

## Chapter 2

# Synthesis of Organic and Bioorganic Nanoparticles: An Overview of the Preparation Methods

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**Abstract** Since the emergence of Nanotechnology in the past decades, the development and design of organic and bioorganic nanomaterials has become an important field of research. Such materials find many applications in a wide range of domains such as electronic, photonic, or biotechnology, which contribute to impact our society and our way of life. The improvement of properties and the discovery of new functionalities are key goals that cannot be obtained without a well controlled and a better understanding of the preparation methods which constitute the starting point of the design of a specific organic material. In this context, this chapter gives a general but non-exhaustive overview of the methods of preparation of organic and bioorganic nanoparticles. Some general definitions about organic nanoparticles and description of organic compounds are given before describing the most common methods used divided into two families, the two-step and one-step procedures. The major part of the two-step procedures is based on an emulsification step followed by generation of nanoparticles through different mechanisms such as precipitation, gelation, or polymerization. The one-step procedures are founded on generation of nanoparticles through different techniques such as nanoprecipitation, desolvation, or drying processes without preliminary emulsification step. For each method, the description is supported by several examples and focused on the explanation of the general mechanisms and of the major key parameters involved in the control

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of the nanoparticles formation. In addition, since emergence and improvement of syntheses are often associated to development of experimental setups, technological aspects are also mentioned.

## 2.1 Introduction

Organic nanoparticles can be commonly described as solid particles composed of organic compounds (mainly lipids or polymeric) ranging in diameter from 10 nm to 1  $\mu\text{m}$  [1, 2]. Over the past decades, this type of nanoparticles has met a great expansion and intensive investigations due to their high potentialities in a wide spectrum of industrial areas ranging from electronic to photonic, conducting materials to sensors, medicine to biotechnology, and so forth [3–13]. Therefore, the choice of the synthetic route is central to optimize the final properties of nanoparticles designed for a specific application. This choice has to be guided by a series of factors such as physico-chemical parameters of the organic compound, chemical composition, nanoparticles diameter, structure, morphology, or environmental considerations which constitute definitely an increasingly incontrovertible criterion. Consequently, a suitable preparation method cannot be dissociated from a real compromise chosen in function of the different constraints which have to be overcome to design well-controlled organic nanomaterials.

This chapter focuses on the description of the most used preparation methods reported in the literature for the preparation of organic nanoparticles. It starts by giving some general definitions on the different types of nanoparticles and features of organic compounds. The second part is devoted to the description of the preparation methods highlighting the general principles and mechanisms involved and the parameters governing the particles formation and their properties.

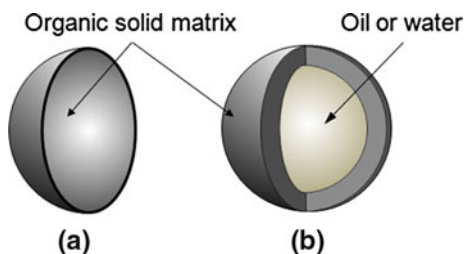
## 2.2 Definition and Description of the Different Types of Particles

### 2.2.1 Structure and Morphology

Nanoparticles can be divided into main two groups: nanospheres and nanocapsules (Fig. 2.1). Nanospheres are considered as matrix particles whose entire mass are solid whereas nanocapsules are composed of a liquid or empty core surrounded by an organic solid shell. Nanospheres and nanocapsules are generally spherical but non-spherical shape can be encountered. The obtaining of the different types of nanoparticles depends evidently on the methods selected for the preparation.

**Fig. 2.1** The different types of nanoparticles.

**a** Nanospheres,  
**b** nanocapsules



## 2.2.2 Organic Materials Composing the Particles

### 2.2.2.1 Polymeric Nanoparticles


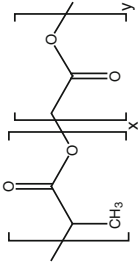
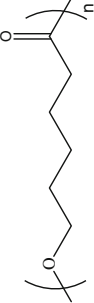
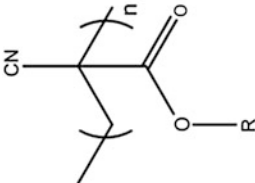
Polymeric nanoparticles constitute by far the most studied organic particles in the literature [14–18]. Although polymeric nanoparticles can be designed for a wide spectrum of applications, two major families can be distinguished. The first one is related to nanoparticles elaborated for drug delivery and/or for biomedical purposes [19–22]. In this case, the macromolecules require biodegradable or biocompatible properties. Number of synthetic or natural polymers can be used and the most widely used are reported in Table 2.1. Despite the great potential of polymer chemistry today, one can observe that only a limited number of molecules can be used as constituents of drug delivery nanocarriers. This is particularly due to the drastic constraints and requirements which characterized *in vivo* applications in terms of toxicity and biocompatibility.

The second family of polymeric particles is constituted of conjugated polymeric nanoparticles that exhibit electronic or opto-electronic properties [15, 23]. Among these conjugated polymers; polyaniline, polypyrrole, polyacetylene, and their derivatives have been widely studied for their intrinsic conductivity [24–30], while polythiophenes, polyfluorenes, poly(p-phenylenevinylene)s, and poly(p-phenyleneethynylene)s derivatives [31–38] have rather been studied for their electro-optical and photoluminescence behaviors.

### 2.2.2.2 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are composed of lipid matrices derived generally from glycerol esters of fatty acids [39–41]. The lipid compound is characterized by a melting temperature above 37 °C in order to ensure solidification at physiological temperature. Due their high stability (several years), good biocompatibility and low toxicity, SLNs are considered as promising drug delivery systems and a good alternative to polymeric nanoparticles especially for parenteral method. But the limit of this type of particles lies in the fact that the majority of drugs have a poor solubility in lipids [42]. Ongoing investigations are conducted to increase encapsulations rates using nanostructured lipids matrices or lipid-drug conjugates [43, 44].

**Table 2.1** Description of the most widely used polymers in the synthesis of organic nanoparticles

Materials	Full name	Abbreviation or commercial name	Molecular structure
Synthetic Homopolymers	Poly(lactide)	PLA	
	Poly(lactide-co-glycolide)	PLGA	
	Poly(epsilon-caprolactone)	PCL	
	Poly(isobutylcyanoacrylate)	P(CBA)	
	Poly(isohexylcyanoacrylate)	PIHCA	
	Poly(n-butylcyanoacrylate)	PBCA	
			R = CH <sub>2</sub> CH (CH <sub>3</sub> ) <sub>2</sub> for P(CBA) R = (CH <sub>2</sub> ) <sub>3</sub> CH(CH <sub>3</sub> ) <sub>2</sub> for PIHCA R = (CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> for PBCA

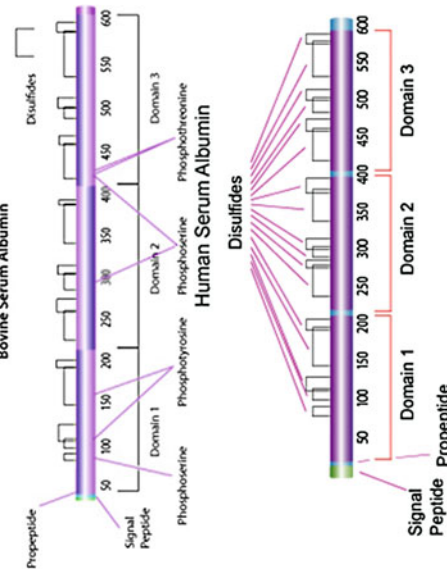
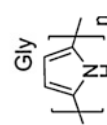
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Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
	Poly(acrylate) and poly(methacrylate)	Eudragit®	<p><math>R_1 = \text{CH}_3, \text{H}</math> <math>R_2 = \text{CH}_3, \text{CH}_3\text{CH}_2-</math> <math>R_3 = \text{COOH}</math> (Eudragit® L and S) <math>R_3 = \text{COOCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3\text{Cl}^-</math> (Eudragit® RL and LS)</p>
Copolymers	Poly(lactide)-poly(ethylene glycol)	PLA-PEG	
	Poly(lactide-co-glycolide)-poly(ethylene glycol)	PLGA-PEG	
Natural polymers and biopolymers	Chitosan		
	Alginate		

(Continued)

Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
Gelatin <sup>a</sup> Albumin <sup>b</sup>	-Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-		
	Fibroin <sup>a</sup>		
Conjugated polymers	Polypyrrole		
	Polyproline		

(Continued)

Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
	Polyaniline	PANI	
	Polyacetylene		
	Poly[3,4-(ethylenedioxy)thiophene]	PEDOT	
	Poly(dialkylfluorene)		
	Poly(p-phenyleneethynylene)	PPE	
	Poly(p-phenylenevinylene)	PPV	

<sup>a</sup> Typical amino acids sequence<sup>b</sup> Structures taken from the Sigma-Aldrich® website

## 2.3 Methods of Preparation of Nanoparticles

The preparation of organic and bioorganic nanoparticles is divided into two main methods [14, 16, 17]. The first approach is based on a two-step procedure involving generally the preparation of an emulsification system during the first step carried out to generate nanodroplets of definite sizes wherein organic compounds (polymer, monomer, lipid) are previously solubilized. The strategies of emulsification developed in the literature differ from their high- or low-energy stirring procedures. The nanoparticles are formed in the second step of the process by various mechanisms such as precipitation, gelation, or polymerization.

The second approach consists in conduction of one-step procedures where emulsification is not required prior to the formation of nanoparticles. The methods are generally based on the precipitation of organic compounds in solution occurring through different routes including nanoprecipitation by solvent displacement or self-assembly mechanisms induced by ionic gelation or by the formation of polyelectrolyte complexes. A few other methods have also been reported recently based on strategies involving spray-drying [45, 46], supercritical fluid technologies [14, 47, 48], or piezoelectrical ways [49].

### 2.3.1 Two-Step Procedures Based on Emulsification

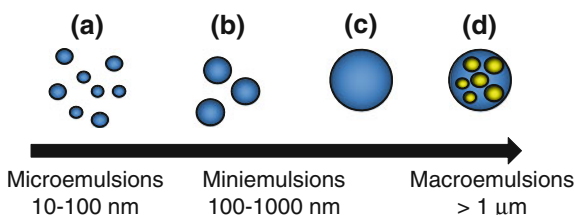
#### 2.3.1.1 Emulsions and Methods of Emulsification

The term emulsion is defined basically as a mixing of two or more totally or partially immiscible liquids obtained in the presence or absence of a surface active agent. Generally, depending on the type of dispersed phase and of the dispersion medium, o/w (oil in water) direct emulsion or w/o (water in oil) inverse emulsion can be formed but more complex systems such as O/O (oil in oil) or multiple emulsions of different kinds (W/O/W, O/W/O, W/O/O) can also be obtained (Fig. 2.2). Depending on the sizes of droplets, the emulsion formed can be classified into three main categories: a microemulsion type which is characterized by a thermodynamically stable behavior with droplet diameters ranging from 10 to 100 nm and a miniemulsion or a macroemulsion systems that are both thermodynamically unstable with drop sizes comprised between 100 nm and 1  $\mu\text{m}$  and up to 1  $\mu\text{m}$ , respectively [50–52].

Over the past decade, methods to prepare suitable emulsions with nanoscaled droplets to design organic nanoparticles have been considerably evolved due to the technological development of emulsification devices and due to the expansion of low-energy stirring routes in constant progress, thanks to environmental constraints. Indeed, low- and high-energy emulsification techniques constitute two strategies to obtain nanodroplets and consequently nanoparticles.



**Fig. 2.2** Different types of emulsions. **a** Microemulsion, **b** nanoemulsion, **c** simple macroemulsion, **d** multiple emulsion



### Low-energy Emulsification Methods

Nanoemulsions can be generated by low-energy emulsification techniques classified into two groups in the literature. The first one is the so-called spontaneous emulsification [50, 51, 53–55] obtained by the rapid diffusion of a water-soluble solvent, solubilized first in the oily phase, moving toward the aqueous one when the two phases are mixed. This phenomenon, presented as a good alternative of high-energy methods, has been described in several works as a solvent displacement method [56–61] (also called the “Ouzo effect”) where nanoemulsion is obtained by a rapid diffusion of an organic solvent generally acetone or ethanol from the oily phase to the aqueous phase. Direct O/W emulsion as well as inverse W/O emulsion can be produced by this way. Spontaneous emulsification mechanism originates from interfacial turbulence related to surface tension gradient produced by the diffusion of solutes between two phases [54]. It is assumed that drops are created by interfacial corrugations caused by a Marangoni effect causing severe interfacial fluctuations. In the case of the presence of surfactants, fluctuations of the interfacial amphiphile concentration create local supersaturation of the surfactant at interface resulting in the nucleation and growth of drops [62–64]. The study of the basic mechanism involved can be illustrated by a simple ternary water/alcohol/oil ternary system through the determination of a phase diagram which is essential to describe the diffusion path and to target the spontaneous emulsification domain [54]. In such diagram, as illustrated in Fig. 2.3, a two-phase equilibrium region occurs corresponding to the spontaneous emulsification (SE) domain. Upon dilution, the diffusion path of the water phase crosses the SE region inducing spontaneous emulsification and the formation of nanoemulsion.

In the field of nanoparticles synthesis, more complex systems are involved and the ternary diagram has to be adjusted to take into account the potential influences of additional components such as monomers, polymers, or surface active agents on the modification of the diffusion pathway. Among all key parameters modifying the droplet sizes and stability of nanoemulsions (pH, water and oil proportions, solvent type and content, etc.), temperature strongly affects the solubility of the organic solvent in the water and the oil phase and modifies consequently the diffusion process.

Another route to produce spontaneous emulsification is the so-called emulsion inversion point method (EIP) carried out at constant temperature. The method is

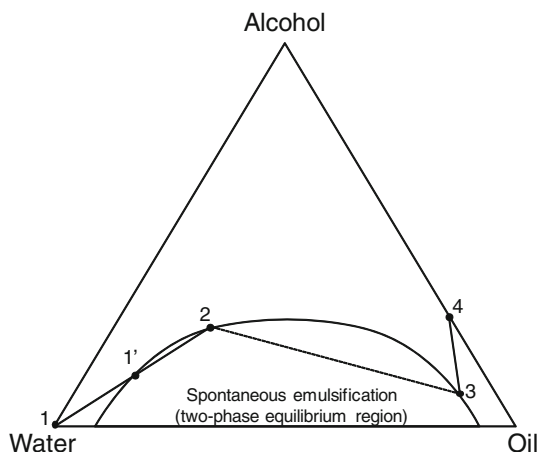
**Fig. 2.3** Diffusion path in a water/alcohol/oil system.

Segment (1–2): diffusion path of the aqueous phase.

Segment (2–3): interfacial equilibrium. Segment (3–4):

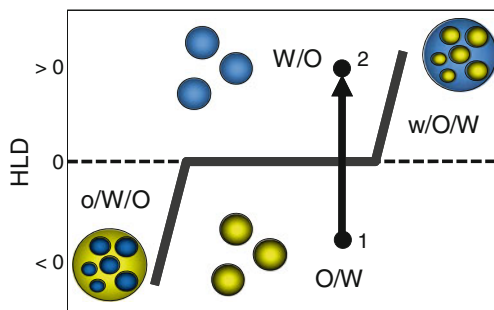
diffusion path of the oily phase. Segment (1'–2):

crossing of the two-phase equilibrium region induces spontaneous emulsification



based on a progressive dilution with water or oil of a microemulsion or liquid crystals leading kinetically stable nanoemulsions [65–73]. Indeed, by changing the water and oil proportion in microemulsion network, interfacial instabilities are occurring resulting in the destabilization of the thermodynamically stable microemulsion structure into nanoemulsions. Keeping in mind that the best conditions have to be found to obtain the smallest drop sizes, determination of phase diagrams is also required in this case and have to be adjusted carefully in function of the formulation used for the design of nanoparticles (type of monomers, polymers, initiators, drug encapsulated, etc.).

The third group of low-energy emulsification methods is the so-called phase inversion temperature (PIT) method [74, 75] offering the main advantages to obtain nanoemulsions with a potentially low amount of surfactant (typically less than 5 wt %), a reduced toxicity since no organic solvent is needed, and a relatively easy handling. It makes the method suitable for biotechnology applications (nanomedicine, pharmaceutical science or cosmetics) since degradation of drug to be encapsulated is avoided. This versatile way uses the ability of polyethylene oxide (PEO)-based surfactants to change their affinity for water and oil in function of temperature leading to a so-called “transitional phase inversion” of emulsions. Typically, when temperature increases, the PEO blocks undergo dehydration which modify the amphiphilic character of surfactants toward higher lipophilic behavior. Consequently, an O/W emulsion produced at low temperature inverts into a W/O one upon a temperature rising. Likewise, in the transitional region at temperatures for which the surfactant exhibits similar affinity for the two immiscible phases, ultralow interfacial tension and low curvature create bicontinuous microemulsion nanostructures. Therefore, the principle of the PIT method is to suddenly breakup such structures maintained at the PIT temperature by rapid cooling or dilution generating immediately kinetically stable nanoemulsions [76–81]. Once again, phase diagram has to be established to determine the transitional inversion region in function of the formulation parameters (temperature,



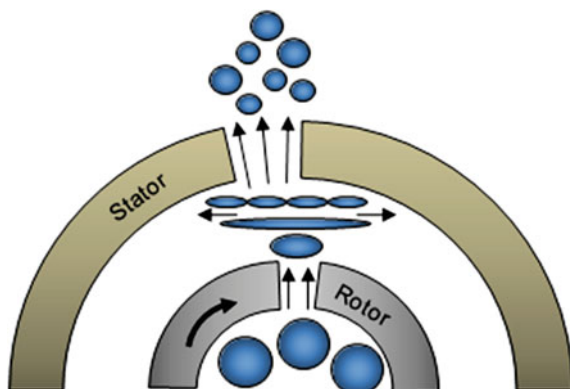
**Fig. 2.4** Typical ‘formulation-composition’ map for a given water/surfactant/oil system showing the emulsion inversion zones and the different types of emulsions. Path (1–2) illustrates a possible transitional inversion from O/W emulsion to nano-W/O emulsion inducing by the crossing of the ultra-low interfacial tension region  $HLD = 0$

oil type, salinity, etc.) and of the water to oil ratio (WOR). This is suitable to target the inversion path which has to be followed to produce nanoemulsions. The group of Salager et al. has developed an empirical “Hydrophilic Lipophilic Deviation” (HLD) expression (Eq. 2.1) based on the difference of the chemical potential of surfactants in the two phases [82–86].

$$HLD = \alpha - EON + bS - kACN + t\Delta T + aA \quad (2.1)$$

where EON is the number of ethylene oxide groups for surfactants,  $S$  is the weight percentage of electrolytes in the aqueous phase, ACN the amount of carbon numbers of the  $n$ -alkane composing the oily phase,  $\Delta T$  the temperature difference from the reference temperature (25 °C),  $A$  the weight percentage of alcohol potentially added,  $\alpha$ ,  $k$ ,  $t$  the parameters in function of the used surfactant,  $a$ , a constant function of the types of alcohol and surfactants, and finally  $b$  a constant function of the nature of the added electrolytes. It comes that HLD can be calculated and its value depends on the different formulation parameters of the system representing by the terms of the equation.  $HLD = 0$  corresponds to the optimum formulation where the surfactant has equal affinity to water and oil whereas  $HLD > 0$  and  $HLD < 0$  represent higher lipophilic or hydrophilic behaviors, respectively. Moreover, formulation-composition maps can be constructed as illustrated in Fig. 2.4 [83, 87–91]. For a given system at constant stirring conditions and for a fixed surfactant concentration, zones of macroemulsion, multiple emulsions, and transitional region appear. It allows the identification of the best path conditions (especially the temperature range) to produce transitional inversion of emulsion [91–96]. Although transitional inversion presents many advantages for the preparation of nanoparticles, this method is still anecdotal and very recently developed in the literature for the production of lipid nanocarriers and nanocapsules or polymer nanoparticles compared to high-energy emulsification techniques.

**Fig. 2.5** Scheme of the principle of emulsification with a rotor–stator device



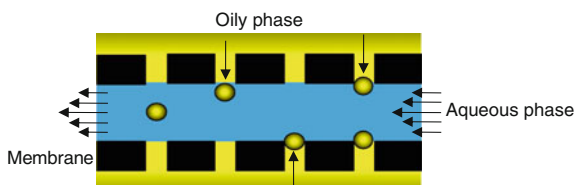
### High-Energy Emulsification Methods

Most of methods of preparation of nanoemulsions are based on mechanical processes related to high-energy stirring techniques. In this field, the most common device consists in a rotor–stator apparatus in which a shear stress is applied to induce deformation of pre-emulsion droplets leading to their breaking into smaller ones of uniform size (Fig. 2.5). The final diameter of the daughter droplets is mainly determined by the applied stress and only weakly depends on the viscosity ratio between the dispersed and the continuous phase [97–102].

Sonication is also a process widely used in the literature for nanoemulsification [50, 103–114] and for the generation of polymer or lipid nanoparticles [115–127]. This process of ultrasound emulsification is performed under high frequency where large drops are generated by the instability of interfacial waves [104, 105]. The drops are subsequently broken into smaller ones through a cavitation mechanism. Some authors have shown that optimal conditions for sizes reduction and better nanoemulsion stability are obtained using high-power setting for short exposure times [103]. Indeed, longer exposure times produce degradation of surfactant by radicals which form during the thermal decomposition of water [128, 129].

In the past few years, others machines have been designed to obtain droplets with more reproducible calibrated sizes and well-defined characteristics in the view of large-scale production. These machines have been related to microfluidic techniques that constitute at the moment an intense research area in progress [130–137]. The principle is based on an extrusion mechanism where the dispersed phase is forced to permeate through a microfiltration device to calibrate droplets in the continuous phase (Fig. 2.6). The microfiltration units differ from their technological design and can be engineered as porous membranes, flow-focusing, Y-shaped or T-type microchannels [138]. Polymeric particles of PLGA–PEG [139], PCL [140], or alginate [141] have for example already been produced by this technique.

**Fig. 2.6** Scheme of the principle of emulsification using a microfiltration device based on a porous membrane



### 2.3.1.2 Generation of Nanoparticles from Emulsion

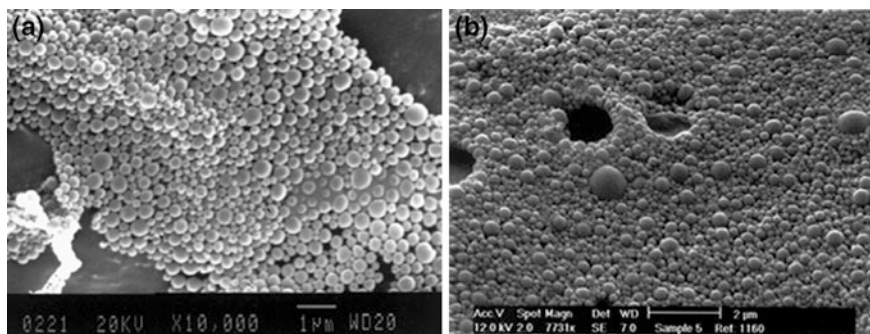
#### Precipitation Induced by Solvent Removal

Macromolecules dissolved in the dispersed phase (mainly the oily one) of the emulsion can undergo precipitation upon removal of the organic, often volatile solvent. To perform this solvent extraction, several methods such as solvent evaporation, solvent diffusion, or salting-out procedures have been developed and constitute by far the most famous routes carried out in the field of organic nanoparticles formation from emulsified systems. It comes from the versatility character of this way that can be applied to a wide range of organic compounds including synthetic polymers and natural bioorganic macromolecules such as chitosan, polysaccharides, alginate, or gelatin.

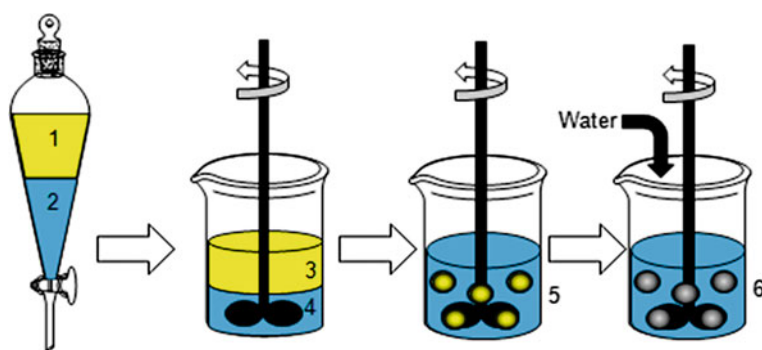
#### *Solvent Evaporation*

The method consists in the preparation of nanoemulsion formulated with a polymer dissolved in a volatile solvent solution [142]. Dichloromethane and chloroform are the most widely used solvents but are often replaced by ethyl acetate, less toxic and hence much more adapted to the synthesis of controlled release systems where drug encapsulation is generally involved. In this method performed under vacuum, suspension is produced by evaporation of the polymer solvent from emulsion droplets which is allowed to diffuse through the continuous phase [19]. This slow procedure involves first a fast evaporation period during which at least 90 % of the polymer solvent is evacuated followed by a slow evaporation period where the few percent of the remaining solvent is extracted. During the first step, droplets sizes dramatically decrease to reach a minimum value due to the high solvent lost. In contrary, the second step is characterized by a significant increase of the droplet diameters in reason to coalescence. This coalescence process can be accentuated in the case of a polymer having interfacial adsorption properties whereas for polymers characterized by poor surface active properties, the coalescence is reduced. In addition, partially miscible solvents in the preparation of emulsion can be used and change the conditions of evaporation. The volatile solvent removal can be in this case realized by distillation [143].

Numerous examples of nanoparticles preparations by solvent evaporation can be found in the literature with various polymers such as PLGA [144–147], PLA [148, 149], or PCL [150] (Fig. 2.7). Also, amphiphilic copolymers (PEG-PLA



**Fig. 2.7** Examples of nanoparticles produced by the emulsion–solvent evaporation method. **a** PLGA nanoparticles (adapted with permission from [145]). **b** PCL nanoparticles (adapted with permission from [150])



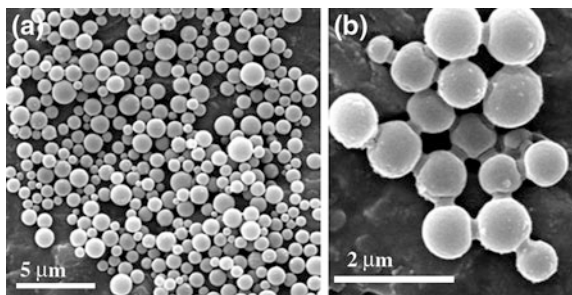
**Fig. 2.8** Scheme of preparation of nanoparticles by the emulsion–diffusion procedure. (1) Partially water miscible solvent saturated with water. (2) Water saturated with solvent. (3) Solvent saturated with water + dissolved polymer. (4) Water saturated with solvent + surfactant. (5) Emulsification. (6) Dilution with water and formation of polymeric nanoparticles from emulsion

[151, 152], PEG–PLGA [153], polysaccharides–PCL [154, 155]) nanospheres can be produced by this method with no need of surfactant to ensure the emulsion formation and the stability of the final nanoparticles suspension.

### *Solvent Diffusion*

The solvent diffusion method also called solvent displacement method (Fig. 2.8) requires a polymer solvent partially soluble in water [156–158]. The preparation of emulsion involves two immiscible phases, but where the oil dispersed one is composed of water saturated with the polymer solvent and where the continuous one is composed of oil saturated with water. This can be obtained by mixing the polymer solvent and water and waiting the decantation to get a two-phase system. At the bottom and at the top of the resulting system, the solvent saturated water and the

**Fig. 2.9** Scanning Electron Microscopy images of PLA nanoparticles obtained with the solvent diffusion method (adapted with permission from [161])



water saturated solvent can be collected, respectively. Oil-in-water emulsion is prepared with the previous two immiscible phases and subsequently diluted with a high quantity of water. This leads to the precipitation of the polymer induced by a rapid diffusion of the organic solvent from the oil droplets to the continuous phase. Several polymer solvents can be used such as ethyl acetate [143], isopropyl acetate [159], benzyl alcohol, propylene carbonate [160], and surfactants such as pluronic F68<sup>®</sup> or Polyvinyl alcohol (PVA) is often employed to play a role in the stabilization and sizes of the emulsion droplets. PLA [161] (Fig. 2.9), PLGA [162–164], and PCL [157] are the most common and suitable polymers used but gelatin or chitosan nanoparticles [165–167] have also been synthesized by this method.

In addition, this method involves a pure diffusion mechanism where the droplet sizes drop suddenly in a millisecond time scale during solvent extraction and polymeric nanoparticles formation. Among all factors impacting the reduction of the particle diameters, one can cite the increase of the miscibility of water with the organic solvent or of the stirring rate and the use and concentration of stabilizing agents added in the emulsion. On the contrary, the rise of the polymer concentration leads to significant increase of the particle sizes and polydispersity. Generally, nanospheres are produced by this technique but adding a small amount of oil in the organic phase results in the generation of nanocapsules.

### *Salting-Out*

Very close to the solvent-diffusion method, this process involves emulsification with a polymer solvent generally acetone that is normally totally miscible with water. Actually, the artifice used to emulsify water and acetone is to dissolve high contents of salt or sucrose in the aqueous phase to provoke a strong salting-out effect modifying the solubility of water with the solvent [168]. The emulsion can hence be formed with a polymer dissolved in the solvent droplets. Particles precipitation is induced, as in the solvent-diffusion process, by diluting the emulsion and adding a large amount of water in the continuous phase to drop the salt concentration and to cause extraction of the solvent out of the droplets.

The suitable electrolytes for this process are generally magnesium chloride [169–171] or calcium chloride [172] but salting-out can also be produced by saturation of the aqueous phase by PVA [173] which acts in addition as a



viscosity-increasing agent and emulsion stabilizer. Poly(ethylene oxide) [169], PLGA [172, 174], or poly(trimethylene carbonate) [175] particles, for instance, have been synthesized by this method with diameters in a 100-500 nm range. The stirring energy required for this method is also reduced which provides lower particle diameter and less pronounced influence of the polymer concentration and of the stirring speed on the emulsion droplets in comparison to more conventional emulsification routes.

### Gelation of the Emulsion Droplets

Another method to obtain nanoparticles after nanoemulsification is to gelify polymer or crystallize lipid dissolved in the droplets [39, 176]. For instance, in the case of agarose [177] or gelatin [127], the preparation of the nanoemulsion can be performed at moderate high temperature above the melting point and subsequent cooling down induces gelation of the emulsion droplets and their conversion into nanoparticles. The same procedure can be applied for the production of solid lipid nanoparticles by crystallization of the lipid under the melting temperature [39]. Gelation can be produced by other physicochemical factors such as pH or by adding components like divalent cation (generally calcium) to induce ionic gelation [178]. Polysaccharides biopolymers such as alginate or pectin are particularly adapted since their chemical compositions based on uronic acids functions are responsive to pH or to complexation with cations. In this kind of gelation, two emulsions are generally prepared, the first one containing the dissolved biopolymer and the second one containing the pH controlling agent or the cation. The two emulsions are mixed under strong agitation to provoke droplets collision which is essential to induce gelation and hence formation of nanoparticles.

### Polymerization in Emulsion

Among all techniques used for the generation of nanoparticles from emulsions, polymerization is the subject of the abundant literature since well-defined and desired nanoparticles properties for a particular application can be attained through this process [179–184]. In this case, instead of the previously described techniques for which a solution of a preformed polymer is prepared, macromolecules form through polymerization of monomers. The major emulsion polymerization techniques can be classified into different methods such as conventional emulsion polymerization, surfactant-free emulsion polymerization, as well as mini- (or nanoemulsions) and microemulsions polymerizations which differ from the kinetically and thermodynamically different emulsion behaviors. In addition, we can cite interfacial polymerization, a very useful method for the preparation of nanocapsules and living/controlled radical polymerization process that offers at this moment a much better control of the polymer characteristics in comparison to older conventional polymerization techniques.



### *Conventional Emulsion Polymerization*

This method can be considered as the traditional way to generate nanospheres from emulsion polymerization and is still widely used nowadays. Generally, the components are water, a monomer of low water solubility, a water-soluble initiator which may be an ion or a free-radical and a surfactant. Polymerization starts in this case when a monomer molecule collides with an initiator molecule. Another way consist in initiating radical from the monomer itself using UV irradiation, ultrasonication, or  $\gamma$ -radiation. Before or after the termination of the polymerization, the solid particles can be formed. Various types of polymeric nanoparticles could be produced by this technique such as poly(vinylcarbazole) [185], poly(methyl-methacrylate) [186, 187], polystyrene [188–195], or poly(alkylcyanoacrylate) [196–201] nanoparticles. In the latter case, anionic polymerization for which initiation occurs by any nucleophilic groups like hydroxyl groups of water is the most common way. In addition, performing anionic polymerization in acidic conditions slows down the rate of reaction and hence favors the formation of nanospheres instead of polymer aggregates. The particles sizes depend on the surfactant used and can be obtained generally in a 50–300 nm range.

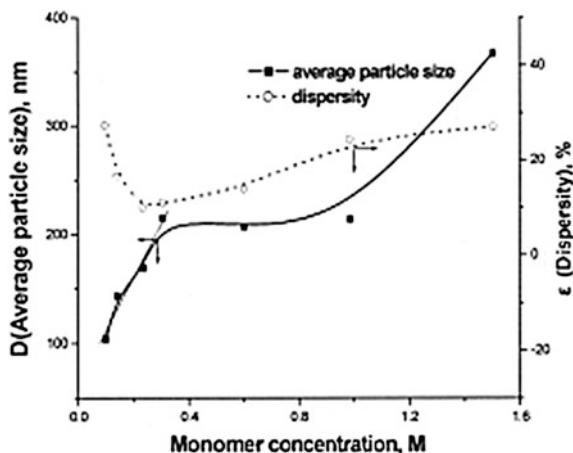
### *Surfactant-Free Emulsion Polymerization*

In contrary to conventional emulsion polymerization, surfactant-free emulsion polymerization is performed without emulsifier offering the major advantage to obtain nanoparticles without any step of surfactant removal [202–208]. This could be environmentally, energetically, and time-consuming advantageous especially for high-scale productions of nanoparticles. The ingredients in such emulsifier-free system are water, a water-soluble initiator (like potassium persulfate), and monomers which are generally vinyl or acrylic. The stabilization of nanoparticles in formation is ensured in this case by the use of ionizable initiators or ionic co-monomers. Two mechanisms of polymerization are involved, micellar-like nucleation [209, 210] and homogeneous nucleation [211–216] that differs from the aqueous solubility of the monomer. PMMA nanoparticles have been obtained by this technique using microwave irradiation [217, 218], redox initiation [219], or laponite clays as stabilizing agent for the emulsion [220]. The general trend is that monomer concentration is a key parameter influencing the particle sizes as illustrated in Fig. 2.10 where the increase of the monomer concentration increases the particle size.

Polyacrylate nanospheres were also obtained and some authors have shown that ultrasonic irradiation or the concentration of a stabilizing agent (4-styrene sulfonic acid) altered the particle sizes and distribution [221]. Polythiophene nanoparticles have been equally successfully prepared by  $\text{Fe}^{3+}$  oxidative polymerization [222] where the difference in the polymerization rates of monomers and the electrostatic attraction between sulfonate and  $\text{Fe}^{3+}$  ions results in the production of core-shell morphology with a size distribution ranging from 300 to 800 nm.

Although surfactant-free emulsion polymerization is considered as a simple and “green” way for polymeric nanoparticles preparation, improvements have to be

**Fig. 2.10** Influence of monomer concentration on dispersity and average particle size of PMMA nanoparticles obtained through surfactant-free emulsion polymerization (reprinted with permission from [217])

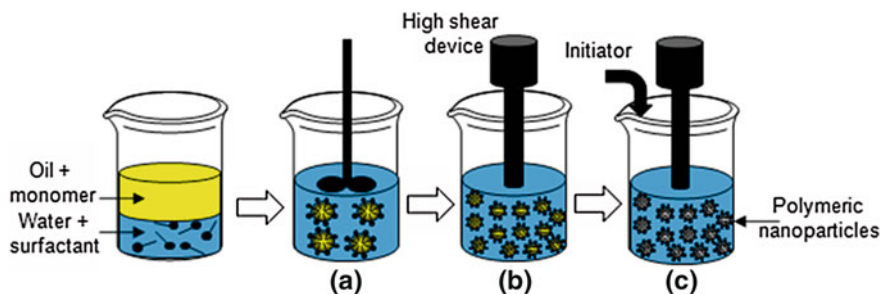


conducted in the future to obtain monodisperse and more precisely controlled particle sizes.

### *Miniemulsion Polymerization*

This method can be distinguished from conventional and surfactant-free emulsion polymerization by the generation of nanoemulsions using high-energy methods (Fig. 2.11) and generally a low molecular mass compound as co-stabilizer prior initiating polymerization [223–231]. The typical formulation consists in water, monomer mixture, co-stabilizer, surfactant, and initiator. The droplets of the nanoemulsion are generally composed of a pure monomer phase stabilized by a suitable adsorbed surfactant. The polymerization mechanism widely employed is radical polymerization which is initiated in the emulsions droplets via the incorporation, in most cases, of the initiator in the continuous phase. The general mechanism assumed in the literature is the droplet nucleation mechanism suggesting that radicals are generating in each monomer droplet taken as individual reaction site [180, 232]. The number and size of particles do not consequently vary during the polymerization process. The choice of the initiator and its solubility has evidently a great influence on the final particles properties especially the particle size. Even if inverse nanoemulsion polymerization is possible using hydrophilic ingredients [233–235], in most cases, the hydrophobic character of the dispersed monomer phase requires an oil-soluble initiator which is more suitable to obtain well-defined nanoparticles.

Although radical polymerization is often selected for the generation of nanoparticles in miniemulsion polymerization, non-radical polymerization methods such as polyaddition [236, 237], anionic polymerization [238], or metal-catalyzed reactions [239] are less aggressive for drug encapsulation applications and can also be used. Some authors [236, 237] have demonstrated for instance the preparation



**Fig. 2.11** Scheme of nanoparticles preparation from a typical miniemulsion polymerization method. **a** Pre-emulsification **b** nanoemulsification using a high shear device **c** formation of polymeric nanoparticles upon addition of initiator

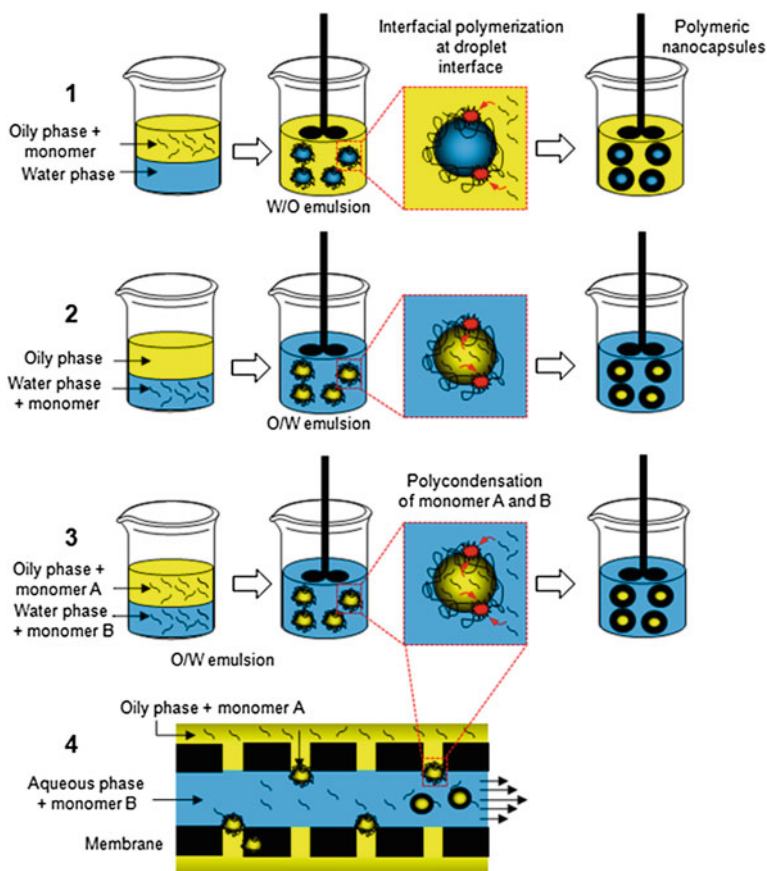
of polyurethane latex nanospheres using reaction between diisocyanate and activated diol in nanoemulsion droplets. The low water solubility of reactants and the slower polymerization kinetics than emulsification are the main key factors inducing the successful synthesis.

### *Microemulsion Polymerization*

The main difference between microemulsion and miniemulsion polymerization methods relies on the kinetic character of the dispersed phase produced in the emulsified system. Indeed, in contrary to miniemulsion, microemulsion is a thermodynamically and spontaneous stable system prepared with a high quantity of surfactant and characterized by an interfacial tension at the oil/water interface close to zero [52, 64]. The generation of nanoparticles through microemulsion polymerization generally results in smaller particle size (typically less than 80 nm) than in miniemulsion polymerization processes. A water-soluble initiator is introduced in the aqueous phase initiating the polymerization in only some swollen micelles of the microemulsion containing the monomer. As time elapses, the osmotic and elastic influence of the polymeric chains in formation destabilizes the microemulsions leading to an increase in the particle size, to the formation of empty micelles, and to a secondary nucleation [232, 240].

In the literature, various formulations have been investigated to prepare for instance nanoparticles of polyvinyl acetate [241, 242], polyaniline [243–246], polyacrylamide, or polypyrrole [247]. The studies highlighted the critical factors that influence the final particle properties as the type of initiator and concentration, the surfactant, the concentration of monomer, and the reaction temperature.

Microemulsion polymerization has a great potential in many applications but retains important drawbacks relating to its high dilute formulation and its high amount of surfactant which limit in a large extent the commercial use of this technique.

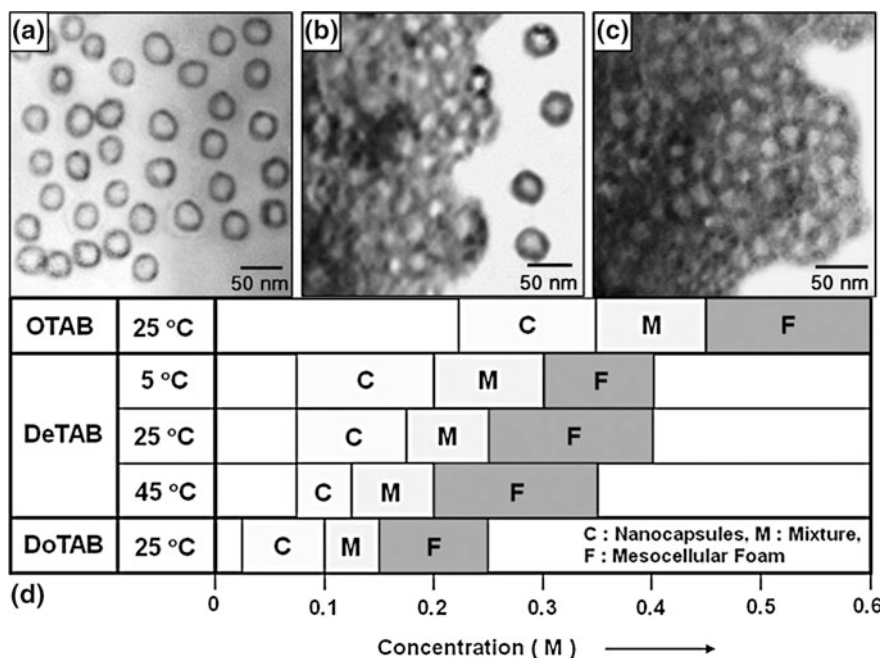


**Fig. 2.12** Scheme of the different strategies of interfacial polymerization. (1) Introduction of the monomer in the oily phase. (2) Introduction of monomer in the aqueous phase. (3) Introduction of monomers in the oily and aqueous phase. (4) Polymerization using a membrane reactor device

### *Interfacial Polymerization*

Interfacial polymerization is characterized by the polycondensation of monomers at the droplet interface leading to the generation of mainly nanocapsules instead of nanospheres. Different strategies have been developed (Fig. 2.12) which depend on the formulation selected and the choice of monomers introduced either in the continuous and/or in the dispersed phase.

The first way consists in introducing the monomer in the continuous phase of the emulsified system. The monomer can subsequently react with the emulsion droplets to form nanocapsules. Due to the hydrophobic character of the majority of monomers used, the emulsions prepared in this case are generally of the W/O type. Thus, a good solubility of the monomer for the external phase and its sufficient



**Fig. 2.13** TEM images of PEDOT nanocapsules and mesocellular foams. **a** Nanocapsules prepared using 0.15 M of DeTAB. **b** Mixture of nanocapsules and mesocellular foams obtained using 0.20 M of DeTab. **c** Mesocellular foams prepared using 0.30 M of DeTab. **d** The concentration ranges for formation of PEDOT nanomaterials as a function of the surfactant hydrocarbon length and polymerization temperature. *DeTab* Decyltrimethylammonium bromide, *OTAB* Octadecyltrimethylammonium bromide, *DoTAB* Dodecyltrimethylammonium bromide. (adapted with permission from [257])

reactivity toward the aqueous phase are critical factors that influence the success of the procedure. For instance, in the case of the polymerization of alkylcyanoacrylate such as isobutyl-cyanoacrylate, hydroxyl ions catalyze the reaction and w/o nanocapsules can be generated [248–255]. Lipophilic diisocyanate can also be used due to its high hydrolyzable properties which induce conversion of the isocyanate functions into amine [256]. The amine function can react subsequently with another monomer molecule leading to polymerization at droplet interface. Another interesting work showed by Jang et al. [257] is the possibility to obtain from this first strategy different architectures of poly(3,4-ethylenedioxythiophene) (PEDOT) nanomaterials. The method is based on a so-called “Surfactant Mediating Interfacial Polymerization (SMIP)” method allowing selective synthesis of either nanocapsules or mesocellular foams in function of the concentration of quaternary ammonium-based surfactants (Fig. 2.13).

In the second strategy, the monomer is introduced in the droplet phase and polymerization occurs by reaction with the continuous phase or by adding initiator in the external phase [258, 259]. Initiator can also be added in the dispersed phase

and polymerization is initiated by temperature. In this case, as polymerization proceeds; gradual segregation of the polymer in formation toward the water/oil interface creates nanocapsules. Another interesting procedure that could be possible is the simultaneous generation of interfacial polymerization and nanoemulsion using solvent diffusion. For instance, alkyl cyanoacrylate monomers can be introduced in the oil dispersed phase containing a water miscible organic solvent [62, 260–263]. Polymerization can thus be initiated along with the rapid solvent diffusion to the aqueous continuous phase. Nanocapsules are hence formed at the same time than the nanoemulsion.

Finally, the third route involves two reactive species of different solubilities introduced, respectively, in the continuous and dispersed phases. The reaction takes place at the interface of the two liquids. This method is the most commonly used for the generation of nanocapsules. Evidently, the type of monomers added defines the nature of the final polymer shell. For instance, polyamides [264], polyurea [265], polyurethanes [57] nanocapsules can be produced by this technique using generally the reaction of isocyanate functions with activated diol. Some authors have shown that the thickness of the polymer wall is independent of the concentration of the lipophilic monomer but varies with the amount of the hydrophilic monomer added [264].

From the strategies described above, different methods have been developed to design nanoparticles by interfacial polymerization but a main problem inherent to the well control of the particle size remains. In the recent past years, the use of membrane reactors has been developed to overcome this problem since a better controlled addition of one reactant to another reactant can be achieved [266, 267]. Indeed, this versatile technique allows the preparation of either nanospheres or nanocapsules and offers the possibility to target the nanoparticle size by a suitable choice of the membrane parameters (membrane pore radius, cross-flow velocity, shape of the pore opening, transmembrane pressure, etc.) and the formulation factors (viscosity of the dispersed and continuous phase, type of surfactant, etc.) [268, 269]. However, nanoparticles preparation by membrane reactors is often considered as an expensive process due to a complicated technological development which constitutes a major drawback to its widespread utilization.

### *Controlled/Living Radical Polymerization*

Main limitations caused by fast radical–radical terminations are inherent to radical polymerization including the control of the molar mass and mass distribution, the end-functionalities and the macromolecular architecture. To obtain a better control of such parameters, the controlled/living radical polymerization [270–272] has emerged as a new field in the recent past years helped by the industrial production of hydrophilic polymeric nanoparticles designed specifically for biomedical applications and by environmental concern with the development of the so-called “green chemistry”. The principal methods of controlled/living radical polymerization are nitroxide-mediated polymerization (NMP) [273–277], atom transfer radical polymerization (ATRP) [278–284], and reversible addition and fragmentation transfer

chain polymerization (RAFT) [285–287]. Suitable properties of nanoparticles can be obtained by optimizing different parameters such as the nature and concentration of the monomer, surfactant, initiator, and the type of emulsion but above all the type and concentration of the mediating (control) agent. Several kinds of polymeric nanoparticles have been synthesized by this technique such as for instance poly(butyl acrylate) [275, 276, 288], poly(styrene) [277, 286], or poly(methyl methacrylate) [282, 284] nanoparticles with typical particle size in a 30–400 nm range, using either NMP, ATRP, or RAFT approaches and different formulations. The presence of residual control agent is at this moment the major problem of controlled/living radical polymerization. For environmental purpose, the removal of control agent needs to be performed which caused additional difficulties and cost for this process.

### 2.3.2 *One-Step Procedures*

#### 2.3.2.1 Nanoprecipitation

Nanoprecipitation method also called solvent displacement method was developed by Fessi et al. [63] in the end of 1980s. It is one of the easiest, most economic, and reproducible routes to produce nanospheres using preformed polymers instead of monomers. This method, very close to the previous described spontaneous emulsification technique, is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Three ingredients are required to achieve the process: the polymer, the polymer solvent, and the non-solvent of the polymer. The polymer can be synthetic, semisynthetic, or natural and the most frequently used polymer solvents are ethanol, acetone, hexane, methylene chloride, or dioxane. The choice of the polymer solvent is guided by two factors: a high solubility in water and an easy removal by evaporation. To satisfy these conditions, acetone is often selected [63, 289, 290] but a binary blend of solvent like acetone with a small amount of water or blends of acetone and ethanol [291–293] or methanol [294] can be used. The non-solvent phase is composed of one or a mixture of nonsolvent of the polymer with eventually the addition of surfactants. The nanoparticles are generated by a rapid diffusion of the polymer solvent in the non-solvent phase by mixing the polymer solution with the latter one. This results in a drop of the interfacial tension between the two phases causing an increase of the surface area and the instantaneous precipitation of polymeric nanoparticles. The lipophilic polymer solution is generally added slowly to the non-polymer solvent but the reverse order also produces nanoparticles. Smaller, more well-defined and narrower distribution of the nanoparticle sizes, typically in a 75–900 nm range, can be obtained than those obtained by emulsification solvent evaporation technique. Many parameters are conditioning the final nanoparticle properties such as the organic phase injection rate, the agitation during addition of the polymer solution, the miscibility of the organic solvent with the non-solvent phase, or the nature of



the polymer/solvent interactions. The type and concentration of added surface active agents also influence the nanoprecipitation process [157, 295] since surfactants help in the stabilization of the nanoparticles prevented from aggregation which is especially useful for long storage periods of suspensions.

The method of nanoprecipitation can be performed with various formulation including a wide range of polymers such as poly( $\epsilon$ -caprolactone) [295–299], polylactide [300, 301], poly(lactide-co-glycolide) [302, 303], poly(hydroxyl butyrate) [304], or even peptides [305]. Moreover, the process can be applied to non-polymeric compounds such as cyclodextrin [306] and drug [307].

Finally, nanoprecipitation is a method widely used for the preparation of polymeric or non-polymeric nanospheres due its simplicity, rapidity, and reproducibility even though the low polymer concentration required limits the recovering yield of nanoparticles.

### 2.3.2.2 Dialysis

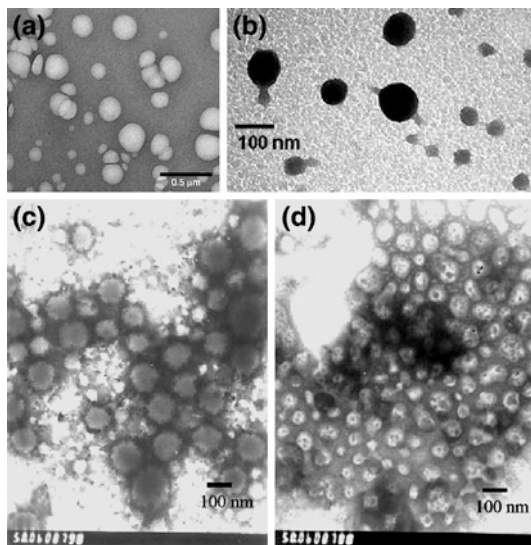
Very close to the above described method, dialysis is based on a solvent displacement mechanism but includes, in contrary to the conventional nanoprecipitation technique, additional tools such as dialysis tubes or semi-permeable membranes with suitable molecular weight cutoff which serve as a physical barrier for the polymer [308–310]. Thus, dialysis is performed against a nonsolvent of the polymer miscible with the polymer solvent. The displacement of the polymer solvent through the membrane induces a progressive loss of solubility of the polymer leading to the formation of homogeneous suspensions of nanoparticles. According to the solvents used, the morphology and size of the particles can be affected [311]. Various formulations have been investigated and nanoparticles of different types of polymers such as poly(lactide)-*b*-poly(ethylene oxide) [312], polystyrene [313], poly(L-lactic acid)-*b*-poly(ethylene glycol) [314], poly( $\gamma$ -glutamic acid) [311], or cellulose-derived polymers [315] can be obtained by dialysis.

### 2.3.2.3 Desolvation

The desolvation method is based on a slow addition of a desolvation factor such as salts, alcohols, or solvents in a solution of macromolecules to provoke the precipitation of the polymer [316, 317]. This method, rather similar than other nanoprecipitation methods based on a loss of solubilization of the polymer, is however often associated to the generation of biopolymeric nanoparticles. Indeed, desolvation process is generally employed for the production of nanoparticles of different types of proteins (Fig. 2.14) such as human serum albumin [318–323], bovine serum albumin [324, 325], gliadin [326–328], or gelatin [318, 329–335]. The desolvation is often followed by a cross-linking step performed with the addition of a certain amount of aldehyde (typically glutaraldehyde) to stabilize the formed nanoparticles. In the case of gelatin (type A), a two-step desolvation route



**Fig. 2.14** TEM images of Gelatin (a), Human serum albumin (b), and Bovine serum albumin (c, d; high and low magnification, respectively) nanoparticles prepared by the desolvation method (reprinted with permission from [323, 324, 334] respectively)



has been developed by Coester et al. [329, 330, 336]. In the first step, the low molecular gelatin fractions present in the supernatant is removed by decanting. The sediment is then redissolved and desolvated in a second step at pH 2.5. This two-step process can also be applied to type B gelatin but the pH is adjusted at 12. Polysaccharide particles of chitosan [337] or hyaluronic acid [338] can also be obtained by desolvation but using sodium sulfate as desolvating agent in these cases.

### 2.3.2.4 Self-Assembly and Gelation

#### Polyelectrolytes Complexation

In this case, the organic nanoparticles are obtained based on the association of oppositely charged macromolecules forming, when mixed in specific conditions, polyelectrolyte complexes. Such particles are widely used and developed as *in vivo* drug delivery carrier of nucleic acids [339, 340]. In this case, nucleic acids play the role of drug as well as a component of the drug delivery system. The polycations, typically poly(ethylenimine), poly(lysine) or chitosan, are generally employed as the opposite (positive) charged compound able to interact with the negative charges of the nucleic acid phosphate groups [340]. One of the key parameters to optimize the nanoparticles formation is the ratio N/P of the positive amine groups noted “N” (as nitrogen) to the nucleic acid negative phosphate groups noted “P”. Thus, a value of N/P above one means a polyelectrolyte complex positively charged where the internal polyelectrolyte chains of the system are able to be swollen by water.

Other types of complexes can be form based on the association of alginate, a negatively charged polysaccharide, and poly(lysine), a positively charged peptide [341]. Dextran sulfate and chitosan can also interact to form complexes [342–344].

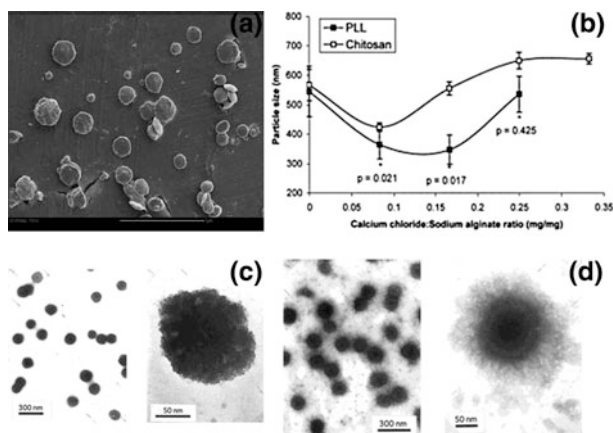
An interesting feature of polyelectrolyte complexes is the possibility to add one polyelectrolyte in excess compared to the other in order to modulate the net charge of the nanoparticles and to induce colloidal stabilization. Indeed, the excess of component is segregated at the outer shell of the complex which produces a core-shell structure where the surface charge of the nanosphere is conferred by the polyelectrolyte in excess. In addition, the nanoparticle size is influenced by the chain length ratio of the macromolecules which conditions the mutual role of each electrolyte in the complex. Indeed, the highest molecular weighted compound serves as host for the lowest weighted one which is defined as the guest [342].

### “Lock and Key” Nanogels

Supramolecular nanoassemblies based on a “lock and key” concept lead nanospheres formed by neutral association of a certain type of macromolecules that have been developed recently [345, 346]. These polymers are composed of dextran modified by the grafting of alkyl chains and a poly(beta-cyclodextrin). The assembly occurs spontaneously in aqueous medium forming a hydrogel since the alkyl chains of the modified dextran are incorporated as a guest in the hydrophobic cavities of the host poly(beta-cyclodextrin). One of the main advantages of this system is the efficiency of the association phenomena which can lead 95 % of incorporation of the guest in the nanogel as well as a nondependence of the protocol followed (order of introduction of the polymer solutions, method of mixing, temperature, etc.). However, the final properties of nanogels depend on various factors such as the polymer concentrations, the weight ratio between the host and the guest, the number of carbons in the alkyl chains, or the percentage of substitution of the glucose units of dextran by the alkyl chains. Finally, these nanogels used as drug delivery devices of hydrophobic drugs such as benzophenone and tamoxifen exhibit excellent loading efficiencies (at least 90 %) and a well- controlled release of the drugs over a period of 16 days.

### Ionic Gelation

Synthesis of nanoparticles by ionic gelation is commonly performed with biopolymers especially charged polysaccharides in aqueous medium in very dilute solution (Fig. 2.15). Indeed, the polymer is dissolved in water with a concentration below the gel point and can react with small ions of the opposite charges to form clusters. These clusters can be stabilized subsequently using oppositely charged polyelectrolytes. For instance, using alginate, gelation is typically carried out in the presence of calcium ions leading to a pre-gel phase which is then stabilized



**Fig. 2.15** Examples of polysaccharides-based nanoparticles prepared by ionic gelation. **a** Alginate/chitosan nanoparticles (adapted with permission from [357]). **b** Influence of calcium chloride/sodium alginate ratio on alginate/polylysine and alginate/chitosan nanoparticles (Reprinted with permission from [348]). **c** Chitosan nanoparticles. **d** Chitosan-coated PEO-PPO diblock copolymer (adapted with permission from [359])

with polycations such as polylysine [341, 347–350] or chitosan [348, 351–357]. It is noted that alginate can react with polylysine without addition of cations to form a simple polyelectrolyte complex but a pre-gel phase ensures a more compact structure of the nanogel. Furthermore, the size of the nanoparticles obtained greatly depends on the concentration of the biopolymers and is also influenced by the molecular weight of the opposite charged macromolecule.

Chitosan nanoparticles can also be produced via ionic gelation. In contrary to alginate, chitosan is positively charged at neutral pH and in consequence, can form nanogels with anionic ions like tri-polyphosphates (TPP). Thus, the pre-gel phase is induced, as for alginate, in diluted solution with the addition of a small amount of TPP. The nanoparticles can be stabilized by copolymers such as pluronic® and their sizes depend on the concentration of chitosan but are not influenced by the TPP concentration. Calvo et al. [358, 359] have demonstrated the possibility to design chitosan nanoparticles and chitosan coated with a diblock PEO-PPO copolymer using this method (Fig. 2.15). The authors have shown the modification of sizes and zeta potential in function of the amount of the copolymer. An interesting feature of chitosan nanoparticles obtained from this method is their ability to swell and shrink upon ionic strength or pH variations. Indeed, an increase of the pH from acidic to basic causes deprotonation of the chitosan glucosamine units and, as a consequence, a gel shrinking due to the reduction of the intramolecular electric repulsions inside the particles [360]. In addition, variation of the ionic strength by increasing the concentration of salt (typically KCl) in the medium drops the chitosan-TPP interactions which favor the particles swelling or even

their complete restructuration. Thus, due to their triggered swelling response upon pH or ionic strength variations, chitosan nanogels are evidently investigated as drug delivery nanocarriers [361–365].

Finally, ionic gelation is a method that offers the main advantages of a solvent-free and a relative simple preparation of organic nanoparticles but suffers from the high diluted conditions which limit the yield of production of particles.

### 2.3.2.5 Organic Nanoparticles Prepared by Drying Processes

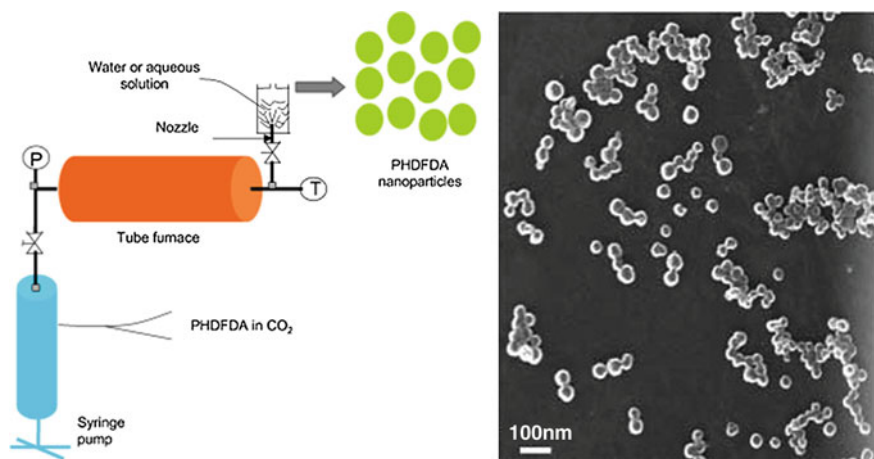
In the past few years, environmental considerations have motivated research on the development of methods of nanoparticles synthesis avoiding the utilization of organic solvents. In this aim, supercritical or spray drying processes offer the possibility to design and prepare nanoparticles without the main drawbacks of the traditional methods [47, 48, 366–370].

#### Supercritical Drying

Two procedures have been developed for the production of nanoparticles using supercritical fluid. The first one is based on a rapid expansion of a supercritical solution and the second one is founded on a rapid expansion of a supercritical solution into liquid solvent.

#### *Rapid Expansion of Supercritical Solution*

In this method, organic macromolecules are solubilized in a supercritical fluid solution which subsequently undergoes a rapid expansion through a nozzle into ambient air. Well-dispersed particles can form resulting from a homogeneous nucleation imposed by the high supersaturation conditions combined with the rapid pressure reduction [371]. Generally, CO<sub>2</sub> is the supercritical fluid used in the majority of studies. A typical experimental apparatus is composed of three units: a high pressure stainless steel mixing cell, a syringe pump, and a pre-expansion unit. The polymer is dissolved in a CO<sub>2</sub> solution at ambient temperature in the mixing cell. The solution moves in the pre-expansion unit with the help of the syringe pump and is heated isobarically to the pre-expansion temperature until it expands through the nozzle at ambient pressure. Poly(heptadecafluorodecyl acrylate) [372] or poly(L-lactic acid) [373] nanoparticles were yet prepared by this technique and it appears that various factors impact the properties of the particles formed such as the concentration and degree of saturation of the polymer, the processing conditions, the molecular mass and the melting point of the polymer, etc. Although the method is performed without organic solvents and produces a majority of nano-sized particles, the main drawback is the generation of micro-sized particles or agglomerates. This is due to a coalescence mechanism involved in the free jet. To overcome this problem, another technology has been developed.



**Fig. 2.16** *Left:* Scheme showing the experimental setup of the rapid expansion of supercritical fluid solution into liquid solvent process. *Right:* SEM images of PHDFDA nanoparticles obtained in presence of NaCl and about 5 min after the rapid expansion process. (adapted with permission from [375])

### Rapid Expansion of Supercritical Solution into Liquid Solvent

In contrary to the above method, the supercritical solution expands in this case into a liquid solvent instead of ambient air [374] (Fig. 2.16). The primary nanosized particles are not allowed to grow in the expansion jet due to the presence of the liquid solvent. For instance, poly(heptafluorodecylacrylate) (PHDFDA) [375] particles were produced using water as the solvent in which were expanded the supercritical solution and precipitated the polymer. It was shown that the particle formation results from the aggregation of initially formed nanoparticles. In addition, the presence of NaCl in the water phase helps to a better stabilization of the nanoparticles due to an increase in the ionic strength.

Poly(methyl methacrylate) and poly(L-lactic acid) nanomaterials were also synthesized by this method using a CO<sub>2</sub>-cosolvent as the supercritical fluid. The cosolvent allows a better solubilization of the polymers in the supercritical solution and the presence of NaCl in the water solution generates only nanosized particles [376].

However, in spite of the wide spectrum of fluids available (carbon dioxide, n-pentane, ammonia, etc.), the poor solubility of polymers in these supercritical fluids remains a main drawback of this technology.

### Spray-Drying

Spray-drying process has been used in the past few years for the production of microsized organic particles or to convert nanoparticle suspensions in dry powder mainly for biomedical and pharmaceutical applications especially in drug

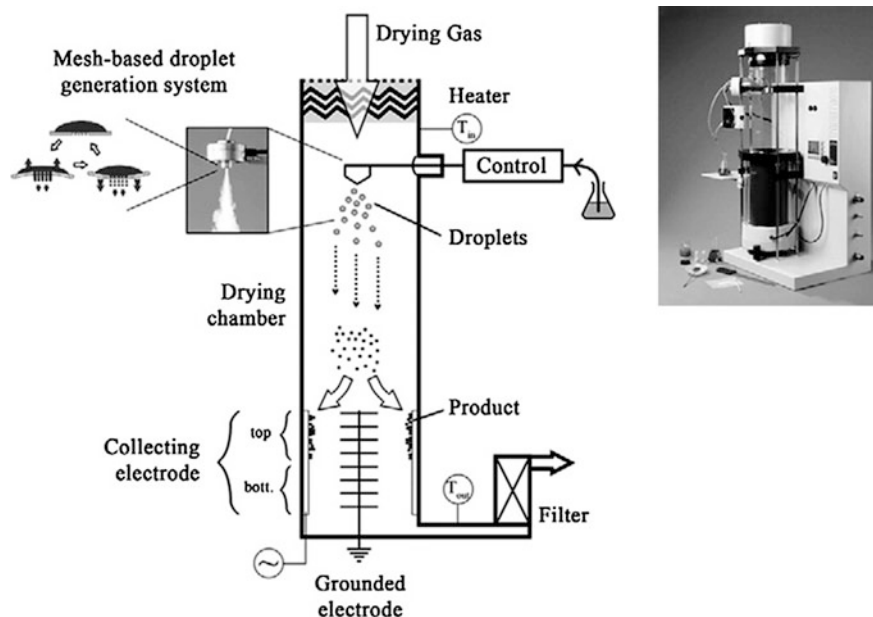


Fig. 2.17 Scheme of the nano Spray-Dryer B-90 (reprinted with permission from [45])

delivery [377–379]. A typical spray-drying process consists in the atomization of a liquid into a spray of fine droplets brought subsequently in contact with a hot drying gas to evaporate the moisture and to form the solid product which is finally recovered via generally a cyclone unit. Spray-drying technology has undergone constant evolution in the past years and the synthesis of polymeric nanosized particles obtained in a one-step procedure by spray-drying a polymer solution has emerged recently. For instance, Li et al. [45] described the preparation of different types of polymeric nanoparticles such as Arabic gum, whey protein, polyvinyl alcohol, modified starch, and maltodextrin based on a “nano spray dryer”, an innovative new spray-drying technology developed by Büchi® (Fig. 2.17).

Very recently, the group of Lee et al. [46] has used the same technology to produce bovine serum albumin nanoparticles. In contrary to conventional spray dryer, the “nano spray dryer” is characterized by a vibration mesh spray technology creating tiny droplets in a range of a smaller order of magnitude than the conventional apparatus. The generation of droplets is based on a piezoelectric actuator driven at an ultrasonic frequency (i.e., 60 kHz) ensuring vibration of a thin perforated membrane with micron-sized holes which can vary from 4 to 7  $\mu\text{m}$  in diameter. The membrane vibration causes ejection of millions of nanodroplets per second with a very narrow size distribution. The final sizes and standard deviation of the nanoparticles obtained depend on several parameters such as the nature and concentration of the polymer, the spray mesh size, the operating conditions (drying temperature, feed rate, drying gas flow rate, etc.), or the concentration of surfactant, if present in the formulation. Finally, another advantage of this novel technology is the high yield production of particles that can be in 70–95 % range.

## 2.4 Conclusion

This chapter provides an overview of the main synthesis methods of organic and bioorganic nanoparticles reported in the literature. Two approaches are highlighted based on either one- or two-step procedures. In the case of two-step procedures, a nanoemulsification step is required prior to conversion of nanodroplets into nanoparticles. It constitutes an important part of the challenge to obtain materials with well-defined structures and morphologies. High-energy emulsifications are by far the most widely used methods but low-energy emulsifications, still few reported, are undergoing a great expansion due to their main advantage in terms of environmental impact. Conversion of nanoemulsions into nanoparticles can be done subsequently in the second step through several ways including nanogelation, solvent removal, salting out, or polymerization.

In the case of one-step procedures, no nanoemulsification is required and the nanoparticles can be generated via different mechanisms such as nanoprecipitation, desolvation, self-assembly, nanogelation, or using more technological ways such as supercritical drying or nanospray-drying methods.

In the field of organic nanoparticles, according to the synthesis methods described above, the control of size, morphology, and structure of particles is still submitted to a number of difficulties that have to be overcome to develop new functional nanomaterials based on organic nanoparticles in the future. A better fundamental knowledge of the processes and mechanisms controlling the particles synthesis should be the subject of an intensive research in the next decades. Both technological aspects and precipitation techniques in solution should be developed and improved simultaneously to ensure a wide spectrum of preparation methods easily adapted to a large and increasing range of organic materials available.

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Brayner, R.; Fiévet, F.; Coradin, T. (Eds.)

2013, VIII, 400 p., Hardcover

ISBN: 978-1-4471-4212-6