Pharmaceutical Research, Vol. 26, No. 5, May 2009 (© 2008)
DOI: 10.1007/s11095-008-9800-3

Expert Review

Methods for the Preparation and Manufacture of Polymeric Nanoparticles

Christine Vauthier[1,2] and Kawthar Bouchemal[1]

Received September 17, 2008; accepted December 1, 2008; published online December 24, 2008

Abstract. This review summarizes the different methods of preparation of polymer nanoparticles including nanospheres and nanocapsules. The first part summarizes the basic principle of each method of nanoparticle preparation. It presents the most recent innovations and progresses obtained over the last decade and which were not included in previous reviews on the subject. Strategies for the obtaining of nanoparticles with controlled *in vivo* fate are described in the second part of the review. A paragraph summarizing scaling up of nanoparticle production and presenting corresponding pilot set-up is considered in the third part of the review. Treatments of nanoparticles, applied after the synthesis, are described in the next part including purification, sterilization, lyophilization and concentration. Finally, methods to obtain labelled nanoparticles for *in vitro* and *in vivo* investigations are described in the last part of this review.

KEY WORDS: gel; nanocapsules; nanospheres; polyelectrolyte complex; polymerization; precipitation; purification; scale-up.

INTRODUCTION

Today, polymer nanotechnologies are an important part of the more promising future to achieve drug delivery challenges such as those based on drug targeting and on the delivery of undeliverable molecules such as oligonucleotides or RNA interfering effectors (1–7). Methods for the preparation of nanoparticles are an important part if this challenge. They allow to make polymer nanoparticles with suitable properties to ensure proper drug delivery and targeting. Thanks to progress in polymer chemistry and in polymer colloid physico-chemistry, it is now possible to prepare polymer nanoparticles with a wide range of properties under controlled conditions (8,9). Also the possibility to synthesize polymers with well controlled structures and composition paves the road to the obtaining of nanoparticles with finely tuned properties which are requested to achieve the goal of drug targeting.

The aim of this review is to summarize the different methods of preparation that have been proposed so far to prepare polymer nanoparticles for the *in vivo* delivery of drugs. It starts by giving the general principles of each method and highlighting the main parameters that govern nanoparticle formation and their physico-chemical characteristics. This part of the review updates previous reviews made on the subject and it points out the more recent progresses brought in the different methods (4,10–17). The review also focuses on works which aim to scale up the production of nanoparticles designed for pharmaceutical applications. Another part of the review presents post synthesis treatments that are applied to package nanoparticles as a medicine. For instance, this includes purification, sterilization, lyophilization and concentration methods. Finally, the last part of the review describes how the nanoparticles can be labelled to become detectable during *in vitro* and *in vivo* evaluation experiments. The three last parts include new subjects that were never reviewed before and compose the most original part of this review.

[1] CNRS UMR 8612, Université Paris Sud-11, 92296, Chatenay-Malabry, France.
[2] To whom correspondence should be addressed. (e-mail: christine.vauthier@u-psud.fr)

DEFINITIONS, STRUCTURAL FEATURES AND MATERIAL COMPOSING NANOPARTICLES

Nanocapsules and Nanospheres: Definitions and Structural Features

Nanocapsules differ from nanospheres in that they are a reservoir form, in which a solid material shell surrounds a core which is liquid or semisolid at room temperature (15–25°C). In the first nanocapsule formulations, the core was composed of oil hence allowing a high payload of a liposoluble drug encapsulation. Nanocapsules with an aqueous core able to encapsulate water-soluble compounds were developed more recently. The content of the nanocapsules is determined by the nature of the dispersed phase of the emulsion or of the microemulsion constituting the basis of the formulation (Fig. 1). Generally, the polymer shell surrounding the liquid core is formed thanks to polymerization taking place at the interface between the dispersed and continuous phase of the emulsion (18–26) or by precipitation of a preformed polymer at the surface of emulsion droplets (27–30).

Nanospheres are matrix particles, ie particles whose entire mass is solid. These particulate systems are characterized by a size ranging from several tenths of nanometres to a

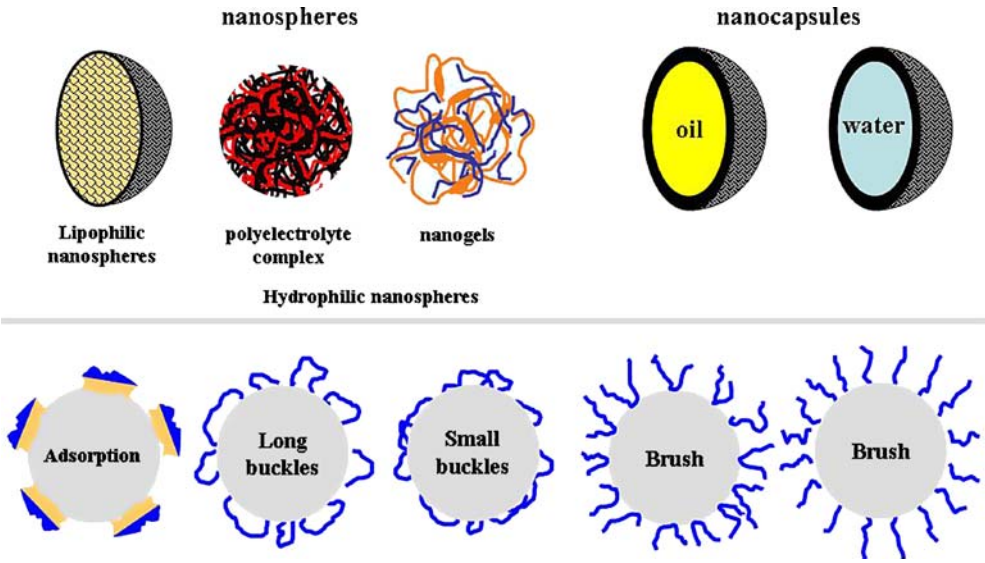

Fig. 1. Structure of the different types of polymer nanoparticles (*upper part*) and steric arrangement of coating material made of amphiphilic block copolymers of different structures (*lower part*).

few hundred of nanometres. In general they are of spherical shape but nanospheres with shape different from a sphere were described in the literature (31–33). To remain well dispersed in liquid dispersion, nanoparticles, like all types of colloids, need to be stabilized using amphiphilic molecules or colloid protecting agents. Nanospheres and nanocapsules designed as drug carriers can be loaded with drugs. Drugs can be either entrapped inside the nanoparticles or adsorbed on their surface. In general, fragile molecules are better preserved from enzymatic degradation occurring in biological medium when they are entrapped in the nanocarrier (20,34). In this case, their association with the drug carrier should be done during the preparation of the nanocapsules or the nanospheres (19,20,35–39). However, when the drug is highly susceptible to degradation which may occur during the preparation process of the drug carrier or when it does not associate during the preparation of the drug carrier, it can be loaded by adsorption on the surface of already prepared carriers (40–42).

Polymers Used to Design Nanoparticles for *in vivo* Delivery of Drugs

Considering the potential offered by polymer chemistry today, there are only a limited number of polymers which can be used as constituent of nanoparticles designed to deliver drugs *in vivo* (15,43–46). To explain this fact, one should consider that a suitable polymer needs to fulfil several requirements to be used in such an application. Firstly, it needs to be biodegradable or at least totally eliminated from the body in a short period of time allowing to repeat administration without any risk of uncontrolled accumulation. Secondly, it must be non toxic and non immunogenic. Its degradation products, if any, must also be non toxic and non immunogenic. Thirdly, it should be formulated under the form of polymer nanoparticles with suitable properties regarding the drug delivery goal for which the nanoparticles are designed. The Table I gives a list of the most widely used polymers in the composition of nanocarriers. At present, only a few of them are accepted by health authorities for parenteral administration. Others received agreements to be used in oral or topical formulations or they are used in the food industry. During the last decade, a large number of copolymers including one part of poly(ethylene glycol) or polysaccharides were developed. The rational behind the development of these copolymers came out from the need of nanoparticles with tuneable surface properties to modulate their interactions with blood proteins and with mucosa hence controlling their *in vivo* fate (6,47–49). The designed copolymers were also proved efficient to be used as stabilizers to insure nanoparticle stability without the need of other additional surfactants (45,50–52).

PRINCIPLE OF METHODS OF PREPARATION OF DRUG-LOADED NANOPARTICLES

Many methods for the preparation of nanoparticles include two main steps. The preparation of an emulsified system corresponds to the first step while the nanoparticles are formed during the second step of the process. This second step is achieved either by the precipitation or the gelation of a polymer or by polymerization of monomers. In general, the principle of this second step gives its name to the method. In a few cases, the nanoparticles form in the same time than the starting emulsified system. Suitable emulsified systems can be emulsions, mini-emulsions, nano-emulsions and microemulsions.

A few other methods do not require the preparation of an emulsion prior to the obtaining of the nanoparticles. They are based on the precipitation of a polymer in conditions of spontaneous dispersion formation or thanks to the self assembly of macromolecules to form nanogels or polyelectrolyte complexes from a polymer solution. These methods occurring in one main step will be explained at the end of this part of the review.

Table I. Most Widely Used Polymers Constituting Nanoparticles Designed as Drug Carriers

Material	Full name	Abbreviation or Commercial names*
Synthetic homopolymers	Poly(lactide)	PLA
	Poly(lactide-co-glycolide)	PLGA
	Poly(epsilon-caprolactone)	PCL
	Poly(isobutylcyanoacrylate)	PICBA
	Poly(isohexylcyanoacrylate)	PIHCA
	Poly(n-butylcyanoacrylate)	PBCA
	Poly(acrylate) and poly(methacrylate)	Eudragit*
Natural polymers	Chitosan	
	Alginate	
	Gelatin	
	Albumin	
Copolymers	Poly(lactide)-poly(ethylene glycol)	PLA-PEG
	Poly(lactide-co-glycolide)-poly(ethylene glycol)	PLGA-PEG
	Poly(epsilon-caprolactone)-poly(ethylene glycol)	PCL-PEG
	Poly(hexadecylcyanoacrylate-co-poly(ethylene glycol) cyanoacrylate)	Poly(HDCA-PEGCA)
Colloid stabilisers	Dextran	F68
	Pluronic F68	PVA
	Poly(vinyl alcohol)	
	Copolymers (see above)	
	Tween® 20 or Tween® 80	

Two Steps Procedure: Preparation of the Emulsified System

During the last decade methods to prepare emulsified systems have significantly evolved. Although, they still require two immiscible phases and a surface active agent, the methods used to achieve the dispersion of one phase in the other were diversified.

A new way to generate nano-emulsions was proposed using a phase inversion temperature method which uses the specific properties of polyethoxylated surfactants to modify their partitioning coefficient as a function of the temperature. Bicontinuous systems formed at a temperature close to the phase inversion temperature are broken up by dilution and temperature drop to generate oil-in-water nano-emulsions (53).

Most of the new methods of emulsifications are based on mechanical processes and are related to the high-energy emulsification techniques. They allow the preparation of emulsions with droplets of uniform size which can easily be scaled up to produce large quantities of well characterized emulsions. For instance, the design of colloidal mills allows to transform a rough emulsion in a thin emulsion with emulsion droplets of very well defined size and a narrow size distribution. The heart of a colloidal mill consists in a rotor stator device in which droplets of the pre-emulsion are deformed under a shear stress into long threads that undergo Rayleigh instability. This instability induced the breaking of the parent droplet in smaller droplets of uniform size (54,55) (Fig. 2). According to the authors, the obtained diameter of the daughter droplets is mainly determined by the applied stress and only weakly depends on the viscosity ratio between the dispersed and continuous phases (55).

Other methods are using different machines suitable to prepare emulsions with calibrated droplet size on methods based on an extrusion process. In these machines, the dispersed phase is forced to permeate through a microfiltration device which allows to extrude calibrated droplets of the dispersed phase in the continuous phase. The different apparatus differ from the design of their microfiltration unit which can take the shape of a porous membrane (56), of a series of small channels (57) or of a grid perforated with calibrated holes (57,58). A couple of works was already devoted to the understanding of the formation of the dispersed phase droplets of the emulsion by the extrusion methods. Numerical methods were applied to simulate the formation of droplets extruded from perforated grids (58). Geerken *et al.* (59) have investigated the mechanism of formation of water droplets at the micro-engineered orifice of the perforated grid during extrusion of an aqueous phase into an organic phase composed of hexane. Such fundamental works are useful to anticipate the characteristics of the emulsion produced by these techniques in the view of large scale production. The experimental set-up used by Charcosset and Fessi (56) for the preparation of nanoparticles includes a pump, a membrane contactor as the microfiltration device equipped with two manometers placed at the module inlet and module outlet, and a valve placed at the module outlet (Fig. 3). The organic phase placed in a thermostated vessel is forced to permeate through the membrane into the continuous aqueous phase by pressurising the vessel using nitrogen gas (1–7 bar) (56).

This set up was used to prepare nanoparticles by nanoprecipitation and nanocapsules by interfacial polycondensation combined with spontaneous emulsification (56). Examples of such preparations are given in Table II (56). In this case, the aqueous phase circulates inside the microfiltration device sweeping away the oil droplets which form at the outlets of the pores of the membrane.

It can be expected that further progresses on these techniques will come from the microfluidic area which is developing very fast at the moment and allows the preparation of emulsion droplets of very small size with well define characteristics.

In parallel to the development of new mechanical emulsification techniques, progresses have also been made

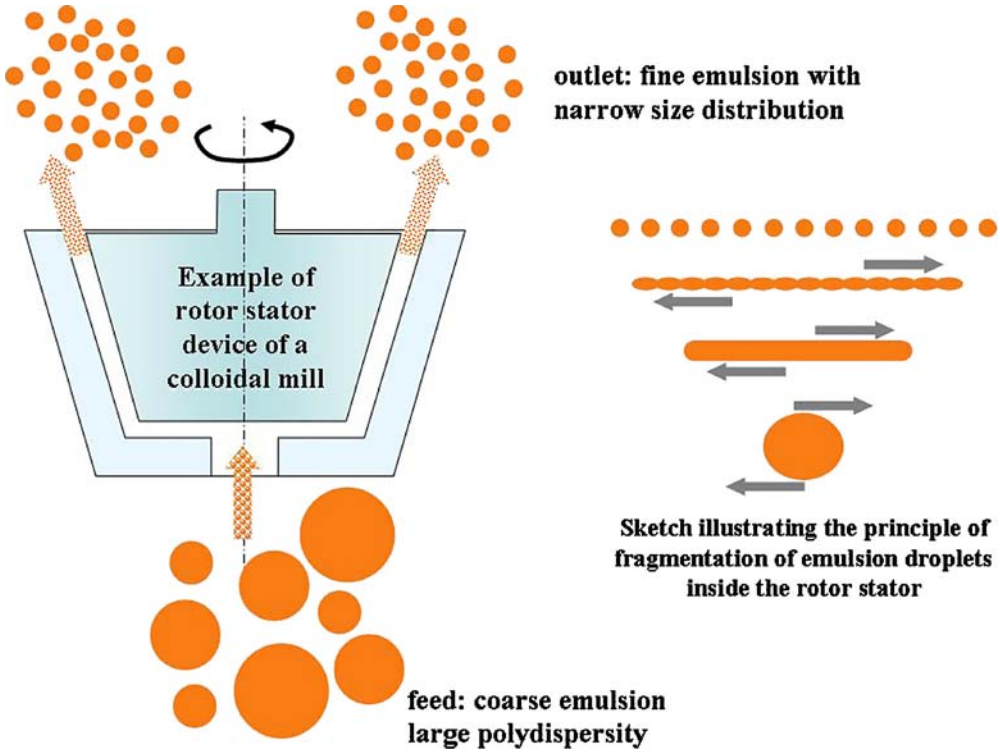

Fig. 2. Scheme of principle of the obtaining of thin emulsions with narrow size distribution using a colloidal mill.

to better understand the existing and well established emulsification methods. For instance, it was shown that the droplet size, D, of an emulsion prepared with a microfluidizer is directly proportional to the viscosity of the organic solution of polymer which is dispersed in an aqueous phase (63) (Eq. 1).

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

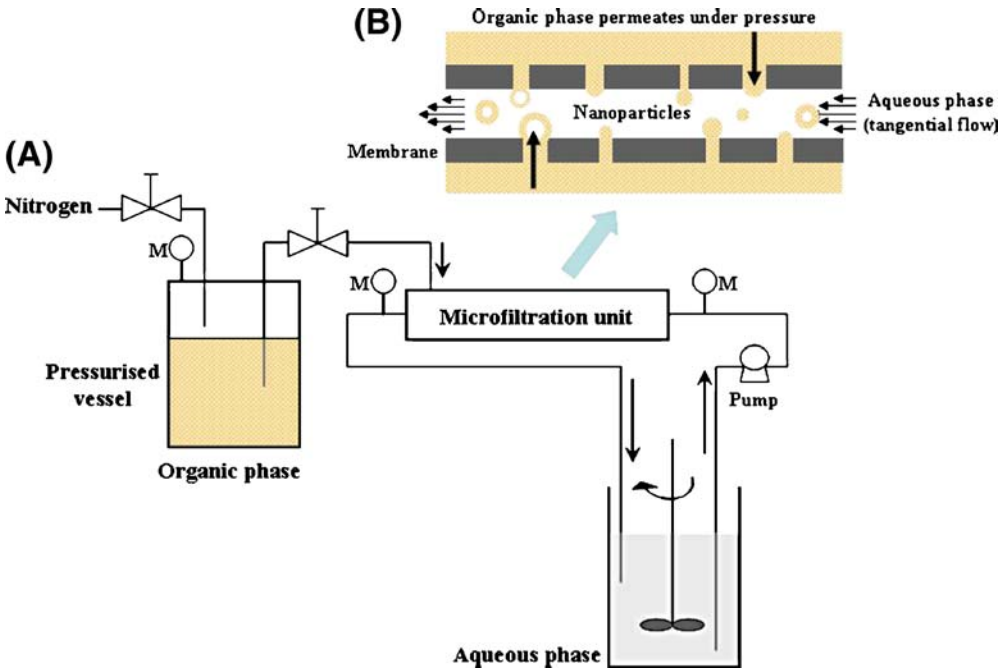

Fig. 3. Experimental set-up for the nanoparticle preparation using membrane contactor. **A** General set up. *M* Manometer. **B** Enlargement of the microfiltration unit. The aqueous phase which circulates in the microfiltration unit sweeps away droplets of organic phase which form at the pore outlet.

Table II. Formulation Used for Nanoparticle Preparations by the Membrane Contactor Technique

Method	Organic phase	Aqueous phase	References	Type of nanoparticles
Nanoprecipitation	Acetone (0.6 L) PCL (15 g)	Water (1.2 L) Tween® 20 (2.04 g)	60	NS
	Acetone (0.6 L) PCL (2.4-3 g) Labrafac® hydro (9.6-12 mL)	Water (1.2 L) Tween® 80 (2.8-4.8 g)	61	NC
	Water pH>5.2 Eudragit® L100	Water pH<2 PVA	62	NS
	Water pH<5.2 Eudragit® E100	Water pH>11 PVA	62	NS
Interfacial polycondensation combined with spontaneous emulsification	Acetone (0.6 L) Span® 80 (1.2 g) Oil: hexyl laurate (6 g) Lipophilic monomer (3.3 g)	Water (1.2 L) Tween® 20 (2.04 g) Hydrophilic monomer (19.65 g)	60	NC

NS nanospheres, *NC* nanocapsules

In this equation, η corresponds to the viscosity of the polymer solution, the α coefficient depends on the nature of the polymer. The value of alpha was found to be 0.05 and 0.28 for solutions of ethylcellulose and PLA prepared in ethylacetate respectively (63). This emulsion procedure is important because it is widely used in the process of the preparation of nanoparticles by solvent evaporation, solvent diffusion and salting out using solutions of preformed polymers.

Several progresses were made for understanding the formation of the oil droplets generated by spontaneous emulsification. This method is based on a solvent displacement phenomenon also called the ouzo or pastis effect which occurs when the two bulk phases are brought into contact without stirring during the very rapid diffusion of the organic phase prepared with water miscible solvent i.e. acetone or ethanol in the aqueous phase (64). Spontaneous emulsification processes are another important emulsification technique used in the formulation of drug carriers because it avoids the use of strong energetic method to achieve the dispersion of one phase in the other (65,66). It can be classified as a low-energy emulsification method (53). It is widely used in the procedures of preparation of nanocapsules by nanoprecipitation and interfacial polymerization/polycondensation (60–62) (Table II).

Apart from progresses coming from the introduction of new processes, novel types of emulsions have appeared composed of dispersions including two immiscible organic phases. They are interesting to promote the encapsulation of various types of compounds. For instance higher encapsulation rates of ciprofloxacine were reported using a multiple emulsion W/O/O compared with protocols based on the use of the better known W/O/W multiple emulsion (67). The use of miniemulsions, nano-emulsions and microemulsions instead of classical emulsions has been suggested (53,65,68). The main advantage of these systems is their higher stability compared with the stability of classical emulsions (Table III) hence leading to the possibility to obtain nanoparticles with better controlled particle size and improving the reproducibility of the methods of fabrication.

Table III. Main Characteristics and Differences Between Major Dispersed Systems used to Prepare Nanoparticles by Two Steps Methods (69,70)

Type	Macroemulsion	Miniemulsion	Microemulsion
Principal characteristics	Non thermodynamically stable system	Non thermodynamically stable system	Thermodynamically stable System with an interfacial tension oil/water close to zero
	Stability seconds to months	Stability hours to months Droplet growth is limited by suppression of Oswald ripening of the droplets Oil/water interfacial tension much above zero	Infinite stability upon constant storage conditions
Droplet size range (diameter)	1–10 µm	20 to 200 nm	~10 nm
	Large polydispersity	Narrow size distribution	Narrow size distribution
Equipement requirements	Simple mechanical stirrer (ultraturrax,)	Required	Form spontaneously
	Membrane contactor	High pressure homogenizeur	Simple mechanical stirrer
	Optional: High pressure homogenizeur	Ultrasounds	
	Ultrasounds	Colloidal mills	
	Colloidal mills	Or spontaneous emulsification process	

The nature of the surfactant has also evolved. The very common pluronic or span are now replaced by new amphiphilic copolymers which are composed of the polymer used to constitute the nanoparticles linked to a hydrophilic moiety chosen among poly(ethylene glycol) (44,48,71,72) or various polysaccharides (51,73). They can be used to stabilize emulsions even if they are not soluble in the liquid phases composing the emulsion (50,52). In this case, they are believed to dissolve at the interface, each part of the copolymer being soluble in one phase of the emulsion. It is noteworthy that the stability of the emulsion may depend on the nature of the solvent. For instance, in a work considering emulsions stabilized with Dextran-PCL copolymers, it was clearly showed that ethyl acetate was a more suitable organic solvent to be used to formulate the emulsion than methylene chloride leading to emulsions with a much higher stability (50). It was suggested that the stabilisation ability of such copolymers depended on the way they arrange at the surface of the emulsion droplet.

All emulsions prepared can serve as basis for the preparation of polymer nanoparticles and also of nanoparticle systems composed of lipids (solid lipid nanoparticles (SLN) and lipid nanocapsules (LNC) (53).

Two Steps Procedures: Formation of Polymer Nanoparticles from an Emulsion

Inducing Polymer Precipitation by Solvent Removal

There are several ways to provoke polymer precipitation in the emulsion droplets by removing the polymer solvent. Solvent can be extracted from the organic phase by different methods such as solvent evaporation, fast diffusion after dilution or salting out (Fig. 4). In general, these methods lead to the production of nanospheres when they are performed on simple oil-in-water emulsions (12,74–76). Oil containing nanocapsules can be obtained simply by adding oil in the polymer solution composing the emulsion droplets (28,30). It is also possible to produce water containing nanocapsules applying the methods to a multiple emulsion (29,77,78). In this case, the multiple emulsion $water_1$-in-oil-in-$water_2$ is formulated with an oil phase, composed of a polymer solution in a solvent which can easily be removed by evaporation or by the other extraction methods. In general, the nanocapsules formulated from multiple emulsions are much larger in size than the oil-containing nanocapsules obtained from simple emulsions (Fig. 5).

The nature of drug molecules which can be encapsulated in the nanoparticles prepared by this group of methods greatly depends on the type of the starting emulsion. In general, lipophilic molecules are encapsulated in nanospheres prepared from the simple oil-in-water emulsions and in oil-containing nanocapsules. Hydrophilic molecules such as peptides, proteins and nucleic acids can be encapsulated in water-containing nanocapsules.

Obtaining nanoparticles by emulsification–solvent evaporation. The obtaining of nanoparticles by emulsification–solvent evaporation was the first method of preparation of nanoparticles developed from a preformed polymer (79). Although this method was originally proposed by polymer chemists, it is in the field of pharmaceutical technology that it founds its main developments thanks to the pioneer work of Gurny *et al.* (74) who applied it to biodegradable polymers to produce drug carriers. In this method, emulsions are formulated with polymer solutions prepared in volatile solvents. Dichloromethane and chloroform were widely used in the past but are now replaced by ethyl acetate which displays a better toxicological profile. Conversion of the emulsion into a nanoparticle suspension occurs by the evaporation of the polymer solvent which is allowed to diffuse through the continuous phase of the emulsion (11,15,53). This is a slow process performed under vacuum. For instance, the complete evaporation of 10 ml of ethyl acetate from a 50 ml emulsion can take 70 to 80 min under well controlled conditions (63). It comprised a fast evaporation period lasting over 40 min and a very slow evaporation period requiring another additional 40 min to remove the last few percent of remaining solvent. As shown in the same report, the size of the emulsion droplets is dropped down during the first period of the solvent evaporation process to reach a minimal value. It corresponds to a loss of more than 90% of the polymer

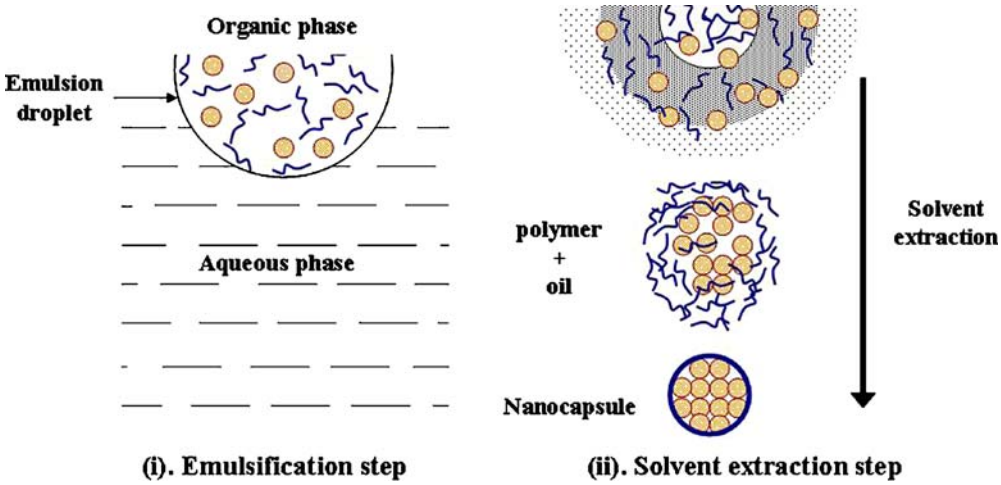

Fig. 4. Schematic description of the proposed formation mechanism of nanocapsules by emulsification (*i*) followed by solvent extraction (*ii*), where the *orange dots* and the *blue lines* are the oil droplets and polymer respectively.

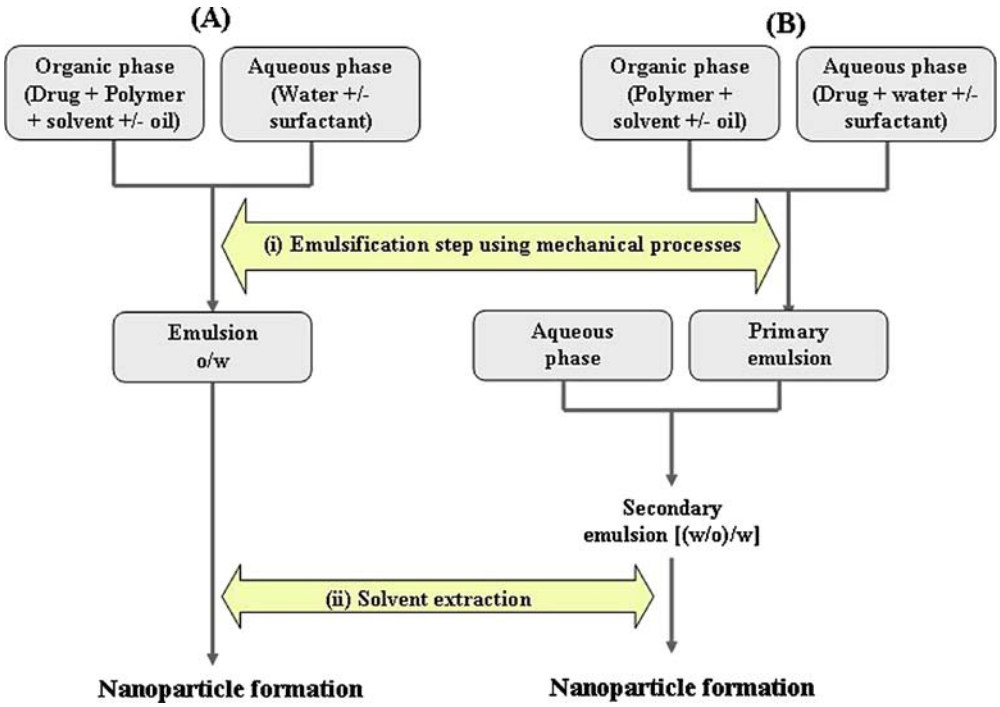

Fig. 5. Examples of single (**A**) and double (**B**) emulsion techniques for nanoparticle preparation.

solvent composing the emulsion droplets of the parent emulsion. During the second phase of the evaporation, the size of the dispersed objects increased again due to coalescence of droplets. The intensity of the coalescence phenomena which define the increase in size occurring during this stage of the process depends greatly on the nature of the polymer and more specifically on its ability to adsorb on the interface between the oil and water phase of the parent emulsion which is generally stabilized by a surfactant. The coalescence was found to be amplified with a polymer like ethyl cellulose which showed surface active properties. In contrast, it is reduced with a polymer like PLA which did not show any interfacial adsorption in emulsions formed between ethyl acetate and a water phase containing 2% *w/w* of sodium dodecyl sulphate (SDS). In this later case the increase in size of the dispersed particles during the second phase of the solvent evaporation is very low.

Solvent evaporation conditions can be modified by preparing the emulsion with partially mutual soluble solvents and removing the volatile organic solvent contained in the oil droplets of the dispersed phase by distillation (80). As the mechanism of formation of the nanoparticles seems to be very different in this case compared with the above described method, the starting emulsions can be prepared with a simple laboratory propeller stirrer working at 1,000 rpm. Nevertheless, it seems that this method provided with nanoparticles with size larger than 250 nm (80).

The emulsification–solvent evaporation method has been widely applied to prepare nanoparticles composed of PLA, PLGA and PCL using pluronic F68 as stabilizing agent (15,48,53,81). It can be apply to formulate nanoparticles with amphiphilic copolymers including PEG-PLA, PEG-PLGA, PEG-PCL, PEG-PACA and polysaccharide-PCL (48,71–73,82). In this case there is no need to add a surfactant to insure the formation of the emulsion and the stability of the final nanoparticle suspension. One advantage of using such copolymers is that their hydrophobic moiety is of the same nature than the polymer generally used to make the core of the nanoparticles, i.e. PLGA, PCL, PACA.

Obtaining nanoparticles by emulsification–solvent diffusion. The emulsification–diffusion of solvent method also called emulsification–solvent displacement has been successfully used to prepare biodegradable nanoparticles in an efficient and reproducible manner (Table IV) (16). For the success of this method, the polymer solvent used to prepare the emulsion needs to be partly soluble in water (83). Then, the emulsion is prepared with water saturated with the polymer solvent composing the oil phase and with an oil phase saturated with water as continuous phase. Mutual saturation of both polymer solvent and water is obtained by mixing the two liquids in equal volume and waiting for phase separation to collect the solvent saturated water at the bottom and the water saturated organic solvent at the top of the

Table IV. Example of Materials and Active Ingredients used in Emulsification–solvent Diffusion Method

Materials	Drug	Diameter (nm)	Reference
PLA	–	~100–450	90
	DNA	<300	35
	p-THPP	~125	93
PLGA	Doxorubicin	<1,000	94
	p-THPP	~117–118	93
PCL	–	~400–800	95
	Indomethacin	~246–671	86
Eudragit® E	–	~300–600	86

p-THPP mesotetra(hydroxyphenyl)porphyrin

resulting two phase system. Once the oil-in-water emulsion is obtained using the previous saturated solvents, it is diluted with an extensive amount of pure water. As a result of this dilution, additional organic solvent from the organic phase contained in the dispersed droplets can diffuses out of the droplets leading to the precipitation of the polymer (Fig. 6). As suggested by the work of Quintanar-Guerrero *et al*. (84), the formation of nanoparticles results from a pure diffusion mechanism. Suitable solvents include benzyl alcohol, propylene carbonate (85), ethyl acetate (80,86), isopropyl acetate, methyl acetate, methyl ethyl ketone (87), benzyl alcohol, butyl lactate (88) and isovaleric acid (89). Surfactants like pluronic F68, PVA and sodium taurodeoxycholate can be added to the aqueous phase (85,86,89,90) while soy lecithin may be added in the organic phase (89). Main suitable polymers are PLA, PLGA, PCL and Eudragit® E (83,86,91). As far as drug encapsulation is aimed, poorly water soluble drug is incorporated in the organic phase of the initial emulsion (91). Using approaches based on the formation of ion-pairs, charged hydrosoluble molecules including oligonucleotides were incorporated in PLA nanoparticles prepared by this method (77,92).

In general, the diameter of the nanoparticles produced by this method is around 150 nm (85,90). During the process of formation of the nanoparticles, the size of the initial emulsion droplets is reduced due to solvent extraction. In contrast with methods based on solvent evaporation, the extraction of the polymer solvent from the emulsion droplets occurs in a time scale of milli-seconds (30). Thus, the drop in size of the dispersed particles is abrupt. For instance, mygliol-containing PCL nanocapsules are formed in 20 ms from an emulsion prepared with a solution of PCL dissolved in ethyl acetate saturated with water in which mygliol was added and an aqueous phase saturated with ethyl acetate containing PVA. Several parameters can affect the size of the nanoparticles. The mean diameter can be significantly lowered when the miscibility of water with the organic solvent is increased (87). Size of the nanoparticles can also be reduced by increasing the stirring rate and the concentration of stabilizing agents added in the emulsion. On the contrary, the size of the nanoparticles is increased by increasing the concentration of polymer in the emulsion droplets (85,90). In this case, it is also accompanied by an increase of the polydispersity of the size distribution. Modification of parameters such as the viscosity, the pH of the external phase and volume ratio between the organic and aqueous phases have a limited influence on the size of the nanoparticles produced by this method (85).

Nanocapsules can be produced by the same method just by adding a small amount of oil in the organic phase (28,30,96).

Obtaining nanoparticles by emulsification–reverse salting-out. The emulsification–reverse salting out method is very close to the emulsification–solvent diffusion method. The main difference comes from the composition of the emulsion. The emulsion is formulated with a polymer solvent which is normally totally miscible with water, i.e. acetone (75,97). The artefact used to emulsify the polymer solution in the aqueous phase consists in dissolving high concentration of salt or sucrose (several mol/L) chosen for their strong salting out effect in the aqueous phase. Examples of suitable electrolytes are magnesium chloride, calcium chloride, and magnesium acetate (97). These components retain the water molecules for their own solubilisation; hence modify the miscibility properties of water with other solvents such as acetone. The precipitation of the polymer dissolved in the droplets of the emulsion can be induced through a reverse salting out effect which is simply obtained by dilution of the emulsion with a large excess of water. Indeed, the dilution produces a sudden drop of the concentration in salt or sucrose in the continuous phase of the emulsion inducing the polymer solvent to migrate out of the emulsion droplets.

In practice, mechanical processes are used to prepare the emulsion. A Colloid stabilizer such as PVA can be added to the aqueous phase to improve the stability of the final polymer nanoparticles produced during the process (75,97). Once the emulsion is done, it is diluted with a sufficient amount of water to provoke acetone diffusion into the aqueous phase, inducing the polymer precipitation resulted in nanosphere formation. The nanoparticles are then purified by eliminating both the polymer solvent and the salting-out agent. This method can be used to encapsulate lipophilic drugs which are generally added in the polymer solution (98,99).

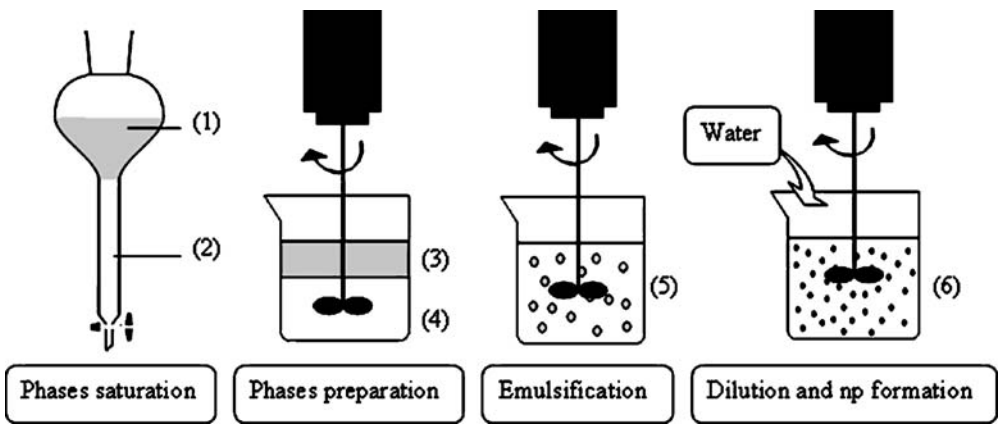

Fig. 6. Sketch of the manufacturing of nanoparticles (*np*) by the emulsification–solvent diffusion method. (*1*) Partially water miscible solvent saturated with water. (*2*) Water saturated with solvent. (*3*) Organic phase composed of polymer (2% *w*/*w*) and oil (5% *w*/*w*) in (10 mL) of phase (*1*). (*4*) Aqueous phase composed of water (40 mL) and surfactant (2.5% *w*/*w*). (*5*) Emulsification. (*6*) Dilution with 200 mL of water.

Obtaining Nanoparticles by Gelation of the Emulsion Droplets

Other methods to produce nanoparticles from emulsion are to gelify the polymer dissolved in the emulsion droplets. This method can be applied with polymers displaying gelling properties. Most of the polymers which were used in such a method of preparation of nanoparticles for drug delivery so far were soluble in water (32,100,101). However, the method can theoretically be applied to gelling polymers solubilizing in organic solvents.

The gelling properties of the polymers defined the procedure which will be followed to obtain nanoparticles from the emulsion. With a polymer like agarose, gels can be formed by cooling down the temperature of the solution which is prepared at a high temperature (100). In this case, emulsions are prepared at high temperature. The nanoparticles form by cooling down the emulsion resulting in the gelation of the emulsion droplets. With other polymers like alginate and pectin, gels form by adding a second component or by modifying the pH of the polymer solution. In this case, two different emulsions will be prepared, one containing the gelling polymer in the dispersed phase and the other containing the gelling agent or the pH controlling agent in the dispersed phase (101). The two emulsions will be mixed together under strong agitation to enhance collisions between droplets which are required to induce the gelation of the polymer; hence formation of nanoparticles. When it is applied with water soluble polymers, this method allows the formation of hydrogel nanoparticles in which hydrophilic drugs such as insulin can be encapsulated (102).

Inducing Nanoparticle Formation by "*in situ*" Polymerization

Polymerization of Alkylcyanoacrylates

To produce nanoparticles by *in situ* polymerization, a monomer is added in the emulsion instead of a polymer solution and the polymer forms by polymerization to give birth of nanoparticles. While very well characterized nanospheres can be produced by polymerization of various types of monomers in emulsions or miniemulsions (68,103–106), nanocapsules are obtained by performing interfacial polymerization or polycondensation reactions in emulsions or in microemulsions (18,20,25,26,107–109). So far, only a few monomers were found suitable to produce nanospheres and nanocapsules for *in vivo* applications as drug delivery carriers. An abundant literature is devoted to nanoparticles made from alkylcyanoacrylates (see for example 110). All kinds of nanoparticles, i.e. nanospheres, oil-containing nanocapsules and water-containing nanocapsules, can be prepared by *in situ* polymerization of such monomers (Fig. 7). This is an advantage because a wide range of drugs can be associated with poly(alkylcyanoacrylate) nanoparticles. This actually includes small and large drug molecules which are either hydrophilic or lipophilic (15,110,111). The different methods of polymerization developed to prepare poly(alkylcyanoacrylate) nanoparticles are now quite well understood leading to the obtaining of nanoparticles with very defined properties. The majority of poly(alkylcyanoacrylate) nanospheres and nanocapsules are obtained by the anionic polymerization of the corresponding monomer which is spontaneously initiated by hydroxyl groups of water or by any types of nucleophilic groups found on molecules dissolved in the polymerization system.

In general, emulsion polymerizations used to produce nanospheres is performed in acidic conditions to slow down the rate of the anionic polymerization and allow nanospheres to form instead of polymer aggregates. Nucleophilic components initiating the polymerization of alkylcyanoacrylates can be included in the structure of the polymer forming the nanospheres. They can be used to functionalize the nanoparticles (112,113) or to synthesize amphiphilic copolymers which in turn serve as stabilizing agents for the nascent polymer nanoparticles (105,114,115). Very strong acidic polymerization media, i.e. nitric acid 0.2 M, are required to initiate redox radical polymerization using a redox couple including cerium IV and polysaccharides (104,116). In this case, the polymerization is initiated on the chain-end of the

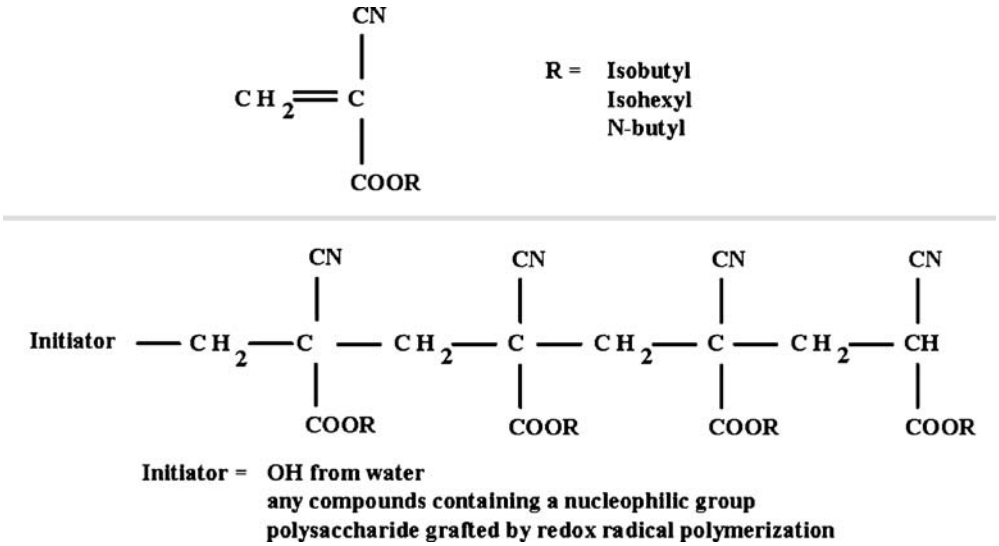

Fig. 7. General structure of alkylcyanoacrylate monomers (*upper part*) and of their corresponding polymer (*lower part*).

polysaccharide. The structure of the resulting amphiphilic copolymers is very different from those obtained by anionic polymerization (105). This is interesting because nanospheres with finely tuned surface properties can be obtained in an easy way simply by changing the conditions of polymerization. Indeed, surface properties can be adjusted by choosing the nature of the polymer stabilizing agent serving as coating material (106,117–121) and by controlling the conformation taken by the polymer chains of the coating material defined by the structure of copolymers and in turn by the polymerization mechanisms, i.e. anionic or redox radicalar (105). Having easy method to modify surface properties of nanoparticles is advantageous because they are critical parameters to control the *in vivo* fate of nanoparticles as drug carrier after *in vivo* administration (106,122–124).

Although to obtain nanospheres it is necessary to slow down the anionic polymerization of the alkylcyanoacrylate monomers, the capacity of these monomers to develop extremely rapid polymerizations is advantageous to achieve successful encapsulation of drugs within nanocapsules. High payload of lipophilic drugs can be encapsulated in oil-containing nanocapsules prepared by interfacial polymerization of alkylcyanoacrylate but hydrophilic macromolecules such as insulin were also well encapsulated in these nanocapsules. The nanocapsules are formed by anionic polymerization of the monomers when the oil-in water emulsion is prepared by the spontaneous emulsification method (18,125). The polymerization occurs at the interface of the fine oil droplets which forms when the organic phase including a water miscible solvent, the oil and the monomer is mixed with the aqueous phase containing a surfactant like pluronic F68. In this method, critical factors are the phase separation of the oil droplets occurring during the diffusion of the water miscible organic solvent in the aqueous phase and the simultaneous precipitation of the nascent polymer at oil/water interface (126). Nanocapsule dispersions may be contaminated with nanospheres. To reduce the contamination, it can be recommended to optimize the ethanol/oil ratio of the organic phase and to acidify the organic phase in order to inhibit the start of the anionic polymerization of the monomer (21,125,126). Improvements can also be obtained by replacing ethanol by aprotic solvents such as acetone and acetonitrile which are not able to initiate the polymerization reaction (127). A wide range of oils can be incorporated in the organic phase, ranging from vegetal and mineral oils to pure compounds including ethyl oleate, the principal criterium of choice being the solubility of the compound to be encapsulated in the nanocapsules.

More recently, the use of a miniemulsion was suggested as an alternative method to prepare oil-containing nanocapsules by interfacial polymerization of alkylcyanoacrylate (128,129). The monomer is directly dissolved in the oil (mygliol), and emulsified in acidified water using ultrasounds to achieve a rapid dispersion of the oily phase. Once the miniemulsion is obtained, the pH of the aqueous phase is raised to boost the anionic polymerization of alkylcyanoacrylates which is initiated at the oil/water interface. Avoiding premature polymerization of the monomer during the preparation of the miniemulsion is a critical step in this process to avoid aggregates and to promote the formation of well characterized nanocapsules. This explains why the continuous phase of the miniemulsion should be acidified. The interfacial polymerization leads to the formation of a thin polymer membrane around the oil droplets of the miniemulsion. Compared with the previous method, oil-containing nanocapsules prepared from miniemulsions are characterized by a smaller size (diameter 100 nm instead of 250 to 300 nm). The main advantage of this technique is that the concentration of nanocapsules in the final suspension can be increased by increasing the volume fraction of the dispersed phase of the starting mini-emulsion without changing the size characteristics of the nanocapsules recovered at the end of the preparation (128).

Water-containing nanocapsules are mostly prepared from inverse emulsions and inverse microemulsions (19,20,24,39,108,129,130). Generally, the emulsions or the microemulsions are prepared without incorporating the monomer which is added to the continuous phase after preparation of the formulation. The anionic polymerization of the monomer is initiated by the contact with water molecules at the surface of the water containing droplets. The diameter of the nanocapsules depends on the type of surfactant added to the polymerization medium. The smallest aqueous containing nanocapsules having a diameter of 50 nm were obtained with poly(ethyleneoxide) lauryl ester (Brij 35) while the addition of an anionic surfactant led to the formation of nanocapsules with diameter around 300 nm (19). With non-ionic surfactants, nanocapsules with diameter ranging from 250 to 350 nm were reported (20,39,129). Aqueous-containing nanocapsules obtained from inverse emulsions and microemulsions are dispersed in oil. They can be transferred in an aqueous phase by centrifugation over a layer of an aqueous solution of surfactant (20,39). In these preparations, drugs to be incorporated in the nanocapsules are dissolved in the aqueous phase of the inverse emulsion or microemulsion. Peptides and short chains of nucleic acids including antisense deoxynucleotides, small interfering RNA can be encapsulated straightforward from an aqueous solution (34,39,108). Small molecules are hardly retained by the polymer membrane of the nanocapsules and tend to escape rapidly the aqueous core of the nanocapsules. An artefact can be used to retain small anionic molecules inside the nanocapsules by forming an ion pair with a polycation dissolved in the aqueous core of the nanocapsules (23).

Obtaining Nanocapsules by Interfacial Polycondensation Reactions

Interfacial polycondensation leads to the formation of a polymer film at the interface between two non miscible phases from the reaction of two monomers each dissolved in one phase. To obtain nanocapsules by interfacial polycondensation, the reaction is carried out at the interface of oil droplets dispersed in the aqueous phase of an emulsion which is obtained by a spontaneous emulsification technique. Typically, the organic phase is composed of a water miscible solvent, the lipophilic monomer, the oil and a lipophilic surfactant while the aqueous phase contains water, the hydrophilic monomer and a hydrophilic surfactant. As the monomers are included in the composition of both phases of the emulsion, the polycondensation can readily occur at the

interface of the oil droplets which form during the mixing of the two phases leading to spontaneous formation of the oil-in-water emulsion (Fig. 8) (25,26,131,132). The nature of the monomers used defines the type of polymer shell which forms the nanocapsule envelope. For instance, the polymeric wall of the nanocapsules can be composed of polyamides, polyurea, polyurethanes and poly(ether urethanes) (Table V) (25,26,109,133).

The nanocapsule wall thickness and porosity which are important parameters influencing loading, protection and release of drugs was found to depend on the monomer concentrations and on their molecular weight (133). Enough experimental and simulated data were collected from nanocapsule preparations obtained by this method to establish a correlation between the monomer molecular weights and concentrations and the membrane properties which include its thickness, its porosity, the polymer swelling and flexibility properties and the resistance to the storage conditions (109,127). The simulated method suggested by Bouchemal *et al.* (127) is capable of predicting the thickness of the nanocapsule wall from the concentration of the monomers. It can be shown that the thickness of the polymer wall of the nanocapsules depends on the concentration of the lipophilic monomer whereas it is independent from the concentration of the hydrophilic monomer.

The method of preparation of oil-containing nanocapsules by interfacial polycondensation is well adapted for the encapsulation of lipophilic drugs. An oily drug such as alpha-tocopherol can be encapsulated with high yield of encapsulation ranging from 65% to 92% (25,26,109). The yield of encapsulation of the nanocapsules was found to be improved by using macromers such as PEG instead of conventional small monomers like ethanediol, butanediol and hexanediol (Table V). Interestingly, the polymer wall of the nanocapsules showed important stability and good protection of alpha-tocopherol against damages caused by the effect of the temperature and the ultraviolet irradiations much better than a nanoemulsion prepared in the same conditions (109).

One Step Procedure: Obtaining Nanospheres by Methods Based on Nanoprecipitation of a Polymer

The nanoprecipitation method also called solvent displacement was developed by Fessi *et al.* (134). It is one of the easiest preparation procedure of nanospheres. Additionally to its simplicity, this procedure is reproducible, fast and economic and it uses preformed polymers as starting materials rather than monomers (15,134–136).

The basic principle of this technique is close to the one described above for the preparation of emulsions by spontaneous emulsification. Nanoprecipitation is performed using systems containing a number of three basic ingredients: the polymer, the polymer solvent and the non-solvent of the polymer. The polymer solvent is chosen among organic solvents being miscible in water and easy to remove by evaporation. For this reason, acetone is the most frequently used polymer solvent in this method (134,137,138). Sometimes, it consists in binary blends of solvents, acetone with small amount of water (137), blends of ethanol and acetone (139). To produce the nanoparticles, the polymer solution is

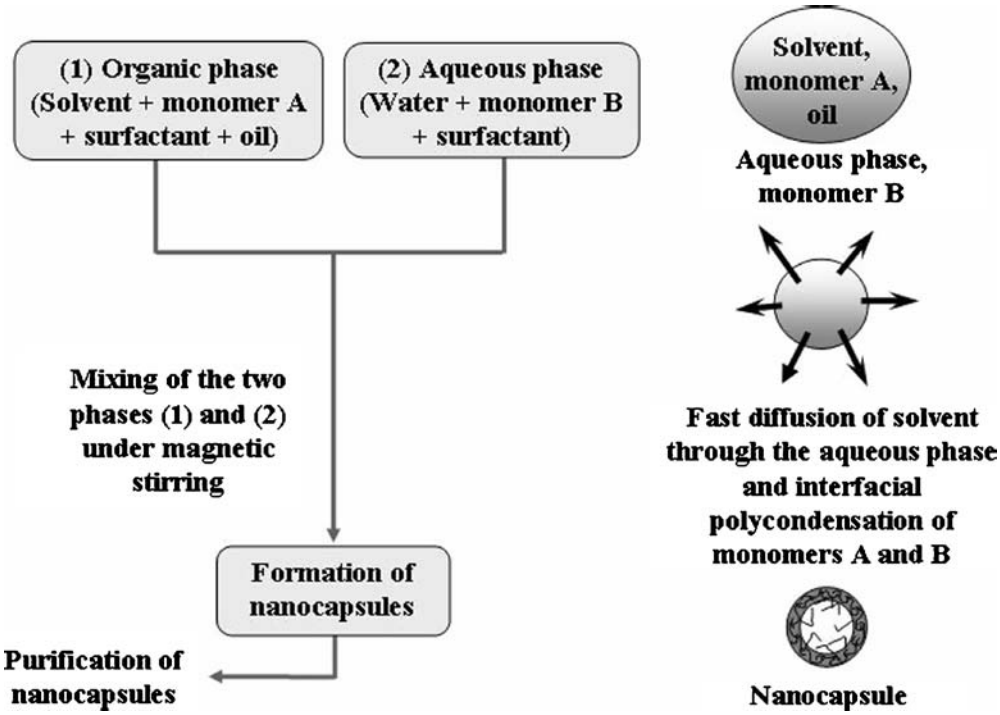

Fig. 8. Preparation of nanocapsules by interfacial polycondensation combined with spontaneous emulsification and hypothesis about the mechanism of formation. [adapted from (25)]. (*1*) Organic phase: organic solvent (40 mL), lipophilic monomer (*A*), oil (400 mg), lipophilic surfactant (86 mg of Span®85). (*2*) Aqueous phase: water (80 mL), hydrophilic monomer (*B*), hydrophilic surfactant (136 mg of Tween®20).

Table V. Nanocapsules Prepared by Interfacial Polycondensation Combined with Spontaneous Emulsification: Characteristics and Yield of Encapsulation of Alpha-Tocopherol (25,26,109,133)

Polymer	Water soluble monomer	Oil soluble monomer	Mean diameter of nanoparticles (nm)	Yield of encapsulation (%)
Polyamides	Diethlenetriamine	Sebacoyl chloride	300–500	75
Polyurea	Dipropylenetriamine	Hexamethylene diisocyanate	250–450	65-70
Polyurethanes	1,2-Ethanediol 1,4-Butanediol 1,6-Hexanediol		230–310	85–90
Poly(ether urethanes)	Poly(ethylene glycol) oligomers (M_w 200, 300, 400 and 600)	Isophorone diisocyanate	218–620	90

added in the non-solvent. The nanoparticles form instantaneously during the rapid diffusion of the polymer solution in the non solvent. The resulting colloidal suspension contains polymer particles with well defined size (typically 200 nm in diameter) characterized by a narrow distribution. They are smaller than those produced by the emulsification solvent evaporation procedure. In general, the organic phase is added to the aqueous phase but reversing this protocol by adding the non solvent phase on the polymer solution also leads to the formation of nanospheres. Important parameters conditioning the success of the method and affecting the physicochemical properties of the nanoparticle preparation are the miscibility of the organic solvent with the non-solvent and the nature of the polymer/solvent interactions. Optimal conditions of nanoprecipitation were described when the polymer was dissolved in a theta solvent and when the concentration in polymer dissolved in the solvent remained below the limit between the semi-dilute and dilute solubilization regime (138). Although a surfactant is not required to insure formation of nanospheres by nanoprecipitation, the amphiphilic character of the polymer may influence the yield of the amount of nanospheres produced during the nanoprecipitation process. For sure, it has been shown that the method is well adapted to be applied on the new generation of amphiphilic copolymers which are synthesized to formulate nanoparticulate drug carriers by nanoprecipitation (140). In previous studies, surfactants added in nanoprecipitation systems help to preserve nanoparticle suspensions from aggregation over long storage periods (134).

The method can be applied to a wide range of polymers (66) including peptides (141) and even to non polymer material such as amphiphilic cyclodextrins (142) and drugs (143,144) (Table VI). Depending on the solubility properties of the material to be nanoprecipitated, water can be replaced by ethanol, methanol or propanol (78). In general, the drug to be encapsulated in the nanospheres produced by this technique is added in the polymer solution (145–147). In general, the polymer solution and the non solvent are mixed together just by pouring one phase in the other under a gentle agitation. It can be achieved in normal laboratory glasswares and does not require any sophisticated material. Limayem *et al.* (148) have suggested to use the ultrafiltration device of a membrane contactor developed to prepare emulsions with well characterized droplets to achieve the dispersion of the polymer solution in the aqueous precipitating medium (Fig. 3). In this device, the polymer solution is forced to pass through the porous membrane to reach the aqueous phase in which precipitation of the polymer occurs in the same time the droplets are formed.

One Step Procedures: Methods Based on Self Assembling Macromolecules

Formation of Polyelectrolyte Complexes

Typical examples of nanospheres obtained from polyelectrolyte complexes are formed between polyamines and nucleic acids thanks to complementary charge annealing (7,158). These nanospheres, also named nanoplexes, are widely developed as drug carriers to make possible the *in vivo* delivery of nucleic acids including plasmid genes, siRNA and antisens oligodeoxynucleotides. In this case, the drug being the nucleic acid is incorporated in the nanocarrier as a constituent of the drug delivery device. The main formulation parameter to take into account to obtain the nanospheres within the desired size range is given by the N/P ratio which is defined as the ratio of the number of amine groups of the polycation divided by the number of phosphate groups of the nucleic acid. Thus, the "*N*" value also corresponds to the number of positive charges involved in the formation of the complex while the "*P*" value gives the number of negative charges. In general, the net charge of the polyplexe appears positive when the *N*/*P* ratio is above 1. The internal structure of polyplexes can be described as a gel like structure in which the complexed polyelectrolyte chains are swollen by water molecules. Typical polycations used to prepare polyplexes are poly(ethylenimine) (PEI), poly (lysine) (PLL), poly(ornithine) or chitosan. Some types of polyamines also include dendrimers which form very small complexes with nucleic acids.

Nanosized polyelectrolyte complexes were reported to form with other types of polyelectrolytes. For instance, Rajaonarivony *et al.* (159) have pointed out the formation of aggregates in the nanosized ranged after the mixing alginate, a negatively charged polysaccharide, with polylysine, a positively charged peptide, in very well defined concentrations of the two polyelectrolytes. A more extensive work has considered the obtaining of nanoparticles by polyelectrolyte complex formation from polysaccharides of complementary charged (160,161). The polysaccharides were dextran sulfate containing negatively charged sulfate groups and chitosan containing positively charged amino groups. In this case, the nanoparticles are elaborated by a one shot addition of the polyelectrolyte used in default in the one used in excess. According to the nature of the polyelectrolyte used in excess, either positively or negatively charged nanoparticles can be synthesized. This is an interesting property because it can affect the way the nanoparticles will interact with cells,

Table VI. Example of Materials and Active Ingredients used in Nanoprecipitation Methods

	Materials	Drug	Diameter (nm)	Reference
Synthetic polymers	PLA	Dexamethasone	~300	134
		Doxorubicin	270	149
		Insulin	~302	150
		Lysozyme	~351	150
		Sodium cromoglycate	~470–780	151
		Taxol	~260	134
		Vitamin K	~270	134
	PLGA	Cyclosporin A	~170	151
		Doxorubicin	274	149
		Indomethacin	~168	151
		Insulin	~105–170	151
			~133–173	150
		Ketoprofen	~167	151
		Lysozyme	~137–570	150
		Valproic acid	~166	151
		Vancomycin	~187	151
	PCL	Cyclosporin A	~100–200	152
	PEG-PCL-PEG	BSA	<100	153
		DMEP		
	Poly(methyl vinyl ether-co-maleic anhydride)	BSA	~279–308	154
Amphiphilic cyclodextrins		Indomethacin	~204–342	155
		Progesterone		156,157
Proteins/Gliadin		–	~100–500	141
Small hydrophobic molecules		Cholesterol acetate		143,144

PEG poly(ethylene glycol), *BSA* bovine serum albumin, *DMEP* 4′-demethylepipodophyllotoxin

hence their *in vivo* fate after administration to animals. The nanoparticles featured a core/shell structure. The hydrophobic core is composed by the complexed segments whereas the excess of component not incorporated in the polyelectrolyte complex is segregated in the outer shell ensuring the colloidal stabilization of the nanoparticles against coagulation and conferring the charge of the nanoparticle surface. The molecular weight of the two polyelectrolytes influences the size of the nanoparticles. A correlation between the chain length ratio of the polyelectrolytes and the size of the nanoparticle formed could be established on the basis of a host/guest concept. According to this concept, the polyelectrolyte of the highest molecular weight serves as host for the guest polyelectrolyte of lower molecular weight (160). The mechanism of formation of the nanoparticles was found to vary according to the polyelectrolyte used in excess during the preparation. This was attributed to the differences in chemical reactivity of the ions of the polycation in excess and to the flexibility of the macromolecular chains.

These nanoparticles were primary designed as antigen presenting device (162). In this purpose, antigenic proteins should be highly purified to insure successful loading on nanoparticles. Indeed, the binding capacity in antigenic proteins of these nanoparticles depends on the degree of purity of the protein antigen. It also depends on the charge of the nanoparticles. For instance the protein P24 from the capside protein of the HIV-1 virus associated up to a concentration of 600 mg/g protein-nanoparticle with negatively charged nanoparticles whereas lower association was reported on the corresponding positively charged nanoparticles.

Formation of Nanoparticles from Neutral Nanogels

Gref *et al.* (163) have developed polymers that can associate together to form supramolecular nanoassemblies of spherical shape (Fig. 9). One polymer consists of dextran on which alkyl chains of different length were grafted. The second polymer includes a polymer of beta-cyclodextrins. Nanoassemblies form instantaneously when the two polymers

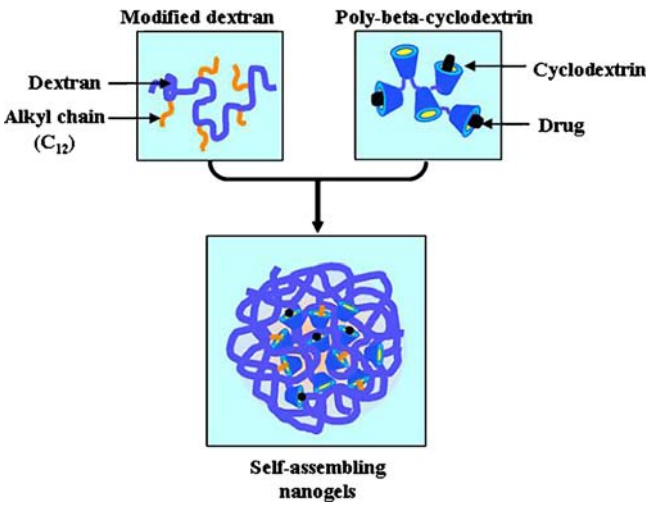

Fig. 9. Schematic representation of formation of nanogels by self assembling of neutral macromolecules. The concentration of the polymer in the suspension of nanogels ranges from 2.5% to 7.5% (*w*/*w*) and the ratio of the two polymers modified dextran/poly-beta-cyclodextrin varied from 20/80 to 80/20 (*w*/*w*) [adapted from (164)].

in solution in water are put together because the hydrophobic cavities of the poly(beta-cyclodextrin) can serve as host for the pendant alkyl chains grafted on the hydrophobised dextran. The cohesion of the whole assembly is based on a "lock and key" scheme. The submicronic nanoparticles that are formed are composed of a hydrogel in which a large amount of water is entrapped in the polymer network formed by the assemblies of the two polymers. For this reason, the resulting nanoparticles which are nanospheres were also named nanogels.

The method for the obtaining of those nanogels is very simple and not very sensitive to the protocol which was followed. Indeed, it was reported that nanogels with the same size form whatever was the manner of mixing the two polymer solutions: order of introduction of the solutions of modified dextran and poly(beta-cyclodextrin), method of mixing, temperature, etc….It seems that the simple diffusion of one solution in the other is sufficient to form the nanogels (164). The production yields can reach 95% of the incorporation of the solubilised polymer in the nanogels, showing the remarkable efficiency of the polymer association phenomena. The feasibility and stability of the nanogels were influenced by the percentage of substitution of the glucose units of dextran by the alkyl chains, the number of carbons in the alkyl chains, the polymer concentration in the solutions, the poly(beta-cyclodextrin) molar mass and the weight ratio between the hydrophobised dextran and the poly(beta-cyclodextrin) (163). The nanospheres can be freeze-dried for long term storage without the necessity to add cryoprotecting agent. Moreover, very encouraging results were recently reported on the sterilization of these nanoparticles (164).

Interestingly, hydrophobic drugs such as benzophenone and tamoxifen can be incorporated in the nanogels. Loading efficiencies up to 90% were reported. The drug can be loaded by formation of an inclusion complexes with beta-cyclodextrin residues of the poly(beta-cyclodextrin) polymer before the preparation of the nanoparticles or after formation of the nanogels. The release of the entrapped drug can be controlled over a period lasting for 16 days (164). Thus, these nanogels appear as remarkable controlled sustained release formulations under the form of nanoparticles.

One Step Procedures: Methods based on Ionic Gelation

Nanoparticles obtained from ionic gelation procedure are synthesized in totally aqueous media. They are included among the few organic solvent free methods. Ionic nanogels can be obtained from aqueous solutions of charged polysaccharides which gel in the presence of small ions of opposite charges. The gelation of the polysaccharide should be performed in very dilute solution using concentrations of the gelling agent below the gel point. This corresponds to the pre-gel phase in which the chains of the polymer reacting with the gelling agent are forming small clusters that can be highlighted by electron microscopy or by a clear reduction of the viscosity of the polysaccharide solution. Clusters formed in the pre-gel phase are stabilized by forming complex with opposite charged polyelectrolytes. Using alginate, the gelation is induced with calcium and the pre-gel phase is then stabilized with polycations like polylysine (159) and chitosan (165–167). Considering the alginate nanoparticles, it can be pointed out that the size of the nanoparticles greatly depends on the concentration of alginate and also on the molecular weight of the polylysine used to stabilize the nanoparticles (168) (Fig. 10). Although alginate complexed with polylysine alone can form nanoparticles based on the formation of a simple polyelectrolyte complex, the formation of a pre-gel phase with calcium before the addition of polylysine allow the obtaining of a more compact structure of the nanogel. This was indicated by the large difference in size between the two nanoparticular systems that formed in each case (159). De and Robinson (165) have identified an optimal mass balance between sodium alginate:$CaCl_2$:cationic polymer (poly-L-lysine [PLL] or chitosan) to obtain nanospheres. This mass balance: 100:17:10 ensured that the calcium alginate is maintained in the pre-gel phase and sufficient cationic polymer is present to form nanospheres. At low cationic polymer concentrations, nanospheres are not formed, whereas microspheres are formed at higher concentrations. Alginate nanoparticles can be loaded with oligonucleotides (169,170) and with peptides (167) by means of ionic interactions with the nanoparticle components. In contrast to what was expected, the loading of the alginate nanoparticles with oligonucleotides was mediated by interactions with calcium ions while interactions with polylysine were only marginal (169). The zeta potential of the oligonucleotides-loaded nanoparticles is negative which is in contrast with most of the other drug carriers of nucleic acids but open better perspectives for their *in vivo* application. Regarding the loading of the nanoparticles with peptides, the association efficiency of insulin into alginate nanoparticles and loading capacity were found to be mainly influenced by the alginate: chitosan mass ratio (167).

Chitosan is the second gelling polysaccharide which was used to produce nanoparticles through a gelling process. Conversely to alginate, it is positively charged in an aqueous solution of neutral pH. Ionic gels can form by adding small polyphosphates ions like tri-polyphosphates. As in the case of alginate, chitosan nanoparticles were obtained from diluted solutions of chitosan which gelation was induced by small amounts of tri-polyphosphate to remain in the pre-gel phase of the gelation process. The nanoparticles are stabilized by copolymers of poly(ethyleneglycol) and poly(propyleneglycol) (pluronic) (171,172). The size of the nanoparticles which form is not influenced by the concentration of the polyphosphate but it increases above a certain concentration of chitosan (172). Such nanoparticles are capable of swelling and shrinking as a function of the pH and ionic strength of the dispersing medium. A modification of pH from acid to basic

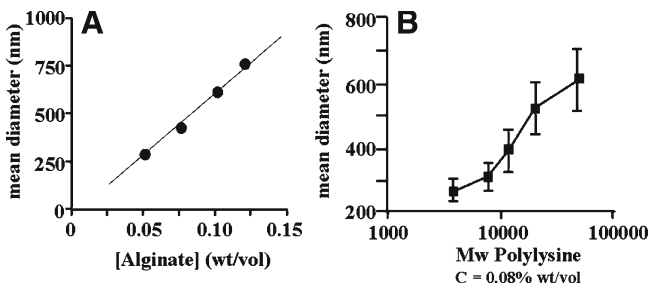

Fig. 10. Effect of the concentration in alginate (*A*) and of the molecular weight of polylysine (*B*) on the diameter of the alginate nanoparticles obtained by ionic gelation (168).

values caused a shrinking on the gel because the intramolecular electric repulsions inside the particle mesh are reduced. Indeed, glucosamine groups of chitosan are deprotonated by raising the pH. Ionic strength variations also induced important structural changes in the nanoparticles. For instance, nanoparticles swell by the addition of low to moderate concentration of KCl in the dispersing medium. The swelling process can even rapidly turn on particle disintegration due to the weakness of chitosan-TPP ionic interactions (173). These properties may be used to trigger the release of a drug encapsulated in the nanoparticles upon the action of a pH or an ion concentration variation stimulus. The chitosan nanoparticles prepared by ionic gelation were mostly investigated for the delivery of therapeutic agents like peptides (174–175) and nucleic acids (176–178) by routes of administrations involving a transmucosal transport of the drug (178–180).

OBTAINING DRUG-LOADED NANOPARTICLES WITH CONTROLLED *IN VIVO* FATE

The most challenging goal motivating the development of nanoparticle drug delivery systems is to achieve a perfectly control of the bioavailability of the drug at the site where the therapeutic activity is needed. This implies that the drug carrier needs to be programmed to reach the desired tissues and cells in the body and even, in certain cases, the relevant intracellular compartment. A way to achieve this goal is to control interactions occurring between drug carriers and biological barriers so that a maximum of the dose of the administered drug will be conveyed down to the target site. The number and types of barriers that the drug needs to cross over to reach their target site depend on the route of administration of the formulation. The physical barriers require that the drug needs to be transported across a physical obstacle such as the digestive epithelium, the endothelium of the blood vessels and the cell membranes. The chemical and biochemical barriers are generally considered as obstacles responsible for premature degradation of drug molecules requesting their association with a drug carrier to improve their stability in the body fluids. However, they are also involved in the opsonisation mechanisms of nanoparticles and in the activation of the complement system which occur in the blood and highly influence the fate of the drug carrier in the blood compartment.

According to these considerations, most studies aiming to design nanoparticles to improve the *in vivo* performance of the drug delivery approach were primary focused on the route of administration. The most advanced studies concerned the delivery of drugs by the intravenous route but much work has also been done to achieve controlled drug delivery through mucosa, especially by the oral and nasal route of administration. As a general rule, small enough particles can diffuse through different biological barriers allowing transport of the drug from the administration site down to the target tissue and/or cells (181). Apart from the size, surface properties are key factors controlling the *in vivo* fate of the nanoparticles. Indeed, the nanoparticle surface is in first line to interact with biological components of the surrounding biological medium. Thus, nanoparticles with controlled *in vivo* fate need to show very well defined surface properties which suit with the drug delivery goal. This may require the design of nanoparticles with different levels of sophistication of the nanoparticle surface which depend on the route of administration and the targeted goal.

As illustrated by the different examples summarized in Table VII and Fig. 1 (lower part), surface properties of nanoparticles will greatly depend on the nature of the component exposed at the nanoparticle surface. For this reason, nanoparticles are now generally prepared using amphiphilic copolymers in which one part is included in the nanoparticle core and the other part confers the nanoparticle surface with the desired properties regarding *in vivo* application requirements. The structure of the copolymer is influencing the spatial arrangement of the chains of the hydrophilic part which are anchored on the nanoparticle surface by the hydrophobic moiety (Fig. 1 lower part). For instance, the grafting of thiomers at the nanoparticle surface improved the interactions of the modified nanoparticles with the intestinal mucosae by enhancing

Table VII. Influence of Nanoparticle Coating Properties on the *in vivo* Fate of Nanoparticles After Intravenous and Mucosal Administration

Coating material	Method of association	Properties and Interest	Reference
Pluronics	Adsorption	Short half life in the blood (several min)	189
		Activation of complement system	
		Targeting of organs of MPS	
PEG	Incorporation as copolymer	Half life in the blood of several hours (Stealth)	
	Brush conformation	Low complement activation capacity	190
	Loop *vs* brush conformation	Targeting of the brain	191
		Modulation of complement activation capacity	121
Polysaccharides	Incorporation in copolymer	Low complement activation	106,118,122
	Brush conformation	Half life in the blood of several hours (Stealth)	73,106,118,192
	Loop conformation	High complement activation	
		Short half life in the blood (several min)	
PEG+targeting moiety	Chemical grafting of targeting moiety	Half life in the blood of several hours (stealth)	183,184,193
		Achievement of targeting of specific cells *in vivo*	
Chitosan	Adsorption	Promote adhesion to mucosae	176,194
	Incorporation in copolymer		
Thiomer	Adsorption	Promote adhesion to mucosae	195
	Incorporation in copolymer	Increase intestinal epithelium permeability by opening of the tight junctions	124 123

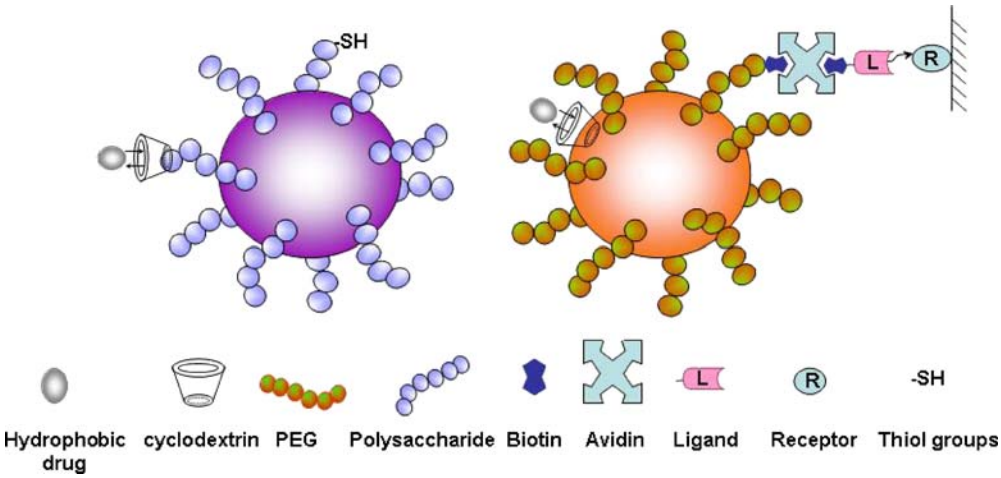

Fig. 11. Schematic representation of different functionalities which can be attributed to nanoparticles (Adapted from 196).

the permeability properties of the epithelium (124). Such a surface modification also induced modification of the bioadhesive properties of the carrier compared with nanoparticles not coated with thiomers. Surface modifications applied to nanoparticles designed for the intravenous route play an important role controlling the biodistribution of the carriers. They regulate protein adsorption phenomena which are involved in the opsonisation of foreign bodies entering in the blood and which are part of the defense mechanisms of the body (118). The coating of nanoparticles with poly(ethyleneglycol) chains considerably reduces the opsonisation of the nanoparticles and the rate of the complement system activation. As a consequence, the nanoparticles are less recognized by the macrophages of the mononuclear phagocyte system and can remain in the blood stream and distribute in tumors located outside organs of the mononuclear phagocyte system (liver and spleen). According to a recent study, the conformation of the hydrophilic polymer chains at the nanoparticle surface can dramatically influence the capacity of the nanoparticles to activate the complement system; hence the *in vivo* fate after intravenous administration (106,182). The more spectacular effect is given by the example of nanoparticles coated with dextran. Although the nanoparticles coated with dextran chains taking the conformation of loops are highly activating the complement system and concentrate in macrophages of the mononuclear phagocyte system, the nanoparticles coated with the same dextran in a hairy conformation are able to escape the intense capture by macrophages and can remain in the blood stream for a longer period of time (122,192).

Additional functionalities and specific targeting ligands can be further added at the nanoparticle surface to promote interactions with very well defined target cells (Fig. 11). In this case, they can be either grafter on already prepared nanoparticles or incorporated in the copolymer used to formulate the nanoparticles (183–185). In the more recent works, it is suggested to prepare nanoparticles with molecules serving as platform to anchor any types of ligands depending on the requirements of the *in vivo* application. Examples of suitable molecules are cyclodextrins and biotin (183,186–188).

Cyclodextrins form inclusion complexes with small hydrophobic molecules. To use cyclodextrins as anchor point of a ligand on the nanoparticle surface, the small hydrophobic molecule making inclusion complex with the cyclodextrin is grafted on the ligand (187,197). In the case the anchorage platform is composed by a biotin residue, the ligand is attached on the nanoparticle surface via avidin-biotin complex formation (188,198). Neutravidin can be grafted at the nanoparticle surface as alternative to biotin (177). The characterization of different functionalities carried by the nanoparticles as well as their interactions with living systems can be achieved using different techniques including isothermal titration calorimetry (196) and Surface Plasmon Resonance (185).

PILOT SCALE PRODUCTION OF NANOPARTICLES

Although the synthesis of nanoparticles by different methods is clearly mastered at a lab scale level as explained above, the transformation from the lab production to an industrial scale lack of information in the literature concerning the manufacture of pharmaceutical grade nanoparticle suspensions. For instance, nothing is known about the transposition of the lab production of doxorubicin loaded poly(alkylcyanoacrylate) nanoparticles, Transdrug®, which are now produced in large quantities to provide with clinical batches used in the phase II/III clinical trials (199). This is also the case with the production of paclitaxel-loaded albumin nanoparticles, nab-paclitaxel or Abraxane®, used in clinics in the USA in treatment of metastatic breast cancer (200). Scale up of methods for the production of pharmaceutical grade nanoparticle suspensions were only described for two types of methods: the emulsification–solvent diffusion method and 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. Going back to the classification of nanoparticle preparation methods proposed above, there are two methods based on two steps procedures requiring the formation of an emulsion, i.e. emulsification–solvent diffusion, emulsification–reverse salting out, and one method classified in the one step procedures, i.e. nanoprecipitation. These three

methods were scaled up to a pilot-scale production which is intermediate between the laboratory and the industrial production. Pilot size production is aimed to simulate as close as possible the industrial production and hence needs to integrate all parameters that need to be optimized before reaching the industrial production.

Pilot-Scale Production of Two Steps Procedures Requiring the Preparation of an Emulsion: Emulsification–Solvent Diffusion and Emulsification–Reverse Salting Out Methods

To develop pilot production of nanoparticles by the emulsification–solvent diffusion method, the whole protocol developed on the lab scale have been reviewed and further decomposed to set up a complete procedure including the preparation of saturated solvents. The Table VIII summarises the differences in the organization and equipments used in laboratory (volume of 60 mL) and pilot scale production of the nanocapsules (volume of 2 L) (201).

The Fig. 12 presents the scheme of the pilot plant assembled to prepare nanocapsules by the emulsification–solvent diffusion technique (201,202).

In this set up, the reservoir A is on the top and the reservoir C of higher capacity is placed at the bottom of the assembly. The reservoir B is placed at an intermediate position. These positions of the three reactors allow the transfer of solution by gravity from A to B and from B to C. In the procedure, the reservoir A is first used to prepare the mutually saturated solvents. Water and partially miscible organic solvent are mixed together to achieve the mass transfer process and reach the thermodynamic equilibrium of both liquids. Then, they are let to separate to give the two mutually saturated solvents required for this method. After phase separation, the lower phase, i.e. water saturated with the organic solvent, is transferred in the reactor B through the valve V1 while the organic solvent saturated with water is retained in the reactor A. Ingredients are then added in A and in B to complete the preparation of the organic and aqueous phases respectively. For instance, polymer, oil and drugs are added in reactor A while a surfactant is added in B. The organic phase prepared in A is then transferred in B by gravity through the valve V1 and the emulsion is prepared in B under vigorous stirring (201). To obtain an emulsion with the desired characteristics, the design of reactor B and the mechanical mixer device were carefully optimized. The rational behind this choice was to reproduce as much as possible fluid motions produced in the laboratory scale set up production glass wares (202). Agitation for the emulsification is maintained over 30 min and the emulsion is then transferred through the valve V2 in the reactor C containing the amount of water required for the dilution step. This reactor has the larger capacity, 6 L in the pilot plant designed by Colombo *et al.* (201) and 15 L in the pilot plant production used by Galindo-Rodriguez *et al.* (202)

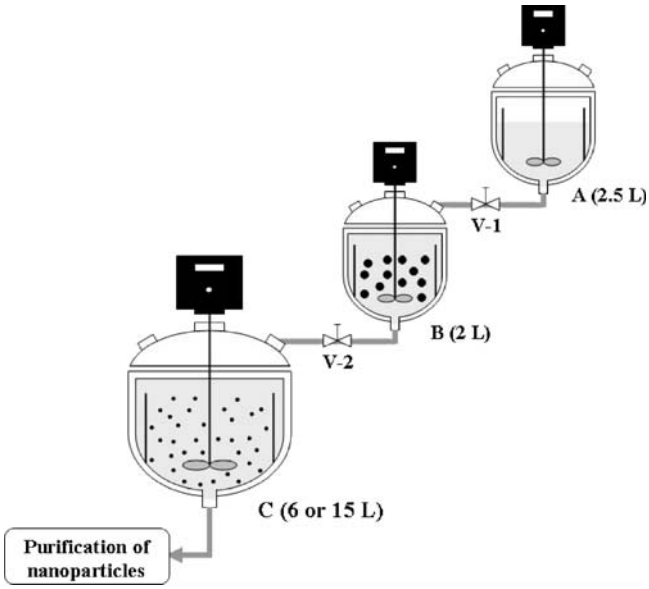

Fig. 12. Pilot set up proposed for the scaling-up of nanoparticle production by the emulsification–solvent diffusion method (Adapted from 202).

Few modifications of the pilot plant developed for the preparation of large batches of nanoparticles by the method of emulsification–solvent diffusion method were introduced to allow large scale production of nanoparticles by the emulsification–reverse salting out method. The scheme given in Fig. 13 clearly highlights these differences. Firstly, the three reactors have almost the same capacity. Secondly, positions of the reactors were rearranged making possible transfer of the content of reactor A in B and content of C in B by gravity. Finally, the procedure itself was also slightly different. The

Table VIII. Comparison of the Equipment used to Prepare Nanoparticles by the Emulsification–solvent Diffusion Method at a Laboratory and Pilot Scale Production [adapted from (201)]

	Laboratory		Pilot	
Steps	Recipient	Agitation	Reactor	Agitation
Saturation	Settling flask	Manual	Double-jacketed reactor (2-2.5 L)	Turbine, 4 paddles
Preparation of the aqueous phase	Round-bottom flask, (100 mL)	Magnetic		
Preparation of the organic phase	Beaker (150–300 mL)	Turbine		
Emulsification		Ultraturrax T25		Ultraturrax T50 + Turbine, 4 paddles
Dilution		Turbine	Reactor, (6 L)	Turbine, 6 paddles
Purification	Evaporation under reduced pressure	–	Evaporation under reduced pressure	-

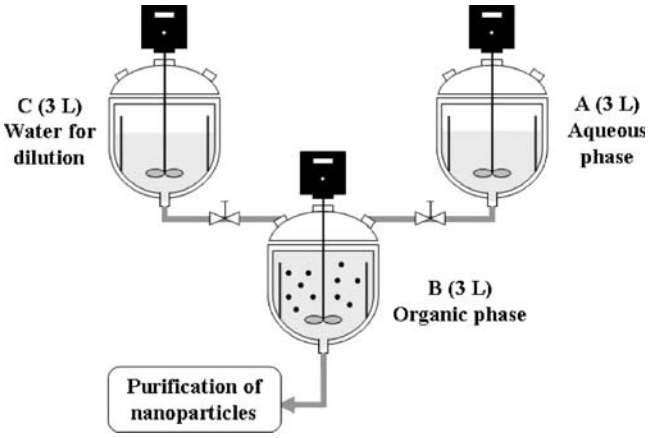

Fig. 13. Configuration of the pilot set up proposed for the scaling-up of nanoparticle production by the reverse salting-out technique (Adapted from 202).

aqueous phase (1,000 g) was prepared in A to be added in the organic phase (600 g) prepared in B. The emulsion obtained in B remained in B for the dilution step was achieved by the addition of the pure water (1,000 g) contained in C.

For these emulsion-based methods, the most important parameter which influences the size of the nanoparticles is the stirring condition applied during the preparation of the emulsion while this parameter has only a marginal effect during dilution step having no influence on the nanoparticle size (201). In the pilot plant designed to produce nanoparticles by the emulsification–reverse salting out method, the size of the produced nanospheres were ranging from 557 to 174 nm using stirring rates of 790–2,000 rpm respectively. The size of nanocapsules produced in the pilot plant set up to applied the emulsification–solvent diffusion method were ranging from 562 to 230 nm. As explained by Galindo-Rodriguez *et al*. (202), an increase in the stirring rate enhances the breakage of the emulsion droplets and leads to the formation of a thinner emulsion which in turn gives smaller nanoparticles. It was pointed out that applying a stirring rate above 1,000 rpm for the emulsification–solvent diffusion method and 790 rpm for the emulsification–reverse salting-out method gives nanoparticles with a reasonably narrow size distribution and a good reproducibility from batch to batch. This revealed that uniform emulsification process can be achieved in these conditions. Below these optimal stirring rates, the batch to batch reproducibility of the preparation of nanoparticles is poor which is believed to result from a rather heterogeneous dispersion of the organic liquid phase in the aqueous phase during the emulsification step (201).

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

In the nanoprecipitation method, the organic and the aqueous phases which are miscible are allowed to mix together producing the precipitation of the polymer as nanoparticles. In this method a critical point is the mixing of the two phases which is driven by the miscibility of the solvents. Thus, designing the pilot plant, efforts were stressed on the development of a specific mixing device allowing the two phases to come in contact and mix together with a continuous feeding process. This mixing device, which T shape was optimized, is the central piece of the pilot plant set up (202,203). The whole pilot plant includes a total of three reactors with capacities of 3 L. The reactors A and B are filled out with the stock solutions of the aqueous and organic phases respectively which are used to feed the T-shape mixing device at flow rates perfectly monitored under the control of peristaltic pumps. The outlet of the T shaped mixing device is connected with reactor C used as a receiver of the nanoparticle suspension which actually formed in the T-shaped mixing device (Fig. 14). In this configuration, the T-shaped mixing device can be continuously feed with the aqueous and organic phases insuring a continuous production of well characterized nanoparticles. The obtained raw nanoparticle suspensions collected in the reactor C present good inter-batch reproducibility. The size of nanoparticle batches produced in the pilot plant can be up to 20-fold larger than batches produced in the laboratory scale (202,204). One batch corresponding to a total volume of 1.5 L nanoparticle suspension, i.e. 7 g polymer, needs about 2 h to be produced (202). The only drawback inherent to the method is related to the low polymer concentration in the organic phase which needs to remain in the dilute regime of the polymer solution (138). This significantly limits the amount of nanoparticules recovered in the final raw dispersion.

TREATMENT OF NANOPARTICLES AFTER PREPARATION

Several types of treatments can be applied to nanoparticle suspensions after synthesis. They include purification, sterilization, drying and concentration.

Purification of Nanoparticle Suspensions

Once nanoparticle suspensions are obtained, purification may be further needed to remove impurities and excess of reagents involved during manufacture. Depending on the method of preparation, impurities include organic solvents, oil, surfactants, residual monomers, polymerization initiators,

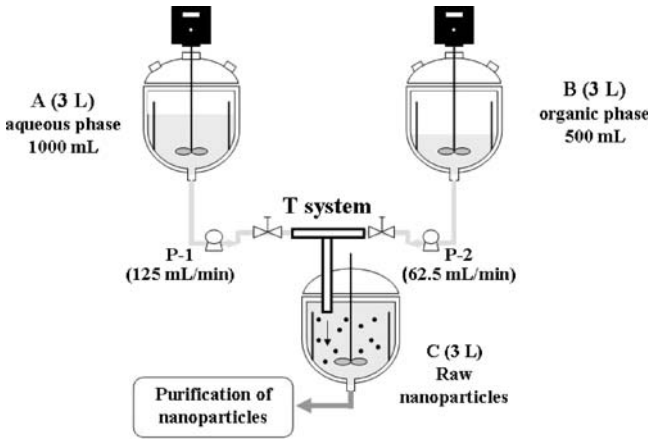

Fig. 14. Pilot set up proposed for the scaling-up of nanoparticle production by the nanoprecipitation method. P-1 and P-2 are peristaltic pumps working at flow rates indicated in the parenthesis [adapted from (202,204)].

salts, excess of surfactants or stabilizing agents and large polymer aggregates. Although, it seems obvious to obtain highly purified nanoparticle suspensions to be used as pharmaceutics, the purification is also needed to obtain nanoparticle suspensions which can be administered *in vivo* by a specific route. For instance, nanoparticles synthesized by the reverse salting-out method are provided in suspensions containing high concentrations in salt (83). They must be desalted before they can be administered *in vivo* by the intravenous route. In another example, water containing nanocapsules dispersed in oil as provided from the synthesis can be administered by the oral route (205). However, they need to be cleaned and transferred in an aqueous medium before they will suit prerequisites for an administration by the intravenous route (20).

There are several suitable methods that can be applied to purify nanoparticle dispersions. They include evaporation under reduced pressure, centrifugation, ultracentrifugation techniques (20,25,109,171,206–208) filtration through mesh or filters (139,206), dialysis (51,209), gel filtration (210), ultrafiltration (99,211), diafiltration (212,213) and cross-flow microfiltration (90,99,148).

Evaporation under reduced pressure is the most common approach to remove large quantities of volatile organic solvents and a part of water. This process is usually used after the obtaining of nanoparticle suspensions by nanoprecipitation (138,141,147,153), emulsification–reverse salting-out, emulsification–solvent diffusion (30,95) and interfacial polycondensation combined with spontaneous emulsification (25,109). Filtrations through mesh or filters are applied to remove large particles or polymer aggregates which formed during preparations (139,206). Such purification is systematically applied on nanoparticle suspensions designed for intravenous injections.

A centrifugation at low gravity force can also be applied to remove aggregates and large particles on most of the polymer nanoparticle suspensions. However, it does not warranty the elimination of all particles with a diameter above a very define size as filtration on calibrated membrane does. Moreover, it is not suitable to purify nanoparticles having a high density because they will sediment with aggregates. For instance, this restriction applies in the case of metal colloids containing nanoparticles which are designed for applications in diagnosis by imaging techniques or in techniques based on thermal treatments applied in cancer therapy.

Ultracentrifugation methods consist in very high speed centrifugations. In general, nanospheres, even those having a slightly higher density than water, can sediment and concentrate in a pellet which is then separated from the dispersing medium found in the supernatant. Oil-containing nanocapsules having a lower density than the aqueous dispersing media are creaming (126). In both cases, the dispersing medium containing all the unwanted impurities can be removed and replaced by a new medium free from impurities. Nanoparticles can be washed by applying several cycles (generally three) of ultracentrifugation followed by dispersion in a new dispersing medium of pellets containing the nanospheres or creams containing nanocapsules (105,125,138,141,214–218). For most of nanospheres and oil containing nanocapsules developed so far as drug carriers, ultracentrifugations are performed at 100,000–110,000×*g* for 30 to 45 min. The main problem of this technique is that nanospheres are not always easy to re-disperse after ultracentrifugation (106,219–221). Aggregates may remain and the uses of vortex or ultrasounds are often mentioned as methods used to re-dispersed pellets after ultracentrifugation (39,217). Interestingly, Alphandary *et al.* (219) showed that purified nanospheres under a fully dispersed form can be collected after ultracentrifugation of the original suspension over a gradient of sucrose. The nanospheres concentrate in the gradient as a band of suspension in region of the same density than themselves while impurities remained in the upper phase of the lower density. Nanocapsules are more difficult to separate from the dispersing medium because the cream remains semi-liquid. In addition, they are fragile and the application of several cycles of ultracentrifugation is hazardous because they can break easily (14,28). Despite these drawbacks, ultracentrifugation appeared as a method of choice to facilitate the transfer of water-containing nanocapsules from the oil dispersion medium of their synthesis to an aqueous phase required for their *in vivo* administration by intravenous injections (20,39). In this case the ultracentrifugation is performed by overlaying the oil dispersion of nanocapsules on a cushion of an aqueous solution of surfactant. The nanocapsules are transferred in the aqueous phase during the ultracentrifugation crossing the interface between the oil and the aqueous solution of surfactant. Then, they concentrate at the bottom of the ultracentrifuge tube to form a pellet. Although the method for the re-dispersion of the pellet obtained still needs to be improved, this is the only method that was found suitable to overcome this difficult task.

Purification by dialysis can be performed using different kinds of cellulose membranes of various molecular weight cut off allowing substances having low or high molecular weight to diffuse toward the counter dialysing medium thanks to the concentration gradient of solutes. Although dialysis is a simple and common method used to purify nanoparticles, premature release of nanoparticle payload can occur during the long purification period it requires and, because large volume of counter dialysing medium are required to make the purification efficient. Furthermore, the application of dialysis in a large-scale is disputable from an economical point of view and from the high risk of microbial contamination of the final product due to the long duration of the process. Alternative methods based on cross-flow filtration, diafiltration, ultrafiltration and microfiltration were suggested (96,148,212,213,221,222). For instance, batches of nanoparticles can be purified completely in less than 3 h by cross-flow filtration using a microfiltration membrane (11). The method of cross-flow filtration can also be applied with ultrafiltration membrane and it generally requires only very little manipulations. In addition to their rapidity, these purification procedures are more efficient than dialysis and can be applied with minimal detrimental impact on nanoparticle size and drug-loading capacity (148). Although these methods show many advantages, caking is a common problem found when a colloidal suspension is pressed through a filtration membrane. This corresponds to the formation of a precipitate of particles on the filtration membrane surface. If the adhesion of the particles is stronger than the repulsion, the cake formed is irreversible and membrane permeability decreases dramatically (223). The cake formation can be reduced by using the

cross-flow filtration mode, by using hydrophilic membranes (224), or by using diafiltration with suction (213). The purification of nanoparticle suspensions by cross-flow microfiltration was investigated at a large scale (90,96). The process involves two steps, a concentration step in which the nanoparticle suspension is concentrated by a factor of 1/5, followed by a diafiltration step in which the volume of the feed suspension is kept constant by continuous addition of pure water (148).

As another purification process, gel filtration can be used (210). It is also much faster than methods based on simple dialysis but it is greatly limited by the relatively small volume of sample which can be processed at a time. In addition, irreversible adsorption of actives onto the column stationary phase and the poor resolution between large impurities and small nanoparticles can restrict the use of this technique for purification of drug-loaded nanoparticulate formulations.

Among all the purifications methods described in this part of the review, it can be concluded that the methods based on cross flow filtration and diafiltration present many advantages. They combine a high efficacy with the possibility to operate a concentration of the suspension without causing aggregation of the nanoparticles. Moreover, these methods are applicable in large scale productions suitable for industrial developments.

Sterilization

For clinical uses, parenteral drug delivery systems have to meet the pharmacopeia requirements of sterility. Sterilization techniques include autoclaving, gamma irradiation, membrane filtration, high hydrostatic pressure sterilization and sterilization by using ethylene oxide or formaldehyde.

The more convenient and first tested method is heat sterilization also called moist sterilization or autoclaving which is an effective method accepted by most Pharmacopeias. However, heat sterilization by autoclaving involves high temperatures (120°C), which may influence decomposition or degradation of active ingredient as well as of the nanoparticle material. Specifically, mechanical properties of polymers having a glass transition or/and a melting point below 120°C are strongly diminished after autoclaving. It was reported that nanocapsule size increased from 200 to 500 nm after sterilization. This effect was attributed to either the swelling of polymeric membrane composed of poly(isobutylcyanoacrylate) or the expansion of oily phase (225). It was also shown that autoclaving can catalyse some reactions with additives such as surfactants which can modify the polymers constituting the nanoparticles (226). A significant increase of poly(butylcyanoacrylate) nanosphere size was reported by Sommerfield *et al.* (227). Finally, autoclaving of polymer based gene transfer complexes may result in total loss of transfection capacity (228).

Gamma irradiation is another effective method of polymer sterilization accepted by European Pharmacopeia which can be applied on heat-sensitive materials. This process has the advantage to insure a homogeneous sterilization. It can be applied on packaged products avoiding further risk of microbial contamination. However, gamma irradiation may affect the performance of a drug delivery system because energy transfer may induce fragmentation of covalent bonds and produce free radicals which, in turn, can damage the polymer forming the nanoparticles or induce production of compounds with toxicological hazard (226,229,230). Gamma irradiations of PCL nanoparticles resulted in an increase of molecular weight due to cross-linking reactions occurring between polymer chains or between PCL and surfactants (226). The nanoparticle size was not altered after application of this type of treatment on the PCL nanoparticles. The size of PLA nanoparticles was also unchanged after gamma irradiation, but polymeric chain scissions were reported. As a consequence to the modification of the polymer composing the nanoparticles during gamma irradiation, the release properties of a drug, e.g savoxepine, entrapped in the nanoparticles and the degradation rate of the nanoparticles were accelerated (98,231). In another example, gamma-irradiation and electron beam irradiation at a dose of 15 KGy were sufficient to sterilize doxorubicin-loaded poly (butyl cyanoacrylate) (PBCA) nanoparticles. The formulation showed excellent stability to irradiation. The drug was stable to radiolysis while the molecular weights of the PBCA polymer remained nearly unchanged (232).

Sterile filtration may be considered as an alternative method for chemically or thermally sensitive material since no adverse effect on the polymer or the drug was reported (233). This technique involves a filtration of the nanoparticles on membrane filters with pore diameters of 0.22 μm which represents a limitation for many types of nanoparticle suspensions. Indeed, this technique of sterilization can only be applied on nanoparticle suspension of a low viscosity and containing nanoparticles with size having a diameter below the pore size. It is not suitable for nanoparticles showing a diameter larger or in the range of 220 nm. If the size distribution of small nanoparticles is too large, this method of sterilization is also hardly applicable. When the drug is adsorbed on the particle surface and when the nanoparticle dispersion is too viscous are other cases in which sterile filtration cannot be applied (98,228,234). In general, these unfavourable factors induced a clog of the membrane making the filtration of the samples impossible.

Finally, High Hydrostatic Pressure (HHP) treatment was tested for the sterilization of poly(alkylcyanoacrylate) nanoparticles (235). It is a method which can be applied on large batch of nanoparticles by circulation of the nanoparticles through the high hydrostatic pressure apparatus. The data obtained with this method demonstrated that the HHP treatments did not induce physical damage on the different nanoparticle suspensions and did not modify physically or chemically the poly(alkylcyanoacrylate) nanospheres, and nanocapsules, regardless of their preparation method and their surface characteristics (235). Although this method proved its efficacy to destroy all vegetative microorganisms, improvements are needed to completely eliminated bacteria spores which resisted to the treatments applied in this first work considering such a sterilization method.

Drying of Nanoparticles

As in case of many pharmaceutical preparations, storage of nanoparticles as suspensions presents many disadvantages such as risk of microbiological contamination, premature polymer degradation by hydrolysis, physicochemical instabil-

ity due to particle aggregation and sedimentation and loss of the biological activity of the drug (234). To circumvent such problems, pharmaceutical preparations are stored under a dry form. In general, the transformation of a liquid preparation into a dry product can be achieved using freeze-drying (235–241) or spray-drying processes (204,242). Both processes can be used at an industrial scale and their applications were tested on nanoparticle suspensions.

Freeze Drying of Nanoparticles

Freeze drying, also known as lyophilization, is a very common technique of conservation used to ensure long term stability of pharmaceutical and biological products preserving their original properties (243). A review on the application of the freeze drying process to nanoparticle suspensions was proposed by Abdelwahed *et al.* (240). The basic principle of this process consists on removing water content of a frozen sample by sublimation and desorption under vacuum. In general, freeze-drying processes can be divided in three steps: freezing of the sample (solidification), primary drying corresponding to the ice sublimation and secondary drying corresponding to desorption of unfrozen water. In the case of nanoparticle suspensions, the cryo-concentrated phase includes nanoparticles, remaining free surfactants, buffer and free drugs. During the freeze drying process, several problems may arise which can lead to a loss of integrity of the nanoparticle characteristics. For instance, crystallization of ice may exert a mechanical stress on nanoparticles leading to their destabilization. This effect is more critical during the lyophilization of nanocapsules which are very fragile upon lyophilization (240). The high concentration in nanoparticles in the final dried product may favour aggregation and even in some cases irreversible coalescence of nanoparticles. The addition of cryoprotectants can improve the resistance of nanoparticles toward freezing and drying stresses and also increase stability during long term storage (240). In general, the type of cryoprotectant is selected in the aim to ensure a maximum of stabilization of nanoparticles. Sugars including trehalose, mannose, sucrose, glucose, lactose, maltose and mannitol are often used but the level of stabilization generally depends on their concentrations (237,241,244,245). The weight ratio cryoprotectant/nanoparticles is an important parameter to consider to preserve the stability of the nanoparticles during freeze drying. For instance, weight ratio trehalose/nanoparticles (1/1) was found as optimal to promote the complete redispersion of freeze-dried PLA-PEG nanoparticles (244). In some cases, increasing cryoprotectant concentration above the optimal value can compromise the stability of the nanoparticles and even promote their destabilization (245). Although sugars are the most popular cryoprotectants, other components can protect nanoparticles during lyophilization. For instance, PCL nanocapsules remained intact after lyophilization in the presence of an excess of PVA added to the preparation medium at concentrations ranging from 2.5% to 5% (246). Similarly, poly (isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) nanoparticles can be freeze-dried without any modification of their size in presence of 2% of pluronic® F68 (247).

Besides excipients, the freeze-drying process parameters can impact the texture of the frozen matrix and the final morphological characteristics of the freeze-dried cake (248). In general, the optimization of freeze-drying cycle is aimed to shorten the sublimation duration which is the longest step of the whole process (235,241).

Finally, freeze-dried nanocapsules should be stored at a temperature below the glass transition temperature of the formulation to maintain the glassy state of cryoprotectant and to prevent aggregation of the nanocapsules. This was the case of freeze-dried nanocapsules made of poly(vinylpyrrolidone) (PVP) which remained stable after six months of storage under accelerated stability conditions (40°C) (249).

Spray-drying

The spray-drying technique transforms liquids into dried particules under a continuous process. It might be an interesting alternative to freeze-drying thanks to advantages like low price, rapid process and possibility to modulate physicochemical characteristics of the produced powders by varying process parameters (250). It is one treatment which suits with heat-sensitive molecules like proteins preserving them from significant degradation (251). Nanoparticle formulations submitted to spray drying are generally aqueous suspensions and contain one soluble compound added as drying auxiliary. Examples of drying auxiliaries are colloidal silicon dioxide (242,252), lactose, mannitol and PVP (204). Spray-drying process includes four important steps: (1) atomisation of the feed, i.e. nanoparticle suspension, into a spray, (2) spray-air contact, (3) drying of the spray and (4)

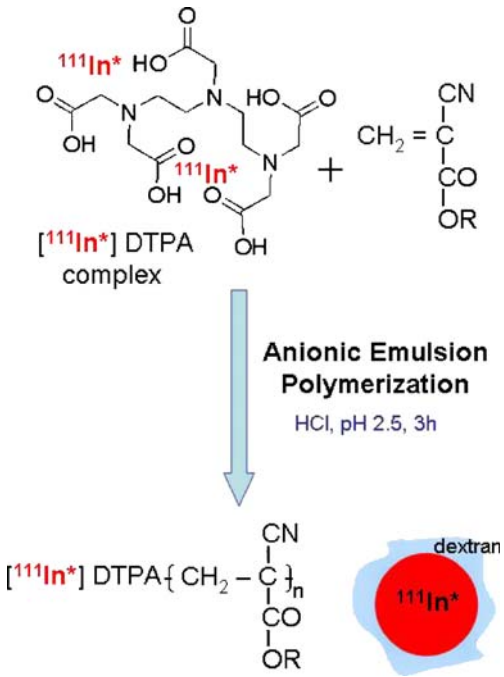

Fig. 15. Method of radiolabelling of PACA nanoparticles by the formation of a complex between gamma emitting isotopes and diethylene triamine penta-acetate (DTPA) which is entrapped in the nanoparticles during the preparation by anionic emulsion polymerization (112). Other radiolabelled isotopes used with this method were ^{99m}Tc, ^{125}I, ^{131}I.

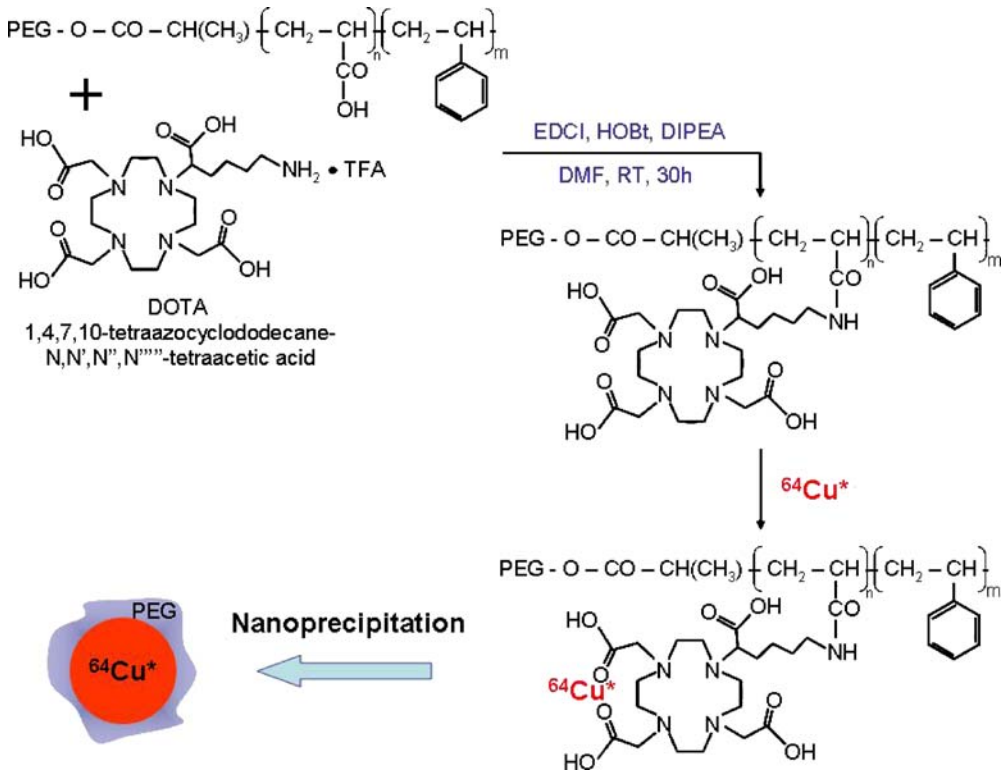

Fig. 16. Method of coupling of a complexing agent for radioactive cations to polymer bearing carboxylic acids (265). *DIPEA N,N*-diisopropylethylamide, *DMF* dimethylformamide, *EDCI* 1-[3′-(dimethylamino) propyl]-3-ethylcarbodiimide methiodide, *HOBt* 1-hydroxybenzotriasole.

separation of the dried product from the drying gas (242,253). Similarly to freeze drying techniques, the nanoparticle suspension is converted into a dry formulation. The powder form should be stored at temperature under the glass transition temperature of the polymer forming the nanoparticles. The main problem can be found during redispersion of the powder where aggregates may be difficult to dissociate.

Concentrating Nanoparticle Dispersions

Many of the methods of preparation of nanoparticles to be used as drug carriers are providing with suspensions of low solid content (63,110,138). Therefore, the clinical use of the drug formulation can be seriously hampered if the volume of suspension that has to be administered in order to reach

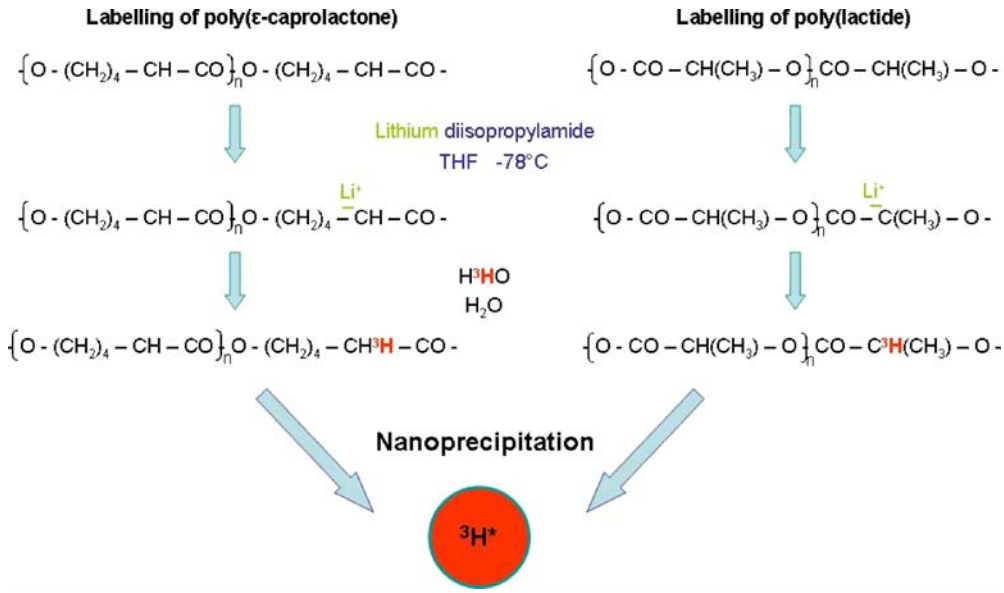

Fig. 17. Method of radiolabelling of polyesters by introducing a tritium atom in the polymer structure (267,268).

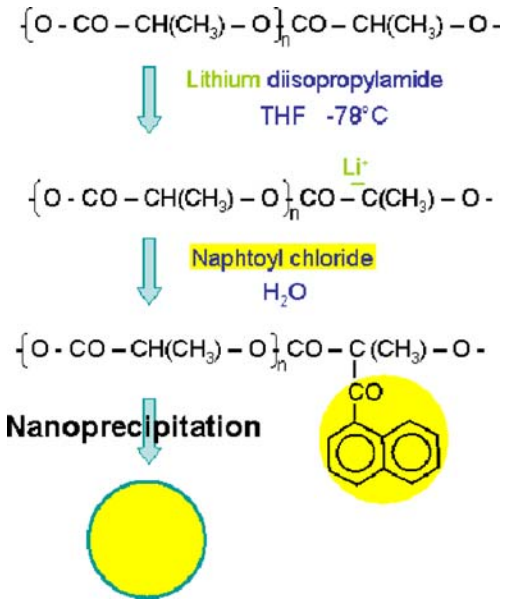

Fig. 18. Method of preparation of fluorescent poly(lactide) from preformed polymer (267).

therapeutic concentrations is too high. For this reason, drug-loaded nanoparticles often need to be concentrated before they can be administered *in vivo*.

Concentrated suspensions of nanoparticles can be obtained through different methods. For instance, dried preparations obtained either by freeze drying or spray drying can be reconstituted in a much smaller volume than the original volume. In the same way, nanoparticles separated by ultracentrifugation can be redispersed in a smaller volume than the parent suspensions. The main problem encountered with these techniques is the difficulty to fully redisperse the nanoparticles especially when the volume of the final suspension should be much smaller than the initial volume of the parent suspensions (48,240,254,255). Additionally, aggregates are difficult to dissociate because they can be formed during the concentration process due to over-concentration of nanoparticles which appears in some regions of the suspensions during the drying process or the ultracentrifugation. It should also be mentioned that these method are not suitable to be applied to all types of nanoparticles. For instance nanocapsules are very fragile and they do not always resist to such treatments (240).

Concentration by evaporation of part of the dispersion medium is adapted to remove volatile organic solvents involved in several preparation methods, i.e. emulsification–solvent evaporation (63), emulsification–solvent diffusion (86,95), nanoprecipitation (138) and interfacial polycondensation combined with spontaneous emulsification (26,65,109,133,256). In contrast, this method is difficult to apply to remove part of the water contained in nanoparticle suspensions. The temperature needs to be kept below the glass transition temperature of the polymer forming the nanoparticles and as with the previous methods, aggregates can form due to loci of over concentrated region of nanoparticles which forms in the suspension during water evaporation (255).

Methods based on ultrafiltration have been proposed as alternative methods to avoid problems of aggregation of nanoparticles. However, simple ultrafiltration also presents difficulties because membranes can be clogged by nanoparticles. The problem can be solved using diafiltration or tangential filtration methods (148).

Very recently, a new method based on a dialysis achieved by application an osmotic stress on the nanoparticle suspen-

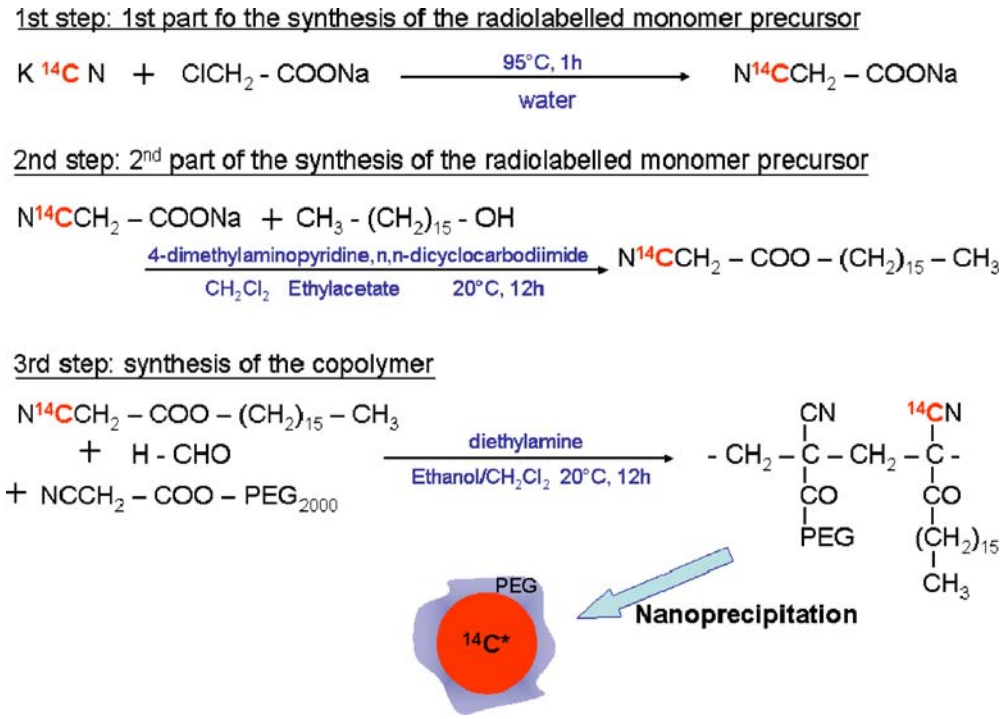

Fig. 19. Radiolabelling of poly(alkylcyanoacrylate) during the synthesis of the polymer (258).

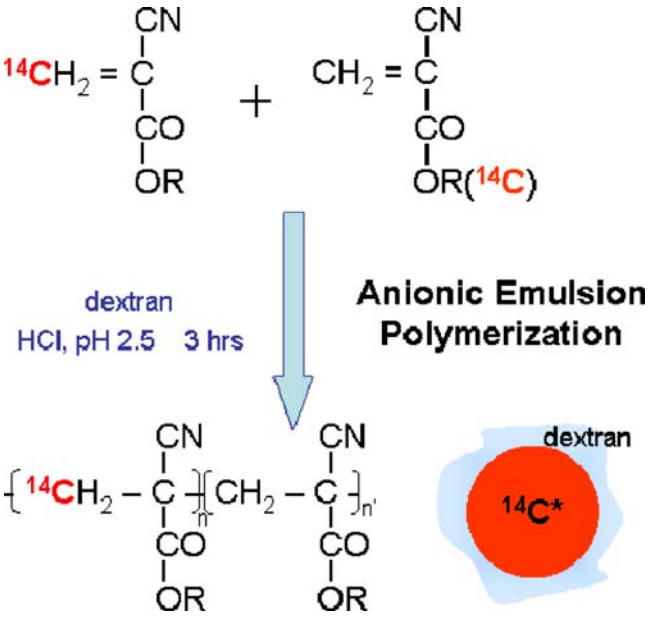

Fig. 20. Preparation of radiolabelled PACA nanoparticles by copolymerization of a radiolabelled monomer (192).

sion entrapped in a dialysis bag gives very promising perspectives (255). Through the osmotic stress applied on the nanoparticle suspension, water molecules are displaced from the inside of the dialysis bag towards the outside dialysis solution until equilibrium is reached from both side of the dialysis membrane. Because the amount of water removed from the nanoparticle suspension can be controlled by the polymer concentration in the counter-dialysis medium, it is possible to predict the concentration in nanoparticles that will be reached in the final suspension. The method presents many advantages. It is simple to apply, does not require any sophisticated material and is safe for both types of nanoparticles including nanocapsules that are notoriously fragile objects. The concentration of nanoparticles in pharmaceutical suspensions can be increased from initial formulation values by factors ranging from 10 to 50 without causing any aggregation of the nanoparticles.

METHODS FOR NANOPARTICLE LABELLING

Labelling of nanoparticles is required as soon as one wants to study the fate of nanocarriers *in vivo* and in cells during *in vitro* investigations (see for instance 122,192,257–260). The choice of the labelling method depends greatly on the type of method of detection that is expected to be used and on the aim of the work. Three types of labelling were suggested including the use of radioisotope containing compounds, fluorescent molecules and metal colloids.

To label nanoparticles, the easiest way to proceed is to encapsulate the label-bearing probe in the nanoparticles. This can be a specific compound like technicium or gadolidium complexes which can be detected by gamma scintigraphy or magnetic resonance imaging techniques (MRI), a drug such as doxorubicin which is fluorescent or a metal colloid having superparamagnetic properties making them detectable by MRI. Using this approach, it is necessary to control carefully the stability of the assemblies in the biological media to be used (261). Indeed, as the probe will not be a component taking part of the structure of the nanoparticles, dissociation of the probe from the drug carrier may lead to misinterpreted results. This approach is very popular and was used in many works (see for instance: 218,259,262–264). The entrapment of the labelled species can be promoted using chelating agent bearing polymers (112,265). For instance, a chelating agent can be grafted on PACA polymer during the initiation stage of the anionic polymerization of the alkylcyanoacrylate

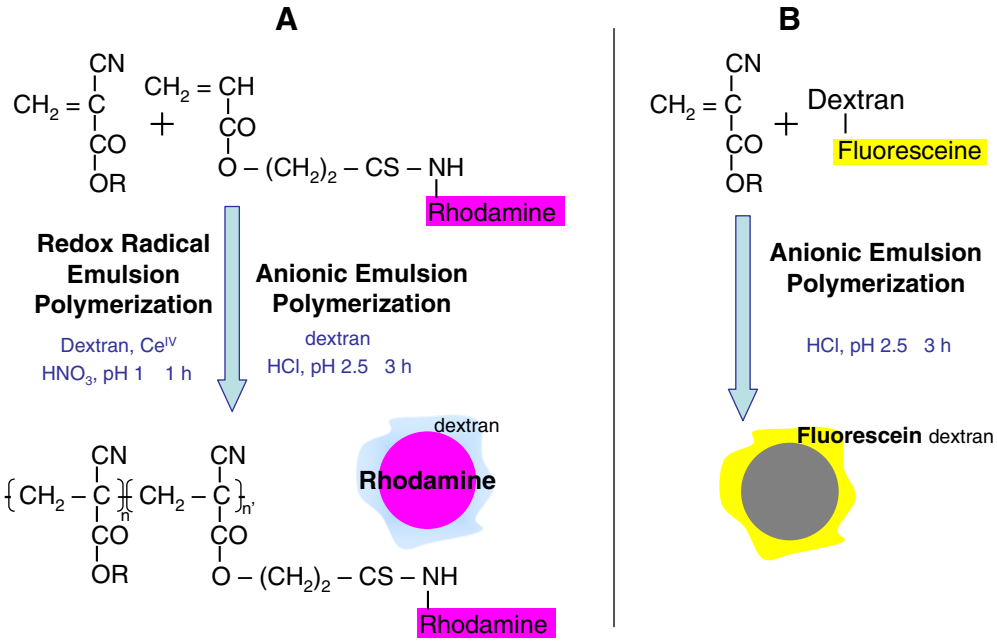

Fig. 21. Preparation of fluorescent labelled PACA nanoparticles by copolymerization of a fluorescent comonomer (119) (**A**) or by using fluorescent dextran (219) (**B**).

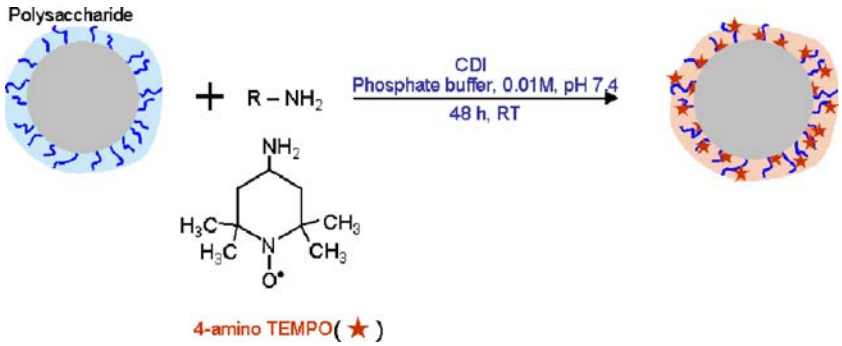

Fig. 22. Method of labelling PACA nanoparticles by grafting the probe on polysaccharide-coated nanoparticles. *CDI* carbonyl diimidazole (269,270).

monomers. By this way, it is also incorporated in the PACA nanoparticles which form during the emulsion polymerization process. This technique was used to associate gamma emmitors radioisotopes including ^{111}In, ^{99m}Tc, ^{125}I, ^{131}I with the nanoparticles (Fig. 15). The labelled nanoparticles can be detected *in vivo* by gamma scintigraphy imaging after intravenous administration in rabbits and in man (112). Chelating agents can also be grafted on a preformed polymer bearing free carboxylic groups in their structure (265). An example of the chemistry developed to achieve such a grafting is given in Fig. 16. Using ^{64}Cu as the radiolabelled compound, the nanoparticles prepared from the radiolabelled polymer can be detected by positon emission tomography.

When the purpose of the work is to follow the drug carrier, the most relevant methods of labelling are those considering the labelling of the polymer composing the nanoparticles. For nanoparticles prepared from preformed polymers, the polymer can be labelled either by grafting a label on the polymer or by incorporating it during the

Table IX. General Advantages and Disadvantages of the Nanoparticle Preparation Methods

Method	Advantages	Disadvantages
Nanoparticles obtained using colloidal mill	Production of well characterized emulsions, uniform size, Easy to scale-up	High energy for the emulsification process
Emulsification–solvent evaporation	Possibility to encapsulate both hydrophilic and lipophilic drugs	Possible coalescence of the nanodroplets during the evaporation process
Emulsification–solvent diffusion	Possibility to control the size of the nanoparticles Easy to scale-up	High volumes of water to be eliminated Leakage of water-soluble drug into the saturated-aqueous external phase
Emulsification–reverse salting-out	Minimization of the stress to fragile drugs, High loading efficiency, Easy to scale-up	Possible incompatibility between the salts and the drugs Purification is needed to remove electrolytes
Obtaining nanoparticles by gelation of the emulsion droplets	Possibility to use natural macromolecules, hydrophilic and biocompatible	Limited to the encapsulation of hydrophilic drugs
Polymerization of alkylcyanoacrylates	Easy method to obtaining core-shell tuned nanoparticles Control the size of the nanoparticles by using surfactant	Possible reaction between the drug and CeVI in the case of radical emulsion polymerization Purification is needed
Interfacial polycondensation reactions	Low concentrations of surfactants Modulation of the nanocapsule thickness by varying the monomer concentration	Limited to the encapsulation of lipophilic drugs Purification is needed
Nanoprecipitation of a polymer	High simplicity, fast and reproducible Low concentrations of surfactants Easy to scale-up	Low polymer concentration in the organic phase
Formation of polyelectrolyte complexes	Easy to achieve According to the nature of the polyelectrolyte used in excess, either positively or negatively charged nanoparticles can be synthesized	Necessity to optimize the ratio between negatively and positively charged molecules
Formation of nanoparticles from neutral nanogels	Organic solvent free method Controlled release of the drug	Is not yet applicable to hydrophilic drugs
One step procedures: Methods based on ionic gelation	Organic solvent free method Possibility to control the release of a drug encapsulated in the nanoparticles upon the action of a pH or an ion concentration variation stimulus	Possible particle disintegration due to the weakness of the ionic interactions

synthesis of the polymer chain. For instance, PLA and PCL can be tritiated post synthesis using a rapid method occurring in organic solution at low temperature and which can be applied even on high molecular weigh polymers. It consists on a substitution of a proton of the polymer by a tritium from tritiated water under the presence of lithium diisopropylamide (266–268) (Fig. 17).

It is interesting to point out that the same approach can be used to achieve a fluorescent labelling of these polymers by substituting one proton of the polymer by a fluorescent residue (267) (Fig. 18). PEG-containing poly(alkylcyanoacrylate) copolymers can be radiolabelled with [^{14}C] isotope during the synthesis of the copolymer (258) (Fig. 19). The labelling was achieved on the nitrile group of hexadecylcyanocrylate residues of the copolymer. Although the labelling procedure required several steps, this method had the advantage to provide with a polymer in which the labelled is well associated with the polymer without risk to be removed during the degradation of the polymers produced by esterases.

Easier labelling procedures were suggested to label PACA nanoparticles prepared by emulsion polymerization. In these cases, either a radioactive monomer or a fluorescent monomer can be incorporated in the polymerization medium as a co-monomer (Figs. 20 and 21a). The co-monomer is then spontaneously incorporated in PACA chains during the polymerization leading to the labelling of the nanoparticles (119,122,192). It is noteworthy that the location of the labelling in the structure of the labelled monomer is important to know. In the case of PACA nanoparticles, the labelling should preferentially be located on a stable position like in the main polymer carbon chain or on the nitrile group to avoid removal during degradation of the polymer by esterases. Another option is to use a labelled comonomer which will not be degraded by esterases (Fig. 21a). By using labelling approaches based on the incorporation of a labelled co-monomer in the PACA chain, the label is incorporated in the core of the nanoparticles (Figs. 20 and 21a). As an alternative method, a fluorescent label can also be incorporated during nanoparticle preparation by using fluorescent dextran, In this case, the labelling will be located on the surface of the PACA nanoparticles (219) (Fig. 21b). The location of the probe in the structure of the nanoparticles is important to consider especially in the case of the use of a fluorescent labelling. Indeed, this type of labelling often implies the incorporation of quite large molecules in the structure of the nanoparticles which can modify the original properties of the nanoparticles. Taking place at the nanoparticle surface, such changes may disturb interactions of the nanoparticles with proteins hence modify the fate of the nanoparticles either *in vivo* or in their interactions with cells.

Finally, nanoparticles can be labelled after their synthese when they display suitable functional groups on their surface. The methods of coupling which can be applied are very similar to those used to attach ligands at the nanoparticles as targeting moieties (183). The Fig. 22 illustrates the labelling of polysaccharide-coated PACA nanoparticles with a spin probe (4-amino TEMPO). Although this approach of nanoparticle labelling method can easily be achieved by rather simple chemistry, modifications of the nanoparticle surface properties may be induced (269). It can also be pointed out that the colloidal stability of the nanoparticles in suspension may be compromised by the introduction of new chemical groups on the nanoparticle surface.

CONCLUSION

This review highlights the diversity of methods that can be applied to produce polymer nanoparticles either from preformed polymers or by *in-situ* polymerization together with their advantages and disadvantages, are summarized in Table IX.

Although a low number of polymers were taken in the examples because the scoop of this review was limited to the production of nanoparticulate drug carriers, the methods described in this paper can be applied to a much larger range of polymers. It is noteworthy that during the last decades, progresses were mainly focused on the improvement of existing methods thanks to innovation coming out from the emulsification methods. Another important achievement was the appearance of methods which allow the production of large batches of nanoparticles in a reproducible manner. Post treatment methods have also greatly evolved especially for the obtaining of sterile and lyophilized nanoparticle preparations. Finally, methods of labelling of the nanoparticles were diversified.

Future evolutions will probably come from the introduction of new types of polymers fulfilling the requirements to be used as constituent of a drug delivery system. For instance, they will include stimuli responding polymers which can confer triggered released properties to drug nanocarriers. With most of these polymers, nanospheres and nanocapsules should easily be obtained by applying the existing methods with only a few adjustments. New structures like polymersomes found in the domain of polymer colloids are waiting to join the family of nanoparticulate drug delivery systems. The method for their production is more likely the method of production of liposomes but polymers to built suitable polymersomes for *in vivo* applications still need to be developed.

REFERENCES

1. J. C. Allémann Leroux, and R. Gurny. Polymeric nano-and microparticles for the oral delivery of peptides and peptidomimetics. *Adv. Drug Deliv. Rev.* **34**(2–3):171–189 (1998). doi:10.1016/S0169-409X(98)00039-8.
2. P. Couvreur, and C. Vauthier. Nanotechnology: intelligent design to treat complex disease. *Pharm. Res.* **23**:1417–1450 (2006). doi:10.1007/s11095-006-0284-8.
3. R. Juliano, M. R. Alam, V. Dixit, and H. Kang. Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. *Nucleic Acids Res.* **36**(12):4158–4171 (2008). doi:10.1093/nar/gkn342.
4. C. Pinto-Reis, R. J. Neufeld, A. J. Ribeiro, and F. Veiga. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine*. **2**:8–21 (2006).
5. A. F. Soares, R. A. de Carvalho, and F. Veiga. Oral administration of peptides and proteins: nanoparticles and cyclodextrins as biocompatible delivery systems. *Nanomedicine*. **2**(2):183–202 (2007). doi:10.2217/17435889.2.2.183.
6. C. Vauthier, and D. Labarre. Modular biomimetic drug delivery systems. *J. Drug Deliv. Sci. Technol.* **18**(1):59–68 (2008).
7. H. de Martimprey, C. Vauthier, C. Malvy, and P. Couvreur. Polymer nanocarriers for the delivery of small fragments of nucleic acids: Oligonucleotides and siRNA. *Eur. J. Pharm. Biopharm.*, in press (2008)

8. C. Pichot, and J. C. Daniel (eds.), *Latex Synthétiques : Elaboration-Propriétés-Applications*, Lavoisier, Paris, France, 2006.
9. W. A. Braunecker, and K. Matyjaszewski. Controlled/living radical polymerization: features, developments and perspectives. *Prog. Polymer. Sci.* **33**:93–146 (2007). doi:10.1016/j.progpolymsci.2006.11.002.
10. C. Vauthier-Holtzscherer, S. Benabbou, G. Spenlehauer, M. Veillard, and P. Couvreur. Methodology for the preparation of ultradispersed polymer systems. *STP Pharma Sci.* **1**:109–116 (1991).
11. E. Allémann, R. Gurny, and E. Doelker. Drug loaded nanoparticles. Preparation, methods and drug targeting issues. *Eur. J. Pharm. Biopharm.* **39**:173–191 (1993).
12. D. Quintanar-Guerrero, E. Allémann, H. Fessi, and E. Doelker. Preparation techniques and mechanism of formation of biodegradable nanoparticles from preformed polymers. *Drug Dev. Ind. Pharm.* **24**:1113–1128 (1998). doi:10.3109/03639049809108571.
13. F. De Jaeghere, E. Doelker, and R. Gurny. Nanoparticles. In E. Mathiowitz (ed.), *The Encyclopedia of Controlled Drug Delivery*, Wiley, New York, 1999, pp. 641–664.
14. P. Couvreur, G. Barratt, E. Fattal, P. Legrand, and C. Vauthier. Nanocapsule technology: a review. *Crit. Rev. Ther. Drug. Carrier Syst.* **19**(2):99–134 (2002). doi:10.1615/CritRevTherDrugCarrierSyst.v19.i2.10.
15. C. Vauthier, E. Fattal, and D. Labarre. From polymer chemistry and physicochemistry to nanoparticular drug carrier design and applications. In M. J. Yaszemski, D. J. Trantolo, K. U. Lewamdrowski, V. Hasirci, D. E. Altobelli, and D. L. Wise (eds.), *Biomaterial Handbook-Advanced Applications of Basic Sciences and Bioengineering*, Marcel Dekker, New York, 2004, pp. 563–598.
16. D. Moinard-Chécot, Y. Chevalier, S. Briançon, H. Fessi, and S. Guinebretière. Nanoparticles for drug delivery: review of the formulation and process difficulties illustrated by the emulsion-diffusion process. *J. Nanosci. Nanotechnol.* **6**(9–10):2664–2681 (2006). doi:10.1166/jnn.2006.479.
17. C. Vauthier. Généralités sur les techniques d'émulsification et d'obtention de dispersions de particules polymère : avantages et inconvénients. In C. Pichot et, and J. C. Daniel (eds.), *Latex Synthétiques : Elaboration-Propriétés-Applications*, Lavoisier, Paris, 2006, pp. 291–317.
18. N. Al Khoury-Fallouh, L. Roblot-Treupel, H. Fessi, J. P. Devissaguet, and F. Puisieux. Development of a new process for the manufacture of poly(isobutylcyanoacrylate) nanocapsules. *Int. J. Pharm.* **28**:125–136 (1986). doi:10.1016/0378-5173(86)90236-X.
19. H. Vranckx, M. Demoustier, and M. Deleers. A new nanocapsule formulation with hydrophilic core: Application to the oral administration of salmon calcitonin in rats. *J. Pharm. Pharmacol.* **42**:345–347 (1996).
20. G. Lambert, E. Fattal, H. Pinto-Alphandary, A. Gulik, and P. Couvreur. Polyisobutylcyanoacrylate nanocapsules containing an aqueous core as a novel colloidal carrier for the delivery of oligonucleotides. *Pharm. Res.* **17**(6):707–714 (2000). doi:10.1023/A:1007582332491.
21. M. Wohlgemuth, W. Mächtle, and C. Mayer. Improved preparation and physical studes of polybutylcyanoacrylate nanocapsules. *J. Microencapsulation*. **17**:437–448 (2000). doi:10.1080/026520400405697.
22. C. Mayer. Nanocapsules as drug delivery systems. *Int. J. Artif. Organs*. **28**(11):1163–1171 (2005).
23. H. Hillaireau, T. Le Doan, M. Appel, and P. Couvreur. Hybrid polymer nanocapsules enhance *in vitro* delivery of azidothymidine-triphosphate to macrophages. *J. Control. Release*. **116**:346–352 (2006). doi:10.1016/j.jconrel.2006.09.016.
24. S. Watnasirichaikul, T. Rades, I. G. Tucker, and N. M. Davies. Effects of formulation variables on characteristics of poly (ethylcyanoacrylate) nanocapsules prepared from w/o microemulsions. *Int. J. Pharm.* **235**:237–246 (2002). doi:10.1016/S0378-5173(02)00002-9.
25. K. Bouchemal, S. Briançon, E. Perrier, H. Fessi, I. Bonnet, and N. Zydowicz. Synthesis and characterization of polyurethane and poly (ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification. *Int. J. Pharm.* **269**(1):89–100 (2004). doi:10.1016/j.ijpharm.2003.09.025.
26. K. Bouchemal, S. Briançon, H. Fessi, Y. Chevalier, I. Bonnet, and E. Perrier. Simultaneous emulsification and interfacial polycondensation for the preparation of colloidal suspension of nanocapsules. *Mater. Sci. Eng. C.* **26**:472–480 (2006). doi:10.1016/j.msec.2005.10.022.
27. N. Ammoury, H. Fessi, J. P. Devissaguet, F. Puisieux, and S. Benita. *In vitro* release kinetic pattern of indomethacin from poly(D,L-lactide) nanocapsules. *J. Pharm. Sci.* **79**(9):763–767 (1990). doi:10.1002/jps.2600790902.
28. D. Quintanar-Guerrero, É. Allémann, É. Doelker, and H. Fessi. Preparation and characterization of nanocapsules from preformed polymers by a new process based on emulsification–diffusion technique. *Pharm. Res.* **15**:1056–1062 (1998). doi:10.1023/A:1011934328471.
29. M. F. Zambaux, F. Bonneaux, R. Gref, P. Maincent, E. Dellacherie, M. J. Alonso, P. Labrude, and C. Vigneron. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. *J. Control. Release*. **50**(1–3):31–40 (1998). doi:10.1016/S0168-3659(97)00106-5.
30. D. Moinard-Chécot, Y. Chevalier, S. Briançon, L. Beney, and H. Fessi. Mechanism of nanocapsules formation by the emulsion–diffusion process. *J. Colloid Interface Sci.* **317**:458–468 (2008). doi:10.1016/j.jcis.2007.09.081.
31. M. Skiba. Developpement pharmacotechnique et biopharmaceutique de nouveaux vecteurs colloidaux : nanoparticles à base de cyclodextrines modifiées. Ph.D. Université de Paris Sud-11, décembre 1994.
32. C. Vauthier, and P. Couvreur. Development of nanoparticles made of polysaccharides as novel drug carrier systems. In D. L. Wise (ed.), *Handbook of Pharmaceutical Controlled Release Technology*, Marcel Dekker, New York, 2000, pp. 413–429.
33. E. Martinez-Barbosa. Synthèse de dérivés de poly(L-glutamate de γ-benzyle). Préparation et caractérisation de nanoparticules multifonctionnelles. Ph. D. Université Paris Sud-11. 2006.
34. N. Toub, C. Malvy, E. Fattal, and P. Couvreur. Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. *Biomed. Pharmacother.* **60**(9):607–620 (2006). doi:10.1016/j.biopha.2006.07.093.
35. C. Perez, A. Sanchez, D. Putnam, D. Ting, R. Langer, and M. J. Alonso. Poly(lactic acid)-poly(ethylene glycol) nanoparticles as new carriers for the delivery of plasmid DNA. *J. Control. Release*. **75**:211–224 (2001). doi:10.1016/S0168-3659(01)00397-2.
36. C. G. Oster, M. Wittmar, F. Unger, L. Barbu-Tudoran, A. K. Schaper, and T. Kissel. Design of amine-modified graft polyesters for effective gene delivery using DNA-loaded nanoparticles. *Pharm. Res.* **21**(6):927–931 (2004). doi:10.1023/B:PHAM.0000029279.50733.55.
37. V. Vogel, D. Lochmann, J. Weyermann, G. Mayer, C. Tziatzios, J. A. van den Broek, W. Haase, D. Wouters, U. S. Schubert, J. Kreuter, A. Zimmer, and D. Schubert. Oligonucleotide–protamine–albumin nanoparticles: preparation, physical properties and intracellular distribution. *J. Control. Release*. **103**(1, 2):99–111 (2005).
38. N. Nafee, S. Taetz, M. Schneider, U. F. Schaefer, and C. M. Lehr. Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. *Nanomedicine*. **3**(3):173–183 (2007).
39. H. Hillaireau, T. Le Doan, H. Chacun, J. Janin, and P. Couvreur. Encapsulation of mono-and oligo-nucleotides into aqueous-core nanocapsules in presence of various water-soluble polymers. *Int. J. Pharm.* **331**(2):148–152 (2007). doi:10.1016/j.ijpharm.2006.10.031.
40. C. Chavany, T. Le Doan, P. Couvreur, F. Puisieux, and C. Hélène. Polyalkylcyanoacrylate nanoparticles as polymeric carriers for antisense oligonucleotides. *Pharm. Res.* **9**(4):441–449 (1992). doi:10.1023/A:1015871809313.
41. P. Zobel, M. Junghans, V. Maienschein, D. Werner, M. Gilbert, H. Zimmermann, C. Noe, J. Kreuter, and A. Zimmer. Enhanced antisense efficacy of oligonucleotides adsorbed to monomethylaminoethylmethacrylate methylmethacrylate co-

polymer nanoparticles. *Eur. J. Pharm. Biopharm.* **49**(3):203–210 (2000). doi:10.1016/S0939-6411(00)00080-1.
42. H. de Martimprey, J. R. Bertrand, A. Fusco, M. Santoro, P. Couvreur, C. Vauthier, and C. Malvy. siRNA nanoformulation against the ret/PTC1 junction oncogene is efficient in an *in vivo* model of papillary thyroid carcinoma. *Nucleic Acids Res.* **36**(1): e2 (2008). doi:10.1093/nar/gkm1094.
43. M. Okada. Chemical synthesis of biodegradable polymers. *Prog. Polym. Sci.* **27**:87–133 (2002). doi:10.1016/S0079-6700(01)00039-9.
44. L. Y Qiu, and Y. H. Bae. Polymer architecture and drug delivery. *Pharm. Res.* **23**(1):1–30 (2006). doi:10.1007/s11095-005-9046-2.
45. S. Slomkovski. Biodegradable nano-and microparticles as carriers of bioactive compounds. *Acta Pol. Pharm.* **63**(5):351–358 (2006).
46. L. S. Nair, and C. T. Laurencin. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* **32**:762–789 (2007). doi:10.1016/j.progpolymsci.2007.05.017.
47. V. P. Torchilin, and V. S. Trubetskoy. Which polymers can make nanoparticulate drug carriers long-circulating? *Adv. Drug Deliv. Rev.* **16**:141–155 (1995). doi:10.1016/0169-409X(95)00022-Y.
48. K. Avgoustakis. Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: preparation, properties and possible applications in drug delivery. *Curr. Drug Deliv.* **1**(4):321–333 (2004). doi:10.2174/1567201043334605.
49. V. C. Mosqueira, P. Legrand, J. L. Morgat, M. Vert, E. Mysiakine, R. Gref, J. P. Devissaguet, and G. Barratt. Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. *Pharm. Res.* **18**(10):1411–1419 (2001). doi:10.1023/A:1012248721523.
50. C. Lemarchand, P. Couvreur, C. Vauthier, D. Costantini, and R. Gref. Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *Int. J. Pharm.* **254**(1):77–82 (2003). doi:10.1016/S0378-5173(02)00687-7.
51. C. Chauvierre, D. Labarre, P. Couvreur, and C. Vauthier. Novel polysaccharide-decorated poly(isobutyl cyanoacrylate) nanoparticles. *Pharm. Res.* **20**:1786–1793 (2003). doi:10.1023/B:PHAM.0000003376.57954.2a.
52. C. Chauvierre, D. Labarre, P. Couvreur, and C. Vauthier. A new approach for the characterization of insoluble amphiphilic copolymers based on their emulsifying properties. *Colloid Polym. Sci.* **282**:1097–1104 (2004). doi:10.1007/s00396-003-1040-9.
53. N. Anton, J. P. Benoit, and P. Saulnier. Design and production of nanoparticles formulated from nano-emulsion templates-A review. *J. Control. Release.* **128**:185–199 (2008). doi:10.1016/j.jconrel.2008.02.007.
54. M. Stork, R. L. Tousain, J. A. Wieringa, and O. H. Bosgra. A MILP approach to the optimization of the operation procedure of a fed-batch emulsification process in a stirred vessel. *Comp. Chem. Eng.* **27**:1681–1691 (2003). doi:10.1016/S0098-1354(03)00135-2.
55. C. Mabille, F. Leal-Calderon, J. Bibette, and V. Schmitt. Monodisperse fragmentation in emulsions: Mechanisms and kinetics. *Europhys. Lett.* **61**(5):708–714 (2003). doi:10.1209/epl/i2003-00133-6.
56. C. Charcosset, and H. Fessi. Preparation of nanoparticles with a membrane contactor. *J. Membrane Sci.* **266**:115–120 (2005). doi:10.1016/j.memsci.2005.05.016.
57. S. Freitas, H. P. Merkle, and B. Gander. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *J. Control. Release*. **102**(2):313–332 (2005). doi:10.1016/j.jconrel.2004.10.015.
58. I. Kobayashi, S. Mukataka, and M. Nakajima. Effects of type and physical properties of oil phase on oil-in-water emulsion droplet formation in straight-through microchannel emulsification, experimental and CFD studies. *Langmuir.* **21**(13):5722–2730 (2005). doi:10.1021/la050039n.
59. M. J. Geerken, R. G. H. Lammertink, and M. Wessling. Interfacial aspects of water drop formation at micro-engineered orifices. *J. Colloid Interface Sci.* **312**(2):460–446 (2007). doi:10.1016/j.jcis.2007.03.074.
60. C. Charcosset, A. El-Harati, and H. Fessi. Preparation of solid lipid nanoparticles using a membrane contactor. *J. Control. Release*. **108**:112–120 (2005). doi:10.1016/j.jconrel.2005.07.023.
61. I. Limayem Blouza, C. Charcosset, S. Sfar, and H. Fessi. Preparation and characterization of spironolactone-loaded nanocapsules for paediatric use. *Int. J. Pharm.* **325**:124–131 (2006). doi:10.1016/j.ijpharm.2006.06.022.
62. N. Sheibat-Othman, T. Burne, C. Charcosset, and H. Fessi. Preparation of pH-sensitive particles by membrane contactor. *Colloid Surf. A*. **315**:13–22 (2008). doi:10.1016/j.colsurfa.2007.07.003.
63. S. Desgouilles, C. Vauthier, D. Bazile, J. Vacus, J.-L. Grossiord, M. Veillard, and P. Couvreur. The design of nanoparticles obtained by solvent evaporation: a comprehensive study. *Langmuir.* **19**(22):9504–9510 (2003). doi:10.1021/la034999q.
64. S. A. Vitale, and J. L. Katz. Liquid droplet dispersions formed by homogeneous liquid-liquid nucleation: "The ouzo effect". *Langmuir.* **19**(10):4105–4110 (2003). doi:10.1021/la026842o.
65. K. Bouchemal, S. Briançon, E. Perrier, and H. Fessi. Nano-emulsion formulation using spontaneous emulsification: Solvent, oil and surfactant optimization. *Int. J. Pharm.* **280**(1–2): 241–251 (2004). doi:10.1016/j.ijpharm.2004.05.016.
66. F. Ganachaud, and J. Katz. Nanoparticles and nanocapsules created using the ouzo effect: spontaneous emulsification as an alternative to ultrasonic and high-shear devices. *Chem. Phys. Chem.* **6**:209–216 (2005). doi:10.1002/cphc.200400527.
67. J. W. Nah, T. R. Jung, Y. L. Jeong, M. K. Jang. Biodegradable nanoparticles of poly(DL-lactide-co-glycolide) encapsulating ciprofloxacin HCl having an extended-release property and manufacturing method thereof. World Patent 054042 (2008).
68. C. K. Weiss, U. Ziener, and K. Landfester. A route to nonfunctionalized and functionalized poly(n-butylcyanoacrylate) nanoparticles: preparation in miniemulsion. *Macromolecules*. **40**(4):928–938 (2007). doi:10.1021/ma061865l.
69. K. Landfester. Polyreactions in miniemulsions. *Macromol. Rapid Comm.* **22**:896–936 (2001). doi:10.1002/1521-3927(20010801)22:12<896::AID-MARC896>3.0.CO;2-R.
70. J. Qiu, B. Charleux, and K. Matyjaszewski. Controlled/living radical polymerization in aqueous media: homogeneous and heterogeneous systems. *Prog. Polym. Sci.* **26**:2083–2134 (2001). doi:10.1016/S0079-6700(01)00033-8.
71. R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer. Biodegradable long-circulating polymeric nanospheres. *Science*. **263**:1600–1603 (1994). doi:10.1126/science.8128245.
72. D. Bazile, C. Prud'homme, M. T. Bassoulet, M. Marlard, G. Spenlehauer, and M. Veillard. Stealth Me-PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. *J. Pharm. Sci.* **84**:493–498 (1995). doi:10.1002/jps.2600840420.
73. C. Lemarchand, P. Couvreur, M. Besnard, D. Costantini, and R. Gref. Novel polyester-polysaccharide nanoparticles. *Pharm. Res.* **20**(8):1284–1292 (2003). doi:10.1023/A:1025017502379.
74. R. Gurny, N. A. Peppas, D. D. Harrington, and G. S. Banker. Development of biodegradable and injectable lattices for controlled release potent drugs. *Drug. Dev. Ind. Pharm.* **7**:1–25 (1981). doi:10.3109/03639048109055684.
75. E. Allémann, R. Gurny, and E. Doelker. Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: influence of process parameters on particle size. *Int. J. Pharm.* **87**(1–3):247–253 (1992). doi:10.1016/0378-5173(92)90249-2.
76. W. Y. Dong, M. Körber, V. López Esguerra, and R. Bodmeier. Stability of poly(D,L-lactide-co-glycolide) and leuprolide acetate in *in-situ* forming drug delivery systems. *J. Control. Release*. **115**(2):158–167 (2006). doi:10.1016/j.jconrel.2006.07.013.
77. F. Delie, M. Berton, E. Allémann, and R. Gurny. Comparison of two methods of encapsulation of an oligonucleotide into Poly (D,L-Lactic Acid) particles. *Int. J. Pharm.* **214**:25–30 (2001). doi:10.1016/S0378-5173(00)00627-X.
78. U. Bilati, E. Allémann, and E. Doelker. Poly(D,L-lactide-co-glycolide) protein-loaded nanoparticles prepared by the double emulsion method—processing and formulation issues for enhanced entrapment efficiency. *J. Microencapsul.* **22**(2):205–214 (2005). doi:10.1080/02652040400026442.
79. J. W. Vanderhoff, M. S. El Aasser, and J. Ugelstad. Polymer emulsification process. US Patent 4,177,177 (1979).
80. D. Quintanar-Guerrero, E. Allémann, H. Fessi, and E. Doelker. Pseudolatex preparation using a novel emulsion-diffusion

process involving direct displacement of partially water-miscible solvents by distillation. *Int. J. Pharm.* **188**(2):155–164 (1999). doi:10.1016/S0378-5173(99)00216-1.

81. R. C. Mundargi, V. R. Babu, V. Rangaswamy, P. Patel, and T. M. Aminabhavi. Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. *J. Control. Release*. **125**(3):193–209 (2008). doi:10.1016/j.jconrel.2007.09.013.
82. I. Brigger, P. Chaminade, D. Desmaële, M. T. Peracchia, J. d'Angelo, R. Gurny, M. Renoir, and P. Couvreur. Near infrared with principal component analysis as a novel analytical approach for nanoparticle technology. *Pharm. Res.* **17** (9):1124–1132 (2000). doi:10.1023/A:1026465931525.
83. J. C. Leroux, E. Allemann, E. Doelker, and R. Gurny. New approach for the preparation of nanoparticles by an emulsification–diffusion method. *Eur. J. Pharm. Biopharm.* **41**(1):14–18 (1995).
84. D. Quintanar-Guerrero, E. Allémann, E. Doelker, and H. Fessi. A mechanistic study of the formation of polymer nanoparticles by the emulsification–diffusion technique. *Colloid Polym. Sci.* **275**:640–647 (1997). doi:10.1007/s003960050130.
85. D. Quintanar-Guerrero, É. Allémann, H. Fessi, and E. Doelker. Influence of stabilizing agents and preparative variables on the formation of poly(-lactic acid) nanoparticles by an emulsification–diffusion technique. *Int. J. Pharm.* **143**:133–141 (1996). doi:10.1016/S0378-5173(96)04697-2.
86. S. Guinebretière. Nanocapsules par émulsion–diffusion de solvant: obtention, caractérisation et mécanisme de formation. Ph.D. Université Claude Bernard Lyon 1 (2001).
87. D. Quintanar-Guerrero, D. Tamayo-Esquivel, A. Ganem-Quintanar, E. Allémann, and E. Doelker. Adaptation and optimization of the emulsification–diffusion technique to prepare lipidic nanospheres. *Eur. J. Pharm. Sci.* **26**:211–218 (2005). doi:10.1016/j.ejps.2005.06.001.
88. M. Trotta, F. Debernardi, and O. Caputo. Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique. *Int. J. Pharm.* **257**:153–160 (2003). doi:10.1016/S0378-5173(03)00135-2.
89. L. Battaglia, M. Trotta, M. Gallarate, M. E. Carlotti, G. P. Zara, and A. Bargoni. Solid lipid nanoparticles formed by solvent-in-water emulsion–diffusion technique: Development and influence on insulin stability. *J. Microencapsul.* **24**:672–684 (2007). doi:10.1080/02652040701532981.
90. D. Quintanar-Guerrero, A. Ganem-Quintanar, E. Allemann, H. Fessi, and E. Doelker. Influence of the stabilizer coating layer on the purification and freeze-drying of poly(D,L-lactic acid) nanoparticles prepared by an emulsion–diffusion technique. *J. Microencapsul.* **15**:107–119 (1998). doi:10.3109/02652049809006840.
91. F. F. De Jaeghere, E. Allémann, F. Kubel, B. Galli, R. Cozens, E. Doelker, and R. Gurny. Oral bioavailability of a poorly water soluble HIV-1 protease inhibitor incorporated into pH-sensitive particles: effect of the particle size and nutritional state. *J. Control. Release*. **68**(2):291–298 (2000). doi:10.1016/S0168-3659(00)00272-8.
92. M. Berton, E. Allemann, C. A. Stein, and R. Gurny. Highly loaded nanoparticulate carrier using an hydrophobic antisense oligonucleotide complex. *Eur. J. Pharm. Sci.* **9**(2):163–170 (1999). doi:10.1016/S0928-0987(99)00049-4.
93. Y. N. Konan, R. Cerny, J. Favet, M. Berton, R. Gurny, and E. Allémann. Preparation and characterization of sterile sub-200 nm meso-tetra(4-hydroxylphenyl)porphyrin-loaded nanoparticles for photodynamic therapy. *Eur. J. Pharm. Biopharm.* **55**:115–124 (2003). doi:10.1016/S0939-6411(02)00128-5.
94. H. S. Yoo, J. E. Oh, K. H. Lee, and T. G. Park. Biodegradable nanoparticles containing PLGA conjugate for sustained release. *Pharm. Res.* **16**:1114–1118 (1996). doi:10.1023/A:1018908421434.
95. S. Guinebretière, S. Briancon, H. Fessi, V. S. Teodorescu, and M. G. Blanchin. Nanocapsules of biodegradable polymers: preparation and characterization by direct high resolution electron microscopy. *Mater. Sci. Eng. C.* **21**:137–142 (2002). doi:10.1016/S0928-4931(02)00073-5.
96. D. Quintanar, H. Fessi, É. Doelker, and E. Allémann, Procédé de préparation de nanocapsules de type vésiculaire. World Patent 004766 (1999).
97. H. Ibrahim, C. Bindschaedler, E. Doelker, P. Buri, and R. Gurny. Aqueous nanodispersions prepared by a salting-out process. *Int. J. Pharm.* **87**:239–246 (1992). doi:10.1016/0378-5173(92)90248-Z.
98. E. Allémann, J. C. Leroux, R. Gurny, and E. Doelker. *In vitro* extended-release properties of drug-loaded poly(DL-lactic acid) nanoparticles produced by a salting-out procedure. *Pharm. Res.* **10**:1732–1737 (1993). doi:10.1023/A:1018970030327.
99. E. Allémann, E. Doelker, and R. Gurny. Drug loaded poly (lactic acid) nanoparticles produced by a reversible salting-out process: purification of an injectable dosage form. *Eur. J. Pharm. Biopharm.* **39**:13–18 (1993).
100. N. Wang, and X. S. Wu. Preparation and characterization of agarose hydrogel nanoparticles for protein and peptide drug delivery. *Pharm. Dev. Technol.* **2**:135–142 (1997). doi:10.3109/10837459709022618.
101. H. Tokumitsu, H. Ichikawa, Y. Fukumori, J. Hiratsuka, Y. Sakurai, and T. Kobayashi. Preparation of gadopentenate-loaded nanoparticles for gadolinium neutron capture therapy of cancer using a novel emulsion droplet coalescence technique. *Proc. 2nd world meeting APGI/APV*, Paris, France, 25–28 Mai 1998, pp. 641–642 (1998).
102. C. Pinto-Reis, A. J. Ribeiro, F. Veiga, R. J. Neufeld, and C. Damgé. Polyelectrolyte biomaterial interactions provide nanoparticulate carrier for oral insulin delivery. *Drug. Deliv.* **15** (2):127–139 (2008). doi:10.1080/10717540801905165.
103. P. Couvreur, B. Kante, M. Roland, P. Guiot, P. Baudhuin, and P. Speiser. Poly(cyanoacrylate) nanoparticles as potential lysosomotropic carriers: preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.* **31**:331–332 (1979).
104. C. Chauvierre, D. Labarre, P. Couvreur, and C. Vauthier. A radical emulsion polymerization of alkylcyanoacrylates initiated by the redox system dextran–cerium IV in acidic aqueous conditions. *Macromolecules*. **36**:6018–6027 (2003). doi:10.1021/ma034097w.
105. I. Bertholon, S. Lesieur, D. Labarre, M. Besnard, and C. Vauthier. Characterization of dextran-poly(isobutylcyanoacrylate) copolymers obtained by redox radical and anionic emulsion polymerization. *Macromolecules*. **39**:3559–3567 (2006). doi:10.1021/ma060338z.
106. I. Bertholon, C. Vauthier, and D. Labarre. Complement Activation by core-shell poly(isobutylcyanoacrylate)-polysaccharide nanoparticles: influences of surface morphology, length and type of polysaccharide. *Pharm. Res.* **23**:1313–1323 (2006). doi:10.1007/s11095-006-0069-0.
107. M. R. Gasco, and M. Trotta. Nanoparticles from microemulsions. *Int. J. Pharm.* **29**:267–268 (1986). doi:10.1016/0378-5173(86)90125-0.
108. S. Watnasirichaikul, M. N. Davies, R. Rades, and I. G. Tucker. Preparation of biodegradable insulin nanocapsules from biocompatible microemulsions. *Pharm. Res.* **17**:684–689 (2000). doi:10.1023/A:1007574030674.
109. K. Bouchemal, F. Couenne, S. Briançon, H. Fessi, and M. Tayakout. Stability studies on colloidal suspensions of polyurethane nanocapsules. *J. Nanosci. Nanotechno.* **6**:3187–3192 (2006). doi:10.1166/jnn.2006.468.
110. C. Vauthier, D. Labarre, and G. Ponchel. Design aspects of poly (alkylcyanoacrylate) nanoparticles for drug delivery. *J. Drug. Targeting*. **15**:641–663 (2007). doi:10.1080/10611860701603372.
111. C. Pinto-Reis, R. J. Neufeld, A. J. Ribeiro, and F. Veiga. Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. *Nanomedicine*. **2**(2):53–65 (2006).
112. G. E. Ghanem, C. Joubran, R. Arnould, F. Lejeune, and J. Fruhling. Labelled polycyanoacrylate nanoparticles for human *in vivo* use. *Appl. Radiat. Isotopes*. **44**(9):1219–1224 (1993). doi:10.1016/0969-8043(93)90068-L.
113. R. K. Kulkarni, D. E. Bartak, and F. Leonard. Initiation of polymerization of alkyl 2-cyanoacrylates in aqueous solutions of glycine and its derivatives. *J. Polym. Sci. A. Polym. Chem.* **9** (10):2977–2981 (1971).
114. S. J. Douglas, L. Illum, and S. S. Davis. Particle size and size distribution of poly(butyl 2-cyanoacrylate) nanoparticles. II. Influence of stabilizers. *J. Colloid Interface Sci.* **103**:154–163 (1985). doi:10.1016/0021-9797(85)90087-6.

115. M. T. Peracchia, C. Vauthier, M. Popa, F. Puisieux, and P. Couvreur. An investigation on the formation of sterically stabilized PEG-PIBCA nanoparticles by chemical grafting of PEG during the polymerization of isobutylcyanoacrylate. *STP Pharma Sci.* **7**:514–521 (1997).
116. C. Chauvierre, D. Labarre, P. Couvreur, and C. Vauthier. Plug-in spectrometry with optical fibers as a novel analytical tool for nanoparticles technology: application to the investigation of the emulsion polymerization of the alkylcyanoacrylate. *J. Nanopart. Res.* **5**:365–371 (2003). doi:10.1023/A:1025575730542.
117. S. C. Yang, H. X. Ge, Y. Hu, X. Q. Jiang, and C. Z. Yang. Formation of positively charged poly(butyl cyanoacrylate) nanoparticles stabilized by chitosan. *Colloid Polym. Sci.* **278**:285–292 (2000). doi:10.1007/s003960050516.
118. D. Labarre, C. Vauthier, C. Chauvierre, B. Petri, R. Müller, and M. M. Chehimi. Interactions of blood proteins with poly (isobutylcyanoacrylate) nanoparticles decorated with a polysaccharidic brush. *Biomaterials*. **26**(24):5075–5084 (2005). doi:10.1016/j.biomaterials.2005.01.019.
119. I. Bravo-Osuna, G. Ponchel, and C. Vauthier. Tuning of shell and core characteristics of chitosan-decorated acrylic nanoparticles. *Eur. J. Pharm. Sci.* **30**:143–154 (2007). doi:10.1016/j.ejps.2006.10.007.
120. I. Bravo Osuna, C. Vauthier, and G. Ponchel. Core-shell polymer nanoparticle formulations for the oral administration of peptides and proteins. In A. O. Hartmann, and L. K. Newmann (eds.), *Drugs: Approval, Evaluation, Delivery and Control*, Novapublishers, New York, 2008, pp. 35–71.
121. M. T. Peracchia, C. Vauthier, C. Passirani, P. Couvreur, and D. Labarre. Complement consumption by poly(ethylene glycol) in different configurations chemically coupled to polyisobutylcyanoacrylate nanoparticles. *Life Sci.* **61**:749–761 (1997). doi:10.1016/S0024-3205(97)00539-0.
122. C. Passirani, G. Barratt, J. P. Devissaguet, and D. Labarre. Long-circulating nanoparticles bearing heparin or dextran covalently bound to poly(methyl methacrylate). *Pharm. Res.* **15**(7):1046–1050 (1998). doi:10.1023/A:1011930127562.
123. I. Bravo-Osuna, C. Vauthier, A. Farabollini, G. F. Palmieri, and G. Ponchel. Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles. *Biomaterials*. **28**(13):2233–2243 (2007). doi:10.1016/j.biomaterials.2007.01.005.
124. I. Bravo-Osuna, C. Vauthier, H. Chacun, and G. Ponchel. Specific permeability modulation of intestinal paracellular pathway by chitosan-poly(isobutylcyanoacrylate) core-shell nanoparticles. *Eur. J. Pharm. Biopharm.* **69**:436–444 (2008). doi:10.1016/j.ejpb.2007.12.012.
125. M. Aboubakar, F. Puisieux, P. Couvreur, M. Deyme, and C. Vauthier. Study of the mechanism of insulin encapsulation in poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial polymerization. *J. Biomed. Mater. Res.* **47**:568–576 (1999). doi:10.1002/(SICI)1097-4636(19991215)47:4<568::AID-JBM14>3.0.CO;2-X.
126. M. Gallardo, G. Couarraze, B. Denizot, L. Treupel, P. Couvreur, and F. Puisieux. Study of the mechanism of formation of nanoparticles and nanocapsules of poly(isobutyl-2-cyanoacrylate). *Int. J. Pharm.* **100**:55–64 (1993). doi:10.1016/0378-5173(93)90075-Q.
127. G. Puglisi, M. Fresta, G. Giammona, and C. A. Ventura. Influence of the preparation conditions on poly(ethylcyanoacrylate) nanocapsule formation. *Int. J. Pharm.* **125**:283–287 (1995). doi:10.1016/0378-5173(95)00142-6.
128. N. Altinbas, C. Fehmer, A. Terheiden, A. Shukla, H. Rehage, and C. Mayer. Alkylcyanoacrylate nanocapsules prepared from mini-emulsions: a comparison with the conventional approach. *J. Microencapsul.* **23**(5):567–581 (2006). doi:10.1080/02652040600776424.
129. C. Y. Huang, C. M. Chen, and Y. D. Lee. Synthesis of high loading and encapsulation efficient paclitaxel-loaded poly(n-butyl cyanoacrylate) nanoparticles via miniemulsion. *Int. J. Pharm.* **338**(1–2):267–275 (2007). doi:10.1016/j.ijpharm.2007.01.052.
130. K. Krauel, N. M. Davies, S. Hook, and T. Rades. Using different structure types of microemulsions for the preparation of poly(alkylcyanoacrylate) nanoparticles by interfacial polymerization. *J. Control. Release*. **106**:76–87 (2005). doi:10.1016/j.jconrel.2005.04.013.
131. I. Montasser, H Fessi, S. Briançon, and J. Lieto. Procédé de préparation de particules colloïdales sous forme de nanocapsules. *World Patent* 0168235 (2001).
132. I. Montasser, S. I. Briançon, and H. Fessi. The effect of monomers on the formulation of polymeric nanocapsules based on polyureas and polyamides. *Int. J. Pharm.* **335**(1–2):176–179 (2007). doi:10.1016/j.ijpharm.2006.11.011.
133. K. Bouchemal, F. Couenne, S. Briançon, H. Fessi, and M. Tayakout. Polyamides nanocapsules: modelling and wall thickness estimation. *AIChE J.* **52**(6):1–10 (2006).
134. H. Fessi, F. Puisieux, J.-P. Devissaguet, N. Ammoury, and S. Benita. Nanocapsule formation by interfacial deposition following solvent displacement. *Int. J. Pharm.* **55**:R1–R4 (1989). doi:10.1016/0378-5173(89)90281-0.
135. R. A. Jain. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials*. **21**:2475–2490 (2000). doi:10.1016/S0142-9612(00)00115-0.
136. T. Delair. Colloidal particles: elaboration from preformed polymers. In A. Elaissari (ed.), *Colloidal Biomolecules, Biomaterials and Biomedical Applications*, Marcel Dekker, New York, 2004, pp. 329–347.
137. O. Thioune, H. Fessi, J. P. Devissaguet, and F. Puisieux. Preparation of pseudolatex by nanoprecipitation: Influence of the solvent nature on intrinsic viscosity and interaction constant. *Int. J. Pharm.* **146**:233–238 (1997). doi:10.1016/S0378-5173(96)04830-2.
138. P. Legrand, S. Lesieur, A. Bochot, R. Gref, W. Raatjes, G. Barratt, and C. Vauthier. Influence of polymer behaviour in organic solution on the production of polylactide nanoparticles by nanoprecipitation. *Int. J. Pharm.* **344**:33–43 (2007). doi:10.1016/j.ijpharm.2007.05.054.
139. H. Murakami, M. Kobayashi, H. Takeuchi, and Y. Kawashima. Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *Int. J. Pharm.* **187**(2):143–152 (1999). doi:10.1016/S0378-5173(99)00187-8.
140. M. T. Peracchia, C. Vauthier, D. Desmaël, A. Gulik, J. C. Dedieu, M. Demoy, J. D'Angelo, and P. Couvreur. Pegylated nanoparticles from a novel methoxypolyethyleneglycol cyanoacrylate-hexadecyl cyanoacrylate amphiphilic copolymer. *Pharm. Res.* **15**:550–556 (1998). doi:10.1023/A:1011973625803.
141. C. Duclairoir, E. Nakache, H. Marchais, and A. M. Orecchioni. Formation of gliadin nanoparticles: influence of the solubility parameter of the protein solvent. *Colloid Polym. Sci.* **276**:321–327 (1998). doi:10.1007/s003960050246.
142. M. Skiba, D. Wouessidjewe, F. Puisieux, D. Duchêne, and A. Gulik. Characterization of amphiphilic fl-cyclodextrin nanospheres. *Int. J. Pharm.* **142**:121–124 (1996). doi:10.1016/0378-5173(96)04653-4.
143. H. Lannibois-Drean. Des molécules hydrophobes dans l'eau: fabrication de nanoparticles par precipitation. Ph.D. Université Pierre et Marie Curie, Paris, France (1995).
144. H. Lannibois, A. Hasmy, R. Botet, O. Aguerre Chariol, and B. Cabane. Surfactant limited aggregation of hydrophobic molecules in water. *J. Phys. II*. **7**:319–342 (1997). doi:10.1051/jp2:1997128.
145. T. Niwa, T. Takeuchi, T. Hino, N. Kunou, and Y. Kawashima. Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with d,llactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method and the drug release behavior. *J. Control. Release*. **25**:89–98 (1993). doi:10.1016/0168-3659(93)90097-O.
146. H. Murakami, M. Kobayashi, H. Takeuchi, and Y. Kawashima. Further application of a modified spontaneous emulsification solvent diffusion method to various types of PLGA and PLA polymers for preparation of nanoparticles. *Powder Technol.* **107**:137–143 (2000).
147. L. Peltonen, J. Anitta, S. Hyvönen, M. Kajalainen, and J. Hirvonen. Improved entrapment efficiency of hydrophilic drug substance during nanoprecipitation of poly(l)lactide nanoparticles. *AAPS PharmSciTech*. **5**(1):1–6 (2004). doi:10.1007/BF02830584.

148. I. Limayem, C. Charcosset, and H. Fessi. Purification of nanoparticle suspensions by a concentration/diafiltration process. *Sep. Purif. Technol.* **38**:1–9 (2004). doi:10.1016/j.seppur.2003.10.002.
149. F. Némati, C. Dubernet, H. Fessi, A. C. Verdière, M. F. Poupon, F. Puisieux, and P. Couvreur. Reversion of multidrug resistance using nanoparticles *in vitro*: influence of the nature of the polymer. *Int. J. Pharm.* **138**:237–246 (1996). doi:10.1016/0378-5173(96)04559-0.
150. U. Bilati, E. Allémann, and E. Doelker. Nanoprecipitation *Versus* Emulsion-based techniques for the encapsulation of proteins into biodegradable nanoparticles and process-related stability issues. *AAPS PharmSciTech.* **6**(4):E594–E604 (2005). doi:10.1208/pt060474.
151. J. M. Barichello, M. Morishita, K. Takayama, and T. Nagai. Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. *Drug. Dev. Ind. Pharm.* **25**:471–476 (1999). doi:10.1081/DDC-100102197.
152. J. Molpeceres, M. Guzman, M. R. Aberturas, M. Chacon, and L. Berges. Application of central composite design to the preparation of polycaprolactone nanoparticles by solvent displacement. *J. Pharm. Sci.* **85**:206–13 (1996). doi:10.1021/js950164r.
153. Y. Zhang, and R.-X. Zhuo. Synthesis and *in vitro* drug release behavior of amphiphilic triblock copolymer nanoparticles based on poly (ethylene glycol) and polycaprolactone. *Biomaterials*. **26**:6736–6742 (2005). doi:10.1016/j.biomaterials.2005.03.045.
154. P. Arbós, M. A. Campanero, M. A. Arangoa, M. J. Renedo, and J. M. Irache. Influence of the surface characteristics of PVM/MA nanoparticles on their bioadhesive properties. *J. Control Release*. **89**:19–30 (2003). doi:10.1016/S0168-3659(03)00066-X.
155. M. Skiba, C. Morvan, D. Duchene, F. Puisieux, and D. Wouessidjewe. Evaluation of gastrointestinal behaviour in the rat of amphiphilic β-cyclodextrin nanocapsules, loaded with indomethacin. *Int. J. Pharm.* **126**:275–279 (1995). doi:10.1016/0378-5173(95)04121-4.
156. E. Lemos-Senna, D. Wouessidjewe, S. Lesieur, F. Puisieux, G. Couarraze, and D. Duchêne. Evaluation of the hydrophobic drug loading characteristics in nanoprecipitated amphiphilic cyclodextrin nanospheres. *Pharm. Dev. Technol.* **3**(1):85–94 (1998). doi:10.3109/10837459809028482.
157. E. Lemos-Senna, D. Wouessidjewe, S. Lesieur, and D. Duchêne. Preparation of amphiphilic cyclodextrin nanospheres using the emulsification solvent evaporation method. Influence of the surfactant on preparation and hydrophobic drug loading. *Int. J. Pharm.* **170**:119–128 (1998). doi:10.1016/S0378-5173(98)00147-1.
158. K. A. Howard, and J. Kjems. Polycation-based nanoparticle delivery for improved RNA interference therapeutics. *Expert. Opin. Biol. Ther.* **7**(12):1811–1822 (2007). doi:10.1517/14712598.7.12.1811.
159. M. Rajaonarivony, C. Vauthier, G. Couarraze, F. Puisieux, and P. Couvreur. Development of a new drug carrier made from alginate. *J. Pharm. Sci.* **82**:912–918 (1993). doi:10.1002/jps.2600820909.
160. C. Schatz, A. Domard, C. Viton, C. Pichot, and T. Delair. Versatile and efficient formation of colloids of biopolymer-based polyelectrolyte complexes. *Biomacromolecules*. **5**(5):1882–1892 (2004). doi:10.1021/bm049786+.
161. A. Drogoz, L. David, C. Rochas, A. Domard, and T. Delair. Polyelectrolyte complexes from polysaccharides: formation and stoichiometry monitoring. *Langmuir*. **23**(22):10950–10958 (2007). doi:10.1021/la7008545.
162. A. Drogoz, S. Munier, B. Verrier, L. David, A. Domard, and T. Delair. Towards biocompatible vaccine delivery systems: interactions of colloidal PECs based on polysaccharides with HIV-1 p24 antigen. *Biomacromolecules*. **9**(2):583–591 (2008). doi:10.1021/bm701154h.
163. R. Gref, C. Amiel, K. Molinard, S. Daoud-Mahammed, B. Sébille, B. Gillet c, J-C. Beloeil, C. Ringard, V. Rosilio, J. Poupaert, and P. Couvreur. New self-assembled nanogels based on host–guest interactions: Characterization and drug loading. *J. Control. Release*. **111**:316–324 (2006). doi:10.1016/j.jconrel.2005.12.025.
164. S. Daoud-Mahammed, C. Ringard-Lefebvre, N. Razzouq, V. Rosilio, B. Gillet, P. Couvreur, C. Amiel, and R. Gref. Spontaneous association of hydrophobized dextran and poly-β-cyclodextrin into nanoassemblies. Formation and interaction with a hydrophobic drug. *J. Colloid Interface Sci.* **307**(1):83–93 (2007). doi:10.1016/j.jcis.2006.10.072.
165. S. De, and D. Robinson. Polymer relationships during preparation of chitosan-alginate and poly-l-lysine-alginate nanospheres. *J. Control. Release*. **89**(1):101–12 (2003). doi:10.1016/S0168-3659(03)00098-1.
166. K. L. Douglas, and M. Tabrizian. Effect of experimental parameters on the formation of alginate-chitosan nanoparticles and evaluation of their potential application as DNA carrier. *J. Biomater. Sci. Polym. E.* **16**(1):43–56 (2005). doi:10.1163/1568562052843339.
167. B. Sarmento, A. J. Ribeiro, F. Veiga, D. C. Ferreira, and R. J. Neufeld. Insulin-loaded nanoparticles are prepared by alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation. *J Nanosci. Nanotechnol.* **7**(8):2833–2841 (2007). doi:10.1166/jnn.2007.609.
168. I. Aynié. Vectorisation d'oligonucleotides antisens par des nanoparticules d'alginate. Ph.D. Université Paris Sud-11. 01 February 1999.
169. I. Aynié, C. Vauthier, H. Chacun, E. Fattal, and P. Couvreur. Sponge-like alginate nanoparticles as a new system for the delivery of antisense oligonucleotides. *Antisense Nucleic Acid Drug Dev.* **9**:301–312 (1999).
170. M. González Ferreiro, L. Tillman, G. Hardee, and R. Bodmeier. Characterization of alginate/poly-L-lysine particles as antisense oligonucleotide carriers. *Int. J. Pharm.* **239**(1–2):47–59 (2002). doi:10.1016/S0378-5173(02)00030-3.
171. P. Calvo, C. Remuñan-Lopez, J. L. Vila-Jato, and M. J. Alonso. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharm. Res.* **14**:1431–1436 (1997). doi:10.1023/A:1012128907225.
172. P. Calvo, C. Remuñan-Lopez, J. L. Vila-Jato, and M. J. Alonso. Novel hydrophilic chitosan-polyethylene ocide nanoparticles as protein carriers. *J. Appl. Polym. Sci.* **63**:125–132 (1997). doi:10.1002/(SICI)1097-4628(19970103)63:1<125::AID-APP13>3.0.CO;2-4.
173. T. López-León, E. L. Carvalho, B. Seijo, J. L. Ortega-Vinuesa, and D. Bastos-González. Physicochemical characterization of chitosan nanoparticles: electrokinetic and stability behavior. *J. Colloid Interf. Sci.* **283**(2):344–351 (2005). doi:10.1016/j.jcis.2004.08.186.
174. R. Fernández-Urrusuno, P. Calvo, C. Remuñán-López, J. L. Vila-Jato, and M. J. Alonso. Enhancement of nasal absorption of insulin using chitosan nanoparticles. *Pharm. Res.* **16**(10):1576–1581 (1999). doi:10.1023/A:1018908705446.
175. M. Cetin, Y. Aktas, I. Vural, Y. Capan, L.A. Dogan, M. Duman, and T. Dalkara. Preparation and *in vitro* evaluation of bFGF-loaded chitosan nanoparticles. *Drug. Deliv.* **14**(8):525–529 (2007). doi:10.1080/10717540701606483.
176. K. A. Janes, P. Calvo, and M. J. Alonso. Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv. Drug Deliv. Rev.* **47**(1):83–97 (2001). doi:10.1016/S0169-409X(00)00123-X.
177. H. Kastar, and H. O. Alpar. Development and characterisation of chitosan nanoparticles for siRNA delivery. *J. Control. Release*. **115**(2):216–225 (2006). doi:10.1016/j.jconrel.2006.07.021.
178. T. H. Dung, S. R. Lee, S. D. Han, S. J. Kim, Y. M. Ju, M. S. Kim, and H. Yoo. Chitosan-TPP nanoparticle as a release system of antisense oligonucleotide in the oral environment. *J. Nanosci. Nanotechnol.* **7**(11):3695–3699 (2007). doi:10.1166/jnn.2007.041.
179. C. Pegro, D. Torres, and M. J. Alonso. The potential of chitosan for the oral administration of peptides. *Expert. Opin. Drug. Deliv.* **2**(5):843–854 (2005). doi:10.1517/17425247.2.5.843.
180. N. Csaba, M. Garcia-Fuentes, and M. J. Alonso. The performance of nanocarriers for transmucosal drug delivery. *Expert. Opin. Drug. Deliv.* **3**(4):463–478 (2006). doi:10.1517/17425247.3.4.463.
181. S. M. Moghimi, A. C. Hunter, and J. C. Murray. Nanomedicine: current status and future prospects. *FASEB J.* **19**:311–330 (2005). doi:10.1096/fj.04-2747rev.

182. A. Vonabourg, C. Passirani, P. Saulnier, and J. P. Benoit. Parameters influencing the stealthiness of colloidal drug delivery systems. *Biomaterials*. **27**:4356–4373 (2006). doi:10.1016/j.biomaterials.2006.03.039.
183. L. Nobs, F. Buchegger, R. Gurny, and E. Allemann. Poly(lactic acid) nanoparticles labeled with biologically active Neutravidin for active targeting. *Eur. J. Pharm. Biopharm.* **58**(3):483–490 (2004). doi:10.1016/j.ejpb.2004.04.006.
184. L. Nobs, F. Buchegger, R. Gurny, and E. Allemann. Current methods for attaching targeting ligands to liposomes and nanoparticles. *J. Pharm. Sci.* **93**(8):1980–1992 (2004). doi:10.1002/jps.20098.
185. B. Stella, S. Arpicco, M. T. Peracchia, D. Desmaële, J. Hoebeke, M. Renoir, J. D'Angelo, L. Cattel, and P. Couvreur. Design of folic acid-conjugated nanoparticles for drug targeting. *J. Pharm. Sci.* **89**(11):1452–1464 (2000). doi:10.1002/1520-6017(200011)89:11<1452::AID-JPS8>3.0.CO;2-P.
186. Y. De Kozak, K. Andrieux, H. Villarroya, C. Klein, B. Thillaye-Goldenberg, M. C. Naud, E. Garcia, and P. Couvreur. Intraocular injection of tamoxifen-loaded nanoparticles: a new treatment of experimental autoimmune uveoretinitis. *Eur. J. Immunol.* **34**(12):3702–3712 (2004). doi:10.1002/eji.200425022.
187. D. W. Barlet, and M. E. Davis. Physicochemical and biological characterization of targeted nucleic acid-containing nanoparticles. *Bioconjug. Chem.* **18**:456–468 (2007). doi:10.1021/bc0603539.
188. R. Gref, P. Couvreur, G. Barratt, and E. Mysiakine. Surface-engineered nanoparticles for multiple ligand coupling. *Biomaterials*. **24**(24):4529–4537 (2003). doi:10.1016/S0142-9612(03)00348-X.
189. L. Illum, L. O. Jacobsen, R. H. Müller, R. Mak, and S. S. Davis. Surface characteristics and the interaction of colloidal particles with mouse peritoneal macrophages. *Biomaterials*. **8**:113–117 (1987). doi:10.1016/0142-9612(87)90099-8.
190. P. Calvo, B. Gouritin, H. Chacun, D. Desmaële, J. D'Angelo, J. P. Noel, G. Georgin, E. Fattal, J. P. Andreux, and P. Couvreur. Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. *Pharm. Res.* **18**(8):1157–1166 (2001). doi:10.1023/A:1010931127745.
191. J. Kreuter. Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. *J. Nanosci. Nanotechnol.* **4**:484–488 (2004). doi:10.1166/jnn.2003.077.
192. L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, D. Deprez-Decampeneere, and P. Speiser. Pharmacokinetics and distribution of a biodegradable drug-carrier. *Int. J. Pharm.* **15**:335–345 (1983). doi:10.1016/0378-5173(83)90166-7.
193. A. Béduneau, P. Saulnier, and J. P. Benoit. Active targeting of brain tumors using nanocarriers. *Biomaterials*. **28**(33):4947–4967 (2007). doi:10.1016/j.biomaterials.2007.06.011.
194. I. Bertholon, G. Ponchel, D. Labarre, P. Couvreur, and C. Vauthier. Bioadhesive properties of poly(alkylcyanoacrylate) nanoparticles coated with polysaccharide. *J. Nanosci. Nanotechnol.* **6**(9–10):3102–3109 (2006). doi:10.1166/jnn.2006.418.
195. K. Albrecht, and A. Bernkop-Schnürch. Thiomers: forms, functions and applications to nanomedicine. *Nanomedicine*. **2**(1):41–50 (2007). doi:10.2217/17435889.2.1.41.
196. K. Bouchemal. New challenges for pharmaceutical formulations and drug delivery systems characterization using isothermal titration calorimetry. *Drug Discov. Today.* **13**(21–22):960–972 (2008). doi:10.1016/j.drudis.2008.06.004.
197. M. E. Martinez-Barbosa, L. Bouteiller, S. Cammas-Marion, V. Montembault, L. Fontaine, and G. Ponchel. Synthesis and ITC characterization of novel nanoparticles constituted by poly (gamma-benzyl L-glutamate)-beta-cyclodextrin. *J. Mol. Recognit.* **21**(3):169–178 (2008). doi:10.1002/jmr.882.
198. Y. Aktaş, M. Yemisci, K. Andrieux, R. N. Gürsoy, M. J. Alonso, E. Fernandez-Megia, R. Novoa-Carballal, E. Quiñoá, R. Riguera, M. F. Sargon, H. H. Celik, A. S. Demir, A. A. Hincal, T. Dalkara, Y. Capan, and P. Couvreur. Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26. *Bioconjug. Chem.* **16**(6):1503–1511 (2005). doi:10.1021/bc050217o.
199. Bioalliance Pharma: Doxorubicin Transdrug®: Phase II/III http://www.bioalliancepharma.com Assessed 28 August 2008.
200. M. J. Hawkins, P. Soon-Shiong, and N. Desai. Protein nanoparticles as drug carriers in clinical medicine. *Adv. Drug. Deliv. Rev.* **60**:876–885 (2008). doi:10.1016/j.addr.2007.08.044.
201. A. P. Colombo, S. Briancon, J. Lieto, and H. Fessi. Project, design and use of a pilot plant for nanocapsule production. *Drug. Dev. Ind. Pharm.* **27**(10):1063–1072 (2001). doi:10.1081/DDC-100108369.
202. S. A. Galindo-Rodriguez, F. Puel, S. Briançon, E. Allémann, E. Doelker, and H. Fessi. Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. *Eur. J. Pharm. Sci.* **25**:357–367 (2005). doi:10.1016/j.ejps.2005.03.013.
203. S. Briançon, H. Fessi, F. Lecomte, and J. Lieto. Study of an original production process of nanoparticles by precipitation, second ed. European Congress of Chemical Engineering, Montpellier, France (1999).
204. P. Tewa-Tagne, S. Briançon, and H. Fessi. Preparation of redispersible dry nanocapsules by means of spray-drying: development and characterisation. *Eur. J. Pharm. Sci.* **30**:124–135 (2007). doi:10.1016/j.ejps.2006.10.006.
205. S. Watnasirichaikul, T. Rades, I. G. Tucker, and N. M. Davies. *In-vitro* release and oral bioactivity of insulin in diabetic rats using nanocapsules dispersed in biocompatible microemulsion. *J. Pharm. Pharmacol.* **54**:473–480 (2002). doi:10.1211/0022357021778736.
206. T. Govender, S. Stolnik, M. C. Garnett, L. Illum, and S. S. Davis. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. *J. Control. Release*. **57**:171–185 (1999). doi:10.1016/S0168-3659(98)00116-3.
207. S. K. Sahoo, J. Panyam, S. Prabha, and V. Labhasetwar. Residual polyvinyl alcohol associated with poly (DL,-lactide-coglycolide) nanoparticles affects their physical properties and cellular uptake. *J. Control. Release*. **82**:105–114 (2002). doi:10.1016/S0168-3659(02)00127-X.
208. C. A. Nguyen, E. Allemann, G. Schwach, E. Doelker, and R. Gurny. Synthesis of a novel fluorescent poly (DL,-lactide) endcapped with 1-pyrenebutanol used for the preparation of nanoparticles. *Eur. J. Pharm. Sci.* **20**:217–222 (2003). doi:10.1016/S0928-0987(03)00196-9.
209. I. Bravo-Osuna, T. Schmitz, A. Bernkop-Schnürch, C. Vauthier, and G. Ponchel. Elaboration and characterization of thiolated chitosan-coated acrylic nanoparticles. *Int. J. Pharm.* **316**:170–175 (2006). doi:10.1016/j.ijpharm.2006.02.037.
210. P. Beck, D. Scherer, and J. Kreuter. Separation of drug-loaded nanoparticles from free drug by gel filtration. *J. Microencapsul.* **7**:491–496 (1990). doi:10.3109/02652049009040471.
211. J. Zahka, and L. Mir. Ultrafiltration of latex emulsions. *Chem. Eng. Prog.* **73**:53–55 (1977).
212. G. Tishchenko, K. Luetzow, J. Schauer, W. Albrecht, and M. Bleha. Purification of polymer nanoparticles by diafiltration with polysulfone/hydrophilic polymer blend membranes. *Sep. Purif. Technol.* **22–23**:403–415 (2001). doi:10.1016/S1383-5866(00)00177-5.
213. G. Tishchenko, R. Hilke, W. Albrecht, J. Schauer, K. Luetzow, Z. Pientka, and M. Bleha. Ultrafiltration and microfiltration membranes in latex purification by diafiltration with suction. *Sep. Purif. Technol.* **30**:57–68 (2003). doi:10.1016/S1383-5866(02)00120-X.
214. M. T. Peracchia, C. Vauthier, F. Puisieux, and P. Couvreur. Development of sterically stabilized poly(isobutyl 2-cyanoacrylate) nanoparticles by chemical coupling of poly(ethylene glycol). *J. Biomed. Mater. Res.* **34**(3):317–326 (1997). doi:10.1002/(SICI)1097-4636(19970305)34:3<317::AID-JBM6>3.0.CO;2-N.
215. U. B. Kompella, N. Bandi, and S. P. Ayalasomayajula. Poly (lactic acid) nanoparticles for sustained release of budesonide. *Drug Deliv. Technol.* **1**:1–7 (2001).
216. S. Prabha, W. -Z. Zhou, J. Panyam, and V. Labhasetwar. Size dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *Int. J. Pharm.* **244**:105–115 (2002). doi:10.1016/S0378-5173(02)00315-0.
217. S. Dreis, F. Rothweiler, M. Michaelis, J. Cinatl Jr, J. Kreuter, and K. Langer. Preparation, characterisation and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles. *Int. J. Pharm.* **341**(1–2):207–214 (2007). doi:10.1016/j.ijpharm.2007.03.036.
218. M. Hamoudeh, A. Al Faraj, E. Canet-Soulas, F. Bessueille, D. Léonard, and H. Fessi. Elaboration of PLLA-based super-

paramagnetic nanoparticles: characterization, magnetic behaviour study and *in vitro* relaxivity evaluation. *Int. J. Pharm.* **338** (1–2):248–257 (2007). doi:10.1016/j.ijpharm.2007.01.023.
219. H. Pinto-Alphandary, O. Balland, and P. Couvreur. A new method to isolate poly(alkylcyanoacrylate) nanoparticle preparations. *J. Drug. Target.* **3**:167–169 (1995). doi:10.3109/1061186950905921 6.
220. E. Chiellini, L. M. Orsini, and R. Solaro. Polymeric nanoparticles based on polylactide and related co-polymers. *Macromol. Symp.* **197**:345–354 (2003). doi:10.1002/masy.200350730.
221. K. Bouchemal, G. Ponchel, S. Mazzaferro, V.-H. Campos-Requena, C. Gueutin, G.-F. Palmieri, and C. Vauthier. A new approach to determine loading efficiency of Leu-enkephalin in poly(isobutylcyanoacrylate) nanoparticles coated with thiolated chitosan. *J. Drug. Del. Sci. Tech.* **22**(12):2152–2162 (2008).
222. G. Dalwadi, H. A. Benson, and Y. Chen. Comparison of diafiltration and tangential flow filtration for purification of nanoparticle suspensions. *Pharm. Res.* **22**(12):2152–2162 (2005). doi:10.1007/s11095-005-7781-z.
223. P. Harmant, and P. Aimar. Materials, Interfaces and Electrochemical Phenomena coagulation of colloids retained by porous wall. *AIChE J.* **42**:3523 (1996).
224. S. S. Madaeni, and A. G. Fane. Microfiltration of very dilute colloidal mixtures. *J. Membr. Sci.* **113**:301–312 (1996). doi:10.1016/0376-7388(95)00129-8.
225. J. Rollot, P. Couvreur, L. Roblot-Treupel, and F. Puisieux. Physicochemical and morphological characterization of polyisobutylcyanoacrylate nanocapsules. *J. Pharm. Sci.* **75**:361–364 (1986). doi:10.1002/jps.2600750408.
226. V. Masson, F. Maurin, H. Fessi, and J. P. Devissaguet. Influence of sterilization processes on poly(ε-caprolactone) nanospheres. *Biomaterials*. **18**:327–335 (1997). doi:10.1016/S0142-9612(96)00144-5.
227. P. Sommerfeld, U. Schroeder, and B. A. Sabel. Sterilization of unloaded polybutylcyanoacrylate nanoparticles. *Int. J. Pharm.* **164**:113–118 (1998). doi:10.1016/S0378-5173(97)00394-3.
228. G. W. Bos, A. Trullas-Jimeno, W. Jiskoot, D. J. A. Crommelin, and W. E. Hennink. Sterilization of poly(dimethylamino) ethyl methacrylate-based gene transfer complexes. *Int. J. Pharm.* **211**:79–88 (2000). doi:10.1016/S0378-5173(00)00593-7.
229. C. Boess, and K. W. Bögl. Influence of radiation treatment on pharmaceuticals—a review: alkaloids, morphine derivatives and antibiotics. *Drug. Dev. Ind. Pharm.* **22**(6):495–529 (1996). doi:10.3109/03639049609108354.
230. M. B. Sintzel, A. Merklia, C. Tabatabay, and R. Gurny. Influence of irradiation sterilization on polymers used as drug carriers : A review. *Drug. Dev. Ind. Pharm.* **23**(9):857–878 (1997). doi:10.3109/03639049709148693.
231. K. A. Athanasiou, G. G. Niederauer, and C. M. Agrawal. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials*. **17**:93–102 (1996). doi:10.1016/0142-9612(96)85754-1.
232. O. Maksimenko, E. Pavlov, E. Toushov, A. Molin, Y. Stukalov, T. Prudskova, V. Feldman, J. Kreuter, and S. Gelperina. Radiation sterilisation of doxorubicin bound to poly(butyl cyanoacrylate) nanoparticles. *Int. J. Pharm.* **356**(1–2):325–332 (2008). doi:10.1016/j.ijpharm.2008.01.010.
233. E. Memisoglu-Bilensoy, and A. A. Hincal. Sterile, injectable cyclodextrin nanoparticles: Effects of gamma irradiation and autoclaving. *Int. J. Pharm.* **311**:203–208 (2006). doi:10.1016/j.ijpharm.2005.12.013.
234. B. Magenheim, and S. Benita. Nanoparticle characterization: a comprehensive physicochemical approach. *STP Pharma. Sci.* **1**:221–241 (1991).
235. I. Brigger, L. Armand-Lefevre, P. Chaminade, M. Besnard, Y. Rigaldie, A. Largeteau, A. Andremont, L. Grislain, G. Demazeau, and P. Couvreur. The stenlying effect of high hydrostatic pressure on thermally and hydrolytically labile nanosized carriers. *Pharm Res.* **20**(4):674–683 (2003). doi:10.1023/A:1023267304096.
236. F. Nemati, G. N. Cavé, and P. Couvreur. Lyophilization of substances with low water permeability by a modification of crystallized structures during Freezing. Proceedings of the 6th International Congress of Pharmaceutical Technology Assoc. Pharm. Galénique Ind., Châtenay Malabry, APGI, Paris-France. (3):487–493 (1992).
237. S. De Chasteigner, H. Fessi, G. Cavé, J. P. Devissaguet, and F. Puisieux. gastro-intestinal tolerance study of a freeze-dried oral dosage form of indomethacin-loaded nanocapsules. *S.T.P. Pharma Sci.* **5**:242–246 (1995).
238. S. De Chasteigner, G. Cavé, H. Fessi, J. P. Devissaguet, and F. Puisieux. Freeze-drying of Itraconazole-loaded nanosphere suspensions : a feasibility study. *Drug. Dev. Res.* **38**:116–124 (1996). doi:10.1002/(SICI)1098-2299(199606)38:2<116::AID-DDR6>3.0.CO;2-M.
239. M. Auvillain, G. Cavé, H. Fessi, and J. P. Devissaguet. Lyophilisation de vecteurs colloïdaux submicroniques. *STP Pharma. Sci.* **5**:738–744 (1989).
240. W. Abdelwahed, G. Degobert, S. Stainmesse, and H. Fessi. Freeze-drying of nanoparticles: Formulation, process and storage considerations. *Adv. Drug. Deliv. Rev.* **58**:1688–1713 (2006). doi:10.1016/j.addr.2006.09.017.
241. W. Abdelwahed, G. Degobert, and H. Fessi. Freeze-drying of nanocapsules: Impact of annealing on the drying process. *Int. J. Pharm.* **324**:74–82 (2006). doi:10.1016/j.ijpharm.2006.06.047.
242. P. Tewa-Tagne, S. Briançon, and H. Fessi. Spray-dried microparticles containing polymeric nanocapsules: Formulation aspects, liquid phase interactions and particles characteristics. *Int. J. Pharm.* **325**:63–74 (2006). doi:10.1016/j.ijpharm.2006.06.025.
243. A. M. Layre, P. Couvreur, J. Richard, D. Requier, N. E. Ghermani, and R. Gref. Freeze-drying of composite core-shell nanoparticles. *Drug. Dev. Ind. Pharm.* **32**(7):839–846 (2006). doi:10.1080/03639040600685134.
244. F. De Jaeghere, E. Allémann, J. -C. Leroux, W. Stevels, J. Feijen, E. Doelker, and R. Gurny. Formulation and lyoprotection of poly (Lactic acid-co-ethylene oxide) nanoparticles: influence on physical stability and *in vitro* cell uptake. *Pharm. Res.* **16**:859–866 (1999). doi:10.1023/A:1018826103261.
245. M. Sameti, G. Bohr, M. N. V. Ravi Kumar, C. Kneuer, U. Bakowsky, M. Nacken, H. Schmidt, and C. -M. Lehr. Stabilization by freeze-drying of cationically modified silica nanoparticles for gene delivery. *Int. J. Pharm.* **266**:51–60 (2003). doi:10.1016/S0378-5173(03)00380-6.
246. W. Abdelwahed, G. Degobert, and H. Fessi. A pilot study of freeze drying of poly(epsilon-caprolactone) nanocapsules stabilized by poly(vinyl alcohol): Formulation and process optimization. *Int J Pharm.* **309**:178–188 (2006). doi:10.1016/j.ijpharm.2005.10.003.
247. B. Seijo, E. Fattal, L. Roblot-Treupel, and P. Couvreur. Design of nanoparticles of less than 50 nm diameter: preparation, characterization and drug loading. *Int. J. Pharm.* **62**:1–7 (1990). doi:10.1016/0378-5173(90)90024-X.
248. T. W. Patapoff, and D. E. Overcashier. The importance of freezing on lyophilization cycle development. *Biopharm.* **3**:16–21 (2002).
249. W. Abdelwahed, G. Degobert, and H. Fessi. Investigation of nanocapsules stabilization by amorphous excipients during freeze-drying and storage. *Eur. J. Pharm Biopharm.* **63**:87–94 (2006). doi:10.1016/j.ejpb.2006.01.015.
250. J. Broadhead, S. K. Edmond Rouan, and C. T. Rhodes. The spray drying of pharmaceuticals. *Drug. Dev. Ind. Pharm.* **18**:1169–1206 (1992). doi:10.3109/03639049209046327.
251. M. Adler, M. Unger, and G. Lee. Surface composition of spray-dried particles of bovine serum albumin/trehalose/surfactant. *Pharm. Res.* **17**:863–870 (2000). doi:10.1023/A:1007568511399.
252. C. R. Müller, V. L. Bassani, A. R. Pohlmann, C. B. Michalowski, P. R. Petrovick, and S. S. Guterres. Preparation and characterization of spray-dried nanocapsules. *Drug. Dev. Ind. Pharm.* **26**:343–347 (2000). doi:10.1081/DDC-100100363.
253. K. Master. Spray Drying Handbook. Longman Scientific and Technical, New York, 1991.
254. S. Bozdag, K. Dillen, J. Vandervoort, and A. Ludwig. The effect of freeze drying with cryoprotectants and gamma-irradiation sterilization on the characteristics of ciprofloxacin HCl-loaded poly(D,L-lactideglycolide) nanoparticles. *J. Pharm. Pharmacol.* **57**:699–707 (2005). doi:10.1211/0022357056145.
255. C. Vauthier, B. Cabane, and D. Labarre. How to concentrate nanoparticles and avoid aggregation ? *Eur. J. Pharm. Biopharm.* **69**:466–475 (2008). doi:10.1016/j.ejpb.2008.01.025.
256. F. Cournarie, M. Chéron, M. Besnard, and C. Vauthier. Evidence for restrictive parameters in formulation of insulin-

loaded nanocapsules. *Eur. J. Pharm. Biopharm.* **57**(2):171–179 (2004). doi:10.1016/S0939-6411(03)00191-7.

257. D. V. Bazile, C. Ropert, P. Huve, T. Verracchia, M. Marlard, A. Frydman, M. Veillard, and G. Spenlehauer. Body distribution of fully biodegradable [14C]-poly(lactic acid) nanoparticles coated with albumin after parenteral administration to rats. *Biomaterials*. **13**(15):1093–1102 (1992). doi:10.1016/0142-9612(92)90142-B.
258. M. T. Peracchia, E. Fattal, D. Desmaële, M. Besnard, J. P. Noël, J. M. Gomis, M. Appel, J. d'Angelo, and P. Couvreur. Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. *J. Control. Release*. **60**(1):121–128 (1999). doi:10.1016/S0168-3659(99)00063-2.
259. H. Pinto-Alphandary, M. Aboubakar, D. Jaillard, P. Couvreur, and C. Vauthier. Visualization of insulin-loaded nanocapsules: *in vitro* and *in vivo* studies after oral administration to rats. *Pharm. Res.* **20**(7):1071–1084 (2003). doi:10.1023/A:1024470508758.
260. B. Weiss, U. F. Schaefer, J. Zapp, A. Lamprecht, A. Stallmach, and C. M. Lehr. Nanoparticles made of fluorescence-labelled poly(L-lactide-co-glycolide): preparation, stability and biocompatibility. *J. Nanosci. Nanotechnol.* **6**(9–10):3048–3056 (2006). doi:10.1166/jnn.2006.424.
261. M. A. Pereira, V. C. Mosqueira, J. M. Vilela, M. S. Andrade, G. A. Ramaldes, and V. N. Cardose. PLA-PEG nanocapsules radiolabelled with 99 m Technitium-HMPAO: release properties and physicochemical characterization by atomic force microscopy and photon correlation spectroscopy. *Eur. J. Pharm. Sci.* **33**:42–51 (2008).
262. M. Simeonova, T. Ivanova, Z. Raikov, and H. Konstantinov. Tissue distribution of polybutylcyanoacrylate nanoparticles loaded with spin-labelled nitrosourea in Lewis lung carcinoma-bearing mice. *Acta Physiol. Pharmacol. Bulg.* **20**(3–4):77–82 (1994).
263. M. Tobio, A. Sanchez, A. Vila, I. I. Soriano, C. Evora, J. L. Vila-Jato, and M. J. Alonso. The role of PEG on the stability in digestive fluids and *in vivo* fate of PEG-PLA nanoparticles following oral administration. *Colloids Surf. B. Biointerfaces*. **18**(3–4):315–323 (2000). doi:10.1016/S0927-7765(99)00157-5.
264. P. Prabu, A. A Chaudhari, N. Dharmaraj, M. S. Khil, S. Y. Park, and H. Y. Kim. Preparation, characterization, *in-vitro* drug release and cellular uptake of poly(caprolactone) grafted dextran copolymeric nanoparticles loaded with anticancer drug. *J. Biomed. Mater. Res: A*. (2008). [doi:10.1002/jbm.a.32163]
265. G. Sun, A. Hagooly, J. Xu, A. M. Nyström, Z. Li, R. Rossin, D. A. Moore, K. L. Wooley, and M. L. Welch. Facile, efficient approach to accomplish tunable chemistries and variable biodistributions for shell cross-linked nanoparticles. *Biomacromolecules.* **9**(7):1997–2006 (2008). doi:10.1021/bm800246x.
266. S. Ponsart, J. Coudane, J. L. Morgat, and M. Vert. Synthesis of [^{3}H]-labeled poly(ε-caprolactone). *J. Labelled Compd Rad.* **43**:271–281 (2000).
267. S. Ponsart, J. Coudane, J. L. Morgat, and M. Vert. Synthesis of [^{3}H] and fluorescence-labeled poly(lactide). *J. Labelled Compd Rad.* **44**(10):677–687 (2001).
268. S. Ponsart, J. Coudane, B. Saulnier, J. L. Morgat, and M. Vert. Biodegradation of [(3)H]poly(ε-caprolactone) in the presence of active sludge extracts. *Biomacromolecules*. **2**(2):373–377 (2001). doi:10.1021/bm015549k.
269. I. Bertholon, H. Hommel, D. Labarre, and C. Vauthier. Properties of Polysaccharides Grafted on Nanoparticles Investigated by EPR. *Langmuir.* **22**:5485–5490 (2006). doi:10.1021/la060570y.
270. C. Chauvierre, C. Vauthier, D. Labarre, and H. Hommel. Evaluation of the surface properties of dextran coated poly (isobutylcyanocrylate) nanoparticles by Spin-labelling coupled with electron resonance spectroscopy. *Colloid Polym. Sci.* **282**:1016–1025 (2004). doi:10.1007/s00396-003-1027-6.