Processing and Scale-up of Polymeric Nanoparticles

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Abstract This chapter presents methods of nanoparticle processing based on the use of preformed polymers. It will discuss the basic principle of the different methods, the scale up and the methods for preparing the polymer nanoparticles for storage including purification, drying sterilization and eventually concentration. The last part of the chapter will discuss the performance, the application and the present limitation of the processing methods considered here.

Keywords Emulsification-solvent diffusion • Emulsification-solvent evaporation • Gelation • Nanocapsules • Nanoprecipitation • Nanospheres • Polymer micelles • Scale-up

Abbreviations

ACN	acetonitrile
CMC	critical micellar concentration
DMAc	N-N-dimethylacetamide
DMF	dimethylformamide
O/O	Oil in oil emulsion
O/W	oil in water emulsion
PEC	polyelectrolyte complexes
PEG	poly(ethylene glycol)
PEI	poly(ethylenimine)

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A. Prokop (ed.), *Intracellular Delivery: Fundamentals and Applications*, Fundamental Biomedical Technologies 5, DOI 10.1007/978-94-007-1248-5_16,

PEO	poly(oxyethylene)
PLL	poly(lysine)
THF	tetrahydrofuran
W/O/O	water in oil in oil multiple emulsion
W/O/W	water in oil in water multiple emulsion
W/O	water in oil emulsion

1 Introduction

Nanoparticles have become common tools in research to improve drug efficiency in vivo by a better control of the biodistribution. Proof of concept is now well established and the use of nanomaterials to deliver drugs is about to revolution treatments of severe diseases. It is noteworthy that a few systems have reached the clinics for the treatment of cancer including TransDrug® and one already marketed formulation, Abraxane®. Due to the variety of drug candidates, many polymer nanoparticles were developed requiring different methods of processing. The aim of this chapter is to summarize the different methods of processing polymer nanoparticles and to discuss their performance regarding their potential and limitations for a scale up and for drug encapsulation. The principles of the preparation methods will be described first. Considerations about the scaling up will be presented in the next part followed by general methods of treatments applied after preparation to purify, sterilize, dry and condition the nanoparticles. The different functionalities which may be interested to integrate in the nanoparticles will be discussed in the last part of the chapter.

2 Principles of Methods of Preparation of Polymer Nanoparticles

Two classes of methods can be identified depending whether the obtaining of nanoparticles is achieved from a polymer solution or from an emulsion. The following presentation of the general principles for nanoparticle preparation from polymers will include this subdivision. Only methods of nanoparticle preparations from polymers will be presented in this chapter. The methods based on polymerizations are discussed in another chapter (See **chapter by Nicolas and Vauthier in this book**). Schemes of the different types of nanoparticles are illustrated in Fig. 1.

2.1 Methods Based on the Conversion of a Polymer Solution into Nanoparticle Dispersions

Dilute solutions of polymers can be converted into nanoparticle dispersions taking advantages of solubility properties of the dissolved polymer to precipitate as

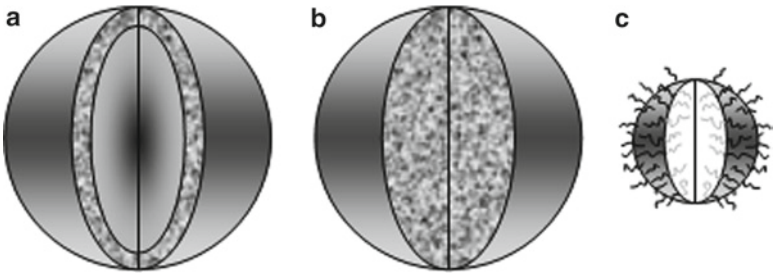


Fig. 1 Scheme of the different types of nanoparticles including nanocapsules (**a**), nanospheres (**b**) and polymer micelles (**c**). Nanocapsules are reservoir type particles including a cavity surrounded by a polymer envelope. Nanospheres are plain nanoparticles. Polymer micelles are formed by self association of amphiphilic polymers

nanoparticles (nanoprecipitation) or of its capacity to form nanogels, nanosized polyelectrolyte complexes (PEC) and micelles. In all cases, formation of particles in the nanometer size range occurred in well controlled conditions which can be studied through systematic approaches including the establishment of a phase diagram.

2.1.1 Obtaining Nanoparticles by Nanoprecipitation or Solvent Displacement

Nanoprecipitation or solvent displacement methods are now well established methods producing nanoparticles or polymer micelles from a polymer solution. They are based on the induction of the precipitation of a polymer thanks to the displacement of the polymer solvent by a non solvent which is miscible with the polymer solvent (Fessi et al. 1989; Thioune et al. 1997; Murakami et al. 1999; Legrand et al. 2007). In practice, a solution of polymer is prepared by dissolving the polymer in one of its solvent. Then, the polymer solution is added in a non solvent which is miscible to the polymer solvent allowing polymer colloids to form. Polymers with no or poor amphiphilic properties precipitate to form nanoparticles in a narrow window of the phase diagram considering the composition in polymer-solvent- non solvent of the nanoprecipitation system (Stainemesse et al. 1995). In general, the produced nanoparticles are well defined in size with a narrow size distribution. As requirements for the success of the methods, (i) the solution of polymer should be rather diluted, (ii) the polymer solvent and the non solvent should be miscible, (iii) the polymer solvent should easily be removed at the end of the preparation. Thus, the preferred polymer solvents are acetone, ethanol and THF while the preferred non solvent is water. Suitable polymers include many of the polymers suggested as material for the development of nanoparticulate drug delivery systems including main types of polyesters (poly(lactic acid), poly(lactide-co-glycolide), poly(epsilon caprolactone)), ethylcellulose, new polymer candidates like poly(benzylglutamic acids) and all the corresponding copolymers with poly(ethyleneglycol) moiety (Avgoustakis

2004; Barbosa et al. 2008). At the end of the preparation, the polymer solvent is removed by evaporation or ultrafiltration. Nanoparticles produced by nanoprecipitation are generally characterized by a diameter ranging from 200 to 300 nm. This size range is rather narrow and was found to result from the precipitation of polymer chains with well define molecular weight (Legrand et al. 2007).

Several theoretical works have been developed to explain the formation of the nanoparticles from the nucleation of supersaturated zones in the solution which form during mixing the polymer solution with the non solvent (Johnson and Prud'homme 2003; Ganachaud and Katz 2005; Lince et al. 2008; Aubry et al. 2009). Optimized conditions for the production of nanoparticles with the highest yield of nanoparticle production are fulfilled when the polymer is dissolved in a teta solvent at a concentration comprised in the dilute regimen which means that the polymer chains are surrounded by enough solvent so that they remain separated in the solution (Legrand et al. 2007). In other words, in the optimal conditions polymer molecules do not overlap with each other and remain independent in the solution. By increasing polymer concentration in the solution above the critical interpenetrating concentration C*, polymer molecules overlap each others promoting the formation of aggregates instead of individual nanoparticles. To adjust solvency properties some authors have suggested to use binary blends of solvents such as acetone with small amount of added water (Thioune et al. 1997), or blends of ethanol and acetone (Murakami et al. 1999). Another optimization parameter includes the molecular weight of the polymer. Indeed, nanoparticles were found to be formed by polymer chains with define molecular weight. It was shown that all polymer chains with a mean molecular weight outside the optimal molecular weight precipitate as aggregates. Thus, by choosing polymers with optimal molecular weight, i.e. molecular weight of polymer chains found in nanoparticles, yield of nanoparticle production can be optimized while formation of aggregates can be avoided. Nanoprecipitation methods suit well to prepare drug carriers incorporating lipophilic drugs. Indeed, in general, the drug to be encapsulated in the nanospheres produced by this technique is simply added in the polymer solution (Niwa et al. 1993; Murakami et al. 2000; Chorny et al. 2002; Peltonen et al. 2004).

Although the main type of nanoparticles prepared by nanoprecipitation is nanospheres, the method can easily be adapted to produce nanocapsules by adding a small amount of oil in the polymer solution (Fessi et al. 1989). During mixing of the polymer solution with the non solvent of the polymer, the oil splits as tiny droplets around which the polymer precipitates to form the nanocapsule shell. The dispersion phenomenon of the oil was explained by the "Ouzo or Pastis effect" elucidated by Vitale and Katz (2003).

It is noteworthy that polymer micelles can be prepared by nanoprecipitation of amphiphilic copolymers (Trivedi and Kompella 2010). In general, polymer micelles are formed spontaneously in a solution just because the concentration of the amphiphilic molecule is above the Critical Micellar Concentration (CMC). Although polymer micelles form spontaneously, artefacts can be used to promote their formation from amphiphilic polymers with low CMC in water and to favour drug entrapment with a high payload (Fournier et al. 2004; Gaucher et al. 2010a, b;

Joralemon et al. 2010). The two major methods of preparation of drug loaded polymer micelles are based either on nanoprecipitation or on dialysis. The polymer micelles are to be produced in a continuous aqueous phase. In general, the solubility of the amphiphilic polymer is higher in organic solvents compared to water. The use of an organic solvent miscible with water promotes the dissolution of unimers of the polymer helping the formation of well structured polymer micelles when solubility conditions change by addition of large amount of water such as in the nanoprecipitation method (Johnson and Prud'homme 2003; Forrest et al. 2008) or by the progressive replacement of the organic solvent by water through a dialysis against water (Kim et al. 1999; Lee et al. 2003; Huh et al. 2005; Park et al 2005). Eventually, the polymer solution is slightly diluted by the addition of a limited amount of water prior to dialyse against water (Jie et al. 2005; He et al. 2007; Kang and Leroux 2004). Typical organic solvents are chosen among acetonitrile (ACN), tetrahydrofuran (THF), dimethylformamide (DMF), or N-N-dimethylacetamide (DMAc). The drug to be encapsulated in the polymer micelles is added in the polymer solution before induction of the micelle formation achieved by modifications of polymer solubility conditions.

2.1.2 Obtaining Nanoparticles Through Controlled Gelation

Gels are a three-dimensional polymer network swollen by solvent. Several polymers form gels either by cooling down a solution prepared at hot temperature or by adding small molecules which crosslink polymer chains through chemical linkage or by physical interactions. Obtaining nanoparticles from a polymer solution through a gelation process was described inducing gel formation by physical interactions. Typical polymers are charged polysaccharides dissolved at a low concentration in an aqueous solvent. The addition of a small molecule bearing a low number of opposite charges induces formation of gel nanoparticles through ionic gelation. This can easily be followed by measuring the viscosity of the polysaccharide solution which dropped down when the nanoparticles formed, by electron microscopy and by light scattering allowing to measure the size of the nanoparticles formed (Vauthier et al. 1994; Vauthier and Couvreur 2000). In general, the nanosized gels form at low concentrations of both polymer and gelling agent.

This method was applied with alginate which gelify with calcium ions. Consolidation of nanogels formed with calcium can be achieved by addition of a positively charged polyelectrolyte. Poly-lysine was used in the earlier development but most of the recent works are considering chitosan as another suitable polyelectrolyte candidate (Rajaonarivony et al. 1993; Vauthier and Couvreur 2000; Douglas and Tabrizian 2005). Alginate nanoparticles produced by controlled gelation can easily be loaded with oligonucleotides. They protect oligonucleotides from degradation by nucleases (Aynié et al. 1999). This system was also highly investigated for the delivery of oral formulation of peptides including insulin (Li et al. 2007; Sarmento et al. 2007). Chitosan based nanoparticles can be obtained by gelation

with tripolyphosphate in presence of PEG to achieve stability of the nanoparticles (Calvo et al. 1997). Chitosan nanoparticles were designed as delivery systems for macromolecules (Janes et al. 2001; Brunel et al. 2010).

2.1.3 Formation of Nanoparticles from Polyelectrolyte Complexes

Another method for the production of nanoparticles is based on formation of polyelectrolyte complexes (PEC). In this method, two polymers of opposite charges are brought together to interact and form aggregates in the nanosize range (Berger et al. 2004; Schatz et al. 2004; Goycoolea et al. 2009; Oyarzun-Ampuero et al. 2009; Woitiski et al. 2009a, b). Originally, this approach was suggested to develop drug carriers for nucleic acid delivery. Complexes were prepared by mixing polycations like poly(lysine) (PLL) or poly(ethylenimine) (PEI) and nucleic acids which are negatively charged macromolecules (Boussif et al. 1995; Coll et al. 1999; Jeong et al. 2007; Sun and Zhang 2010). The main difficulty with this method was to produce nanoparticles which remained stable over time. Stability of nanoparticles can be improved by using block copolymers including a poly(ethylene glycol) (PEG) moiety combined with either the polycation or the nucleic acid. The nanoparticles obtained with these copolymers are sterically stabilized. The PEG chains form a corona at the surface of the core of the nanoparticles formed by the poly(electrolyte) complex between the nucleic acid and the polycation (Kabanov et al. 2005; Jeong et al. 2007; Joralemon et al. 2010).

Recent studies provide relevant methodologies to prepare stable and well define nanoparticles by mixing polyelectrolytes of opposite charges. A pioneer work was given by the group of Delair who drawn a concept in which one polyelectrolyte is a guest for the second polyelectrolyte of opposite charge which is the host. They clearly demonstrated that the host and guest balance control the formation of well define nanoparticles with either positive or negative charges (Schatz et al. 2004; Drogoz et al. 2007). This approach is now successfully applied by many authors using various polymers (Berger et al. 2004; Schatz et al. 2004; Oyarzun-Ampuero et al. 2009; Goycoolea et al. 2009; Woitiski et al. 2009a, b; Mao et al. 2006; Sun et al. 2008). Briefly, the polyelectrolyte with the larger molecular weight is considered as the host while the polyelectrolyte of opposite charge with a much lower molecular weight is consider as the guest. To obtain nanoparticles, the guest polyelectrolyte is added into a solution containing the host polyelectrolyte. Parameters such as the respective concentration of the two polyelectrolytes, and the ratio of their molecular weight are affecting the characteristics of the produced nanoparticles (Drogoz et al. 2007).

Nanoparticles made of polyelectrolyte complexes are largely developed for hydrophilic macromolecular drugs. These nanoparticles are suitable for all types of nucleic acids (Brunel et al. 2010; Sun and Zhang 2010). Peptides and proteins which are ampholyte molecules are a second category of drug molecules which are associated with such nanoparticles (Mao et al. 2006; Woitiski et al. 2009b). Several works are considering their use for the development of an oral formulation of

insulin while others are considering these nanoparticles for the delivery of antigens entering the formulation of vaccines (Mao et al. 2006; Weber et al. 2010; Woitiski et al. 2009b).

2.2 *Methods Based on the Formation of an Emulsion*

The second class of methods requires the preparation of an emulsion prior to the formation of nanoparticles. This part of the chapter will present methods for the preparation of the required emulsion and then the different methods used to convert the emulsion into dispersion of polymer nanoparticles.

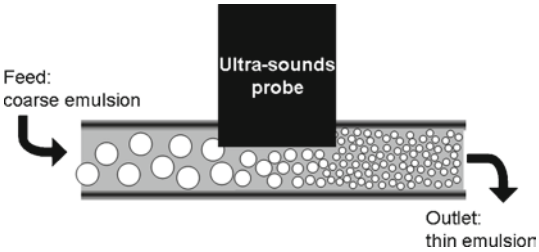
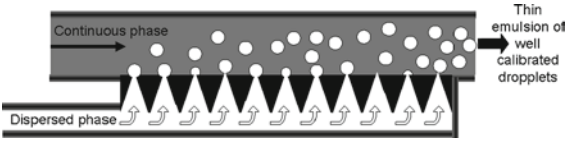
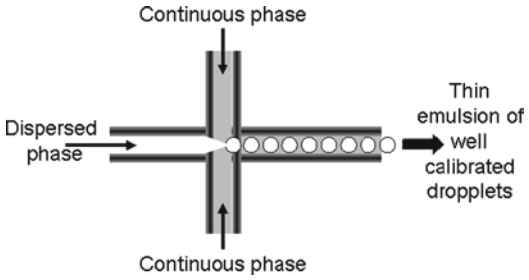
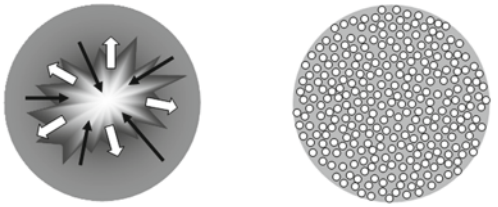
2.2.1 Preparation of Emulsions Suitable for Nanoparticle Preparation

The preparation of the emulsion is critical in the sense that it will influence the size of the polymer particles at the end of the preparation. It consists in mixing two immiscible phases together, the polymer solution being the dispersed phase of the emulsion. The sense of the emulsion, i.e. water in oil (W/O) or oil in water (O/W), depends on the nature of the polymer and solubility properties. Oil in oil (O/O) emulsions were introduced to improve drug incorporation performance in nanoparticles (Nah et al. 2008). In general, simple emulsions are used to prepare nanospheres. Preparation of nanocapsules can be achieved from multiple emulsions water in oil in water (W/O/W) (Lu et al. 1999; Bilati et al. 2003). In this latter case, the polymer is dissolved in the intermediate oily phase while the drug is dissolved in the aqueous inner phase. More complex emulsions were recently proposed to produce double walled particles (Zheng 2009). Although the technique is presently applied to produce microparticles which are larger particles than that of the nanometer range but it can be expected that such methods of emulsification will soon be applied to produce nanoparticles as well.

Several methods are suitable to prepare the required simple emulsions straight from the two separate phases. This includes ultrasounds, extrusion through microporous and nanoporous materials, microfluidic systems and co-solvent assisted emulsification (Ouzo effect) (Table 1).

For all other methods, the two phases are premixed before being submitted to specific treatments providing the emulsion of the desired characteristics. For instance, very small size emulsions, i.e. nano-emulsion can be generated from a bi-continuous system prepared with poly(ethylene glycol) (PEG) containing surfactants through a phase inversion method (Anton et al. 2008). The conversion of the bi-continuous system into an oil-in-water nano-emulsion is induced by simultaneous dilution and temperature drop. This method requires a very low energy to achieve the formation of the desired emulsion. Depending on the characteristics of the PEG surfactant, it can be applied at temperature ranging from 40°C to 90°C.

Table 1 Methods used to prepare thin emulsions straight from the two phases

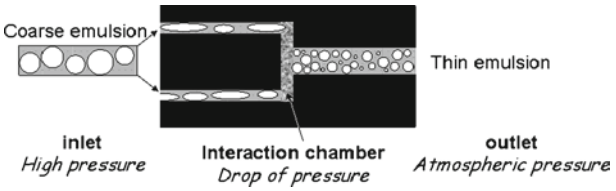
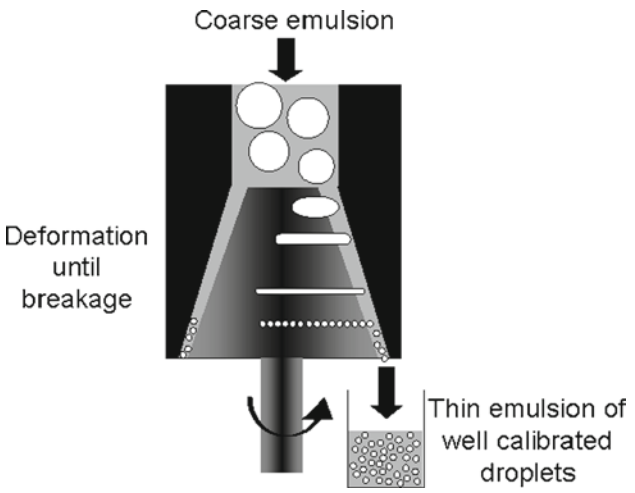
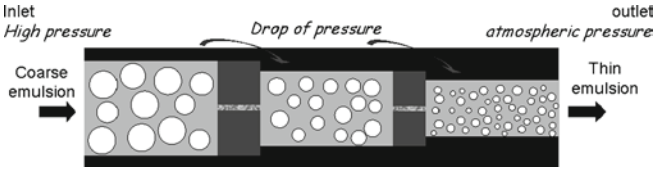
Ultrasounds (Vila et al. 2002; Perez et al. 2001; Freitas et al. 2005)	An ultrasound probe is used to mix together the oil and water phases of the emulsion. 
Extrusion through porous materials (Charcosset and Fessi 2005)	The polymer and drug containing phase is extruded through a porous membrane into the continuous phase placed on the other side of the membrane. Although this method is suitable to prepare simple emulsions, double emulsions can also be prepared from simple emulsions extruded into a new continuous phase. 
Microfluidic systems (Abate and Weitz 2009)	The two phases are mixed together through a well organized circulation in a microfluidic device composed of nanochannels. Emulsion droplets of well define diameter are formed. This method is suitable to produce double emulsion by adding more channel in the microfluidic device. 
Co-solvent assisted emulsification: Ouzo effect (Vitale and Katz 2003; Ganachaud and Katz 2005)	The polymer, the drug and a small amount of oil are dissolved in an organic solvent miscible with water. By mixing this organic phase with water under gentle conditions, oil droplets form while the solvent diffuses in the aqueous phase. 

Other methods consist in reducing size of the droplets of a rough emulsion using special machines including colloidal mills, microfluidizer and high pressure homogeniser (Table 2) (Urban et al. 2006; Anton et al. 2008). By using a microfluidizer, the diameter of the droplets, D, formed in the final emulsion depends on the viscosity of the organic solution of polymer as indicated by the Eq. 1 (Koshy et al. 1988; Walstra 1983).

$$D = \eta^{\alpha} \tag{1}$$

In this equation, η corresponds to the viscosity of the polymer solution, α is a coefficient which depends on many parameters of the system including the nature of the polymer. The alpha coefficients were determined for ethylcellulose and poly(lactic acid) using ethylacetate as the organic solvent. The values were 0.05 and 0.28 respectively (Desgouilles et al. 2003).

Table 2 Apparatus used to reduce size of emulsion droplets from a rough emulsion (Adapted from Urban et al. 2006)

Microfluidizer (Bodmeier and Huagang 1990; Desgouilles et al. 2003)	
Colloidal mill (Stork et al. 2003; Mabile et al. 2003)	
High pressure homogenizer (Shegokar et al. 2010)	

Multiple emulsions can be prepared with microfluidizer (Sani et al. 2009), high pressure homogeniser (Lamprecht et al. 1999, 2000) and ultrasounds (Perez et al. 2001) by re-emulsifying the simple emulsion prepared above into a new continuous phase.

Surfactants such as pluronic and span are often used to prepare stable emulsions. However new polymer surfactants were synthesized to formulate suitable emulsions for nanoparticle preparation. In general these polymer surfactants include polymer chains of the same nature than that composing the nanoparticle. The hydrophilic moiety required to achieve the stability of the nanoparticles which protruded in the aqueous phase is composed either of polyethylene glycol (Gref et al. 1994; Bazile et al. 1995; Avgoustakis 2004; Qui and Bae 2006) or of various polysaccharides (Lemarchand et al. 2003; Chauvierre et al. 2004).

2.2.2 Conversion of an Emulsion into Nanoparticle Dispersion

Several artefacts can be used to convert the emulsion into nanoparticle dispersion. For instance, it can be achieved by inducing the precipitation of the polymer dissolved in the emulsion droplets. The simplest methods inducing polymer precipitation consists in removing the solvent contained in the emulsion droplets by evaporation of the solvent composing the dispersed phase of O/W and W/O/W emulsions (Avgoustakis 2004; Vauthier and Bouchemal 2009). This corresponds to the original method of preparation of pseudolatexes by Gurny et al. (1981). To achieve this, it is required that the emulsion was prepared with an organic volatile solvent such as dichloromethane and ethyl-acetate. Because solvent evaporation is a slow process, formation of nanoparticles takes more then a few minutes. Polymer concentration increases gradually over time in the emulsion droplets thanks to solvent removal until polymer starts precipitation (Desgouilles et al. 2003). Diameter and size distribution of the nanoparticles depends on the diameter and size distribution of the emulsion droplets. However, no general relationship can be drawn between droplet and nanoparticle size because this depends on the extend of emulsion droplet coalescence occurrence during the solvent removal step which in turn depends on surface active properties of the polymer and may be affected by the presence of surfactant.

A second method consists in a rapid extraction of the solvent contained in the emulsion droplets by diffusion toward the continuous phase of the emulsion. This method was named the emulsification-solvent extraction method or the emulsification-solvent diffusion method. Typically, suitable conditions are obtained by dilution of the emulsion by adding more continuous phase if the polymer solvent contained in emulsion droplets is partly miscible with the continuous phase (i.e. ethylacetate for instance). Instead of diluting the continuous phase, solvents in which both the continuous and the dispersed phase are miscible can be added to displace the polymer solvent from the dispersed phase towards the continuous phase causing polymer precipitation (Leroux et al. 1995; Quintanard-Guerrero et al. 1999; Perez et al. 2001; Guinebretiere et al. 2002; Moinard-Chécot et al. 2006, 2008).

In contrast with the previous methods, nanoparticles form within milliseconds (Moinard-Chécot et al. 2008). The so called emulsification-inverse salting out method is a specific case of preparation of nanoparticles based on solvent extraction. The emulsion is prepared with a solution of polymer dissolved in acetone which is dispersed in a concentrated aqueous solution of electrolytes or mono/disaccharides. After simple dilution of the emulsion with an excess of water, the electrolyte or saccharide concentration in solute dropped down inducing instantaneous diffusion of acetone out of the emulsion droplets towards the continuous phase of the emulsion. As a consequence, the polymer precipitates as nanoparticles (Allémann et al. 1992; Ibrahim et al. 1992).

Alternative methods to polymer precipitation are based on gelation of polymers contained in the emulsion droplets of water-in-oil emulsions. Suitable polymers used to prepare nanoparticles by this method are polysaccharides. For instance, agarose nanoparticles are obtained by decreasing the temperature of the emulsion prepared at high temperature allowing the polysaccharide to solubilise (Wang and Wu 1997). Pectine nanoparticles are prepared from two emulsions, one containing the polysaccharide and the second a basic aqueous dispersed phase. By mixing the two emulsions together, gelation of pectine contained in the emulsion droplets of the first emulsion is promoted thanks to an increase of pH of the emulsion droplets. This gelation produces the nanospheres (Tokumitsu et al. 1998).

3 Pilot Scale Production of Nanoparticles

Only few articles report preparation of large amount of nanoparticles for drug delivery applications. Indeed, nothing is known about transposition from laboratory preparation to production of clinical batches for doxorubicin loaded poly(alkylcyanoacrylate) nanoparticles, Transdrug®, used in phase II/III clinical trials (BioAlliance Pharma 2010). No more is known about the production of a marketed formulation of paclitaxel-loaded albumin nanoparticles, nab-paclitaxel or Abraxane®, used in clinics in the USA for the treatment of metastatic breast cancer (Hawkin et al. 2008; Petrelli et al. 2010). The most detailed data about the scale up of production of pharmaceutical grade nanoparticle dispersions are only available on the pilot scale production of nanoparticles prepared by the emulsification-solvent diffusion method and by the nanoprecipitation method. By extension, the scale up approach developed for the method of emulsification-solvent diffusion was also applied to produce large batches of nanoparticles by the emulsification-reverse salting out method. As a reminder, a pilot-scale production is intermediate between the laboratory and the industrial scale production. Pilot size production is aimed to simulate the closest as possible industrial production and hence needs to integrate all parameters that need to be optimized before reaching the industrial production. This topic was recently extensively reviewed by the authors (Vauthier and Bouchemal 2009).

3.1 Pilot-Scale Production of Nanoparticles by Emulsification-Solvent Diffusion Method

Large scale production of nanocapsules by emulsification-solvent diffusion method was developed up to a production of batches of 15 L each (Colombo et al. 2001; Galindo-Rodriguez et al. 2005). The pilot set up comprises several vessels for the preparation of the different solutions which are connected through a system of flexible tubing to a main reactor. Transfer of solutions from the vessels to the reactor occurred by simple gravity. The rational behind this design was to reproduce as much as possible fluid motions produced in the laboratory scale set up (60 mL) (Galindo-Rodriguez et al. 2005). By introducing a few modifications, this pilot set up can be used for the production of nanoparticles by the emulsification-reverse salting out method (Galindo-Rodriguez et al. 2005).

In both cases, agitation is an important process parameter to consider during the preparation of the emulsion (Colombo et al. 2001). It greatly influences the size of the nanoparticles produced at the end of the procedure. Optimal conditions of agitation were found by using stirring rates above 1,000 rpm for the emulsification–solvent diffusion method and 790 rpm for the emulsification-reverse salting-out method. By applying the optimal conditions, the methods are reproducible and provide with nanoparticle dispersions with a narrow size distribution (Galindo-Rodriguez et al. 2005).

3.2 Pilot-Scale Production of Nanoparticles by a One Step Procedure Based on the Nanoprecipitation of a Polymer

Success of the nanoprecipitation method resides on the way the organic and the aqueous phases mix together resulting in the precipitation of the polymer as nanoparticles. Progresses in the comprehension of nanoprecipitation phenomena highlighted that it is controlled by a few key parameters. It was established that the time of mixing must be faster than the time required to induce nanoparticle formation (Johnson and Prud'homme 2003). Ideal conditions are obtained at the beginning of the mixing of the two phases. In conditions found in the lab scale production, the amounts of organic solution of polymer and that of the non-solvent are small. Ideal conditions of nanoprecipitation are fulfilled at the beginning of the mixing and are almost maintained during mixing of the total amounts of solutions used to produce small batch of nanoparticles in small reactors (lab scale production of a few mL). However, production of large batches of nanoparticles by nanoprecipitation in a reactor is not suitable as the conditions for nanoprecipitation change from the beginning to the end of the two phases mixing. To maintain a homogenous condition for nanoprecipitation along mixing time, the two phases need to be brought together in the same conditions all along the process. Briançon et al. (1999) have suggested to inject the two phases in a mixing device which is continuously feed at

specific flow rates with each phase including the polymer solution and the non solvent of the polymer. Simultaneously, the nanoparticle dispersion flows out of the mixing device through the outlet. The mixing conditions provided by the mixing device are constant whatever the volume of nanoparticle dispersion produced. This can be achieved thanks to the small volume of each phase processed instantaneously and continuously in the mixing device. Therefore, the mixing device is the central piece of the production set-up and allows to maintain homogenous starting condition all along the addition of the two phases whatever the total volume is. This first mixing device was designed on the basis of a T-shape tube (Briançon et al. 1999; Tewa-Tagne et al. 2007; Colombo et al. 2001; Galindo-Rodriguez et al. 2005). Several mixing devices have been designed since then by different groups with capacities varying from a few microlitres to a few millilitres (Fig. 2) (Liu et al. 2008; Akbulut et al. 2009; Lince et al. 2009; Tuereli et al. 2009). Simulations of the mixing dynamic flow occurring in the mixing device help optimizing operating conditions of nanoprecipitation (Gavy et al. 2009; Lince et al. 2009). The whole

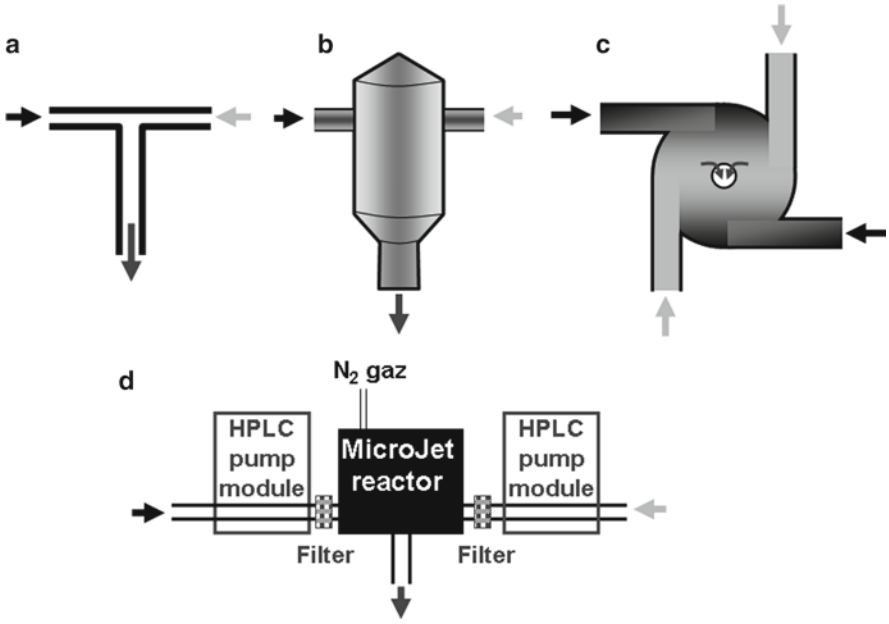


Fig. 2 Mixing devices used to produce large batches of nanoparticles by nanoprecipitation or solvent displacement methods. (**a**) T shape mixing device according to Briançon et al. (1999), diameters of the inlets can vary; (**b**) Smart mixer and reactor according to Lince et al. (2009), diameters of the inlets can vary; (**c**) Vortex jet mixer according to Liu et al. (2008) and Akbulut et al. (2009), the nanoparticle dispersion is evacuated from the center of the device; (**d**) Microjet reactor set-up according to Tuereli et al. (2009). *Black arrows* indicate the inlet of the mixing device feed with polymer solution, *light gray arrows* indicate the inlet of the mixing device feed with the polymer non solvent and medium *gray arrows* indicate the outlet of the mixing device where the nanoparticle dispersions are collected

pilot plant for the production of nanoparticles by nanoprecipitation includes one reservoir (can be several litres) for the polymer solution, one reservoir (can be several litres as well) with the polymer non-solvent, a receiver of large capacity (can be several litres), the mixing device and pumps used to feed the mixing device with the polymer solution on the one hands and the non-solvent on the other hands with perfectly controlled flow rates. A pilot plan built with reservoirs of 3 L each and a T-shape mixing device can convert 5 g of polymer into nanoparticles in 2 h with a very good reproducibility (Galindo-Rodriguez et al. 2005; Tewa-Tagne et al. 2007)

4 Treatment of Nanoparticles and Preparation for Storage

After synthesis, several types of treatments may be necessary to apply on nanoparticle dispersions including purification, sterilization and preparation for storage.

Purification is often required to remove traces of impurities such as residual organic solvent, excess of surfactants, salts and large polymer aggregates (Vauthier and Bouchemal 2009). Volatile organic solvents can be removed by evaporation under reduced pressure. Although this method can easily be applied on small amounts of nanoparticle dispersions, ultrafiltration (Allemann et al. 1993), diafiltration (Tishchenko et al. 2003) and cross-flow microfiltration (Allemann et al. 1993; Limayem et al. 2004; Quintanar-Guerrero et al. 1998) are suitable methods to treat large volumes of nanoparticle dispersions. Filtration can be used to remove aggregates (Govender et al. 1999; Murakami et al. 1999). Centrifugations and ultracentrifugations can be used to separate nanoparticles from the dispersing medium which retains all excess of reagent not included in the nanoparticles during preparation (Bouchemal et al. 2004, 2006; Govender et al. 1999; Calvo et al. 1997; Sahoo et al. 2002; Nguyen et al. 2003; Lambert et al. 2000). Dialysis (Chauvierre et al. 2003) and gel filtration (Beck et al. 1990) are alternative methods when difficulties of dispersion of nanoparticles arise after centrifugation.

In general, the solid content of nanoparticle dispersions as prepared by the previously described methods is rather low (Desgouilles et al. 2003; Legrand et al. 2007). In some case, it is so low that it compromises the application as drug delivery system as the volume to administer to reach a therapeutic concentration in drug in vivo is much above the maximal volume tolerated for the administration. Therefore, nanoparticle dispersions may need to be concentrated prior to their in vivo administration. Straightforward methods to increase nanoparticle concentration are freeze drying, spray drying, ultracentrifugation and solvent evaporation. Although these methods are suitable for several types of nanoparticles, the main problem resides in the formation of aggregates which form when nanoparticles come in contact with each other (Abdelwahed et al. 2006; Avgoustakis 2004; Bozdag et al. 2005; Vauthier et al. 2008). Methods given the more satisfactory results without any risk of causing nanoparticle aggregation are based on dialysis and ultrafiltration. Difficulties which might be encountered with simple ultrafiltration (i.e. clogged membranes with nanoparticles) can be resolved using diafiltration

or tangential filtration methods (Limayem et al. 2004). Concentration of nanoparticle dispersion based on dialysis is a gentle method in which the water from the nanoparticle dispersion is removed thanks to the application of an osmotic pressure on the dialysing bag using a solution of high molecular weight polymer as counter dialysing medium. The dispersions can be concentrated up to factors of 50 without producing aggregation of the nanoparticles and in a couple of hours (Vauthier et al. 2008).

Nanoparticle dispersions for parenteral administration need to be sterilized. Autoclaving is not always suitable because nanoparticles may be modified and drug may loose their activity (Rollot et al. 1986; Masson et al. 1997; Boess et al. 1996). Autoclaving can be used to sterilize nanoparticles made of chitosan-carboxymethyl dextran polyelectrolyte complexes (Lin et al. 2009). Gamma irradiations also need to be applied with caution because it can significantly modify the characteristics of the polymer composing the nanoparticles and the drug hence the initial performance of the drug delivery system (Sintzel et al. 1997; Masson et al. 1997; Athanasiou et al. 1996). However, this technique was found suitable to sterilize doxorubicin-loaded poly(butyl cyanoacrylate) (PBCA) nanoparticles (Maksimenko et al. 2008) and vaccine nanoparticles made of poly(anhydride) and containing Brucella ovis antigen (Da Costa Martinez et al. 2009). Both types of nanoparticles showed an excellent stability to irradiation without radiolysis of the polymer and with good performance preserving the integrity of the drug/antigen activity. Sterilization by filtration can be applied only on nanoparticle dispersions with a diameter below 0.22 μm which is a major limitation (Memisoglu-Bilensoy and Hincal 2006). High Hydrostatic Pressure treatment (HHP) proved its efficacy to destroy vegetative forms of microorganisms found in nanoparticle dispersions (Brigger et al. 2003). However, this technique still needs improvement to make possible elimination of bacteria spores before if can be validated as a suitable method of sterilization for nanoparticles. It could be interesting to also explore other sterilization methods such as those based on the use of gaz plasma and ethylene oxide which were recently applied on gold nanoparticles developed for biomedical applications (França et al. 2010). In the absence of suitable satisfactory method of sterilization, preparations must be done in sterile environment which complicates the development for clinical applications.

As valuable for many pharmaceutical products, storage of nanoparticle drug delivery systems under a dried form would be the preferred method. In general, nanoparticles are obtained in a liquid medium after preparation. Transformation of the liquid dispersion into a dried powder can be achieved either by lyophilisation (Nemati et al. 1992; De Chasteigner et al. 1996; De Jaeghere et al. 1999; Abdelwahed et al. 2006) or spray-drying (Tewa-Tagne et al. 2007) which are both suitable at industrial scale. Considering freeze drying, it may be required to add cryoprotectants to the nanoparticle dispersion to prevent aggregation of nanoparticles during the reconstitution of the dispersion from the dried powder prior to use. Optimization of the freeze drying cycle improves the quality of the freeze dried product (Patapoff and Overcashier 2002; Abdelwahed et al. 2006). In contrast with freeze drying, spray drying is a continuous process. Other advantages are the low

prize and the speed compared to the freeze drying process. Spray drying required addition of drying auxiliary compounds in the nanoparticle dispersion prior to be processed in the spray drier (Müller et al 2000; Tewa-Tagne et al. 2007).

5 Integration of Required Functionalities

Challenging functions need to be associated to nanoparticles to be applied as drug carriers. (1) The polymers should be non toxic, biocompatible and biodegradable if the nanoparticles are designed for parenteral administration. This considerably limits the number of suitable polymers (Vauthier and Bouchemal 2009). (2) Drug association must be efficient. Many types of drugs are interesting to associate with nanoparticle drug carriers. This includes small and large molecules as well as hydrophilic and hydrophobic compounds. By combining the choice of polymer composing nanoparticles and method of nanoparticle preparation almost all types of drugs can be associated with nanoparticles based on their physico-chemical properties. Use of cyclodextrin may improve drug loading if necessary (Duchene et al. 1999). However, remaining challenges are to increase the amount of drug actually associated with the nanoparticles (drug payload) and to reduce drug leakage from the nanoparticles before they reach the target site (Li and Huang 2008). (3) Nanoparticles should transport active drug from the site of administration to target site. In general, drugs associated with nanoparticles are well protected against degradation. However, the most challenging and motivating part of the development of nanoparticle drug carriers is to achieve transport to a specific target. The method is to add specific equipments on the drug carrier surface. This equipment has a dual role. First, it insures nanoparticles to remain well dispersed including in biological media. Second, it confers specific capacity of the carrier to recognize the pharmacological target. As obvious, one of these equipments is the targeting moiety which allows the nanoparticles to recognize target tissue and target cells with a high specificity. For instance, nanoparticles can be decorated with folic acid to target cancer cells over-expressing the folic acid receptor at the cell surface (Xia and Low 2010) but antibodies can also be used to this aim (Nobs et al. 2004a). Nanoparticle platforms with cyclodextrin and biotin residues at the surface were created to facilitate further attachment of targeting moieties (Gref et al. 2003; Nobs et al. 2004b). In addition to the targeting moiety and for all nanoparticles designed to target tissues outside the mononuclear phagocyte system, nanoparticle surface must be masked with a protective coating conferring the nanoparticles the required stability in the blood and a low capacity to activate the complement system (Vonabourg et al. 2006; Vauthier et al. 2011). Typical material used are poly(ethylene glycol) and polysaccharides (Li and Huang 2010; Romberg et al. 2008; Labarre et al. 2005). The chain density should be high enough to hamper accessibility of the nanoparticle surface to large proteins such as those implicated in the activation of the complement system (Gref et al. 2000; Vauthier et al. 2011). With polysaccharide coatings, an additional effect of the chain conformation was highlighted

(Labarre et al. 2005; Bertholon et al. 2006). (4) Once the drug carrier has reached the target site, the cargo should be released. The main challenge in this task is that the drug will be retained by the formulation all along the way from the site of administration down to the target site. Indeed, burst release from nanoparticles is often pointed out as a problem which compromises the delivery of enough amount of drug to the target because of the premature leak of the carrier. New formulation approaches integrate stimuli responsive materials allowing triggered drug release. Thus, the drug remains associated with the carrier until it is triggered by local in vivo variation of either temperature, pH or electrolyte concentrations due to the physiopathology of the targeted area. The drug release can also be triggered from the outside of the body using the action of a magnetic field or of the illumination by light (Bawa et al. 2009). Design of nanoparticles with such properties are feasible with the recent development of stimuli-responding polymers (Stuart et al. 2010) but lots of efforts are still needed to make these materials acceptable for a safe use in drug delivery formulations. (5) By integrating both drug delivery properties and contrast agent properties in a single nanoparticle, this more complex nanoparticulate drug delivery system is ready to enter the era of theragnostic. A couple of prototypes have been designed and it can be expected that multifunctional nanoparticles will be the next step in the development of drug delivery systems (Schärtl 2010).

6 Conclusion and Perspectives

Numerous methods can be used to produce nanoparticles from preformed polymers to improve drug delivery. Several methods are ready for large scale production. Purification, freeze drying and sterilization still need improvements to make possible treatment of large batches and to prevent aggregation. Future developments of nanoparticles for drug delivery are going towards multifunctional nanoparticulate systems eventually integrating stimuli responsive functionalities. The methods described in this chapter should easily be applicable with minor modifications with the new materials to produce the next generation of nanocapsules and nanospheres.

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