion

Drug delivery systems have an important role in disease treatment or prevention, constituting an important area where recently research is highly focused. Research, development and the sales of drug delivery systems are increasing in a rapid way. Even taking into account the increased demand for new healthcare treatments to satisfy worldwide necessities, existing drugs have been commercialized and will continue being formulated in order to achieve an optimal molecular activity. The major driving force of this area has been focused on the search for drug encapsulated systems with high efficiency and ability to target the release of the drug to specific sites. To achieve this, an intensive research to develop new drugs and also to use new materials is ongoing. Furthermore, more complex systems composed by micro and nanoparticles for multiple drug release with molecules to target the delivery of the active compound in a desired site or to a specific type of cells/organ have been studied.

The most widely used applications of particulate delivery systems are focused in oral, nasal, injectable and topical applications in order to enhance a sustained and controlled release of the encapsulated compound.

Depending on the drug, encapsulation material and final application of the system, the appropriate encapsulation methodologies may be selected. The encapsulation enhances the material stability, extends the release of the drug and also reduces its adverse toxic effects. Focusing on the most used encapsulation methodologies in food and pharmaceutical industry, such as spray drying, emulsions and coacervation (previously described), some major disadvantages are found and addressed in this review. In a significant part of these methodologies, aggressive environments are required during intermediate steps, including extreme temperatures or pressure and direct contact with organic solvents, which affect the stability of the bioactive compounds or even the biological activity of encapsulated cells or microorganisms. Until now, there is no single encapsulation technique able to encapsulate all the drugs loaded in the process without some loss of material. This is the reason for the continuous search for improvements of the existing techniques and also to develop new ones based on innovative concepts.

The materials to be used in the fabrication of particles should be also not limited by the traditional synthetic polymers currently used. For example, marine origin macromolecules, such as chitosan or alginate, are very promising polymers obtained from renewable sources able to be chemically modified and processed by different techniques.

Natural polymers constitute a very interesting class of materials due to their low/non-cytotoxicity, low immunogenicity, good biocompatibility and easy availability from renewable sources. The design, synthesis of new hydrogels or other polymers and search for new applications are important issues in polymeric science research applied to biomedicine [40].

Modifications of hydrogel properties are important in some aspects of delivery systems mechanisms: permeability (sustained release), environment responsive nature (pulsatile release), surface functionality (e.g., PEG coatings for stealth release), biodegradability (bioresorbable applications) and surface biorecognition sites (target release and bioadhesion applications). However, improvements at the chemical architecture point of view should be made together with technological developments related to particle design and processing, for example, particles able to release multiple molecules with distinct release profiles should possess a special compartmental organization that requires the use of non-conventional fabrication techniques. The precise control of the release kinetics should also be possible with the production of more complex structures, such as hierarchical or property gradient systems. In this context, the involvement of a multidisciplinary field, namely, materials science, engineering, nanotechnology, chemistry, health sciences and pharmaceutics, is crucial.

Beyond bioactive compounds encapsulated in polymeric particles, cells and genes have been studied as potential participants in therapeutic applications. In this field, more restricted regulatory issues should be considered and the processing technologies to be used must also be more specific to accommodate the use of biomedical materials.

In the future, an improvement of the existing delivery systems and the development of innovative systems are expected: more accurate, more efficient and obtained through better methodologies. The main aim of this review paper is to draw attention to the high number of innovations in encapsulation technologies described in patents and research papers. Nevertheless, in most of the cases, it remains difficult to implement all the ideal properties of drug delivery systems in a single processing methodology. Therefore, strategies could also include the combination of different techniques and the use of different materials.

## Declaration of interest

The authors declare no conflict of interest. The authors are grateful for financial support from Portuguese Foundation for Science and Technology (FCT) through the grants SFRH/BD/71395/2010 and SFRH/BPD/489948/2008.

## Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


1. Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 2001;9:321-39

•• **A comprehensive review regarding the potencial of thermo, pH, glucose, electric, pressure, ionic, and other responsive hydrogels for applications in drug delivery.**

2. Kim S, Kim JH, Jeon O, et al. Engineered polymers for advanced drug delivery. Eur J Pharm Biopharm 2009;71:420-30

3. Wang W, Liu XD, Xie YB, et al. Microencapsulation using natural polysaccharides for drug delivery and cell implantation. J Mater Chem 2006;16:3252-67

4. Davis SS, Illum L. Polymeric microspheres as drug carriers. Biomaterials 1988;9:111-15

• **A discussion about the role of microspheres, their properties and effects when administered through a parental route.**

5. Okada H, Toguchi H. Biodegradable microspheres in drug-delivery. Crit Rev Ther Drug Carrier Syst 1995;12:1-99

6. Varde NK, Pack DW. Microspheres for controlled release drug delivery. Expert Opin Biol Ther 2004;4:35-51

7. Freiberg S, Zhu X. Polymer microspheres for controlled drug release. Int J Pharm 2004;282:1-18

8. Langer R. Polymer-controlled drug delivery systems. Acc Chem Res 1993;26:537-42

•• **An overview of the main drug release mechanisms from polymeric matrices.**

9. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 2001;53:283-318

•• **A very good review focusing important aspects related to the long circulation and targeting of polymeric nanoparticles.**

10. Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. J Pharm Sci 2008;97:71-87

• **An overview through polyester micro and nanoparticle properties for controled drug release.**

11. Mano JF. Stimuli-responsive polymeric systems for biomedical applications. Adv Eng Mater 2008;10:515-27

• **An interesting review about smart polymeric devices for biomedical applications, namely, delivery of therapeutic molecules and actuators for tissue engineering and regenerative medicine.**

12. Kent TB, Johnson J, Rudge SR. Magnetically guided particles for radiative therapies. WO2004034876A2; 2004

13. Tapolsky GH, Li Y, Jiao Y. Magnetically targetable particles comprising magnetic components and biocompatible polymers for site-specific delivery of biologically active agents. WO2005025508; 2005

14. Mandal TK, Praetorius NP. Engineered nanoparticles in cancer therapy. Recent Pat Drug Deliv Formul 2007;1:37-51

• **An overview of US and World patents in development and modification of nanoparticles for cancer detection, analysis and treatment.**

15. Alley SC, Okeley NM, Senter PD. Antibody-drug conjugates: targeted drug delivery for cancer. Curr Opin Chem Biol 2010;14:529-37

16. Shubayev VI, Pisanic TR II, Jin S. Magnetic nanoparticles for theragnostics. Adv Drug Deliv Rev 2009;61:467-77

17. Allegra A, Penna G, Alonci A, et al. Nanoparticles in Oncology: the new theragnostic. Molecules. Anticancer Agents Med Chem 2011;11:669-86

•• **A very complete review about nanoparticle applications in drug delivery, namely gene therapy, their action on the immune system and apoptosis and their ability to detect and characterize circulating tumor cells.**

18. Pillai O, Panchagnula R. Polymers in drug delivery. Curr Opin Chem Biol 2001;5:447-51

19. Bravo SA, Lamas MC, Salomon CJ. In-vitro studies of Diclofenac Sodium controlled-release from biopolymeric hydrophilic matrices. J Pharm Pharm Sci 2002;5:213-19

20. Yang LB, Fassihi R. Examination of drug solubility, polymer types, hydrodynamics and loading dose on drug release behavior from a triple-layer asymmetric configuration delivery system. Int J Pharm 1997;155:219-29

21. Davis SS, Illum L, Stolnik S. Polymers in drug delivery. Curr Opin Colloid Interface Sci 1996;1:660-6

22. Heller J. Controlled release of biologically-active compounds from bioerodible polymers. Biomaterials 1980;1:51-7

23. Jalil R, Nixon JR. Biodegradable poly (lactic acid) and poly(lactide-co-glycolide) microcapsules - problems associated with preparative techniques and release properties. J Microencapsul 1990;7:297-325

24. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 2000;21:2475-90

25. Ranade VV, Hollinger MA. Drug Delivery Systems. Florida: CRC Press, 2004

26. Ibim SM, Uhrich KE, Bronson R, et al. Poly(anhydride-co-imides): in vivo biocompatibility in a rat model. Biomaterials 1998;19:941-51

27. Mathiowitz E, Jacob JS, Jong YS, et al. Biologically erodable microsphere as potential oral drug delivery system. Nature 1997;386:410-14

28. Bala I, Hariharan S, Kumar M. PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst 2004;21:387-422

29. Langer R. Selected advances in drug delivery and tissue engineering. J Control Release 1999;62:7-11

30. Mano JF, Silva GA, Azevedo HS, et al. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. J R Soc Interface 2007;4:999-1030

31. Thomson RC, Wake MC, Yaszemski MJ, et al. Biodegradable polymer scaffolds to regenerate organs. Adv Polym Sci 1995;122:254-74

32. Johnston APR, Cortez C, Angelatos AS, et al. Layer-by-layer engineered capsules

and their applications. Curr Opin Colloid Interface Sci 2006;11:204-9

• **An overview focusing on the potential of LbL capsules as drug delivery vehicles, methods for encapsulation and release and targeting applications.**

33. De Geest BG, Sanders NN, Sukhorukov GB, et al. Release mechanisms for polyelectrolyte capsules. Chem Soc Rev 2007;36:636-49

34. Deluca P, Jiang G, Woo B. Poly (acryloyl-hydroxyethyl starch)-plga composite microspheres. WO2004084819; 2004

35. Champion JA, Mitragotri S, Tezel A. Coated hyaluronic acid particles. WO2008147817; 2008

36. Sprincl L, Kopecek J, Vacik J, et al. Biological tolerance of poly(N-substituted methacrylamides). J Biomed Mater Res 1971;5:197-205

37. Sprincl L, Vacik J, Kopecek J. Biological tolerance of ionogenic hydrophilic gels. J Biomed Mater Res 1973;7:123-36

38. Wang C, Lau TT, Loh WL, et al. Cytocompatibility study of a natural biomaterial crosslinker–genipin with therapeutic model cells. J Biomed Mater Res B Appl Biomater 2011;97:58-65

39. Hennink WE, van Nostrum CF. Novel crosslinking methods to design hydrogels. Adv Drug Deliv Rev 2002;54:13-36

•• **A very important paper that revised the hydrogel crosslinking methods.**

40. Peppas NA, Hilt JZ, Khademhosseini A, et al. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Adv Mater 2006;18:1345-60

41. Gupta P, Vermani K, Garg S. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today 2002;7:569-79

42. Ahn SK, Kasi RM, Kim SC, et al. Stimuli-responsive polymer gels. Soft Matter 2008;4:1151-7

43. Bajpai AK, Shukla SK, Bhanu S, et al. Responsive polymers in controlled drug delivery. Prog Polym Sci 2008;33:1088-118

44. Song F, Zhang LM, Shi JF, et al. Novel casein hydrogels: Formation, structure and controlled drug release. Colloids Surf B Biointerfaces 2010;79:142-8

45. Oh JK, Drumright R, Siegwart DJ, et al. The development of microgels/nanogels for drug delivery applications. Prog Polym Sci 2008;33:448-77

46. Kost J, Langer R. Responsive polymeric delivery systems. Adv Drug Deliv Rev 2001;46:125-48

47. Langer R. Drug delivery systems. MRS Bull 1991;16:47-9

48. Silva GA, Ducheyne P, Reis RL. Materials in particulate form for tissue engineering. 1. Basic concepts. J Tissue Eng Regen Med 2007;1:4-24

•• **A very good review article that covers the important characteristics of micro and nanoparticles for tissue engineering and drug delivery applications.**

49. Soppimath KS, Aminabhavi TM, Kulkarni AR, et al. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 2001;70:1-20

50. Ryan SM, Mantovani G, Wang X, et al. Advances in PEGylation of important biotech molecules: delivery aspects. Expert Opin Drug Deliv 2008;5:371-83

•• **A paper focusing on the importance of PEGylation and its most important advances.**

51. Liu Z, Jiao Y, Wang Y, et al. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 2008;60:1650-62

52. Shine AD, Gelb JJ. Microencapsulation process using supercritical fluids. WO1998015348; 1998

53. Obeidat WM. Recent patents review in microencapsulation of pharmaceuticals using the emulsion solvent removal methods. Recent Pat Drug Deliv Formul 2009;3:14

•• **A very interesting review paper focused on patents related to emulsion solvent removal methods.**

54. Bansode SS, Banarjee SK, Gaikwad DD, et al. Microencapsulation : a review. Int J Pharm Sci Rev Res 2010;1:38-43

•• **General overview regarding microencapsulation methods.**

55. Yeo Y, Baek N, Park K. Microencapsulation methods for delivery of protein drugs. Biotechnol Bioprocess Eng 2001;6:213-30

•• **A general overview of the existing methods for proteins encapsulation.**

56. Oliveira MB, Mano JF. Polymer-based microparticles in tissue engineering and regenerative medicine. Biotechnol Prog 2011;27:897-912

• **A review paper focusing on the production and application of polymer microparticles in tissue engineering and regenerative medicine.**

57. Cleland JL. Solvent evaporation processes for the production of controlled release biodegradable microsphere formulations for therapeutics and vaccines. Biotechnol Prog 1998;14:102-7

58. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: state of the art for process engineering approaches. Int J Pharm 2008;363:26-39

• **State of the art of microencapsulation using solvent evaporation methods.**

59. Vuaridel E, Orsolini P. Microparticles of biodegradable polymer encapsulating a biologically active substance. WO2002058672; 2002

60. Laakso T, Reslow M. Encapsulation method. US6861064; 2005

61. Walstra P. Principles of emulsion formation. Chem Eng Sci 1993;48:333-49

62. Kino S, Osajima T, Mizuta H. Sustained release microsphere preparation containing antipsychotic drug. US5871778; 1999

63. Mandal TK, Graves R. An improved method of micro-encapsulation. WO2008054659; 2008

64. Yoshimoto T, Tajima M, Watabe K. Delayed drug-releasing microspheres. US5993855; 1996

65. Meinel L, Illi OE, Zapf J, et al. Stabilizing insulin-like growth factor-I in poly(D,L-lactide-co-glycolide) microspheres. J Control Release 2001;70:193-202

66. Diwan M, Park TG. Pegylation enhances protein stability during encapsulation in PLGA microspheres. J Control Release 2001;73:233-44

67. Dai CY, Wang BC, Zhao HW. Microencapsulation peptide and protein drugs delivery system. Colloid Surf B 2005;41:117-20

68. Furtado S, Abramson D, Simhkay L, et al. Subcutaneous delivery of insulin loaded poly(fumaric-co-sebacic anhydride) microspheres to type 1 diabetic rats. Eur J Pharm Biopharm 2006;63:229-36

69. van de Weert M, Hennink WE, Jiskoot W. Protein instability in poly (lactic-co-glycolic acid) microparticles. Pharm Res 2000;17:1159-67

70. Phan VA, Godinot N, Sagalowicz L, et al. Oil-in-water emulsion and its use for the delayed release of active elements. WO2008145744; 2008

71. Masao K, Tsutomu Y, Asaji K, et al. Encapsulation method. US3691090; 1972

72. Takehiko S, Yasuhisa M, Akira S. Method for preparing microsphere. US7323039; 2008

73. Babtsov V, Shapiro Y, Kvitnitsky E. A method of microencapsulation. WO2001035933; 2001

74. Calvo AMG, Banderas LM, Mosquera MF, et al. Method and device for obtaining micro and nanometric size particles. WO2006082263; 2006

75. Little SR, Anderson DG, Langer RS. High-throughput fabrication of microparticles. WO2007078765; 2007

76. Anton N, Vandamme TF. Nano-emulsions and Micro-emulsions: clarifications of the critical differences. Pharm Res 2011;28:978-85

77. Landfester K. Miniemulsion polymerization and the structure of polymer and hybrid nanoparticles. Angew Chem Int Ed 2009;48:4488-507

• **Description of the miniemulsion process for fabricating polymeric nanoparticles.**

78. Siebert JM, Baumann D, Zeller A, et al. Synthesis of polyester nanoparticles in miniemulsion obtained by radical ring-opening of BMDO and their potential as biodegradable drug carriers. Macromol Biosci 2011;published online 15 November 2011; doi: 10.1002/mabi.201100236

79. Heller PF. Coacervate of anionic and cationic polymer forming microparticles for the sustained release of therapeutic agents. WO2006023207; 2006

80. Gheith KM, Fang Y-L, Wong MS. Method for nanoencapsulation. WO2008063158; 2008

81. Kumar R, Troiano G, Ramstack JM, et al. Coacervation process. WO2008118712; 2008

82. Dardelle G, Normand V. Method for preparing microcapsules by coacervation. WO2007113706; 2007

83. Lee PK. Heat stable microcapsules and methods for making and using the same. WO2009054841; 2009

84. Trapani K, Herbert P. Quench liquids and washing systems for production of microparticles. WO2008157540; 2008

85. Re MI. Formulating drug delivery systems by spray drying. Dry Technol 2006;24:433-46

86. Vehring R. Pharmaceutical particle engineering via spray drying. Pharm Res 2008;25:999-1022

• **A review covering recent developments in the area of particle engineering via spray drying.**

87. Ozeki T, Beppu S, Mizoe T, et al. Preparation of polymeric submicron particle-containing microparticles using a 4-fluid nozzle spray drier. Pharm Res 2006;23:177-83

88. Takada S, Uda Y, Toguchi H, et al. Application of a spray drying technique in the production of TRH-containing injectable sustained-release microparticles of biodegradable polymers. PDA J Pharm Sci Technol 1995;49:180-4

89. Trophardy G. One step spray-drying process. WO2007135583; 2007

90. Johansen P, Merkle HP, Gander B. Technological considerations related to the up-scaling of protein microencapsulation by spray-drying. Eur J Pharm Biopharm 2000;50:413-17

91. Gombotz WR, Healy MS, Brown LR. Very low temperature casting of controlled release microspheres. WO199013780; 1990

92. Rizi K, Green RJ, Donaldson M, et al. Production of pH-responsive microparticles by spray drying: Investigation of experimental parameter effects on morphological and release properties. J Pharm Sci 2011;100:566-79

93. Houben RJ, Browers LAM, Oosterhuis G, et al. Effective droplet drying. WO2010085143; 2010

94. Koch W, Holfeld J, Krug N, et al. Method for producing an inhalation atmosphere for provoking allergens. WO2010063714; 2010

95. Origuchi T, Nakayama Y, Kurahashu T, et al. Cosmetic. WO2010082530; 2010

96. D'Souza MJ. Nanospheres encapsulating bioactive material and method for formulation of nanospheres. WO2010037142; 2010

97. Kondo M, Niwa T, Okamoto H, et al. Particle characterization of poorly water-soluble drugs using a spray freeze drying technique. Chem Pharm Bull 2009;57:657-62

98. Costantino HR, Firouzabadian L, Wu C, et al. Protein spray freeze drying. 2. Effect of formulation variables on particle size and stability. J Pharm Sci 2002;91:388-95

99. Gombotz WR, Wee S. Protein release from alginate matrices. Adv Drug Deliv Rev 1998;31:267-85

100. Tracy MA. Development and scale-up of a microsphere protein delivery system. Biotechnol Prog 1998;14:108-15

101. Sakai S, Ito S, Kawakami K. Calcium alginate microcapsules with spherical liquid cores templated by gelatin microparticles for mass production of multicellular spheroids. Acta Biomater 2010;6:3132-7

102. Dudhani AR, Kosaraju SL. Bioadhesive chitosan nanoparticles: preparation and characterization. Carbohydr Polym 2010;81:243-51

103. Ko JA, Park HJ, Hwang SJ, et al. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. Int J Pharm 2002;249:165-74

104. Gonzalez-Rodriguez ML, Holgado MA, Sanchez-Lafuente C, et al. Alginate/chitosan particulate systems for sodium diclofenac release. Int J Pharm 2002;232:225-34

105 Kwon S-Y, Kochinke F. Alginate particle formulation. WO2002019989; 2002

106. Melvik JE, Gaserod O. Hydrogels containing low molecular weight alginates and biostructures made therefrom. WO2008006658; 2008

107. Janes KA, Calvo P, Alonso MJ. Polysaccharide colloidal particles as delivery systems for macromolecules. Adv Drug Deliv Rev 2001;47:83-97

108. Van Tomme SR, van Steenbergen MJ, De Smedt SC, et al. Self-gelling hydrogels based on oppositely charged dextran microspheres. Biomaterials 2005;26:2129-35

109. Santo VE, Frias AM, Carida M, et al. Carrageenan-based hydrogels for the controlled delivery of PDGF-BB in bone tissue engineering applications. Biomacromolecules 2009;10:1392-401

110. Pereira DR, Silva-Correia J, Caridade SG, et al. Development of gellan gum-based microparticles/hydrogel matrices for application in the intervertebral disc regeneration. Tissue Eng Part C Methods 2011;17:961-72

111. Shi J, Alves NM, Mano JF. Drug release of pH/temperature-responsive calcium alginate/poly(N-isopropylacrylamide) semi-IPN beads. Macromol Biosci 2006;6:358-63

112. Shi J, Alves NM, Mano JF. Chitosan coated alginate beads containing poly(N-isopropylacrylamide) for dual-stimuli-responsive drug release. J Biomed Mater Res B Appl Biomater 2008;84B:595-603

113. Zhao Y, Carvajal MT, Won Y-Y, et al. Preparation of calcium alginate microgel beads in an electrodispersion reactor using an internal source of calcium carbonate nanoparticles. Langmuir 2007;23:12489-96

114. Ramasubramanian MK. Methods and devices for microencapsulation of cells. WO2005071060; 2005

115. Park JY, Jeong CM, Lee MK, et al. Sustained-release chitosan capsules comprising chitosan and phytic acid. WO2008156250; 2008

116. Reis ACBP, Veiga FJB, Ribeiro AJ, et al. Production and recovery of polymeric micro- and nanoparticles containing bioactive macromolecules. WO2007129926; 2007

117. Kauper P, Laue C. Chitosan-based particles. WO2007031812; 2007

118. Grenha A, Gomes ME, Rodrigues M, et al. Development of new chitosan/carrageenan nanoparticles for drug delivery applications. J Biomed Mater Res A 2010;92A:1265-72

119. Wohl BM, Engbersen JF. Responsive layer-by-layer materials for drug delivery. J Control Rel 2011;published online 6 September 2011; doi: 10.1016/j.jconrel.2011.08.035

120. Costa NL, Sher P, Mano JF. Liquefied capsules coated with multilayered polyelectrolyte films for cell immobilization. Adv Eng Mater 2011;13:B218-24

121. Becker AL, Johnston APR, Caruso F. Layer-by-layer-assembled capsules and films for therapeutic delivery. Small 2010;6:1836-52

122. Duarte ARC, Mano JF, Reis RL. Supercritical fluids in biomedical and tissue engineering applications: a review. Int Mater Rev 2009;54:214-22

• **A comprehensive review that covers the biomedical and tissue engineering applications of supercritical fluids.**

123. Smith RD. Supercritical fluid molecular spray film deposition and powder formation. US4582731; 1986

124. Tandya A, Mammucari R, Dehghani F, et al. Dense gas processing of polymeric controlled release formulations. Int J Pharm 2007;328:1-11

125. Reverchon E, Adami R, Caputo G, et al. Spherical microparticles production by supercritical antisolvent precipitation: interpretation of results. J Supercrit Fluids 2008;47:70-84

126. Garay I, Pocheville A, Madariaga L. Polymeric microparticles prepared by supercritical antisolvent precipitation. Powder Technol 2010;197:211-17

127. Kunastitchai S, Pichert L, Sarisuta N, et al. Application of aerosol solvent extraction system (ASES) process for preparation of liposomes in a dry and reconstitutable form. Int J Pharm 2006;316:93-101

128. Reverchon E, Porta GD. Continuous process for microspheres production by using expanded fluids. WO2009016677; 2009

129. Tu LS, Regtop HL, Foster NR, et al. Method of particle formation. WO2007009164; 2007

130. Wu M-H, Pan W-C. Development of microfluidic alginate microbead generator tunable by pulsed airflow injection for the microencapsulation of cells. Microfluid Nanofluidics 2010;8:823-35

131. Capretto L, Mazzitelli S, Luca G, et al. Preparation and characterization of polysaccharidic microbeads by a microfluidic technique: Application to the encapsulation of Sertoli cells. Acta Biomater 2010;6:429-35

132. Oh JK, Drumright R, Siegwart DJ, et al. The development of microgels/nanogels for drug delivery applications. Prog Polym Sci 2008;33:448-77

133. Huang S-B, Wu M-H, Lee G-B. Microfluidic device utilizing pneumatic micro-vibrators to generate alginate microbeads for microencapsulation of cells. Sens Actuators B Chem 2010;147:755-64

• **Microfluidic systems for continuous cell encapsulation in alginate microparticles.**

134. Liao C-Y, Su Y-C. Formation of biodegradable microcapsules utilizing 3D, selectively surface-modified PDMS microfluidic devices. Biomed Microdevices 2010;12:125-33

135. Nie Z, Li W, Seo M, et al. Janus and ternary particles generated by microfluidic synthesis: design, synthesis, and self-assembly. J Am Chem Soc 2006;128:9408-12

136. Lee AP, Tan YC. Microfluidic devices and method for controlled viscous shearing and formation of amphiphilic vesicles. WO2004071638; 2004

137. Mathies RA, Kumaresan P, Yang C, et al. Microfabricated dropley generator. WO2009015296; 2009

• **A high-throughput microfluidic device for DNA/RNA or cell encapsulation capable of crontroling the size of the particles obtained by emulsion processes.**

138. Song W, Lima AC, Mano JF. Bioinspired methodology to fabricate hydrogel spheres for multi-applications using superhydrophobic substrates. Soft Matter 2010;6:5868-71

•• **A general overview of the potential of superhydrophobic surfaces in production of particles for drug delivery and cell encapsulation, cell carriers and supports for cell expansion.**

139. Lima AC, Song W, Blanco-Fernandez B, et al. Synthesis of Temperature-Responsive Dextran-MA/PNIPAAm particles for controlled drug delivery using superhydrophobic surfaces. Pharm Res 2011;28:1294-305

140. Kamei S, Ohta T, Saikawa A, et al. Sustained-release microspheres, their production and use. WO1998032423; 1998

141. Jonsson M, Larsson K, Gustafsson NO, et al. Vaccine composition comprising an immunologically active substance embedded in microparticles consisting of starch with reduced molecular weight. WO2002028371; 2002

142. Hural J, Johnson ME, Spies AG. Microparticles and methods for delivery of recombinant viral vaccines. WO2002092132; 2002

143. Hughes PM, Olejnik C. Delivery of a drug via subconjuctival or periocular delivery of a prodrug in a polymeric microparticle. WO2005011741; 2005

144. Lavik E, Kwon YH, Kuehn M, et al. Sustained intraocular delivery of drugs from biodegradable polymeric microparticles. WO2008157614; 2008

145. Futo T, Saito K, Hoshino T, et al. Sustained-release composition and method for producing the same. WO2008075762; 2008

146. Jason ME, Kalota DJ. Microencapsulation process. WO1996032191; 1996

147. Lumsdon SO, Friedmann TE, Green JH. Encapsulation of oils by coacervation. WO2005105290; 2005

148. Forster T. New type of phase separation of micellar colloid solutions. WO2006109104; 2006

149. Ruiz J-M. Preparation comprising microparticles of an insoluble polysaccharide resin with a biopolymeric active ingredient and method for production thereof. WO2006072613; 2006

150. Lai J-W, Qian K, Venkatesh G. Taste-masked multiparticulate pharmaceutical compositions comprising a drug-containing core particle and a solvent-coacervated membrane. WO2006055142; 2006

151. Bouquerand P-E, Dardelle G, Gouin S, et al. Encapsulated active ingredients, methods of preparation and their use. WO2007026307; 2007

•• **A successful application of superhydrophobic surfaces in producing thermo responsive hydrogel beads for the controlled delivery of bioactive compounds.**

## Affiliation

Ana Catarina Lima[1,2] MSc,
Praveen Sher[1,2] PhD & João F Mano[†1,2] PhD
[†]Author for correspondence
[1]University of Minho,
Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine,
3B's Research Group-Biomaterials, Biodegradables and Biomimetics,
AvePark, Zona Industrial da Gandra,
S. Cláudio do Barco,
4806-909 Caldas das Taipas – Guimarães, Portugal
Tel: +351 253 510 900; Fax: +351 253 510 909;
E-mail: jmano @dep.uminho.pt
[2]ICVS/3B's – PT Government Associate Laboratory, Guimarães, Portugal