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Polymer-based nanocapsules for drug delivery

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ABSTRACT

A review of the state of knowledge on nanocapsules prepared from preformed polymers as active substances carriers is presented. This entails a general review of the different preparation methods: nanoprecipitation, emulsion–diffusion, double emulsification, emulsion-coacervation, polymer-coating and layer-by-layer, from the point of view of the methodological and mechanistic aspects involved, encapsulation of the active substance and the raw materials used. Similarly, a comparative analysis is given of the size, zeta-potential, dispersion pH, shell thickness, encapsulation efficiency, active substance release, stability and *in vivo* and *in vitro* pharmacological performances, using as basis the data reported in the different research works published. Consequently, the information obtained allows establishing criteria for selecting a method for preparation of nanocapsules according to its advantages, limitations and behaviours as a drug carrier.

Contents

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1. Introduction

Generally, nanoparticles are defined as solid colloidal particles that include both nanospheres and nanocapsules. They can be prepared by both polymerization methods and synthesis with preformed polymers (Fattal and Vauthier, 2002; Vauthier and Bouchemal, 2008). One of their fundamental characteristics is their size, which is generally taken to be around 5–10 nm with an upper size limit of ~1000 nm, although the range generally obtained is 100–500 nm (Quintanar et al., 1998a).

As asserted by different authors, nanoparticulated systems show promise as active vectors due to their capacity to release drugs (Cruz et al., 2006; Amaral et al., 2007); their subcellular size allows relatively higher intracellular uptake than other particulate systems (Furtado et al., 2001a,b); they can improve the stability of active substances (Ourique et al., 2008) and can be biocompatible with tissue and cells when synthesized from materials that are either biocompatible or biodegradable (Guinebretière et al., 2002).

Other advantages of nanoencapsulated systems as active substance carriers include high drug encapsulation efficiency due to optimized drug solubility in the core, low polymer content compared to other nanoparticulated systems such as nanospheres, drug polymeric shell protection against degradation factors like pH and light and the reduction of tissue irritation due to the polymeric shell (Pinto et al., 2006a; Anton et al., 2008).

Polymeric nanoparticles have been extensively studied as drug carriers in the pharmaceutical field (Legrand et al., 1999; Barratt, 2000; Chaubal, 2004; Sinha et al., 2004; Letchford and Burt, 2007) and different research teams have published reviews about the nanoparticle formation mechanisms (Quintanar et al., 1998a; Moinard-Checot et al., 2006), the classification of nanoparticulated systems (Letchford and Burt, 2007) and the techniques for preparation of nanocapsules (Moinard-Checot et al., 2006; Pinto et al., 2006a; Vauthier and Bouchemal, 2008). As a contribution to updating the state of knowledge, the present review focuses on nanocapsules obtained from preformed polymers, using prototype cases, among others, to provide illustrations. The aspects studied are mean size, zeta-potential, encapsulating efficiency, active release, nanodispersion stability and *in vivo* and *in vitro* pharmacological performance behaviours.

2. Nanocapsule definition

First of all the nanocapsules can be likened to vesicular systems in which a drug is confined in a cavity consisting of an inner liquid core surrounded by a polymeric membrane (Quintanar et al., 1998a). However, seen from a general level, they can be defined as nano-vesicular systems that exhibit a typical core-shell structure in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating (Letchford and Burt, 2007; Anton et al., 2008). The cavity can contain the active substance in liquid or solid form or as a molecular dispersion (Fessi et al., 1989; Devissaguet et al., 1991; Radtchenko et al., 2002b). Likewise, this reservoir can be lipophilic or hydrophobic according to the preparation method and raw materials used. Also, taking into account the operative limitations of preparation methods, nanocapsules can also carry the active substance on their surfaces or imbibed in the polymeric membrane (Khoee and Yaghoobian, 2008) (Fig. 1).

3. Methods for the preparation of nanocapsules and their fundamental mechanisms

Generally, there are six classical methods for the preparation of nanocapsules: nanoprecipitation, emulsion–diffusion, double emulsification, emulsion-coacervation, polymer-coating and layer-by-layer (Fig. 2). Nevertheless, other methods have been used such as emulsion–evaporation and the methodologies for the preparation of polymer liposomes.

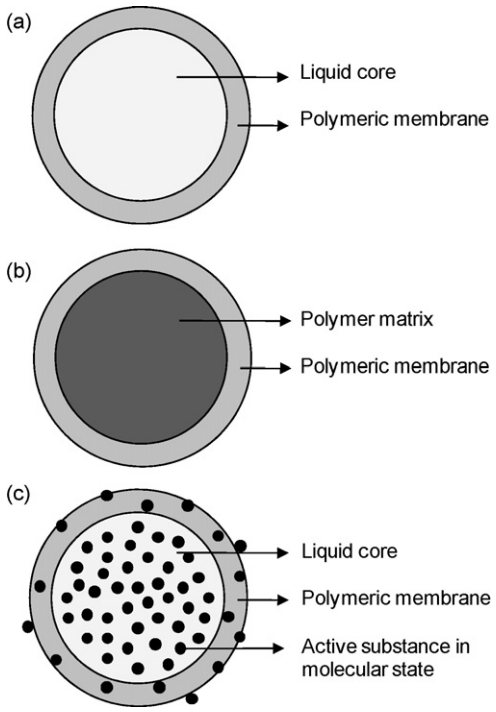

Fig. 1. Different nanocapsular structures: (a) liquid core, (b) polymer matrix and (c) active substance in molecular dispersion.

Regarding to the solvent emulsion–evaporation method, it has been used for the preparation of nanocapsules (Pisani et al., 2008). However, the latter research showed that several apparently different interfacial organizations coexist between the organic and aqueous phases at the same time within a single emulsion. Therefore the presence of compounds with high molecular weights, such as the polymers, can restrict solvent diffusion, which, when removed rapidly during the evaporation step, makes nanocapsule formation difficult.

Although Pisani et al. obtained preparation of nanocapsules by optimising the parameters of emulsion–evaporation process, according to Moinard-Chécot et al. (2008) this method is often performed using microencapsulation technology and is not recommended for nanoencapsulation. They suggest that the nanocapsules do not resist direct evaporation of the solvent, possibly due to the mechanical stress caused by the gas bubbles formed inside the aqueous suspension.

Thus, in agreement with the previous arguments, the emulsion–evaporation method is not currently recognized as feasible, thereby opening the path for other research works to provide options for nanocapsule synthesis.

On the other hand, regarding block copolymer-based vesicles, also called polymer-based liposomes or polymersomes, they appear to be promising for drug encapsulation because their double layer recalls the structure of lipids in membrane cells which could facilitate their biological performance and the design of targeted nanoparticles (Meng et al., 2005; Rodríguez-Hernández et al., 2005). They can be obtained from amphiphilic di-block, tri-block, graft or charged copolymers by means of self-assembled or covalently-assembled strategies. Among the copolymers used are PEG or PEO biodegradable derivatives, although researches has been developed using new materials as polypeptides and choles-

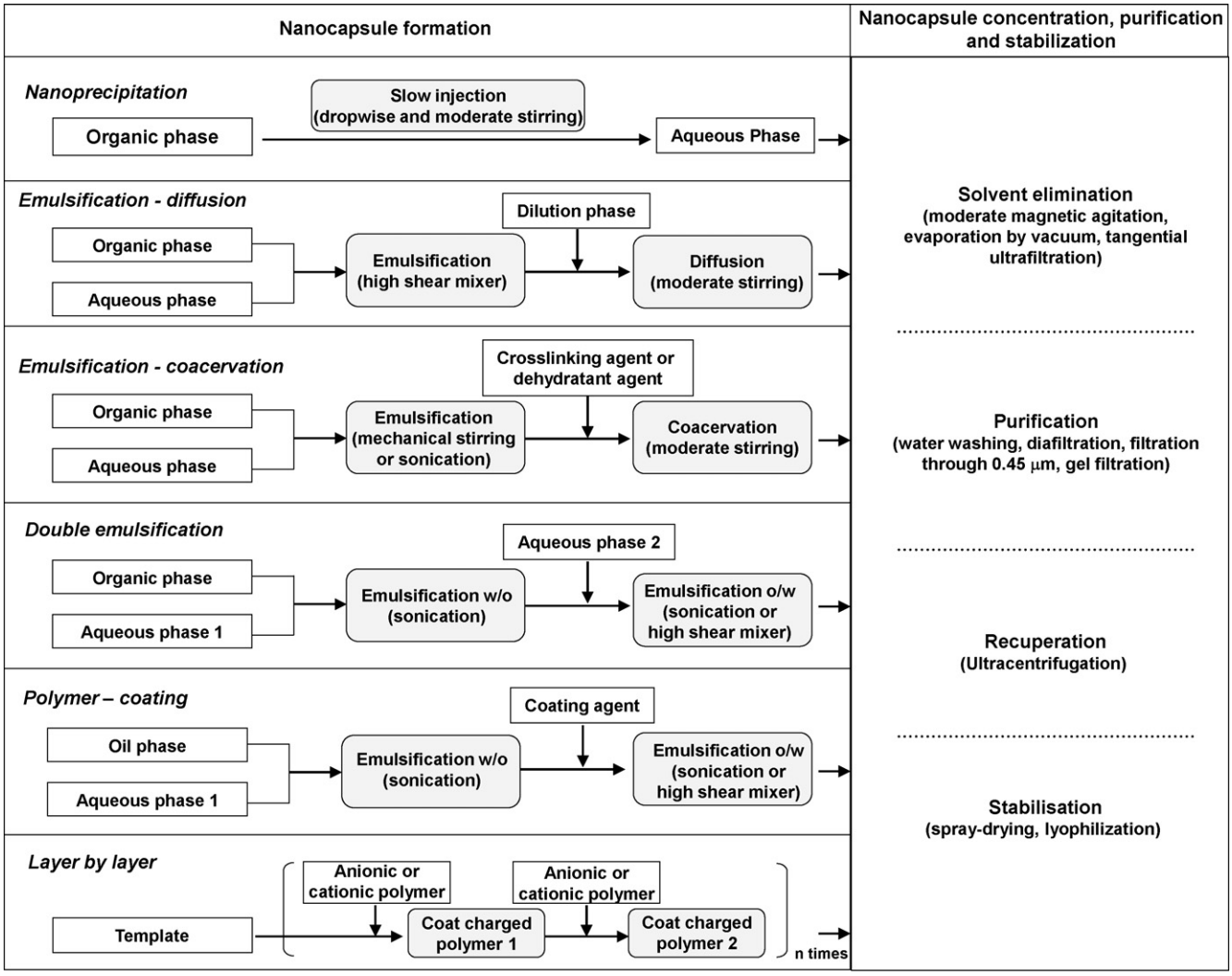

Fig. 2. General procedure of the different methods for the preparation of nanocapsules.

terol derivates (Chécot et al., 2003; Photos et al., 2003; Xu et al., 2005; Zhou et al., 2006).

Typically, the procedures for the polymersome preparation can be classified as solvent free and solvent displacement techniques. In the first method, the dried amphiphile polymer is brought in contact with the aqueous medium and then is hydrated to form vesicles. In the second method, the block copolymer is dissolved in organic solvents, then water is added and subsequently the organic solvent is eliminated. In order to reach monodisperse size distributions of the polymer vesicles, the obtained suspension can be treated by sonication, vortexing, extrusion or freeze-thaw cycles or a combination of these techniques (Kita-Tokarczyk et al., 2005). The cross-linking process of the block polymers allows optimizing the vesicular membrane properties associated with active substance protection and release effect (Chécot et al., 2003).

The encapsulation of active substances inside the polymer vesicles is obtained by incubation based techniques. The hydrophilic or lipophilic nature of the active molecule determines the choice of the polymersome core nature which in turn is obtained according to the block polymer chosen and to the assembly technique. Some examples of active substances encapsulated are mainly anticancer drugs as adriamycin (Xu et al., 2005), paclitaxel (Ahmed et al., 2006) and doxorubicin (Ahmed and Discher, 2004; Zheng et al., 2009), therapeutic proteins and antisense molecules for gene therapy (Christian et al., 2009; Kim et al., 2009).

Table 1
Suggested composition for preparation of nanocapsules by the nanoprecipitation method.

Material	Suggested composition
Active substance	10–25 mg
Polymer	0.2–0.5% of solvent
Oil	1.0–5.0% of solvent
w/o surfactant	0.2–0.5% of solvent
Solvent	25 ml
Stabilizer agent	0.2–0.5% of non-solvent
Non-solvent	50 ml

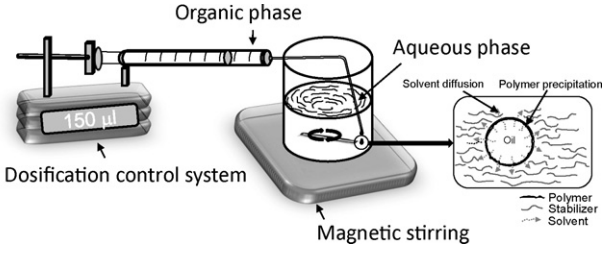

Fig. 3. Set-up used for preparation of nanocapsules by the nanoprecipitation method.

Table 2
Examples of raw materials used for preparation of nanocapsules by the nanoprecipitation method.

Active ingredient	Therapeutic activity	Polymer	Oil core	Solvent	Stabilizer agent	Non-solvent	Reference
Gemcitabine 4-(N)-stearoylgemcitabine 4-(N)-valeroylgemcitabine 4-(N)-lauroylgemcitabine	Antineoplastic	PACA or Poly[H_2NPEGCA-co-HDCA]	Caprylic/capric triglyceride	Acetone ethanol		Water	Stella et al. (2007)
		PLA[a]	Benzyl benzoate	Acetone	Poloxamer 188	Water	Fessi et al. (1989)
		PLA Mw 60 kDa PCL Mw 65 kDa PCL Mn 60 kDa	Phospholipids Capric/caprylic triglycerides Sorbitan monoestearate	Acetone	Polysorbate 80	Water	Fawaz et al. (1996) Pohlmann et al. (2008) Cattani et al. (2008)
Indomethacin	Anti-inflammatory, analgesic Selective cytotoxicity	PCL Mw 60 kDa or PLA[a]	Mineral oil Sorbitan monostearate	Acetone	Polysorbate 80	Water	Pohlmann et al. (2002)
		PCL Mw 40 kDa	Propylene glycol dicaprylate/dicaprate Lecithin	Acetone	Poloxamer 188 Chitosan	Water	Calvo et al. (1997)
		PCL Mw 40 kDa	Propylene glycol dicaprylate/dicaprate Lecithin	Acetone	Poloxamer 188	Water	Calvo et al. (1997)
Indomethacin ethyl ester	Anti-inflammatory, analgesic	PCL Mw 65 kDa	Capric/caprylic triglycerides Sorbitan monostearate	Acetone	Polysorbate 80	Water	Cruz et al. (2006) Cattani et al. (2008) Poletto et al. (2008a,b)
		PLA Mw 200 kDa, PCL Mw 65 or 100 kDa, PLGA Mw 40 kDa	Benzyl benzoate Soybean lecithine	Acetone	Poloxamer 188	Water	Cauchetier et al. (2003)
Atovaquone	Antipneumocystic		Capric/caprylic triglycerides Benzyl benzoate				
		PLA Mw 88 kDa	Caprylic/capric triglycerides PEG-4 complex Oleic acid Phospholipids Capric/caprylic triglycerides Benzyl benzoate	Acetone	Poloxamer 188	Water	Dalençon et al. (1997)
Rifabutine	Antibacterial (tuberculostatic)	PLA Mw 88 kDa	Caprylic/capric triglycerides PEG-4 complex Phospholipids	Acetone	Poloxamer 188	Water	Dalençon et al. (1997)
Tretinoin	Topical treatment of different skin diseases (acne vulgaris, ichtiosys, psoriasis), antineoplastic (hormonal)	PCL[a]	Capric/caprylic triglycerides Sunflower seed oil. Sorbitan monooleate	Acetone	Polysorbate 80	Water	Ourique et al. (2008)
Fluconazole labeled with ^{99m}Technetium	Antifungal	PLA Mw 75 kDa or PLA–PEG (90% PLA Mw 49 kDa–10% PEG Mw 5 kDa)	Capryc/caprylic triglycerides Soybean lecithin	Methanol Acetone	Poloxamer 188	Water	Nogueira de Assis et al. (2008)
Primidone	Anticonvulsant	PCL Mw 64 kDa	Benzyl alcohol	Acetone	Poloxamer 188	Water	Ferranti et al. (1999)
Vitamin E	Vitamin antioxidant	PCL Mn 10 kDa		Acetone	Polysorbate 20	Water	Charcosset and Fessi (2005)
Spironolactone	Diuretic	PCL Mw 10 and 80 kDa	Caprylic/capric triglycerides PEG-4 complex Sorbitan monooleate Sorbitan monolaurate	Acetone	Poloxamer 188 Polysorbate 80 Polysorbate 20	Water	Limayem et al. (2006)
Griseofulvine	Antifungal	PCL Mw 80 kDa	Benzyl benzoate Sorbitan monooleate	Acetone	Polysorbate 80	Water	Zili et al. (2005)
^{99m}Tc-HMPAO complex	Radiotracer	PLA MW/5 kDa or PLA–PLG (90% PLA Mw 49 kDa–10% PEG Mw 5 kDa)	Capric/caprylic triglycerides Soybean lecithin	Acetone	Poloxamer 188	Water	Pereira et al. (2008)
Melatonin	Antioxidant	Eudragit S100	Capric/caprylic triglyceride Sorbitan monooleate	Acetone	Polysorbate 80	Water	Schaffazick et al. (2008)

Diclofenac	Anti-inflammatory	PCL Mw 80 or Eudragit S90	Capric/caprylic triglyceride Benzyl benzoate Sorbitan monostearate	Acetone	Polysorbate 80	Water	Schaffazick et al. (2003)
Diclofenac	Anti-inflammatory	PLA Mw 88 kDa	Benzyl benzoate Capric/caprylic triglyceride Phospholipids	Acetone	Poloxamer 188	Water	Guterres et al. (1995)
Benzathine penicillin G	An bacterial	PLGA 50/50[a]	Sunflower oil Soybean oil Capric/caprylic triglyceride Benzyl benzoate Soy phosphatidylcholine	Acetone	Poloxamer 188	Phosphate buffer solution (pH 7.4)	Santos-Magalhães et al. (2000)
Xanthone 3-methoxyxanthone	Antiinflamatory Antitumoral	PLGA 50/50 Mw 50–75PLA	Soybean lecithine Capric/caprylic acid trygliceride	Acetone	Poloxamer 188	Water	Texeira et al. (2005)
Usnic acid	Antineoplastic	PLGA 50/50[a]	Soybean oil Soy phosphatidylcholine	Acetone	Poloxamer 188 Trehalose	Phosphate buffer solution (pH 7.4)	Pereira et al. (2006)
Tacrolimus	Immunosuppressant	Eudragit RS or Eudragit L100-55	Argan oil Oleoyl polyoxyglycerides	Acetone Absolute ethanol		Water	Nassar et al. (2009)
RU58668	Antiestrogen	PLA Mw 42 kDa PLGA Mw 75 kDa PCL Mw 40 kDa PLA–PEG (45–5 and 45–20 kDa) PLGA-PEG (45–5 kDa) PCL-PEG (40–5 kDa)	Capric/caprylic triglycerides Soy phosphatidylcholine	Acetone	Poloxamer 188	Water	Ameller et al. (2003)
Muramyltripeptide cholesterol (MTP-Chol)	Immunomodulator	PLA Mw 100 kDa	Soybean lecithin Ethyl oleate	Acetone	Poloxamer 188	Water	Seyler et al. (1999)
Benzazole dyes. O-aminophenol 1,2-phenylenediamine 5-aminosalicylic acid 4-aminosalicylic acid		Poly(N-acryloylamide) or Poly(vinylene) or Poly(methyl methacrylate)	Capric/caprylic triglyceride Sorbitan mo n oestea rate	Acetone	Polysorbate 80	Water	Jäger et al. (2007)
		PCL Mn 42.5 kDa	Capric/caprylic triglycerides Sorbitan monostearate	Acetone	Polysorbate 80	Water	Tewa-Tagne et al. (2006) Tewa-Tagne et al. (2007a)
		PCL Mn 42.5 kDa	Capric/caprylic triglycerides Sorbitan monostearate	Acetone	Polysorbate 80	Water	Tewa-Tagne et al. (2007b)
		PLA Mw 42 kDa, PLGA 75/25 Mw 75–120 kDa, PCL Mw 42.5 kDa, PLA–PEG 45-5 kDa, PLGA-PEG 45–20 kDa or PCL-PEG 45-5 kDa.	Capric/caprylic triglycerides Lecithin	Acetone	Poloxamer 188	Water	Furtado et al. (2001a,b)
		PLA Mw 9 kDa	Capric/caprylic triglycerides	Acetone	Poloxamer 188	Water	Rübe et al. (2005)

PACA: poly(alkylcyanoacrylate) derivate; [poly(H2NPEGCA-co-HDCA]: poly[aminopoly(ethylene glycol)cyanoacrylate-co-hexadecyl cyanoacrylate]; PLA: poly(lactide); PCL: poly(e-caprolactone); PLGA: poly(lactide-co-glycolide); PEG: poly(ethylene glycol); HPMC: hydroxypropylmethylcellulose; HPC: hydroxypropylcellulose; PVP: polyvinyl pyrrolidone.

[a] Molecular weight (Mw) non-specified.

In the current review polymer vesicles are not included though active substances have been encapsulated and polymersomes promising to be versatile nanocarriers. They are considered as new polymer therapeutics with profitable and triggered biopharmaceutic behaviours, which are more comparable with liposomal systems (Batrakova et al., 2006; Betancourt et al., 2007).

In what follows, a general review is provided of the methodologies, raw materials and mechanistic fundamentals of each classical method for the preparation of nanocapsules. Furthermore, considerations on aspects regarding the purification, concentration and stabilization of nanoencapsulated systems will be given.

3.1. Nanoprecipitation method

The nanoprecipitation method is also called solvent displacement or interfacial deposition. According to Fessi et al. (1988), the nanocapsule synthesis needs both solvent and non-solvent phases. The solvent phase essentially consisting of a solution in a solvent or in a mixture of solvents (i.e. ethanol, acetone, hexane, methylene chloride or dioxane) of a film-forming substance such as a polymer (synthetic, semi-synthetic or naturally occurring polymer), the active substance, oil, a lipophilic tensioactive and an active substance solvent or oil solvent if these are needed. On the other hand, the non-solvent phase consisting of a non-solvent or a mixture of non-solvents for the film-forming substance, supplemented with one or more naturally occurring or synthetic surfactants.

In most cases, the solvent and non-solvent phases are called organic and aqueous phases, respectively. As a general tendency, the solvent is an organic medium, while the non-solvent is mainly water. However, it is possible to use either two organic phases or two aqueous phases as long as solubility, insolubility and miscibility conditions are satisfied.

A composition base for 150–200 nm preparation of nanocapsules at laboratory-scale using the nanoprecipitation method is shown in Table 1. Likewise, Table 2 shows different examples of solvents, non-solvents, polymers, oils, surfactants and stabilizer agents used in this method. As it can be seen, although an extensive range of raw materials (Devissaguet et al., 1991) can be used in theory, in practice research has been performed with only a limited number of them.

The polymers commonly used are biodegradable polyesters, especially poly-e-caprolactone (PCL), poly(lactide) (PLA) and poly(lactide-co-glicolide) (PLGA). Eudragit can also be used as may other polymers such as poly(alkylcyanoacrylate) (PACA). Synthetic polymers have higher purity and better reproducibility than natural polymers (Khoee and Yaghoobian, 2008). On the other hand, some polymers are PEG copolymerized in order to decrease nanocapsule recognition by the mononuclear phagocyte system (Nogueira de Assis et al., 2008).

Besides the lipophilic active substance, the nanocapsule core is composed by a w/o surfactant and oil chosen having as criterion the highest possible drug solubility, absence of toxicity, low solubility of oil in the polymer and vice-versa, and the absence of risk of polymer degradation (Limayem et al., 2006). It is emphasized that the different capric/caprylic triglyceride types are often used because of their wide range of solubility for active substances. Although other oils such as benzyl benzoate, benzyl alcohol, oleic acid, ethyl oleate, argan oil, sunflower seed oil and soybean oil have not been used frequently, they can nonetheless give good results. Regarding w/o surfactants, sorbitan esters and phospholipids are preferred.

Regarding the polymer solvent, acetone is chosen in all cases. Other solvents such as ethanol are used in order for active substance or oil dissolution. Water or buffer solutions can be used as the non-solvent while the stabilizer agent is poloxamer 188 or polysorbate 80.

Table 3
Suggested composition for preparation of nanocapsules by emulsion–diffusion method.

Material	Suggested composition
Active substance	10–50 mg
Polymer	1.0–2.0% of inner phase solvent
Oil	2.5–5.0% of inner phase solvent
Inner phase solvent	10 ml
Stabilizer agent	2.0–5.0% of external phase solvent
External phase solvent	40 ml
Dilution phase	200 ml

In the nanoprecipitation method, the nanocapsules are obtained as a colloidal suspension formed when the organic phase is added slowly and with moderate stirring to the aqueous phase (Fig. 3). The key variables of the procedure are those associated with the conditions of adding the organic phase to the aqueous phase, such as organic phase injection rate, aqueous phase agitation rate, the method of organic phase addition and the organic phase/aqueous phase ratio. Likewise, nanocapsule characteristics are influenced by the nature and concentration of their components (Plasari et al., 1997; Chorny et al., 2002; Legrand et al., 2007; Lince et al., 2008).

Although disagreement exists regarding the mechanism of nanocapsule formation using this technique, research into polymer precipitation (Lince et al., 2008) and solvent diffusion (Quintanar et al., 1998a) have proved useful in this regard.

On the basis of Sugimoto's theory on polymer precipitation (Sugimoto, 1987), Lince et al. (2008) indicated that the process of particle formation in the nanoprecipitation method comprises three stages: nucleation, growth and aggregation. The rate of each step determines the particle size and the driving force of these phenomena is supersaturation, which is defined as the ratio of polymer concentration over the solubility of the polymer in the solvent mixture. The separation between the nucleation and the growth stages is the key factor for uniform particle formation. Ideally, operating conditions should allow a high nucleation rate strongly dependent on supersaturation and low growth rate.

On the other hand, in line with the research carried out by Davies on mass transfer between two liquids and the Gibbs–Marangoni effect (McManamey et al., 1973; Davies, 1975), Quintanar et al. explained rapid nanoparticle formation as a process due to differences in surface tension. Since 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 causes interfacial turbulence and thermal inequalities in the system, leading to the continuous formation of eddies of solvent at the interface of both liquids. Consequently, violent spreading is observed due to mutual miscibility between the solvents, the solvent flows away from regions of low surface tension and the polymer tends to aggregate on the oil surface and forms nanocapsules. According to this explanation, nanocapsule formation is due to polymer aggregation in stabilized emulsion droplets, while apparently the nucleation and growth steps are not involved.

3.2. Emulsion–diffusion method

According to Quintanar et al. (1998b, 2005), preparation of nanocapsules by the emulsion–diffusion method allows both lipophilic and hydrophilic active substance nanoencapsulation. The experimental procedure performed to achieve this requires three phases: organic, aqueous and dilution.

When the objective is the nanoencapsulation of a lipophilic active substance, the organic phase contains the polymer, the active substance, oil and an organic solvent partially miscible with water, which should be water-satured. This organic medium acts

Table 4
Examples of raw materials used for preparation of nanocapsules by the emulsification – diffusion method – oil core.

Active ingredient	Therapeutic activity	Inner phase (active ingredient + polymer + core + solvent 1)			External phase		Dilution phase	Reference
		Polymer	Core	Solvent 1	Stabilizer agent	Solvent 2		
		PCL Mw 80 kDa	Capric/caprylic triglyceride	Ethyl acetate	PVA Poloxamer 188	Water	Water	Guinebretière et al. (2002)
Indomethacine	Anti-inflamatory	PCL Mw 10 and 80 kDa	Capric/caprylic triglyceride	Ethyl acetate	PVA	Water	Water	Limayem et al. (2004)
	Analgesic	PLA[a] Eudragit E	Capric/caprylic triglyceride	Ethyl acetate, propylene carbonate or benzyl alcohol	PVA	Water	Water	Quintanar et al. (1998b)
Progesterone Estradiol	Progestogen Estrogen							
ChlorambucilClofibrateVitamin E	Antineoplastic Antilipemic Vitamin antioxidant	PLA[a] Eudragit E	Capric/caprylic triglyceride	Ethyl acetate, propylene carbonate or benzyl alcohol	PVA	Water	Water	Quintanar et al. (1998b)
Eugenol	Analgesic	PCL Mw 80 kDa		Ethyl acetate	Poloxamer 188	Water	Water	Choi et al. (2009)
Hinokitiol	Antibacterial	PCL Mw 40–60 kDa	Octyl salicylate	Ethyl acetate	SLS or CTAC or CTAC:gelatin	Water	Water	Joo et al. (2008)
4-Nitroanisole		PLA 70:30 Mw 1500 kDa	Hexane	DCM Acetone	PVA	Water	PVA aqueous solution	Romero-Cano and Vincent (2002)
Sudan III		PLA[a] Eudragit E PCL[a]	Capric/caprylic triglycerides	Ethyl acetate, propylene carbonate or benzyl alcohol	PVA	Water	Water	Quintanar et al. (1998b)
		PCL Mw 80 kDa	Capric/caprylic triglyceride	Ethyl acetate	PVA	Water	Water	Moinard-Chécot et al. (2008)
		PHBHV Mw 23 or 300 kDa	Caprylic/capric triglyceride or mineral oil	Chloroform:ethanol	PVA	Water	PVA aqueous solution	Poletto et al. (2008a,b)
		PCL Mw 14 kDa	Capric/caprylic triglycerides	Ethyl acetate	PVA	Water		Abdelwahed et al. (2006a,b,c)

PLA: poly(lactide); PCL: poly(e-caprolactone); PHBHV: poly(hydroxybutyrate-co-hydroxyvalerate); DCM: dichloromethane; PVA: poly(vinyl alcohol); SLS: sodium lauryl sulfate; CTAC: cetyltrimethylammonium chloride; PVP: polyvinyl pyrrolidone.

[a] Molecular weight (Mw) non-specified.

as solvent for the different components of the organic phase. If it is required, the organic phase can also include an active substance solvent or oil solvent. The aqueous phase comprises the aqueous dispersion of a stabilizing agent that is prepared using solvent-saturated water while the dilution phase is usually water.

A prototype composition for preparation of nanocapsules at laboratory-scale using the emulsion–diffusion method is shown in Table 3 (nanocapsule size: approximately 150–200 nm). Likewise, Table 4 shows different examples of polymers, oils, inner phase solvent, stabilizer agent, external phase solvent and dilution phase used in nanoencapsulation research with this method. As with the nanoprecipitation method, although an extensive range of raw materials can be used in theory (Quintanar et al., 2005), research has been performed with a only limited number of them in practice.

As can be observed, the polymers commonly used are biodegradable polyesters, especially PCL, PLA and eudragit. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBHV) may also be used. The inner phase contains the oil in addition to the active substance and solvent. In line with what has been mentioned previously about nanoprecipitation method, also different capric/caprylic triglyceride types are frequently used. Regarding the solvents, ethyl acetate is the first option, though propylene carbonate, benzyl alcohol and dichloromethane can also be chosen.

In regarding to the external phase, the solvent used is water and poly(vinyl alcohol) (PVA) is preferred as the stabilizing agent. Other stabilizing agents such as poloxamer and ionic emulsifiers have been used. The dilution phase is often water; nevertheless, in order to obtain better nanodispersion stability stabilizer agents may be used in diluted solutions.

For preparation of nanocapsules using the emulsion–diffusion method, the organic phase is emulsified under vigorous agitation in the aqueous phase (Fig. 4). The subsequent addition of water to the system causes the diffusion of the solvent into the external phase, resulting in nanocapsule formation. This can be eliminated by distillation or cross-flow filtration depending on the boiling point of the solvent. It has been shown that nanocapsule size is related to the shear rate used in the emulsification process, the chemical composition of the organic phase, the polymer concentration, the oil-to-polymer ratio and the drop size of the primary emulsion (Guinebretière, 2001; Moinard-Chécot et al., 2008).

The nanocapsule formation mechanism suggested by Quintanar et al. (1998a) is based on the theory that each emulsion droplet produces several nanocapsules and that these are formed by the combination of polymer precipitation and interfacial phenomena during solvent diffusion. Consequently, solvent diffusion from the globules carries molecules into the aqueous phase forming local regions of supersaturation from which new globules or polymer aggregates (not totally desolvated) are formed and stabilized by the stabilizer agent which prevents their coalescence and the formation of agglomerates. Then, if the stabilizer remains at the liquid–liquid interface during the diffusion process and if its protective effect is adequate, the nanocapsules will be formed after the complete diffusion of the solvent.

Guinebretière et al. (2002) demonstrated that mean nanocapsule size is always smaller than that of the emulsion droplets, in agreement with the diffusion theory proposed by Quintanar. In this sense, nanocapsule formation is a dynamic process associated with the diffusion of the solvent from the droplet to the external phase caused by the addition of water to the emulsion and resulting in the transformation of each droplet into a particle of smaller size.

In order to better understand nanocapsule formation, Hassou (2007) and Moinard-Chécot et al. (2008) had modeled the different intermediate states that take place during solvent diffusion at the dilution stage, by a step-by-step diffusion study and determined its duration by using the stopped-flow technique. According to these researches, diffusion of the solvent from the droplets takes place too fast (duration less than 20 ms) and as a continuous process. There are no discontinuities that reveal a transition from homogeneous droplets to heterogeneous nanocapsules.

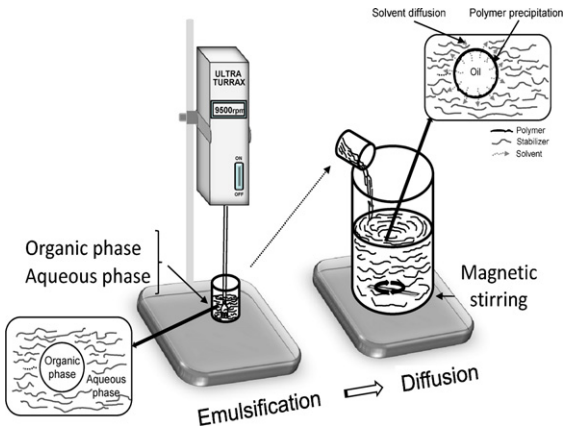

Fig. 4. Set-up used for preparation of nanocapsules by the emulsion–diffusion method.

Perez et al. (2001) and Ma et al. (2001) have modified the process proposed by Quintanar et al. (2005) in order to nanoencapsulate hydrophilic active substances. In this case, a stabilizer agent such as PVA or poly(vinylpirrolidone) (PVP) is present in the aqueous inner phase in addition to the active substance (Table 5), while the external phase is composed of the polymer and an organic solvent (methylene chloride or acetone). The dilution of the emulsion is made first by solvent addition (ethanol) which leads to organic solvent migration. Then, water addition is made in order to facilitate the collection of the particles. The aqueous dilution phase may or may not include a stabilizer agent.

3.3. Double emulsification method

Double emulsions are complex heterodisperse systems called "emulsions of emulsions", that can be classified into two major types: water-oil-water emulsion (w/o/w) and oil-water-oil emulsion (o/w/o) (Garti, 1997; Grigoriev and Miller, 2009). Thus the dispersed phase is itself an emulsion and the inner dispersed globule/droplet is separated from the outer liquid phase by a layer of another phase. Double emulsions are usually prepared in a two-step emulsification process using two surfactants: a hydrophobic one designed to stabilize the interface of the w/o internal emulsion and a hydrophilic one to stabilize the external interface of the oil globules for w/o/w emulsions.

For preparation of nanocapsules, the principle of double emulsion formation, specifically of the w/o/w type, is associated with the principles of both nanoprecipitation and emulsion–diffusion methods. In this case, in the primary w/o emulsion the oil is changed by an organic phase containing a solvent that is totally or partially miscible in water, the film-formed polymer and a w/o surfactant. Then the water containing a stabilizing agent is added to the system to obtain the water in organic in water emulsion. However in this step, particle hardening is obtained through solvent diffusion and polymer precipitation (Bilati et al., 2005c; Khoee and Yaghoobian, 2008). Water is frequently added to the double emulsion in order to achieve full solvent diffusion.

According to Khoee and Yaghoobian (2008), surfactants play a dual role in emulsions: as a film former and a barrier to drug release at the internal interface, and as a steric stabilizer on the

Table 5
Examples of raw materials used for preparation of nanocapsules by the emulsion–diffusion method—aqueous core.

Active ingredient	Therapeutic activity	Inner phase		External phase		Dilution phase	Reference
		Core	Solvent 1	Polymer	Solvent 2		
Plasmid DNA plasmid DNA–PVA plasmid DNA–PVP	Gene therapy	Active ingredient PVA or PVP (stabilizer agent)	Water	PLA–PEG46–5 kDa	Methylene chloride	Ethanol Water	Perez et al. (2001)
Insulin	Antidiabetic	Active ingredient	Hydrochloric acid	PLA–PEG–PLA copolymers (PLA from 2 to 45 kDa; PEG variable PLA (Mn 32 kDa) in glycerol trioleate	Acetone	Polysorbate 20, dextrin and water	Ma et al. (2001)

DNA: deoxyribonucleic acid; PLA: poly(lactide); PVA: poly(vinyl alcohol); PEG: poly(ethylene glycol); PVP: polyvinyl pyrrolidone.

Table 6
Suggested composition for preparation of nanocapsules by the double emulsification method.

Material	Suggested composition
Inner aqueous phase	
Active substance	Variable (0.5–25 mg)
Water	0.15–0.5 ml
Organic phase	
Polymer	5–10% of organic phase solvent
w/o surfactant	5–7% of organic phase solvent
Solvent	1.5–5 ml
External aqueous phase	
Stabilizer agent	1–5% of external aqueous phase solvent
Water	2–5 ml
Dilution phase (optional)	
Stabilizer agent	1–5% of dilution phase solvent
Water	50–100 ml

external interface. It was found that drug encapsulation efficiency and average particle size are affected by changing the type and concentration of both the w/o emulsion and the stabilizing agent.

A composition base for preparation of nanocapsules at laboratory-scale by the double emulsification method (size about 150–200 nm) is provided in Table 6.

As can be seen in Table 7, at present, the inner aqueous phase is composed only for the active substance, in some cases forming complexes, and water. In the organic phase, ethyl acetate, methylene chloride and dichloromethane have been used as solvents and biodegradable polyesters, such as PCL, PLA and PLGA have been frequently used. Regarding o/w surfactants, sorbitan esters are preferred.

Regarding the external aqueous phase, the stabilizing agents most frequently used are PVA and polysorbates. To contribute to nanocapsule dispersion, the same external aqueous phase composition is used for the dilution phase if the procedure used involves a final dilution stage.

In a typical procedure for preparation of nanocapsules by double emulsification, the primary emulsion is formed by ultrasound and the w/o surfactant stabilizes the interface of the w/o internal emulsion (Fig. 5). The second emulsion is also formed by ultrasound and nanocapsule dispersion is stabilized by the addition of the stabilizing agent. Finally, the solvents are removed by evaporation or extraction by vacuum, leaving hardened nanocapsules in an aqueous medium. As mentioned previously, as an optional step, nanocapsule dispersion can be diluted before extraction under vacuum to ensure full solvent diffusion.

On the other hand, Bilati et al. (2005a) (Table 8), showed that it is possible to obtain solid-organic–water systems by following the same method.

3.4. Emulsion-coacervation method

The emulsion-coacervation process is mainly presented as a strategy for nanocapsules preparation from naturally occurring polymeric materials. Up to now, sodium alginate and gelatin have been used though synthetic polymeric materials could be used for this purpose.

The procedure involves the o/w emulsification of an organic phase (oil, active substance and active substance solvent if necessary) with an aqueous phase (water, polymer, stabilizing agent) by mechanical stirring or ultrasound. Then, a simple coacervation process is performed by using either electrolytes as done by Lertsutthiwong et al. (2008a,b) with a sodium alginate–calcium chloride system, by the addition of a water miscible non-solvent or a dehydration agent as done by Krause and Rohdewald (1985) with a gelatin–isopropanol–sodium sulfate system or by temperature

Table 7
Examples of raw materials used for preparation of nanocapsules by the double emulsification method—liquid core.

Active ingredient	Therapeutic activity	W1 phase	Organic phase	W2 phase	Reference
Insulin	Antidiabetic	Active ingredient Water	PLA Mw 10 kDa DCM Sorbitan monooleate Sorbitan monostearate Sorbitan monolaurate	Polysorbate 80, 60 or 20 glycerin:water (1:1)	Zhu et al. (2005)
		Protein–SLS 0.1 M HCl solution	PLA Mw 28 kDa or PLGA 50/50 Mw 34 kDa Ethyl acetate or methylene chloride	PVA Water	Bilati et al. (2005a)
Ciprofloxacin.HCl	Antibacterial	Active ingredient Water	PLGA[a] DCM	PVA Water	Jeong et al. (2008)
Bovine serum albumin	Protein	Protein Water	PLA Mw 16 and 51 kDa or PCL–PEO block copolymer 60/40 Mw 79 Kd Sorbitan monooleate Methylene chloride	Polysorbate 80 glycerin:water (1:1)	Lu et al. (1999)
		Protein Water	PLGA Mw 40 kDa or PCL Mw 42 kDa Methylene choride	PVA Water	Lamprecht et al. (2000)
Penicillin G	Antibacterial	Active ingredient Water	PBA Mw 10 kDa Sorbitan esters 60 or 20. DCM	Polysorbate 60 or 20. glycerin:water (1:1)	Khoee and Yaghoobian (2008)
Plasmid DNA Plasmid DNA–PVAPlasmid DNA–PVP	Gene therapy	Active ingredient PVP or PVA Water	PLA 46 kDa–PEG 5 kDa Ethyl acetate:methylene chloride (1:1)	PVA Water	Perez et al. (2001)
Tetanus toxoid Lysozyme	Antigen Mucolytic enzyme Antiviral	Protein Water Protein–sodium oleate Water	PLA Mw 28 kDa or PLGA Mw 34 kDa Ethyl acetate or methylene chloride	PVA Water	Bilati et al. (2005a)

DNA: deoxyribonucleic acid; PVA: poly(vinyl alcohol); PVP: polyvinyl pyrrolidone; SLS: sodium lauryl sulfate; HCl: hydrochloric acid; PLA: poly(lactide); DCM: dichloromethane; PLGA: poly(lactide-co-glycolide); PEG: poly(ethylene glycol); PCL: poly(e-caprolactone); PEO: poly(ethylene oxide); PBA: polybutyl adipate.

[a] Molecular weight (Mw) non-specified.

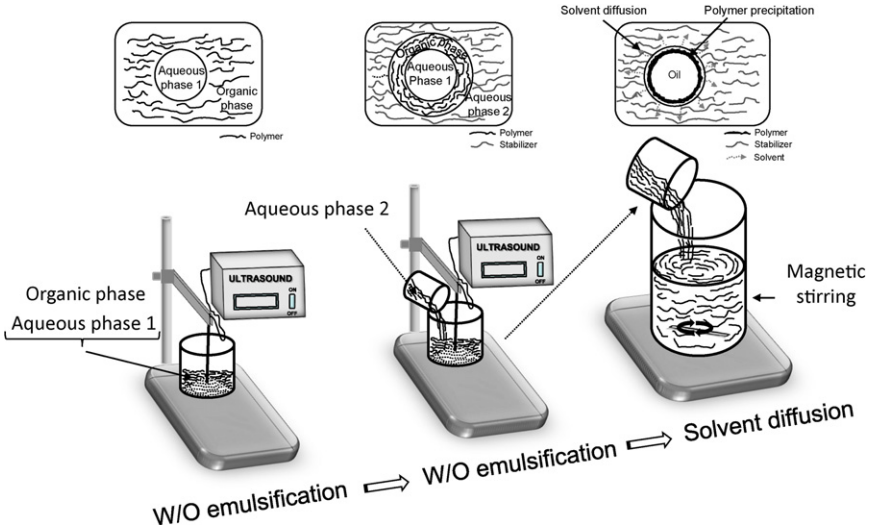

Fig. 5. Set-up used for preparation of nanocapsules by the double emulsification method.

modification as done by Lutter et al. (2008) with the application of triblock terpolymer in gold nanocapsule synthesis. Finally, the coacervation process is complemented with additional cross-linked steps that make it possible to obtain a rigid nanocapsule shell structure (Fig. 6).

Nanocapsule formation by the emulsion-coacervation method uses the emulsion as a template phase and the formation of a coacervate phase that causes polymer precipitation from the continuous emulsion-phase to form a film on the template forming the nanocapsule. Additionally, it can be stabilized by physical intermolecular or covalent cross-linking, which typically can be achieved by altering pH or temperature, or by adding a cross-linking agent.

Probably the critical stage in preparation of nanocapsules by the emulsion-coacervation method is coacervate phase formation. As explained by Gander et al. (2002), the polymer dissolved in water is enclosed by water molecules that solvate its functional groups, typically through hydrogen-bonding and van der Waals forces that prevent attraction among chain segments in close proximity by interchain H-bonds, or van der Waals or opposing ionic forces. Thus, the coacervating agents lower the solvation of dissolved polymers and induce thin solvated shell. It may also allow the attraction among contiguous chains via secondary valence bonds to form an entangled network or even non-covalent weak cross-links as the polymer concentration gradually increases in the coacervated phase.

The use of electrolytes for polymer desolvation is known as salting-out and the electrolytic efficiency for this process follows the Hofmeister or lyotropic series, which arranges ions in increasing order according to their capacity to immobilize water molecules in solvation in the ternary polymer–water–salt system. A practice demonstration of polymer coacervation behaviour according to the lyotropic series was performed by Yin et al. (2008) in their work on konjac glucomannan.

On the other hand, in the case where a dehydrating agent is used, the ternary system formed (polymer – dehydrating agent – water) allows the increase of polymer concentration due to solvent–solvation competition process. This results in the desolvation of the polymer chains, leading to phase separation.

Regarding the use of temperature changes to trigger polymer precipitation, it is essential to bear in mind the theories of Flory and Huggins on the interaction of parameter χ, which predicts that a polymer will dissolve in a solvent only if the interaction parameter is lower than a critical value χ_c, which, at a given temperature, depends on the degree of polymerization of the polymer.

Although electrolytes, dehydration and temperature modification are frequently used to reduce polymer solvation, other factors such as changing pH and adding other materials that are incompatible with the polymer solution can also be used.

Table 9 gives a non-exhaustive list of different raw materials used in research using emulsion-coacervation for preparation of nanocapsules. It is noteworthy that research conducted by Lutter et al. (2008) which, contrary to work done elsewhere, used the principle of emulsion-coacervation to prepare aqueous core nanocapsules.

Taking into account the limited amount of research and particularly the different methodological strategies followed by each team, it appears premature to establish general criteria regarding the materials and compositions that can be employed.

3.5. Polymer-coating method

References on the use of the polymer-coating method for preparation of nanocapsules are provided in Table 10. As can be seen, different methodological strategies can be used to deposit a thin layer of polymer on the nanoparticle surface. This can be achieved by adsorbing the polymer onto the preformed uncoated

Table 8
Examples of raw materials used for preparation of nanocapsules by the double emulsification method—solid core.

Active ingredient	Therapeutic activity	S phase	Organic phase	W phase	Reference
Tetanus toxoid Lysozyme Insulin	Antigen Mucolytic enzyme Antiviral Antidiabetic	Protein Protein–sodium oleato Protein–SLS	PLA Mw 28 kDa or PLGA Mw 34 kDa Ethyl acetate or methylene chloride	PVA Water	Bilati et al. (2005a)

Mw: molecular weight; SLS: sodium lauryl sulfate; PLA: poly(lactide); PLGA: poly(lactide-co-glycolide); PVA: poly(vinyl alcohol).

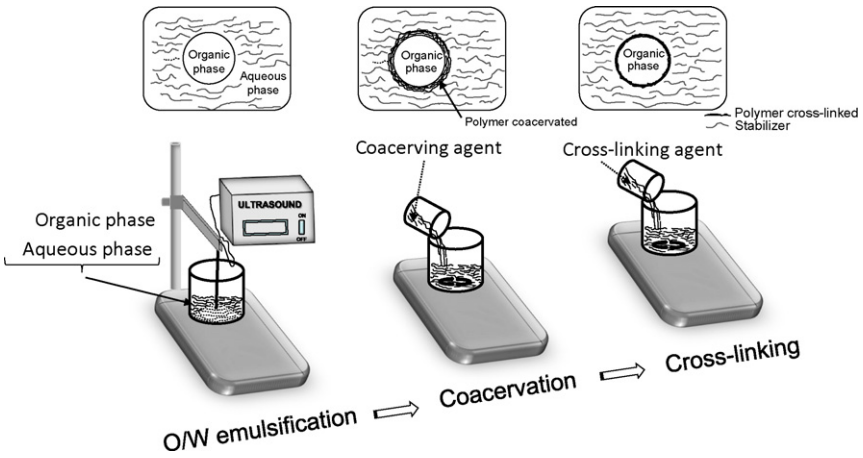

Fig. 6. Set-up used for preparation of nanocapsules by the emulsion-coacervation method.

nanocapsules when the latter are incubated in polymer dispersion under predetermined stirring and time conditions (Calvo et al., 1997).

Likewise, layer-formed polymer can be added during the final stage of conventional methods for the preparation of nanocapsules such as nanoprecipitation and double emulsification. Thus, these methods have been modified in order to add a layer of polymer to the external aqueous medium and allow to simultaneous layer formation due to the precipitation of the charged polymer (mainly negatively in nature) and to the diffusion of the solvent (Calvo et al., 1997; Vila et al., 2002).

On the other hand, Prego et al. (2006) propose a polymer-coating method in which the first step is to prepare the nanoemulsion template and then coat it by polymer deposition on the water/oil nanoemulsion surface. The polymers are added in the continuous phase and their precipitation onto the nanoemulsion droplets is triggered by solvent evaporation, as opposed to the emulsion-coacervation method.

Prego et al. (2006) have encapsulated salmon calcitonin using chitosan and PEG chitosan. In their procedure (Fig. 7), they start from an organic phase composed of the active substance, oil, surfactant (lecithin) and acetone as solvent; an aqueous phase containing the stabilizing agent and an aqueous polymer-coating solution. The organic and aqueous phases are mixed under moderate stirring and the o/w nanoemulsion is formed by solvent displacement. The solvents are subsequently evaporated under vacuum until reaching a specific volume and the nanoemulsion is finally coated by the polymer by simple incubation in the polymer solution.

The nanocapsule formation mechanism is mediated by the ionic interaction between the negatively charged phospholipids and the positively charged chitosan molecules. As established by Prego et al. (2006), the use of high lecithin concentrations affects the amount of chitosan associated with the surface of the nanocapsules while the chain length of chitosan molecules determines nanocapsule size.

Likewise, Anton et al. (2008) report a method used by Paiphansiri et al., based on the formation by sonication of a w/o nanoemulsion followed by coating with a solution composed of polymer and dichloromethane gradually added in the continuous organic phase of the nanoemulsion. The layer-formed polymers used by them are poly(methyl methacrylate) (PMMA), poly(methacrylate) (PMA) and PCL. Nanocapsule formation is based on the mechanism of engulfment in three-phase systems (Torza and Mason, 1970). When two drops of liquids miscible with each other are brought together in a third liquid phase that forms a film between them, the third phase drains until a hole suddenly forms in the same way as when two identical drops coalesce to form one drop. Since one of the drops comprises the polymer, when the two drops fuse a third interface is formed at the expanding hole and engulfment occurs via a combination of simultaneous penetration processes driven by the difference of capillary pressure between the two drops and the spreading of the polymer phase over the aqueous phase. Thus, when the solvent is finally evaporated, the polymer precipitates onto the nanoemulsion water droplets to form the nanocapsules.

As in the emulsion-coacervation method, taking into account the limited amount of research and their different methodological strategies, it is premature to establish general criteria for the materials and compositions that could be employed.

3.6. Layer-by-layer method

The layer-by-layer assembly process developed by Sukhorukov et al. (1998) for colloidal particle preparation makes it possible to obtain vesicular particles, called polyelectrolyte capsules, with well-defined chemical and structural properties. To sum up, the mechanism of nanocapsule formation is based on irreversible electrostatic attraction that leads to polyelectrolyte adsorption at supersaturating bulk polyelectrolyte concentrations.

This method requires a colloidal template onto which is adsorbed a polymer layer either by incubation in the polymer solution, subsequently washed, or by decreasing polymer solubility by drop-wise addition of a miscible solvent (Radtchenko et al., 2002a). This procedure is then repeated with a second polymer and multiple polymer layers are deposited sequentially, one after another.

As shown in Tables 11 and 12, the solid form of the active substance can be used as a template (Chen et al., 2009; Agarwal et al., 2008), as can inorganic particles and biological cells (Krol et al., 2004). The use of dyes, compact forms of DNA, protein aggregates and gel beads (Radtchenko et al., 2002b) have also been reported.

Likewise, the adsorption of oppositely charged polyelectrolytes can be done on the surface of colloidal particles with subsequent core dissolution. The hollow nanocapsules are then loaded with the substance of interest (Antipov et al., 2002; Fan et al., 2002; Radtchenko et al., 2002b; Ai and Gao, 2004; Krol et al., 2004; Cui et al., 2009).

According to Radtchenko et al. (2002b), "large macromolecules cannot penetrate polyelectrolyte multilayers whereas small solutes like ions or drug molecules can do so readily. As a result the presence of macromolecules only inside the capsules leads to a difference in physicochemical properties between the bulk and capsule interior and makes it possible to establish a polarity gradient across the capsule wall that could be used to precipitate poorly

Table 9
Examples of raw materials used for preparation of nanocapsules by the emulsion-coacervation method.

Active ingredient	Therapeutic activity	Polymer	Core	Organic phase	Aqueous phase	Cross-linking agent	Other components	Reference
Turmeric oil	Antifungal Antibacterial Antioxidant Antimutagenic Anticarcinogenic	Sodium alginate Mw 80–120 kDa	Organic	Turmeric oil ethanol or acetone	Sodium alginate Polysorbate 80 Water			Lertsuttiwong et al. (2008a)
		Sodium alginate Mw 80–120 kDa – chitosan Mn 41 and 72 kDa	Organic	Turmeric oil ethanol	Sodium alginate Polysorbate 80 Water Chitosan acetic acid Water	Calcium chloride		Lertsutthiwong et al. (2008b)
Triamcinolone acetonide	Glucocorticoid Antiasthmatic Antiallergic	Swine skin gelatin type II	Organic	Chloroform	Desolvation agents: sodium sulfate and isopropanol	Glutaraldehyde	Sodium metabisulfate	Krause and Rohdewald (1985)
Hydrogen tetrachloroaureate $HAuCl_4$.[a]		Poly (1,4 butadiene) (PB)-block-polystyrene (PS)-block-poly(ethylene oxide) (PEO) triblock terpolymer Mn 76–86 kDa	Aqueous	w/o microemulsion of the pseudo-ternary system water/SDS/xylene–pentanol		Sodium borohydride		Lutter et al. (2008)

[a] Precursor gold nanoparticle synthesis. Mw: molecular weight.

Table 10
Examples of raw materials used for preparation of nanocapsules by the polymer-coating method.

Active ingredient	Therapeutic activity	Organic phase	Aqueous phase	Coating	Reference
Nanoemulsion					
Salmon calcitonin	Calcium regulator	Active ingredient capric/caprylic triglycerides Ethanol Soybean lecithin Acetone	Poloxamer188 Water	Chitosan oligomers[a] or medium molecular weight chitosan[a] Water	Prego et al. (2006)
Modified nanoprecipitation					
Indomethacin	Anti-inflammatory Analgesic	PCL Mw 40 kDa Capric/caprylic triglycerides Lecithin Acetone	Poloxamer 188Water	Chitosan[a] or Poly-L-lysine[a]	Calvo et al. (1997)
Modified double emulsification					
Tetanus toxoid	Antigen	Aqueous phase 1: active ingredient/water Organic phase: PLA Mw 28 kDa/lecithin/ethyl acetate or PLGA[a]/lecithin/ethyl acetate Aqueous phase 2: PVA/water		PEG Mn 5 kDa or Chitosan Mw Mn >50 kDa	Vila et al., 2002

PCL: poly(e-caprolactone); PEG: poly(ethylene glycol); PLA: poly(lactic acid); PLGA: poly(lactic acid-glycolic acid); PVA: poly(vinyl alcohol).
[a] Molecular weight (Mw) non-specified.

water-soluble materials (like most drugs) within them". In line with this approach, the permeability properties of hollow polyelectrolyte multilayer nanocapsules as a function of pH and the reversible behaviour of the open and closed states of the capsule wall have been demonstrated (Antipov et al., 2002). Also, this shift from "open" to "closed" nanocapsule and vice-versa, may happen through changes in environmental conditions such as temperature or the presence of organic solvents (Ai and Gao, 2004).

On the other hand, Preetz et al. (2008) have made methodological modifications in order to prepare oil-loaded polyelectrolyte nanocapsules (Fig. 8). Firstly, an emulsion containing modified starch (octenyl succinic anhydride-modified starch) and oil was prepared by high-pressure homogenization. The modified starch was used both as an emulsifier of the oily phase and as the first negatively charged polyelectrolyte layer of the shell. Then, the solution of the second polyelectrolyte was added under stirring and when adsorption had terminated, a solution of a third polyelectrolyte was injected into the system under the same conditions. Once the polyelectrolyte addition had ended, nanocapsule dispersion was again treated by high-pressure homogenization and the dispersion was finally centrifuged.

As reported in different research works, the layer-by-layer method makes used of polycations such as polylysine, chitosan, gelatin B, poly(allylamine) (PAA) poly(ethyleneimine) (PEI), aminidextran and protamine sulfate. The following polyanions are used: poly(styrene sulfonate) (PSS), sodium alginate, poly(acrylic acid), dextran sulfate, carboxymethyl cellulose, hyaluronic acid, gelatin A, chondroitin and heparin (Agarwal et al., 2008).

According to Radtchenko et al. (2000), the key issue of layer-by-layer assembly is the need for surface recharging at each adsorption step. The molecules employed for assembly should have a sufficient number of charged groups to provide stable adsorption on an oppositely charged surface and non-compensated charges exposed to the exterior. Nevertheless, taking into account energetic considerations, the possibility that the sequential adsorption of the following polyelectrolyte may remove the contrapolyion deposited instead of adsorbing onto it cannot be excluded (Sukhorukov et al., 1998).

Furthermore, this method raises other difficulties such as the formation of contraion aggregates, the separation of the remaining free polyelectrolyte from the particles prior to the next deposition cycle and polyelectrolyte-induced bridging during centrifugation. Close particle–particle encounters may cause unfavorable inter-

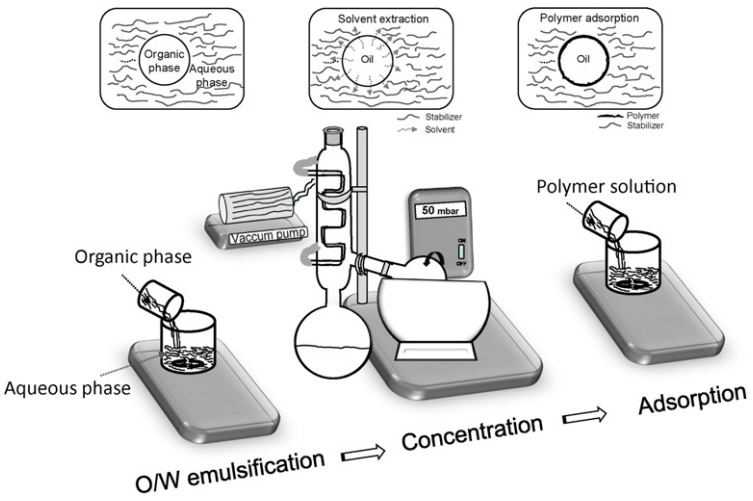

Fig. 7. Set-up used for preparation of nanocapsules by the polymer-coating method.

Table 11
Examples of raw materials used for preparation of nanocapsules by the layer-by-layer method—non-removable template.

Active ingredient	Therapeutic activity	Core	Cationic polymer	Anionic polymer	Solvent LbL procedure	Reference
Artemisinin	Antineoplastic	Artemisinin solid	Chitosan Mw 250 kDa, PDDA Mw 200 kDa or gelatin Mw 500 kDa	Sodium alginate Mw 70 kDa	Water	Chen et al. (2009)
Tamoxifen	Antineoplastic	Tamoxifen solid	PAH[a]	PSS[a]	Water and PBS	Agarwal et al. (2008)
Paclitaxel	Antineoplastic	Paclitaxel solid	PDDA[a]			
Sacharomyces cerevisiae *Neurospora crassa*		*Sacharomyces cerevisiae* *Neurospora crassa*	PAH Mw15 kDa	PSS Mw 70 kDa	NaCl aqueous solution	Krol et al. (2004)
		Medium-chain triglycerides OSA starch	Chitosan Mw 400 g/mol	Lambda-carrageenan[a]	Acetate buffer (pH 4.5) for cationic polymer Water for anionic polymer	Preetz et al. (2008)

OSA starch: octenyl succinic anhydride-modified starch; PAH: poly(allylamine hydrochloride); PDDA: poly(dimethyldiallylamide ammonium chloride); PSS: sodium poly(styrene sulphonate); PBS: sodium phosphate buffer.
[a] Molecular weight (Mw) non-specified.

actions with the polyelectrolyte films, possibly leading to film destruction and aggregate formation (Sukhorukov et al., 1998).

In addition, another difficulty is the particle sizes obtained which are higher than 500 nm (Sukhorukov et al., 1998; Chen et al., 2009). Although these particle sizes are at submicronic scale, they are obviously larger than the size commonly accepted for nanocapsules. However, this problem has been overcome by ultrasonic treatment of aqueous suspensions to decrease the size of individual drug particles to nano-scale (100–200 nm). They are then stabilized in solution by applying layer-by-layer coating by ultrasonic treatment and thin polyelectrolyte shells are assembled on their surfaces (Agarwal et al., 2008).

Consequently, although research using this strategy has greatly improved the technique, it is acknowledged that the high number of assembly steps involved is quite complex and time consuming, particularly for the synthesis of thick walled polymer nanocapsules (Sablon, 2008). In addition, taking into account that research into this method of nanoencapsulation of active substances has only just begun, it is not possible to propose formulations that can be used as a model.

3.7. Strategies for the concentration, purification and stabilization of nanoencapsulated systems

There are different reasons for ensuring the concentration, purification and stabilization of nanocapsule dispersions. In relation to the need of concentration, the different methods used for preparation of nanocapsules frequently produce dispersions with low drug carrying contents which is a serious disadvantage when the aim is to obtain therapeutic concentrations. This information is limited in reviews of research so it is difficult to make comparisons between works due to the different volumes used and the different encapsulation efficiencies reported by each team. Table 13 shows an approximation of dispersion concentrations before and after their concentration.

With regard to the need for purification, the initial nanocapsule dispersions obtained from preformed polymers can be contaminated by solvents, salts, stabilizers and cross-linking agents that must be eliminated in order to guarantee the purity required for in vivo nanocapsule administration.

Likewise, regarding stabilization, although nanocapsule dispersions are catalogued as stable systems due to Brownian motion, they can be subject to non-stability phenomena due to, among other things, polymer degradation, migration of the active substance from the inner liquid and microbiological contamination of aqueous systems. Indeed, one of the things limiting the industrial development of polymeric nanocapsule suspensions as drug delivery systems is the problem encountered in maintaining the stability of suspensions (Pohlmann et al., 2002).

As shown in Fig. 2, different options exist for the concentration, purification and stabilization of nanoencapsulated systems that can be used independently or combined sequentially. Evapo-

Table 12
Examples of raw materials used for preparation of nanocapsules by the layer-by-layer method—removable template.

Template	Cationic polymer	Anionic polymer	Solvent LbL procedure	Core removed solvent	References
Polystyrene latex particles	PHA Mw 8–11 kDa	PSS Mw 70 kDa	NaCl aqueous solution		Sukhorukov et al. (1998)
$CaCO_3$ particles	PHA Mw 15 kDa	PSS Mw 70 kDa	NaCl aqueous solution	HCl solution	Krol et al. (2004)
$CdCO_3$ particles	PHA Mw 50 kDa	PSS Mw 70 kDa	NaCl aqueous solution	HCl solution	Antipov et al. (2002)
MFparticles	PDDA Mw 200 kDa in water	Gelatin negatively charged Mw 50 kDa	PBS (pH 7.4)	HCl solution	Ai and Gao (2004)
MFparticles	PHA Mw 50 kDa	PSS Mw 70 kDa	NaCl aqueous solution	HCl solution	Radtchenko et al. (2002a)
$PSS^{-}/7Y^{3+}$ complex-MFparticles	PHA Mw 50 kDa	PSS Mw 70 kDa	NaCl aqueous solution	HCl solution and NaCl/EDTA	Radtchenko et al. (2002a)

MFparticles: melamine formaldehyde colloidal particles; PSS^{-}/Y^{3+} complex-MFparticles: poly(styrene sulfonate)/Yttrium3+ ions complex onto the surface of the melamine formaldehyde colloidal particles; PAH: poly(allylamine hydrochloride); PDDA: poly(dimethyldiallylamide ammonium chloride); PSS: sodium poly(styrene sulphonate); PBS: sodium phosphate buffer; EDTA: ethylenediaminetetraacetic acid.

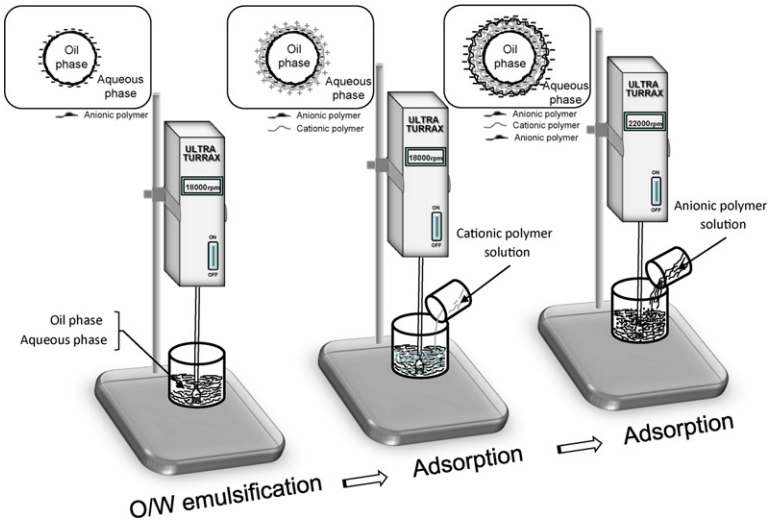

Fig. 8. Set-up used for preparation of nanocapsules by the layer-by-layer method.

ration under reduced pressure, water washing, ultracentrifugation and lyophilization are undoubtedly the methods used most. However, they are often inapplicable due to the aggregates formed (Duclairoir et al., 1998; Vauthier et al., 2008) and they are currently only adapted for purifying small batches (Limayem et al., 2004).

Among the strategies used for nanocapsule purification, the literature reports the use of dialysis against water (Schaffazick et al., 2003; Stella et al., 2007), dialysis against a polymer solution (Vauthier et al., 2008), filtration through 0.45 μm (Stella et al., 2007), cross-flow microfiltration and diafiltration, which efficiently eliminates surfactants and solvents (Limayem et al., 2004). Nevertheless, it is important to note that techniques such as filtration, dialysis, and ultracentrifugation do not provide efficient separation for small nanocapsule sizes (80–150 nm). In these cases, methods such as gel permeation chromatography have proved to be efficient (Ma et al., 2001).

Likewise, in an attempt to find alternatives for nanocapsule stabilization, the spray-drying technique using lactose or colloidal silicon dioxide as nanocapsule protectors has been proposed instead of lyophilization (Pohlmann et al., 2002; Tewa-Tagne et al., 2007a,b). However, research into optimizing the latter technique is still in progress and the use of cryoprotectants and lyoprotectants is necessary since the thin polymeric envelope of the nanocapsules may not withstand the stress of this process. Nanocapsules can be destabilized by the crystallization during freezing, dessication or storage of certain cryoprotectants such as mannitol, sucrose or glucose (Abdelwahed et al., 2006c). However, the behaviour of other protectants such as povidone and colloidal silicon dioxide appears to be acceptable (Schaffazick et al., 2003; Abdelwahed et al., 2006b). Table 14 provides a summary of research into nanocapsule lyophilization and spray-drying.

4. Behaviour of nanocapsules as drug delivery systems

The current section of this review will focus on the behaviour of nanocapsules in relation to their size, zeta-potential, dispersion pH, shell thickness, encapsulation efficiency, drug release, stability and *in vivo* and *in vitro* performances as a function of their preparation method. These properties have been chosen because they are those most frequently sought.

To this end, more than seventy research works available in electronic databases (Science direct® and Springerlink®) have been studied. The data analysis performed was confined to the comparison of methods and identification of trends in order to contribute to the state of knowledge. Hence, it is clear that comparing data from the literature is difficult when differences exist in the experimental methods used and in the specific aims of each research team. Likewise, generalizations are limited because the studies chosen represent only a sample of the universe of research performed in this field as many works may remain unpublished or hard to obtain.

4.1. Mean nanocapsule size

The mean particle sizes of nanocapsules prepared from preformed polymers are in general between 250 and 500 nm (Fig. 9). Exceptions stem from research in which the solid active substance has been encapsulated directly (s/o/w emulsification and layer-by-layer methods). However, as mentioned previously, in these cases it is possible to obtain low mean particle sizes by using ultrasound in the initial steps of the procedure.

Fig. 9 shows the range of sizes that can be obtained by each method while an explanation is provided in Table 15. This table summarizes research illustrating the impact of changes made to composition parameters on nanocapsule sizes. As can be seen, such changes are significant for most nanoencapsulation methods. For example, in regarding to nanoprecipitation, the nature and concentration of the polymer in the organic phase, solvent polarities, the nature and ratio of internal/external phases and the nature and concentration of surfactants are essential factors in determining nanocapsule size (Santos-Magalhães et al., 2000; Zili et al., 2005).

With regard to emulsion–diffusion method, parameters such as the nature and the volume of the organic and aqueous phase, the nature and concentration of surfactants and polymers have rele-

Table 13
Drug encapsulation in diluted and concentrated dispersions as a function of nanoencapsulation method.

Method	Drug concentration in diluted dispersions (mg/ml)	Drug concentration in concentrated dispersions (mg/ml)
Nanoprecipitation	0.002–0.09	0.15–6.5
Emulsification–diffusion	~0.2	~50
Double emulsification	2–5	20–50
Emulsification–coacervation	~0.24	~12

This data corresponds to a general estimate taking as base different information available in the researcher works that supported this review.

Table 14
Summary of research into the stabilization of nanoencapsulated systems by lyophilization and spray-drying.

Method	Material evaluated	Conclusion	Reference
Spray-drying	Colloidal silicon dioxide	Yield about 70%. The nanocapsule drug recovery and their morphological characteristics presented stable after 5 months of storage at room temperature.	Pohlmann et al. (2002)
Spray-drying	Colloidal silicon dioxide	The concentrations of both NC and excipient, and the mixing procedure are crucial parameters for the NC spray-drying. Nanocapsule concentration suggest: 1% (w/v). Excipient concentration suggest: 10% (w/v).	Tewa-Tagne et al. (2006, 2007a)
Spray-drying	Lactose, mannitol, dextrose, maltodextrine, PVP, HPC, HPMC	Lactose allows a desirable powder morphology and favouring NC suspension reconstitution with only ~2% of agglomerates. Mannitol and PVP allow the particle redispersion in the range of the original particle size	Tewa-Tagne et al. (2007b)
Lyophilization	Colloidal silicon dioxide	It is required an excipient for the successful NC lyophilization. The microparticle surface of the freeze-dried powders showed NC with size range similar to that observed for the corresponding original suspensions with SiO_2.	Schaffazick et al. (2003)
Lyophilization	HPbCD, sucrose, glucose, anhydrous glucose, trehalose, mannitol, PVP	Nanocapsule aggregation and the formation of macroscopic particles were noticed after the freeze-drying without cryoprotectant. Nanocapsule sizes are conserved after freeze-drying when sucrose, HPbCD, glucose and PVP are used. Nanocapsule freeze-drying with mannitol produces aggregates.	Abdelwahed et al. (2006a,b,c)

PVP: poly(vinylpyrrolidone); HPC: hydroxypropylcellulose; HPMC: hydroxypropylmethylcellulose; SiO_2: colloidal silicon dioxide; HPbCD: hydroxypropylbeta-cyclodextrine

vant implications on particle size distribution. Likewise, the control of nanocapsule mean diameter can be achieved by the intensity and duration of homogenization, in other words, the shear rate of the emulsification process (Ma et al., 2001; Joo et al., 2008; Moinard-Chécot et al., 2008).

Research into the double emulsification method has concluded that particle size depends on the balance between the types and concentrations of the internal and external surfactants that determine droplet size, the interactions at the interface and the structural conformation of the nanocapsule wall (Khoee and Yaghoobian, 2008).

On the other hand, it has been observed that the nature of concentration of drugs does not appear to influence the size of nanocapsules when the latter are prepared by nanoprecipitation or emulsion–diffusion methods (Guterres et al., 1995; Pereira et al., 2006; Joo et al., 2008). However, research elsewhere has reported contrasting conclusions (Fessi et al., 1989; Dalençon et al., 1997; Quintanar et al., 1998b; Stella et al., 2007).

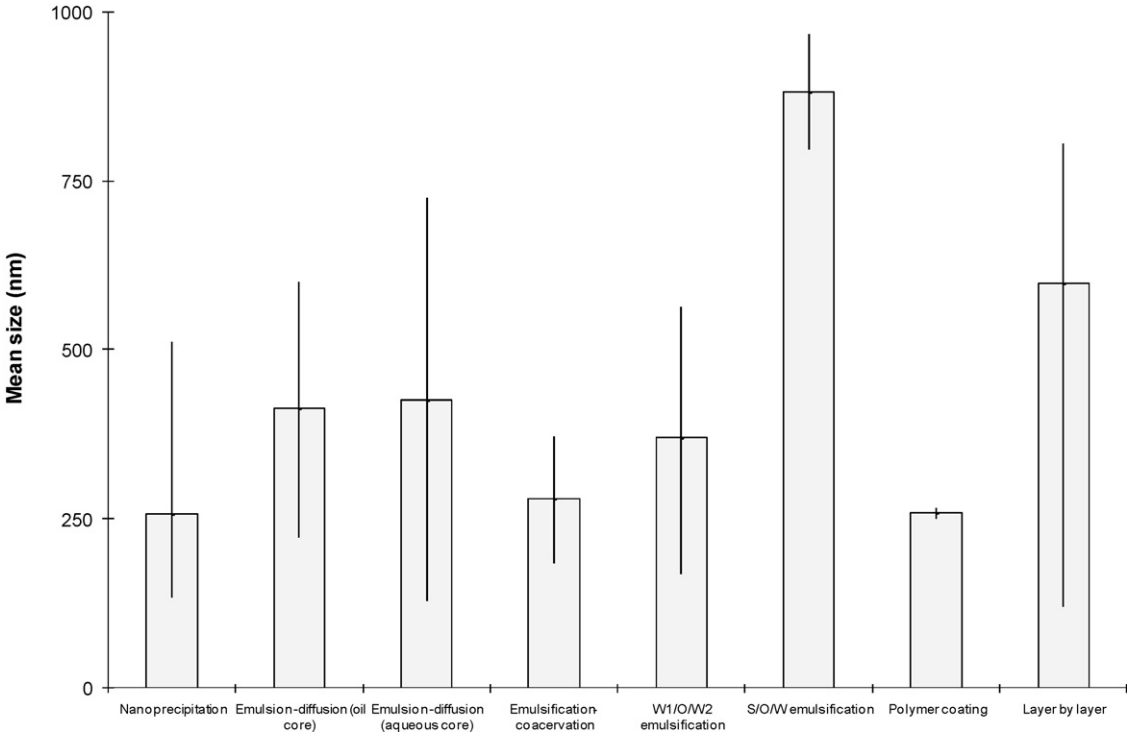

Fig. 9. Size behaviour obtained as a function of method for preparation of nanocapsules.

Table 15
The effect of various parameters on the size of formed nanocapsules.

Variable	Method	Evaluated materials	Work conditions	Mean size range (nm)	Behaviour*	Reference
Active principle nature	Nanoprecipitation	Gemcitabine derivates	Variable	182–301	Significant	Stella et al. (2007)
		Taxol, dexamethasone, vitamin K	Variable	260–300	Significant	Fessi et al. (1989)
		DNA, DNA–PVP, DNA–PVA	Variable	127–132		Perez et al. (2001)
	Emulsion–diffusion	Indomethacine – Progesterone – Estradiol	20 mg	335–510	Significant	Quintanar et al. (1998b)
	Double emulsification	DNA, DNA–PVP, DNA–PVA	Variable	272–296		Perez et al. (2001)
Active principle concentration	Nanoprecipitation	Rifabutine	0.32–0.8 mg/ml solvent	205–512	Significant	Dalençon et al. (1997)
Oil nature	Nanoprecipitation	Capric/caprylic triglycerides–benzyl benzoate	0.012 ml/ml acetone	225–202		Schaffazick et al. (2003)
	Emulsion–diffusion	Mineral oil–capric/caprylic triglycerides	0.25 ml/ml AcEt	303–340	Significant	Moinard-Chécot et al. (2008)
	Layer-by-layer	Capric/caprylic triglycerides, sesame oil, olive oil	5%	150–200		Preetz et al. (2008)
Oil concentration	Emulsion–diffusion	Capric/caprylic triglycerides	5–25%	360–483	Significant	Moinard-Chécot et al. (2008)
	Emulsion-coacervation	Turmeric oil	0.5–10%	87–739	Significant	Lertsutthiwong et al. (2008a)
Oil viscosity	Emulsion–diffusion	Capric/caprylic triglycerides	Viscosity variable	358–702	Significant	Moinard-Chécot et al. (2008)
		PCL, Eudragit	3.7 mg/ml acetone	327–225	Significant	Schaffazick et al. (2003)
		PLA, PLA–PEG	Variable	218–277	Significant	Furtado et al. (2001b)
	Nanoprecipitation	PLA, PCL	3.7 mg/ml acetone	197–182		Pohlmann et al. (2002)
		PLA, PCL	4 mg/ml acetone	228–241		Cauchetier et al. (2003)
		PLA, PLGA	4 mg/ml acetone	206–205		Dalençon et al. (1997)
Polymer nature		PLGA, PLA, PCL, PEG–PLGA, PEG–PLA, PEG–PCL	5 mg/ml acetone	210–287	Significant	Ameller et al. (2003)
	Emulsion–diffusion	PLA-Tone P-700	10 mg/ml AcEt	340–346		Fessi et al., 1989
		PLA, PLA–PEG	Variable	726–133	Significant	Ma et al. (2001)
	Double emulsification	PLA, PCL	Variable	890–317	Significant	Lu et al., 1999
Polymer concentration	Nanoprecipitation	PCL 10000	4–10 mg/ml acetone	741–924	Significant	Limayem et al., 2006
	Emulsion–diffusion	PCL 80000	20–50 mg/ml	585–1329	Significant	Guinebretière et al. (2002)
		PCL 80000	10–80 mg/ml AcEt	465–483		Moinard-Chécot et al. (2008)
		PLA	6.25–30 mg/ml AcEt	319–614	Significant	Quintanar et al. (1998b)
		PLA	20–35 mg/ml solvent	549–601	Significant	Romero-Cano and Vincent (2002)
	Double emulsification	PLGA or PCL	Variable	300–600	Significant	Lamprecht et al. (2000)
		PLGA	25–51 mg/ml DCM	130–353	Significant	Jeong et al. (2008)
Polymer molecular weight	Nanoprecipitation	PCL	10,000–80,000	741–924	Significant	Limayem et al. (2006)
	Emulsion–diffusion	PCL	14,000–80,000	420–483	Significant	Moinard-Chécot et al. (2008)
	Double emulsification	PLA	16,000–51,000	563–890	Significant	Lu et al. (1999)
Surfactant nature	Double emulsification	Sorbitan esters–Polysorbates (20, 60, 80)	Variable	169–254	Significant	Zhu et al. (2005)
		Sorbitan esters–Polysorbates (20, 60, 80)	Variable	75–621	Significant	Khoee and Yaghoobian (2008)
Surfactant concentration	Double emulsification	Sorbitan esters–polysorbates (20, 60, 80)	Variable	75–621	Significant	Khoee and Yaghoobian (2008)
Solvent nature	Emulsion–diffusion	Ethyl acetate–propylene carbonate–benzyl alcohol	20 ml	332–239	Significant	Quintanar et al. (1998b)
Solvent volume	Nanoprecipitation	Acetone	Solvent/water ratio: 0.5–0.8	352–308	Significant	Ferranti et al. (1999)
	Double emulsification	Ethyl acetate–methylene chloride	Water/organic solvent ratio: 1:2; 1:7	425–1402	Significant	Bilati et al. (2005a)
Stabilizer nature	Nanoprecipitation	Polysorbate 20, Polysorbate 80, PLX 188	Variables	320–825	Significant	Moinard-Chécot et al. (2008)
	Emulsion–diffusion	SDS, CTAC: gelatin, CTAC	0.20%	223–598	Significant	Joo et al. (2008)
Stabilizer concentration	Nanoprecipitation	PLX 188	0.1–0.5% of aqueous phase	814–725	Significant	Limayem et al. (2006)
	Emulsion–diffusion	PVA 88000	0.5–3.75% of aqueous phase	365–1247	Significant	Moinard-Chécot et al. (2008)
	Double emulsification	PVA	0–0.4%	300–275	Significant	Lamprecht et al. (2000)
Stabilizer molecular weight	Emulsion–diffusion	PVA	31,000–88,000	456–483	Significant	Moinard-Chécot et al. (2008)
Water volume	Nanoprecipitation	Water	Ratio solvent/water: 1:2–1:4	320–536	Significant	Limayem et al. (2006)

* Significant behaviour exists when the nanoparticle size difference among evaluated conditions is greater than 20 nm.

Table 16
Zeta-potential of nanoencapsules as a function of preparation method.

Polymer	Stabilizer agent	Z-Potential (mV)	Reference
Nanoprecipitation			
PCL	Polysorbate 80	−50.7	Cruz et al. (2006)
PCL	Polysorbate 80	−7.3	Ourique et al. (2008)
PCL	Polysorbate 80	−31	Tewa-Tagne et al. (2007a)
PCL	Polysorbate 80	−27.9	Tewa-Tagne et al. (2006)
PCL	Poloxamer 188	−39.9	Calvo et al. (1997)
PCL	Poloxamer 188/Chitosan	37.1	Calvo et al. (1997)
PLA	Poloxamer 188	−62.0	Pereira et al. (2008)
PLA–PEG	Poloxamer 188	−60.3	Pereira et al. (2008)
PLGA	Poloxamer 188	−39.5	Texeira et al. (2005)
PLGA	Poloxamer 188 + trehalose	−28.4	Pereira et al. (2006)
Eudragit	Polysorbate 80	−33	Schaffazick et al. (2008)
Emulsion-diffusion aqueous core			
PCL	Poloxamer 188	−5.9	Choi et al. (2009)
Aqueous core			
	PVA or PVP	30.9	Perez et al. (2001)
Emulsion-coacervation			
Sodium alginate	Sodium alginate/polysorbate 80 calcium chloride—cross-linking agent	−17.4	Lertsutthiwong et al. (2008a)
Double emulsification (W/O/W)			
PLA	Polysorbate 80/glycerin	−38.9	Zhu et al. (2005)
PLA–PEG	PVA	−18.6	Perez et al. (2001)
PLGA	PVA chitosan	21.8	Vila et al. (2002)
Polymer-coating			
Chitosan	Poloxamer 188	+34.8	Prego et al. (2006)
Poly-L-lysine	Poloxamer 188	27.9	Calvo et al. (1997)
Layer-by-layer			
Chitosan/Alginate		−30	Chen et al. (2009)
Chitosan/lambda-carraaeenan		−21.1	Preetz et al. (2008)

All measures have been realized "after adequate dilution of an aliquot of the suspension in water".

4.2. Nanocapsule zeta-potential

No specific trend regarding nanocapsule zeta-potential behaviour has been brought to light as yet (Table 16). Taking into account the author's experience, nanocapsule zeta-potential mainly depends on the chemical nature of the polymer, the chemical nature of the stabilizing agent and pH of the medium. Therefore when nanocapsules are prepared from polyester polymers or methacrylate derivates using non-ionic stabilizing agents, negative zeta-potential values are obtained due to the presence of polymer terminal carboxylic groups. Likewise, positive zeta-potential values are obtained when cationic polymers and non-ionic stabilizing agents are used.

On the other hand, when nanocapsules are prepared by using negatively charged polymers and negatively charged stabilizing agents (i.e. sodium lauryl sulphate), negative zeta-potential values are obtained with absolute values higher than when non-charged stabilizers are used. Similarly, the zeta-potential is positive if a positively charged stabilizing agent is chosen. This behaviour is due to the adsorption of the stabilizing agent onto the nanocapsule surface, which, for example in the case of PCL, can be explained by its hydrophobic nature. Consequently, the hydrocarbon chains of the

Table 17
The effect of various parameters on the zeta-potential of the formed nanocapsules.

Variable	Method	Materials evaluated	Work conditions	Z-Potential range (mV)	Behaviour*	Reference
Active principle nature	Double emulsification	DNA, DNA–PVP, DNA–PVA	Variable	(−29)–(−34)		Perez et al. (2001)
Active principle concentration	Nanoprecipitation	4-(N)-stearoylgemcitabine	100–1000 mcg/ml	27–44	Significant	Stella et al. (2007)
Oil nature	Layer-by-layer	Medium-chain triglycerides, sesame oil, olive oil	5%	(−12.7)–(−21.1)		Preetz et al. (2008).
Oil concentration	Emulsion-coacervation	Turmeric oil	0.5–10%	(−17)–(−19)		Lertsutthiwong et al. (2008a)
Polymer nature	Nanoprecipitation	PLA, PLA–PEG	Composition variable	(−50)–(−56)		Furtado et al. (2001a)
		PLGA, PLA, PCL, PEG–PLGA, PEG–PLA, PEG–PCL	Variable	(−42)–(−57)		Ameller et al. (2003)
	Emulsion–diffusion	DNA, DNA–PVP, DNA–PVA	Composition variable	(−29)–(−33)		Perez et al. (2001)
Polymer concentration	Emulsion–diffusion	PLA	6.25–30 mg/ml AcEt	(−15)–(−20)		Quintanar et al. (1998b)
Stabilizer nature	Double emulsification	Sorbitan esters–polysorbates (20, 60, 80)	Variable	(−34)–(−39)		Zhu et al. (2005)

* Significant behaviour exists when the Z-potential difference among evaluated conditions is greater than 15 mV.

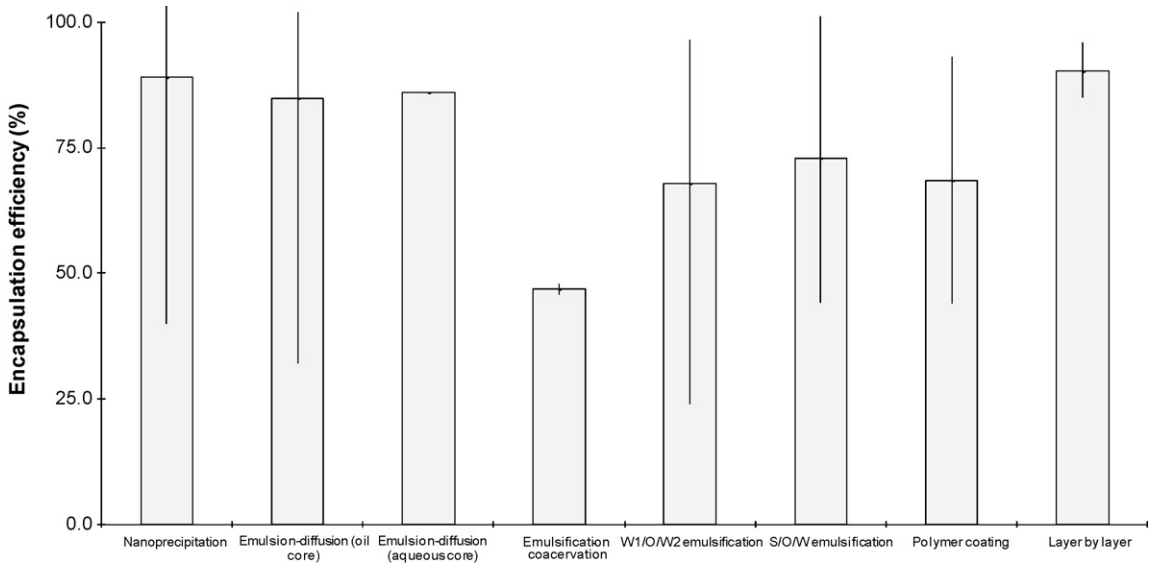

Fig. 10. Encapsulation efficiency behaviour obtained as a function of method for preparation of nanocapsules.

surfactant interact with the hydrophobic regions of the PCL wall and the surfactant head facing aqueous phase, which induces negative or positive zeta-potentials depending on its chemical nature (Joo et al., 2008).

In addition, the magnitude of the zeta-potential depends on the dispersion pH regardless of the nature of the stabilizing agent (Joo et al., 2008). Unfortunately, the literature reports no specific value for zeta-potential measurement, which is frequently expressed as "all measurements have been performed after adequate dilution of an aliquot of the suspension in water". With unknown pH and salinity, it is difficult to propose general behaviour. However, it can be stated that in most cases, zeta-potential values lower than −10 mV (usually between −25 and −30 mV, Table 16) are reported, which allows predicting good colloidal stability due to the high-energy barrier between particles.

Furthermore, the studies reported in Table 17 which were developed with 4-(N)-stearoylgemcitabine nanocapsules prepared by nanoprecipitation (Stella et al., 2007), indomethacine and DNA nanocapsules obtained by emulsion–diffusion (Quintanar et al., 1998b; Perez et al., 2001) and turmeric oil and DNA nanocapsules prepared by the emulsion-coacervation and double emulsification methods, respectively (Perez et al., 2001; Lertsutthiwong et al., 2008a), suggest that the zeta-potential of the nanocapsules shows no dependence on the nature of the active molecule, polymer concentration or stabilizer concentration. According to the conclusions of these studies and taking into account that the active substance may be entrapped within the nanocapsule core, the resulting zeta-potential probably depends on the combination of materials and maybe on certain process conditions such as those that determine molecular organization when the polymer is re-precipitated.

4.3. Nanocapsule dispersion pH

In general terms, nanocapsule dispersion pH-values fall within a range of 3.0–7.5 when nanoprecipitation, emulsion–diffusion or layer-by-layer methods are applied. No information is available in the literature for the other methods for preparation of nanocapsules.

As mentioned previously, dispersion pH determines the zeta-potential of colloidal dispersions which can impact on their stability. For example, it has been reported that PLA hydrolysis is non-enzymatic and depends on the temperature and pH of the medium, accelerated under both acidic and basic conditions. Therefore when PLA nanocapsules were prepared with benzyl benzoate, pH-dispersion was more acidic than with capric/caprylic triglycerides, probably because of traces of free acids in the central oil core. The stability study of these nanocapsule dispersions shows considerable polymer degradation in the formulations with benzyl benzoate after 8 months storage, whereas minimal PLA breakdown was seen in the preparations containing capric/caprylic triglycerides (Guterres et al., 1995; Dalençon et al., 1997).

The pH of the dispersion medium seems to be a key factor controlling the size of nanoparticles and thus their biodistribution. In fact the nanoparticles in the circulation can leak from endothelial barrier openings named fenestrations (Gaumet et al., 2008). Unfortunately in the current review, it was not possible to identify studies illustrating the impact of pH on nanocapsules biodistribution.

4.4. Nanocapsule shell thickness

As will be discussed later, in the case of nanocapsules the polymeric shell plays a predominant role in protecting the active substances incorporated and probably in the release profile (Rübe et al., 2005; Poletto et al., 2008a). According to different authors, shell thickness values are about 10 nm (Rübe et al., 2005) and 20 nm (Cauchetier et al., 2003) when PCL is selected as polymer by the nanoprecipitation method and 10 nm when PLGA is chosen (Nassar et al., 2009). The differences observed between studies are probably due to the methods used for each one. Whereas Cauchetier et al. (2003) make theoretical approaches based on the hypothesis that the polymer is the unique component of the nanocapsules wall, Rübe et al. (2005) and Nassar et al. (2009) estimate shell thickness by using TEM photomicrographs of nanocapsules. The over-estimation of shell thickness obtained by Cauchetier et al. (2003) suggests that probably not all the polymer forms nanocapsules, meaning that nanosphere formation may also occur.

For nanocapsules prepared by emulsion–diffusion method had been reported shell thickness values between 1.5 and 2 nm (Guinebretière et al., 2002). At present, there is not enough experimental evidence to explain the huge difference between the shell thicknesses obtained when nanoprecipitation and emulsion–diffusion methods are used.

On the other hand, it has been reported that in both nanoprecipitation and emulsion–diffusion methods, the higher polymer

Table 18
The effect of various parameters on the encapsulation efficiency of the formed nanocapsules.

Active	Polymer	Oil	Nanocapsule preparation method	Variable of interest	Work conditions	Encapsulation efficiency (%)	Reference
Primidone	PCL	Benzyl alcohol	Nanoprecipitation	Ratio solvent/water	0.5–0.8	75–67	Ferranti et al. (1999)
Spironolactone	PCL	Capric/caprylic triglyceride PEG-4 esters	Nanoprecipitation	Ratio solvent/water	0.25–0.5	16–96	Limayem et al. (2006)
Xanthone	PLGA	Capric/caprylic triglyceride	Nanoprecipitation	Active concentration	200–600 mcg/ml	85–89	Texeira et al. (2005)
3-Methylxhantone	PLGA	Capric/caprylic triglyceride	Nanoprecipitation	Active concentration	1000–1400 mcg/ml	77–89	Texeira et al. (2005)
RU 58668	PLA, PLGA, PCL, PEG–PLA, PEG–PLGA, PEG–PCL	Capric/caprylic triglyceride	Nanoprecipitation	Polymer type	5 mg/ml acetone	94–99	Ameller et al. (2003)
	PLA		Emulsion–diffusion	Polymer type	PLA	18.5	
Insuline	PLA–PEG copolymers				PLA–PEG	32–38	Ma et al. (2001)
DNA	PLA–PEG copolymer		Emulsion–diffusion	Complex active-polymer	DNA	87	Perez et al. (2001)
					DNA–PVP or DNA–PVA	82–89	
Insulin	PLA		Double emulsification	Tensioactive—stabilizer ratio, tensioactive type, stabilizer type, stabilizer concentration	Sorbitan ester 80: Polysorbate 80, low concentration. Sorbitan ester 80: Polysorbate 80, high concentration.	34 66	Zhu et al. (2005)
Bovine serum albumin	PLA or PCL–PEO		Double emulsification	Polymer type	PLA	51–60	Lu et al. (1999)
					PCL–PEO	29	
Penicillin G	PBA		Double emulsification	Tensioactive—stabilizer ratio, tensioactive type, stabilizer type, stabilizer concentration	Sorbitan ester 60: Polysorbate 60, 1:2.8 ratio, low concentration. Sorbitan ester 60: Polysorbate 60, 1:3.8 ratio, high concentration.	22 76	Khoee and Yaghoobian (2008)
DNA	PLA–PEG copolymer		Double emulsification	Complex active-polymer	DNA	72	Perez et al. (2001)
					DNA–PVP or DNA–PVA	79–59	

concentration in the oil phase leads to an increase in the shell thickness of the nanocapsules obtained (Romero-Cano and Vincent, 2002; Cauchetier et al., 2003).

Regarding shell thickness for nanocapsules prepared by the layer-by-layer method, it depends on the number of layers, the measurement conditions and possibly the conditions for preparation of nanocapsules. Consequently, the value estimated is between 1.5 and 1.7 nm per polycation/polyanion bilayer in dry state (Radtchenko et al., 2002a; Agarwal et al., 2008). Furthermore, the research performed by Agarwal et al. (2008) shows that shell thickness is almost twice these values when the measurements are carried out in water. According to other studies, the mean increase of the particle diameter per cationic/anionic layer is 5 nm; however, the first layer has an apparent thickness of 8–11 nm (Sukhorukov et al., 1998).

Unfortunately, there does not appear to any information about this parameter for nanocapsules prepared by double emulsification, emulsion-coacervation and polymer-coating, which makes a global comparison of all the methods problematic.

4.5. Nanocapsule encapsulation efficiency

As shown in Fig. 10, nanoprecipitation, emulsion–diffusion and layer-by-layer methods currently give the best results for nanocapsule encapsulation (80% or more). In the case of the layer-by-layer method the fact that the solid drug is the template ensures high encapsulation efficiency. Nevertheless, as shown in Table 18, for the nanoprecipitation and emulsion–diffusion methods, different determinant factors of drug encapsulation efficiency exist. For example, the active chemical nature of the drug and its polarity in particular, determine encapsulation efficiency. In this sense, hydrophilic drugs can reach maximum values of 10% and in cases of lipophilic compounds major encapsulation efficiency is getting (higher than 70%) (Ma et al., 2001; Stella et al., 2007).

On the other hand, as mentioned previously, in these methods (nanoprecipitation and emulsion–diffusion) the maximum solubility of the active substance in oil is one of the criteria for oil selection and defining initial concentration when starting preparation of nanocapsules. Therefore it is logical to assume that systems in which the concentration of the active substance is close to the saturation concentration can give better results. However, it is necessary take into account that when using saturation concentrations, the active substance may precipitate easily due to process conditions. Consequently, drug nanocrystals can be present in the drug-loaded polymeric nanocapsule aqueous suspensions. This phenomenon can have a big impact on the drug release profile (Pohlmann et al., 2008).

Regarding the double emulsification method, it was found that drug mean encapsulation efficiency ranges from 65% to 75% (Fig. 10). This parameter may well be influenced by both the polymers and the surfactants used. Therefore when polymers are used with hydrophilic groups in their structure, for example the polycaprolactone-poly(ethylene oxide) block copolymer, these groups tend to enter the aqueous phase which might facilitate leakage of the drug from the nanocapsule to the outer aqueous solution and, as a result, provide the lowest encapsulation efficiency (Lu et al., 1999).

With regard to the surfactant effect when the double emulsification method is performed, it has been evaluated for sorbitan ester–poly(ethylene oxide) ester systems whose aggregation is controlled by a balanced molecular geometry determined by the packing parameter of each surfactant. Thus systems with good packing between the pair of surfactant, high emulsifying power and a high concentration, give better encapsulation efficiency results since they contribute towards obtaining more tightly sealed barrier structures with an inner aqueous phase capable of improving drug residence (Zhu et al., 2005; Khoee and Yaghoobian, 2008).

Finally, as shown in Fig. 10, regarding the other nanoencapsulation methods, the encapsulation efficiency obtained with the polymer-coating method is within the ranges obtained when using nanoprecipitation or double emulsification, depending on the method used for nanocapsule template preparation. In relation to the emulsion-coacervation method, its encapsulation efficiency is obviously low in comparison to other nanoencapsulation methods. According to the scanning electron microphotography of nanocapsules obtained by this method, holes due to solvent migration from the inner core can be seen at their surface. These holes probably allow drug leakage (Krause and Rohdewald, 1985).

4.6. Nanocapsule active substance release

It is rash to make generalizations about active substance release as a function of preparation method due to the limited number of available case studies. However, by way of illustration, Fig. 11 shows the results obtained by different studies while Table 19 provides a comparative summary of the results of different methods.

As can be observed, active substance release is the faster from nanocapsules prepared by emulsion–diffusion and emulsification coacervation methods. They are followed in descending order by nanoprecipitation, polymer-coating, layer-by-layer and double emulsification.

Some cases can be considered as exceptions because of their marked difference from the overall data. They are atovaquone nanocapsules prepared by nanoprecipitation and 4-nitroanisole nanocapsules obtained by emulsification diffusion. In the case of atovaquone, only between 20% and 25% of active substance was released within 4 months. This was assumed by researchers to be due to the capacity of the polymer or phospholipids to retain the active substance (Cauchetier et al., 2003). On the other hand, with regard to 4-nitroanisole, the results of slow release allow observing the effect of the nature and concentration of the polymer, likewise with the influence of the organic phase composition, which in this case is PLA, the active substance, hexane and DCM (Romero-Cano and Vincent, 2002).

In vitro active substance release behaviours of nanocapsules depends on a great variety of factors, such as the concentration and physicochemical characteristics of the active substance (particularly its solubility and oil/water partition coefficient); the nature, degradability, molecular weight and concentration of the polymer; the polymer solid microstructure when re-precipitated, the nature of the oil, nanocapsule size, the conditions of the *in vitro* release test (medium pH, temperature, contact time, among others) and the conditions of the preparation method. Therefore, the different active release behaviours seen in Fig. 11 are determined by the conditions established for carrying out each study. Likewise, each study has provided explanations for the behaviours observed in relation to the underlying theory used and additional tests carried out in the framework of the same research. Consequently this review compiles these explanations in order to provide better understanding of the general behaviours obtained.

Firstly, there is evidence of either modification of the release effect attributed to nanoencapsulation or its effect as a dissolution enhancer. Therefore, when the release profiles of non-encapsulated active substances are compared with those of the same active substance encapsulated by nanoprecipitation or layer-by-layer, a significant reduction of amounts released by unit of time is displayed from nanoencapsulated systems. This is because the presence of oil may increase the half-life of the sustained phase (Ferranti et al., 1999; Texeira et al., 2005; Agarwal et al., 2008; Poletto et al., 2008a). Likewise, the drug release behaviour observed when polymer-coating and double emulsification methods are per-

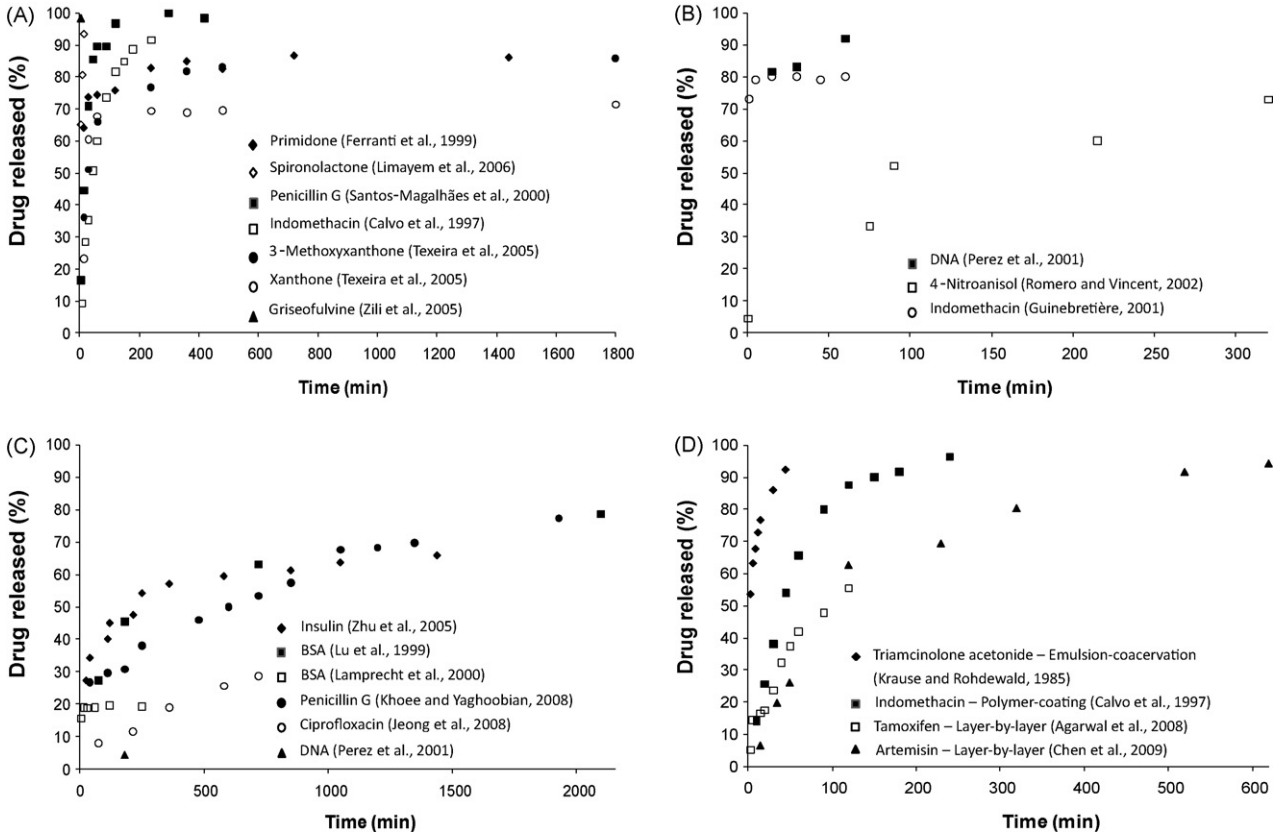

Fig. 11. Drug release behaviour of nanocapsules obtained by: (A) nanoprecipitation, (B) emulsion–diffusion, (C) double emulsification, and (D) emulsion-coacervation, polymer-coating and layer-by-layer.

formed demonstrates modified release (Lamprecht et al., 2000; Prego et al., 2006). On the other hand, it has also been reported that active substance dissolution rate is enhanced by encapsulation (Zili et al., 2005).

Furthermore, it has been proposed that nanocapsules obtained by nanoprecipitation, emulsion–diffusion, emulsion-coacervation and polymer-coating are biphasic systems with a fast initial release phase followed by a slower second release phase (Fig. 11A, B and D) (Cauchetier et al., 2003). The initial phase, called burst effect, can be attributed either to desorption of the drug located on the nanocapsule surface (Ferranti et al., 1999; Perez et al., 2001; Cruz et al., 2006), or to the degradation of the thin polymeric membrane (Cauchetier et al., 2003). Its behaviour is exhibited by apparent zero order kinetics (Santos-Magalhães et al., 2000).

The second phase corresponds to the diffusion of the drug molecules from the inner compartment, the reservoir core, to the outer phase. This diffusion process seem to be determined by the partition coefficient of the drug between the oily core and the aqueous external medium, the relative volumes of both phases, the existence of active substance-polymer interactions and the concentration of surfactants (Calvo et al., 1997; Zili et al., 2005; Texeira et al., 2005; Limayem et al., 2006).

In this diffusion process, the drug diffusion rate through the thin polymeric barrier does not seem to be a limiting factor (Krause and Rohdewald, 1985; Calvo et al., 1997; Zili et al., 2005). Nevertheless, it has been demonstrated that increasing the amount of polymer used significantly reduces the release rate (Romero-Cano and Vincent, 2002) and in these cases, the possibility that the polymer erosion could contribute to facilitating drug release has been considered by some researchers (Poletto et al., 2008a). This apparently contradiction could be explained by the fact that at low polymer concentrations (between 0.5% and 1% of the organic phase), the polymer-coating of nanocapsules does not form a consistent polymer wall but rather a thin polymer film possibly without impact on drug release (Cruz et al., 2006). It is probable that the walls of polymers at increased concentrations and high molecular weights, as in the case of the studies carried out by Romero-Cano and Vincent (2002), are more consistent, thereby having an impact on the release of the active substance.

On the other hand, nanoparticle size can influence the nanocapsule dissolution rate which increases as particle size decreases, due to an increase of available surface area (Zili et al., 2005). Likewise, the incomplete active substance release observed in most cases may be attributed to the retention capacity of the active substance by the polymer or surfactants such as phospholipids (Cauchetier et al., 2003).

With regard to the double emulsification process, which is the method preferred for water-soluble active substance nanoencapsulation, the drug release behaviour of the nanocapsules was different from that described for the other methods. According to Fig. 11C,

Table 19
General trend of active substance released from nanocapsules as a function of preparation method.

Method	Active substance release time (min)[a]			
	25%	50%	75%	90%
Nanoprecipitation	10	45	75	750
Emulsion–diffusion	<2	<2	10	60
Emulsion-coacervation	<4	<4	15	45
Double emulsification	145	1000	>2000	>2000
Polymer-coating	20	40	60	150
Layer-by-layer	40	85	320	510

[a] Time and percentaje release values estimated taking into account the data general trend.

the profiles show active substance releases higher than 70% within 30 h of beginning the test. According to some researchers, the active substance release follows a typical biphasic release model. The first phase is probably due to surface molecules and to molecule diffusion through the aqueous pores or channels created during particle preparation. The second phase corresponds to the release following the degradation–erosion of the particles (Perez et al., 2001). However, other researchers have proposed a model with three phases for drug release: an initial burst release, a plateau phase for a certain period resulting from the diffusion of the drug dispersed in the polymer matrix and, finally, a constant sustained release of the drug due to drug diffusion through the polymer wall and the erosion of the latter (Lamprecht et al., 2000).

According to Perez et al., and bearing in mind that the polymer concentration used for preparation of nanocapsules by double emulsification is higher than that used for the other methods (concentrations suggested in relation to the solvent used: Nanoprecipitation: 0.2–0.5%; emulsion–diffusion: 1–2% and double emulsification: 5–10%) it seems that nanocapsules prepared by double emulsification may have a compact structure so release is mainly controlled by the degradation and erosion of the polymer.

Therefore, release behaviour can be determined by parameters such as polymer molecular weight, nanocapsule inner core composition and particularly the nature of the w/o surfactant (Lu et al., 1999; Perez et al., 2001; Zhu et al., 2005). Moreover, it is important to take into account that drug encapsulation efficiency with double emulsification is lower than that obtained by the nanoprecipitation and emulsion–diffusion methods, which can also influence active substance release (Lu et al., 1999). Differences of particle size and drug content do not seem to affect the kinetic release of nanocapsules (Perez et al., 2001; Jeong et al., 2008).

4.7. Nanocapsule stability

Many factors, combined with nanocapsule composition, the parameters used in the preparation method and nanocapsule storage conditions, may affect the stability of nanoencapsulated systems. Therefore in most cases, it is difficult to identify specific determinants and the behaviours observed are the consequences of combinations that necessarily lead to general conclusions.

Consequently, researchers have focused on studying the stability of nanoencapsulated systems and seek to identify properties recognized as "instability tracers". Thus visual appearance can highlight advanced instability and particle size can reflect presence of aggregation while pH and active molecule quantification can permit the detection of chemical degradation for example.

In general terms, from the point of view of visual appearance and nanocapsule size, there are no variations under the different conditions studied (Cauchetier et al., 2003; Zili et al., 2005; Pereira et al., 2006; Limayem et al., 2006; Pohlmann et al., 2008; Lertsutthiwong et al., 2008a,b). In cases where variation has been detected 6 months after starting the study due to unknown storage conditions, polymer degradation is given as the reason (Dalençon et al., 1997).

In relation to pH variations, these have been detected in some cases when PLA or PCL are used (Pohlmann et al., 2002; Cauchetier et al., 2003) and this behaviour has been attributed to polymer degradation. Thus it has been reported that hydrolytic degradation of low molecular weight PLA polymers starts within a few days, whereas for high molecular weights this takes much longer (Romero-Cano and Vincent, 2002).

Table 20 summarises the results of stability studies developed with nanocapsules prepared by the nanoprecipitation method (only available information) taking as "instability tracer" the variation of the active substance concentration. As can be seen, storage of nanocapsules dispersion under high temperature conditions (above 40 °C) affects the stability of the system. Probably it is due to weakness of the polymeric structure, which facilitates the migration of the active substance from the inner core oil.

Likewise, studies of atovaquone, indomethacine, tretinoin and diclofenac nanocapsules have illustrated the impact of variables such as polymer molecular weight, active substance concentration, polymer nature and oil nature. Thus as an example, the photodegradation study of tretinoin nanocapsules shows the importance of the polymer in preventing active photodegradation. In this case, according to the researchers, the better protection obtained could be due to the crystallinity of the polymer, as it can reflect and scatter UV radiation. In the same study it was concluded that the use of different oily phases did not show any effect in this respect (Ourique et al., 2008).

In addition, a study of rifabutine nanocapsule stability exemplified another common instability factor of nanoencapsulated systems. Here, drug instability had been explained by the relative solubility of its ionized form in water and the suspension pH which increased rifabutine migration from the nanocapsule oily core to the aqueous medium (Dalençon et al., 1997).

4.8. Nanocapsule performance evaluation

Among the main challenges of administering nanocapsules as carriers of active molecules are the targeting of specific organs, allowing site-selective action of the compounds, minimizing their side effects, and providing sustained drug delivery in order to increase therapeutic availability, modification of tissue drug distribution, transmucosal delivery, gastrointestinal mucosal protection and simply to obtain significant therapeutic activity (Fawaz et al., 1996; De Jaeghere et al., 1999; Whelan, 2001; Prego et al., 2005; Pinto et al., 2006b; Singh and Lillard, 2009; De Martimprey et al., 2009).

Indeed, these objectives are not easy to achieve because when the nanocapsules enter the blood, they are quickly removed by the action of the mononuclear phagocytic system (MPS). Also, the extent and nature of nanocapsule opsonization, which is the first step of phagocytosis, depends on nanocapsule physicochemical properties such as size, surface charge and surface hydrophobicity. Consequently, the opzonization preferentially occurs in hydrophobic rather than hydrophilic surfaces, the negative surface charge increases the clearance of nanocapsules in relation to neutral or positively charged surfaces and particles less than 100 nm can leave the circulation through gaps or fenestrations in the endothelial cells lining the blood vessels (De Jaeghere et al., 1999).

Taking the above into consideration, some researchers have advanced towards the corroboration of their research expectations by using *in vitro* or *in vivo* models. A summary of the conclusions obtained is shown in Table 21. As can be seen, the results are promising. The role of nanocapsules used as active substance carriers is highlighted in drug pharmacokinetic modification (Fawaz et al., 1996; Furtado et al., 2001b; Vila et al., 2002; Prego et al., 2006; Jeong et al., 2008), increased drug bioavailability (Calvo et al., 1997; Vila et al., 2002; Nassar et al., 2009), modification of drug biodistribution (Furtado et al., 2001b; Vila et al., 2002), the capacity to increase therapeutic effects (Dalençon et al., 1997; Vila et al., 2002; Prego et al., 2006; Pereira et al., 2006; Jeong et al., 2008; Schaffazick et al., 2008), the hepatotoxicity reduction (Pereira et al., 2006), biocompatibility with ocular mucosa (Calvo et al., 1997) and skin-barrier permeation (Joo et al., 2008). Likewise, surface modification achieved by hydrophilic copolymers shows a reduction of opsonization (Furtado et al., 2001a) whereas size reduction facilitates phagocytosis in view to attacking tumor cells (Seyler et al., 1999).

On the other hand, the results of the above mentioned research has also shown limitations of nanocapsules such as their lim-

Table 20
Nanocapsule stability studies as a function of preparation method.

Active	Polymer	Oil	Stability study conditions		Active concentration variation (%)	Reference
			Storage temperature	Sampling (months)		
Nanoprecipitation						
Ato-vaquone	PLA 200000	Benzyl benzoate	4 °C	0 and 4	27	Cauchetier et al. (2003)
	PLGA 40000			0 and 3	18	
	PCL 65000			0 and 4	10	
	PCL100000			0 and 4	3	
Indomethacin (1 mg/ml)	PCL 65000	Capric/caprylic triglycerides	Room temperature and protected from light	0 to 5	0	Pohlmann et al. (2008)
Indomethacin (3 mg/ml)				0 to 0.6	52	
Indomethacin (1.5 mg/ml)	PCL 60000	Mineral oil	Room temperature	0 to 3	5	Pohlmann et al. (2002)
			50 °C	0 to 3	50	
	PLA[a]		Room temperature	0 to3	10	
			50 °C	0 to 3	30	
Tretinoin	PCL[a]	Capric/caprylic triglycerides	UV radiation exposition	1 h	32	Ourique et al. (2008)
		Sunflower oil	UV radiation exposition	1 h	34	
Spironolactone	PCL 10000	Capric/caprylic triglycerides PEG esters	25 °C	0 and 6	0	Limayem et al. (2006)
Griseofulvine	PCL 80000	Benzyl benzoate	4 °C	0 and 6	0	Zili et al. (2005)
Diclofenac	PLA 88000	Benzyl benzoate	Room temperature and protected from light	0 to 8	10	Guterres et al. (1995)
		Capric/caprylic triglycerides			40	
Emulsion–diffusion						
Indomethacin	PCL 80000	Capric/caprylic triglycerides	25 °C	0–2.5	Remain stable	Limayem et al. (2004)
Eugenol	PCL 80000			0–2	Remain stable	Choi et al. (2009)
	PCL 80000	Capric/caprylic triglycerides	Protected from light 4 °C and 40 °C	0–5	Remain stable	Moinard-Chécot et al. (2008)

[a] Polymer molecular weight non-specified.

Table 21
In vitro and *in vivo* performance of nanocapsules.

Active	Test	Conclusion	Reference
Indomethacin	Pharmacokinetic study and potential irritant effect on the rectal mucosa in rabbits.	Nanocapsules enhance the extravascular distribution by enhancing the capture of the colloidal carrier by the liver and at the same time, increases the active elimination rates compared to active solutions. A limited protective effect on the rectal mucosa was shown.	Fawaz et al. (1996)
Indomethacin	Active ocular distribution and acute ocular tolerance studies in rabbits.	The nanocapsules displayed a good ocular tolerancy and an ocular bioavailability of indomethacin higher than for control solution.	Calvo et al. (1997)
Atovaquone	Antiparasitic activity.	Nanocapsules increases the therapeutic effect compared with active suspension.	Dalençon et al. (1997)
Muramyltripeptide cholesterol (MTP-Chol)	Immunomodulating capacity towards a mouse macrophage cell line *in vitro*.	MTP-chol included within biodegradable polymeric nanocapsules can activate mouse macrophages.	Seyler et al. (1999)
–	Biodistribution studies in mice of PEG–PLA nanocapsules against PLA nanocapsules.	Covalent attachment of PEG to the nanocapsule surface led to significant changes in the body distribution of the particles, the AUC and the mean residence time are higher that PLA nanocapsules.	Furtado et al. (2001b)
Tetanus toxoid	Absorption, biodistribution and inmunologic test in mice after oral and nasal administration.	PEG or chitosan coated nanocapsules were able to enhance the behaviour of absorption, biodistribution and inmunologic responses than PLA nanocapsules.	Vila et al. (2002)
Salmon calcitonin	Hypocalcemic effect in rats.	Pulsatile pharmacological profile and enhancement of the hypocalcemic effect when compared to the peptide solution.	Prego et al. (2006)
Usnic acid	Antitumor activity in Sarcoma 180-bearing mice and subchronic toxicity in healthy animals.	Nanoencapsulation was able to maintain and improve the usnic acid antitumor activity and considerably reduce the hepatotoxicity of this drug.	Pereira et al. (2006)
4-(N)-stearoylgemcitabine	Cytotoxic activity on human cancer cell lines.	Active incorporation in nanocapsules did not change the IC_{50} compared to the free active.	Stella et al. (2007)
Indomethacin ethyl ester	Antiedematogenic activity study in rats.	Nanoencapsulation was not able to target the pro-drug to the site of action and the antiedematogenic effect observed was exclusively due the metabolite formed *in vivo*.	Cattani et al. (2008)
Hinikitiol	Permeation study in hairless mice.	The active nanoencapsulated was more skin-permeable than active in propyleneglycol.	Joo et al. (2008)
Ciprofloxacin	*In vitro* and *in vivo* antibacterial activity test with *E. coli*.	*In vitro*, antibacterial activity of active nanoencapsulated shows less cytotoxic response than that of active free due probably to the nanocapsules sustained release behaviour. *In vivo*, the active nanoencapsulate can inhibit the growth of bacteria for a longer period rather than active free.	Jeong et al. (2008)
Melatonin	Acute antioxidant effect of intra-peritoneal administration in mice.	Increase in the antioxidant effect of the melatonin-loaded nanocapsules.	Schaffazick et al. (2008)
Tacrolimus	Pharmacokinetic study in rats and pigs.	Nanocapsules yielded significantly higher drug levels than an active emulsion, resulting in a more enhanced bioavailability.	Nassar et al. (2009)

ited protective effect on rectal mucosa (Fawaz et al., 1996); the non-reduction of certain toxic effects (Stella et al., 2007) and the non-achievement of expectations regarding their drug targeting performance (Cattani et al., 2008). Obviously, as has been mentioned, these results should be considered within the context of each research.

5. Discussion and concluding remarks

Nanoencapsulation is an attractive strategy for the vectorization of a variety of active substances. As is shown in Table 2, although with different objectives, research has been focused on antineoplastics, antiinflammatories, immunosupresants, antigens,

hormones, antivirals, antibacterials, antifungals, diuretics, antipneumocystics and vitamins, among others.

According to different authors, nanocapsules used as drug carriers can mask unpleasant tastes, provide controlled release properties and protect vulnerable molecules from degradation by external factors such as light or by enzymatic attack in their transit through the digestive tract (Furtado et al., 2001b; Whelan, 2001; Ourique et al., 2008). Likewise, they can increase the therapeutic efficacy of active molecules because their biodistribution follows that of the carrier, rather than depending on the physicochemical properties of the active molecule itself (Barratt, 2000). Additionally, although nanoencapsulated systems have a relatively higher intracellular uptake compared with microparticles, this behaviour can be modified depending on nanocapsule surface charges and the hydrophilic or hydrophobic nature of the polymer used in shell formation (Pinto et al., 2006a).

Therefore, research into nanocapsules obtained by nanoprecipitation, emulsion–diffusion, double emulsification, emulsion-coacervation, polymer-coating and layer-by-layer methods support some of these assertions. There is evidence of increased therapeutic efficacy and the role of nanoencapsulation in both drug release modification and absorption enhancement. What is more, it has been shown that strategies such as polymer modification in order to obtain more hydrophilic surfaces or polymer coatings to obtain positively charged surfaces could provide better *in vivo* performance. In addition, some studies have verified favorable behaviour regarding active substance stability in the case of encapsulation. Unfortunately, no experimental data on important aspects such as nanocapsule behaviour in masking unpleasant tastes was found in the literature.

Also, as with all nanoparticulated delivery systems, the nanosize range obtained for nanocapsules produced by all methods except layer-by-layer (all method between 250 and 500 nm, layer-by-layer upper 500 nm) allows their administration by different routes: oral, rectal, transdermal, ocular, nasal, subcutaneous, intraperitoneal and intramuscular and they can be injected directly into the systemic circulation without the risk of blocking blood vessels as suggested by some researchers (Barratt, 2000; Fattal and Vauthier, 2002; Letchford and Burt, 2007). However, it has been asserted that nanocapsules reduce the systemic toxicity of active substances (Whelan, 2001) and numerous reviews focusing on the state of knowledge of their behaviour and interaction with biological systems have been published and much concern remains on this subject (FDA, 2007).

On the other hand, bearing in mind that there are different alternatives for nanocapsule synthesis by using preformed polymers, the choice of a specific method is usually determined by the drug's physicochemical characteristics, particularly its solubility and the therapeutic objective of nanocapsule administration, for example the route chosen and drug release profile. Nevertheless, it is important to take into account that the method chosen should also considerer other aspects such as active substance stability under operational conditions, particularly stirring, encapsulation efficiency, method feasibility, the generation of contaminants and the need for subsequent purification steps, solvent nature, the water volume required and time consumption. Likewise, the feasibility of scaling-up and cost should be considered. However at the moment, there is not enough information to back up judgement on this matter.

Table 22 shows a comparative analysis of some of the criteria mentioned previously taking into account the author's experience and the information on nanoencapsulation research available in databases. Most of the research has been done at laboratory-scale.

As can be observed, there is no ideal method because each one has its advantages and limitations. In general terms, for example, all the methods allow lipophilic active substance encapsulation, excluding the double emulsification method which had been developed for hydrophilic active substances such as proteins. In their majority, all procedures can be used with solvents with low toxic potential and without the addition of other chemical substances that allow an easy purification. However, emulsion-coacervation is excluded and the polymer-coating and layer-by-layer methods require particular considerations on their procedure. From the point of view of water consumption, emulsion–diffusion is undoubtedly disadvantageous. Nevertheless this condition represents an advantage in terms of purification steps.

In relation to method feasibility and time consumption, it is only possible to make an approximation taking into account laboratory experiment and pilot scales. In principle, all the methods are feasible at laboratory scale and as is logical, some difficulties are predictable in their scaling-up. Nevertheless, since the time for assembly preparation is approximately the same for all the methods, nanoprecipitation, which requires the slow addition of the organic phase, provides poor results in terms of time consumption. Consequently, research into the use of a membrane contactor at the pilot scale is being performed to find a more efficient alternative (Charcosset and Fessi, 2005; Limayem et al., 2006). In spite of the method's advantages and limitations mentioned above, it is possible to identify trends in research into nanoencapsulation method selection. Therefore, taking into account a general review of the available information in electronic databases (Science direct® and Springerlink®) on nanoencapsulation research, the nanoprecipitation method patented by Fessi et al. (1988) is the most used (Fig. 12). It is valued for the simplicity of its procedure, low cost, reproducible carrier size and high encapsulation efficiency (Leroueil-Le Verger et al., 1998; Lamprecht et al., 2001; Chorny et al., 2002; Cauchetier et al., 2003; Pinto et al., 2006a). Approximately 50% of research has been developed in line with this method followed by emulsion–diffusion and double emulsification methods. Nevertheless, it is important to take into account that if the objective of research is hydrosoluble molecule encapsulation, the method preferred is double emulsification.

In view to obtaining the best results as a function of the target design of the nanocapsules, besides the researches developed on polymeric vesicles or polymersomes, the classical methods can be modified or combined as described in the methodologies proposed by Calvo et al. (1997), Bilati et al. (2005a,b,c) and Nassar et al. (2009) on nanoprecipitation method; Ma et al. (2001) and Perez et al. (2001) on emulsion–diffusion method and Perez et al. (2001), Romero-Cano and Vincent (2002), Vila et al. (2002) and Béduneau et al. (2006) on modified double emulsification methods. Likewise, the literature reports research on scaling-up nanocapsule production using membrane contactor based on the nanoprecipitation principle, after substantial modification of operational conditions (Charcosset and Fessi, 2005; Limayem et al., 2006).

The other methodologies are not used very often. Emulsion-coacervation historically was the first methodological approximation for preparation of nanocapsules through the research done by Krause and Rohdewald (1985) on triamcinolone acetonide nanoencapsulation using gelatine as a polymer. However, as already

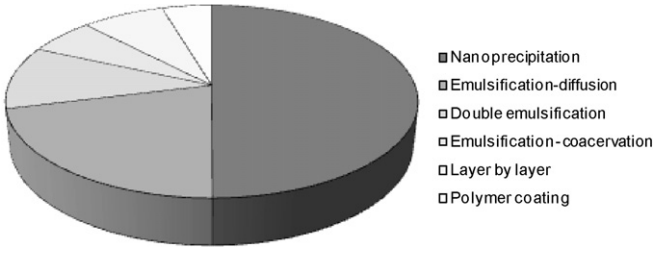

Fig. 12. Method selection trends in nanocapsule research.

Table 22
Comparative analysis of criteria suggested for the selection of nanoencapsulation methods.

Criterions	Nanoprecipitation	Emulsion–diffusion	Double emulsification	Emulsion-coacervation	Polymer-coating	Layer-by-layer
Active substance nature	Oil core: lipophilic	Oil core: lipophilic Aqueous core: hydrophilic	Aqueous core: hydrophilic Solid core: solid	Oil core: lipophilic Aqueous core: hydrophilic	Oil core: lipophilic	Oil core: lipophilic Solid core: solid
Active substance stability	High	High	Proteins can be denatured by high shear rate.	High	High	High
Solvent nature	Class 3	Class 3	Class 3/Class2	Class 3	Class 3	No required
Water volume consumption	Moderate	High	Moderate	Moderate	Moderate	Moderate
Method feasibility	High	High	High	High	High	High
Generation of contaminants	Low	Low	Low	Moderate	Low	Low
Purification steps	Low	Low	Low	High	Moderate	Moderate
Time consuming	High	Moderate	Low	Moderate	No reference available	No reference available

mentioned, this method requires an exhaustive purification process due to its inherent generation of nanocapsule dispersion contaminants, which is a major disadvantage in comparison with other alternatives.

On the other hand, the nanoencapsulation strategies such as polymer-coating and the layer-by-layer technique have shown interesting results, particularly in relation to *in vivo* nanocapsule behaviours since the final nanocapsule positive charge reduces their enzymatic degradation (Calvo et al., 1997). Such method is promising but needs more systematic and fundamental investigations.

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