

Composite soy lecithin–decylpolyglucoside vesicles: A theoretical and experimental study

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Abstract

In the present work, vesicles made with soy lecithin and a commercial mixture of alkyl polyglucosides were prepared and characterized. Vesicles with a constant amount of soy lecithin and an increasing amount of a decylpolyglucoside surfactant, (OrNS10), were formulated and their physicochemical properties were studied with the aim to design a drug delivery system suitable for different applications. To this purpose, morphology, size distribution, ζ -potential and apparent viscosity of the prepared vesicles were studied. Vesicles were also characterized by using optical and light polarized microscopy, transmission electron microscopy and photon correlation spectroscopy. A stability study was also performed by checking mean size and ζ -potential value variation of the several formulations during 4 weeks.

Moreover, theoretical advances on geometric and thermodynamic aspects related to lipid vesicle formation were applied to this study.
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1. Introduction

Classic liposomes are not efficient enough for delivering molecules to their target site *in vivo*. To overcome these problems, new liposomal formulations have been developed modifying surface properties or bilayer fluidity of vesicles. Any change in formulation components affects physical and chemical properties of liposomes as well as other aspects like longer *in vivo* circulation, targeting to a specific tissue, improvement of drug delivery and toxicity decrease.

Liposomes have a short circulation half life as they are eliminated very rapidly from circulation by a complement-mediate phenomenon and end up in the organs of the reticulo endothelial system (RES). Surface modification of liposomes, such as pegylation and polymer coating, could change their *in vivo* biodistribution improving their half life. To this aim, polysaccharides, hydrophilic polymers, glycoproteins, peptides, antibodies,

and surfactants have been used. To increase the circulation time and the stability of liposomes PEG-lipids can be incorporated in the liposome bilayer. PEG-lipids mediate steric stabilization. They act as a hydrophilic barrier and reduce surface–surface interactions including aggregation of liposomes and adsorption of various molecules, such as plasma proteins, onto the particle surface in biological environments. PEG interferes with the binding of serum proteins and inhibits the adhesion to cell of RES reducing the clearance of liposomes [1]. Another way to obtain a significantly longer circulation in blood is the use of polymers such as chitosan, polyvinyl alcohol or polyaminoacids grafted onto the lipid bilayers of liposomes. Coating is obtained by just mixing a liposome suspension and a polymer solution without chemically linking the polymers to the lipid molecules [2,3].

In the present work, vesicles made with soy lecithin and decylpolyglucoside have been prepared and characterized. Soy lecithin (SL) is a natural mixture of phosphatidylcholine (90%) with other phospholipids and fatty acids. It forms vesicles in water spontaneously [4]. Oramix[®] NS10 (OrNS10) is the commercial name of a mixture of decylpolyglucoside

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surfactant in water (60%), which has been employed as a co-surfactant in emulsion and detergent for pharmaceutical and cosmetic uses. It exhibits peculiar interfacial properties; in water at low concentrations, it forms isotropic micelles while at higher concentrations (more than 70%) it exists in lamellar liquid crystalline phases [5,6]. In a previous work, OrNS10 capability to form non-ionic surfactant vesicles in water with and without cholesterol was studied [7]. We found that OrNS10 can form vesicles only in presence of cholesterol.

During this work, vesicles with a constant amount of soy lecithin and an increasing amount of OrNS10 were prepared and their physicochemical properties were studied to design new hydrophilic liposomal formulations as drug delivery systems. Morphology, size distribution, ζ -potential and viscosity of the prepared vesicles were measured to characterize the systems.

Moreover, theoretical advances on geometric and thermodynamic aspects related to lipid vesicle formation were applied to this study. Energy required to form vesicles with amphiphilic molecules has three contributors, which are related to surface energy, mechanical energy due to overpressure and chemical potential excess. This parameterization has been extensively applied to vesicle formation process of phospholipid systems such as dilauroyl-, dimyristoyl-, dipalmytoyl and distearoyl-phosphatidylcholine [8,9] with excellent agreement between theoretical and experimental results. From a theoretical point of view, application of this parameterization does not depend on the type of the employed amphiphile. In a previous work, for the first time this model was applied to non-phospholipidic surfactants, in particular to OrNS10 and OrNS10/cholesterol associations [10,11]. We found that this parameterization is also suitable for the evaluation of non-ionic surfactant self-assembly. In the present study, using this parameterization, the energy balance (δ) of SL/OrNS10 was calculated for the critical conditions of formation.

2. Experimental

2.1. Materials

Oramix[®] NS10 (OrNS10), a 60% solution of decylpolyglucosides, was kindly supplied by Seppic (Italy). Soy lecithin (SL) was purchased from Guinama S.L.U. (Valencia, Spain). Cholesterol and all the other products were of analytical-grade and were purchased from Aldrich (Italy).

2.2. Vesicle preparation

Liposome dispersions of soy lecithin combined at several molar ratios with decylpolyglucoside were prepared (SL/OrNS10 1/0; 1/0.25; 1/0.50; 1/0.75; 1/1; 1/1.25; 1/1.5 molar ratio).

Multilamellar vesicles (MLV) were prepared according to the film hydration method [12,13], using cholesterol in order to increase sample homogeneity. The amount of used cholesterol was constant in each formulation (5%, w/w of soy lecithin

amount). Surfactants and cholesterol in chloroform solution were mixed in the appropriate amount. Chloroform was removed under vacuum and the thin film was hydrated at $25 \pm 2^\circ\text{C}$ with bi-distillate water under mechanical stirring. The final SL concentration was always 50 mM.

Sonicated vesicles (SV) were prepared starting from the MLV dispersions by sonication (with 14 μm wave amplitude) using a Soniprep 150 apparatus (MSE, Crowley), under a nitrogen stream, for 10 min (10 times for 1 min), at $25 \pm 2^\circ\text{C}$.

2.3. Vesicle size characterization

Vesicles were characterized by transmission electron microscopy (TEM), and optical microscopy for vesicle formation and morphology, by dynamic laser light scattering (DLS) for mean size and size distribution, and by Zetasizer (ZS) for ζ -potential.

A drop of vesicle dispersion was applied to a carbon film-covered copper grid and was stained with a 1% phosphotungstic acid. Then samples were examined and photographed with a Zeiss EM 109 transmission electron microscope at an accelerating voltage of 80 kV.

Optical micrographs were obtained with an optical microscope, Olympus AT70TF, equipped with a camera Olympus C-2000 zoom.

Liposome size distribution was determined by DLS (N4 plus, Beckman Coulter) at 25°C . Samples were scattered (633 nm) at an angle of 90° . Data were fitted by the method of inverse “Laplace transformation” and Contin.

Liposome ζ -potential was determined by Zetasizer (Nanoseries, Malvern) at 25°C . Data were worked out by Smoluchowski equation for illustrative purpose.

PH values of dispersions were measured at 25°C using a pH-meter, pH 212 (Hanna Instruments, Italy). Vesicle dispersions were stored in glass vials at 4°C for 4 weeks. Size and ζ -potential of liposomes were monitored each week for 4 weeks. All measures were made three times at least.

2.4. Turbidity and viscosity measurement

Effects of Oramix NS10 addition in lecithin vesicle dispersions were assessed according to the turbidity measurements of their diluted suspensions. Sonicated vesicles were assayed by UV, 24 h after sonication. Turbidity was measured spectrophotometrically at 550 nm and 25°C using an UV/visible spectrophotometer (UV-Visible, Hitachi-U200). Viscosity measures were performed using a Gemini 150 NanoViscosimeter, Bohlin Instruments (Worcestershire, UK) with a torque range of speeds ranging from 0.05 $\mu\text{N}\cdot\text{m}$ to 200 $\mu\text{N}\cdot\text{m}$ and connected with a computer equipped with Bohlin software package.

Analyses were performed at controlled temperature of 25°C , at controlled shear stress of 0.500 Pa with variable shear rate, using a cone plate. Measures were provided at steady state. All the turbidity and viscosity measures were made three times.

2.5. Theoretical evaluation of parameters. Energy balance (δ) calculation

In each case, the energy balance was calculated for the critical conditions of formation according to eq. (1), as reported in previous papers [10,11]:

$$\delta = \frac{2\Delta\mu'}{\gamma_{\text{ccf}}} S_m \quad (1)$$

where $\Delta\mu'$ is the chemical potential excess that was evaluated from the chemical potential at the critical concentration of vesicle formation (ccf) with the opposite sign [14]; γ_{ccf} is the surface tension at ccf. Finally, S_m is the mean molar surface of the SL/OrNS10 samples that was theoretically estimated, in any case, by means of a procedure used by other authors [15,16] and based on bibliographic data of phosphatidylcholine and polyglycosides [17,18].

To measure the critical concentration of formation, SL and OrNS10 aqueous dispersions were prepared at several molar ratios (formulations 1–7). In each dispersion, SL concentration was left constant (1 mol/L) while OrNS10 concentration was increased. After, each suspension was diluted in bi-distillate water to obtain different SL concentrations ranging between 1 mol/L and 10^{-8} mol/L. Obtained samples were sonicated under a nitrogen stream, for 10 min (60 times for 10 s). Then, 24 h later, the samples were assayed spectrophotometrically at 550 nm and 25 °C. Surface tension of these samples was measured at 25 °C using a Tensimeter K12 (Krüss, GmbH) interfaced to a processor (Krüss, GmbH). The tensimeter K12 provided standard deviation of 10 measurements for each sample, which was prepared in triplicate. The error was the highest standard deviation of the three samples. Surface tension at ccf concentration, γ_{ccf} , was determined by interpolation at the plot γ versus concentration, according to the method developed previously [19].

2.6. Statistical analysis

Each experiment was performed in triplicate and values expressed as mean \pm S.D. Statistical analysis were performed using Student's *t*-test.

3. Results and discussion

Optical microscopy and transmission electron microscopy allowed visualization of vesicles, confirmed their formation and

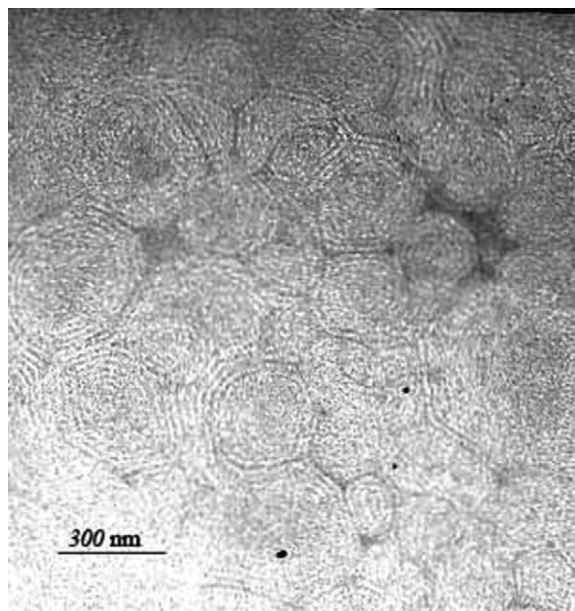


Fig. 1. TEM pictures of formulation 4 of SL/OrNS10.

showed their morphology. Fig. 1 show TEM picture of formulation 4 of SL/OrNS10 of multilamellar vesicles.

Dynamic laser light scattering (DLS) allowed determination of size and size distribution while Zetasizer (ZS) provided zeta-potential values of vesicle dispersions. Values are shown in Table 1. As can be seen, mean size of vesicles decreased slightly (more significantly in formulation 4) and zeta-potential values decreased (in absolute value) from formulation 1 to 4 because of OrNS10 molecules intercalation into the phospholipid bilayer. Therefore, their big uncharged hydrophilic heads split off the phosphatidylcholine charged molecules causing a better packing of the bilayer and a reduction of surface charge density. In formulation 5, OrNS10 reached a critical concentration value into the phosphatidylcholine bilayer and its molecules shifted on the bilayer surface. The presence of OrNS10 molecules on the bilayer surface led to vesicle fusion causing an enlargement of structure size.

It is important to remind that formulations were not prepared in a buffer medium but in bi-distilled water and their pH values decreased from 6.62 to 5.23 as OrNS10 concentration increased in the formulations. However, the reduction was slight (from 6.62 to 6.35) from formulation 1 to formulation 4 because in these cases, the OrNS10 molecules were intercalated into the bilayer, while in formulations 5–7, pH values decreased strongly

Table 1

Influence of surfactant addition on the physicochemical properties of SVs: mean size, polydispersity index (PI) and ζ -potential at 25 °C.

Formulation	SL/OrNS10 molar ratio	Diameter (nm)	PI	ζ -Potential (mV)	pH
1	1.00/0.00	390 \pm 20	0.22	-52 \pm 9	6.62
2	1.00/0.25	370 \pm 15	0.20	-50 \pm 7	6.56
3	1.00/0.50	340 \pm 20	0.21	-45 \pm 10	6.42
4	1.00/0.75	220 \pm 20	0.06	-44 \pm 10	6.35
5	1.00/1.00	310 \pm 15	0.04	-44 \pm 10	5.84
6	1.00/1.25	610 \pm 30	0.16	-54 \pm 8	5.57
7	1.00/1.50	600 \pm 30	0.17	-69 \pm 7	5.23

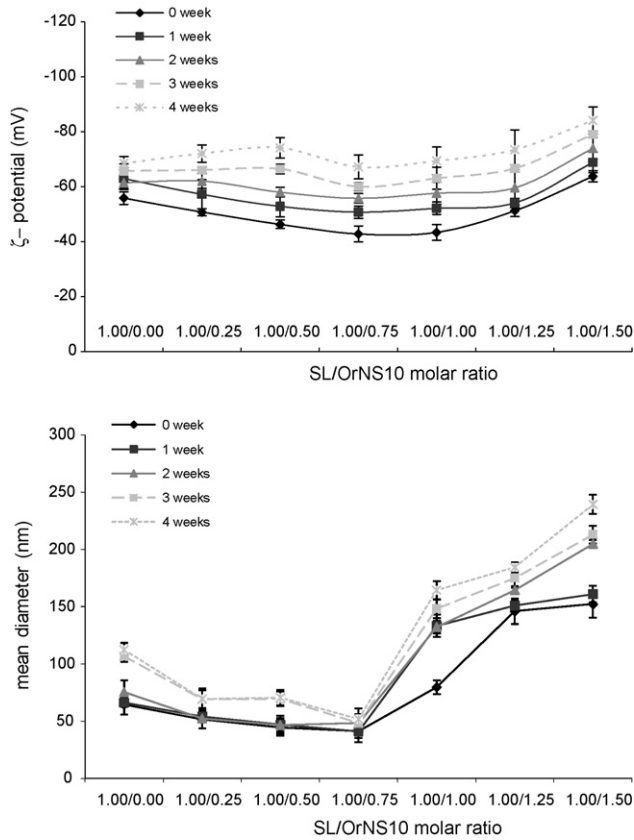


Fig. 2. Mean size and ζ -potential of the dispersions after 4 weeks. Vesicles dispersions were stored in glass vials at 4 °C for 4 weeks.

because of the surfactant excess motion from bilayer or to vesicle surface or to the aqueous medium. In this last case, it is possible to observe an increase of the absolute value of the zeta-potential. Changes of pH and zeta-potential values indicates the formation of different aggregation structures (i.e. hexagonal, hexagonal reverse or cubic phases), with a different behaviour compared to multilamellar vesicles because of the different molecular assembly.

Vesicle dispersions were stored in glass vials at 4 °C for 4 weeks. Fig. 2 shows mean size and ζ -potential value variation of the different formulations during the 4 weeks. With the exception of formulation 4 (1.00/0.75 SL/OrNS10 molar ratio), the z-average diameter of the other vesicular formulations showed a significant increase. For formulations from 5 to 7, this fact

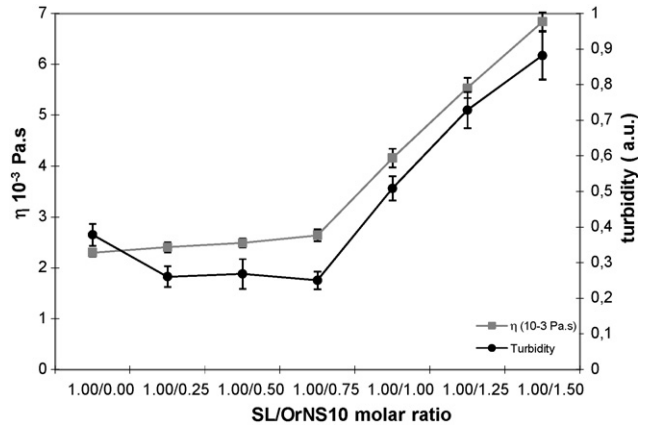


Fig. 3. Viscosity values (η) and turbidity measures vs. SL/OrNS10 molar ratio.

is due to the tendency of vesicles to fuse and form different self-assembly structures. The increase of the absolute value of ζ -potential is a common event when the samples are prepared in a non-buffered medium where some degradation processes such as lipid peroxidation can occur. However, in our study there is a small variation that could be accepted.

Turbidimetry has been frequently used to characterize surfactant and phospholipid molecule aggregates in supramolecular assembly that are associated with a strong change of dispersion turbidity. Using turbidity measures, it is also possible to monitor surfactant interactions with phospholipid vesicles. In fact, surfactants can intercalate into liposomal bilayers causing a change in liposome size and ζ -potential or may lead to phospholipid solubilisation with a sharp variation in turbidity [20,21]. Fig. 3 shows results obtained by plotting turbidity and viscosity values versus SL/OrNS10 molar ratio. As can be seen, dispersion turbidity decreased from formulation 1 (SL alone) to formulation 4 (1/0.75 SL/OrNS10 molar ratio) as a consequence of the surfactant molecule intercalation into the liposomal bilayer, which caused a vesicle size decrease. A sharp increase in turbidity was observed in formulations 5–7 because of the presence of the OrNS10 excess that could not intercalate into the bilayer but moved on the vesicle surface causing vesicle fusion.

Viscosity values were very similar in the formulations 1–4, but they increased significantly in formulation 5 (1.00/1.00 SL/OrNS10 molar ratio) because free water decreased and dispersed particles were larger and interacted each other. Turbidity

Table 2

Estimated, experimental and calculated magnitudes of critical concentration of vesicle formation (ccf), surface tension, molar surface area (S_m), energy balance (δ) of the diverse SL/OrNS10 molar ratio mixtures at 25 °C

Formulation	SL/OrNS10 molar ratio	Experimental values		Estimated values	Calculated values
		ccf (mol/L)	Surface tension ($N m^{-1}$)		
1	1.00/0.00	5.26×10^{-7}	0.062	300000	4.92
2	1.00/0.25	6.49×10^{-5}	0.055	322730	3.81
3	1.00/0.50	5.82×10^{-5}	0.052	345460	3.79
4	1.00/0.75	5.27×10^{-5}	0.051	368190	3.66
5	1.00/1.00	6.49×10^{-4}	0.048	390920	3.00
6	1.00/1.25	6.10×10^{-3}	0.049	397730	2.31
7	1.00/1.50	7.14×10^{-3}	0.049	422163	2.14

and viscosity results show that a change exists in formulation 5 corresponding to a critical concentration of OrNS10 into the phosphatidylcholine bilayer.

In each case, the energy balance, $\delta = 2\Delta\mu'/\gamma_{\text{cf}}S_m$, was calculated for the critical conditions of formation [5,6] and the obtained results are shown in Table 2. When the energy balance, δ , is between 10/3 and 5, vesicle geometries are obtained. Therefore, $\delta = 10/3$ represents the lowest limit of vesicle existence. Micelle aggregates are present for $5 \leq \delta \leq 10$, while for $\delta < 10/3$, other aggregation structures can form (hexagonal phase, cubic phase, etc.). Therefore, the formulations 1–4 show energy balance values related to vesicular geometry, but formulations 5–7 have energy balance values corresponding to other topologies. In these last formulations, the amount of OrNS10 is very high and, probably, it does not allow the vesicles to form.

4. Conclusions

For vesicles formation, there is a critical SL/OrNS10 molar ratio corresponding to formulation 4 (1.00/0.75 SL/OrNS10 molar ratio). The increase of OrNS10 amount leads to structures with other topologies. Theoretical studies and experimental data confirm this conclusion. The capability of formulation from 1 to 4 to form multi or unilamellar vesicles and for formulation from 5 to 7 to form other structures is supported by the energy balance (δ) values, viscosity and turbidity results.

Physical stability studies of the formulations were carried out for 4 weeks. During this period of storage at 4 °C, size and zeta-potential were monitored. Results of this study have shown that formulation 4 was the most stable. In fact, during the 4 weeks, the size did not vary demonstrating that aggregation had not occurred. Similar results were obtained for formulation from 1 to 3 during the first 3 weeks of the study. Moreover, the ζ -potential of all formulations showed no significant difference and a good electrokinetic behavior during this period. Zeta-potential determination during different times is a good method to evaluate possible degradation processes. In this case, value variations were minimal, therefore, indicating that OrSN10 addition to soy lecithin is able to improve liposome stability.

Finally, the results of this work show an excellent way to modify the physicochemical properties of the liposomes in order to design a drug delivery system suitable for different applications.

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