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Preparation Techniques and Mechanisms of Formation of Biodegradable Nanoparticles from Preformed Polymers

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ABSTRACT

The techniques available to prepare biodegradable nanoparticles (nanospheres and nanocapsules) from preformed polymers are reviewed. Although there is abundant literature on this topic, only a few focus on the thorough analysis of preparative procedures. In particular, four techniques are discussed in terms of their technological advantages and drawbacks: emulsification evaporation, solvent displacement, salting-out, and emulsification diffusion. The proposed mechanism of nanoparticle formation for each technique is described from a physicochemical perspective. The effects of preparative variables on nanoparticle size and drug-entrapment efficiency are also discussed.

Key Words: *Nanoparticles; Nanospheres; Nanocapsules; Preparation methods; Mechanism of formation.*

INTRODUCTION

Ehrlich's idea about developing tiny particles (''magic bullets'') able to carry active molecules to specific sites in the body, where the therapeutic effect is required, has been retained as one of the principal goals in the pharmaceutical field. Various authors (1–4) have agreed that colloidal drug delivery systems hold great promise for reaching this idealistic goal. Colloidal carriers may take many forms: liposomes, niosomes, nanoparticles (NPs), and

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microemulsions. They are similar in their size, shape, and mode of administration (1,5). The systems most studied until now have been liposomal in nature. Since 1968, extensive research has been dedicated to their potential use as biocompatible drug-carrier systems, even though some fundamental problems, such as sensitivity of the phospholipid membranes to environmental degradation, rapid drug leakage across the phospholipidic bilayers, unreliable reproducibility, and difficult large-scale manufacture, have limited their development and commercialization (3,6,7). NPs appear to offer an interesting alternative. They possess better stability in biological fluids and during storage and their preparation is more amenable to scaling-up. In fact, a clear shift toward the NP domain has been taking place in the last decade. This is reflected by the increasing number of research articles, symposia, and patent applications involving the pharmaceutical use of NPs. In general, these have touched on three main aspects: preparative methods, physicochemical characterization and release properties, and drug-targeting issues. This review focuses only on the methods of preparation of NPs, because excellent reviews about the other two aspects are already available (8,9).

The materials used to prepare NPs may be classified broadly in two groups: synthetic polymers and natural compounds, such as proteins and lipids. Different methods have been proposed to prepare NPs of natural compounds, involving the use of heat or sonication, high volumes of organic solvents or oils, and toxic chemical crosslinking agents. Moreover, uncertainty of the source and purity of the macromolecule and potential antigenicity restrict the use of this type of NPs (3–5). Similar drawbacks have been reported for polymeric NPs prepared by polymerization of dispersed monomers. Not only are most NPs formed from these monomers not biodegradable, but byproducts may not be totally biocompatible, and toxic residues such as monomers, oligomers, and catalysts may persist. Furthermore, cross-reactions with the drug or degradation of the NP components, when radiation is used to induce polymerization, are probable (8,10). Considering the limitations of NPs obtained from natural molecules and by polymerization techniques, particularly if the NPs are designed for parenteral administration, we focus our attention on describing the methods involving synthetic-preformed biodegradable polymers. We look closely at the polyesters, such as poly(lactic acid) (PLA), poly(β-hydroxybutyrate) (PHB), poly(lactide-*co*-glycolide) (PLGA), and poly(ϵ-caprolactone) (PCL), which have shown good histocompatibility, biodegradability, and nontoxic byproducts. Furthermore, the safety of these polymers for human use has been extensively documented during the last three decades, and several drug-delivery systems designed with polyesters have been approved and commercialized (11–14).

CONCEPTS

NPs can be defined as solid colloidal particles containing an active substance that are produced by mechanical or chemical means. In terms of size alone, the lower limit of NPs is generally taken to be in the neighborhood of 5–10 nm and the upper size limit of ~1000 nm (1 μm), although the range generally obtained, in particular for the methods discussed here, is 100–500 nm. Some authors associate NPs only with nanospheres; however, for purposes of this review, the term NP is used as the collective name to describe both nanospheres and nanocapsules (1,6,8,15). The difference between these two forms lies in their morphology and body architecture. Nanospheres are formed by a dense polymeric matrix, whereas nanocapsules are composed of an oil core surrounded by a polymeric membrane (16–18).

PREPARATION METHODS

The methods for preparing NPs from preformed polymers can be classified into four categories: emulsion evaporation, solvent displacement, salting-out, and emulsification diffusion. These techniques are similar in that they involve an organic solution containing the NP components that functions as an internal phase during preparation and an aqueous solution containing stabilizers that constitute the dispersion medium for the NPs. Another similarity between the techniques is the poor encapsulation efficiency of moderately water-soluble and freely water-soluble drugs (including peptides and proteins), which partition out from the organic phase into the aqueous continuous phase. Despite considerable attempts, the techniques remain efficient only for lipophilic drugs. It is worth noting that although all the methods enable the preparation of nanospheres, only solvent displacement (19) and, more recently, the emulsification-diffusion technique (20) have enabled the preparation of nanocapsules.

Emulsification Evaporation

Emulsification evaporation is a well-established method based on the classical procedure patented by Vanderhoff et al. (21) for the preparation of pseudolatexes or artificial latexes. The preformed polymer and the

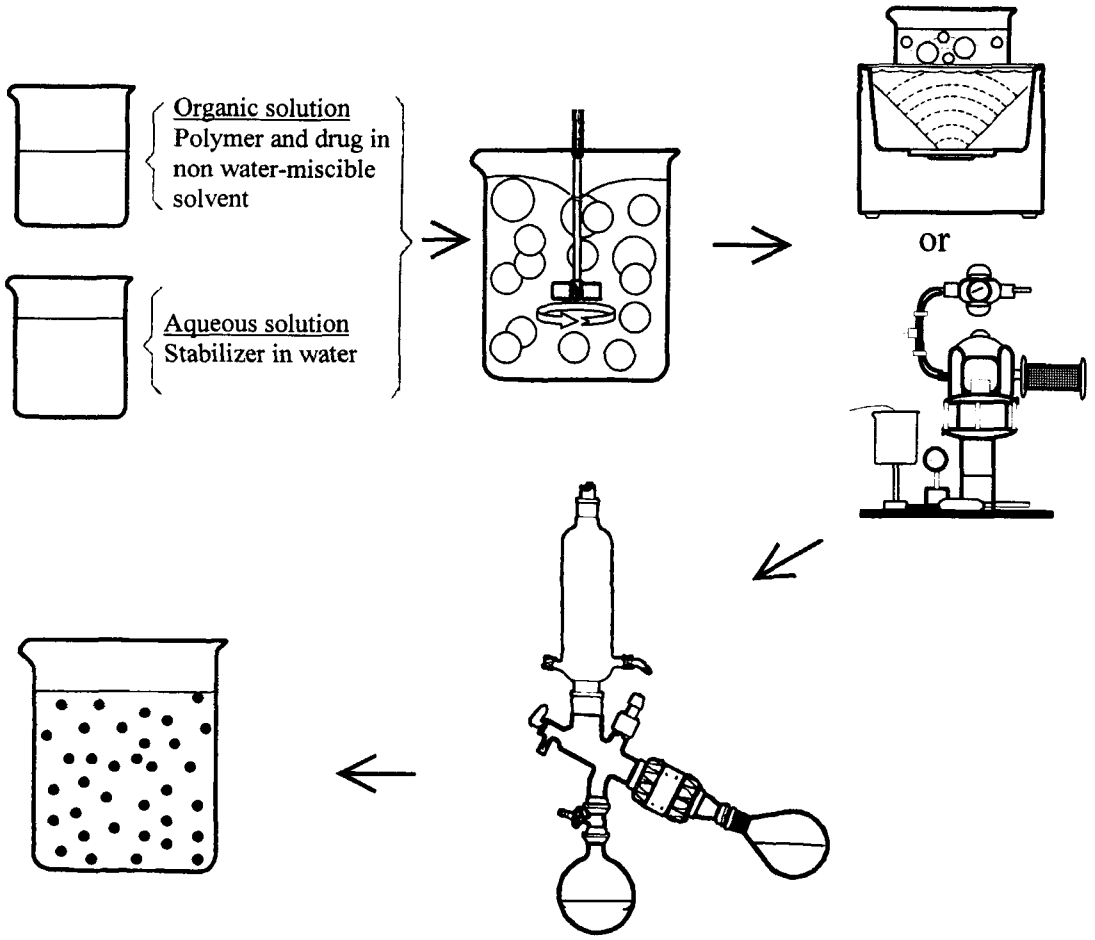

Figure 1. Schematic representation of the nanoparticle preparation by the emulsification-evaporation technique.

drug are dissolved in a water-immiscible organic solvent, which is emulsified in an aqueous solution. This crude emulsion is then exposed to a high-energy source such as ultrasonic devices or is passed through homogenizers, colloid mills, or microfluidizers to reduce the globule size. The subsequent removal of the organic solvent, by heat, vacuum, or both, results in the formation of a fine aqueous dispersion of nanospheres. A schematic representation of this method is shown in Fig. 1. The homogenization step is the determining factor in obtaining submicronic particles. Then, it is well known that the emulsification of oil and water by mechanical shear produces, in most cases, a droplet whose size is 2–5 μm and down to 1 μm in exceptional circumstances (22). On the other hand, the diffusional motion of water-immiscible solvents into the aqueous phase is slow (23), and once the limiting concentration for polymer precipitation is reached, phase separation occurs from the interface. Thus, each emulsion droplet forms one polymer particle when the solvent is removed. In general, a good emulsion homogenization produces droplets with a diameter below 0.5 μm (generally 0.1–0.3 μm) and thus a similar size is yielded for the nanospheres.

Ultrasonication is a convenient homogenization procedure; however, it presents some drawbacks: potential titanium contamination, high polydispersity of NPs, induction of chemical reactions or degradation, and difficult scaling-up (24–26). To overcome these problems, Bodmeier and Chen (27), and subsequently other authors, proposed the use of high-pressure homogenizers or microfluidizers. The principle of these devices is based on a submerged jet in which a single-feed stream is split into two fluidized streams, which are caused to interact with each other at ultrahigh velocities (pressures) in precisely

defined microchannels within an interaction chamber. This impingement is so arranged that it allows each unit volume of emulsion to be exposed to consistent forces (turbulence and cavitation), resulting in extremely fine droplets with narrow size distributions and better reproducibility. Control of the process results is achieved by alterations in stream velocities, residence time, and channel design. An interesting option with this technology is the possibility to scale-up to industrial size (28,29).

Table 1 lists some typical examples of NPs prepared by emulsification evaporation. In most cases, chlorinated solvents (chloroform and methylene chloride) were used because of their water insolubility, easy emulsification, solubilizing properties, and low boiling point. However, the disadvantage of these solvents is their toxicity (class 2 in the International Conference on Harmonization [ICH] guidelines for residual solvents [43]), and their use should be limited to protect patients from potential adverse effects. Poly(vinyl alcohol) (PVAL) and albumin have been preferentially used as colloidal stabilizers. PVAL has been shown to be an excellent stabilizer to prepare biodegradable NPs, not only by emulsification evaporation but with all techniques discussed here. Furthermore, it is one of the few stabilizers that avoids NP aggregation during postpreparative steps (e.g., purification and freeze drying), enhancing the yield of dry NP product without addition of other adjuvants (44). Nevertheless, PVAL has the disadvantage of not being accepted for i.v. administration. Although its i.v. safety has been revised recently and compared with that of poly(ethylene glycol) (45), use of PVAL for this route is not recommended. Albumin has been proposed as a biodegradable substitute for PVAL. Solvent evaporation and microfluidization appeared not to damage the albumin molecules, and the immunogenicity of the albumin adsorbed on NPs is the same as that of native albumin solution (39). However, the source (human or bovine) and the purity of this macromolecule are aspects that could limit its utilization.

The type and concentration of colloidal stabilizer and the phase volume ratio have been reported to affect NP size and polydispersity for this technique. Julienne et al. (46) reported that nanospheres could be formed with high-speed stirring (10,000 rpm/10 min), using 0.5% w/v of PVAL, whereas with methylcellulose at the same concentration, particles greater than 1 μm were obtained. The authors stated that this difference was due to the more efficient reduction of interfacial free energy produced with PVAL. The same study showed a significant influence of the volume phase ratio and the polymer concentration on the mean particle size and the coefficient of variation, whereas the polydispersity was also affected by the homogenization pressure.

Other workers using PLGA (36,38) and PLA (25) found that a particle-size reduction and narrow polydispersity were obtained by lowering the polymer concentration, and they attributed this effect to a decrease of the dispersed phase viscosities, which caused the formation of smaller emulsified droplets and hence produced NPs. Scholes et al. (36) showed that increasing the stabilizer concentration had a biphasic effect on the particle size of PLGA nanospheres, causing first a decrease for concentrations of PVAL between 1 and 8% w/v, followed by an increase for concentrations up to 15% w/v PVAL. Thus, it seems that a balance exists between an enhanced stabilization and an optimal mixing efficiency under a given set of emulsification conditions with higher PVAL concentrations. Apparently, there exists a critical continuous phase viscosity, beyond which homogeneous emulsification is not possible, and particle size and polydispersity increase again. On the other hand, Scholes et al. (36) reported a decrease on particle size by lowering PVAL molecular weight, which was attributed to a decrease in the viscosity and an apparent enhanced stabilizing ability of the lower molecular weights of PVAL. This effect may give rise to the increasing number of polymer chains with decreasing PVAL molecular weight for a constant surfactant concentration. Verrecchia et al. (33) also found that the size of PLA NPs depended on the concentration of albumin used. At concentrations below 0.25% w/v, the emulsion was unstable, precluding the formation of NPs. Above 0.25% w/v, the size decreased rapidly as a function of the albumin concentration and reached a value close to 100 nm for a concentration of 1.5% w/v.

Jalil and Nixon (47) pointed out that successful entrapment of drug within the biodegradable microspheres prepared by emulsification evaporation is highly dependent on solubility in the aqueous phase. This statement is also applicable to NPs. Drugs with low water solubility, such as testosterone, triamcinolone acetonide, and cyclosporin A, were successfully retained within NPs (25,30,34). In contrast, water-soluble drugs could not be entrapped. Interestingly, the entrapment of loperamide hydrochloride in PLA NPs can be markedly improved by adjusting the pH (41). The higher pH of the aqueous phase prevented ionization of loperamide hydrochloride (pK_a = 8.7), resulting in an increase in partitioning of drug into the organic phase. Batches prepared with pH 4.0 acetate buffer gave an entrapment efficacy of 1.1%, whereas with pH 7.4 phosphate buffer, the entrapment efficacy increased up to 64.7%. These workers also re-

Table 1

Examples of Nanospheres Prepared by the Emulsion-Evaporation Procedure

Polymer	Solvent (v/v)	Emulsifiers (% w/v)[a]	Homogenization Device	Size ± SD (nm)	Drug (% w/w)[b]	Entrapment Efficiency (%)	Reference	Year
PLA	nr	Poloxamer 188 or polysorbate 80 or sodium lauryl sulphate (nr)	nr	450 ± 16	Testosterone (nr)	nr	30	1981
PLA	CH_2Cl_2	Gelatin (0.5)	Ultrasonication (45 min)	476 ± 410 to 710 ± 406	Triamcinolone acetonide (nr)	64.5–75.4	25	1985
PLA	$CHCl_3$	PVAL (0.5)	Mechanical stirrer (25 000 rpm)	216 ± 54 to 316 ± 41	Roxithromycin	4.4	31	1989
PCL	CH_2Cl_2	PVAL (0.5)	Microfluidizer (4000 psi/8 cycles)	314 ± 26	ni	—		
PLA and PCL	$CHCl_3/CH_3COOC_2H_5$ (1:1)	Poloxamer 188 or potassium oleate or sodium dodecyl sulphate or polysorbate 20 (0.3–1.0)	Microfluidizer (6000 psi/4 cycles)	227–405	ni	—	32	1992
PLA	CH_2Cl_2	Albumin (0.25–1.5)	Microfluidizer	100–425	ni	—	33	1992
PLGA	CH_2Cl_2	PVAL (0.5)	High-speed homogenizer (10,000 rpm)	298 ± 7	Cyclosporin A (10)	59.8	34	1993
PLA, PLGA, and PHB	CH_2Cl_2	PVAL (0.5) or sodium dodecyl sulphate (0.2)	Ultrasonication	nr	ni	—	35	1993
PLGA	CH_2Cl_2	PVAL (2–15)	Ultrasonication	90–500	ni	—	36	1993
PLA	$CH_3COO\ C_2H_5$	Poloxamer 188 or polysorbate 60, polyoxyl 40 stearate, or mixtures (0.3–2.0)	Ultrasonication (1 min)	250–386	ni	—	37	1994
PLA	CH_2Cl_2	Polysorbate 80 (1.0)	Microfluidizer (10, 000 psi/25 cycles)	175 ± 10	ni	—	38	1994
PLGA	CH_2Cl_2	Albumin (0.3–1.5)	Microfluidizer	nr	IBP 5823 antisubstance P drug (20)	nr	39	1995
PLA	CH_2Cl_2	Albumin (1.0)	Microfluidizer (6000 psi/2 min)	100	ni	—	40	1996
PLA	CH_2Cl_2	PVAL (0.3–2.0)	Microfluidizer (6000/3 cycles)	183 ± 7 to 265 ± 5	Loperamide (10)	1.3–69.2	41	1985
PLA	CH_2Cl_2	Albumin or PVAL (1.0)	Microfluidizer (6000 psi)	nr	Prodan (0.07)	nr	42	1997

[a] In the aqueous phase.
[b] Referred to the amount of polymer.
nr, not reported; ni, not incorporated; PHB, poly(β-hydroxybutyrate).

ported that an evaporation of the organic phase under reduced pressure and the addition of ethanol in the organic phase yielded a high drug entrapment, presumably because of the rapid polymer desolvation, which prevents or slows down the diffusion of the drug into the aqueous phase. Furthermore, the addition of lipophilic surfactants, such as sorbitan fatty acid esters, increased the drug entrapment even at higher drug concentrations. Recently, Blanco and Alonso (48) used the double emulsion (water-in-oil-in-water) technique to develop protein-loaded PLGA nanospheres. Although the results are encouraging (entrapment efficiencies up to 90%), further studies are necessary to establish the real applicability of this technique to encapsulation of hydrophilic biomolecules.

Solvent Displacement

This technique was first described and patented by Fessi et al. (19). In this process, polymer, drug, and optionally a lipophilic stabilizer (e.g., phospholipids) are dissolved in a semipolar water-miscible solvent, such as acetone or ethanol. This solution is poured or injected into an aqueous solution containing a stabilizer (e.g., PVAL or poloxamer 188) under magnetic stirring. NPs are formed instantaneously by rapid solvent diffusion, which is then eliminated from the suspension under reduced pressure. This simple technique is shown schematically in Fig. 2. The mechanism of formation of NPs by this technique has been explained by the interfacial turbulence generated during the solvent displacement (49,50). Subsequently, a violent spreading is observed because of mutual miscibility between the solvents. Droplets of solvent, probably of nanometric size, are torn from the interface. These droplets are rapidly stabilized by the stabilizing agent, until diffusion of the solvent is complete and polymer aggregation has occurred.

Davies and Rideal (51) suggested that the interfacial turbulence is caused by localized lowering of the interfacial tension where the oil phase undergoes rapid and erratic pulsations or "kicks," each of which is quickly damped out by viscous drag. The energy necessary for these jerky movements comes from the free energy re-

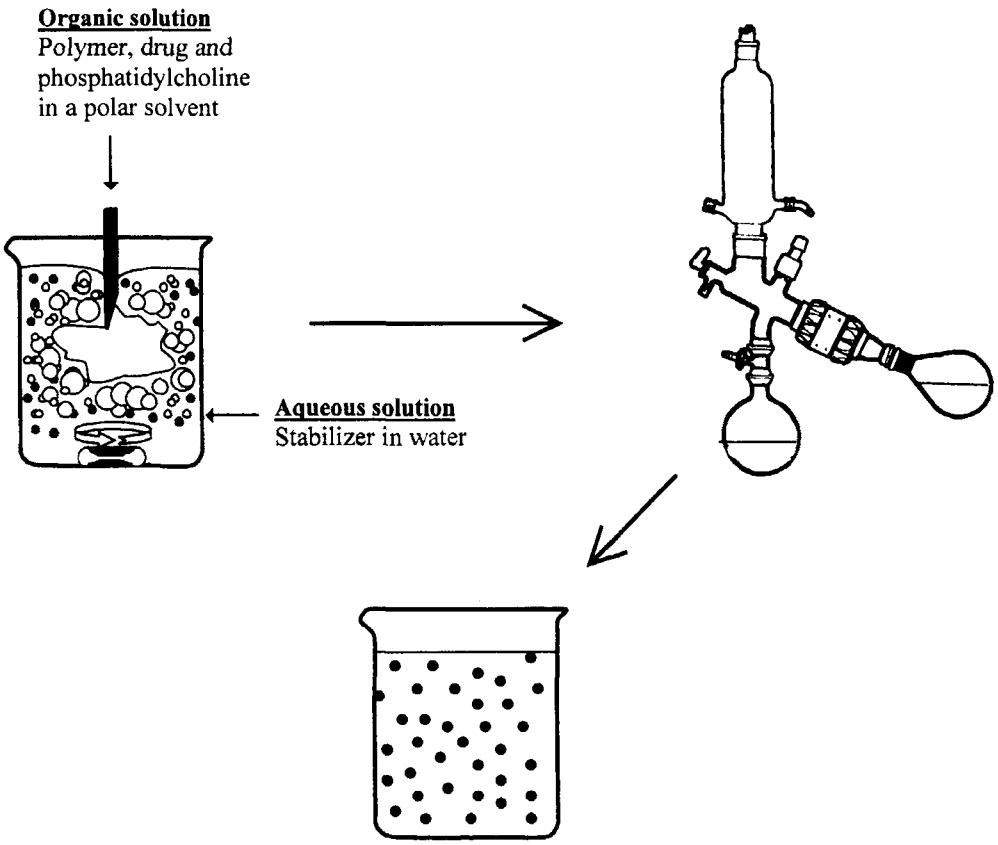

Figure 2. Schematic representation of the nanoparticle preparation by the solvent displacement technique.

leased as the solvent is redistributed to its equilibrium state. The molecular mechanism of interfacial turbulence could be explained by the continuous formation of eddies of solvent (e.g., acetone) at the interface. Such eddies may originate either during drop formation or in thermal inequalities in the system. Thus, once the process has started, movements associated with previous kicks change the pressure inside the solvent by increasing the surface pressure or decreasing the interfacial tension. Thus, if the solvent droplets formed contain polymer, these will tend to aggregate and form NPs because of the continuous diffusion of solvent and because of the presence of a nonsolvent medium. These events are represented in Fig. 3.

The term nanoprecipitation (26,52,53) is frequently used to define this process; however, it is important to point out that according to the mechanism described, the formation of NPs is due to polymer aggregation in stabilized emulsion droplets, and the nucleation and growth steps are not apparently involved. The usefulness of this technique is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvents produce a certain instability when they are mixed in water, no spontaneous emulsification is observed if the coalescence rate of the formed droplets is high enough (54). Also, the technique can be used only for drugs soluble in this type of solvents. Although some authors (Table 2) used acetone/dichloromethane to dissolve and increase the entrapment of drugs, the presence of dichloromethane increased the mean particle size (58), and as previously mentioned, this solvent is considered toxic.

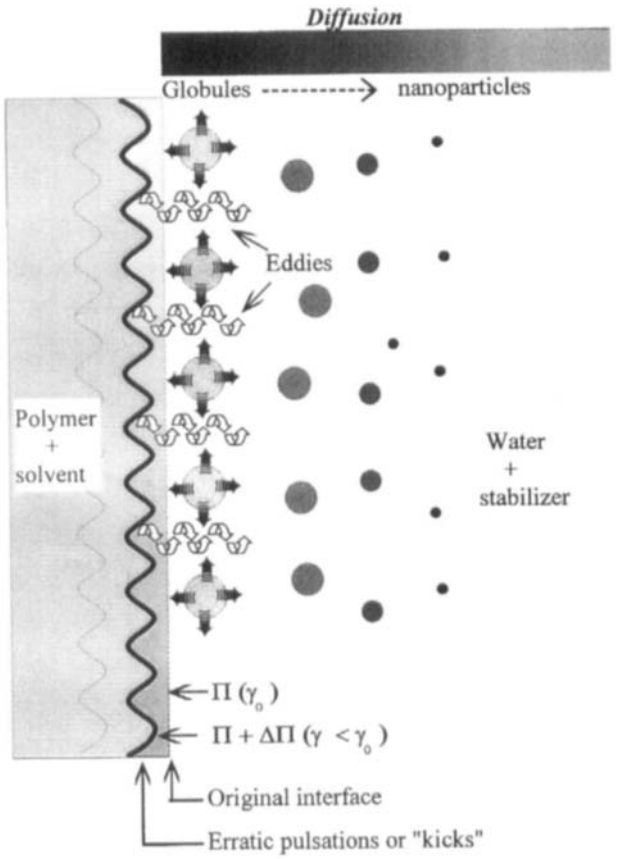

Figure 3. Schematic representation of nanoparticle formation by solvent displacement based on the interfacial turbulence mechanism, where π and γ represent the surface pressure and the interfacial tension, respectively.

A major drawback of this technique is the difficulty to choose a drug/polymer/solvent/nonsolvent system in which NPs would be formed and the drug efficiently entrapped (8,59). Stainmesse et al. (53) found that PCL NPs could be prepared under restricted conditions, corresponding to a very narrow area of the PLC/acetone/water-phase diagram. Other authors (57–59) showed that the mean size and the recovery of nanospheres were significantly affected by the concentration of polymer. This behavior was attributed to the huge increase in the viscosity of the organic phase. On the other hand, Niwa et al. (56) showed that the recovery of PLGA nanospheres increased with increasing stabilizer concentration, whereas the mean size was independent of PVAL concentration. Similar results were obtained by Molpeceres et al. (52) for PCL nanospheres, using poloxamer 188 as the stabilizer. In contrast, Murakami et al. (61) found recently that the mean diameter of PLGA NPs tended to increase when the concentration of PVAL was increased. Apparently, this effect was related to the high concentrations of PVAL (2–6% w/v), which increased the viscosity of the aqueous medium, decreasing the diffusion rate of the solvent. The authors also reported that the yield, particle size, and redispersibility of the NPs varied drastically, depending on the PVAL grade used. Low-hydrolyzed PVAL was noticeably more efficient than its highly hydrolyzed counterpart.

Solvent displacement is not an efficient means to encapsulate water-soluble drugs. Niwa et al. (56) studied the efficiency of this technique to entrap indomethacin and 5-fluorouracil as examples of poorly water-soluble and water-soluble drugs, respectively. They found that indomethacin was efficiently encapsulated in PLGA NPs and that its entrapment can be increased up to 75% by controlling the pH of the aqueous medium to a lower value than the pK_a of indomethacin (4.5). On the contrary,

Table 2

Examples of Nanospheres Prepared by the Solvent-Displacement Procedure

Polymer	Solvent (v/v)	Emulsifiers (% w/v)[a]	Size ± SD (nm)	Drug (% w/w)[b]	Entrapment Efficiency (%)	Reference	Year
PCL	CH_3COCH_3	Poloxamer 188 (nr)	95 ± 25	Cyclosporin A (4.6)	84.9	55	1993
PLGA	CH_2Cl_2/CH_3COCH_3 (1.0:50.0)	PVAL (2.0)	338 ± 67 to 637 ± 40	Indomethacin (10)	33.0–50.0	56	1993
PLGA	$CH_2Cl_2/CH_3COCH_3/CH_3OH$ (1.0:25.0:5.0)	PVAL (2.0)	195 ± 34 to 207 ± 13	5-Fluorouracil (10)	1.6–15.0	56	1993
PLGA	CH_3COCH_3	ni	208 ± 4	ni	—	57	1995
PLA	CH_2Cl_2/CH_3COCH_3 (1:20)	Poloxamer 188 (0.5)	75–600	Indomethacin (6.2)	nr	58	1995
PLA	CH_2Cl_2/*N*-methyl-2-pyrrolidone (1:25)	PVAL (2.0)	163–188	Zinc phthalocyanine (2.5–4.8)	51.7-56.7	59	1996
PCL	CH_3COCH_3	Poloxamer 188 (0.1–0.4)	113 ± 10 to 215 ± 17	Cyclosporin A (0.6–2.5)	90.5–98.0	52	1996
PLGA	CH_3COCH_3	Poloxamer 407 or poloxamine 904 or 908 (1.0)	83.4 ± 2 to 192.9 ± 3	Indium-111 (nr)	80	60	1997

[a] In the aqueous phase.
[b] Referred to the amount of polymer.
nr, not reported; ni, not incorporated.

5-fluorouracil was poorly encapsulated because of considerable leakage of drug into the aqueous phase during preparation. They proposed that a moderate increase in the 5-fluorouracil entrapment efficiency can be obtained by accelerating the deposition rate of polymeric film on the droplet, by modifying the solvent composition, or by increasing the molecular weight of PLGA. In a similar study (62), these authors proposed that the encapsulation of nafarelin acetate (a water-soluble peptide drug) was improved by blending low-molecular-weight PLGA with higher-molecular-weight PLGA because of the synergistic effect of the rapid deposition of polymer and the ionic interaction between nafarelin acetate and PLGA. Furthermore, they claimed that the leakage of drug into the aqueous phase can be decreased by compounding phospholipids such as dipalmitoylphosphatidylglycerol or diacetyl phosphate, which are negatively charged. Apparently, these charges are capable of forming ion pairs with the positively charged groups (histidil and arginyl residues) of nafarelin, making it more lipophilic.

The solvent-displacement technique allows the preparation of nanocapsules when a small volume of a nontoxic oil is incorporated in the organic phase. Two aspects seem important in the selection of this oil: first, it must not degrade the polymer and second, it must be a good solvent for the drug to prevent drug leakage and to reduce spontaneous crystallization of the drug during preparation (63,64). Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. Some examples are summarized in Table 3.

Salting-Out

In 1988, Bindschaedler et al. (65) patented a new procedure to prepare pseudolatexes, which was subsequently adapted and optimized by Allémann (66) and Leroux (67), for drug-loaded biodegradable nanospheres. This method is based on the separation of a water-miscible solvent from aqueous solutions via a salting-out effect. Acetone is generally chosen as the water-miscible solvent because of its solubilizing properties and its well-known separation from aqueous solutions by salting-out with electrolytes (68). Polymer and drug are thus dissolved in acetone, and this solution is emulsified under vigorous mechanical stirring in an aqueous gel containing the salting-out agent and a colloidal stabilizer. This oil-in-water emulsion is diluted with a sufficient volume of water or

Table 3

Examples of Nanocapsules Prepared by the Solvent-Displacement Procedure

Polymer	Solvent	Oil (% v/v)[a]	Emulsifiers (% w/v)[b]	Size ± SD (nm)	Drug (% w/w)[c]	Entrapment Efficiency (%)	Reference	Year
PLA	CH_3COCH_3	Benzyl benzoate (1:50)	Poloxamer 188 (0.5)	229 ± 29	Indomethacin (10)	~100	49	1989
				260 ± 20	Taxol (10)	~100		
				300 ± 25	Dexamethasone (10)	40		
				270 ± 30	Vitamine K (10)	~100		
PLA	CH_3COCH_3	Benzyl benzoate (1:50)	Poloxamer 188 (0.5)	229 ± 29	Indomethacin (10)	96	65	1990
PLA	CH_3COCH_3	Ethyl oleate (1:50)	Poloxamer 188 (0.5)	250 ± 30	MDP-L-alanyl-cholesterol (5.7)	96	66	1993
PLA	CH_3COCH_3	Benzyl benzoate or Myg-liol 810 (1:30)	Poloxamer 188 (0.8)	nr	Diclofenac (nr)	~100	63	1995
PLA	CH_3COCH_3	Soybean oil (1:60)	Poloxamer 188 (0.3–0.5)	215–310	Clofibrate (10–60)	~100	64	1995

[a] Refered to the volume of solvent.
[b] In the aqueous phase.
[c] Referred to the amount of polymer.
nr, not reported.

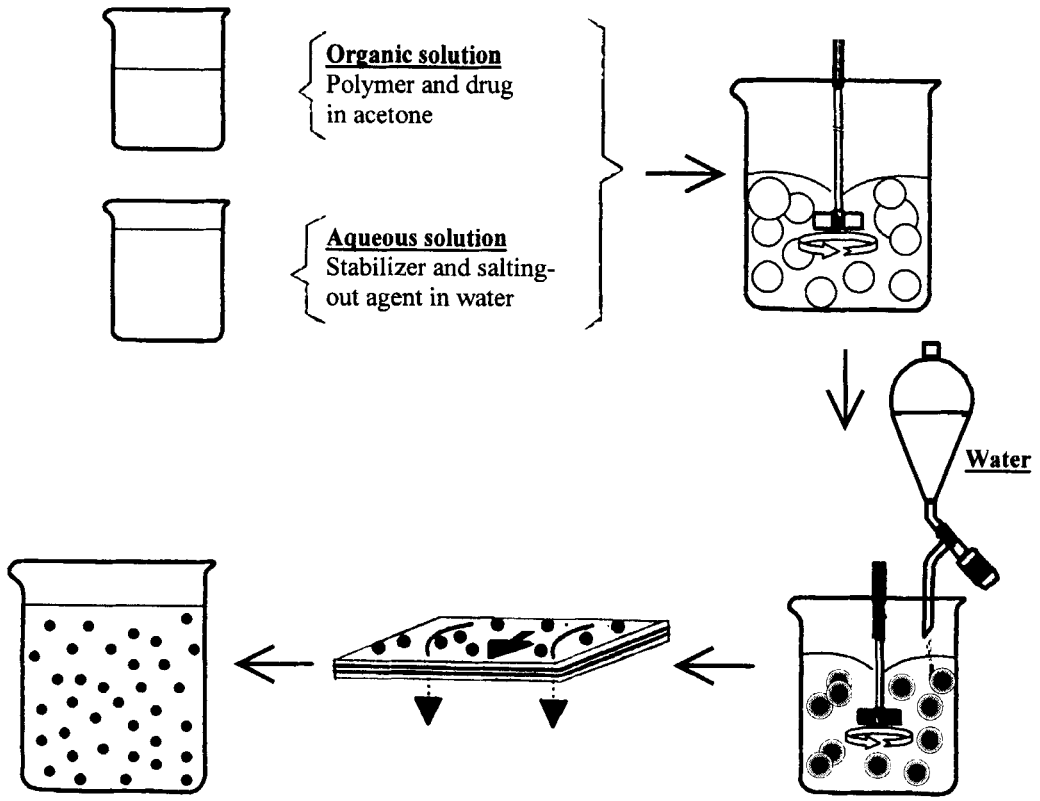

Figure 4. Schematic representation of the nanoparticle preparation by the salting-out technique.

aqueous solutions to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. Solvent and salting-out agent are then eliminated by cross-flow filtration. The preparative steps of this procedure are shown in Fig. 4. Although the mechanism of formation has not been proposed, this can have a certain similarity to that observed for solvent displacement. Thus, the diffusion of acetone from the droplets during the dilution step can generate interfacial turbulence and polymer aggregation in nanospheres.

The major advantages of this technique are the possible incorporation of high amounts of polymer and drug, the excellent yields generally obtained, and the easy scaling-up of the process. Furthermore, once the solvent/salting-out agent/stabilizer system has been found, it is not necessary to search for specific proportions to obtain drug-loaded nanospheres.

On the other hand, the technique is limited to lipophilic drugs, salting-out agents that enable phase separation without precipitation, and soluble stabilizers compatible with saturated aqueous solutions and not coacervating in the presence of solvent. Salting-out agents for acetone are the electrolytes magnesium chloride, sodium chloride, calcium chloride, and magnesium acetate (69,70) and one nonelectrolyte, sucrose (68). The selection of the salting-out agent is important because it can play an important role in the drug-entrapment efficiency. For example, Allémann et al. (71) reported that the entrapment efficiency of savoxepin (pK_a = 8.3) can be significantly improved by using a basic salt (magnesium acetate) instead of neutral or acid salts. Leroux et al. (72) showed the opposite case for a novel human immunodeficiency virus type 1 protease inhibitor. With regard to the stabilizers, only three have been successfully used, namely PVAL, polyvinyl pyrrolidone, and hydroxyethyl cellulose (69).

Several factors have been reported as influencing the mean size of the NPs. Allémann et al. (70) found that when increasing the PVAL concentration in the external phase of the emulsion, a decrease in particle size is observed. An increase in stirring rate and polymer concentration allow only a slight reduction of particle size. In contrast, the internal-external phase ratio seems not have significant influence on the size.

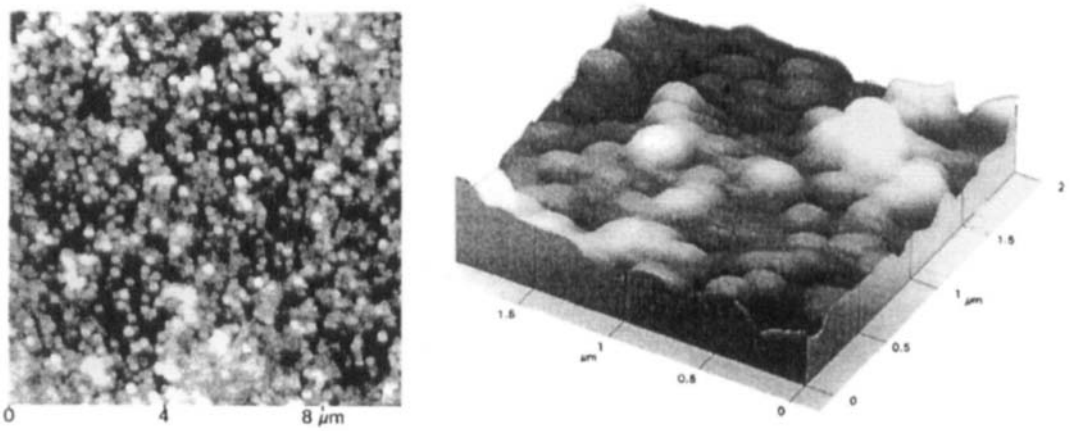

Figure 5. Images obtained by atomic force microscopy (noncontact mode) of PLA nanospheres prepared by emulsification-diffusion using ethyl acetate and PVAL as solvent and stabilizer, respectively.

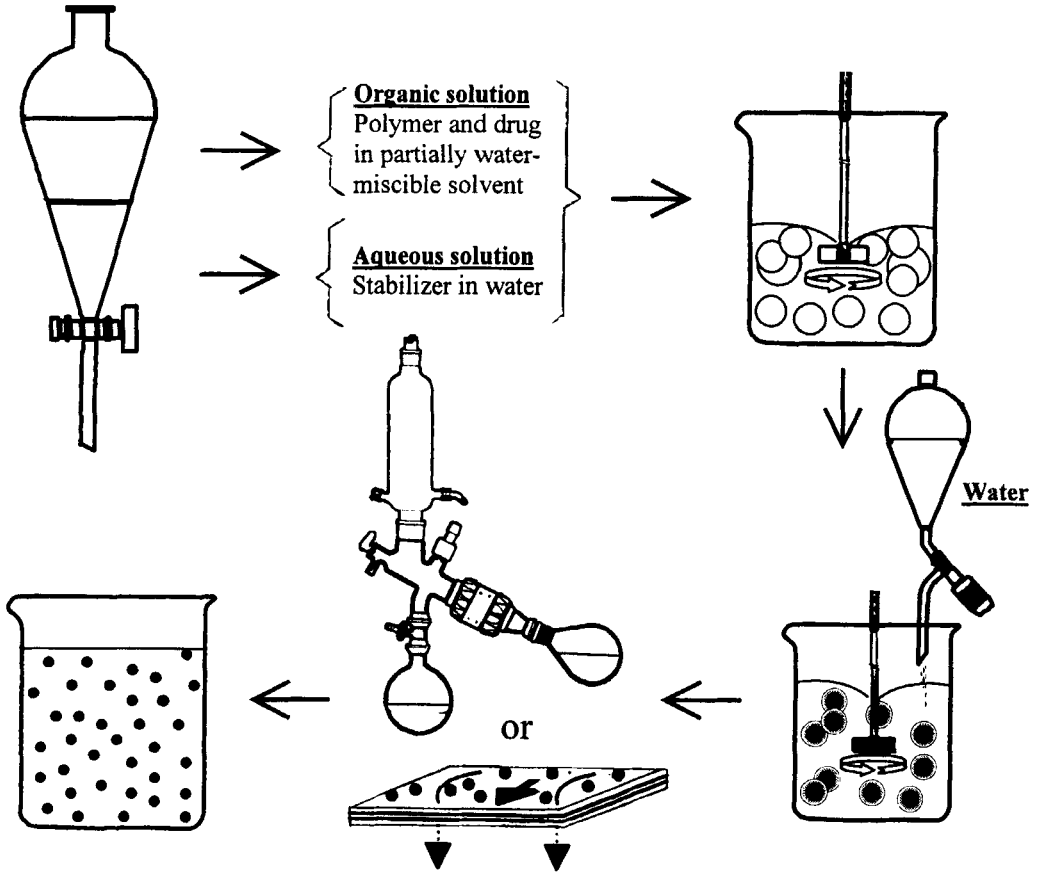

Figure 6. Schematic representation of the nanoparticle preparation by the emulsification-diffusion technique.

Emulsification-Diffusion

This method can be considered as a modification of the salting-out procedure, avoiding the use of salts and hence intensive purification steps. It involves the use of a partially water-soluble solvent, which is previously saturated in water to ensure the initial thermodynamic equilibrium of both liquids. Polymer is dissolved in the water-saturated solvent, and this organic phase is emulsified, under vigorous agitation, in an aqueous solution containing a stabilizer. The subsequent addition of water to the system causes the solvent to diffuse into the external phase, resulting in the formation of NPs (Fig. 5). Depending on the boiling point of the solvent, this can be eliminated by distillation or cross-flow filtration. The procedure is illustrated in Fig 6.

The mechanism of formation by this method has been reviewed under different preparation conditions and by turbidimetry measurements (50). It has been shown that each emulsion droplet produces several NPs and that these are formed by interfacial phenomena during solvent diffusion. However, these phenomena cannot be entirely explained by the convection effects caused by interfacial turbulence. Therefore, it is suggested that NPs are formed because of a physicochemical instability produced by solvent transport by a similar mechanism to that used to explain spontaneous emulsification processes (diffusion-stranding mechanism). The basic idea is that diffusion of solvent 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. The stabilization of these "protonanoparticles" by the presence of a stabilizer is very important to avoid their coalescence and the formation of agglomerates. Thus, if the stabilizer remains at the liquid–liquid interface during the diffusion process and if its protective effect is adequate, then NPs will form after complete diffusion of solvent (Fig. 7).

Benzyl alcohol, propylene carbonate, and ethyl acetate have been successfully used to prepare biodegradable NPs by this technique. Regarding the stabilizer, its selection will depend on its ability to form stable emulsions

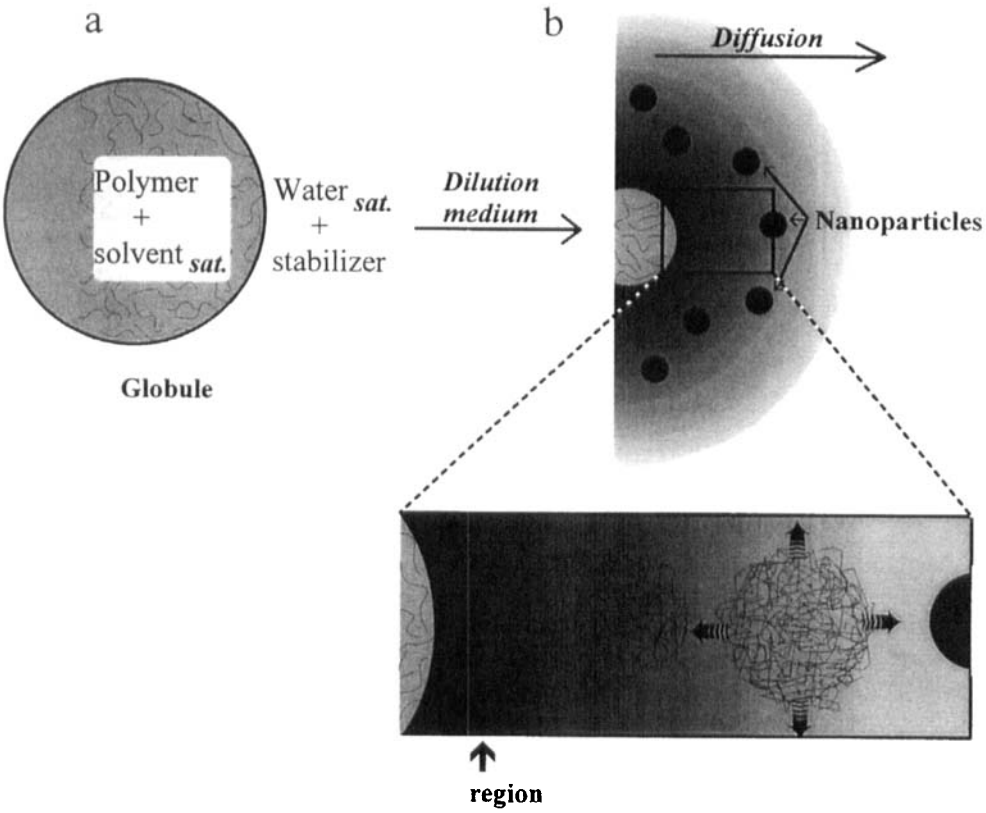

Figure 7. Schematic description of the proposed formation mechanism of nanoparticles by the emulsification-diffusion based on the diffusion-stranding mechanism. (a) Before the diffusion step; (b) during the diffusion step.

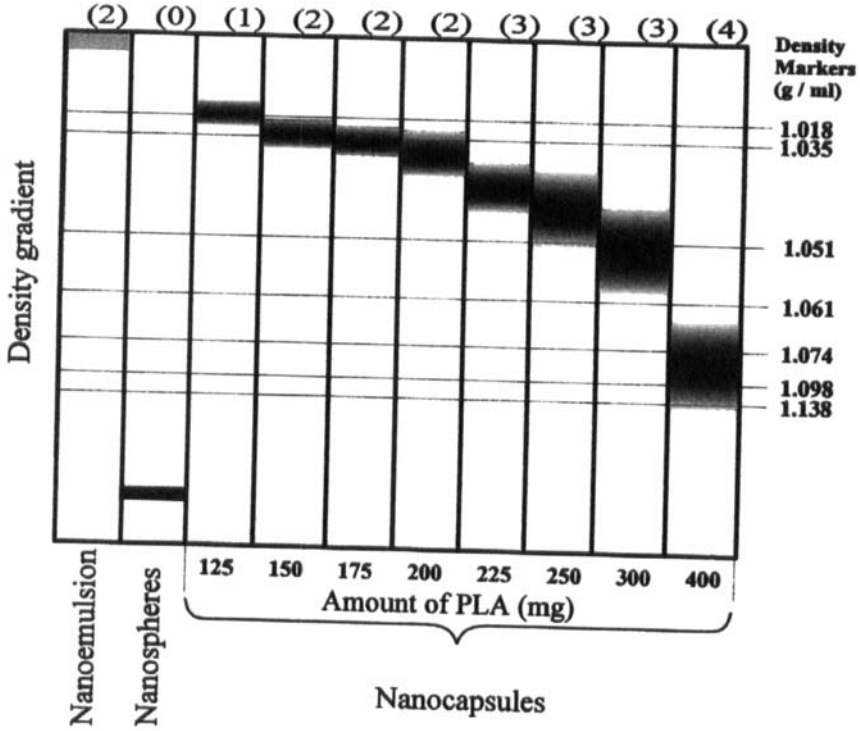

Figure 8. Bands of nanocapsules, nanospheres, nanoemulsion, and external standards (g/ml) generated in gradients of Percoll® by ultracentrifugation. Batches of nanocapsules were prepared using 0.5 ml of Myglio®l and different amounts of PLA. The numbers in parentheses represent the polydispersity of the systems.

to ensure the NP formation. For example, we reported that PLA NPs can be prepared using PVAL or poloxamer 188 as stabilizer and propylene carbonate as solvent. However, large aggregates were obtained when polysorbate 80 or dextran was used, suggesting that these agents did not form stable emulsions nor did they prevent globule coalescence during the diffusion (44). This technique presents some advantages over the other methods described above, such as the use of pharmaceutically acceptable organic solvents, no need of homogenization steps, high yields generally obtained, high batch to batch reproducibility, and ease of scaling-up.

Two drawbacks need to be mentioned: the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification.

As with the other techniques, emulsification-diffusion is efficient in encapsulating lipophilic drugs. We tried to encapsulate a hydrophilic dipeptide (Trp-Leu) in PLA NPs using the Trp-Leu in the form of an ion pair in propylene carbonate. A low entrapment efficiency (<3%) was always obtained (73). This result was attributed to the leakage of the peptide contained in the globules into the aqueous phase during solvent diffusion. Apparently, the velocity of ion pair dissociation was faster than polymer precipitation.

Several preparative variables have been reported to affect the size of the NPs. We found that a high concentration of polymer and a low internal-external ratio rapidly increase the size and polydispersity of PLA NPs prepared using propylene carbonate. This behavior was explained by the greater probability that the desolvated macromolecules (or small aggregates formed from these molecules) coalesce in a more concentrated solution, thereby forming larger particles. Similar results have been reported by Leroux et al. (74) using benzyl alcohol. The concentration of stabilizer (PVAL or poloxamer 188) influenced NP size only at low concentrations (<5% w/v in the external phase). Other process parameters, such as viscosity and pH of the external phase, had limited effect on NP size.

Recently, we reported that emulsification diffusion can be used to prepare biodegradable nanocapsules (20). Density gradient centrifugation was used to confirm the formation of nanocapsules (Fig. 8). The density was found to be intermediate between those of nanoemul-

Table 4

Properties and Entrapment Efficiency for Nanocapsules Prepared Using the Emulsification-Diffusion Procedure with Different Oil Core/Polymer/Drug Systems

Example	Oil[a]	Polymer[b]	Subtance (mg)	Mean size ± SD[c] (nm)	PI[d]	Entrapment Efficiency (%)
1	Mineral oil	PLA	Sudan III (5)	303 ± 3	2	98.0
2	Mygliol	PLA	Sudan III (5)	340 ± 4	2	100.8
3	Mygliol	PCL	Sudan III (5)	346 ± 4	2	98.9
4	Mygliol	PLA	Indomethacin (20)	335 ± 3	2	102.0
5	Mygliol	PCL	Indomethacin (20)	314 ± 2	2	94.4
6	Mygliol	PLA	Progesterone (20)	510 ± 3	1	98.7
7	Mygliol	PLA	Estradiol (20)	340 ± 2	1	52.0
8	Mygliol	PLA	Chlorambucil (20)	335 ± 3	2	32.1
9	None	PLA	Clofibrate (400)	370 ± 5	4	95.3
10	None	PLA	Vitamin E (470)	360 ± 3	4	92.2

[a] Incorporated in a ratio 1:40 with respect to the solvent.
[b] 1% w/v in 20 ml of ethyl acetate.
[c] $n = 3$.
[d] PI: Polydispersity index expressed using a 0–9 scale.

sions and nanospheres prepared under the same conditions. The existence of a unique density band indicated high yields. Nanocapsule density was a function of the original oil-polymer ratio, revealing that the polymer content, and consequently the wall thickness, can be controlled by this method. High entrapment efficiencies were obtained for different lipophilic substances (Table 4).

CONCLUSIONS

Several techniques to prepare biodegradable NPs have been developed during the last two decades. Their evolution has been marked by three aspects: search for less toxic ingredients, simplification of the procedure to make it susceptible to scaling-up, and optimization of the technique in terms of yields and entrapment efficiency. Great technological advances have been achieved; simple, safe, and reproducible techniques are now available to prepare drug-loaded nanospheres and nanocapsules. Therefore, from an industrial point of view, transition to large scale is potentially possible. Nevertheless, there are several issues that remain to be solved, principally the encapsulation of hydrophilic drugs (including peptides and proteins). In this respect, the development of a technique that enables the incorporation of biomolecules without affecting their activity constitutes a fundamental goal for the pharmaceutical nanotechnologist. In addition, the postpreparative steps, such as purification and preservation (in particular for nanocapsules) and residual solvent analyses, have not been extensively investigated. It is clear that additional efforts in the NP field are necessary to develop systems for human use.

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