J. Cosmet. Sci., **65**, 225–238 (July/August 2014)

Development of a new resistant liposome coated with polysaccharide film for cosmetic application

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Accepted for publication May 22, 2014.

Synopsis

The aim of our study was to elaborate a resistant liposome that can be used in cosmetic formulations containing high amounts of surfactants and electrolytes. The stability of liposomes was increased via hydrophobized polysaccharide (Stearoyl Inulin) by anchoring its stearic acid tail into liposome bilayer. Coated and noncoated liposomes were prepared under the same conditions and their morphology, size, and resistance to surfactants and electrolytes were evaluated. We established that coated liposomes were more resistant to surfactants and electrolytes. It seems that a coating of polysaccharides prevents liposome destabilization in the presence of high amounts of surfactants and electrolytes. Moreover, the ability of coated liposomes to improve the skin delivery of active molecules was evaluated. Coated liposomes increased the efficacy of magnesium chloride by improving its skin availability.

INTRODUCTION

Liposomes are mainly used for the encapsulation of bioactive molecules in cosmetics, pharmaceutics, nutraceutics, and in food sciences. Both hydrophilic and lipophilic bioactive molecules can be incorporated into liposomes to enhance their skin penetration and thus to improve their efficacy. Because of their biocompatibility with skin composition, liposomes have been principally used in the cosmetic industry since the eighties (1–3). Liposomes are incorporated into different cosmetic products such as creams, lotions, and gels. However, even if they are very interesting delivery systems for the cosmetic industry, they still have some limitations. For example, various cosmetic ingredients decrease the stability of liposomes and therefore their efficacy to deliver bioactive molecules. Maherani et al. (4) have reported that physicochemical stability of liposomes depends mainly on the lipid composition, the rigidity of the membrane, and the ability of liposomes to maintain the entrapment efficiency despite changing external conditions (pH, electrolytes, surfactants, and so on).

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In cosmetics and toiletries, electrolytes have an important role on the sensorial properties of final products because they influence the gel formation. They are largely used in shampoo, deodorants, and as water in oil emulsion stabilizers. Also, several studies reported the beneficial effect of some salts on human cutaneous barrier functions (5,6) and on biological mechanisms (7).

Moreover, surfactants are widely used in personal care products for emulsion stabilization and for their several properties such as detergency, solubilization, conditioning, thickening, and emolliency (8). Surfactants are also used in toiletries formulations as rheology controllers to modify the viscoelastic properties and thus the sensorial characteristics of the final product (9). Deo *et al.* (10) have reported that liposomes made of phosphatidylcholine and phosphatidic acid are very sensitive to surfactants. In the presence of dodecyl sulfonate, the size of liposomes increased until complete solubilization.

It has been reported from several studies that the stability of liposomes can be enhanced by modification of their surfaces using a coating process with natural polysaccharides (i.e., mannan, pullulan, amylopectin, dextran, chitosan) (11). However, the adsorption of these polysaccharides to the liposome membrane seemed to be thermodynamically unstable (12). The use of chemically modified polysaccharides for coating liposomes may avoid this stability problem (13–15). Good stability could be obtained by coating the outermost surface of liposomes with derivatives of polysaccharides (16–18). Fatty acids are chemically grafted to natural polysaccharides to make them amphiphilic. The hydrophobic alkyl chains of modified polysaccharides were then anchored into the bilayer membrane of liposomes.

The first part of our study was the physicochemical characterization of modified polysaccharide coated liposomes compared to classical noncoated liposomes. Then, we evaluated the resistance of coated liposomes versus noncoated ones to different concentrations of ionic/nonionic surfactants and electrolytes. In order to evaluate the delivery property of coated liposomes, magnesium chloride ($MgCl_2$), a divalent salt, was chosen as the bioactive molecule which is implicated in P-glycoprotein (P-gp) transporter activity. Natural $MgCl_2$ has interesting cosmetic properties as it plays an important role in the expulsion of wastes out of cells and then avoids the accumulation of reactive oxygen species. Detoxification of cells was regulated by P-gp transporter; it is a magnesium-dependent transmembrane pump.

In this way, 12% of $MgCl_2$ was entrapped in coated liposomes. These coated liposomes loaded with $MgCl_2$, called magnesium chloride-coated liposomes (MCCL), were physicochemically characterized. The $MgCl_2$ release from MCCL was then measured in an acrylate gel formula whose viscosity is known to be very sensitive to divalent ions. The delivery property of this resistant liposome was evaluated by an *ex vivo* study on human skin explants.

MATERIALS AND METHODS

MATERIALS

Two soybean lecithins (Emulmetik™ 930 and Emulmetik™ 900) distributed by Lucas Meyer Cosmetics were used for the preparation of liposomes. They are composed of about 92% and 45% of phosphatidylcholine, respectively.

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1,3-propanediol was purchased from DuPont Tate & Lyle (Loudon, TN). Stearoyl Inulin was obtained from Miyoshi (Saitama, Japan), and α-tocopherol from VitaeCaps (Talavera de la Reina, Spain). Dermosoft® 1388 and polyglyceryl-10-laurate were supplied by Dr. Straetmans (Hamburg, Germany).

Natural $MgCl_2$ was purchased from Celnat (Saint-Germain Laprade, France) and acrylates/C10-30 alkyl acrylate cross-polymer from Lubrizol (Wickliffe, OH).

Sodium lauryl ether sulfate (SLES) was purchased from Cognis (Monheim, Germany) and Polysorbate 20 was obtained from Kolb (Hedingen, Switzerland). Behenylalcoholethoxylate, Triton X-100, and sodium chloride (>99%) were provided by Prolabo (Darmstadt, Germany). Phosphate-buffered solution (PBS), ascorbic acid, bicinchoninic acid, glutathione, and sodium dodecyl sulfate (SDS) (≥99%) were purchased from Sigma (Saint-Quentin Fallavier, France). Pgp-Glo™ Assay Systems were purchased from Promega (Charbonnieres, France). Parabens and phenoxyethanol were provided from Jan Dekker (Langenfeld, Germany). Xanthan gum and sodium hydroxide were purchased from Cargill (Hamburg, Germany) and Le Comptoir Français Interchimie (Compans, France), respectively.

LIPOSOME PREPARATION

Natural polysaccharides are chemically hydrophobized by grafting stearic acid chains via an ester bond. Coated liposomes were prepared by dissolving phospholipids in 1,3-propanediol (7:20; w/w) at 70°C. Then, polysaccharide-fatty acid complex (Stearoyl Inulin) was incorporated at 75°–80°C to the homogenous mixture. After adding 0.1% of α-tocopherol to the previous mixture, the aqueous phase was vigorously homogenized with lipid phase using a rotor stator for 20 min at 1500 rpm. Finally, the coated liposome suspension was homogenized using Turrax for 5 min at 3000 rpm to reduce the polydispersity of vesicles. At the end of the process, 0.5% of Dermosoft® 1388 was added as antimicrobial system.

Noncoated or classical liposomes were prepared under the same conditions (process and composition) without adding polysaccharide-fatty acid complex.

MAGNESIUM CHLORIDE ENTRAPMENT

Coated and noncoated liposomes entrapped $MgCl_2$ were prepared by adding 12% (w/w) of $MgCl_2$ into the aqueous phases before their homogenization with the lipid phases.

LIPOSOME CHARACTERIZATION

Structural characterization. Optical microscope and freeze-fracture electron microscopy were used to determine the morphology of liposomes.

Vesicles were observed under an optical microscope (Nikon Eclipse 50i from Nikon, Kanagawa, Japan) immediately after their preparation. Coated and noncoated liposomes were deposited between two glass lamellas and observed using a phase contrast

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mode at a magnification ×1000. Immersion oil was deposited between the objective lens and the upper glass lamella.

Coated and noncoated liposomes were observed in parallel using freeze-fracture electron microscopy. About 1 µl of liposome suspension was placed on a copper base. The sample was rapidly frozen with a cryoprotectant (glycerol) in liquid nitrogen (-196°C) cooled propane.

After storage in liquid nitrogen, samples were placed on the support immersed in liquid nitrogen which was inserted into the cryoscouring device (BAL-TEC BAF 060 (Nanterre, France)) and then cooled to -150°C. When a vacuum of 10^{-7} torr was reached, samples were fractured using a scalpel blade cooled to -150°C. The fracture surface was shadowed by a spray of platinum, and a layer of 4 nm was filled in one direction at an angle of 45°C to reveal the relief and surface structures. The thin layer was consolidated with vertically sprayed carbon, which is transparent to electrons. A quartz oscillating measuring device measured the final thickness; it was about 30 nm. The replicas were cleaned with a mixture of solvents and/or sulfochromic acid, rinsed with distilled water, and examined under a transmission electron microscope (FEI CM120, Philips, Cambridge, UK).

Vesicle size measurement. The empty noncoated liposomes size was measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcester, UK). The samples were diluted (1:400) with ultrafiltrated distilled water. Droplets sizes were obtained from the correlation function calculated by the dispersion technology software using various algorithms. The apparatus is equipped with a 4 mW He/Ne laser, emitting 633 nm, measurement cell, photomultiplier, and correlator. The refractive index and absorbance were set at 1.47 and 0.01 respectively at 25°C. The measurements were performed in five replicates (19).

DLS is not adapted to polydisperse suspensions and important size vesicles. Therefore coated liposomes sizes were determined using static light scattering.

The size distribution of empty coated liposomes and MCCL was determined using static light scattering (Beckman Coulter LS230 instrument, Brea, CA) according to Fraunhofer diffraction theory. By applying the polarization intensity differential scattering, this apparatus can detect particles from 0.04 to 2000 µm using up to 116 size classes and a helium neon laser with a wavelength of 750 nm.

Empty coated liposomes and MCCL suspensions were dispersed in distilled water under agitation into the measuring cell to be in the optimal obscuration range (8%–20%). Particle size distribution was expressed in volume units.

STABILITY EVALUATION

Resistance to surfactants and electrolytes. Since most cosmetic formulations are emulsions, the improvement of liposome stability against surfactants is a very important aspect for their use in such cosmetic formulations.

Empty coated and noncoated liposomes were incubated for 30 days at 25°C with different percentages of ionic surfactants (SDS, SLES), nonionic surfactants (Triton X-100, Polysorbate 20, polyglyceryl-10-laurate, Behenyl alcohol ethoxylate), and salts (NaCl, $MgCl_2$).

As high as 90% of liposome suspensions were mixed with 10% of surfactant solutions at different concentrations. Surfactant solutions were prepared in distilled water at different

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concentrations from 1% to 10%. Regarding the salts resistance test, 70% of coated and noncoated liposome suspensions were mixed with 30% of various salt solutions. The final concentrations of salts in liposome suspensions were set at 5%, 10%, 15%, 20%, 25%, and 30%. All fractions were expressed as w/w.

During a period of 30 days, daily optical microscope observations were performed at a magnification ×1000 at 25°C. We believe that the liposome suspension is unstable from the first signs of destabilization, such as aggregation and destruction of the phospholipid bilayer.

PERMEABILITY EVALUATION

Magnesium chloride release from coated liposomes. Twelve percent of $MgCl_2$ was entrapped in coated liposomes. The permeability of coated liposome membrane to $MgCl_2$ was evaluated in acrylates/C10-30 alkyl acrylate cross-polymer hydrogels (0.8%, w/w) neutralized with sodium hydroxide. Being sensitive to electrolytes, the viscosity of hydrogels decreases by increasing the concentration of $MgCl_2$. The release of $MgCl_2$ from coated liposomes was then evaluated by measuring the viscosity evolution of hydrogels containing 2% (w/w) of entrapped $MgCl_2$ in coated liposomes (MCCL) and an equivalent amount of free $MgCl_2$ (0.24% w/w). The viscosity measurement was performed using a rheometer apparatus (Rheomat RM 200, Lamy Rheology, Champagne au Mont d'Or, France). The effect of coated liposome dilution (1/2, 1/10) on the release of $MgCl_2$ was carried out. We supposed that dilution of the external medium of coated liposomes (distilled water) could increase the diffusion of $MgCl_2$ from their internal core.

EVALUATION OF THE DELIVERY EFFICACY OF COATED LIPOSOMES

In order to bring out the delivery efficacy of coated liposomes, the latter were used to improve the detoxification activity of $MgCl_2$. Human skin explants were used to evaluate the effect of entrapped and free $MgCl_2$ on P-gp transporter activity. Human normal skin explants were obtained from a piece of surgical resection of 37-year-old subjects. Hydrogel composed of 1% (w/w) of acrylates/C10-30 alkyl acrylate cross-polymer neutralized with sodium hydroxide, 0.5% (w/w) of xanthan gum and preserved with 0.8% (w/w) of parabens and phenoxyethanol's mixture was used. Deionized water was added to complete the formulation (100%; w/w). Entrapped and free $MgCl_2$ was incorporated into the hydrogel at 3% (i.e., 0.36% of $MgCl_2$) and 0.36%, respectively. Hydrogel without coated liposomes and $MgCl_2$ was used as placebo. A mixture of ascorbic acid (100 µg/ml) and glutathione (100 µg/ml) was tested as a positive control for the evaluation of P-gp activity. Skin explants were incubated during 24 h at 37°C in contact with positive controls, entrapped $MgCl_2$, nonentrapped $MgCl_2$, and hydrogel as placebo. All formulations were applied to the skin explants' surface except the reference product, which was directly diluted in the culture medium. At the end of the incubation period, skin explants were rinsed with a PBS and stored at -196°C. The assay was performed in triplicate.

Proteins assay. At the end of the incubation period, proteins were quantified in cell lysates by a spectrocolorimetric method using bicinchoninic acid assay (20).

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P-gp activity assay. At the end of the incubation period, P-gp activity was assessed in cell lysates using a sensitive and specific assay kit. For P-gp analysis, results are expressed as nmol of ATP consumed by P-gp per µg of proteins (mean ± S.D.).

Statistical analysis. Levels of significance were assessed using Student *t*-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

LIPOSOMES CHARACTERIZATION

Structural characterization. Coated and noncoated liposomes used for the entrapment of $MgCl_2$ (12%) were observed by optical microscope immediately after being prepared (Figure 1).

Because of the high ionic strength of the medium, a decomposition and aggregation of liposome vesicles was observed attesting that noncoated liposomes are not resistant to 12% of $MgCl_2$ (Figure 1A). These data are in agreement with those reported by Crommelin (21), which supported that high ionic strength dispersions predicted an irreversible aggregation of liposomes.

However, we clearly observed in Figure 1B that coated liposomes are more resistant and can entrap 12% of $MgCl_2$. Polysaccharide coating allowed the entrapment of high amount of electrolytes into liposomes represented by spherical vesicles with good membrane integrity (Figure 1B).

In Figure 2B, we confirmed by freeze-fracture electron microscopy the spherical morphology of coated liposomes with entrapped $MgCl_2$ (Figure 2B). These microscope images revealed that the coating procedure did not modify the vesicular structure and the morphology of liposomes (Figures 2B and C).

Vesicle size measurement. The presence of polysaccharide coating was evaluated by vesicle size analysis of empty coated and noncoated liposomes. The mean vesicle size of noncoated liposomes was about 251.03 ± 15.56 nm. However, coated liposomes had a mean

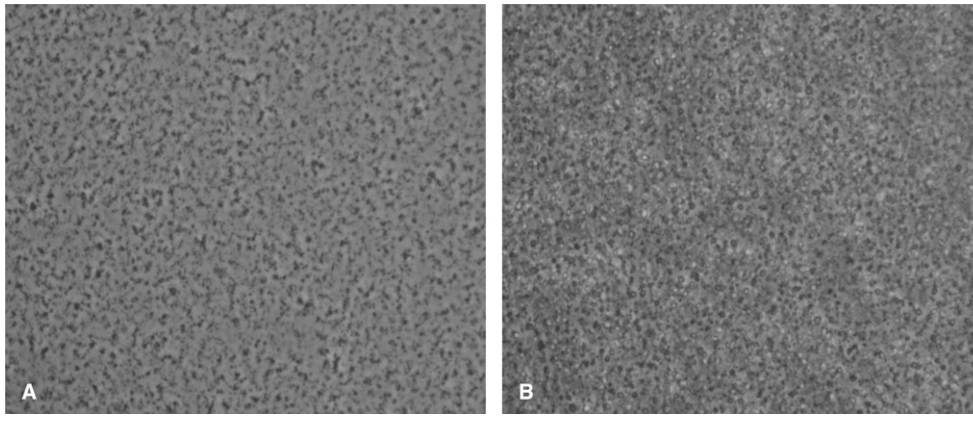

Figure 1. Optical microscope observation of liposomes (×1000): (A) Noncoated liposomes in the presence of 12% of magnesium chloride and (B) 12% of magnesium chloride entrapped in coated liposomes.

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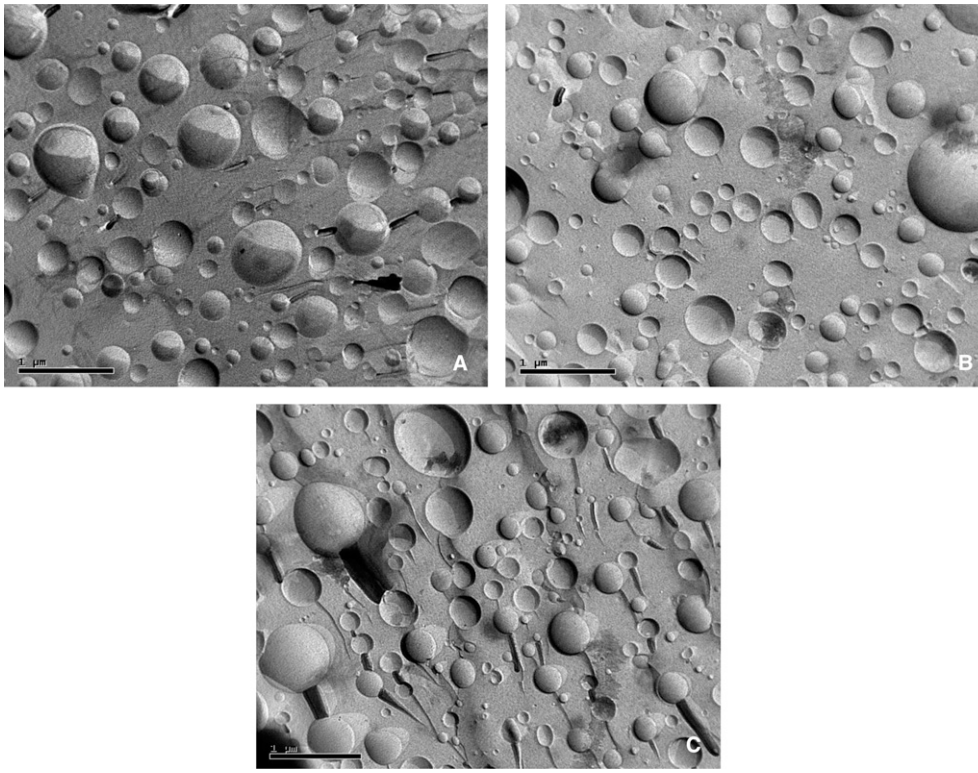

Figure 2. Freeze-fracture electron microscopy (×41,100): (A) Empty noncoated liposomes, (B) Magnesium chloride entrapped in coated liposomes, and (C) Empty coated liposome.

size of 514.00 ± 79.00 nm and thus are two times larger than noncoated liposomes. This size difference could be explained by the presence of a polysaccharide film (11–16). The stearic acid from Stearoyl Inulin would be anchored inside the liposomal membrane with the inulin surrounding the outer bilayer.

On the other hand, the entrapment of $MgCl_2$ into coated liposomes (MCCL) affected their size and their size distribution. In the presence of $MgCl_2$, coated liposome size was increased without impacting their morphology (Figures 2A, B, and C). The mean size of MCCL was about 1.37 µm, and 90% of vesicles had a size inferior to 3.14 µm. The increase in liposome size might be linked to the swelling of polysaccharide film in contact with high amounts of salts (22).

Beyond size parameter modification, the introduction of a hydrophobic chain into liposome membrane may affect the physicochemical properties of liposomes. According to Carafa *et al.* (11), coating by hydrophobic chain grafted polysaccharides decreased transition temperature of liposome phospholipids compared to nongrafted polysaccharides. We also supposed that hydrogen bonds and hydrophobic interactions between carbon chain of phospholipids and stearic acid from hydrophobized polysaccharides could limit the release of the encapsulated molecules by forming a very tight embedment in the membrane.

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STABILITY OF LIPOSOMES

Resistance of coated and noncoated liposomes to surfactants. The physicochemical stability of empty coated and noncoated liposomes was evaluated in the presence of ionic (SDS, SLES) and nonionic surfactants (Triton X-100, Polysorbate 20, polyglyceryl-10-laurate, Behenyl alcohol ethoxylate).

The maximum percentages of ionic surfactants which do not destabilize empty coated and noncoated liposomes are shown in Table I. The shape of liposomes was observed after each period of time (Days 1, 7, 15, and 30) by optical microscope (×1000) at different ionic surfactant concentrations (1%–10%). Coated liposomes resisted up to 3% of both ionic surfactants until 30 days of storage in the presence of SDS and SLES. However, noncoated liposomes are less resistant and remained stable at 1% and 2% of SDS and SLES during the period of storage, respectively. These observations allowed us to conclude that the coating process increases the stability of liposomes against ionic surfactants.

The effect of different nonionic surfactant concentration (1%–10%) on the stability and shape of empty coated and noncoated liposomes was summarized in Table II. Firstly, coated and noncoated liposomes are more resistant to nonionic surfactants than to ionic ones. Whatever the kind of nonionic surfactant, the coating process improved the stability of liposomes. Our results showed that coated liposomes remained stable with good integrity in the presence of 9% of polysorbate 20 and 9% of polyglyceryl-10-laurate for 30 days. Coated liposomes resisted up to 9% of behenyl alcohol 25 EO for 15 days but only up to 6% after 30 days. However, Triton X-100 is the most solubilizable nonionic surfactant as coated liposomes were stable until 30 days of storage in the presence of 3% of Triton X-100. Noncoated liposomes are more resistant to behenyl alcohol 25 EO and polyglyceryl-10-laurate than to polysorbate 20 and Triton X-100.

Surfactants are widely used for the solubilization of phospholipid membrane. Mady *et al.* (23) have reported that the solubilization process is divided into three stages.

(i) Stage I: corresponds to the insertion of surfactants into the bilayer membrane.

(ii) Stage II: reached when the phospholipid membrane is saturated with surfactants, and followed by the formation of micelles.

(iii) Stage III: Mixed lipid-surfactant micelles enriched in surfactant.

Table I
Resistance Kinetics of Coated and Noncoated Liposomes to Ionic Surfactants during 30 Days of Storage at 25°C

	T0	Day 1	Day 7	Day 15	Day 30
			Percentage of SDS		
Noncoated liposome	1.0	1.0	1.0	1.0	1.0
Coated liposome	4.0	4.0	4.0	4.0	3.0
			Percentage of SLES		
Noncoated liposome	2.0	2.0	2.0	2.0	2.0
Coated liposome	4.0	4.0	4.0	3.0	3.0

SDS: Sodium dodecyl sulfate, SLES: Sodium lauryl ether sulfate.

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Table II
Resistance Kinetics of Coated and Noncoated Liposomes to Nonionic Surfactants during 30 Days of Storage at 25°C

	T0	Day 1	Day 7	Day 15	Day 30
	Percentage of polysorbate 20				
Noncoated liposome	5.0	5.0	4.0	4.0	3.0
Coated liposome	9.0	9.0	9.0	9.0	9.0
	Percentage of polyglyceryl-10-laurate				
Noncoated liposome	7.0	7.0	7.0	7.0	7.0
Coated liposome	9.0	9.0	9.0	9.0	9.0
	Percentage of behenyl alcohol 25 EO				
Noncoated liposome	8.0	5.0	4.0	3.0	3.0
Coated liposome	9.0	9.0	9.0	9.0	6.0
	Percentage of Triton X-100				
Noncoated liposome	3.0	3.0	3.0	2.5	2.5
Coated liposome	4.0	4.0	4.0	3.5	3.0

At stages I and II, liposomes inflated by increasing the surfactant concentration. At the third stage, there is a drastic decrease in size due to the formation of hybrid micelles (8). By increasing the concentration of detergent, the latter integrates phospholipid membrane and then accelerates the destabilization of liposomes. As already described by Mady *et al.* (23), we confirmed that coating of liposomes by Stearoyl Inulin decreased their destabilization by ionic and nonionic surfactants. Surfactants are firstly incorporated in the polysaccharide film, which delays the arrival of surfactant to the liposome phospholipid bilayer. Moreover, due to the stearic acid and phospholipid interactions, coated membranes became less soluble in contact with surfactants. However, this resistance depends on the concentration and the kind of surfactant.

Resistance of coated and noncoated liposomes to electrolytes. Monovalent (NaCl) and divalent ($MgCl_2$) salts were used to evaluate the influence of coating process on the resistance of liposomes against the ionic strength of salts.

The maximum percentages of NaCl and $MgCl_2$, which do not destabilize coated and noncoated liposomes were summarized in Table III. Noncoated liposomes resisted up to 10% of NaCl until 15 days and 5% until 30 days. The difference is even more pronounced in the presence of $MgCl_2$ where coated liposomes were stable up to 20% in comparison with noncoated liposomes, which were immediately destabilized in contact with this divalent salt. However, coated liposomes resisted surprisingly to 20% of NaCl and $MgCl_2$ over the entire storage period.

The presence of salts in the medium destabilizes the liposomes by modifying the structure of phospholipid head groups (24). Furthermore, entrapment of high concentrations of $MgCl_2$ inside liposomes suspended in low concentration water outside leads to the internalization of water until complete rupture of the phospholipid membrane (25). By the hydrophobized polysaccharide coating process, liposomes became four times more resistant to electrolytes and then to ionic strength. The insertion and interaction of Stearoyl Inulin's alkyl chains with phospholipid membrane guaranteed the presence of

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Table III
Resistance Kinetics of Coated and Noncoated Liposomes to Electrolytes during 30 Days of Storage at 25°C

	T0	Day 1	Day 7	Day 15	Day 30
	Percentage of NaCl				
Noncoated liposome	20.0	10.0	10.0	10.0	5.0
Coated liposome	20.0	20.0	20.0	20.0	20.0
	Percentage of $MgCl_2$				
Noncoated liposome	20.0	0.0	0.0	0.0	0.0
Coated liposome	20.0	20.0	20.0	20.0	20.0

polysaccharide film at the surface of liposomes, thus protecting phospholipid head groups against electrolytes. Hydrophobic interactions between alkyl chains of the hydrophobized polysaccharide and phospholipids fatty acids probably stabilized the coated liposomes more than nonmodified polysaccharide.

MAGNESIUM CHLORIDE RELEASE FROM COATED LIPOSOMES

The membrane permeability of coated liposomes was evaluated by studying the release of $MgCl_2$ from coated liposomes to the external acrylate gel medium, reducing its viscosity. Depending on the shear rate (0–70 s^{-1}), acrylate gel viscosity was measured at Day 0 and after 30 days at 25°C. The results are presented in Figure 3.

After adding 0.24% of free $MgCl_2$ to the acrylates gel and an equivalent amount of entrapped $MgCl_2$ in coated liposomes (2%), we observed a visual viscosity difference compared to the control acrylates gel. The gel containing free $MgCl_2$ was completely liquid, the one containing MCCL was soft, and the control gel was hard.

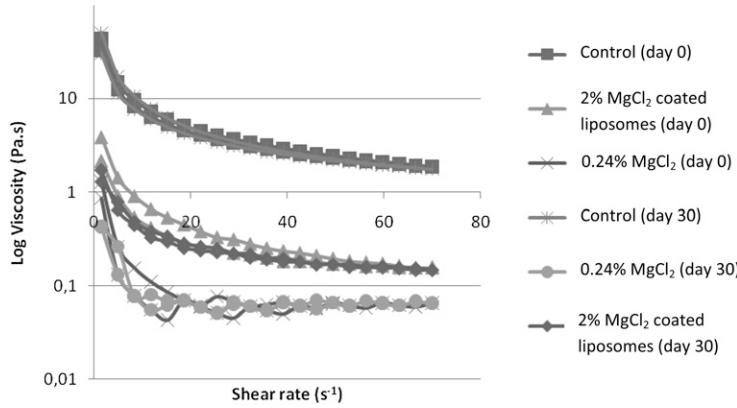

Figure 3. Comparison of the viscosity of acrylates gel containing coated liposome magnesium chloride (2%; w/w), free magnesium chloride (0.24%; w/w), and control acrylates gel at Day 0 and after 30 days of storage at 25°C.

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Our observations were confirmed by the viscosity values presented in Figure 3. Compared to the control acrylate gel, the incorporation of free and entrapped $MgCl_2$ decreased the acrylate gel viscosity. There was an important difference between the effects of $MgCl_2$ whether in its entrapped or free form. 0.24% of free $MgCl_2$ decreased the viscosity of acrylate gel by about 41 times. However, when the same amount of $MgCl_2$ was entrapped in coated liposomes (2%), the viscosity of acrylate gel decreased about 14 times compared to the control. These results were considerably different and showed that $MgCl_2$ was partly entrapped into coated liposomes and some $MgCl_2$ remained outside the coated liposome. Consequently, only nonentrapped $MgCl_2$ decreases the viscosity of acrylates gel.

Figure 3 shows that viscosities of the control and acrylate gel containing free $MgCl_2$ were stable for 30 days of storage at 25°C.

Because of the strong encapsulation efficiency of our technology, the viscosity of coated $MgCl_2$ gel at Day 0 and Day 30 is not considerably different (Figure 3). This result revealed that coated liposome membrane is very resistant and does not allow the release of $MgCl_2$ during the period of storage.

Aqueous suspensions of MCCL were diluted with distilled water (1/2, 1/10) and then introduced into an acrylates gel to potentially induce a diffusion of $MgCl_2$ through the coated liposome membrane. An equivalent amount of free $MgCl_2$ was incorporated into acrylate gel. Figure 4 presents the viscosity of acrylate gels containing 2% of MCCL (i.e., 0.24% $MgCl_2$), 4% of MCCL suspension diluted by half to maintain an equivalent amount of $MgCl_2$ (i.e., 0.24% $MgCl_2$), and 20% of MCCL suspension diluted to 1/10 (i.e., 0.24% $MgCl_2$). Our results showed that there was no effect on the $MgCl_2$ release after the external medium dilution of coated liposomes. If the membrane permeability of coated liposomes depended on the osmotic gradient, distilled water dilution would have induced the release of $MgCl_2$ from coated liposomes. Our $MgCl_2$ equilibrium hypothesis was then refuted because all acrylate gels containing different concentrations of entrapped $MgCl_2$ had the same viscosity profile. This study confirmed that coated liposome membrane is very resistant to $MgCl_2$ release. Liposome membranes are semipermeable to molecules depending on different characteristics. The permeability of liposome

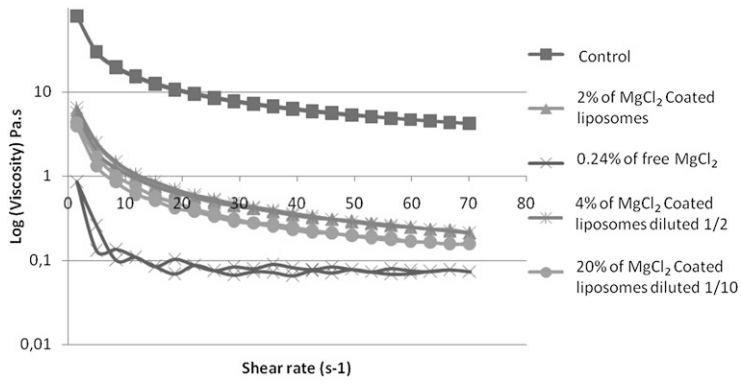

Figure 4. Effect of dilution on magnesium chloride release from coated liposomes (Acrylates gels viscosity was measured at 25°C).

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membrane to ions varies considerably (25). Protons and hydroxyl ions pass rapidly across liposomal membrane. However, the permeability of liposome membranes to divalent and multivalent ions is slower than monovalent ions. This could be due to the charge increasing and the hydration shell of ions.

IMPROVEMENT OF MAGNESIUM CHLORIDE DETOXIFICATION ACTIVITY

Ex vivo study of the MCCL efficacy to improve the skin availability of active molecules was performed. Because of its destabilization in contact with 12% of $MgCl_2$, noncoated liposomes were not tested in this *ex vivo* test. Indeed, $MgCl_2$ was entrapped in coated liposomes, and its effect on P-gp skin explants activity was evaluated versus non-entrapped $MgCl_2$. Acrylates/xanthan (1:0.5 w/w) gel containing 3% of MCCL or an equivalent amount of free $MgCl_2$ (0.36%) was used to evaluate the P-gp activity in a model of normal human skin explants. Figure 5 presents the quantity of ATP consumed by P-gp per µg of proteins. Compared to the nontreated explants (control), placebo and free $MgCl_2$ did not have significant effect on the consumption of ATP by P-gp transporter. However, MCCL increased the consumption of ATP ($p = 0.06$) significantly, followed by the activity of P-gp transporter. P-gp is a transmembrane biological target involved in cell detoxification system and waste elimination (26–28). Hamada and Tsuruo (7) found that magnesium is essential for P-gp ATPase activity. However, high concentrations of Mg^{2+} inhibited the ATPase activity of P-gp (7). According to this finding, we can conclude that 0.36% of $MgCl_2$ inhibited the ATPases activity of P-gp transporter. Figure 5 shows that coated liposomes improved the penetration of $MgCl_2$ through skin explants significantly compared to nonentrapped $MgCl_2$. We can suppose that the entrapment of $MgCl_2$ decreased its concentration in external aqueous medium of coated liposome suspension and then increased the activity of ATPase. During the incubation time, the long-lasting release of $MgCl_2$ reduced potential metabolism inhibition of P-gp ATPase in the presence of high salt contents.

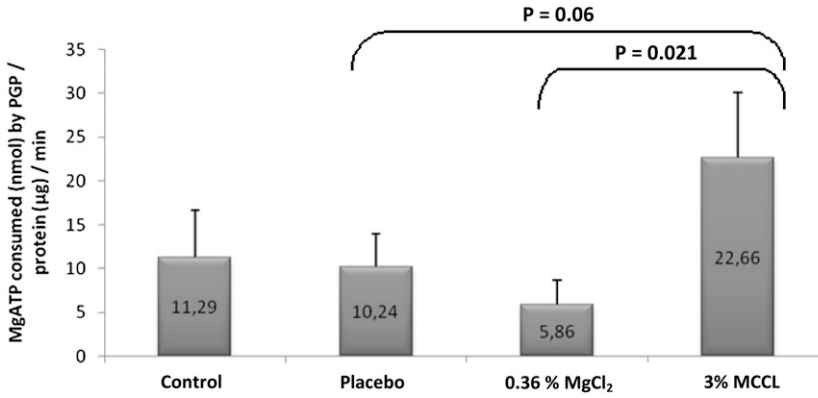

Figure 5. P-gp activity in human normal skin explants model (MCCL: Magnesium chloride coated liposomes).

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CONCLUSION

The development of a resistant liposome coated with hydrophobized polysaccharide increased its stability in cosmetic formula composed of high amounts of surfactants and/or electrolytes. Alkyl chains of hydrophobized polysaccharides were inserted into the bilayer membrane of liposomes, and then the polysaccharide surrounded the vesicle surface. We conclude that polysaccharide film surrounded liposomes did not reduce their delivery property and then the penetration of $MgCl_2$ into the skin. Unlike noncoated liposomes, the coated ones allowed entrapment of high amounts of $MgCl_2$ (12%) and then to limit the decrease of viscosity in cosmetics formulas. Compared to the free $MgCl_2$, the entrapment into coated liposomes increased the P-gp activity implicated in cell detoxification significantly and then its skin bioavailability. Thus, the coating maintains delivery property of liposomes. With this new generation of biomimetic vector, new cosmetic formulations can be achieved with higher efficacy and stability.

ACKNOWLEDGMENTS

The authors would like to thank Barbara Trouiller for her technical support in this project.

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