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Influence of Surfactant Structure in the Encapsulation and Stability of Amphotericin B in Niosomes

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***SUMMARY*. Bilayer vesicles, such as liposomes and niosomes, are widely known as efficient delivery systems for drugs. Spherical vesicles consisting of amphipatic non-ionic surfactants named niosomes are arranged in one or more concentric bilayers. They can entrap both water and oil soluble substances in the inner aqueous phase and in the vesicular membrane, respectively. Niosomes are studied as an alternative to liposomes because they overcome the disadvantages associated with liposomes. The present study aimed to evaluate noisome formation from different surfactants and the encapsulation of amphotericin B as an amphiphilic model drug. Niosomes of Span 60®, Span 80®, Glyceryl monooleate, Dehydol® LS 2 HN (lauric alcohol 2EO) or Brij ®72 (Polyoxyethylene (2) Stearyl Ether) were prepared with the inclusion of cholesterol (ratio 1:1) by a modified ether injection technique. Two concentrations, 20 and 30 mM were studied. Span 60® and Span 80® 30mM formulations were the most stable and also the ones with higher entrapment capacity.**

INTRODUCTION

Niosomes are vesicles formed from the self-assembly of non-ionic amphiphiles in aqueous medium. Niosomes, are now widely studied as an alternative to liposomes with lower cost, because they overcome the disadvantages associated with liposomes, such as chemical instability and variable purity of phospholipids. There is also a greater variety of surfactants available and they are easy to store. Niosomes can be prepared by the same procedures as of liposomes e.g. conventional chloroform film method, reverse phase evaporation, ethanol injection, sonication and heating method [1-5]. As liposomes, niosomes can be uni or multilamellar rounded vesicles.

Vesicles were considered primitive cell models and matrices for bioencapsulation. Drug encapsulation in addition to being modulated release systems also serves to protect bioactive substances from degradation; for example doxorubicin encapsulation in C16 triglyceryl ester niosomes was found to partially protect the drug from photodegradation [6].

Niosomes can entrap water-soluble substances in the inner aqueous phase and oil soluble substances in the vesicular membrane. Encapsulation efficiency depends on several factors; intrinsic properties of the vesicles such as vesicle size, cholesterol content and nature of the membrane components and also the nature of the solute, the hydration temperature and the loading method used rule the encapsulation. The vesicle structure and drug retention capability contribute to formulation stability [1,2,6].

The first niosomes were formulated using cholesterol and single-chain surfactants such as alkyl oxyethylenes with the alkyl chain usually C12–C18 long. The hydrophilic- lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. Uchegbu et al. reported that the sorbitan esters surfactants (Span®) with HLB values between 4 and 8 were compatible with vesicle formation [7]. Monoalkyl

KEY WORDS: Amphotericin B, Niosomes, Non- ionic surfactant, Vesicular systems.

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or dialkyl chain etheric surfactants have also been used to form niosomes, the latter are similar to phospholipids and possess higher encapsulation efficiency. Ester type surfactants were also used for niosome formulation; although these surfactants are less stable than ether type, they possess less toxicity. Furthermore, glucosides of myristil, cethyl and stearyl alcohols were reported to form niosomes [3].

With some surfactants vesicles can only be formed with the inclusion of cholesterol in the bilayer. Moreover, cholesterol enhances solute retention and stabilizes the system by preventing the formation of aggregates by repulsive steric or electrostatic effects [3,6-9].

In recent years, nonionic surfactant vesicles received great attention as a potential alternative drug delivery system for both hydrophilic (*e.g.* colchicine, enoxacin, salbutamol) and lipophilic drugs (*e.g.* finasteride, nimesulide, dithranol). However, the encapsulation of an amphiphilic molecule of high molecular weight has not been reported yet [5].

Amphotericin B (AmB) belongs to the class of polyene antibiotics, which are characterized by a very low solubility of less than 1µg / mL in water or water-free alcohols at physiological pH (pH 6–7). AmB is water-soluble at a pH below 2 or above 11, but under such extreme conditions the molecule is not stable. Due to its amphiphilic structure (Fig. 1), AmB forms aggregates in water at concentrations around 2×10–M by interaction between neighboring polyene chains. The drug exists as a combination of monomers, soluble and non-water soluble aggregates [10,11]. For parenteral injections, AmB is often given together with sodium desoxycholate as a solubilizing agent. In water, these two molecules form micellar colloidal complexes. However, only a fraction of AmB is solubilized [10]. Non-selective toxicity can be attributed to the aggregated form of the drug. Surfactants and some amphiphilic polymers have been demonstrated to reduce the toxicity of AmB by decreasing the extent of aggregation while retaining its antifungal activity [11].

In the present work we investigated the influence of different surfactants, with a constant ratio surfactant: cholesterol (1:1), in the encapsulation of AmB as an amphiphilic model drug using a simple and inexpensive technique. Span 60® (sorbitan monostearate; Sp60), Span 80® (sorbitan monooleate; Sp80), Monomuls® (glyceryl monooleate; Glyc), Dehydol® LS 2 HN (lauric alcohol 2EO; Dehy) and Brij 72® (Polyoxyethylene (2) Stearyl Ether; Brij) were used. We also evaluated the influence of cholesterol content in the systems that showed higher entrapment.

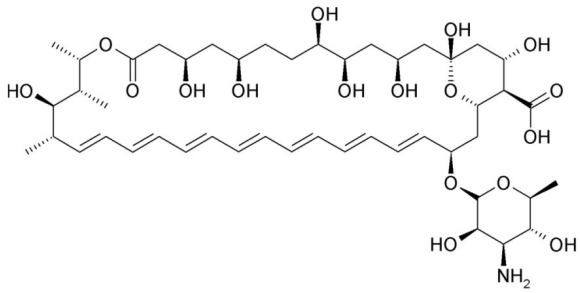

Figure 1. Amphotericin B structure.

MATERIALS AND METHODS

Materials

Amphotericin B was a gift from Alpharma, Argentina. Monomuls® (Glyceryl monooleate) and Dehydol® LS 2 HN (lauric alcohol 2EO) were kind gift from Cognis, Argentina. Brij 72® (Polyoxyethylene (2) Stearyl Ether) was a gift from Croda, Argentina. Cholesterol was supplied by Sigma, Argentina. Span 60® and Span 80® were purchased from Parafarm, Argentina and Sephadex G50 Sigma-Aldrich, USA. All the other reagents were of analytical grade.

Niosomes preparation

Niosomes were prepared by a modified ethanol injection method for liposomes described by Batzri & Korn [3,12,13]. The surfactant and cholesterol were mixed with ethanol and heated in a water bath until they were melted. Then, the drug (10 mg), previously dissolved in methanol, was added to the ethanolic solution and the mixture was injected into a warm aqueous phase (40 mL) using a 14-gauge needle syringe, with continuous magnetic stirring for 20 min at 75 °C. Then the preparations were sonicated for 1 h, using a bath sonicator filled with water at 60 °C. The evaporation of the solvents (BP 78.45 and 64.65 °C for ethanol and methanol, respectively) was evaluated by weighing the preparations at the end of the process. The systems were filtrated and stored in dark glass containers.

Characterization of the systems

Microscopy and physical stability of unloaded systems

The presence of vesicles in the samples was observed by optical microscope (40 X) at the

time of preparation. The formulations were stored at different temperature conditions, 4, 25, and 40 °C, and evaluated by visual observation for macroscopic changes.

Determination of vesicle size

The particle size of niosomes was measured at room temperature using a Sympatec HELOS Particle size analyser with a CUVETTE adapter for the dispersion of the samples. The lens used was R4, with a particle size range from: 0.5/1.8 up to 350 µm). Samples were diluted with Milli-Q water to a proper optical saturation of the equipement. Measurements were made in triplicate.

Determination of entrapment efficiency

The AmB containing vesicles were separated from the unencapsulated drug by a Sephadex G 50 column. An aliquot of 200 µL was placed and the niosomal fraction was recovered using distilled water as eluent solvent. Vesicles were lysed with methanol and the entrapment of the drug was determined by UV spectrophotometry (UV-VIS Spectrophotometer Shimadzu UV-260) at 406 nm, using a calibration curve of AmB in methanol covering the range 0.5-10 µg/mL (correlation factor 0.9995-1.000) using methanol as blank. Assays were done in triplicate. A solubility factor was calculated as $F_s=S_a/S_w$, where S_a and S_w are the apparent solubility of AmB in the noisome system and solubility in water, respectively.

Stability of the vesicle dispersions and drug leakage

Three samples of each system were kept under different storage conditions: 4, 25, and 40 °C, and at fixed times they were evaluated for drug content. Aliquots were lysed with methanol and sonicated for 5 min. The concentration of the drug was determined by UV spectrophotometry (see above).

Data Analysis

Data were analyzed statistically by unpaired T-student Test, two-tailed using GraphPad InStat (level of significance for $p < 0.05$).

RESULTS AND DISCUSSION

Niosomes Preparation

Niosomes of Span 60®, Span80®, Dehydol® LS 2 HN, Glyceryl monooleate, and Brij 72® were prepared. Figure 2 and Table 1 show structures and physicochemical data of the surfactants, respectively. After preliminary studies on total concentration of surfactants and cholesterol, ranging from 15 to 60 mM (data not shown), we included two final concentration of the mixture in this study, 20 mM and 30 mM, because they allowed proper formation of niosomes without sediment at the time of preparation.

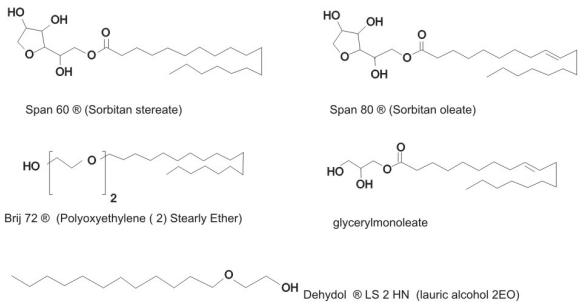

Figure 2. Surfactants structures.

Based on previous reports we assayed 1:1 cholesterol:surfactant ratio [1,2,17,18,20]. All the surfactants tested in the present work were able to form vesicles in the presence of this cholesterol ratio. The physical aspect of the formulations is shown in Figure 3. Most vesicles were spherical in shape (Fig. 4).

As Uchegbu and Florence [6] layed out, the association of non-ionic surfactant monomers into vesicles upon hydratation is a result of the high interfacial tension between water and the hydrocarbon portion of the amphiphile which causes the association of these groups. Simultaneously, the hydrophilic and/ or ionic repulsion between the head groups ensures that these groups are in contact with water. These two opposing

Surfactants	Molecular weight (g/mol)	MP (°C)	HLB
Cholesterol	386.67 [a]	148–150 [a]	2.7 [b]
Span® 60 (sorbitan monostearate)	430.62 [a]	54-57 [a]	4.7 [a]
Span® 80 (sorbitan monooleate)	428.61 [a]	----	4.3 [a]
Glyceryl monooleate	356.54 [a]	36-40 [a]	3.8 [a]
Dehydol® LS 2 HN (lauric alcohol 2EO)	274.33 [c]	---	6.4 [d]
Brij ®72 (Polyoxyethylene (2) Stearyl Ether)	358.60 [a]	44-45 [a]	4.9 [a]

Table 1. Molecular weight,melting point and HLB of surfactants. [a] Kibbe [14]; [b] Pasquali & Bregni [15]; [c] supplied by Cognis Argentina (http://www.cognis.com/countries/Argentina/sp/); [d] calculated according to Pasquali *et al.* [16].

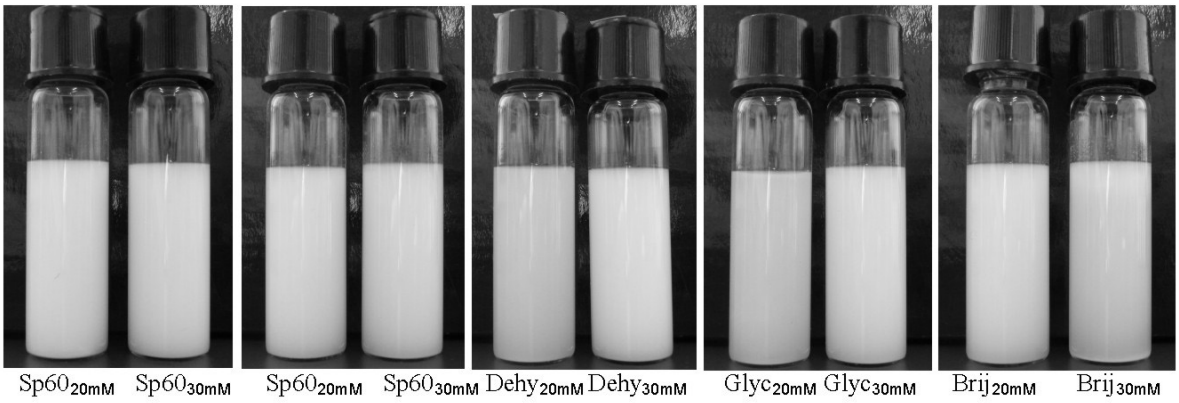

Figure 3. Macroscopic aspect of niosomes formulations.

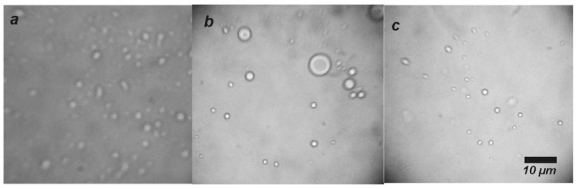

Figure 4. Optical microscope images of Sp60$_{30\ mM}$ (**a**), Sp80$_{30\ mM}$ (**b**) and Dehy$_{30\ mM}$ (**c**).

forces result in a supramolecular assembly. Factors such as surfactant structure, concentration and temperature of the medium are important determinants of vesicle formation. The type of the surfactants influences encapsulation efficiency and stability of niosomes. The geometric features of amphiphilic monomer aggregation have been analyzed by Israelachvili and a critical packing parameter (CPP) was proposed. The CPP is a dimensionless number that describes the tendency of any amphiphile to form aggregates, whether micellar or vesicular. According to Eq. [**1**].

$$CPP = V / I_c . a_o \qquad [1]$$

where a_o = hydrophilic head group area, v = hydrophobic chain volume, I_c = critical hydrophobic chain length (the length above which hydrocarbon chain fluidity may no longer be guaranteed), a calculated value of CPP below 0.5 indicates the spherical micelle formation, values between 0.5 and 1 indicate that the surfactant monomers assemble preferentially into vesicles, while a CPP of above 1 would predispose a compound to form inverted micelles [6,8].

Characterization and stability of unloaded systems

Large unilamellar vesicles with diameters 1-10 µm were observed as expected, since no additional step was used to reduce size of the vesicles (Table 2).

In the present work Sp60 and Sp80, which are well known to form stable vesicles, could form niosomes with the lowest size dispersity. In our study size was not related to length of the alkyl chain as reported elsewhere; moreover, Dehy (C12) vesicles were slightly larger than the ones in Sp60 and Sp80 (C18) formulations; larger vesicles are formed when hy-

Formulation	Composition	SMD ± SD (µm)	VMD ± SD (µm)
Sp60$_{20mM}$	Span60®:chol 1:1 20mM	2.79 ± 0.02	2.90 ± 0.06
Sp60$_{30mM}$	Span60®:chol 1:1 30mM	2.92 ± 0.00	3.10 ± 0.00
Sp80$_{20mM}$	Span80®:chol 1:1 20mM	1.10 ± 0.00	1.64 ± 0.00
Sp80$_{30mM}$	Span80®:chol 1:1 30mM	3.09 ± 0.00	3.33 ± 0.00
Dehy$_{20mM}$	Dehydol®LS2:chol 1:1 20mM	4.00 ± 0.00	5.87 ± 0.01
Dehy$_{30mM}$	Dehydol®LS2:chol 1:1 30mM	3.17 ± 0.00	3.40 ± 0.01
Glyc$_{20mM}$	Glyceryl monooleate:chol 1:1 20mM	1.80 ± 0.15	7.01 ± 4.91
Glyc$_{30mM}$	Glyceryl monooleate:chol 1:1 30mM	2.53 ± 0.06	14.57 ± 1.05
Brij$_{20mM}$	Brij 72®:chol 1:1 20mM	3.78 ± 0.03	11.96 ± 0.14
Brij$_{30mM}$	Brij 72®:chol 1:1 30mM	8.39 ± 0.08	21.00 ± 0.22

Table 2: Size of unloaded vesicles at time of preparation. Chol: cholesterol; SMD: Sauter mean diameter; VMD: Volume median diameter.

	4 °C					25 °C					40 °C				
Formulation	**Time (days)**														
	0	**7**	**15**	**30**	**60**	**0**	**7**	**15**	**30**	**60**	**0**	**7**	**15**	**30**	**60**
$Sp60_{20mM}$	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
$Sp60_{30mM}$	--	--	--	--	S	--	--	--	--	--	--	--	--	--	C
$Sp80_{20mM}$	--	--	--	--	--	--	--	--	--	--	--	--	--	--	C
$Sp80_{30mM}$	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
$Dehy_{20mM}$	--	--	--	--	C	--	--	--	--	fs	--	C	C	C	C
$Dehy_{30mM}$	--	--	--	--	--	--	--	--	fs	fs	--	fs/C	fs/C	fs/C	fs/C
$Glyc_{20mM}$	--	--	--	--	S	--	fs	--	fs	fs	--	C	C	C	C
$Glyc_{30mM}$	--	--	--	S	S	--	fs	--	--	--	--	C	C	C	C
$Brij_{20mM}$	--	--	--	S	S	--	--	fs	fs	S	--	fs/C	fs/C	fs/C	fs/C
$Brij_{30mM}$	--	S	S	S	S	--	--	fs	fs	S	--	fs/C	fs/C	fs/C	fs/C

Table 3. Physical appearance of niosomes at different storage conditions. -- = no change observed; S = Sedimentation; fs = fase separation C change in color.

drophilic portion of the molecule is decreased relative to hydrophobic portion. Thus a larger head group would also increase membrane curvature and decrease vesicle size, which also explains the bigger size for Brij (C18) niosomes compared with Sp60 and Sp80. It has been reported that single alkyl tail surfactants with higher HLB and smaller CPP can only form vesicles in the presence of suitable amounts of cholesterol, so in this case the ratio surfactant:cholesterol 1:1 might have rendered larger vesicles [2,3,5,18-21].

For the case of Glyceryl monooleate (C18) we observed two populations of vesicles with high dispersion in size (x_{10}- x_{90}: 0.79 ± 0.03 - 57.59 ± 0.96 µm for 20 mM formulation and x_{10}- x_{90}: 0.97± 0.02 - 58.75 ± 3.94 µm for 30 mM formulation); microscopic observation showed that part of the surfactant was unable to form vesicles. Among C18 surfactants used in this study glyceryl monooleate has the lowest HLB and also according to Oda *et al.* C18 amphiphiles with chiral carbons form helical ribbons [22]. In Figure 5 polarized light microscopy image of glyceryl monooleate show part of the surfactant in the unloaded system without forming vesicles but ribbons.

Sp60 and Sp80 showed to be the most stable systems when stored at 4, 25 and 40 °C. Niosomes have been shown to be more stable when stored under refrigerated conditions; however, these systems remained macroscopically stable for at least one month at 40 °C and two months at room temperature. Table 3 reports visual changes observed. The formulations containing Brij 30mM were the ones that first

Formulation	**AmB (mg)**	**% Entrapment**	**Drug:excipient ratio**	
			µg/mg	**mol/10^3mol**
$Sp60_{20mM}$	10	82.87 ± 8.83	25.43	11.21
$Sp60_{30mM}$	10	96.74 ± 10.82	19.70	8.73
$Sp80_{20mM}$	10	63.67 ± 8.63	19.30	8.61
$Sp80_{30mM}$	10	82.24 ± 4.34	16.57	7.42
$Dehy_{20mM}$	10	69.86 ± 7.73	31.83	9.45
$Dehy_{30mM}$	10	78.25 ± 3.18	23.77	7.06
$Glyc_{20mM}$	10	57.29 ± 4.41	20.08	7.75
$Glyc_{30mM}$	10	61.28 ± 8.75	14.32	5.53
$Brij_{20mM}$	10	73.36 ± 10.11	24.60	9.92
$Brij_{30mM}$	10	76.88 ± 8.89	17.12	6.93

Table 4. Entrapment efficiency. AmB: Amphotericin B; Drug MW: 924.02.

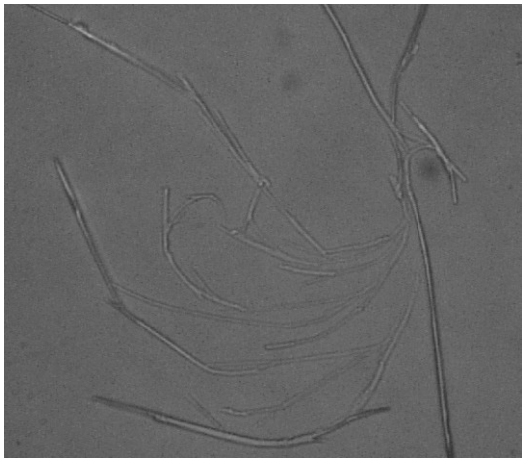

Figure 5. Glyceril monooleate (polarized light microscopy; 100 x)

Formulations	ratio
$Sp60_{30mM}$ / $Sp60_{20mM}$	1.17
$Sp80_{30mM}$ / $Sp80_{20mM}$	1.29
$Dehy_{30mM}$ / $Dehy_{20mM}$	1.12
$Glyc_{30mM}$ / $Glyc_{20mM}$	1.07
$Brij_{30mM}$ / $Brij_{20mM}$	1.05
$Sp60_{30mM}$ / $Glyc_{30mM}$	1.58

Table 5. Entrapment Ratios.

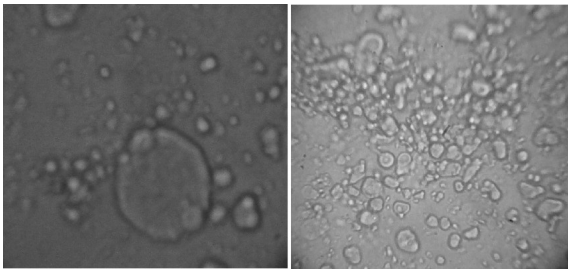

Figure 6. $Brij_{30mM}$ vesicles after a month of storage at room temperature.

showed phase separation or sedimentation, even at 4 °C. Glyceryl monooleate systems showed separation in the form of flakes and became more translucent at room temperature whereas sedimentation could be observed at 4 °C. Sedimentation was greater for Brij 72® than Glyceryl monooleate. Figure 6 shows Brij 72® vesicles aggregated and fused after a month at room temperature. In all cases the more concentrated systems showed more signs of destabilization.

Entrapment efficient, size and stability of loaded systems

Table 4 shows the entrapment efficiency of AmB, being systems $Sp60_{30mM}$ and $Sp80_{30mM}$ the ones with more entrapment capacity.

The average ratio of increase in the encapsulation efficiency for the more concentrated systems (30mM/20mM) was 1.14, without statistically significant difference between both concentrations, except for $Sp80_{30mM}/Sp80_{20mM}$ ($p < 0.05$) (Table 5). There was a significant difference in the encapsulation between of Sp60 formulation and Glyc (ratio $Sp60_{30mM}/Glyc_{30mM}$ = 1.58) ($p<0.05$). The solubility factors considered as increment in water solubility ranged from 143.23±4.41 to 241.85 ± 10.82.

We found that vesicles obtained from long alkyl chain (C18) surfactants are more stable and give higher entrapment efficiency than those obtained from shorter alkyl chain (C12) surfactants. Surfactants with the larger hydrophilic head group and long alkyl chain such as Sp60 significantly gave higher entrapment efficiency than those with the same alkyl chain but smaller hydrophilic head group. These results are similar to the ones reported by Manosroi *et al.* [18], despite they encapsulated a water soluble substance. It is remarkable that C18 unloaded formulations were more stable, but with the inclusion of an amphiphilic drug the content of drug was similar for both C18 and C12 systems at the end of our study.

$Sp80_{20mM}$ entrapped 19.2% less than $Sp60_{20mM}$. This may be attributed to the fact that Sp60 is solid at room temperature with a higher phase transition temperature (Tc) [23]. Sp60 and Sp80 have the same head group, but Sp80 has an unsaturated alkyl chain. The presence of a double bond in the alkyl chain causes a marked increase in the permeability of the vesicles because the molecules are bent and more separated, which might be the reason for the lower entrapment efficiency of Sp80 system [24]. This would also explain the case of Glyceryl monooleate systems, which entrapped 25.6 and 35.5 % (20 and 30 mM, respectively) less than Sp 60. Glyceryl monooleate has the same alkyl chain length but with a double bond and also the polar head is smaller than that of Spans.

$Dehy_{30mM}$ and $Brij_{30mM}$ entrapped 18.5 and 19.9% less than Sp6030mM, respectively, which can be explained by the shorter alkyl chain and much smaller polar head of Dehydol®. Although Brij 72® is a C18 surfactant, its polar head is smaller than Sp60. $Dehy_{30mM}$ and $Brij_{30mM}$ systems entrappment capacity was similar, although the latter has a longer alkyl chain and its polar head is twice the one of Dehydol®. Polyglycerol monoalkyl and polyoxylate ethers are the most widely used single-chain surfactants used, however, they have less encapsulation efficiency even in presence of cholesterol [3].

As mentioned, cholesterol has an important role in vesicle formation and drug loading. It is known that cholesterol alters the fluidity of the chains in the bilayer when present in sufficient concentration and eliminates the gel to liquid phase transition endotherm of surfactant bilayers [6-9]. For sorbitan monoesters we studied the influence of cholesterol ratio in the entrapment efficiency. Figure 7 shows that for both Sp60 and Sp80 the maximum loading capacity is for 1:1 surfactant: cholesterol ratio. Balakrishnan P *et al.* studied the influence of cholesterol for mi-

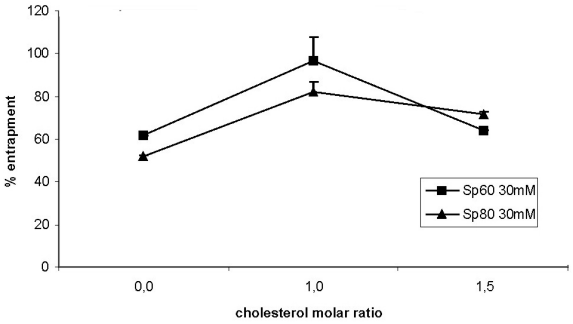

Figure 7. Influence of surfactant:cholesterol ratio in the encapsulation of AmB for systems $Sp60_{30mM}$ and $Sp80_{30mM}$.

noxidil niosomes, and showed similar results. The increment in the encapsulation in presence of cholesterol may be due to increase in the lipophilic behavior of the lipid bilayer which may lead to better trapping of hydrophobic or amphiphilic drugs. However, at higher concentration of cholesterol (1:1.5), it may compete with the drug for packing space within the bilayer as amphiphilic moieties assemble into vesicles [5,25].

Table 6 shows sizes of loaded vesicles. Loaded niosomes of $Sp60_{30mM}$ and $Sp80_{30mM}$ were 3.04 and 2.84 µm, respectively which could be explained by the amphiphilic structure of AmB with polar groups located along one side of the molecule. Because of the influence of HLB value and CPP in a potential niosome system the presence of an amphipathic or hydrophobic drug must be taken into account as both these substances are to be incorporated into the vesicle membrane [8,25]. For AmB molecules the ability to form cylindrical molecular aggregates follows directly from its stereochemical structure (Fig. 1). Gruszecki *et al.* showed modified packing of the drug in the presence of a lipid component. Self-organization of AmB in a polar and hydrophobic environment was the subject of numerous recent theoretical and experimental studies. In particular, the organization of AmB in the environment of lipid membranes has been recently addressed as directly relevant to the pharmacological efectiveness of the drug [26].

Furthermore, Dehydol® and Brij 72® loaded vesicles were smaller than unloaded ones, which may be explained by the influence of the addition of an amphipathic drug, similar to what Pardakhty *et al.* reported for the effect of adding cholesterol to Brij 52® (C16) niosomes, increasing hydrophobicity of the surfactant mixture led to a smaller vesicle size [24,27]. In Figure 8 the image of AmB loaded $Glyc_{30mM}$ shows irregular vesicles without spherical shape; interestingly, loaded system had only one vesicles population, showing the effect of the loaded drug in the assembly of the bilayer.

Formulation	SMD ± SD (µm)	VMD ± SD (µm)
$Sp60_{20mM}$	1.08 ± 0.04	2.68 ± 0.02
$Sp60_{30mM}$	3.04 ± 0.02	3.28 ± 0.03
$Sp80_{20mM}$	1.35 ± 0.05	3.05 ± 0.04
$Sp80_{30mM}$	2.84 ± 0.10	3.04 ± 0.08
$Dehy_{20mM}$	2.87 ± 0.00	3.09 ± 0.01
$Dehy_{30mM}$	1.04 ± 0.01	2.73 ± 0.02
$Glyc_{20mM}$	2.98 ± 0.05	3.26 ± 0.91
$Glyc_{30mM}$	1.44 ± 0.12	1.71 ± 0.04
$Brij_{20mM}$	2.99 ± 0.05	3.25 ± 0.09
$Brij_{30mM}$	3.40 ± 0.01	4.39 ± 0.06

Table 6. Size of loaded vesicles at time of preparation. SMD: Sauter mean diameter; VMD: Volume median diameter.

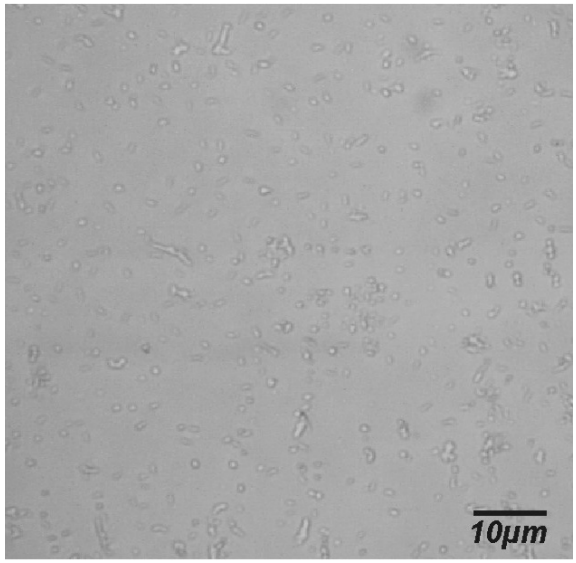

Figure 8. $Gly30_{mM}$ AmB loaded vesicles (polarized light microscopy).

Figures 9, 10 and 11 show the stability of loaded formulations at the different storage conditions 4, 25 and 40 °C. As expected systems were more stable at refrigerated conditions both because of the vesicles and the drug stability itself, but it is worth noting that drug content in Dehydol® vesicles remain stable after 90 days in a similar extent to Span 60® and Span 80®. This might be explained by the stabilization effect of the amphiphilic drug compared with the

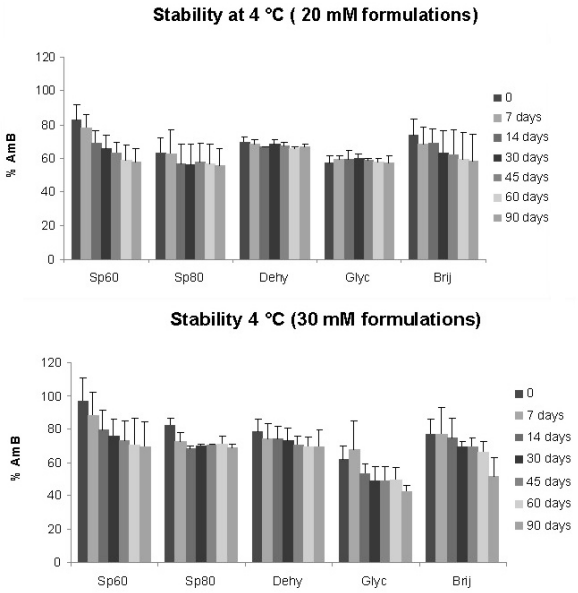

Figure 9. Stability of loaded systems at 4 °C.

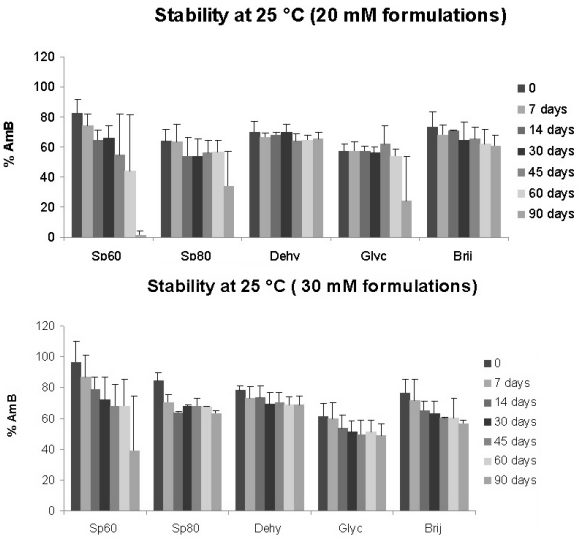

Figure 10. Stability of loaded systems at 25 °C.

empty vesicles. Even more, Dehydol® vesicles were more stable than sorbitan monoesters formulations at 25 °C. Glyceryl monooleate and Brij 72® resulted to be the least stable systems.

The results of drug retention studies show greater drug leakage at higher temperature. This may be due to the increased fluidity of lipid bilayers at higher temperature resulting in greater drug leakage [7]. The stability of niosomes systems might be improved by incorporating into a gel base which may prevent of fusion of vesicles and at the same time the formulation would be appropriate for topical use.

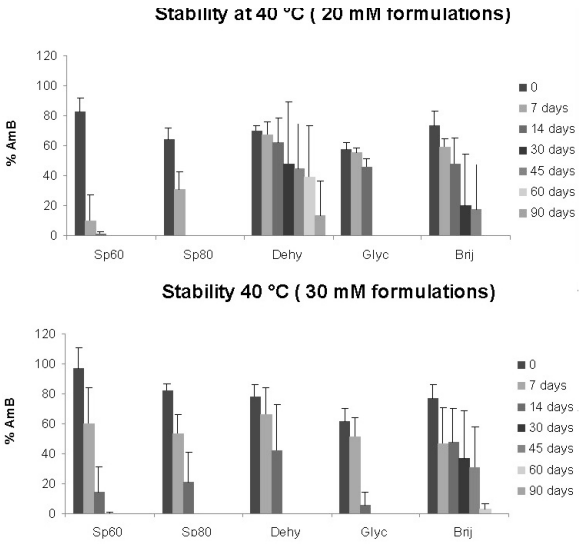

Figure 11. Stability of loaded systems at 40 °C.

CONCLUSION

Amphotericin B as an amphiphilic model drug could be entrapped in various non-ionic surfactants vesicles in the presence of cholesterol, with a simple technique. Sorbitan monoestearate vesicles showed the highest trapping efficiency. The systems could retain 70% of the loaded drug at 4°C for three months. Further investigations incorporating a gelling agent are to be performed to improve drug retention and to evaluate topical application.

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