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Research Paper

Influence of Lipid Nanocapsules Composition on Their Aptness to Freeze-Drying

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Purpose. To link the aptness to freeze-drying and the stability under storage to the topology of lipid nanocapsules.
Methods. The aptness to freeze-drying and the stability under storage of lipid nanocapsules prepared from different compositions with a lecithin content in the 2–20% range were estimated from the preservation of the physical structure, preventing the leakage of the oily phase. The influence of the outer shell composition and of the physical characteristics (investigated by photon correlation spectroscopy and differential scanning calorimetry) on the physical stability was correlated to the topology of the nanoparticulate carrier.
Results. Confirming the assumption that lecithin confers hardness to the outer shell of lipid nanocapsules, this study shows that the aptness to freeze-drying and the stability under storage depend on the Solutol®/lecithin (S/L) ratio in the formulation. The DSC study points out a complexation between lecithin and trehalose, the cryoprotectant, reinforcing the stabilising properties of lecithin.
Conclusions. This paper is a contribution to methodological development of the formulation of lipid nanocapsules, with a special emphasis on the aptness to freeze-drying and the stability under storage.

KEY WORDS: formulation; freeze-drying; lecithin; lipid nanocapsules; stability under storage; thermal analysis.

INTRODUCTION

Lipid nanocapsules (LNs) are submicronic particles made of an oily liquid core surrounded by a solid (or semi-solid) shell. These nanoparticulate carriers were primarily developed to combine the colloidal stability of solid particles suspensions in biological fluids and the solubilizing properties of liquids (1,2).

Polymeric nanocapsules were first prepared by solubilization of the outer shell material in an organic solvent (3). Interesting biopharmaceutical performances of drugs encapsulated in polymeric nanocapsules have been reported for the oral (4), the parenteral (5,6), and the ocular (7) routes. However, the industrial constraints of solvent handling, the limited scale and the particular efforts needed to decrease residual solvent down to few ppm induced high manufacturing costs.

More recently, a lipid-based solvent-free formulation process was developed to prepare lipid nanocapsules in the nanometer range (1,8). This process takes advantage of the variation of the hydrophilic/lipophilic balance of an ethoxylated hydrophilic surfactant (polyethylene glycol-660 hydroxystearate, i.e., Solutol HS15) as a function of the temperature, leading to inversion phase. In a first step, several temperature cycles applied around the inversion phase temperature lead to droplets size decrease and homogenization (9). In a second step, fast cooling leads to the crystallization of the lecithin (introduced in the formulation both as lipophilic co-surfactant and constituting material of the rigid shell), and to the formation of a stable lipid nanocapsules suspension. This suspension can be freeze-dried and resuspended in an aqueous medium, extemporaneously.

Due to the relationship between the structure of the lipid nanocapsules and their biopharmaceutical performances, particular attention should be paid to the topology of the carrier. This topology is a consequence of the physicochemical features of nanocapsules formation driven by the formulation and process parameters (10,11). The composition is of prior importance, as only defined mixtures lead to nanocapsules formation. In a previous work, the exploration of the behavior of the mixtures was monitored using a ternary diagram including the water phase, the hydrophilic surfactant, and the oily phase. The hydrophilic surfactant had a major effect on the average diameter, whereas the lipophilic surfactant had no effect on particle size distribution (8).

Once the topology is controlled from the formulation and process parameters, the challenge of the freeze-drying process development is to manage water removal in such a way that the structure of lipid nanocapsules is preserved. In this context, the axis of exploration combines the identification of the key parameters conditioning the aptness to freeze-drying of the lipid nanocapsules suspensions (concentration,

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ABBREVIATIONS: DSC, differential scanning calorimetry; ICH, International Committee of Harmonization; LNs, lipid nanocapsules; PEG, polyethylene glycol; PI, polydispersity index; RH, relative humidity; SD, standard deviation.

size, shell composition, and thickness) and the definition of the freeze-drying conditions (cryoprotectant, pressure, and temperature cycle). The leakage of the oily phase is, on the short term, the main drawback resulting from the processing of a starting material exhibiting a poor aptness to be freeze-dried or from the use of inappropriate freeze-drying conditions. As far as the mechanical properties of the outer shell are concerned, the lecithin is considered as a major component. As a result of the fast cooling, its solidification prevents the coalescence of the droplets and leads to stable colloidal suspensions at room temperature. At the same time, the increase of the percentage of lecithin in the outer layer leads to a thicker and more rigid shell, with anticipated improvement of the aptness to freeze-drying and the prevention of the leakage of the oily phase (12).

This paper describes the preparation, the aptness to freeze-drying, and the stability under storage of lipid nanocapsules as a function of the composition, with a focus on lecithin-enriched mixtures. Because the experimental domain explored here was changed as compared to the domain previously explored (8), the influence of the composition on the size distribution was first checked. In a second step, the aptness to freeze-drying estimated from i) the processability (i.e., absence of oil leakage) and ii) the stability under storage was explored, as a function of the outer shell composition. For that purpose, the exploration of the behavior of the mixtures was monitored using a ternary diagram based on the lipid components of the nanocapsules, namely the lipophilic surfactant (lecithin), the hydrophilic surfactant (Solutol HS15) and the oily phase.

MATERIALS AND METHODS

Materials

Neutral oil (Captex 8000, tricaprylin), soybean lecithin (Lipoid S 100-3, 94% phosphatidylcholine), and polyethylene glycol-660 hydroxystearate (*European Pharmacopeia*, IVth, 2002, brand name Solutol HS15) were kind gifts from Abitec (Columbus, OH, USA via Unipex, Rueil Malmaison, France), Lipoïd GmbH (Ludwigshafen, Germany) and BASF AG (Ludwigshafen, Germany, via Laserson, Etampes, France), respectively. Due to the complex composition of polyethylene glycol-660 hydroxystearate, the brand name Solutol HS15 is used in this paper. Trehalose was purchased from Amcan Ingredients (Le Chesnay, France). All other reagents were of analytical grade.

Lipid Nanocapsules Preparation

The preparation of lipid nanocapsules was based on the reported method of phase inversion processing (1). Briefly, all components (water, sodium chloride, oil, lecithin, and polyethylene glycol-660 hydroxystearate) were mixed and heated under magnetic stirring up to 92°C, above the phase inversion temperature. In the next step, the temperature was decreased down to 70°C, below the inversion zone. This temperature cycle was applied two other times. The last cooling ramp was performed down to a tremp temperature, defined as the lower limit of the phase inversion zone, measured by conductimetry. The tremp (fast cooling) was operated by addition of a volume of water at 0–2°C. The LN concentration in the final dispersion was approximately 15% w/w.

Freeze-Drying

Before freeze-drying, a cryoprotectant (trehalose) was added to the suspension, in a 1:1(w:w) ratio as compared to the lipid nanocapsules. The suspension was frozen in liquid nitrogen prior to freeze-drying. The suspensions were freeze-dried either in 10 ml glass containers (type I white glass, with rubber stoppers, *European Pharmacopeia*, IVth, 2002) or in stainless steel beakers, with a height of suspension inferior to 1.5 cm. Freeze-drying was operated in a Lyovac GT2 (Steris, Germany)/Phoenix P2 C75P (ThermoHaake, Germany) system. The freeze-drying cycle is described in Table I.

Size Measurements

The average volume diameter, the polydispersity index (PI), and standard deviation (SD) of LNs were determined by photon correlation spectroscopy using a N4 + system (Coulter Corp., Miami, FL, USA) at a fixed angle (90°) at 25°C. Samples of LNs were diluted in distillated water prior to triplicate measurements. The dust value was <5% for all measurements.

Differential Scanning Calorimetry (DSC)

The DSC measurements were performed using a Mettler Toledo DSC 822 (Mettler Toledo, Viroflay, France). Each sample was analyzed at 5°C/min in the –50°C to 150°C range.

Pure components were analyzed as raw material. Lipid nanocapsules were analyzed as freeze-dried powders. Binary mixtures were prepared in the ratios of the formulation, and were submitted to the same temperature cycles before freeze-drying and DSC analysis. Samples were analyzed in 40-μl pinhole aluminium pans. Transition temperatures were determined from the peak minimum (T_p). Enthalpies were calculated from the integration of DSC peaks, normalized according to the mass of the considered component in the mixture. The peak area of indium was used as a standard for enthalpy determination.

Stability

The freeze-dried lipid nanocapsules were placed in closed glass containers (type I white glass, rubber stoppers, *European Pharmacopeia*, IVth, 2002) in International Committee of Harmonization (ICH) conditions for stability studies in climatic chambers ICH600 (Eratis, Bouloc, France). Two storage conditions were explored: 5 ± 3°C and 40 ± 2°C, 75 ± 5% relative humidity (RH). Samples are analyzed after 0, 1, and 3 months of storage.

Table I. Freeze-Drying Cycle

Starting temperature	Final temperature	Duration
–43°C	–10°C	4 h
–10°C	–10°C	15.5 h
–10°C	+10°C	0.5 h
+10°C	+23°C	0.5 h

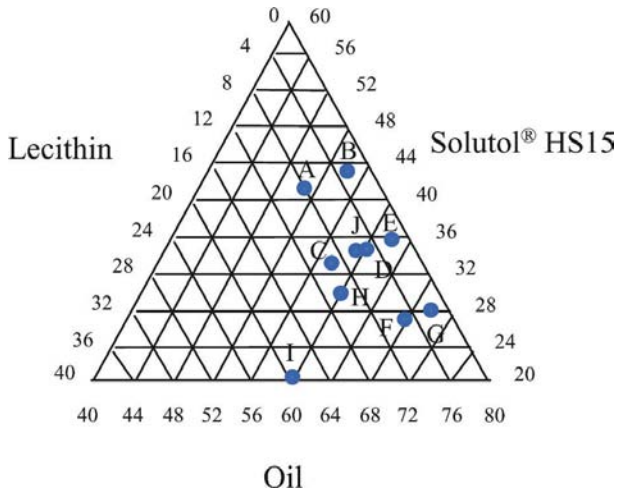

Fig. 1. Ternary diagram used for lipid nanocapsules formulation. Each point corresponds to a LN formulation (see Table III).

RESULTS

One of the objectives of the formulation studies is to compromise the drug payload capacity and the stability of the nanoparticulate carrier. Because the drug is solubilized in the oily phase, the increase of the fraction of oily phase in the composition leads to a corresponding increase of the payload. However, a high amount of oily phase may lead to a more fragile shell and, as a consequence, can alter the aptness to freeze-drying of lipid nanocapsules, or induce oil leakage under storage.

In order to investigate the influence of lipid nanocapsules composition on their aptness to freeze-drying and to be stored in a dried form, it can be assumed that the physical stability of lipid nanocapsules is primarily dependent on i) the thickness and ii) the rigidity (i.e., the crystallinity) of the shell. The shell consists of a lipophilic surfactant, Lipoid S 100, and a hydrophilic surfactant, Solutol HS15. More specifically, if all the surfactant molecules are located at the surface of the lipid nanocapsules, the thickness of the shell is governed by both the amount of surfactants in the formulation and the surface area of the emulsion droplets or, for a given oily phase concentration, the diameter of the lipid nanocapsules. According to the rheological behavior of lipid-based mixtures (12), it can be assumed that the viscosity of the surfactant mixture and, as a consequence, the rigidity of the shell, depends on the composition of the surfactants mixture and the gap between the storage temperature and the melting temperatures of the individual components. Therefore, because the melting temperatures of Solutol HS15 components are 18.4°C and 31.0°C while the melting temperature of Lipoid S100 is 83.5°C, it can be assumed that, at the storage temperatures explored (5 ± 3°C and 40 ± 2°C), the rigidity of the shell increases when the Solutol HS15/Lipoid S100 ratio decreases.

Relationship Between the Composition and the Mean Diameter of Lipid Nanocapsules

The experimental domain investigated for lipid nanocapsules formation is shown on a ternary diagram in Fig. 1. Lipid nanocapsules were prepared according to the procedure described under "Materials and Methods" for all the tested points. The size of the lipid nanocapsules varied from approximately 40 nm to more than 300 nm (Table II). As previously reported (13), the mean size of a particulate carrier prepared according to an emulsification process can be modulated by the amount of surfactant, when it is the limiting factor of the droplet size decrease. In these conditions, the available surface area per weight of oily phase, must be proportional to the concentration of surfactant in the emulsion, C_{surf}, which gives: $S_{oil}/C_{oil} \alpha C_{surf}$, where S_{oil} and C_{oil} are the surface area and the concentration of the oily phase, respectively. This leads to $d \alpha C_{oil}/C_{surf}$, where d is the mean diameter of lipid nanocapsules, and, when the water is removed, to $d \alpha F_{oil}/F_{surf}$, where F_{oil} and F_{surf} are the fractions of oil and surfactant in the dried powder. In Fig. 2, the mean size of lipid nanocapsules was plotted vs. $F_{oil}/F_{solutol}$, where $F_{solutol}$ is the fraction of Solutol HS15 in the ternary composition diagram shown in Fig. 1. No satisfactory linear fit can be observed when the tested formulations are taken as a whole. Nevertheless, the linear fitting is significantlty improved when the formulations are considered in three groups, according to the Solutol HS15/Lipoid S 100 (S/L) ratio. It is worth noting that for low values of S/L (lecithin-enriched formulations) the linear fitting is satisfactory considering the fraction of Solutol HS15 whereas no linear fit can be drawn from the data when the total amount of surfactant (i.e., Solutol HS15 + Lipoid S 100) is considered (data not shown). At the same time, the

Table II. Lipid Nanocapsules Characteristics

	In lipid nanocapsules			In emulsion	Tremp	Before freeze-drying		After freeze-drying	
Batch	Oil (%)	Solutol (%)	Lecithin (%)	NaCl (%)	temperatue (°C)	Mean size (nm)	PI	Mean size (nm)	PI
A	51	41	8	4.4	66	43	0.09	—	—
B	54	43	3	4.4	71	82	0.19	—	—
C	57	33	10	1.6	78	53	0.13	157	0.32
D	60	35	5	1.8	78	73	0.28	142	0.28
E	62	36	2	1.9	78	82	0.17	—	—
F	68	27	5	3.7	69	170	0.18	—	—
G	70	28	2	3.9	71	320	1.14	—	—
H	60	30	10	1.8	71	51	0.11	116	0.46
I	60	20	20	2.7	78	125	0.20	212	0.44
J	59	35	6	1.7	78	60	0.08	N.D.	N.D.

Tremp temperature, temperature at which the fast cooling is operated; PI, polydispersity index; N.D., not determined.

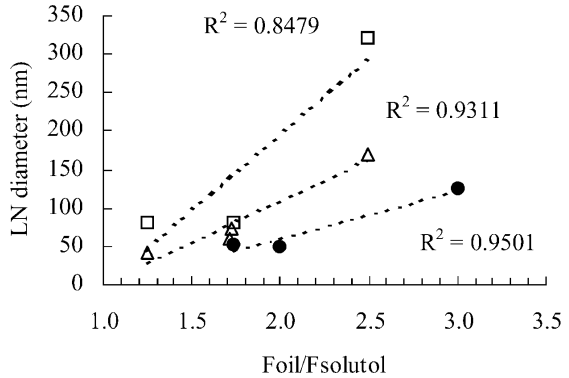

Fig. 2. Mean particle sizes as a function of $F_{oil}/F_{solutol}$. ●: 1 < S/L < 3; Δ: 5 < S/L < 7 ; □: S/L ~ 13.

Lipoid S 100 has an influence on the lipid nanocapsules diameter since the slope of the linear fit decreases when S/L decreases.

Aptness to Freeze-Drying and Stability of Lipid Nanocapsules

Freeze-drying of the lipid nanocapsules formulations was performed introducing trehalose as cryoprotectant in the suspension. Other cryoprotectants were tested, but trehalose gave the best results in terms of polydispersity index of the sample after freeze-drying and resuspending (unpublished results).

The relationship between the composition of the dried lipid nanocapsules, the aptness to freeze-drying and the stability of the powder under storage at 5°C and 40°C, 75% RH are shown in Fig. 3. Low lecithin content formulations exhibit a poor aptness to freeze-drying, while formulations with a lecithin content of 5% or more can be freeze-dried. This result is consistent with the assumption that lecithin, the constituting surfactant of the shell with a high melting point (T_m = 83°C), should be in sufficient amount to confer the appropriate rigidity to the nanoparticulate carrier (12).

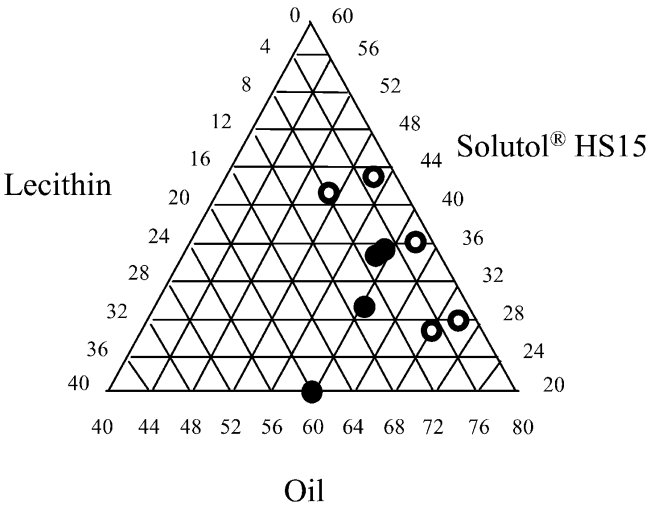

Fig. 3. Aptness to freeze-drying and physical stability upon storage at 5°C and at 40°C, 75% RH of lipid nanocapsules. ○: Oil leakage during freeze-drying or after few days under storage. ●: No oil leakage after 3 months storage.

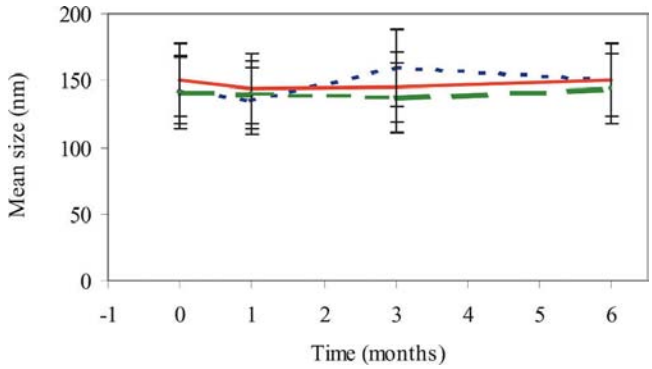

Fig. 4. Size of freeze-dried lipid nanocapsules upon storage at 5°C. Mean size ± SD (n = 3). The three batches correspond to point D from Fig. 1.

It can be noticed that for all the formulations investigated, the mean particles size increased significantly after freeze-drying (Table II). Nevertheless, once freeze-dried, the mean particle size of lipid nanocapsules was not significantly changed under storage (Fig. 4).

From macroscopic examination reported in the Fig. 3, the best stability was observed for formulations J, D, H, and I, which did not exhibit any oil leakage after 3 months storage at 5°C or at 40°C, 75% RH.

To get a deeper insight into the influence of the topology on the aptness to freeze-drying and on the physical stability in the dried form, the composition and the size parameters were combined to probe the influence of the shell thickness. In the one hand, if it is considered that all the surfactant molecules are located at the surface of the lipid nanocapsules, the thickness of the shell (i.e., the density of the surfactants at the surface of the nanoparticulate carrier) depends on the fraction of surfactants in the formulation, and on the mean diameter. Because the hydrophilic surfactant, Solutol HS15, is a mixture of components, it is difficult to propose a relevant evaluation of the surface density in terms of molecule per surface area unit. Nevertheless, based on the assumption that the densities of lipid components are close to the same, an estimation of the thickness of the shell, t, can be proposed, the calculation of which is detailed in the Appendix.

In the other hand, under the assumption that the physical quality of the shell is dependent on the respective amount of Solutol HS15, driving the physicochemistry of the lipid nanocapsules formation (6), and of Lipoid S 100, conferring the rigidity to the shell, it can be anticipated that a critical value of the S/L ratio (Solutol HS15/Lipoid S 100) is needed to compromise lipid nanocapsules formation, aptness to freeze-drying and stability under storage.

The relationships between the shell thickness, the ratio S/L, the aptness to freeze-drying, and the stability of the formulations under storage at 40°C, 75% RH are combined in Fig. 5. The highest value of S/L ~13 (formulations B, E, and G) led to oil leakage during freeze-drying or few days after freeze-drying, even when the calculated thickness was significantly higher than those of lecithin-enriched formulations (S/L values in the 1–3 range) which exhibited satisfactory aptness to freeze-drying. An intermediate behavior was observed for the formulations with S/L values in the 5–7 range. This result suggests that the aptness to freeze-drying and the physical stability under storage of the dried form depend not

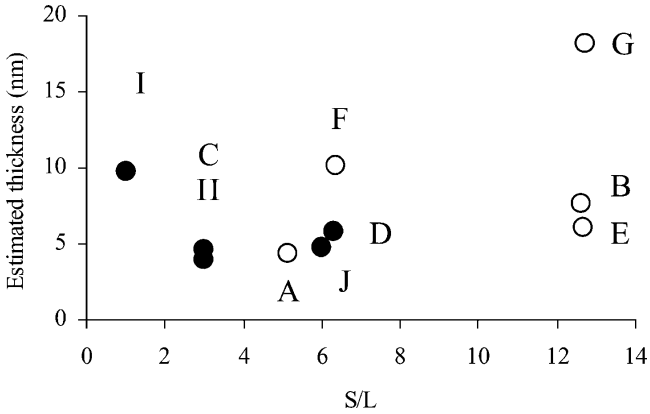

Fig. 5. Relationship between stability of lipid nanocapsules upon storage at 40°C, 75% RH, shell thickness, and shell composition. All LN formulations are composed of 60 ± 3% of oil. ○: Oil leakage during freeze-drying or after few days under storage. ●: No oil leakage after 3 months storage.

only on the thickness but also on the physical quality of the shell.

DSC Analysis of Lipid Nanocapsules

LN are analyzed in DSC as freeze-dried powders, taking into account that when LNs are freeze-dried without cryoprotectant an oil leakage is observed.

The DSC profile of freeze-dried lipid nanocapsules (formulation D in Fig. 1) is shown in Fig. 6. The oil used to prepare lipid nanocapsules (Captex 8000), a pure triglyceride (tricaprylin), exhibits polymorphic transition peaks in the –20°C to 10°C range. Because these peaks are associated to high enthalpy values, further studies were performed from 0°C to 150°C at a 5°C/min rate in order to improve the resolution of the thermograms. It is important to note that, using this temperature ramp, the oily core is kept in the liquid state intended for LN use (solubilization of drugs).

As raw material, Lipoid S 100 exhibits a transition peak at 83.5°C (Table III). In the binary mixture prepared from lecithin and tricaprylin as described under "Materials and Methods" (curve 1 in Fig. 7), the transition temperature of lecithin is identified, and the fusion energy is not decreased as compared to the raw material (Table III). Therefore, the lecithin does not dissolve in the oil, at this formulation ratio.

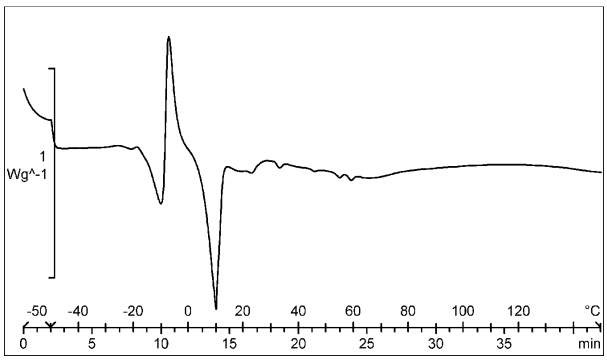

Fig. 6. DSC heating curve of lipid nanocapsules freeze-dried with trehalose (formulation D from Fig. 1). Analysis performed from –50°C to 150°C at a 5°C/min rate. Endothermic transitions are down.

Two main fusion peaks at 18°C and 31°C can be observed on the thermogram of Solutol HS15 (Table III). The first peak at 18°C is attributed to free PEG (32% in Solutol HS15) and the second one, at 31°C, is attributed to the diester of polyethylene glycol-660 hydroxystearate (30–34% in Solutol HS15). In the binary mixture prepared from Solutol HS15 and tricaprylin (curve 2 in Fig. 7), the two transition peaks of Solutol HS15 can be observed. The enthalpy increases for the first peak (78.7 J/g vs. 65.9 J/g for raw material) and decreases for the second peak (4.5 J/g vs. 19.9 J/g for raw material). Therefore, Solutol HS15 does not dissolve in the oil, at this formulation ratio.

For binary mixture of Solutol HS15 and lecithin (curve 3 in Fig. 7), the transition peaks of both components can be observed, and enthalpies are close to those reported for raw materials. When lipid nanocapsules are freeze-dried without trehalose (curve 4 in Fig. 7), the transition peaks of both Solutol HS15 and lecithin are observed, showing that a phase separation occurs in the outer shell.

When lipid nanocapsules are freeze-dried with trehalose (curve 5 in Fig. 7), the transition peak of Solutol HS15 is observed. However, the transition peak of lecithin disappears, while two other peaks are observed at 55°C and 59°C, respectively. These transition temperatures shifts were attributed to the formation of a complex between trehalose and lecithin, as previously described with freeze-dried liposomes (15).

DISCUSSION

Relationship Between Formulation, Aptness to Freeze-Drying, and Stability upon Storage

From a formulation point of view, the selection of the oily phase is dictated by the solubility of the drug in the lipid vehicle. Therefore, the other formulation parameters should be adapted to compromise the payload and the stability of the particulate carrier.

In this study, a relationship between the composition of lipid nanocapsules, the shell thickness, the aptness to freeze-drying and the stability under storage was established *via* the exploration of the ternary diagram of the lipid components.

As mentioned above, considering that the aptness to freeze-drying is related to the thickness and the physical quality of the shell, a lecithin-enriched formulations domain was explored. In agreement with the data reported in pioneering experiments (8), the mean size of lipid nanocapsules described here is proportional to the $F_{oil}/F_{solutol}$ ratio (Fig. 2), whereas especially for the lecithin-enriched formulations, the points cannot be fitted by a linear function when the fraction of the whole amount of surfactants is considered. The major influence of the Solutol HS15 content on the LN diameter, as compared to the Lipoid S 100, can be explained comparing the chemical structures of the surfactants. The Solutol HS15 exhibits high surfactive properties due to its amphiphilic structure consisting of a big hydrophilic polyethylene glycol moiety associated to a big hydrophobic hydroxystearate moiety. The Lipoid S 100 is lipophilic and exhibits lower surfactive properties. Moreover, since it is an ionic surfactant, the presence of sodium chloride in the water phase screens the electric charges of the molecule, leading to a decreased surfactive power. Nevertheless, the Lipoid S 100 has an influence on the lipid nanocapsules diameter, as the slope of the linear

Table III. DSC Analysis of Pure Components, Binary Mixtures, and Lipid Nanocapsules

Samples	Solutol HS15		Lecithin		Trehalose		
	T_p (°C)	Enthalpy (J/g)	T_p (°C)	Enthalpy (J/g)	T_p (°C)	Enthalpy (J/g)	Corresponding curve in Fig. 7
Solutol HS15	18.4	65.9					
	31.0	19.9					
Lecithin			83.5	35.8			
Trehalose					98.9	142.1	
Tricaprylin + lecithin			79.1	51.9			1
Tricaprylin + Solutol HS15	20.1	78.7					2
	31.3	4.5					
Solutol HS15 + lecithin	19.5	71.4	82.0	22.0			3
	33.5	12.7					
Lipid nanocapsules without trehalose	23.3	50.0	77.2	20.3			4
	29.2	11.8					
Lipid nanocapsules with trehalose	21.7	29.9	55.2	16.3			5
	31.2	9.6	58.8	19.4			

Note: Shaded area represents transition temperature shifts attributed to the formation of a complex between trehalose and lecithin.

fit decreases when S/L decreases. Interestingly the mean diameter of lipid nanocapsules is smaller for lecithin-enriched formulations. A possible explanation is that, when introduced in higher amount in the formulation, the Lipoid S 100 may be better combined to Solutol HS15 to stabilize the emulsion as a co-surfactant. Comparison of suspensions suggests that the predictability of the LN mean diameter based on the linear fit should be restricted to a given range of S/L values.

According to the lipid nanocapsules manufacturing process, the fast cooling of the suspension leads to the crystallization of the Lipoid S 100, which prevents the coalescence of the droplets and stabilize the suspension. The Lipoid S 100 content and the tremp temperature are key parameters to obtain the appropriate topology. As shown here, the aptness to freeze-drying and the stability of the dried powder under storage introduced further constraints. Oil leakage during freeze-drying was observed for the low lecithin content formulations. It is important to note that the stability of the LN may also be dependent on the viscosity of the oily phase. The physical state of the surfactant (pasty vs. solid) and its corresponding solubility in the oily phase it also anticipated to be of prior importance because, when the water is removed, the constituting surfactants of the shell may move from the surface of the LN to the core of the oily phase. As concluded from DSC experiments Lipoid S 100 and Solutol HS15 are not solubilized in tricaprylin in the experimental conditions explored here.

The physical quality of the shell related to the S/L ratio appears as a factor at least as important as the evaluated thickness, proportional to the density of the surfactants on the nanoparticulate carrier surface. At high S/L ratio, the leakage of the oily phase during freeze-drying may be due to the softness of the shell of the nanocapsules. At the same time, as Solutol exhibits interesting P-glycoprotein inhibition properties (14), S/L should be high enough to preserve the biopharmaceutical performances of the carrier. Two formulations (A and F) out of the four tested with S/L in the 5–7 range exhibit poor aptness to freeze-drying (Fig. 5), suggesting that the physical quality and the thickness of the shell should be compromised. Finally, the lecithin-enriched formulations (S/L<3) exhibit the best stability performances (points G, H, and I). This is in accordance with the assumption that lecithin confers rigidity to the shell, made at the beginning of the study.

Physical Characterization of LNs by DSC

The DSC analysis is consistent with the expected topology of the lipid nanocapsules. The oily phase is liquid whereas the constituents of the shell are either pasty or solid at ambient temperature (Fig. 6). From the thermal analyses of the binary mixtures, it can be concluded that the surfactants, Lipoid S 100, and Solutol HS15 are not solubilized in the oil phase.

As shown in a previous paper by DSC analysis and X-ray diffraction (15), the interaction between trehalose and a lecithin (L-α-dipalmitoyl phosphatidylcholine) in dried multilamellar dispersions may lead to the integration of trehalose into the lamellar structure. For samples containing approximately 5% water, the ratio trehalose:lecithin in the complex is 1:2. Based on this result, a molecular structure of the complex was proposed. As observed for lipid nanocapsules freeze-dried with trehalose in our experiments, the complex between

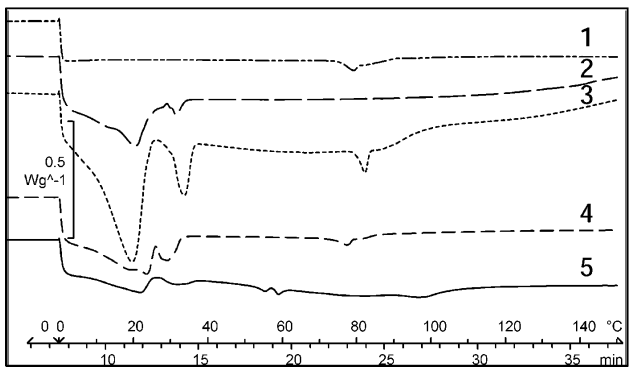

Fig. 7. Comparative DSC heating curves of binary mixtures and lipid nanocapsules. Analysis performed from 0°C to 150°C at a 5°C/min rate. Endothermic transitions are down. See "Materials and Methods" for sample preparation. 1, Tricaprylin + lecithin; 2, tricaprylin + Solutol; 3, Solutol + lecithin; 4, LN freeze-dried without trehalose; 5, LN freeze-dried with trehalose.

trehalose and lecithin in freeze-dried liposomes can be identified by the occurrence of a double peak in DSC, with decreased transition temperatures as compared to lecithin as raw material (15). The proposed molecular mechanism involves hydrogen bonding between the phosphate group of phospholipid (lecithin) and the trehalose hydroxyl groups. This bonding expands the phospholipid headgroups, decreasing the van der Waals interactions between the acyl chains of the phospholipid, and lowering the phase transition temperature (16).

It is interesting to note that these DSC observations suggest that the complexation between trehalose and phospholipids favor the stability of the lamellar structure of the shell in lipid nanocapsules, as described for liposomes (17). Interesting results have also been obtained for preservation of structure and behaviors of biological systems as complex as human platelets, rehydrated after freeze-drying in the presence of (cytoplasmic and external) trehalose (18). The glass transition temperature T_g of trehalose is low, and it is more likely to be in a glass state than other cryoprotectants such as sucrose, a physical state favorable for maintaining the molecular structures (19).

This interaction between lecithin and trehalose, can be considered as a stabilizing factor for freeze-dried LN under storage. Therefore, the influence of the S/L ratio on the stability of the dried form of LN should be considered not only in terms of rigidity improvement of the shell due to a lecithin enrichment of the formulations but also in terms of availability of phosphate groups interacting with thehalose. At the same time, if the trehalose:lecithin complex plays a major role in the stabilization of LN in a dried form, it can be anticipated that the shell should not be too thick so that the phospholipid layers are accessible to the cryoprotectant. This may explain the poor aptness to freeze-drying observed for formulation F.

Because the two transition peaks at 55°C and 59°C are attributed to a complex between lecithin and trehalose, a phase separation between Solutol HS15 and this complex within the rigid shell is suggested from the observation of curve 5 in Fig. 7. The mean particles size of lipid nanocapsules increased drastically after freeze-drying, as well as the polydispersity indexes. This phenomenon, also observed when other cryoprotectants are used, is not fully elucidated. The mentioned complexation between trehalose and lecithin at the molecular level may be part of the explanation. Taking into account all these observations, a structure of LN freeze-dried with trehalose is proposed in Fig. 8.

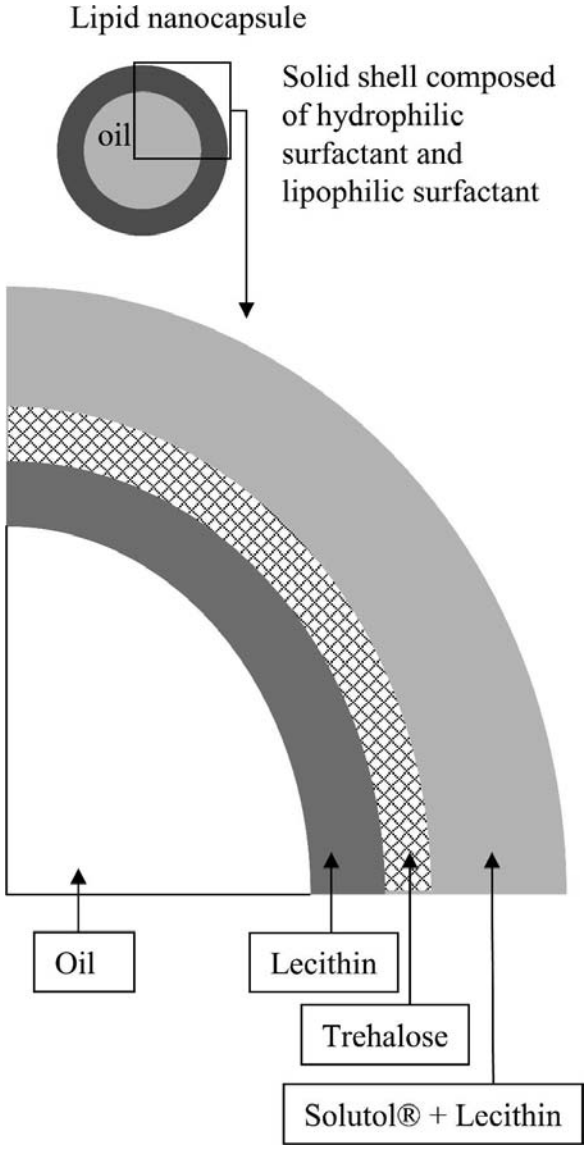

Fig. 8. Proposed topology for lipid nanocapsules freeze-dried in the presence of trehalose.

CONCLUSIONS

This paper is a contribution to the methodological development of lipid nanocapsules formulation. The aptness to freeze-drying and the stability under storage in a dried form is linked to the topology of lipid nanocapsules. The lipid nanocapsules components act as follows:

- the drug payload depends on the oil content,
- the evolution of the hydrophilic/lipophilic balance of Solutol HS15 is the driving force of LN formation,
- the LN diameter depends on both $F_{oil}/F_{Solutol}$, and the Solutol HS15/ Lipoid S 100 ratios,
- Lipoid S 100 is the structure-enforcing component. First, due to its high melting point and its crystallinity, it allows the shell to get hardness and aptness to freeze-drying. Second, the DSC study allowed us to point out a complexation between lecithin and trehalose. This complex was described as a stabilizer of phospholipid bilayers in freeze-dried liposomes formulations. Therefore, the Solutol HS15/Lipoid S 100 ratio should be compromised to preserve both the stability of LN in a dried form and the biopharmaceutical performances of LN related to the P-glycoprotein inhibiting properties of Solutol HS15.

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APPENDIX

Evaluation of t, the Thickness of the LN Shell

In a first approximation, the values of the densities of the oily core and of the shell consisting of Solutol HS15 and Li-

poid S 100 are assumed to be equal. The diameter of the oily core can be calculated from the lipid nanocapsules diameter and the fraction of oil phase.

$$F_{oil} = \frac{d_{oil}^3}{d^3}$$

$$\Rightarrow t = \frac{d - d_{oil}}{2}$$

$$\Rightarrow t = \frac{d}{2}\left(1 - \sqrt[3]{F_{oil}}\right)$$

where F_{oil} is fraction of oily phase, d_{oil} is diameter of the oily core, d is diameter of the lipid nanocapsules, and t is estimated shell thickness of lipid nanocapsules.

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