

Chapter 2

Nanoprecipitation Process: From Particle Preparation to In Vivo Applications

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Abstract Nanoparticles have been widely prepared during the past decades. In fact, encapsulation could provide several advantages over conventional pharmaceutical forms (Miladi et al. in *Int J Pharm* 445(1–2):181–195, 2013; Campos et al. in *J Colloid Sci Biotechnol* 2(2):106–111, 2013; Grando et al. in *J Colloid Sci Biotechnol* 2(2):140–145, 2013; De Melo et al. in *J Colloid Sci Biotechnol* 2(2):146–152, 2013; Mazzaferro et al. in *J Colloid Sci Biotechnol* 1(2):210–217, 2012; Lira et al. in *J Colloid Sci Biotechnol* 2(2):123–129, 2013; Wang et al. in *J Colloid Sci Biotechnol* 1(2):192–200, 2012). Although, several techniques have been used for the preparation of submicron particles from preformed polymers, nanoprecipitation is regarded as a quite simple and reproducible technique that allows the obtaining of submicron-sized polymer particles. Additionally, many research works have focused on the enhancement of the reproducibility of the technique in order to render it more suitable for industrial applications. Nanoprecipitation is still widely used to prepare particulate carriers which are based on various polymers. Biomedical applications of such drug delivery systems are multiple (Rosset et al. in *J Colloid Sci Biotechnol* 1(2):218–224, 2012; Khan et al. in *J Colloid Sci Biotechnol* 1(1):122–128, 2012).

Keywords Supersaturation · Nucleation · Encapsulation · Hydrophilic molecules · PLGA particles · Microfluidics · Bilamination · Anticancer agents ·

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Nanoprecipitation • Flash nanoprecipitation • Solvent displacement • Interfacial deposition • Nanocapsules • Nanospheres

1 Introduction

Nanoprecipitation is also called solvent displacement or interfacial deposition. It is considered as one of the first developed techniques used for the encapsulation of drug molecules. This technique was developed by Fessi et al. (1989). Since its development, the technique has been widely used for the encapsulation of mainly, hydrophobic drugs in either nanocapsules or nanospheres. Many polymers were used for this purpose, especially, biodegradable polyesters such as, poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA), and poly(ϵ -caprolactone) (PCL). Nanocapsules are vesicular forms that exhibit core-shell structure in which the drug is mainly confined to a reservoir or within a cavity surrounded by a polymer membrane. Nanospheres are, however, matrix-type colloidal particles in which the drug is dissolved or dispersed within the polymer matrix. The drug molecule could be also adsorbed on the surface of the nanocarrier (Mora-Huertas et al. 2010; Letchford and Burt 2007). Nanoprecipitation is based on the interfacial deposition of polymers following the displacement of a semi-polar solvent miscible with water from a lipophilic solution (Fessi et al. 1989). It is an easy and reproducible technique that has been widely used in the preparation of nanoparticles. Nanoprecipitation has many advantages over other encapsulation techniques: (1) Simplicity (2) ease of scalability (3) good reproducibility (4) large amounts of toxic solvents are avoided (5) obtaining of submicron particle sizes with narrow size distribution, and (6) no need for using of high energy input (Lassalle and Ferreira 2007). In 2005, Bilati et al. (2005) developed a modified nanoprecipitation method designed for the encapsulation of hydrophilic

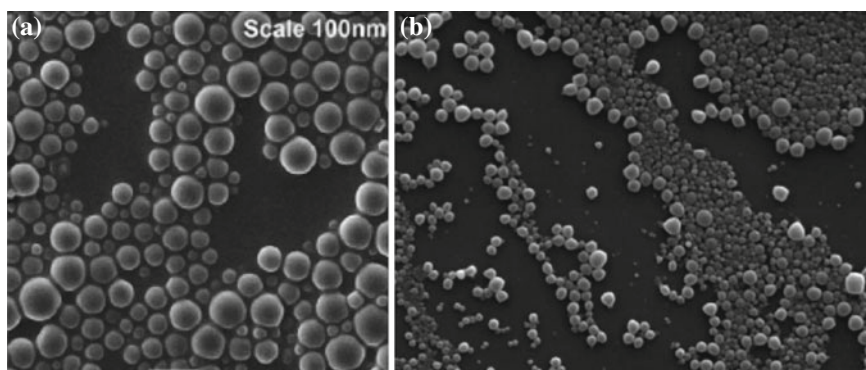


Fig. 1 **a** Scanning electron microscopy (SEM) micrographs of PLGA–PEG nanoparticles (Anand et al. 2010). **b** SEM micrograph of nanoparticles prepared by nanoprecipitation (Costantino et al. 2005). Source: Elsevier

molecules. Figure 1a, b show scanning electron microscopy (SEM) images of nanoparticles prepared by nanoprecipitation.

2 Technical Aspects

2.1 *Mechanism of Particle Formation by Nanoprecipitation*

Nanoprecipitation is a simple and reproducible technique that produces particles with narrow size distribution over a wide range of processing parameters (Budhian et al. 2007). It requires two miscible phases: an organic/oil phase and an aqueous phase (see Fig. 1). Lince et al. (2008) showed that the process of particle formation in the nanoprecipitation method includes three phases: nucleation, growth, and aggregation. Supersaturation was described as the driving force of all these phenomena. It is defined by the ratio of polymer concentration to polymer solubility in the organic solvent. Supersaturation is crucial because it also determines the nucleation rate. Here, fluid dynamics and mixing of phases play an important role. In fact, they influence supersaturation and owing to the rapidity of particle formation process, they determine also the nucleation rate. Consequently, poor mixing produce few big nanoparticles (low nucleation rate) while good mixing conditions give birth to high nucleation rates, i.e., larger population of smaller nanoparticles (Lince et al. 2008). Quintanar-Guerrero et al. (1998), however, explained nanoparticles formation as a result of differences in surface tension. This finding was based on research carried out by Davies on mass transfer between two liquids and on the Gibbs–Marangoni effect (McManamey et al. 1973; Davies 1975). In fact, a liquid with a high surface tension (aqueous phase) pulls more strongly on the surrounding liquid than one with a low surface tension (organic phase solvent). This difference between surface tensions of the aqueous and the oil phase causes interfacial turbulence and thermal inequalities in the system. This leads to the continuous formation of vortices of solvent at the interface of both liquids (Fig. 2). The organic solvent diffuses from regions of low surface tension which causes gradual precipitation of the polymer on the oil surface and forms nanocapsules (Mora-Huertas et al. 2010).

2.2 *Drugs*

Nanoprecipitation technique is essentially used to encapsulate hydrophobic molecules. However, some good results were also obtained with hydrophilic molecules. Table 1 contains some examples of drugs encapsulated by nanoprecipitation and their corresponding nature. More examples will be given in the Chap. 13 by Zandanel and Charrueau. Most of the drug encapsulation studies focused either on poorly water-soluble or amphiphilic compounds that are highly soluble in water

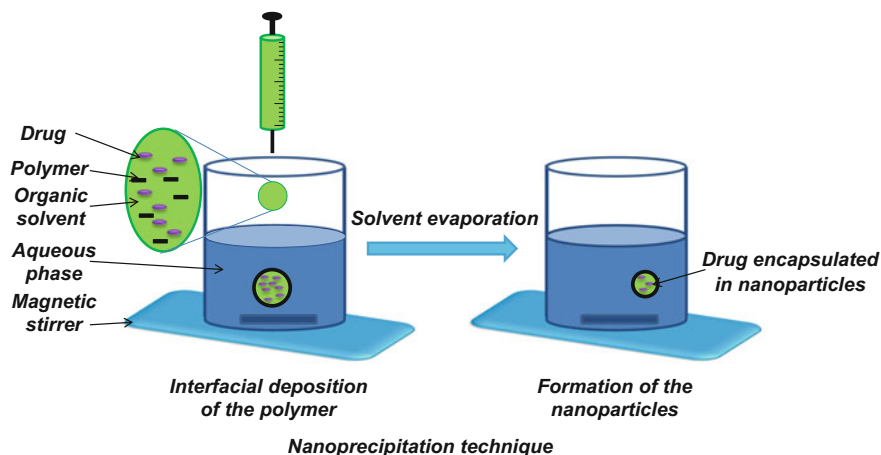


Fig. 2 The nanoprecipitation technique

miscible organic solvents. However, many studies used other approaches to allow the encapsulation of hydrophilic molecules. Three main approaches have been investigated: (1) The dissolving of the hydrophilic molecule in the external aqueous phase, (2) the use of a cosolvent, or (3) the dissolution of small amounts of the molecule in the organic phase. Bilensoy et al. (2009) encapsulated mitomycin *C* in PCL-based nanoparticles coated with chitosan by dissolving the hydrophilic drug in the aqueous phase. Peltonen et al. (2004) used ethanol and methanol as cosolvents and added them to an aqueous solution of cromoglutamate to allow drug dissolution in the organic phase. Govender et al. used nanoprecipitation to prepare PLGA nanoparticles containing the water-soluble molecule, procaine hydrochloride. Experimental procedure consisted on the dissolution of PLGA and a specified quantity of the drug in acetonitrile (Govender et al. 1999).

2.3 Oil Phase

The oil phase consists on an organic solvent which is miscible to water such as, ethanol or acetone. The organic phase contains also the polymer and the hydrophobic drug. Other compounds could be added to the solvent such as triglycerides, mineral or vegetable oils, or hydrophobic surfactants. Addition of mineral or vegetable oils allow obtaining nanocapsules rather than nanospheres. Surfactants hamper the aggregation of the particulate carriers. Table 2 shows some examples of oil phases that could be used in nanoprecipitation. One can notice that acetone is the most commonly used organic solvent in nanoprecipitation.

Table 1 Examples of drugs encapsulated in polymer nanoparticles by nanoprecipitation

Hydrophilic molecules	References	Hydrophobic molecules	References
Cromogluclate	Peltonen et al. (2004)	Olanzapine	Seju et al. (2011)
Doxorubicin	Sanson et al. (2010), Han et al. (2013)	Paclitaxel	Wang et al. (2013)
Bovine Serum Albumin	Gao et al. (2006)	Amphotericin-B	Van de Ven et al. (2012)
Levofloxacin	Cheow and Hadinoto (2010)	Aceclofenac	Katara and Majumdar (2013)
10-Hydroxycamptothecin	Zhang et al. (2007)	Curcumin	Mazzarino et al. (2012)
Mitomycin C	Bilensoy et al. (2009)	Retinoic acid	Almouazen et al. (2012)
Heparin	Eidi et al. (2010)	Naringenin	Krishnakumar et al. (2011)
Stevioside	Barwal et al. (2013)	Efavirenz	Seremeta et al. (2013)
Salbutamol	Hyvönen et al. (2005)	Naproxen	Rosset et al. (2012)
Procaine	Govender et al. (1999)	Chloroaluminum phthalocyanine	Siqueira-Moura et al. (2013)

2.4 Water Phase

The aqueous phase is usually water but some other excipients such as hydrophilic surfactants could be added to avoid particles' aggregation. These surfactants could be natural or synthetic. Likely, some polymers could be added to aqueous phase as coating materials. Hydrophilic drugs could be dissolved in the aqueous phase. Table 3 shows some examples of aqueous phases that could be used in the nanoprecipitation method. As it can be seen, the most used aqueous phase is simply water and the most used surfactant is Pluronic® F68.

2.5 Polymers

Numerous polymers have been used to prepare nanoparticles by nanoprecipitation. To be suitable for in vivo applications, polymers must be biodegradable and biocompatible. The most used materials are biodegradable polyesters such as PLGA, PCL, PLA, and Eudragit®. Coating materials could also be grafted or adsorbed to the initial polymer to confer new surface properties such as, mucoadhesion, protection from reticuloendothelial system (stealth particles) or to tune hydrophilicity.

Table 2 Examples of organic phases used in nanoprecipitation

Composition of the oil phase	References
<i>Oil phases comprising one solvent</i>	
Acetone	Bazylińska et al. (2013), Bernabeu et al. (2013), Shah et al. (2014), Siqueira-Moura et al. (2013), Barwal et al. (2013), Peter Christoper et al., Pavot et al. (2013), Das et al. (2013a), Çirpanlı et al. (2011), Gupta et al. (2010), Liu et al. (2010), Joshi et al. (2010), Cheng et al. (2008), Muthu et al. (2009), Pertuit et al. (2007), Danhier et al. (2009a), Çirpanlı et al. (2009), Yuan et al. (2008), Vila et al. (2004), Fonseca et al. (2002), Leroueil-Le Verger et al. (1998), Nafee et al. (2013), Zili et al. (2005), Yenice et al. (2008), Memisoglu-Bilensoy et al. (2005), Ali et al. (2013), Zhang and Zhuo (2005), Das et al. (2013b), Kumar et al. (2012), Paul et al. (2013), Musumeci et al. (2013), Mazzarino et al. (2012), Eidi et al. (2012)
Ethanol	Ubrich et al. (2005), Perret et al. (2013a, b)
Ehtylacetate	Tao et al. (2013)
Acetonitrile	Wang et al. (2010), Dong and Feng (2004, 2007), Leo et al. (2004)
THF	de Miguel et al. (2013), Peracchia et al. (1999), Kaewprapan et al. (2012)
DMF	Suen and Chau (2013)
DMSO	Esfandyari-Manesh et al. (2013)
PEG	Ali and Lamprecht (2013)
<i>Oil phases comprising solvent mixtures</i>	
Acetone/ethanol	Noronha et al. (2013), das Neves et al. (2013), Le Broc-Ryckewaert et al. (2013)
Acetone/methanol	Das and Suresh (2011)
Acetone/coconut oil	Bazylińska et al. (2013)
Solution of capric/caprylic triglyceride mixture in acetone	Moraes et al. (2009)
Acetone and mixture of chloroform and NEt ₃	Loyer et al. (2013)
Sorbitan monostearate, mineral oil and acetone	Raffin Pohlmann et al. (2002)
thf/water	Kaewprapan et al. (2012)

THF tetrahydrofuran, *DMF* dimethylformamide, *DMSO* dimethylsulfoxide, *PEG* poly(ethylene glycol)

Copolymers could also be used (Miladi et al. 2014). Table 4 contains some examples of polymers used for the preparation of nanoparticles by nanoprecipitation.

Table 3 Examples of aqueous phases used in nanoprecipitation

Composition of the water phase	References
Water	Esfandyari-Manesh et al. (2013), de Miguel et al. (2013), das Neves et al. (2013), Suen and Chau (2013), Das et al. (2013a), Le Broc-Ryckewaert et al. (2013), Le Broc-Ryckewaert et al. (2013), Liu et al. (2010), Danhier et al. (2009a), Dong and Feng (2007), Yuan et al. (2008), Nafee et al. (2013), Loyer et al. (2013), Yenice et al. (2008), Memisoglu-Bilensoy et al. (2005), Peracchia et al. (1999), Zhang and Zhuo (2005), Perret et al. (2013a, b), Kaewprapan et al. (2012)
Aqueous solution of Pluronic® F68	Noronha et al. (2013), Shah et al. (2014), Siqueira-Moura et al. (2013), Barwal et al. (2013), Cırpanlı et al. (2011), Çirpanlı et al. (2009), Dong and Feng (2004), Leroueil-Le Verger et al. (1998), Ubrich et al. (2005), Das et al. (2013b), Kumar et al. (2012), Paul et al. (2013), Eidi et al. (2012)
Aqueous solution of poloxamer 407	Peter Christoper et al., Muthu et al. (2009)
Aqueous PVA solution	Ali and Lamprecht (2013), Gupta et al. (2010), Das and Suresh (2011), Pertuit et al. (2007), Tao et al. (2013),
Aqueous solution of Tween® 80	Moraes et al. (2009), Zili et al. (2005)
Aqueous solution of Cremophor EL	Bazylińska et al. (2013)
Water containing TPGS	Bernabeu et al. (2013)
Water/ethanol	Pavot et al. (2013), Cheng et al. (2008)
Solution of Pluronic® F 127 in phosphate buffer (pH 9.0)	Joshi et al. (2010)
PBS (0.01 M, pH 7.4)	Letchford et al. (2009)
Ethanol	Vila et al. (2004)
Aqueous poloxamer 188 solution	Fonseca et al. (2002)
Aqueous sodium cholate solution	Leo et al. (2004)
Aqueous solution of polysorbate 80	Raffin Pohlmann et al. (2002)
Aqueous sodium taurocholate solution	Ali et al. (2013)
Water/ethanol mixture containing Tween® 80	Musumeci et al. (2013)
Aqueous solution of acetic acid and poloxamer 188	Mazzarino et al. (2012)

PVA poly(vinyl alcohol), TPGS alphasatocopheryl poly(ethylene glycol) 1000 succinate, PBS phosphate buffer saline

2.6 Influence of Operating Conditions

The technique is based on the addition of one phase to the other under moderate magnetic stirring (see Fig. 1). The subsequently obtained suspension of

Table 4 Examples of polymers used in nanoprecipitation

Polymer	References
PLGA	Bazylińska et al. (2013), Shah et al. (2014), Siqueira-Moura et al. (2013), Peter Christopher et al., Ali and Lamprecht (2013), Das et al. (2013a, b), Le Broc-Ryckewaert et al. (2013), Çirpanlı et al. (2011), Gupta et al. (2010), Wang et al. (2010), Joshi et al. (2010), Moraes et al. (2009), Cheng et al. (2008), Muthu et al. (2009), Pertuit et al. (2007), Danhier et al. (2009a), Çirpanlı et al. (2009), Fonseca et al. (2002), Leroueil-Le Verger et al. (1998), Ali et al. (2013), Paul et al. (2013), Tao et al. (2013), Musumeci et al. (2013)
PCL	Noronha et al. (2013), das Neves et al. (2013), Çirpanlı et al. (2011), Çirpanlı et al. (2009), Leroueil-Le Verger et al. (1998), Zili et al. (2005), Yenice et al. (2008), Raffin Pohlmann et al. (2002), Mazzarino et al. (2012)
PLA	Barwal et al. (2013), Pavot et al. (2013), Leroueil-Le Verger et al. (1998), Leo et al. (2004), Raffin Pohlmann et al. (2002)
Eudragit® RL	Ali and Lamprecht (2013), Ubrich et al. (2005)
Eudragit® RS 100	Das and Suresh (2011)
Eudragit® RS	Ubrich et al. (2005)
Eudragit® RS PO	Eidi et al. (2012)
PEG-PLGA	Ali and Lamprecht (2013), Liu et al. (2010), Danhier et al. (2009a), Musumeci et al. (2013)
PEG-b-PCL	Suen and Chau (2013), Danhier et al. (2009a), Nafee et al. (2013)
PEG-PCL-PEG	Zhang and Zhuo (2005)
PLA-PEG	Vila et al. (2004)
PCL conjugated to 5-aminosalicylic acid	Pertuit et al. (2007)
PCL-TPGS	Bernabeu et al. (2013)
mPEG-PLA	Wang et al. (2010), Dong and Feng (2004, 2007)
MePEG-b-PCL	Letchford et al. (2009)
PLA and hydrophobically modified Chitosan	Yuan et al. (2008)
PBLG derivatives	de Miguel et al. (2013)
Amphiphilic derivatives of poly(benzyl malate)	Loyer et al. (2013)
β-CDC6	Memisoglu-Bilensoy et al. (2005)
β-amphiphilic cyclodextrin	Perret et al. (2013a)
PEGylated and non PEGylated PHDCA polymer	Peracchia et al. (1999)
PLGA and DOTAP	Kumar et al. (2012)
Dextran decanoate	Kaewprapan et al. (2012)

PBLG poly(γ -benzyl-L-glutamate), *β-CDC6* cyclodextrin modified on the secondary face with 6C aliphatic esters, *PHDCA* poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecyl-cyanoacrylate), *DOTAP* 1,2-dioleoyl-3-trimethylammonium-propane

nanoparticles is subjected to evaporation of the organic solvent by a rotavapor or at ambient temperature. The next step consists of the removing of the aqueous phase either by ultracentrifugation or freeze drying. The obtained nanoparticles are characterized by the measurement of size, zeta potential, and by transmission electron microscopy (TEM) or scanning electron microscopy (SEM). Many operating conditions could exert important effect on the characteristics of the obtained nanocarriers. Effects of these parameters are summarized in Table 5.

2.6.1 Amount of Polymer

Many studies evaluated the effect of the variation of polymer amount on the characteristics of the nanoparticles. Table 5 presents some examples for the effect of polymer amount on nanoparticle characteristics. As it can be seen, an increase of polymer amount generally increased particle size and encapsulation efficiency. This could be explained by an increase of the viscosity of the oil phase which gives birth to bigger particles and render drug diffusion more difficult. According to Legrand et al. (2007), polymer concentration in organic solvent should remain below the limit between the dilute and semi dilute regime to avoid formation of aggregates.

2.6.2 Molecular Weight of the Polymer

Polymer molecular weight is a crucial parameter that could exert strong influence on particles' properties. Lince et al. evaluated the effect of PCL molecular weight on particle size. The greater the molecular weight, the smaller the size of the particles. An increase of polymer molecular weight led to a decrease of particles size from 144.1 to 93.6 nm. This phenomenon was explained by faster precipitation of the high molecular weight PCL owing to its more limited solubility in the acetone/water medium (Lince et al. 2008; Seremeta et al. 2013). Conversely, Blouza et al. reported an increase of particles size following an increase of polymer molecular weight. This finding was explained by higher viscosity of the organic solution in the case of high polymer molecular weight (Limayem Blouza et al. 2006). In another study, Legrand et al. showed no influence of the molecular weight of PLA on the size of nanoparticles produced in the absence of surfactant. In contrast, they found that the yield of formation of nanoparticles was greatly influenced by the molecular weight of the polymer highlighting that there is an optimal molecular weight of PLA to obtain high production rate of nanoparticles. It was suggested that all PLA chains with molecular weight outside the optimal range are precipitating as aggregates and contribute to reduce the yield of production of nanoparticles (Legrand et al. 2007).

Table 5 Influence of operating conditions on nanoparticles' properties

Operational parameter	Action	Effect	References
Drug amount	Increase	No significant effect on particles size	Chorny et al. (2002)
		Increase of particles size	Govender et al. (1999), Khayata et al. (2012a)
		No significant effect on drug loading	Chorny et al. (2002)
Polymer amount	Increase	Increase of particles size	Chorny et al. (2002), Limayem Blouza et al. (2006), Simsek et al. (2013), Dong and Feng (2004), Ali et al. (2013), Bazylińska et al. (2013), Khayata et al. (2012a), Lince et al. (2008), Plasari et al. (1997), Nehilla et al. (2008) and Guhagarkar et al. (2009)
		Increase of drug loading	Chorny et al. (2002), Dong and Feng (2004)
Polymer molecular weight ^b	Increase	Increase of particles size	Limayem Blouza et al. (2006), Holgado et al. (2012)
		Decrease of particles size	Seremeta et al. (2013)
		No significant effect on particles size	Budhian et al. (2007)
		No significant effect on drug loading	Budhian et al. (2007)
Oil to water phase ratio	Decrease	Decrease of particles size	Budhian et al. (2007), Bazylińska et al. (2013) and Fonseca et al. (2002)
		Increase of particles size	Limayem Blouza et al. (2006), Nehilla et al. (2008)
		No significant effect on particles size	Chorny et al. (2002)
	Increase	Decrease of drug loading	Budhian et al. (2007), Limayem Blouza et al. (2006) and Guhagarkar et al. (2009)
		Decrease of particles size	Dong and Feng (2004)
		Increase of particles size	Stainmesse et al. (1995)
Organic phase addition rate	Increase	Decrease of the particles size	Lince et al. (2008)

(continued)

Table 5 (continued)

Operational parameter	Action	Effect	References
Surfactant amount	Increase	Decrease of the particles size	Contado et al. (2013), Siqueira-Moura et al. (2013) and Guhagarkar et al. (2009)
		Decrease then increase in particles size	Budhian et al. (2007), Limayem Blouza et al. (2006) and Khayata et al. (2012a)
		No significant effect on particles size	Dong and Feng (2004)
		No significant effect on drug loading	Budhian et al. (2007)
Stirring rate	Increase	Decrease	Asadi et al. (2011)
Organic solvent evaporation rate	Increase	No significant effect on particles size	Chorny et al. (2002)
		No significant effect on drug loading	Chorny et al. (2002)

^aYield of nanoparticle formation increases while concentration of polymer remains in the dilute regime (Legrand et al. 2007)

^bYield of nanoparticle production decrease when polymer molecular weight diverge from the optimal value (Legrand et al. 2007)

2.6.3 Amount of Surfactant

Stabilizer amount influence on particle properties has been largely studied. An increase in size of PLGA nanoparticles at high poly(vinyl alcohol) (PVA) concentrations (5–10 %) has been reported by Zweers et al. (2003) and Arica and Lamprecht (2005), while Allemann et al. (1992) reported a continuous decrease in particle size. Lamprecht et al. (2001) noticed also that an increased sodium cholate concentration led to a particle size reduction. In order to explain this contradiction, Budhian et al. (2007) and Arica and Lamprecht (2005) proposed the presence of two competing effects at high PVA concentrations: an enhanced interfacial stabilization that caused a size decrease and an increased viscosity of the aqueous phase which led to a less favorable mixing efficiency and thus, to a size increase. The concentration of PVA at which one effect starts dominating over the other depends on the system and processing parameters. For PLGA nanoparticles, the size first decreased due to better stabilization and then increased at higher PVA concentrations due to high aqueous phase viscosity (Arica and Lamprecht 2005; Budhian et al. 2007). Guhagarkar et al. noticed a sharp decrease in particle size from greater than 1000 nm to around 300 nm as PVA concentration increased from 0.1 to 0.5 %. Further increase in PVA concentration to 4 % resulted in an increase in particle size. In fact, the subsequent increase in viscosity of external aqueous

phase hampered effective diffusion of organic phase leading to larger droplet formation and thus, an increase of mean size (Guhagarkar et al. 2009). Similar results at higher PVA concentrations have been reported (Quintanar-Guerrero et al. 1996; Moinard-Chécot et al. 2008; Murakami et al. 1997).

Stabilizer nature is another crucial parameter that could have an impact on particle size. For instance, Van de Ven et al. (2012) showed that smaller nanoparticles were prepared using Poloxamer 188 in combination with sodium cholate, whereas the largest ones were obtained with PVA. Likely, studies performed by Limayem Blouza et al. (2006) and Khayata et al. (2012a) showed that surfactant type changed the size of vitamin E-loaded nanocapsules as Tween[®] 80 gave the smallest particles.

2.6.4 Oil to Water Phase Ratio

Fonseca et al. (2002) reported that doubling the aqueous phase volume resulted in a significant decrease in the size of PLGA nanoparticles. In fact, in nanoprecipitation, the nanoparticles are formed due to rapid solvent diffusion to the aqueous phase (Quintanar-Guerrero et al. 1997). Consequently, as the volume of the aqueous phase increases, the diffusion of the organic solvent in the aqueous phase increases which decreases particle size. Additionally, an increase of the aqueous phase volume increases the drug amount that can be dissolved in the aqueous phase, which causes more drug loss into the aqueous phase (Budhian et al. 2007).

2.6.5 Solvents Nature and Order of Phases' Addition

Choice of solvents depends on requirements of the method and physicochemical properties of the polymer. In fact, organic solvent must respond to three criteria: (1) dissolving capacity toward polymer (2) miscibility with water, and (3) low boiling point in order to facilitate evaporation. Aqueous phase consists, however, of a nonsolvent for the polymer. This phase would thus cause polymer precipitation to form nanoparticles. It was shown that theta solvent (a solvent in which polymer coils act like ideal chains) tends to give smaller nanoparticles than other solvents (Flory 1969; Legrand et al. 2007). The nature of the aqueous and oil phase and the order of phases' addition could strongly influence nanoparticles' properties. For instance, influence of aqueous phase pH was described by Govender et al. who reported an increasing drug entrapment and drug content trend due to an increase of aqueous phase pH from 5.8 to 9.3. In fact, aqueous phase pH influenced the ionization of the encapsulated drug, procaine hydrochloride and hence, its solubility. Consequently, an increase of the aqueous phase pH decreased the solubility of procaine hydrochloride and enhanced drug entrapment into nanoparticles (Govender et al. 1999). The effect of oil nature was also evaluated by (Khayata et al. 2012a) who noticed that nanoparticles prepared with castor oil were the largest ones. This was explained by the higher viscosity of this oil. In fact, it was shown

that as oil viscosity was higher, dispersed phase viscosity increased. Polydispersity index (PDI) also augmented when the oil viscosity increased. This finding was similar to results reported by Raffin Pohlmann et al. who noticed an increase in particle diameter and PDI with an increase of oil viscosity (Raffin Pohlmann et al. 2002; Khayata et al. 2012a). Effect of organic solvent nature was evaluated by other studies that had shown that solvents of high polarity like acetone gave birth to small nanoparticles by promoting rapid diffusion to the aqueous phase (Legrand et al. 2007; Thioune et al. 1997). It was shown that a lower dielectric constant of the organic solvent resulted in larger particles size (Bilati et al. 2005). Guhagarkar et al. compared particles size and entrapment efficiency of poly(ethylene sebacate) (PES)-based nanoparticles. Particle size decreased significantly when tetrahydrofuran (THF) and acetone were used in combination as solvent compared to THF alone at all polymer concentrations. This was explained by more rapid diffusion of the more polar solvent acetone into the nonsolvent phase that favored the formation of smaller nanoparticles. In fact, the dielectric constant of THF/acetone (1:1) was found to be 14.5 compared to 7.5 for THF alone. In addition, increased diffusivity of the organic solvent due to addition of acetone could cause leaching of the drug into the aqueous phase thus, decreasing encapsulation efficiency (Guhagarkar et al. 2009). The order of phases' addition seems also to exert an effect on particles characteristics. The effect of adding the aqueous phase into the organic phase versus adding the organic phase into the aqueous phase was determined by Khayata et al. who prepared vitamin E-loaded nanocapsules. Obvious aggregation between particles was observed when the aqueous phase was added to the organic phase. This was explained by the presence of the stabilizer in the aqueous phase that plays an important role in stabilizing the nanocapsule formed. This aggregation disappeared when organic phase was added to the aqueous phase (Khayata et al. 2012a). Bilati et al. used proposed a nanoprecipitation technique which is intended to hydrophilic drugs encapsulation. Used solvents consisted of polar aprotic solvents, ketones, or esters. Dimethylsulfoxide was described as an interesting solvent especially for protein dissolution. Nonsolvent was chosen on the basis of its polarity in order to enhance final drug loading. Here, alcohols were shown to be suitable nonsolvents that could provide nanoparticles with different sizes. The same mechanism described previously for the particles formation is involved in particles formation as miscible solvents are always used (Bilati et al. 2005).

2.6.6 Stirring Rate

In nanoprecipitation, the most commonly used stirring method is magnetic stirring. An increase of the stirring rate generally results in a decrease in the particles' size. This is explained by more efficient shear mixing and thus, more rapid diffusion of the organic solvent to the water phase (Asadi et al. 2011).

One can conclude that many operating parameters have to be managed to obtain nanoparticles bearing good characteristics. Table 6 contains some approaches to be followed to monitor major particles properties.

Table 6 Principles and parameters that control particle size and drug content for nanoparticles prepared by nanoprecipitation (from Budhian et al. 2007) with modifications)

	Principles	Parameters
Decrease particle size	Increase shear stress	Increase stirring rate Increase volume of aqueous phase Decrease polymer concentration in organic phase Increase surfactant concentration in aqueous phase Decrease polymer molecular weight
Increase particle size	Increase shear stress	Decrease stirring rate Decrease volume of aqueous phase Increase polymer concentration in organic phase Decrease surfactant concentration in aqueous phase Increase polymer molecular weight
Increase drug loading	Inhibit drug diffusion during organic solvent evaporation Increase drug-polymer interaction	Increase particle size Decrease relative volume of organic solvent Increase polymer concentration in organic phase Intermediate polymer molecular weight Select organic solvent with intermediate drug-solvent interactions Reduce drug solubility in the aqueous phase (alter pH) Include specific interactions between drug and polymer end groups

3 Innovative Approaches Using Nanoprecipitation

Since the first discovery of the technique, many efforts have been made to improve its reproducibility, scalability, and safety. Enhancement of reproducibility could minimize inter-batch variations while improvement of scalability allows the obtaining of formulations which are easily applicable in the pharmaceutical industry. Safety could be provided by avoiding the use of toxic organic solvents. Most common approaches are presented in Table 7. They consisted of the use of innovative mixing devices such as, “T”-shape mixer (Briancon et al. 1999), membrane contactor (Khayata et al. 2012b), microfluidics (Bally et al. 2012) or flash nanoprecipitation technique (D’Addio and Prud’homme 2011).

3.1 Membrane Emulsification

Scalability is one of the major encountered limitations in the manufacture of nanoparticles. Conventional nanoprecipitation did not allow the production of large

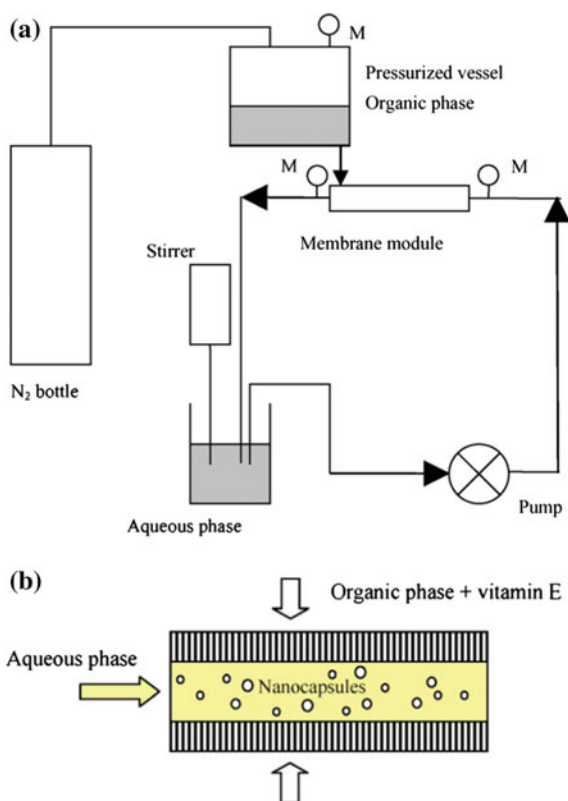
Table 7 Applications of innovative approaches to obtain nanoparticles based on nanoprecipitation carried out with a mixing device

Technique	Drug	Polymer	Oil phase	Water phase	Size (nm)	Zeta potential (mV)	References
"T" shape mixer	–	Eudragit®	Acetone/isopropanol mixture	Aqueous solution of surfactant	100–500	–	Briancon et al. (1999)
Membrane contactor	Vitamin E	PCL	Acetone	Aqueous solution of Tween80	250–353	–20 (–15)	Khayata et al. (2012b)
Membrane contactor	Vitamin E	PCL	Acetone	Aqueous solution of Tween80	170–393	–19.4 (–12.4)	Khayata et al. (2012a)
Microfluidics	–	Linear polymers are poly (methyl methacrylate)s and branched polymers	THF containing a nonionic surfactant (Cremophor ELP®)	Water	76–217	–	Bally et al. (2012)
Flash nanoprecipitation	β-carotene	Poly(styrene)-block-poly (ethylene oxide)	THF	Water	80–1000	–	Johnson and Prud'homme (2003a)
Flash nanoprecipitation	–	PMMA* with coumarin side functionality (PCM)	THF	Water	140–320	–	Chung et al. (2013)
Flash nanoprecipitation	–	Poly(MePEGCA-co-HDCA))*	Acetone	Water	100–300	–50 (–8)	Valente et al. (2012)

*PMMA: poly(methyl-methacrylic acids), poly(MePEGCA-co-HDCA) poly(methoxy poly(ethylene glycol) cyanoacrylate-co-hexadecyl-cyanoacrylate)

scale batches. Membrane contactor could be an interesting alternative in such cases. The technique is relatively simple and could be used to produce large volumes of colloidal dispersions (Yedomon et al. 2013). It has also been shown to be suitable for the preparation of polymer nanoparticles (Charcosset and Fessi 2005; Limayem Blouza et al. 2006; Khayata et al. 2012b). Membrane emulsification involves the permeation of the dispersed phase through a porous membrane into a tangentially moving continuous phase (see Fig. 3a, b). The organic phase is pressed through the membrane pores allowing the formation of small droplets. The precipitation occurs between the droplets of the organic phase and the aqueous phase flowing tangentially to the membrane surface (Khayata et al. 2012b). Khayata et al. performed accelerated stability studies on vitamin E-loaded nanocapsules prepared by conventional nanoprecipitation and by a membrane contactor. These studies showed good physical and chemical stability for both particles. However, nanocapsules prepared by conventional nanoprecipitation were stable for a longer time (Khayata et al. 2012b).

Fig. 3 **a** Experimental setup of the membrane contactor technique (Limayem Blouza et al. 2006). **b** The membrane module (Khayata et al. 2012b). Source: Elsevier



3.2 Microfluidics Device

Nanoprecipitation is usually performed via one-pot pouring of the polymer solution into the nonsolvent, or by dropwise addition of one phase into the other. Microfluidic processes, using a hydrodynamic flow-focusing setup (Karnik et al. 2008; Rhee et al. 2011) or a confined impinging jet reactor (Johnson and Prud'homme 2003b; Lince et al. 2011; Nagasawa et al. 2005) have emerged to improve the mixing of the two phases. Bally et al. used a continuous-flow nanoprecipitation process in which, a diluted polymer solution and water were separately pumped and nanoprecipitation occurred within the micromixer. The latter consisted either of either a T-junction or a High Pressure Interdigital Multilamination Micromixer (HPIMM) (see Fig. 4 for HPIMM). The obtained suspension of nanoparticles could be collected at the outlet of the micromixer (Bally et al. 2012).

Effect of the proportion of solvent and nonsolvent which is defined by the parameter R was investigated by Bally et al.

$$R = \frac{\text{Volume flow rate(water)}}{\text{Volume flow rate(polymer solution)}}$$

It was shown that R managed the number of formed particles whatever was the mechanism considered. In nucleation mechanism, increasing R leads to higher supersaturation and more nuclei, which decrease the final particle size. In the “mechanical” mechanism, a higher value of R increases the potential interface and more droplets are formed during phase separation. As a consequence, the local concentration of the polymer is decreased which leads to smaller nanoparticles. It was shown also that particles size depended both on initial polymer concentration (C) and on the value of R . At low R value, ($R = 3$), particle size did not significantly change at variable C . This was explained by the presence of two competing

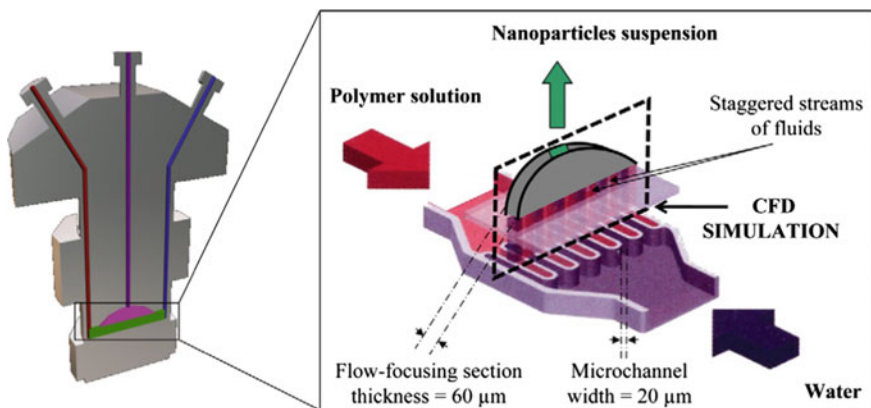


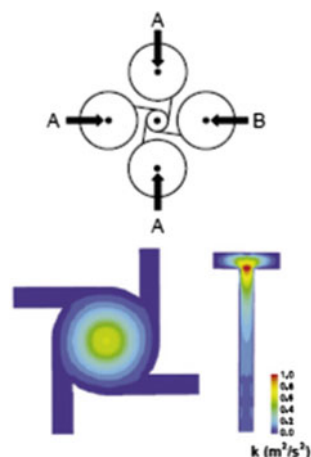
Fig. 4 Overview of HPIMM inner microstructure, used for nanoprecipitation (Bally et al. 2012). Source: Elsevier

mechanisms which are nucleation and growth mechanism. Nucleation rate was shown to increase with C which decreased particle size. Conversely, at high polymer concentrations (≥ 1 wt%), growth phenomena appeared due to proximity of polymer chains. It was concluded that higher nucleation rate finally compensated with higher growth probability when C increases. However, following an increase of R to 10, size of the particles increased from 106 to 210 nm with C . This significant difference was attributed to more aggregation at high polymer concentration. Aggregation of growing particles also contributed to the increase of particle size. The effect of the mixing process on the particles size was also studied as it was previously shown to affect nanoparticles' properties (Lince et al. 2008). Bally et al. compared conventional T-junction, (operating via bilamination mixing) with a multilamination micromixer. Obtained data showed that bilamination mixing gave bigger particles with sizes close to ones obtained by conventional nanoprecipitation. This proves a poor mixing ability. Consequently, fine mixing was described as crucial to produce small nanoparticles at an initial polymer concentration of 1 wt%. Additionally, it was shown that micromixer-assisted nanoprecipitation gave small nanoparticles using less nonsolvent. According to Bally et al, a value of $R = 2$ led to nanoparticles lower than 200 nm whereas at least $R = 10$ is required for conventional nanoprecipitation to obtain the same size. In addition, micromixing allow nanoprecipitation of polymer solution with concentrations up to 5 wt% which is impossible in conventional method in which polydisperse samples were obtained (Bally et al. 2012).

3.3 *Flash Nanoprecipitation (FNP)*

Simple nanoprecipitation carried out with a conventional process results in heterogeneous mixing resulting in polydispersed particle sizes. FNP, however, is a scalable process that could be used to prepare nanoparticles with controlled size distribution and a high drug loading rate. This technique was first described by Johnson and Prud'homme (2003a) to produce nanoparticles encapsulating hydrophobic drugs. FNP produces nanoparticles with a narrow size distribution ranging from 80 to 1 μm . The nanoparticles are obtained via a rapid precipitation process. FNP offers also high loading capacity and the ability to encapsulate multiple drugs in the same nanoparticle. Several successful applications of FNP have been reported for encapsulation of various hydrophobic drugs, peptides, imaging agents, or a combination of both therapeutics and inorganic colloids (Chen et al. 2009; Budijono et al.; Kumar and Adamson 2010; Shi et al. 2012). More information about the potential of this technique is given in Chap. 3 from Tang and Prud'Homme (Fig. 5).

Fig. 5 A schematic representation of multi-inlet vortex mixer used in FNP (D'Addio and Prud'homme 2011). Source: Elsevier



4 In Vivo Applications of Nanoparticles Designed by Nanoprecipitation

Nanoparticles designed by the nanoprecipitation technique were intended to various in vivo applications. Some of these formulations are summarized in Table 8, which also contains some technical aspects of the formulations such as the used polymers, the different phases, and the corresponding in vivo application. Only recent formulations that have been assessed in vivo were taken into account.

4.1 Example of Nanoparticles Developed for Cancer Therapy

Many anticancer agents were encapsulated by the use of the nanoprecipitation technique. Nanoparticles may target cancer cells by passive and active way. Passive way is related to the reduced particles size which allows nanocarriers to cross through fenestrations of endothelial cells and reach tumors. Thanks to the leaky vasculature and the poor lymphatic drainage, Enhanced Permeability and Retention effect (EPR) appears, which enhances the uptake of drugs. Active targeting, however, permits the delivery of the drug to a well-defined tissue or cell by the help of a molecular recognition which occurs between a ligand grafted on the nanoparticles and a receptor exposed on the outside of target cell surface membrane.

Table 8 Examples of nanoparticles prepared by the nanoprecipitation technique and assessed in vivo

Drug	Material	Organic phase	Non organic phase	Size (nm)	Zeta potential (mV)	In vivo application	Reference
Doxorubicin	Gelatin-co-PLA-DPPE	Acetone	Water	131.5	–	Cancer	Han et al. (2013)
Acetofenac	Eudragit RL100	Acetone	0.02 % (w/v) Tween 80 in water	75.52–184.36	22.5–32.6	Ocular inflammation	Katara and Majumdar (2013)
Melatonin	PLGA and PLGA–PEG	Acetone	water/ethanol mixture (1:1 v/v), containing 0.5 % (w/v) of Tween 80 [®]	–	–	Intraocular pressure	Musumeci et al. (2013)
Retinoic acid	PLA	0.75 % Miglyol in acetone	0.05 % of Montanox [®] VG 80 in water	153.6–229.8	–10.4–(–29.4)	Glioma	Almouazen et al. (2012)
Paclitaxel	Hydrophobized pullulan	Acetone Dimethylformamide DMSO Dimethylacetamide	Water	154.6–253 nm 132.6 nm 140.5 nm 127.6 nm	–	Cancer	Lee et al. (2012)
Docetaxel	mPEG–PCL	Acetone	Water	About 70	–	Hepatocellular carcinoma	Liu et al. (2012)
Amphotericin-B	PLGA	DMSO/ acetone (1:1)	Solution of a stabilizer in water	86–153	–31.4–(–9.1)	Invasive fungal infections	Van de Ven et al. (2012)
Insulin	(p (AAPBA-r-MAGA))	DMSO/H ₂ O (1:2) v/v	Water	181.1–220.9	–37.8–(–17.5)	Diabetes	Zhang et al. (2012)

(continued)

Table 8 (continued)

Drug	Material	Organic phase	Non organic phase	Size (nm)	Zeta potential (mV)	In vivo application	Reference
Camptothecin	beta-cyclodextrin PLGA PCL	Ethanol Acetone Acetone	Water	281	-13	Cancer	Cirpanli et al. (2011)
			Water	187	-0.06		
			Water	274	-19		
Olanzapine	PLGA	Acetonitrile	0.25 % (w/v) Poloxamer 407 solution in water	91.2	-23.7	Schizophrenia	Seju et al. (2011)
Curcumin	PLGA-PEG	Acetonitrile	0.1 % pluronic F-68 in water	80.9	-	Cancer	Anand et al. (2010)
Amphotericin-B	Eudragit RL 100	Acetone/methanol (3:1) adjusted to pH4	1 % (w/v) PVA solution in water	134.2-290	22.7-42	Fungal keratitis	Das et al. (2010)
Doxorubicin	Poly(ethylene sebacate)	THF/acetone (1:1)	Solution of 10 % of Tween 80 (v/v) in water	102.8-334.5	-25-(-18)	Hepatic cancer	Guhagarkar et al. (2010)
Sparfloxacin	PLGA	Acetone	1.5 % (w/v) PVA in water	181-232	-22.8-(-22.2)	Bacterial conjunctivitis	(Gupta et al. (2010)
Rivastigmine	PLGA	Acetone	Pluronic F 127 in phosphate buffer pH 9	135.6	-23.7	Alzheimer's disease	Joshi et al. (2010)
Letrozole	PLGA	Acetone	0.5-1 % (w/v) poloxamer-188 in water	15-100	-12- (-19.5)	Breast cancer	Mondal et al. (2010)
Loperamide	SA-GP-PLGA	Acetone	Poloxamer 188 in water	180	-22.8	Chronic neuro-diseases	Tosi et al. (2010)

(continued)

4.1.1 Intravenous Administration

Han et al. formulated Doxorubicin-loaded gelatin-co-PLA-dipalmitoyl-sn-glycero-3-phosphoethanolamine nanoparticles. In vivo experiments showed decreased toxicity of the drug formulated in the developed nanoparticles compared to free Doxorubicin (DOX). In addition, it was shown that developed nanoparticles bore smaller tumor volumes than free doxorubicin when administered to mice. Nanoparticles were then more efficient and less toxic than the free drug (Han et al. 2013). Another alternative was assessed to improve DOX efficacy in liver cancer by enhancing liver targeting. In spite of being a drug of choice for hepatic carcinoma treatment, DOX hydrochloride presents major drawbacks such as the obtaining of low concentrations in the liver. Other limitations consist of cardiotoxicity, nephrotoxicity, myelosuppression, and multiple drug resistance due to P-glycoprotein efflux. To circumvent those shortcomings, the authors aimed to develop long circulating nanocarriers targeted to the liver. The objective was to target Asialoglycoprotein receptor (ASGPR) which is predominantly present in large numbers in the hepatocyte membrane. Polysaccharide including pullulan (PUL), was chosen as a ligand. In fact, pullulan was described to be internalized by hepatocytes via ASGPR mediated endocytosis. Poly(ethylene sebacate) (PES) was used to encapsulate the drug. This polymer presents some advantages such as its ease of synthesis, its good hydrolytic stability, and low cost. In vivo biodistribution studies were performed on healthy female Sprague Dawley rats. Three formulations were assessed: a DOX solution, PES nanoparticles loaded with doxorubicin (PES-DOX), and PES nanoparticles coated with PUL and containing doxorubicin (PUL-PES-DOX). It was shown that PES-DOX and DOX provided higher concentrations of the drug molecule in the liver. Conversely, PUL-PES-DOX gave higher blood concentrations of the drug. These results were explained by a higher uptake of PUL-PES-DOX nanoparticles by Kupffer cells and by the prolonged circulation provided by pullulan. The authors explained lower liver concentration of PES-DOX-PUL by a bypass of kupffer cells. High blood concentrations of PES-DOX-PUL were explained, however, by long circulating property and stealth effect conferred by pullulan. Moreover, PES-DOX and PUL-PES-DOX nanocarriers gave significantly lower heart concentration of DOX which could be interesting to reduce cardiac toxicity (Guhagarkar et al. 2010).

Lee et al. prepared nanoparticles based on hydrophobized pullulan (pullulan acetate) and containing paclitaxel (PTX). An in vivo study using HCT116 human colon carcinoma-bearing mice showed that nanoparticles reduced tumor growth more than free PTX. Efficient accumulation of nanoparticles in tumors was explained by EPR effect and the passive targeting function, although the nanoparticles did not have an active targeting ligand (Lee et al. 2012). Danhier et al. prepared PTX loaded and PEGylated PLGA-based nanoparticles. Tripeptide arginine-glycine-aspartic acid (RGD) has been shown to bind preferentially to particular integrin $\alpha_v\beta_3$ which is highly expressed on tumor cells and neighboring endothelium. RGD pepetidomimetic (RGDp) was developed to mimic the activity RGD. Prepared nanoparticles were grafted either with RGD or RGDp in order to

target tumor endothelium and thus, enhance the antitumor efficacy of PTX. Both of the ligands were grafted on PCL–PEG chains included in the nanoparticles. The used polymers were shown to be safe as drug-free nanoparticles resulted in the same tumor growth profile as Phosphate Buffer Saline solution. In vivo targeting of tumor endothelium was assessed by fluorescence studies. It was shown that fluorescence obtained following the administration RGD conjugated nanoparticles was higher than the fluorescence obtained with RGDp conjugated nanoparticles and nonconjugated nanoparticles. RGDp was, however, higher than in nonconjugated nanoparticles. Furthermore, in vivo antitumor efficacy was evaluated in transplanted liver tumor bearing mice. Obtained data showed that RGD conjugated nanoparticles were more efficient to inhibit tumor growth than RGDp conjugated nanoparticles and nonconjugated nanoparticles. In addition, survival rate provided by RGD conjugated nanoparticles was significantly higher than RGDp conjugated nanoparticles and nontargeted nanocarriers (Danhier et al. 2009b).

Docetaxel (DTX), which is a taxane, possesses an anticancer activity. This drug may cause several side effects due to its nonspecific action. Bone marrow depression, hypersensitivity reactions, and febrile neutropenia are among those toxicological manifestations. PEGylation of carriers has emerged as a smart alternative to prolong circulation time of nanoparticles which facilitates their accumulation in tumors. In fact, stealth surface hampers binding to serum proteins and thus, recognition by reticuloendothelial system. Poly(ϵ -caprolactone)–poly(ethylene glycol) (PEG–PCL) has the advantage of being approved by the Federal Drug Administration to be used clinically. Efficiency of nanoparticles was assessed in H22 tumor bearing mice (a model of hepatic cancer) and compared to the commercialized formulation of DTX Taxotere[®] and DTX solution. Obtained results indicated that nanocarriers significantly reduced tumor growth compared to the other formulations. In addition to enhanced uptake by cancer cells and prolonged circulating time, it was shown by in vivo near-infrared fluorescence imaging that nanocarriers were also eliminated from other normal cells which diminished their toxicity. Penetration studies showed a passive penetration of the nanoparticles through leaky vessels surrounding cancer cells thanks to their submicron size (Liu et al. 2012).

Letrozole (LTZ) is an oral nonsteroidal aromatase inhibitor indicated for the treatment of breast cancer. Mondal et al. prepared PLGA nanoparticles and evaluated them in vivo to see if nanocarriers would provide better tumor targeting. In vivo studies were conducted in normal mice and Ehrlich Ascites tumor-bearing mice by injection in tail vein. The blood concentration of drug-loaded nanocarriers at 24 h post-injection was threefold higher than that of free LTZ. This was explained by a slower blood clearance of the nanoparticles. The tumor uptake of the nanoparticles was significantly higher than the free drug (1.99 % of initial dose/g compared to 0.43 % of initial dose/g) (Mondal et al. 2010).

4.1.2 Local Administration

Another anticancer agent, all trans retinoic acid (TRA), was encapsulated in PLA-based nanocapsules prepared by nanoprecipitation. Retinoic acid is an active derivative of vitamin A which can inhibit the macrophage production of inflammatory cytokines and can, thus, be indicated for some tumors where macrophages play a major role. However, TRA possesses some drawbacks such as poor water solubility and low stability. It was found that nanoparticles injected intratumorally were efficiently phagocytized by glioma infiltrating macrophages (Almouazen et al. 2012). Camptothecin (CPT) is also an efficient anticancer agent. This drug presents, however, some drawbacks such as its extremely high insolubility in water and its chemical instability even in physiological pH which may lead to a loss of the pharmacological activity and cause toxic effects. Cirpanli et al. aimed to develop beta-cyclodextrin nanoparticles and polymer nanoparticles (PLGA and PCL) loaded with CPT for brain cancer treatment. Antitumor efficacy of nanoparticles was assessed on a 9L rat brain tumor model. Cyclodextrin nanoparticles gave the best results (33 and 27 days as median survival time compared to 23.5 and 25.5 days for PLGA and PCL nanoparticles). This significant improvement of survival was explained by the high loading efficiency exhibited by these nanocarriers compared to other formulations (Cirpanli et al. 2011).

4.2 *Example of Nanoparticles Developed for Brain Delivery*

Brain delivery could be alternative to treat central nervous system disorders but passage could be poor because of the presence of the Blood Brain Barrier (BBB). Many nanocarriers have been prepared to circumvent this concern and improve brain targeting. Olanzapine (OLZ), for example, is a second generation antipsychotic which is effective on the associated negative symptoms of schizophrenia. The drug, has, however, low bioavailability due to an important hepatic first-pass metabolism. In addition, OLZ presents low penetration through BBB because of an efflux by P-glycoproteins. Moreover, many side effects may appear such as hypotension, dry mouth, tremor, akathisia and somnolence. Seju et al. assessed nose to brain drug delivery. In vivo efficiency of the prepared PLGA nanoparticles was evaluated versus a drug solution. It was shown that after 3 h of nasal administration, nanoparticles provided a tenfold much higher accumulation of OLZ in the brain compared to the solution form. PLGA nanocarriers showed also no significant toxicity on nasal mucosa, indicating their suitability as carriers for nasal delivery of drugs (Seju et al. 2011). Joshi et al. prepared PLGA nanoparticles loaded with rivastigmine tartrate (RIV) and indicated for the management of Alzheimer disease. Clinical use of RIV has shown a poor entry to the brain from blood circulation due to its hydrophilic nature. In vivo studies were performed in scopolamine-induced amnesic mice. An increase in learning and memory capacities was obtained for RIV solution as well as for the nanocarriers but this improvement was slower in the case

of RIV solution. This was explained by better brain targeting provided by nanoparticles which could present an interesting alternative for better management of Alzheimer disease (Joshi et al. 2010). Loperamide (LOP), an opioid drug, is known to cross blood–brain barrier (BBB) but also to be immediately pumped back out due to the action of the P-gp. The possibility to cross the BBB and to be retained in the brain tissue may make LOP able to exert some opioid effects such as the antinociceptive activity. Tosi et al. prepared LOP loaded nanoparticles in order to target the brain. PLGA nanoparticles were decorated with sialic acid (SA) and/or simil-opioid peptide (g7). Two properties were then allocated to the prepared nanocarriers: First, the ability to cross the BBB due to the presence of g7, (a BBB-penetrating peptide) and second, the capacity to interact with SA receptors in the brain which prolongs the time of residence of the nanoparticles in the brain. This ensured a sustained pharmacological action of the encapsulated drug. In vivo nociceptive study was performed on male albino rats to determine the Maximal Possible Effect (MPE) to measure the intensity of the opioid effect. Two doses of nanoparticles coated with g7 (LOP-PLGA-g7) and nanocarriers coated with SA and g7 (LOP-PLGA-SA-g7) nanoparticles were assessed. It was concluded that, at both doses, nanocarriers reached rapidly the brain (15 min after the injection). After 30–60 min, MPE decreased then increased after 6 h. Obtained values remained then constant for about 15 h but diminished subsequently after 24 h. It was shown also that pharmacological activity of LOP was prolonged compared to other formulations (Tosi et al. 2007). Moreover, LOP-PLGA-SA-g7 nanoparticles exhibited more prolonged pharmacological activity than LOP-PLGA-g7 nanoparticles. In fact, conjugation of SA modified the surface characteristics of the nanoparticles which resulted in a prolongation of the pharmacological action (Tosi et al. 2010). Risperidone (RIS) is an atypical antipsychotic agent which may cause dose-dependent extrapyramidal side effects (EPS). Consequently, the use of low doses is necessary to avoid such manifestations. RIS is practically insoluble in water and undergoes important first-pass metabolism. Long-acting injectable formulations have been already developed but presented poor initial drug release which implied initial oral supplementation. Prepared nanoparticles were assessed in vivo by studying the antagonism of apomorphine-induced climbing and sniffing (antipsychotic activity) in Swiss albino mice. It was shown that PLGA nanoparticles significantly inhibited apomorphine-induced climbing and sniffing up to 72 h while the RIS solution exhibited inhibition up to only 12 h. Furthermore, the incorporation of the nanocarriers in an in situ gel system controlled the initial rapid release of RIS from nanoparticles and showed the maximum inhibition in the apomorphine-induced climbing and sniffing. This was explained by a control of the initial burst by the incorporation in the in situ gel. It was show also that nanoparticles significantly reduced catalepsy which is an EPS (Muthu et al. 2009).

4.3 Example of Nanoparticles Developed to Treat Ocular Diseases

The delivery of drugs into the eye must challenge poor drug ocular bioavailability which is principally caused by precorneal loss. In fact, it was reported that barely 90 % of the applied drug undergoes a precorneal loss by lacrimation and drainage. Precorneal loss ways include rapid tear turnover, nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the drugs to the corneal epithelial membrane (Katara and Majumdar 2013). Nanoparticles have several advantages over conventional drug delivery systems intended to ocular delivery. In fact, they have slower ocular elimination and they could provide sustained release of drugs. While ocular delivery of poly(alkylcyanoacrylate) nanoparticles was described to cause disruption to the corneal epithelium cell membrane, other polymers were shown to be safe such as, PCL and Eudragit® RL. The latter has a positive charge which allows a better adhesion to eye tissue and thus, more prolonged residence time in the cornea (Das et al. 2010).

Encapsulation of melatonin in PLGA and PLGA-PEG nanoparticles was assessed for glaucoma (an optic neuropathy characterized by elevation of intraocular pressure: IOP) treatment by Musumeci et al. Obtained nanoparticles showed ocular tolerability in rabbit eyes. Furthermore, both formulations provided prolonged decrease in IOP but PLGA-PEG-based nanoparticles were more efficient by providing greater decrease. These results were explained by the higher mucoadhesion of the PLGA-PEG nanoparticles thanks to the PEG groups. In addition, the cornea and conjunctiva have a net negative charge. Thus, the lower negative zeta potential of PLGA-PEG nanocarriers allowed a better and more prolonged interaction with the eye (Musumeci et al. 2013). Particulate nanocarriers would be then well-tolerated alternatives to prolong contact with the eye tissue. Eudragit-based nanoparticles containing the anti-inflammatory drug, aceclofenac were prepared and their efficiency was evaluated in vivo by administration to rabbits. Eudragit® RL100 is a positively charged polymer due to many quaternary ammoniums in its structure. This property allows mucoadhesion to the anionic cornea. Katara and Majumdar assessed the effect of the prepared nanoparticles versus an aqueous solution of the drug on arachidonic acid-induced polymorphonuclear leukocytes migration and lid closure in rabbit eyes. Obtained results showed lower lid disclosure for both aceclofenac formulations but nanoparticles provided smaller lid closure compared to the drug solution. Furthermore, more enhanced anti-inflammatory effect was exerted by nanoparticles compared to drug solution (Katara and Majumdar 2013). Das et al. developed Eudragit® RL nanoparticles loaded with amphotericin-B (AmB) which is a polyene antibiotic indicated in fungal keratitis. Other formulations consisting mainly of liposomes and colloidal dispersions were successfully used but presented stability concerns. Stability studies performed at room temperature and at 2–6 °C showed good stability of the nanoparticles during 2 months. Eye irritating effects of the formulation was assessed in vivo in albino rabbits. All the obtained data showed that values of

irritation and opaqueness were almost zero which confirmed the suitability and the safety of the formulation for ocular delivery. Positive charge of the polymer facilitated effective adhesion of the nanocarriers to the corneal surface and ensured a strong interaction with the negatively charged mucosa of the conjunctiva and the anionic mucin present in the tear film (Das et al. 2010).

Yenice et al. prepared hyaluronic coated PCL nanospheres containing cyclosporine A (CyA). CyA is a neutral hydrophobic peptide which is indicated for multiple ocular immune disorders. Systemic use of the drug is limited because of the various significant side effects that may appear such as, hypertension, nephrotoxicity, and hepatotoxicity. Diffusion to the ocular tissue is thought to occur only when the eye is significantly inflamed. Hyaluronic acid (HA) was used due to its mucoadhesion properties which may enhance ocular residence time of cyclosporine A and thus, enhance its ocular bioavailability and prolongs its activity. In vivo studies were performed by topical administration of three different formulations to Male albino New Zealand rabbits: a solution of CyA in castor oil, PCL nanospheres, and PCL nanospheres coated with HA. Obtained corneal concentration of CyA for nanospheres formulations were 6–8 fold higher than those of castor oil solution. HA-coated nanospheres provided significant increase in CyA corneal uptake and similar results were obtained for the conjunctival tissue (Yenice et al. 2008). Sparfloxacin is a newer generation hydrophobic fluoroquinolone used in bacterial conjunctivitis. This drug is poorly water soluble and presents bioavailability concerns. Gupta et al. aimed to enhance sparfloxacin bioavailability by the preparation of PLGA nanoparticles. An in vivo ocular retention study was performed on Male New Zealand albino rabbits. Developed nanocarriers were compared to a marketed formulation. A good spreading was observed over the entire precorneal area for both formulations but the marketed formulation showed rapid clearing from corneal region. PLGA nanoparticles, however, adhered to the cornea for a longer duration providing, thus, a more extended release of drug. Particles size seems to be the key factor to explain this prolonged residence time on the cornea as PLGA is a negatively charged polymer and is not known to be naturally mucoadhesive (Gupta et al. 2010).

4.4 Other Applications

AmB is a polyene antibiotic which is commonly indicated for invasive fungal infections and visceral leishmaniasis. This drug has a poor water solubility which limits its oral bioavailability. In addition, many side effects were described in patients receiving AmB such as fever, chills, vomiting, headache, nausea, and renal malfunctions, especially with the commercialized formulation Fungizone®. Newer lipid-based formulations are more tolerated but their expensiveness and the need of well-defined daily doses limited their success. Van de Ven et al. aimed to develop a more potent and cost-effective formulation of AmB. Hemolysis assay showed that PLGA nanoparticles were less hemolytic than drug solution and some of them were

even not hemolytic at all. A selected formation was evaluated in the acute *A. fumigatus* mouse model and its potency was compared to a nanosuspension of AmB, Fungizone[®], and Ambiosome[®]. Obtained data revealed that PLGA nanoparticles reduced *A. fumigatus* more efficiently than Fungizone[®]. In addition, nanocarriers were about two times more efficient to clear mice organs from the fungi than Ambiosome[®]. The nanosuspension was, however, four times more efficient than Ambiosome[®] (Van de Ven et al. 2012).

The nasal route possesses many advantages over the oral and the parenteral routes in the delivery of biomacromolecules. In fact, it is noninvasive, painless, does not require sterile preparation, and allow self-administration. However, the development of drug delivery systems intended to nasal delivery must challenge poor absorption through the nasal mucosa and eventual enzymatic degradation. New generation phenylboronic acid-functionalized glycopolymers were developed to avoid these shortcomings. Their properties are linked to the presence of boronic acid and its derivatives which could bind to glycoproteins and glycolipids within cell surfaces. Moreover, boronic acid derivatives could resist to enzymatic degradation because they exert potent inhibition toward serine proteases such as trypsin, chymotrypsin, elastase, and leucine aminopeptidase. These properties made interesting the use of these special polymers in the development of nanocarriers, especially in the case of the encapsulation of biomacromolecules. Insulin was encapsulated in poly(3-acrylamidophenylboronic acid-ran-*N*-maleated glucosamine) p(AAPBA-r-MAGA) copolymers and administered to mice by the intranasal route. The potency of the developed nanoparticles was compared to an insulin solution. It was concluded that the insulin solution was not able to reduce significantly glucose blood levels while a significant decrease was provided by nanoparticles. This confirmed enhanced nasal absorption of insulin provided by phenylboronic acid-functionalized glycopolymer nanocarriers (Zhang et al. 2012). Curcumin (CUR) is a yellow pigment in the spice turmeric (*Curcuma longa*). This drug is poorly soluble in water and presents very low oral bioavailability. CUR exhibits antioxidant, anti-inflammatory, anti-survival, antiproliferative, anti-invasive, and antiangiogenic activity. The assessment of the bioavailability of PLGA-PEG nanoparticles was performed in Balb/c mice versus pure CUR. Obtained results showed that serum levels of CUR provided by nanocarriers were almost two times higher than those provided by CUR solution. Moreover, nanoparticles insured a sustained release of the drug (Anand et al. 2010).

5 Conclusion

Several hydrophobic or hydrophilic drugs could present bioavailability, stability, or unpleasant taste concerns. Encapsulation of such molecules in nanoparticles could be a very interesting alternative to solve these problems in order to enhance the efficacy of such molecules and promote patient compliance. Nanoprecipitation is a simple and reproducible technique that has been widely used for the preparation of

polymer nanoparticles intended for several biomedical applications since its first discovery. Operating conditions have to be well managed to obtain nanoparticles with suitable properties for the biomedical applications they are designed for. Several research works have been made to use nanoprecipitation in a conventional way while other works focused on the enhancement of its scalability, reproducibility, and safety. Membrane technology, microfluidics, and flash nanoprecipitation were introduced to achieve such purposes. Advantages of submicron carriers prepared by nanoprecipitation in the biomedical field have been confirmed in vivo by numerous studies. These achievements include enhanced bioavailability, better targeting and tolerance, sustained release, and enhanced absorption of the drug through biological barriers.

References

- Ali H, Kalashnikova I, White MA, Sherman M, Rytting E (2013) Preparation, characterization, and transport of dexamethasone-loaded polymeric nanoparticles across a human placental in vitro model. *Int J Pharm* 454(1):149–157
- Ali ME, Lamprecht A (2013) Polyethylene glycol as an alternative polymer solvent for nanoparticle preparation. *Int J Pharm* 456(1):135–142
- Allémann E, Gurny R, Doelker E (1992) 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
- Almouazen E, Bourgeois S, Boussaïd A, Valot P, Malleval C, Fessi H et al (2012) Development of a nanoparticle-based system for the delivery of retinoic acid into macrophages. *Int J Pharm* 430 (1–2):207–215
- Anand P, Nair HB, Sung B, Kunnumakkara AB, Yadav VR, Tekmal RR et al (2010) Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo. *Biochem Pharmacol* 79 (3):330–338
- Arica B, Lamprecht A (2005) In vitro evaluation of betamethasone-loaded nanoparticles. *Drug Dev Ind Pharm* 31(1):19–24
- Asadi H, Rostamizadeh K, Salari D, Hamidi M (2011) Preparation of biodegradable nanoparticles of tri-block PLA–PEG–PLA copolymer and determination of factors controlling the particle size using artificial neural network. *J Microencapsul* 28(5):406–416
- Bally F, Garg DK, Serra CA, Hoarau Y, Anton N, Brochon C et al (2012) Improved size-tunable preparation of polymeric nanoparticles by microfluidic nanoprecipitation. *Polymer* 53 (22):5045–5051
- Barwal I, Sood A, Sharma M, Singh B, Yadav SC (2013) Development of stevioside Pluronic-F-68 copolymer based PLA-nanoparticles as an antidiabetic nanomedicine. *Colloids Surf B Biointerfaces* 101:510–516
- Bazylińska U, Lewińska A, Lamch Ł, Wilk KA (2013) Polymeric nanocapsules and nanospheres for encapsulation and long sustained release of hydrophobic cyanine-type photosensitizer. *Colloids Surf Physicochem Eng Asp* [Internet]. mars 2013 [cité 1 mai 2013]; Disponible sur: <https://www.sciencedirect.com/frodon.univ-paris5.fr/science/article/pii/S092777571300126X>
- Bernabeu E, Helguera G, Legaspi MJ, Gonzalez L, Hocht C, Taira C et al (2013) Paclitaxel-loaded PCL-TPGS nanoparticles: In vitro and in vivo performance compared with Abraxane®. *Colloids Surf B Biointerfaces* 113C:43–50

- Bilali U, Allémann E, Doelker E (2005) Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. *Eur J Pharm Sci* 24(1):67–75
- Bilensoy E, Sarisozen C, Esendağlı G, Doğan AL, Aktaş Y, Sen M et al (2009) Intravesical cationic nanoparticles of chitosan and polycaprolactone for the delivery of Mitomycin C to bladder tumors. *Int J Pharm* 371(1–2):170–176
- Briancon S, Fessi H, Lecomte F, Lieto J (1999) Study of an original production process of nanoparticles by precipitation. *Récents Prog. En Génie Procédés* [Internet]. 1999 [cité 27 août 2014]. pp 157–164. Disponible sur: <http://cat.inist.fr/?aModele=afficheN&cpsid=1142605>
- Le Broc-Ryckewaert D, Carpentier R, Lipka E, Daher S, Vaccher C, Betheder D et al (2013) Development of innovative paclitaxel-loaded small PLGA nanoparticles: study of their antiproliferative activity and their molecular interactions on prostatic cancer cells. *Int J Pharm* 454(2):712–719
- Budhian A, Siegel SJ, Winey KI (2007) Haloperidol-loaded PLGA nanoparticles: systematic study of particle size and drug content. *Int J Pharm* 336(2):367–375
- Budijono SJ, Shan J, Yao N, Miura Y, Hoye T, Austin RH et al Synthesis of stable block-copolymer-protected $\text{NaYF}_4:\text{Yb}^{3+}$, Er^{3+} up-converting phosphor nanoparticles. *Chem Mater* 22(2):311–318
- Campos EVR, de Melo NFS, de Paula E, Rosa AH, Fraceto LF (2013) Screening of conditions for the preparation of poly(-caprolactone) nanocapsules containing the local anesthetic articaine. *J Colloid Sci Biotechnol* 2(2):106–111
- Charcosset C, Fessi H (2005) Preparation of nanoparticles with a membrane contactor. *J Membr Sci* 266(1–2):115–120
- Chen T, D’Addio SM, Kennedy MT, Swietlow A, Kevrekidis IG, Panagiotopoulos AZ et al (2009) Protected peptide nanoparticles: experiments and brownian dynamics simulations of the energetics of assembly. *Nano Lett* 9(6):2218–2222
- Cheng F-Y, Wang SP-H, Su C-H, Tsai T-L, Wu P-C, Shieh D-B et al (2008) Stabilizer-free poly (lactide-co-glycolide) nanoparticles for multimodal biomedical probes. *Biomaterials* 29 (13):2104–2112
- Cheow WS, Hadinoto K (2010) Enhancing encapsulation efficiency of highly water-soluble antibiotic in poly(lactic-co-glycolic acid) nanoparticles: Modifications of standard nanoparticle preparation methods. *Colloids Surf Physicochem Eng Asp* 370(1–3):79–86
- Chorny M, Fishbein I, Danenberg HD, Golomb G (2002) Lipophilic drug loaded nanospheres prepared by nanoprecipitation: effect of formulation variables on size, drug recovery and release kinetics. *J Control Release* 83(3):389–400
- Chung JW, Neikirk C, Priestley RD (2013) Investigation of coumarin functionality on the formation of polymeric nanoparticles. *J Colloid Interface Sci* 396:16–22
- Contado C, Vighi E, Dalpiaz A, Leo E (2013) Influence of secondary preparative parameters and aging effects on PLGA particle size distribution: a sedimentation field flow fractionation investigation. *Anal Bioanal Chem* 405(2–3):703–711
- Costantino L, Gandolfi F, Tosi G, Rivasi F, Vandelli MA, Forni F (2005) Peptide-derivatized biodegradable nanoparticles able to cross the blood-brain barrier. *J Control Release* 108(1):84–96
- Cirpanlı Y, Allard E, Passirani C, Bilensoy E, Lemaire L, Calış S et al (2011) Antitumoral activity of camptothecin-loaded nanoparticles in 9L rat glioma model. *Int J Pharm* 403(1–2):201–206
- Danhier F, Lecouturier N, Vroman B, Jérôme C, Marchand-Brynaert J, Feron O et al (2009a) Paclitaxel-loaded PEGylated PLGA-based nanoparticles: in vitro and in vivo evaluation. *J Control Release* 133(1):11–17
- Danhier F, Vroman B, Lecouturier N, Crockart N, Pourcelle V, Freichels H et al (2009b) Targeting of tumor endothelium by RGD-grafted PLGA-nanoparticles loaded with Paclitaxel. *J Control Release* 140(2):166–173
- Das S, Das J, Samadder A, Paul A, Khuda-Bukhsh AR (2013a) Strategic formulation of apigenin-loaded PLGA nanoparticles for intracellular trafficking, DNA targeting and improved therapeutic effects in skin melanoma in vitro. *Toxicol Lett* 223(2):124–138
- Das S, Das J, Samadder A, Paul A, Khuda-Bukhsh AR (2013b) Efficacy of PLGA-loaded apigenin nanoparticles in benzo[a]pyrene and ultraviolet-B induced skin cancer of mice: mitochondria

- mediated apoptotic signalling cascades. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc* 62:670–680
- Das Neves J, Amiji M, Bahia MF, Sarmiento B (2013) Assessing the physical-chemical properties and stability of dapivirine-loaded polymeric nanoparticles. *Int J Pharm* 456(2):307–314
- Das S, Suresh PK (2011) Nanosuspension: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to amphotericin B. *Nanomed Nanotechnol Biol Med* 7(2):242–247
- Das S, Suresh PK, Desmukh R (2010) Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. *Nanomed Nanotechnol Biol Med* 6(2):318–323
- Davies JT (1975) Local eddy diffusivities related to «bursts» of fluid near solid walls. *Chem Eng Sci* 30(8):996–997
- Dong Y, Feng S-S (2004) Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. *Biomaterials* 25(14):2843–2849
- Dong Y, Feng S-S (2007) In vitro and in vivo evaluation of methoxy polyethylene glycol-poly(lactide) (MPEG-PLA) nanoparticles for small-molecule drug chemotherapy. *Biomaterials* 28(28):4154–4160
- D'Addio SM, Prud'homme RK (2011) Controlling drug nanoparticle formation by rapid precipitation. *Adv Drug Deliv Rev* 63(6):417–426
- Eidi H, Joubert O, Attik G, Duval RE, Bottin MC, Hamouia A et al (2010) Cytotoxicity assessment of heparin nanoparticles in NR8383 macrophages. *Int J Pharm* 396(1–2):156–165
- Eidi H, Joubert O, Némès C, Grandemange S, Mograbi B, Foliguet B et al (2012) Drug delivery by polymeric nanoparticles induces autophagy in macrophages. *Int J Pharm* 422(1–2):495–503
- Esfandyari-Manesh M, Ghaedi Z, Asemi M, Khanavi M, Manayi A, Jamalifar H et al (2013) Study of antimicrobial activity of anethole and carvone loaded PLGA nanoparticles. *J Pharm Res* 7(4):290–295
- Fessi H, Puisieux F, Devissaguet JP, Ammouy N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm* 55(1):R1–R4
- Flory PJ (1969) Statistical mechanics of chain molecules. Interscience Publishers, New York
- Fonseca C, Simões S, Gaspar R (2002) Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. *J Control Release* 83(2):273–286
- Gao H, Yang Y, Fan Y, Ma J (2006) Conjugates of poly(dl-lactic acid) with ethylenediamino or diethylenetriamino bridged bis(β -cyclodextrin)s and their nanoparticles as protein delivery systems. *J Control Release* 112(3):301–311
- Govender T, Stolnik S, Garnett MC, Illum L, Davis SS (1999) PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. *J Control Release* 57(2):171–185
- Grando CRC, Guimarães CA, Mercuri LP, Matos JDR, Santana MHA (2013) Preparation and characterization of solid lipid nanoparticles loaded with racemic mitotane. *J Colloid Sci Biotechnol* 2(2):140–145
- Guhagarkar SA, Gaikwad RV, Samad A, Malshe VC, Devarajan PV (2010) Polyethylene sebacate–doxorubicin nanoparticles for hepatic targeting. *Int J Pharm* 401(1–2):113–122
- Guhagarkar SA, Malshe VC, Devarajan PV (2009) Nanoparticles of polyethylene sebacate: a new biodegradable polymer. *AAPS PharmSciTech* 10(3):935–942
- Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A, Mittal G (2010) Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. *Nanomed Nanotechnol Biol Med* 6(2):324–333
- Han S, Li M, Liu X, Gao H, Wu Y (2013) Construction of amphiphilic copolymer nanoparticles based on gelatin as drug carriers for doxorubicin delivery. *Colloids Surf B Biointerfaces* 102:833–841
- Holgado MA, Martín-banderas, Álvarez-fuentes, Durán-lobato, Prados J, Melguizo et al (2012) Cannabinoid derivate-loaded PLGA nanocarriers for oral administration: formulation, characterization, and cytotoxicity studies. *Int J Nanomed* 5793
- Hyvönen S, Peltonen L, Karjalainen M, Hirvonen J (2005) Effect of nanoprecipitation on the physicochemical properties of low molecular weight poly(l-lactic acid) nanoparticles loaded with salbutamol sulphate and beclomethasone dipropionate. *Int J Pharm* 295(1–2):269–281

- Johnson BK, Prud'homme RK (2003a) Flash nanoprecipitation of organic actives and block copolymers using a confined impinging jets mixer. *Aust J Chem* 56(10):1021–1024
- Johnson BK, Prud'homme RK (2003b) Chemical processing and micromixing in confined impinging jets. *AIChE J* 49(9):2264–2282
- Joshi SA, Chavhan SS, Sawant KK (2010) Rivastigmine-loaded PLGA and PBCA nanoparticles: preparation, optimization, characterization, in vitro and pharmacodynamic studies. *Eur J Pharm Biopharm* 76(2):189–199
- Kaewprapan K, Inprakhon P, Marie E, Durand A (2012) Enzymatically degradable nanoparticles of dextran esters as potential drug delivery systems. *Carbohydr Polym* 88(3):875–881
- Karnik R, Gu F, Basto P, Cannizzaro C, Dean L, Kyei-Manu W et al (2008) Microfluidic platform for controlled synthesis of polymeric nanoparticles. *Nano Lett* 8(9):2906–2912
- Katara R, Majumdar DK (2013) Eudragit RL 100-based nanoparticulate system of aceclofenac for ocular delivery. *Colloids Surf B Biointerfaces* 103:455–462
- Khan MS, Vishakante GD, Bathool A (2012) Development and characterization of brimonidine tartrate loaded eudragit nanosuspensions for ocular drug delivery. *J Colloid Sci Biotechnol* 1 (1):122–128
- Khayata N, Abdelwahed W, Chehna MF, Charcosset C, Fessi H (2012a) Preparation of vitamin E loaded nanocapsules by the nanoprecipitation method: from laboratory scale to large scale using a membrane contactor. *Int J Pharm* 423(2):419–427
- Khayata N, Abdelwahed W, Chehna MF, Charcosset C, Fessi H (2012b) Stability study and lyophilization of vitamin E-loaded nanocapsules prepared by membrane contactor. *Int J Pharm* 439(1–2):254–259
- Krishnakumar N, Sulfikkarali N, RajendraPrasad N, Karthikeyan S (2011) Enhanced anticancer activity of naringenin-loaded nanoparticles in human cervical (HeLa) cancer cells. *Biomed Prev Nutr* 1(4):223–231
- Kumar V, Adamson DH (2010) Prud'homme RK. Fluorescent polymeric nanoparticles: aggregation and phase behavior of pyrene and amphotericin B molecules in nanoparticle cores. *Small Weinhe Bergstr Ger* 6(24):2907–2914
- Kumar A, Wonganan P, Sandoval MA, Li X, Zhu S, Cui Z (2012) Microneedle-mediated transcutaneous immunization with plasmid DNA coated on cationic PLGA nanoparticles. *J Control Release* 163(2):230–239
- Lamprecht A, Ubrich N, Yamamoto H, Schäfer U, Takeuchi H, Lehr CM et al (2001) Design of rolipram-loaded nanoparticles: comparison of two preparation methods. *J Control Release* 71(3):297–306
- Lassalle V, Ferreira ML (2007) PLA nano- and microparticles for drug delivery: an overview of the methods of preparation. *Macromol Biosci* 7(6):767–783
- Lee SJ, Hong G-Y, Jeong Y-I, Kang M-S, Oh J-S, Song C-E et al (2012) Paclitaxel-incorporated nanoparticles of hydrophobized polysaccharide and their antitumor activity. *Int J Pharm* 433(1–2):121–128
- Legrand P, Lesieur S, Bochot A, Gref R, Raatjes W, Barratt G et al (2007) Influence of polymer behaviour in organic solution on the production of polylactide nanoparticles by nanoprecipitation. *Int J Pharm* 344(1–2):33–43
- Leo E, Brina B, Forni F, Vandelli MA (2004) In vitro evaluation of PLA nanoparticles containing a lipophilic drug in water-soluble or insoluble form. *Int J Pharm* 278(1):133–141
- Leroueil-Le Verger M, Fluckiger L, Kim Y-I, Hoffman M, Maincent P (1998) Preparation and characterization of nanoparticles containing an antihypertensive agent. *Eur J Pharm Biopharm* 46(2):137–143
- Letchford K, Burt H (2007) A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur J Pharm Biopharm* 65(3):259–269
- Letchford K, Liggins R, Wasan KM, Burt H (2009) In vitro human plasma distribution of nanoparticulate paclitaxel is dependent on the physicochemical properties of poly(ethylene glycol)-block-poly(caprolactone) nanoparticles. *Eur J Pharm Biopharm* 71(2):196–206

- Limayem Blouza I, Charcosset C, Sfar S, Fessi H (2006) Preparation and characterization of spirinolactone-loaded nanocapsules for paediatric use. *Int J Pharm* 325(1–2):124–131
- Lince F, Marchisio DL, Barresi AA (2008) Strategies to control the particle size distribution of poly- ϵ -caprolactone nanoparticles for pharmaceutical applications. *J Colloid Interface Sci* 322(2):505–515
- Lince F, Marchisio DL, Barresi AA (2011) A comparative study for nanoparticle production with passive mixers via solvent-displacement: use of CFD models for optimization and design. *Chem Eng Process Process Intensif* 50(4):356–368
- Lira AAM, Cordo PLA, Nogueira ECF, Almeida EDP, Junior RALC, Nunes RS et al (2013) Optimization of topical all-trans retinoic acid penetration using poly-D,L-lactide and poly-D,L-lactide-co-glycolide microparticles. *J Colloid Sci Biotechnol* 2(2):123–129
- Liu Y, Li K, Liu B, Feng S-S (2010) A strategy for precision engineering of nanoparticles of biodegradable copolymers for quantitative control of targeted drug delivery. *Biomaterials* 31(35):9145–9155
- Liu Q, Li R, Zhu Z, Qian X, Guan W, Yu L et al (2012) Enhanced antitumor efficacy, biodistribution and penetration of docetaxel-loaded biodegradable nanoparticles. *Int J Pharm* 430(1–2):350–358
- Loyer P, Bedhouche W, Huang ZW, Cammas-Marion S (2013) Degradable and biocompatible nanoparticles decorated with cyclic RGD peptide for efficient drug delivery to hepatoma cells in vitro. *Int J Pharm* 454(2):727–737
- Mazzaferro S, Bouchemal K, Maksimenko A, Skanji R, Opolon P, Ponchel G (2012) Reduced intestinal toxicity of docetaxel loaded into mucoadhesive nanoparticles, in mouse xenograft model. *J Colloid Sci Biotechnol* 1(2):210–217
- Mazzarino L, Travelet C, Ortega-Murillo S, Otsuka I, Pignot-Paintrand I, Lemos-Senna E et al (2012) Elaboration of chitosan-coated nanoparticles loaded with curcumin for mucoadhesive applications. *J Colloid Interface Sci* 370(1):58–66
- McManamey WJ, Davies JT, Woollen JM, Coe JR (1973) The influence of molecular diffusion on mass transfer between turbulent liquids. *Chem Eng Sci* 28(4):1061–1069
- De Melo NFS, Campos EVR, de Paula E, Rosa AH, Fraceto LF (2013) Factorial design and characterization studies for articaine hydrochloride loaded alginate/chitosan nanoparticles. *J Colloid Sci Biotechnol* 2(2):146–152
- Memisoglu-Bilensoy E, Vural I, Bochot A, Renoir JM, Duchene D, Hincal AA (2005) Tamoxifen citrate loaded amphiphilic beta-cyclodextrin nanoparticles: in vitro characterization and cytotoxicity. *J Control Release* 104(3):489–496
- De Miguel L, Noiray M, Surpateanu G, Iorga BI, Ponchel G (2013) Poly(γ -benzyl-L-glutamate)-PEG-alendronate multivalent nanoparticles for bone targeting. *Int J Pharm* 460(1–2):73–82
- Miladi K, Sfar S, Fessi H, Elaissari A (2013) Drug carriers in osteoporosis: preparation, drug encapsulation and applications. *Int J Pharm* 445(1–2):181–195
- Moinard-Chécot D, Chevalier Y, Brianchon S, Beney L, Fessi H (2008) Mechanism of nanocapsules formation by the emulsion–diffusion process. *J Colloid Interface Sci* 317(2):458–468
- Mondal N, Halder KK, Kamila MM, Debnath MC, Pal TK, Ghosal SK et al (2010) Preparation, characterization, and biodistribution of letrozole loaded PLGA nanoparticles in Ehrlich Ascites tumor bearing mice. *Int J Pharm* 397(1–2):194–200
- Mora-Huertas CE, Fessi H, Elaissari A (2010) Polymer-based nanocapsules for drug delivery. *Int J Pharm* 385(1–2):113–142
- Moraes CM, de Matos AP, de Paula E, Rosa AH, Fraceto LF (2009) Benzocaine loaded biodegradable poly-(D,L-lactide-co-glycolide) nanocapsules: factorial design and characterization. *Mater Sci Eng B* 165(3):243–246
- Murakami H, Kawashima Y, Niwa T, Hino T, Takeuchi H, Kobayashi M (1997) Influence of the degrees of hydrolyzation and polymerization of poly(vinylalcohol) on the preparation and properties of poly(DL-lactide-co-glycolide) nanoparticle. *Int J Pharm* 149(1):43–49

- Musumeci T, Bucolo C, Carbone C, Pignatello R, Drago F, Puglisi G (2013) Polymeric nanoparticles augment the ocular hypotensive effect of melatonin in rabbits. *Int J Pharm* 440(2):135–140
- Muthu MS, Rawat MK, Mishra A, Singh S (2009) PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation. *Nanomed Nanotechnol Biol Med* 5(3):323–333
- Nafee N, Youssef A, El-Gowelli H, Asem H, Kandil S (2013) Antibiotic-free nanotherapeutics: Hypericin nanoparticles thereof for improved in vitro and in vivo antimicrobial photodynamic therapy and wound healing. *Int J Pharm* 454(1):249–258
- Nagasawa H, Aoki N, Mae K (2005) Design of a new micromixer for instant mixing based on the collision of micro segments. *Chem Eng Technol* 28(3):324–330
- Nehilla B, Bergkvist M, Popat K, Desai T (2008) Purified and surfactant-free coenzyme Q10-loaded biodegradable nanoparticles. *Int J Pharm* 348(1–2):107–114
- Noronha CM, Granada AF, de Carvalho SM, Lino RC, de OB Maciel MV, Barreto PLM (2013) Optimization of α -tocopherol loaded nanocapsules by the nanoprecipitation method. *Ind Crops Prod* 50:896–903
- Paul A, Das S, Das J, Samadder A, Khuda-Bukhsh AR (2013) Cytotoxicity and apoptotic signalling cascade induced by chelidonine-loaded PLGA nanoparticles in HepG2 cells in vitro and bioavailability of nano-chelidonine in mice in vivo. *Toxicol Lett* 222(1):10–22
- Pavot V, Rochereau N, Primard C, Genin C, Perouzel E, Lioux T et al (2013) Encapsulation of Nod1 and Nod2 receptor ligands into poly(lactic acid) nanoparticles potentiates their immune properties. *J Control Release* 167(1):60–67
- Peltonen L, Aitta J, Hyvönen S, Karjalainen M, Hirvonen J (2004) Improved entrapment efficiency of hydrophilic drug substance during nanoprecipitation of poly(l)lactide nanoparticles. *AAPS PharmSciTech* 5(1):E16
- Peracchia MT, Fattal E, Desmaële D, Besnard M, Noël JP, Gomis JM et al (1999) Stealth PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. *J Control Release* 60(1):121–128
- Perret F, Duffour M, Chevalier Y, Parrot-Lopez H (2013a) Design, synthesis, and in vitro evaluation of new amphiphilic cyclodextrin-based nanoparticles for the incorporation and controlled release of acyclovir. *Eur J Pharm Biopharm* 83(1):25–32
- Perret F, Marminon C, Zeinyeh W, Nebois P, Bollacke A, Jose J et al (2013b) Preparation and characterization of CK2 inhibitor-loaded cyclodextrin nanoparticles for drug delivery. *Int J Pharm* 441(1–2):491–498
- Pertuit D, Moulari B, Betz T, Nadaradjane A, Neumann D, Ismaïli L et al (2007) 5-amino salicylic acid bound nanoparticles for the therapy of inflammatory bowel disease. *J Control Release* 123(3):211–218
- Peter Christopher GV, Vijaya Raghavan C, Siddharth K, Siva Selva Kumar M, Hari Prasad R (2013) Formulation and optimization of coated PLGA—zidovudine nanoparticles using factorial design and in vitro in vivo evaluations to determine brain targeting efficiency. *Saudi Pharm J* [Internet]. [cité 23 déc 2013]; Disponible sur: <http://www.sciencedirect.com/science/article/pii/S1319016413000406>
- Plasari E, Grisoni PH, Villermaux J (1997) Influence of process parameters on the precipitation of organic nanoparticles by drowning-out. *Chem Eng Res Des* 75(2):237–244
- Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H (1997) A mechanistic study of the formation of polymer nanoparticles by the emulsification-diffusion technique. *Colloid Polym Sci* 275(7):640–647
- Quintanar-Guerrero D, Allémann E, Fessi H, Doelker E (1998) Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug Dev Ind Pharm* 24(12):1113–1128
- Quintanar-Guerrero D, Fessi H, Allémann E, Doelker E (1996) Influence of stabilizing agents and preparative variables on the formation of poly(D,L-lactic acid) nanoparticles by an emulsification-diffusion technique. *Int J Pharm* 143(2):133–141

- Raffin Pohlmann A, Weiss V, Mertins O, Pesce da Silveira N, Stanisçuaski Guterres S (2002) Spray-dried indomethacin-loaded polyester nanocapsules and nanospheres: development, stability evaluation and nanostructure models. *Eur J Pharm Sci* 16(4–5):305–312
- Rhee M, Valencia PM, Rodriguez MI, Langer R, Farokhzad OC, Karnik R (2011) Synthesis of size-tunable polymeric nanoparticles enabled by 3D hydrodynamic flow focusing in single-layer microchannels. *Adv Mater* 23(12):79–83
- Rosset V, Ahmed N, Zaanoun I, Stella B, Fessi H, Elaissari A (2012) Elaboration of argan oil nanocapsules containing naproxen for cosmetic and transdermal local application. *J Colloid Sci Biotechnol* 1(2):218–224
- Sanson C, Schatz C, Le Meins J-F, Soum A, Thévenot J, Garanger E et al (2010) A simple method to achieve high doxorubicin loading in biodegradable polymersomes. *J Control Release* 147(3):428–435
- Seju U, Kumar A, Sawant KK (2011) Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: In vitro and in vivo studies. *Acta Biomater* 7(12):4169–4176
- Seremeta KP, Chiappetta DA, Sosnik A (2013) Poly(ϵ -caprolactone), Eudragit® RS 100 and poly(ϵ -caprolactone)/Eudragit® RS 100 blend submicron particles for the sustained release of the antiretroviral efavirenz. *Colloids Surf B Biointerfaces* 102:441–449
- Shah U, Joshi G, Sawant K (2014) Improvement in antihypertensive and antianginal effects of felodipine by enhanced absorption from PLGA nanoparticles optimized by factorial design. *Mater Sci Eng C* 35:153–163
- Shi L, Shan J, Ju Y, Aikens P, Prud'homme RK (2012) Nanoparticles as delivery vehicles for sunscreen agents. *Colloids Surf Physicochem Eng Asp* 396:122–129
- Simşek S, Eroğlu H, Kurum B, Ulubayram K (2013) Brain targeting of Atorvastatin loaded amphiphilic PLGA-b-PEG nanoparticles. *J Microencapsul* 30(1):10–20
- Siqueira-Moura MP, Primo FL, Esprefaco EM, Tedesco AC (2013) Development, characterization, and photocytotoxicity assessment on human melanoma of chloroaluminum phthalocyanine nanocapsules. *Mater Sci Eng C* 33(3):1744–1752
- Stainmesse S, Orecchioni A-M, Nakache E, Puisieux F, Fessi H (1995) Formation and stabilization of a biodegradable polymeric colloidal suspension of nanoparticles. *Colloid Polym Sci* 273(5):505–511
- Suen W-LL, Chau Y (2013) Specific uptake of folate-decorated triamcinolone-encapsulating nanoparticles by retinal pigment epithelium cells enhances and prolongs antiangiogenic activity. *J Control Release* 67(1):21–28
- Tao Y, Ning M, Dou H (2013) A novel therapeutic system for malignant glioma: nanoformulation, pharmacokinetic, and anticancer properties of cell-nano-drug delivery. *Nanomed Nanotechnol Biol Med* févr 9(2):222–232
- Thioune O, Fessi H, Devissaguet JP, Puisieux F (1997) Preparation of pseudolatex by nanoprecipitation: Influence of the solvent nature on intrinsic viscosity and interaction constant. *Int J Pharm* 146(2):233–238
- Tosi G, Costantino L, Rivasi F, Ruozi B, Leo E, Vergoni AV et al (2007) Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with loperamide and rhodamine-123. *J Control Release* 122(1):1–9
- Tosi G, Vergoni AV, Ruozi B, Bondioli L, Badiali L, Rivasi F et al (2010) Sialic acid and glycopeptides conjugated PLGA nanoparticles for central nervous system targeting: in vivo pharmacological evidence and biodistribution. *J Control Release* 145(1):49–57
- Ubrich N, Schmidt C, Bodmeier R, Hoffman M, Maincent P (2005) Oral evaluation in rabbits of cyclosporin-loaded Eudragit RS or RL nanoparticles. *Int J Pharm* 288(1):169–175
- Valente I, Celasco E, Marchisio DL, Barresi AA (2012) Nanoprecipitation in confined impinging jets mixers: Production, characterization and scale-up of pegylated nanospheres and nanocapsules for pharmaceutical use. *Chem Eng Sci* 77:217–227
- Van de Ven H, Paulussen C, Feijens PB, Matheeußen A, Rombaut P, Kayaert P et al (2012) PLGA nanoparticles and nanosuspensions with amphotericin B: potent in vitro and in vivo alternatives to Fungizone and Am Bisome. *J Control Release* 161(3):795–803

- Vila A, Gill H, McCallion O, Alonso MJ (2004) Transport of PLA-PEG particles across the nasal mucosa: effect of particle size and PEG coating density. *J Control Release* 98(2):231–244
- Wang J, Feng S-S, Wang S, Chen Z-Y (2010) Evaluation of cationic nanoparticles of biodegradable copolymers as siRNA delivery system for hepatitis B treatment. *Int J Pharm* 400 (1–2):194–200
- Wang F, Li J, Wang C (2012) Hydrophilic and fluorescent colloidal nanorods of MWNTs as effective targeted drug carrier. *J Colloid Sci Biotechnol* 1(2):192–200
- Wang G, Yu B, Wu Y, Huang B, Yuan Y, Liu CS (2013) Controlled preparation and antitumor efficacy of vitamin E TPGS-functionalized PLGA nanoparticles for delivery of paclitaxel. *Int J Pharm* 446(1–2):24–33
- Yedomon B, Fessi H, Charcosset C (2013) Preparation of bovine serum albumin (BSA) nanoparticles by desolvation using a membrane contactor: a new tool for large scale production. *Eur J Pharm Biopharm* 85(3):398–405
- Yenice I, Mocan MC, Palaska E, Bochoť A, Bilensoy E, Vural I et al (2008) Hyaluronic acid coated poly-epsilon-caprolactone nanospheres deliver high concentrations of cyclosporine A into the cornea. *Exp Eye Res* 87(3):162–167
- Yuan X-B, Yuan Y-B, Jiang W, Liu J, Tian E-J, Shun H-M et al (2008) Preparation of rapamycin-loaded chitosan/PLA nanoparticles for immunosuppression in corneal transplantation. *Int J Pharm* 349(1–2):241–248
- Zhang X, Wang Y, Zheng C, Li C (2012) Phenylboronic acid-functionalized glycopolymeric nanoparticles for biomacromolecules delivery across nasal respiratory. *Eur J Pharm Biopharm* 82(1):76–84
- Zhang L, Yang M, Wang Q, Li Y, Guo R, Jiang X et al (2007) 10-Hydroxycamptothecin loaded nanoparticles: preparation and antitumor activity in mice. *J Control Release* 119(2):153–162
- Zhang Y, Zhuo R (2005) Synthesis and in vitro drug release behavior of amphiphilic triblock copolymer nanoparticles based on poly(ethylene glycol) and polycaprolactone. *Biomaterials* 26(33):6736–6742
- Zili Z, Sfar S, Fessi H (2005) Preparation and characterization of poly-epsilon-caprolactone nanoparticles containing griseofulvin. *Int J Pharm* 294(1–2):261–267
- Zweers MLT, Grijpma DW, Engbers GHM, Feijen J (2003) The preparation of monodisperse biodegradable polyester nanoparticles with a controlled size. *J Biomed Mater Res B Appl Biomater* 66(2):559–566
- Çirpanlı Y, Bilensoy E, Lale Doğan A, Çaliş S (2009) Comparative evaluation of polymeric and amphiphilic cyclodextrin nanoparticles for effective camptothecin delivery. *Eur J Pharm Biopharm* 73(1):82–89

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