2 Production of Lipospheres for Bioactive Compound Delivery

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2.1 INTRODUCTION

One approach for increasing the beneficial action of drugs and decreasing systemic adverse effects is to deliver the necessary amount of drugs to the diseased sites, where they are most needed, for the appropriate period of time [1–3].

Although the drug delivery system concept is not new, great progress has recently been made in the treatment of a variety of diseases. Particulate carriers (e.g., polymeric nano- and microparticles, fat emulsion, and liposomes) possess specific advantages and disadvantages. For instance, in the case of polymeric microparticles, the degradation of the polymer might possibly cause systemic toxic effects through the impairment of the reticuloendothelial system [4] or by accumulation at the injection site [5]; cytotoxic effects have indeed been observed *in vitro* after phagocytosis of particles by human macrophages and granulocytes [6]. In addition, organic solvent residues deriving from the preparation procedures, such as the solvent evaporation technique often used for liposome [7] and polyester microparticles [8], can be present in the delivery system and could result in severe acceptability and toxicity problems [9].

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To solve these adverse effects, lipid microspheres, often called lipospheres (LS), have been proposed as a new type of fat-based encapsulation system for drug delivery of bioactive compounds (especially lipophilic compounds). LS consist of solid microparticles with a mean diameter usually between 0.2 and 500 μm, composed of a solid hydrophobic fat matrix in which the bioactive compounds are dissolved or dispersed [10–12]. LS have some advantages over other delivery systems, such as good physical stability, low cost of ingredients, ease of preparation and scale-up, and high entrapment yields for hydrophobic drugs. Because of their large range in particle size, LS can be administered by different routes — such as orally, subcutaneously, intramuscularly, or topically — or they can be used in cell encapsulation, thus allowing them to be proposed for treatment of a number of diseases [13–15]. For instance, the *in vivo* distribution of LS demonstrated a high affinity to vascular wells (including capillaries), inflamed tissues, and granulocytes [16–17].

LS have been used for the controlled delivery of various types of drugs, including vasodilator and antiplatelet drugs, antiinflammatory compounds, local anesthetics, antibiotics, and anticancer agents; they have also been used successfully as carriers of vaccines and adjuvants [18].

This chapter will discuss (a) the production and characterization of LS formed by the melt dispersion technique, by the solvent evaporation method, and by the water/oil/water (w/o/w) double-emulsion method; (b) the influence of preparation parameters on liposphere morphology; and (c) the encapsulation efficiency and the release characteristics of two lipophilic model drugs, such as retinyl acetate and progesterone, and one hydrophilic drug, sodium cromoglycate (SCG), from the prepared LS.

For a biocompatible formulation suitable for human administration, triglycerides and monoglycerides have been chosen as the biomaterials for LS because of their high biocompatibility, high physicochemical stability, and drug delivery release. LS were prepared by two alternative approaches, namely, the melt dispersion and the solvent evaporation techniques (Figure 2.1 and Table 2.1).

2.2 MELT DISPERSION TECHNIQUE

The choice of the lipid matrix plays an important role in the morphology of the particles and in the possible formation of aggregates. In a first set of experiments, LS were prepared by the melt dispersion technique, using a lipid mixture constituted of cetyl alcohol and cholesterol (2:1, w/w) and gelatin as the stabilizer. Gelatin was selected from among eight natural or synthetic emulsifiers, namely, gelatin (200 Bloom), pectin, carrageenan κ, carrageenan ι, carrageenan λ, polyvinyl alcohol (PVA), polyoxyethylene 20 sorbitan trioleate (polysorbate 85, Tween 85), and lauryl sarcosine.

The lipidic mixture, both with and without a lipophilic model drug, was melted at 70°C and then emulsified into an external aqueous phase containing a suitable surfactant. The emulsion was mechanically stirred by a stirrer equipped with alternative impellers. Afterward, the emulsion was heated to the same temperature as the melted lipidic phase. The milky formulation was then rapidly cooled to about 20°C by immersing the formulation flask in a cool water bath without stopping the

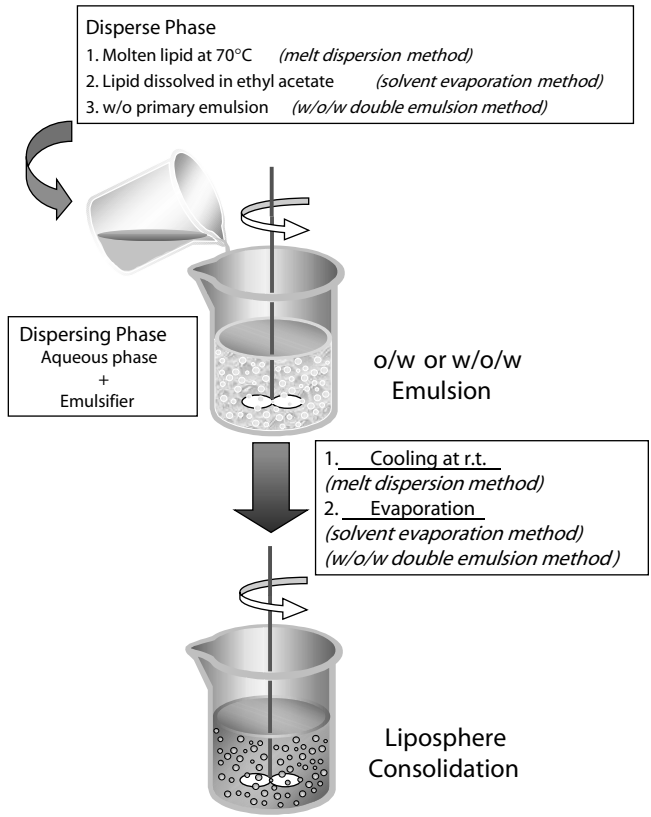

FIGURE 2.1 Schematic representation of the methods of production of LS: melt dispersion and solvent evaporation.

agitation to yield a uniform dispersion of LS. The obtained LS were then washed with water and isolated by filtration through a paper filter.

The morphology of LS was evaluated by optical microscopy (Nikon Diaphot inverted microscope) and scanning electron microscopy observations (Cambridge Stereoscan 360). Microsphere size distributions were determined by photomicrograph analyses, analyzing at least 300 microparticles per sample.

The resulting microparticles (Figure 2.2 and Table 2.2) were characterized by an irregular surface; in addition, some aggregates caused by the fusion of lipid droplets before solidification were present. An improvement of LS features was obtained in terms of recovery, mean diameter, and aggregate formation by decreasing the molecular weight of the gelatin used as a stabilizer. The viscosity of the dispersing phase was, in fact, progressively reduced, passing from gelatin 50 to gelatin 250 Bloom grades.

By adjusting the stirring speed during the emulsification process, it was possible to modify the size of the particles. Increasing the stirring speed from 500 to 1000 rpm, the mean diameter of particles progressively decreased (Table 2.3). With the aim of

TABLE 2.1
Overview of Liposphere Preparation Methods

Method	Process Duration (h)	Stirring Speed (rpm)	Dispersion Medium	Disperse Phase	Particle Isolation
Melt dispersion	1 with rapid cool up to 20°C	500, 750, 1000	Water (150 mL) plus stabilizer[a]	Melted lipid (5 g) at 70°C	Filtration through a glass filter[b]
o/w solvent evaporation	6–8 at room temperature	500, 750, 1000	Water (150 mL) plus polyvinyl alcohol as stabilizer	Dissolved lipids (5 g) in ethyl acetate (10 mL) at 50°C	Filtration through a glass filter[b]
w/o/w double emulsion	3–5 at room temperature	500, 750, 1000	Water (150 mL) plus 0.25% (weight/volume) polyvinyl alcohol as stabilizer	w/o emulsion of melted lipids (5 g) at 70°C stabilized with gelatin or poloxamer 407	Filtration through a glass filter[b]

[a] See Table 2.8.
[b] Glass filters with a maximum nominal pore size of 10–16 µm.

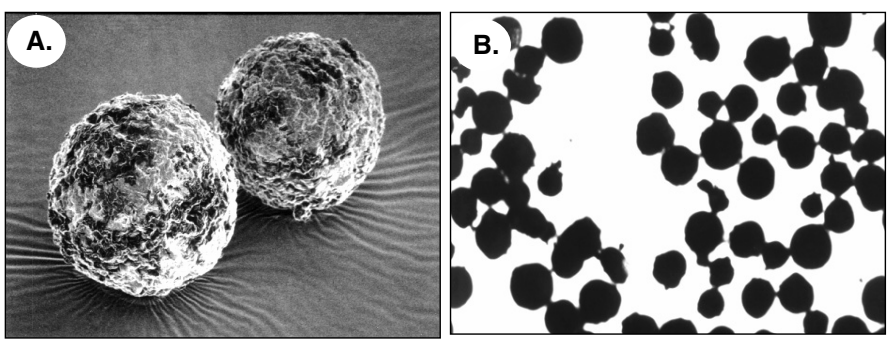

FIGURE 2.2 Effect of gelatin Bloom on morphology and particle size of cetyl alcohol: cholesterol 2:1 (w/w) lipospheres. Scanning electron microscopy **(A)** and optical micrographs **(B)** of microspheres produced with gelatin 200 Bloom grades. Bar corresponds to 76 and 381 µm in panels A and B, respectively.

further improving the characteristics of LS, alternative lipid compositions were considered. For instance, LS were prepared with apolar triglycerides, such as tristearin, tripalmitin, or tribehenin, in combination with other polar (more hydrophilic) lipids, including glyceryl monostearate, glyceryl monooleate, cetyl alcohol, and cholesterol (Table 2.4). As a general consideration, all formulations, apart from those including cholesterol and glyceryl monooleate, were satisfactory in terms of shape, recovery, and size (Figure 2.3 and Figure 2.4).

TABLE 2.2
Effect of Gelatin Type on Liposphere Characteristics

Gelatin (Bloom grades)	Lipid Composition (w/w ratio)	Recovery (%)[a]	Mean Diameter (μm)
250	Cetyl alcohol/cholesterol (2:1)	52	250 ± 12
200	Cetyl alcohol/cholesterol (2:1)	60	205 ± 15
150	Cetyl alcohol/cholesterol (2:1)	61	197 ± 8
50	Cetyl alcohol/cholesterol (2:1)	82	150 ± 21

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor; a 5% gelatin solution; and a 750-rpm stirring speed. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

TABLE 2.3
Effect of Stirring Speed on Characteristics of Cetyl Alcohol/Cholesterol Lipospheres

Stirring Speed (rpm)	Gelatin (Bloom grades)	Recovery (%)[a]	Mean Diameter (μm)
500	50	92	250 ± 14
750	50	82	150 ± 16
1000	50	82	80 ± 24

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor and a 5% gelatin solution. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

Other experiments were undertaken to evaluate the effect of glyceryl monostearate concentration on LS characteristics. Glyceryl monostearate was used in mixture with tristearin (Table 2.5) or tripalmitin (Table 2.6) at different weight ratios. Tables 2.5 and 2.6 report the results of such experiments, in which the percentage of glyceryl monostearate was varied from 0% up to 33% (w/w). In the case of tripalmitin, it was impossible to produce LS without the presence of at least 1% glyceryl monostearate (because of the formation of large blobs), whereas pure tristearin particles were obtained, even if they were of poor quality. By increasing the content of glyceryl monostearate, a progressive decrease in LS size was evident. On the contrary, no effect was detectable on LS recovery.

TABLE 2.4
Effect of Lipid Composition on the Production of Lipospheres

Lipid Composition (w/w ratio)	Recovery (%)[a]	Mean Diameter (μm)
Tristearin:monostearate (2:1)	90	170 ± 19
Tristearin:cetyl alcohol (2:1)	92	200 ± 26
Tristearin:cholesterol (2:1)	98	250 ± 16
Tripalmitin:monostearate (2:1)	82	250 ± 8
Tripalmitin:monooleate (2:1)		Fused mass
Tripalmitin:cholesterol (2:1)		Fused mass
Tripalmitin:cetyl alcohol (2:1)	96	300 ± 15
Tribehenin:monostearate (2:1)	75	200 ± 22

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor; a 1% polyvinyl alcohol solution; and a 750-rpm stirring speed. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

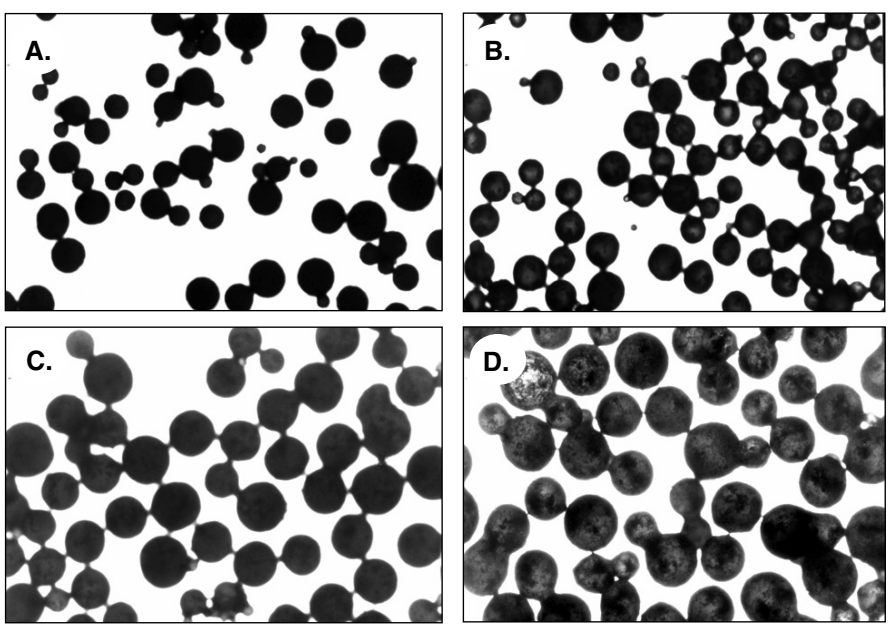

FIGURE 2.3 Effect of lipid composition on the morphology of lipospheres. Lipospheres were prepared with **(A)** cetyl alcohol:cholesterol 2:1 (w/w), **(B)** tripalmitin:glyceryl monostearate 2:1 (w/w), **(C)** tristearin:glyceryl monostearate 2:1 (w/w), and **(D)** tripalmitin:glyceryl monostearate 9:1 (w/w). Bar corresponds to 533, 812, 315, and 487 μm in panels A, B, C, and D, respectively.

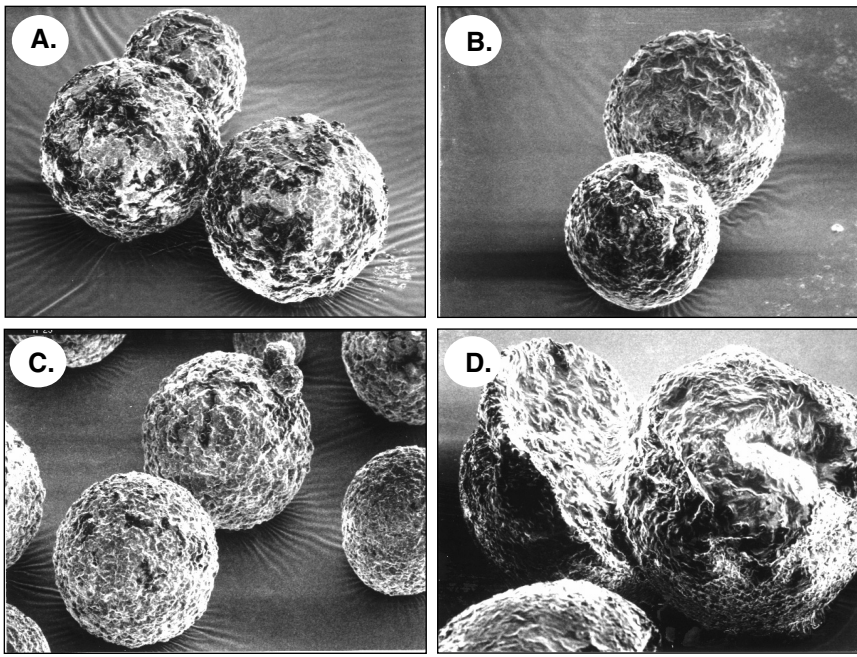

FIGURE 2.4 Scanning electron microscopy photographs showing the effect of lipid composition on the morphology of lipospheres. Lipospheres were prepared with **(A)** tristearin:glyceryl monostearate 2:1 (w/w), **(B)** tristearin:cetyl alcohol 2:1 (w/w), **(C)** tripalmitin:glyceryl monostearate 2:1 (w/w), and **(D)** tripalmitin:cetyl alcohol 2:1 (w/w). Bar corresponds to 67, 87, 101, and 76 μm in panels A, B, C, and D, respectively.

TABLE 2.5
Effect of Monostearate on the Production of Tristearin Lipospheres

Lipid Composition (w/w ratio)	Recovery (%)[a]	Mean Diameter (μm)
Tristearin:glyceryl monostearate 100:0	76	220 ± 31
Tristearin:glyceryl monostearate 98:2	93	200 ± 23
Tristearin:glyceryl monostearate 95:5	90	170 ± 18
Tristearin:glyceryl monostearate 90:10	89	160 ± 15
Tristearin:glyceryl monostearate 80:20	73	158 ± 28
Tristearin:glyceryl monostearate 66:33	90	170 ± 19

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor; a 750-rpm stirring speed; and 1% polyvinyl alcohol solution as the dispersing phase. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

TABLE 2.6
Effect of Monostearate on the Production of Tripalmitin Lipospheres

Lipid Composition (w/w ratio)	Recovery (%)[a]	Mean Diameter (μm)
Tripalmitin:glyceryl monostearate 100:0	Fused mass	
Tripalmitin:glyceryl monostearate 99:1	96	300 ± 9
Tripalmitin:glyceryl monostearate 95:5	75	300 ± 16
Tripalmitin:glyceryl monostearate 90:10	72	300 ± 31
Tripalmitin:glyceryl monostearate 66:33	82	250 ± 21

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor; a 750-rpm stirring speed; and 1% polyvinyl alcohol solution as the dispersing phase. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

The choice and the adjustment of the manufacturing parameters for the production of microspheres of defined size were performed in agreement with the following equation

$$d \propto K \frac{D_v R \upsilon_a \gamma}{D_s N \upsilon_o C_s} \tag{2.1}$$

where

d is the average particle size
K is a variable depending on the apparatus geometry (e.g., type and dimension of stirrer)
D_v and D_s are the diameter of the vessel and of the stirrer, respectively
R is the volume ratio between aqueous and oil phases
υ_a and υ_o are their respective viscosities
N is the stirring speed
γ is the surface tension between the two immiscible phases
C_s is the stabilizer concentration [19]

The influence of some parameters, such as stirring conditions and stabilizer type and concentration, was studied on morphology, mean diameter, dimensional distribution, and recovery of microparticles.

2.2.1 Effect of the Stirring Conditions

The effect of the stirring speed was considered on the production of LS (Table 2.7 and Figure 2.5). LS with dimensions between 90 and 170 μm were obtained by changing the stirring speed from 500 to 1000 rpm. In particular, particles obtained at 1000 rpm presented a spherical geometry with a narrow size distribution; in

TABLE 2.7
Effect of Stirring on Tristearin Liposphere Characteristics

Stirring Speed (rpm)	Impeller Type	Recovery (%)[a]	Mean Diameter (μm)
500	3-blade rotor	90	170 ± 35
750	3-blade rotor	78	120 ± 24
750	4-blade helicoidal rotor	64	50 ± 8
750	double-truncated cone rotor	77	55 ± 19
750	2-blade helicoidal rotor	elliptical particles and filaments	
1000	3-blade rotor	69	90 ± 19

Note: The dispersing phase was a 1% polyvinyl alcohol solution. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

addition, aggregation phenomena were almost absent. The recovery efficiency of particles produced at the higher stirring speed was 69%, whereas for those produced at 500 rpm, it was over 90%.

LS were produced by means of different impellers, namely, (a) a 3-blade rotor with a diameter of 55 mm (taken as reference impeller), (b) a 4-blade helicoidal rotor with a diameter of 50 mm, (c) a double-truncated cone rotor with a diameter of 50 mm, and (d) a 2-blade rotor with a diameter of 50 mm (Figure 2.5, lower panel). The use of rotors (b) and (c) allowed us to obtain smaller particles, with mean diameters of 50 and 55 μm, and a recovery of 64% and 77%, respectively (Table 2.7). However, the use of rotor (d) did not allow the production of lipid particles; in fact, this particular impeller caused the formation of elliptical particles and filaments.

2.2.2 Effect of the Stabilizer Type and Concentration

The effects of different stabilizers on particle morphology and recovery were tested (Table 2.8). As is clearly appreciable from the obtained results, the addition of emulsifiers leads to variable effects on the size of LS droplets during the emulsification step, thus influencing the final microspheres' size. In particular, LS obtained with natural polymers, such as gelatin, pectin, and carrageenans κ and ι allowed the production of spherical particles with an irregular surface and a mean diameter between 150 and 250 μm. In the case of carrageenan λ, it was not possible to isolate the particles because the high viscosity of the suspension did not allow the filtration process. On the contrary, in the cases of gelatin and other carrageenans, the high viscosity was compatible with the separation process, even if it was caused by a lower recovery efficiency (between 62 and 69%) compared to pectin (80%).

The use of synthetic emulsifiers gave different results; for instance, the use of 1% (w/w) of the polyoxyethylene–polyoxypropylene block copolymer Pluronic PE

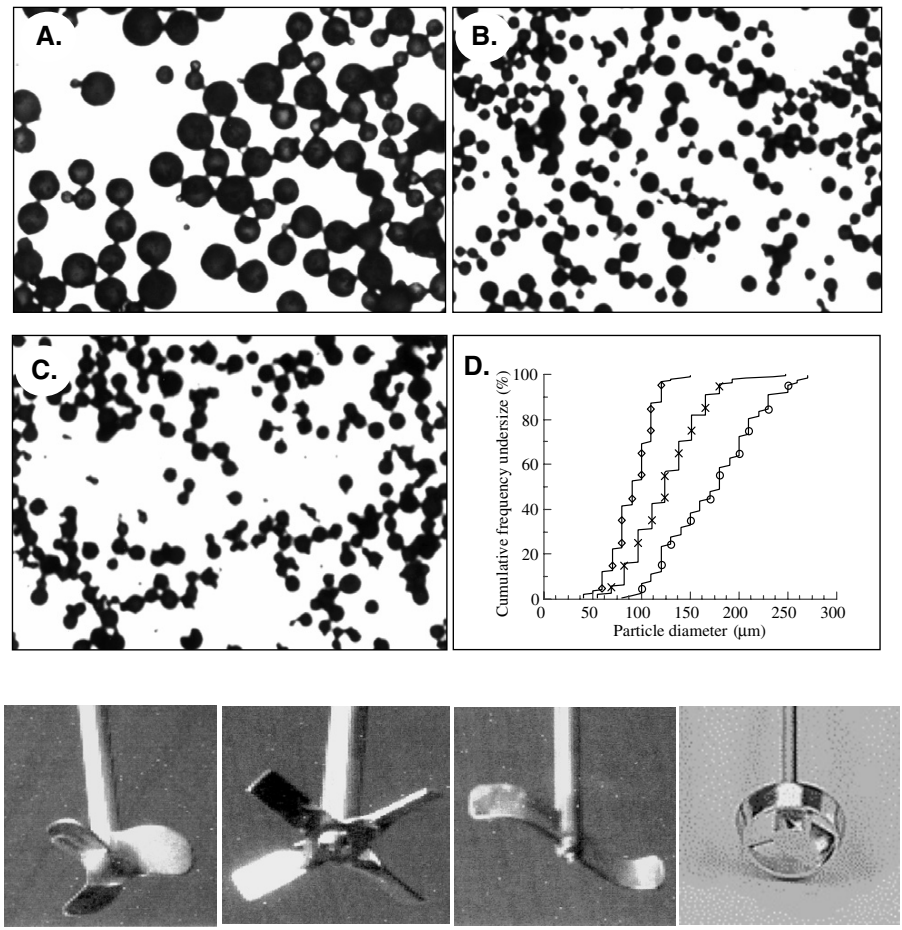

FIGURE 2.5 Optical microscopy photographs showing the effect of stirring speed on morphology and particle size of tristearin: monostearate 2:1 (w/w) produced at **(A)** 500 rpm, **(B)** 750 rpm, and **(C)** 1000 rpm. Bar corresponds to 650, 650, and 347 μm in panels A, B, and C, respectively. **(D)** Frequency distribution plot of microspheres produced at 500 rpm (○), 750 rpm (x), and 1000 rpm (◇). Data are the mean of three different microsphere batches. **Lower panel:** impellers employed for microsphere production, from left to right: a 3-blade rotor with a diameter of 55 mm (taken as reference impeller), a 4-blade helicoidal rotor with a diameter of 50 mm, a 2-blade rotor with a diameter of 50 mm, and finally a double-truncated cone rotor with a diameter of 50 mm.

8100 did not allow the stabilization of the o/w emulsion during the preparation, resulting in the formation of large lipid aggregates. The use of 1% polyoxyethylene sorbitan trioleate or PVA allowed the production of spherical particles with mean diameters of 190 and 120 μm and recoveries of 54% and 78%, respectively. Finally, lauryl sarcosine caused the formation of a very fine o/w emulsion, resulting in the final formation of very small particles (mean diameter 10 ± 4.1 μm) that were isolated

TABLE 2.8
Effect of Stabilizer on Tristearin Liposphere Characteristics

Stabilizer (%, w/v)	Recovery (%)[a]	Mean Diameter (μm)
Gelatin 200 Bloom (8)	69	150 ± 33
Pectin (0.5)	80	250 ± 17
Carrageenan κ (0.5)	62	200 ± 25
Carrageenan ι (0.5)	65	200 ± 31
Carrageenan λ (0.5)	Compromised separation	
Polyvinyl alcohol (1)	78	120 ± 26
Polyoxyethylene sorbitan trioleate (1)	54	190 ± 18
Pluronic PE 8100 (1)	Lipid aggregation	
Lauryl sarcosine (1)	—	10 ± 41

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor and a 750-rpm stirring speed. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

by centrifugation. Further studies are in progress to evaluate the experimental parameters for the production of lipid nanoparticles.

2.3 SOLVENT EVAPORATION TECHNIQUE

As an alternative to the melt dispersion technique, a solvent evaporation method was also tested for the production of LS (Figure 2.1). This approach was considered with the aim of possibly reducing the exposure to the high temperatures of thermolabile compounds, such as proteins and nucleic acids. The solvent evaporation method is based on the evaporation of the organic solvent in which lipids are dissolved, allowing the formation of solid microparticles. Through this technique, LS constituted of tristearin:glyceryl monostearate 2:1 w/w were produced, with 1% PVA as the emulsifier agent. In particular, the lipidic matrix dissolved in an organic solvent such as ethyl acetate at 50°C was emulsified in an external aqueous phase containing the surfactant agent. The resulting oil-in-water emulsion was stirred for 6 to 8 h under ambient conditions to allow the solvent evaporation. LS, after the water rose, were collected by filtration through a paper filter.

The obtained particles (Figure 2.6) were spherical and were characterized by their smaller size with respect to the particles of the same composition that were produced by the melt dispersion technique. Unfortunately, the produced LS showed some poor mechanical properties, including fragility, as well as a higher proportion of interparticellar bridges when compared with the melt dispersion technique.

With the aim of improving the mechanical properties of LS produced by solvent evaporation, as well as the aim of obtaining prolonged-release profiles, the possibility

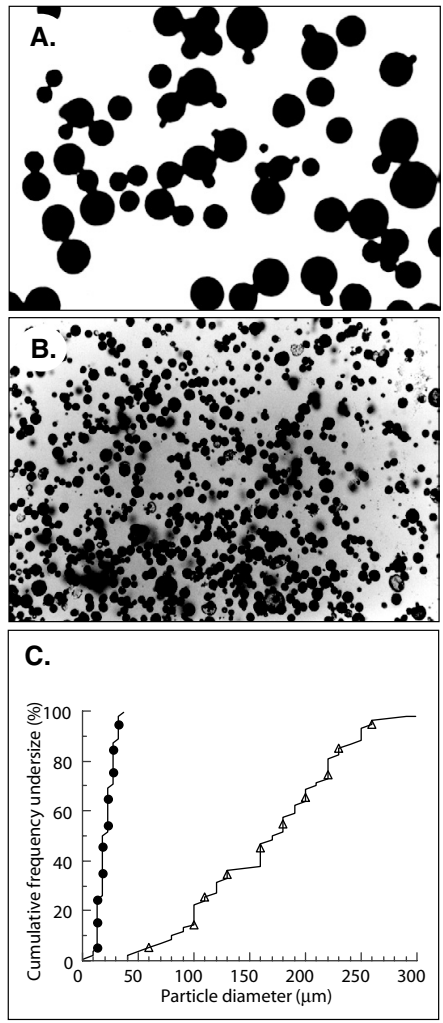

FIGURE 2.6 Effect of the type of the method of preparation on morphology and particle size of lipospheres. Optical micrographs of microspheres produced by **(A)** melt dispersion technique and **(B)** solvent evaporation technique. Lipospheres were constituted of tristearin:glyceryl monostearate 2:1 (w/w) and prepared in the presence of 1% polyvinyl alcohol. Bar corresponds to 292 and 162 μm in panels A and B, respectively. **(C)** Frequency distribution plot of microspheres produced by melt dispersion (△) and solvent evaporation (●) technique. Data are the mean of three different microsphere batches.

of producing particles with a mixed matrix was considered (Table 2.9). LS were produced using lipids in combination with different polymers, in a ratio of up to 20% with respect to the lipid components. Both biodegradable polymers, such as polylactic acid (PLA), and nonbiodegradable polymers, such as Eudragit RS 100, were used. The different polymers allowed the improvement of the mechanical characteristics of the LS and, particularly in the case of Eudragit RS 100, allowed

TABLE 2.9
Effect of Synthetic Polymers on the Production of Lipospheres by Solvent Evaporation

Microparticle Composition (w/w ratio)	Stabilizer (%, w/v)	Recovery (%)[a]	Stirring Speed (rpm)
Tristearin:monostearate (66:34)	PVA (1)	20 ± 4.4	750
Tristearin:monostearate (66:34)	PVA (1)	165 ± 6.2	500
Tristearin:monostearate (66:34)	PVA (0.5)	n.d.	250
Tristearin:monostearate (66:34)	PVA (0.25)	n.d.	250
Tristearin:monostearate:PLA (52:28:20)	PVA (1)	50 ± 11	750
Tristearin:monostearate:PLA (52:28:20)	PVA (0.1)	n.d.	250
Tristearin:monostearate:Eudragit RS (52:28:20)	PVA (1)	15 ± 3.6	750
Tristearin:monostearate:Eudragit RS (52:28:20)	PVA (1)	n.d.	250
Tristearin:monostearate:Eudragit RS (52:28:20)	PVA (0.1)	50 ± 12	500
Tristearin:monostearate:Eudragit RS (52:28:20)	PVA (0.1)	100 ± 9	250

Note: Common experimental parameter was a 55-mm, 3-blade turbine rotor. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation. n.d. = not determined; PVA = polyvinyl alcohol.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

the production of more spherical particles with a narrow size distribution and a mean diameter of 15 μm; in addition, interparticle fusion phenomena were almost absent.

2.4 DRUG-CONTAINING LS

Two hydrophobic compounds, such as retinyl acetate and progesterone, and one hydrophilic drug, SCG, were considered as model drugs. LS were produced by the melt dispersion technique.

LS containing hydrophobic retinyl acetate were yellow (because of the color of the drug) and spherical, with a slightly waved surface (Figure 2.7) and a narrow size distribution (184 ± 6.6 μm). Particles containing progesterone were very similar in shape (data not shown) and were white (mean diameter, 192 ± 11.6 μm).

The amount of encapsulated model drug (retinyl acetate or progesterone) per mg of dried LS was determined through solubilization of LS in ethyl acetate at 60°C. Following filtration, the solution was analyzed by reverse-phase high-performance liquid chromatography (HPLC) to find the drug content.

The HPLC determinations were performed using an HPLC system operating at 215 nm. Samples were chromatographed on a stainless steel C18 reverse-phase column eluted isocratically at room temperature, at a flow rate of 1 mL/min. The mobile phases were 180 mM ammonium acetate (pH 3.0)/methanol (4:96, v/v) for retinyl acetate [20], methanol/water (70:30, v/v) for progesterone [21], and phosphate buffer (pH 2.3)/methanol (50:50, v/v) for cromoglycate [22]. Drug detection was monitored at the λ_{max} characteristic of each compound.

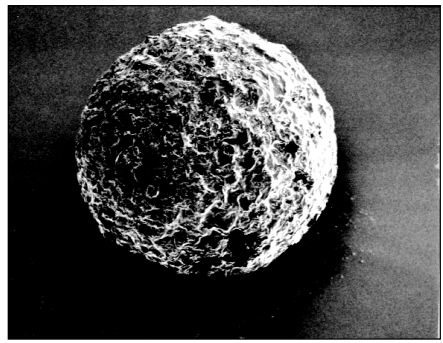

FIGURE 2.7 Scanning electron micrographs of tristearin:glyceryl monostearate 66:33 (w/w) lipospheres containing retinyl acetate. Bar corresponds to 48 μm.

TABLE 2.10
Drug Encapsulation Efficiency and Recovery of Lipospheres

Drug	Encapsulation Yield (%)	Recovery (%)[a]
Retinyl acetate	87.4 ± 1.5	85.4
Progesterone	70.7 ± 2.1	93.0
Sodium cromoglycate (o/w)	2.0 ± 0.6	67.0
Sodium cromoglycate (o/w)	3.0 ± 1.6	63.4
Sodium cromoglycate (w/o/w, gelatin)	22.0 ± 2.4	72.7
Sodium cromoglycate (w/o/w, gelatin)	50.0 ± 8.1	81.0
Sodium cromoglycate (w/o/w, poloxamer)	12.0 ± 3.2	77.0

Note: Experimental parameters were a 55-mm, 3-blade turbine rotor and a 500-rpm stirring speed. Data represent the average of 3 independent experiments on different microsphere batches ± standard deviation.

[a] Percentage (w/w) of liposphere production with respect to the total amount of lipids used for preparation.

As reported in Table 2.10, both LS types are characterized by high encapsulation and recovery efficiencies. In the case of the hydrophilic drug SCG, again the microparticles were morphologically almost identical (data not shown) (mean diameter 234 ± 14.8 μm), but the encapsulation efficiency was, on the contrary, unsatisfactory, being only 2%. This result was partially expected, as hydrophilic drugs can be less efficiently incorporated in LS with respect to hydrophobic ones. In fact, if the drug is too hydrophilic to be soluble in organic solvents, microcrystalline fragments of the drug could be incorporated in the microparticle. The water-soluble drug could then diffuse into the outer continuous aqueous phase, resulting in low trapping of the compound and inducing an initial rapid release of the drug known as "burst effect."

To improve the encapsulation of SCG, LS were produced using a w/o/w double-emulsion strategy, consisting of the solubilization of the drug to be encapsulated in the internal aqueous phase of a w/o/w double emulsion, along with a stabilizer that was able to prevent the loss of drug to the external phase during solvent evaporation [23]. In particular, an aqueous solution of the drug was emulsified in melted lipids at 70°C by an Ultra-Turrax. This primary emulsion was stabilized adding gelatin (250 Bloom grades) or the polyoxyethylene–polyoxypropylene block copolymer, poloxamer 407, as stabilizers solubilized in the aqueous phase. The primary emulsion was then dispersed at 70°C into an aqueous phase containing 0.25% (w/v) of PVA. The obtained double emulsion was stirred at 300 rpm by a four-blade turbine impeller. After 3 to 5 h, microparticles were isolated by filtration.

In particular, the effects of various stabilizers of the primary emulsion on the encapsulation of SCG were studied. Different hydrophilic polymers were employed; namely gelatin (250 Bloom grades) or the polyoxyethylene–polyoxypropylene block copolymer, poloxamer 407. To further optimize the encapsulation yield, some experimental contrivances have been performed: dispersion by turbine of the drug within the lipidic matrix, rapid emulsion cooling using an ice bath, and rapid separation of LS by filtration. The optimized procedure resulted in a final encapsulation of the drug of 50% (Table 2.10).

2.5 *IN VITRO* DRUG RELEASE

To obtain quantitative and qualitative information on drug release from the LS, and possibly to correlate the experimental data with the release mechanism, the complete release profile of LS encapsulated drugs was determined by placing a drug containing LS in a buffer under magnetic stirring at 150 rpm. The buffer was ethanol/water in a 30:70 ratio, with the addition of 0.5% (w/w) polysorbate 20. Following different lengths of time (0 to 8 h), samples of receiving buffer were filtered and analyzed for drug content by reverse-phase HPLC, as previously described.

In Figure 2.8, the release kinetics of retinyl acetate and progesterone (panel A) and of SCG (panel B) is reported. As is noticeable, the release of both hydrophobic drugs was much slower with respect to SCG, especially in the case of retinyl acetate containing LS. For these particles, the drug release efficacy within the first 8 h of release was 27% of the total amount of the drug. In the same period, the amount of progesterone released was 63%. This behavior could be ascribed to the physicochemical characteristics of the drugs, which, as expected, showed a high affinity for the oil phase instead of the aqueous one.

Concerning sodium cromoglycate containing LS, the release of the drug was largely influenced by the type of stabilizer used in the primary emulsion. In the case of LS produced in the presence of gelatin, the shape of the release has a sigmoid form, and, after 8 h, the release reaches 80% of the total amount of the drug. Conversely, LS produced in the presence of poloxamer 407 shows a drug release typified by a biphasic profile. The first part, characterized by rapid drug release, is followed by a slower release rate, during which the drug is released in an approximately

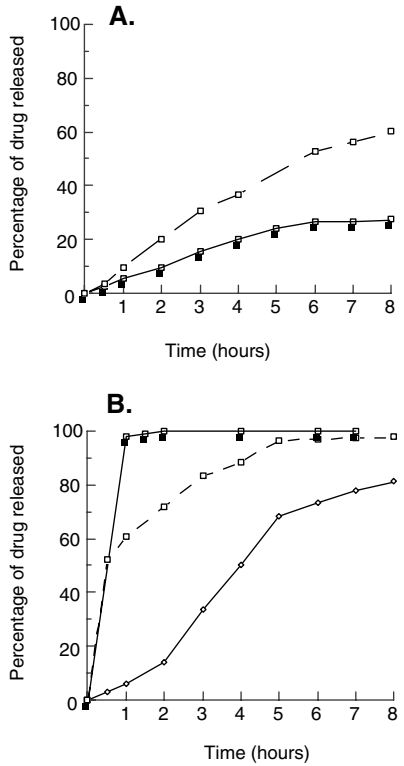

FIGURE 2.8 Release profiles of drugs encapsulated in lipospheres. **(A)** Lipophilic compounds, such as retinyl acetate (■) and progesterone (□). **(B)** Hydrophilic compounds, such as sodium cromoglycate, encapsulated using gelatin (◇) or poloxamer 407 (□) as the stabilizer. As a reference, the release of free SCG is also reported (■). The releases were determined by dialysis method. Data represent the average of five independent experiments on different microsphere batches.

linear mode. In addition, it should be emphasized that the release of cromoglycate reaches 100% of the total amount of the drug 5 h after the experiment begins.

2.6 CONCLUSIONS

Melt dispersion, solvent evaporation, and w/o/w double-emulsion methods enabled us to produce LS whose morphology and size were influenced by the experimental parameters employed.

In particular, in the case of LS prepared by melt dispersion, the use of different lipid mixtures, types of stabilizer, and stirring speeds affected both microparticle shapes and their size distribution. The use of lauryl sarcosine as the stabilizer allowed the formation of very small LS; further experiments will be performed to better investigate the experimental parameters involved in the production of very small LS.

LS, under appropriate experimental conditions, can entrap both hydrophobic and hydrophilic drugs and can control the release of the encapsulated drug. The encouraging results obtained in this study could propose LS for future *in vivo* studies, especially in the delivery of antiinfectives and hormones.

In an earlier paper, we described the production and characterization of biodegradable microparticles containing tetracycline, which were designed for periodontal disease therapy. Microparticles were made by using different preparation procedures and different polyesters: poly(L-lactide), poly(DL-lactide), and poly(DL-lactide-co-glycolide) 50:50. Selection of the appropriate preparation method and polyester enabled us to obtain biodegradable microparticles intended for sustained delivery of tetracycline to the periodontal pocket [23].

Lipid-based microspheres appear to be ideal candidates for administering antibacterial agents for periodontal therapy; because they are biodegradable, they do not have to be removed after the treatment period, and they possess mucoadhesive properties.

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