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Preparation and Characterization of Nanocapsules from Preformed Polymers by a New Process Based on Emulsification-Diffusion Technique

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Purpose. The aim of this study was to investigate whether biodegradable nanocapsules could be obtained by the emulsification-diffusion technique.

Methods. This technique consists of emulsifying an organic solution containing an oil, a polymer, and a drug in an aqueous solution of a stabilizing agent. The subsequent addition of water to the system induces solvent diffusion into the external phase, resulting in the formation of colloidal particles. Nanoparticles obtained in this way were characterized by their particle size, zeta potential, isopycnic density and drug entrapment. The shape, surface and structure of the nanocapsules were evaluated by freeze fracture scanning electron microscopy (SEM) and by atomic force microscopy (AFM).

Results. Density gradient centrifugation confirmed the formation of nanocapsules. The density was found to be intermediate between those of nanoemulsions and nanospheres. 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. SEM and AFM showed the presence of capsular structures with smooth homogeneous walls. The versatility and effectiveness of the method were demonstrated using different lipophilic drug/oil core/wall polymer/partially water-miscible solvent systems. The mechanism of nanocapsule formation was explained as a chemical instability (diffusion stranding) generated during diffusion.

Conclusions. This study demonstrated that the emulsification-diffusion technique enables the preparation of nanocapsules in a simple, efficient, reproducible and versatile manner.

KEY WORDS: nanocapsules; biodegradable polymer; emulsification-diffusion technique; density gradient centrifugation; atomic force microscopy.

INTRODUCTION

The term nanoparticles is typically used to define solid colloidal drug carriers ranging in size from 10 to 1000 nm. These species have received considerable attention in recent years, in particular those prepared with biodegradable polymers, because of their potential use as site-specific drug delivery systems. Nanoparticle is a collective name used to describe both nanospheres and nanocapsules. The difference between these forms lies in the morphology and body architecture. Nanocapsules are composed of a liquid core (generally an oil) surrounded by a polymeric membrane, whereas nanospheres are formed by a dense polymeric matrix (1–4). Nanocapsules are pharmaceutically attractive because of their oil-based central cavities, which allow a high encapsulation level for lipophilic substances and thereby enable improved drug delivery. Furthermore, it is possible to avoid drug precipitation during preparation and subsequent stability problems, caused by the presence of drug on the nanoparticle surface (5,6). To our knowledge, only two techniques are available to prepare biodegradable nanocapsules: (a) Interfacial polymerization of alkylcyanocrylate monomers. In this process the cyanocrylate monomer and the lipophilic drug are dissolved in a mixture of oil and ethanol. This organic solution is then added slowly into water or a buffer solution (pH 3–9) containing surfactants such as poloxamers or phospholipids. Nanocapsules are formed spontaneously by anionic polymerization of the cyanocrylate in the oily phase, after contact with hydroxyl ions which act as initiators. (b) Interfacial deposition of preformed polymers. In this process, the lipophilic drug, oil, polymer and optionally phospholipids, are dissolved in a water-miscible solvent (e.g. acetone). This solution is then poured under stirring into an aqueous solution containing a nonionic surfactant (e.g. poloxamer 188). Nanocapsules are formed instantaneously by the fast diffusion of solvent into water, which provokes the spontaneous emulsification of the oily solution in the form of nanodroplets where the desolvated polymer will form a film around the oily nanodroplets that contain the drug (5,7–9). The method of interfacial polymerization is not ideal for three reasons: i) the probable presence of residual, potentially toxic monomers or oligomers; ii) the possibility of cross-reaction with the drug and iii) the difficulty in predicting the molecular weight of the resulting polymer (4,10,11). Thus, the interfacial deposition technique, involving well-characterized biodegradable polymers such as poly (α-hydroxy) acids, is generally preferred. The principal drawback with this technique is the polymer aggregation that is frequently observed when working with high polymer concentrations, or a low organic solvent/water ratio.

The emulsification-diffusion technique has been used recently to prepare nanospheres from preformed polymers (12). It involves the formation of a conventional oil-in-water emulsion between a partially water-miscible solvent containing the polymer and the drug, and an aqueous phase, 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 nanospheres. In this study the emulsion diffusion-technique was evaluated as a means to prepare biodegradable nanocapsules. A formation mechanism is proposed in terms of a induced chemical instability similar to that observed for the spontaneous emulsification processes.

MATERIALS AND METHODS

Materials

Poly(D,L-lactic acid) (PLA, Medisorb 100 D,L, Cincinnati, OH, USA), Eudragit® E (Röhm, GmbH Darmstadt, Germany) and poly(ϵ-caprolactone) (Tone® 767, Union Carbide,

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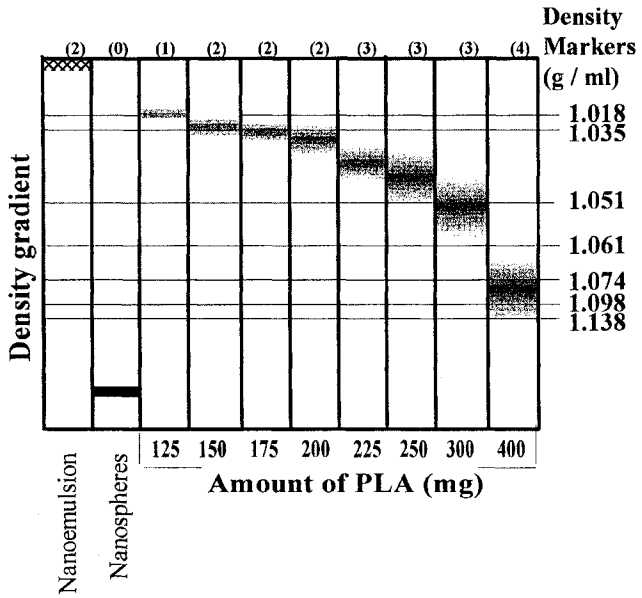

Fig. 1. Bands of nanoemulsion, nanospheres, nanocapsules and external standards (g/ml) generated in gradients of Percoll by centrifugation (45 000 g/30 min). Batches of nanocapsules were prepared using 0.5 ml of Mygliol and differents amounts of PLA. The numbers in parentheses represent the size polydispersity of each system.

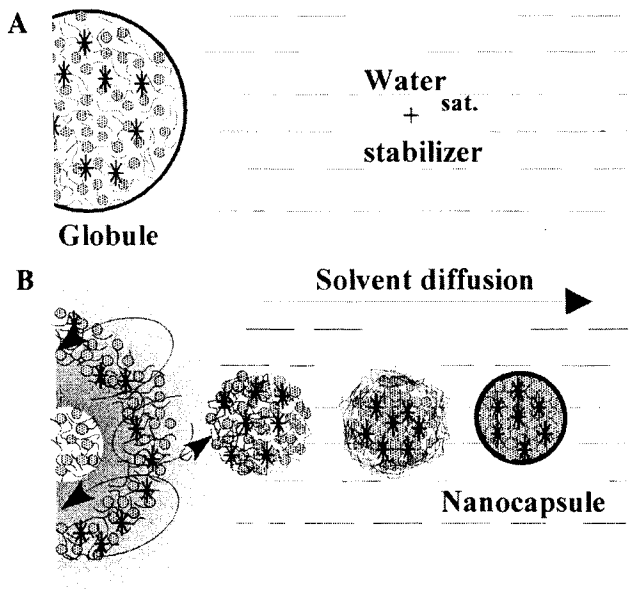

Fig. 2. Schematic description of the proposed formation mechanism of nanocapsules by the emulsification-diffusion technique, where •, ⌋, and ✳ represent the oil, polymer and drug, respectively. A) before the diffusion step; B) during the diffusion step.

Dunbury, USA), were used as envelope polymers. Mygliol® 812 neutral oil (mixed triglycerides from fractioned coconut fatty acids C_8–C_{10}, Dynamit Nobel, Troisdorf, Germany) and mineral oil (Fluka, Buchs, Switzerland) were used as oil cores. Poly(vinyl alcohol) (PVAL) with a Mw = 26 000 (Mowiol® 4-88) was a generous gift from Hoechst (Frankfurt-am-Main, Germany). Ethyl acetate (EtAc) of HPLC grade, propylene carbonate (PC) and benzyl alcohol (BA) of analytical grade (Fluka) were used as partially water-miscible solvents. Sudan III, indomethacin, progesterone, estradiol, chlorambucil, clofibrate and vitamin E were used as lipophilic-model substances (Fluka). Distilled water was purified using a Milli-Q system (Millipore). All other reagents were of analytical grade and used without further purification.

Methods

Nanoparticle Preparation

Nanocapsules were formed by the emulsification-diffusion method described in detail elsewhere (13). Briefly, EtAc and water were mutually saturated for 1 min before use, in order

Table I. Properties of the Systems Prepared with Different Mygliol/PLA Ratios

Batch no.	Mygliol (ml)	PLA (mg)	ζ potential ± SD[a] (mV)	Mean size ± SD (nm)	PI[b]	Ultracentrifugation (45 000 g/30 min) Creaming[c]	Sediment[c]	Supernatant turbidity[c]
1	0.5	0	−18.2 ± 0.2	319 ± 2	2	++++	−	−
2	0.5	20	−18.5 ± 0.6	337 ± 2	2	++++	−	−
3	0.5	30	−15.8 ± 0.2	340 ± 3	2	++++	−	+
4	0.5	50	−18.8 ± 0.3	339 ± 2	2	++++	−	++
5	0.5	80	−20.2 ± 0.2	345 ± 3	2	++	+	++
6	0.5	100	−18.7 ± 0.3	360 ± 3	1	+	++	++
7	0.5	125	−15.1 ± 0.3	319 ± 2	1	−	++++	−
8	0.5	150	−16.7 ± 0.3	346 ± 3	2	−	++++	−
9	0.5	200	−19.9 ± 0.1	332 ± 3	2	−	++++	−
10	0.5	400	−20.3 ± 0.7	378 ± 4	4	−	++++	−
11	0.5	600	−14.8 ± 0.6	614 ± 8	4	−	++++	−
12	0.0	400	−12.4 ± 0.1	174 ± 1	0	−	++++	−

[a] SD: Standard Deviation (n = 3).
[b] PI: Polydispersity index expressed using a 0 – 9 scale.
[c] (++++ : high; +++ : middle; ++ : little; + : very little; − : none).

Table II. Relationship Between the Density Gradient Centrifugation and the Density Calculated of the Original Mygliol/PLA Mixtures

Batch no.	Mygliol (ml)	PLA (mg)	Mean size ± SD[a] (nm)	PI[b]	$\delta_{gradient\ centrif.}$[c] (g/cm^3)	$\delta_{calculated}$[d] (g/cm^3)	Deviation
7	0.5	125	319 ± 2	1	1.018	1.005	0.013
8	0.5	150	346 ± 3	2	1.030	1.014	0.016
13	0.5	175	309 ± 4	2	1.035	1.022	0.013
9	0.5	200	332 ± 3	2	1.037	1.031	0.006
14	0.5	225	316 ± 3	3	1.041	1.038	0.003
15	0.5	250	301 ± 4	3	1.044	1.045	−0.001
16	0.5	300	328 ± 3	3	1.052	1.057	−0.005
10	0.5	400	378 ± 4	4	1.084	1.080	0.004
12	0.0	400	174 ± 1	0	n.d.[e]	1.290	–

[a] SD: Standard deviation (n = 3).
[b] PI: Polydispersity index expressed using a 0–9 scale.
[c] $\delta_{gradient\ centrif.}$, determined from the equation: Distance (mm) = 4.5E5δ^4 − 2E6δ^3 + 3E6δ^2 − 2E6δ + 5.4E5 obtained using the density markers beads at their buoyant density (g/cm^3).
[d] $\delta_{calculated}$, determined considering the δ_{PLA} = 1.29 g/cm^3 (calculated with air comparison pycnometer) and $\delta_{Mygliol}$ = 0.95 g/cm^3 (calculated with glass pycnometer).
[e] n.d.: not determined, the value was out of the calibration curve.

to ensure initial thermodynamic equilibrium of both liquids. Typically, 200 mg of PLA and 0.5 ml of Mygliol 812 were dissolved in 20 ml of water-saturated EtAc and this organic solution was emulsified with 40 ml of a 5% w/v PVAL EtAc-saturated aqueous solution, using a high-speed stirrer (Ultra-Turrax T25, IKA Labotechnik, Staufen, Germany) at 8000 rpm for 10 min. 200 ml of water were subsequently added to the emulsion to induce diffusion of EtAc into the continuous phase,

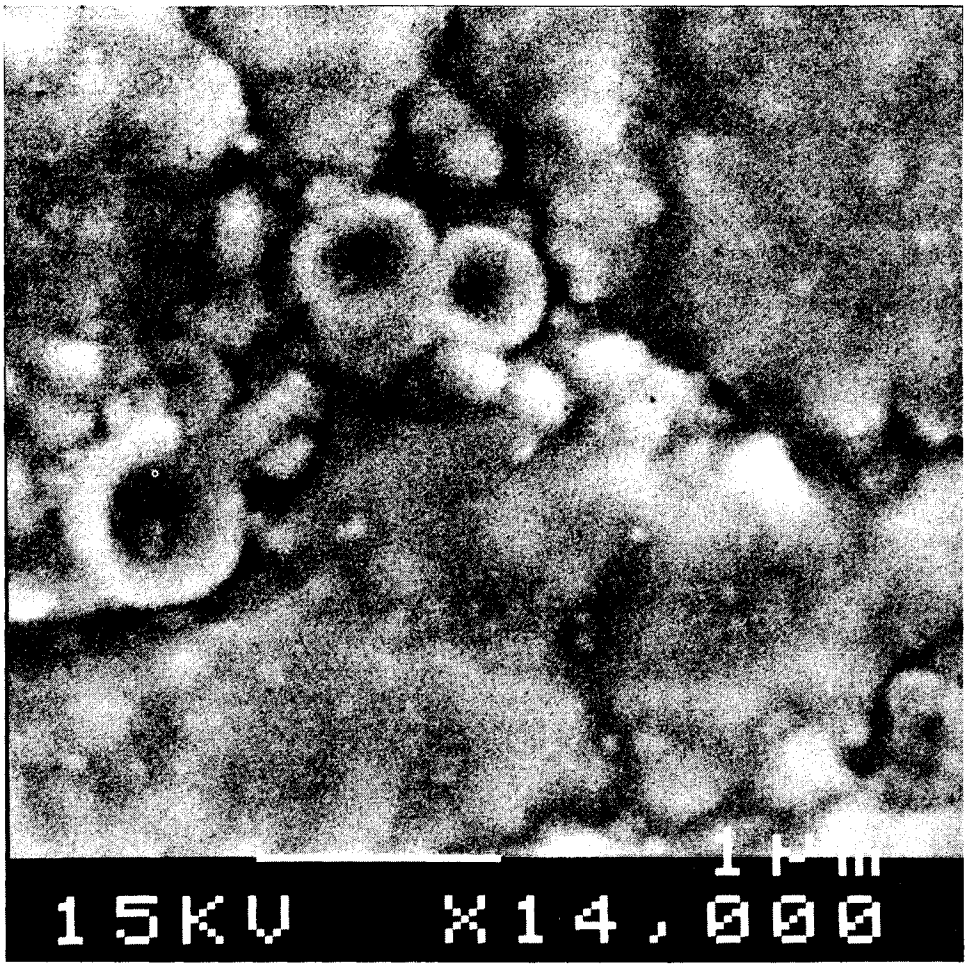

Fig. 3. Scanning electron micrographs of Mygliol/PLA nanocapsules (batch 9) after freeze fracture.

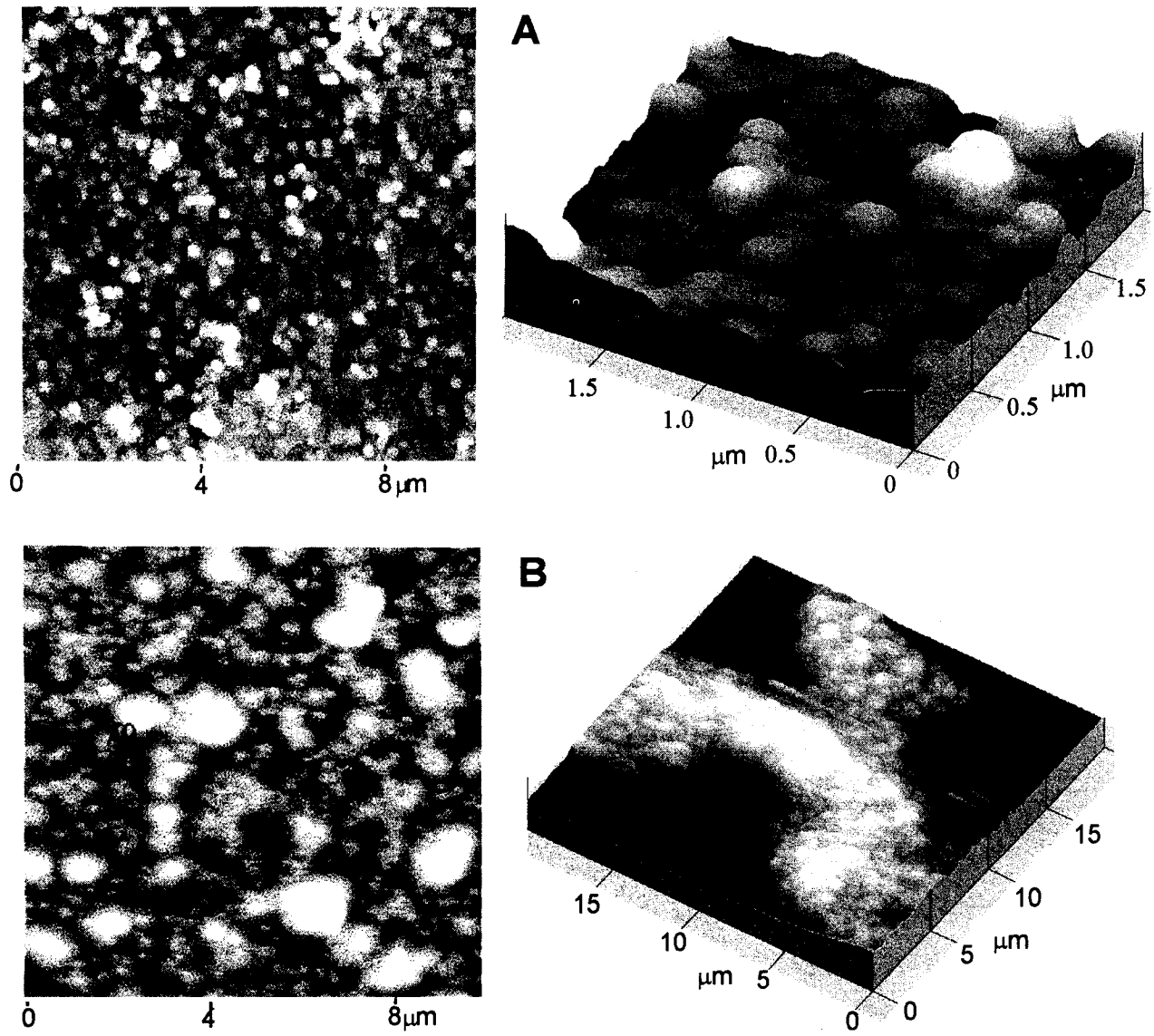

Fig. 4. Images of nanospheres (A) and nanocapsules (batch 9) (B) taken by atomic force microscopy (non-contact mode).

leading to the formation of nanoparticles. EtAc was eliminated from the raw nanoparticle suspension by vacuum steam distillation at 30°C and 70 mm Hg. In order to optimize the process and to determine the effect of PLA/Mygliol ratio, nanoparticles were prepared by using different amounts of polymer. Nanospheres and nanoemulsions were prepared using the same procedure, but without addition of Mygliol and PLA, respectively. All the batches prepared were ultracentrifuged in a 25° angle head rotor (Beckman, Avanti™ 30, CA, USA), at 45 000 g for 30 min.

Particle Size Analysis

The average particle size and polydispersity index (scale from 0 to 9) were determined using a Coulter Nano-Sizer® (Coulter Electronics, Harpenden, UK). Measurements were made in triplicate.

Zeta Potential Determination

The zeta potential of the nanocapsule suspensions was measured using the Zetasizer® 4 with a Series 7032 Multi-8 Correlator (Malvern, Orsay, France), after appropriate dilution in double-distilled water. The results were all normalized with respect to zeta potential of −55 mV for polystyrene standard solution (Malvern). The measurements were made at 25°C in triplicate.

Nanoparticles Density

In order to evaluate nanocapsule density as a function of polymer concentration, isopycnic centrifugation in a density gradient of colloidal silica (Percoll, Pharmacia LKB, Sweden) was used. 10 ml of the nanoparticle suspensions were centrifuged twice at 45 000 g for 30 min and the concentrate was added to 7 ml of Percoll 45% v/v containing 0.15 M NaCl. Centrifugation was performed in a 25° angle head rotor (Beck-

Table III. Size and Entrapment Efficiency for Nanocapsules Prepared Using Lipophilic Model Substances and Differents Oil Core/Polymer/Partially Water-Miscible Solvent Systems

Exemple no.	Oil (ml)	Polymer (mg)	Subtance (mg)	Solvent	Mean size ± SD[a] (nm)	PI[b]	Entrapment efficiency (%)[c]
1	Mineral oil (0.5)	PLA (200)	Sudan III (5)	EtAc	303 ± 3	2	98.0
2	Mygliol (0.5)	PLA (200)	Sudan III (5)	EtAc	340 ± 4	2	100.8
3	Mygliol (0.5)	Eudragit E (200)	Sudan III (5)	PC	239 ± 6	3	88.6
4	Mygliol (0.5)	Eudragit E (200)	Sudan III (5)	BA	287 ± 5	2	92.4
5	Mygliol (0.5)	Tone P-700 (200)	Sudan III (5)	EtAc	346 ± 4	2	98.9
6	Mygliol (0.5)	PLA (200)	Indomethacin (20)	EtAc	335 ± 3	2	102.0
7	Mygliol (0.5)	Tone P-700 (200)	Indomethacin (20)	EtAc	314 ± 2	2	94.4
8	Mygliol (0.5)	PLA (200)	Progesterone (20)	EtAc	510 ± 3	1	98.7
9	Mygliol (0.5)	PLA (200)	Estradiol (20)	EtAc	340 ± 2	1	52.0
10	Mygliol (0.5)	PLA (200)	Chlorambucil (20)	EtAc	335 ± 3	2	32.1
11	None	PLA (200)	Clofibrate (400)	EtAc	370 ± 5	4	95.3
12	None	PLA (200)	Vitamin E (470)	EtAc	360 ± 3	4	92.2

[a] SD: Standard Deviation (n = 3).
[b] PI: Polydispersity index expressed using a 0–9 scale.
[c] Entrapment efficiency (%) = percent drug loading / percent of the initial content × 100.

man). The gradients were generated *in situ* by centrifugation. Density Marker Beads (Pharmacia LKB) of specific density were used as external standards and treated identically to those containing the experimental samples. The height of a band was measured according to the distance from the top of the meniscus to the center of the band using a vernier calliper (Tesa, Digit-Cal SM, Switzerland). The approximate density of the samples was calculated from the polynomial curve obtained by plotting the distance versus the density of the marker beads.

Scanning Electron Microscopy (SEM) After Freeze Fracture

SEM after freeze fracture was performed in order to confirm the "capsular" structure of the nanocapsules. A concentrated aqueous dispersion of nanoparticles was cryofixed under standard conditions, using Balzer-type specimen support plates immersed in liquid nitrogen-cooled Freon (−160°C). Samples were fractured at −150°C and 2×10^{-6} Torr (BAF 400T, Balzers AG, Balzers, Liechtenstein), and the surface was shadowed in a cathodic evaporator. The surface morphology of the nanoparticles was observed by SEM using a JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan).

Atomic Force Microscopy (AFM)

AFM was used to image the shape and surface properties of the nanoparticles prepared by the emulsification-diffusion method. A drop of diluted aqueous dispersion was placed on a washed microscope slide and dried under vacuum for 24 h. The measurements were performed using a commercial AFM (Antrophobe®, Park Scientific Instruments, CA, USA), at room temperature in non-contact mode (frequency ≅ 200 kHz).

Drug Loaded Nanocapsules

A lipophilic colorant (Sudan III) as well as different liquid and solid lipophilic drugs were incorporated to the formulation in order to establish the encapsulation level obtained by the proposed method. Nanocapsules were prepared as described above, incorporating the lipophilic substance as a third solute in EtAc. The nanocapsules were washed three times by centrifugation/redispersion, and sieved through a 300 mesh. A known volume of each batch produced was dried under vacuum for 48 h in a pre-weighed crystallizer. The dry sediment was dissolved in chloroform or chloroform/ethanol (1:1) and assayed at its maximum absorption wavelength (Beckman 35, CA, USA) versus a calibration curve. The entrapment efficiency expressed in percent, was calculated from the percent of drug loading (amount of drug in the sediment/amount of sediment × 100) divided by the percent of the initial drug content in the formulation.

RESULTS AND DISCUSSION

The emulsification-diffusion method has been used successfully to prepare biodegradable nanospheres in an efficient and reproducible manner (12,14). The technique relies on the rapid diffusion of the solvent from the internal into the external phase, which thereby provokes polymer aggregation in the form of solid colloidal particles. Then, when an oil is incorporated in the internal phase, it is proposed that polymer aggregation can follow two probable paths: a) independent aggregation, the dispersion obtained will contain oil globules and nanospheres or polymer particles, and b) aggregation around the oil droplets forming capsules. Table I shows the characteristics of the Mygliol/PLA batches prepared by the emulsion-diffusion technique. All the dispersions (including the emulsion) were of submicrometer size, without polymer flakes or visible oil droplets, and with the exception of batch 11, all were stable for at least one month. Ultracentrifugation results support the hypothesis that PLA aggregates around the Mygliol nanodroplets. The absence of any sediment for batches 2–4 (20, 30 and 50 mg of PLA) revealed the association of polymer with the oil. Ideally, the nanocapsules would have a similar density to that of the original Mygliol/PLA mixture (less than one for these batches); thereby, these systems would behave similarly to the nanoemulsion. It is also probable that a buoyant mixture of nanocapsules and nanoemulsion was formed. In contrast, when the amount of PLA was sufficient to generate nanocapsules of density

greater than one (batches 7–11), sediments without creaming or supernatant turbidity were obtained, indicating that nanoparticles with an oily core were formed. Zeta potential measurements did not allow to demonstrate the presence of a polymeric wall at the oil/water interface. No difference was observed between the zeta potential of the nanoemulsion and the nanocapsules, due probably to the presence of PVAL on the PLA surface. Ideally, a strong negative zeta potential was expected for nanocapsules, due to the carboxyl groups present at the end of PLA chain. PVAL has shown great PLA-surface affinity and tends to form a thick firmly attached layer (14), which provokes a decrease in the electrophoretic mobility, corresponding to a decrease in zeta potential of the nanoparticles. In other words, the PVAL coating layer protects a part of the diffuse layer against displacement by shear forces (15,16).

The results of the density gradient centrifugation, summarized in Fig. 1, confirm the formation of nanocapsules by the proposed method. The set of batches prepared with Mygliol/PLA mixtures had a density intermediate between those of nanoemulsions and nanospheres. Chouinard et al. (17) reported similar results for Mygliol/poly(alkylcyanocrylate) nanocapsules prepared by interfacial polymerization. It is worth noting that only one buoyant band was obtained in each batch, suggesting that the method used in this study, yielded exclusively nanocapsules when Mygliol/PLA mixtures (at least for the ratios used) were present in the internal phase. On the other hand, the density of the nanocapsules and the band thickness increased when the quantity of PLA increased. These observations may be useful for explaining the probable mechanism of formation. We have suggested that nanoparticles prepared using the emulsification-diffusion method are formed due to an instability produced by solvent transport by a similar mechanism to that involved to explain spontaneous emulsification processes (diffusion-stranding mechanism) (18). This hypothesis can be extended to explain the formation of nanocapsules. Thus, solvent diffusion from the globules into the water carries molecules of oil and polymer, forming locally supersaturated regions (19). It is proposed that such supersaturation cannot persist, and that nanodroplets containing oil, polymer drug and probably any remaining solvent are formed and rapidly stabilized by the surface active agent. The importance of this stabilization step has been discussed previously (12). Finally, the molecules of polymer, ideally contained in the nanodroplets, aggregate at the interface of the new phases generated by solvent displacement, which are both non-solvent for the polymer. A schematic representation of this mechanism is shown in Fig. 2. The fact that for the batches prepared with Mygliol/PLA mixtures no band was detected near the nanospheres density range, supports the assumption that polymer aggregation originates from the oil. Furthermore, the nanocapsule densities calculated with the bead markers curve were very close to the original density of the Mygliol/PLA mixtures (Table II), suggesting that nanocapsules were formed from nanodroplets with similar size, but with a polymer content dependent upon the original mixture composition.

SEM examination of the nanocapsules revealed the existence of well-defined capsular structures (Fig. 3) characterized by single central cores which were expected to be filled with tiny volumes of Mygliol. Fig. 4 shows the images of nanospheres and nanocapsules taken by AFM. Nanospheres were observed as spherical units with homogeneous size and smooth surface, whereas nanocapsules showed similar structures but with contrasting larger shiny regions. It is thought that these regions were formed of free oil resulting from the break-up of a certain number of nanocapsules during sample preparation or by the resonance frequencies during the scanning process. This observation reveals two interesting points : a) the differences between nanospheres and nanocapsules with regard to their mechanical properties and b) although it is possible by this emulsification-diffusion technique to control the wall thickness of the nanocapsules and ideally the drug diffusion rate, drug release can be influenced by the break-up of the polymeric envelope. It is clear therefore that these aspects merit further investigation.

Table III lists the characteristics of the batches prepared incorporating lipophilic substances. The aim of these examples was to show: first the versatility of the emulsion-diffusion technique. The components of the oil core/envelope polymer/partially water-miscible solvent system can be changed depending on the aim of the formulation and administration route; second to demonstrate the high entrapment efficiencies when lipophilic molecules are incorporated in nanocapsules.

In conclusion, this study demonstrated that the emulsion-diffusion technique represents a viable new alternative for preparing biodegradable nanocapsules starting from preformed polymers. The technique is efficient, versatile, of simple implementation and permits high efficiency entrapment of lipophilic drugs.

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