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Design of soy lecithin vesicle nanocarriers: impact of DPPC, DOPC and cholesterol on bilayer dynamics and release properties

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INTRODUCTION & AIM

Vesicles are supramolecular systems that can be obtained when molecules with amphiphilic properties (known as surfactants) are suspended in aqueous media. Upon formation, a portion of the solvent becomes entrapped within the vesicles, separated from the external medium by a bilayer structure. Owing to the polarity differences between the bilayer and the internal solvent, these systems are widely used as nanocarriers for hydrophobic and hydrophilic molecules, respectively.

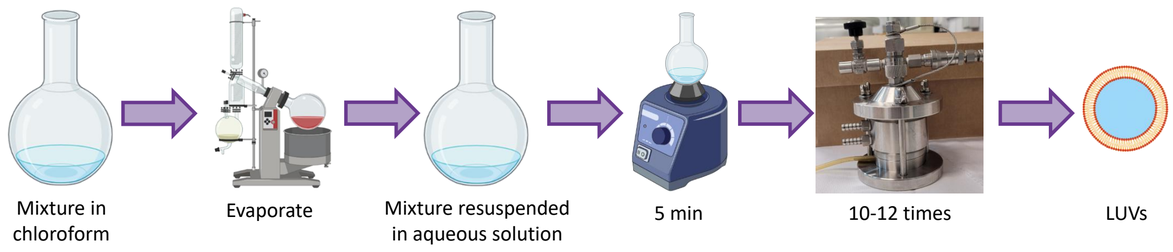
Phospholipids are biocompatible surfactants, as they constitute the main components of cell membranes. It has been shown that vesicles derived from phospholipids exhibit high stability and are therefore often employed to encapsulate hydrophilic drugs. Furthermore, in drug delivery applications, not only encapsulation efficiency but also release rate and release percentage are critical parameters.

Previous studies have demonstrated that phospholipid vesicles can be modified with different compounds, leading to systems with altered bilayer properties. Since drugs must diffuse through this bilayer to be released, modulating its characteristics by incorporating additional components is of great interest.

Following the latest mentioned, this study explores the preparation and characterization of soy lecithin unilamellar vesicles modified with dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC), and cholesterol (CHO), focusing on how these compounds impacts on bilayer fluidity and permeability.

METHODS

Large unilamellar vesicles (LUVs) were successfully obtained by the extrusion method (**Scheme 1**). All the samples were prepared at [lecithin + modifier] = 2 mg/mL. Depending on the experiment, the aqueous phase consisted of either HPLC-grade water, a solution of 6-propionyl-2-(N',N'-dimethyl)aminonaphthalene (PRODAN) in water, or a potassium ferricyanide ($K_4[Fe(CN)_6]$) solution in phosphate buffer (pH 7).



Scheme 1. Extrusion method for the formation of unilamellar lecithin modified vesicles.

Dynamic Light Scattering (DLS) measurements were performed to confirm that all samples consisted of monodisperse unilamellar vesicles. For this purpose, a 1:100 vesicle-to-water dilution was prepared using HPLC-grade water previously filtered through a 0.2 µm nylon membrane. The resulting solution was then transferred to a glass cuvette for analysis.

Bilayer fluidity was evaluated using the fluorescent probe 6-propionyl-2-(N',N'-dimethyl)aminonaphthalene (PRODAN). A stock solution of PRODAN ([PRODAN] = 2 × 10^{-6} M) was prepared in HPLC-grade water, and the vesicle resuspension step was carried out in this medium. Fluorescence emission spectra (λ_exc = 370 nm) were recorded for 2 mL of the PRODAN solution, followed by successive additions of vesicle samples, recording the emission spectrum after each addition.

Bilayer permeability was assessed using the electrochemical probe potassium ferricyanide ($K_4[Fe(CN)_6]$) through square wave voltammetry (SWV). A stock solution of the probe ([$K_4[Fe(CN)_6]$] = 0.2 M) was prepared in phosphate buffer (pH 7), and vesicles were resuspended in this medium. To study the release process, unencapsulated $K_4[Fe(CN)_6]$ was removed by gel filtration chromatography using a Sephadex G-50 column. Fractions containing vesicles were transferred to a conventional three-electrode electrochemical cell consisting of a large-area Pt foil (2 cm^2) as the counter electrode, an Ag/AgCl (saturated KCl) reference electrode, and a gold disk as the working electrode. The gold electrode was cleaned in a 1:10 H_2SO_4:water solution until a typical clean voltammogram was obtained.

RESULTS & DISCUSSION

The size and polydispersity index (PDI) of vesicles suspended in phosphate buffer (pH 7) and in an aqueous $K_4[Fe(CN)_6]$ solution were determined by Dynamic Light Scattering (DLS). In all cases, the PDI values were below 0.2, indicating the formation of monodisperse unilamellar vesicles. This result is relevant since it ensures reproducibility in electrochemical measurements. It is noteworthy that the incorporation of DPPC allowed the formation of stable LUVs at room temperature, well below its gel-to-liquid crystalline phase transition temperature (~41 °C)—a challenging condition when using DPPC alone.

Bilayer fluidity

Bilayer fluidity was then evaluated through changes in the fluorescence spectra of PRODAN as a function of vesicle concentration (**Fig. 1**).

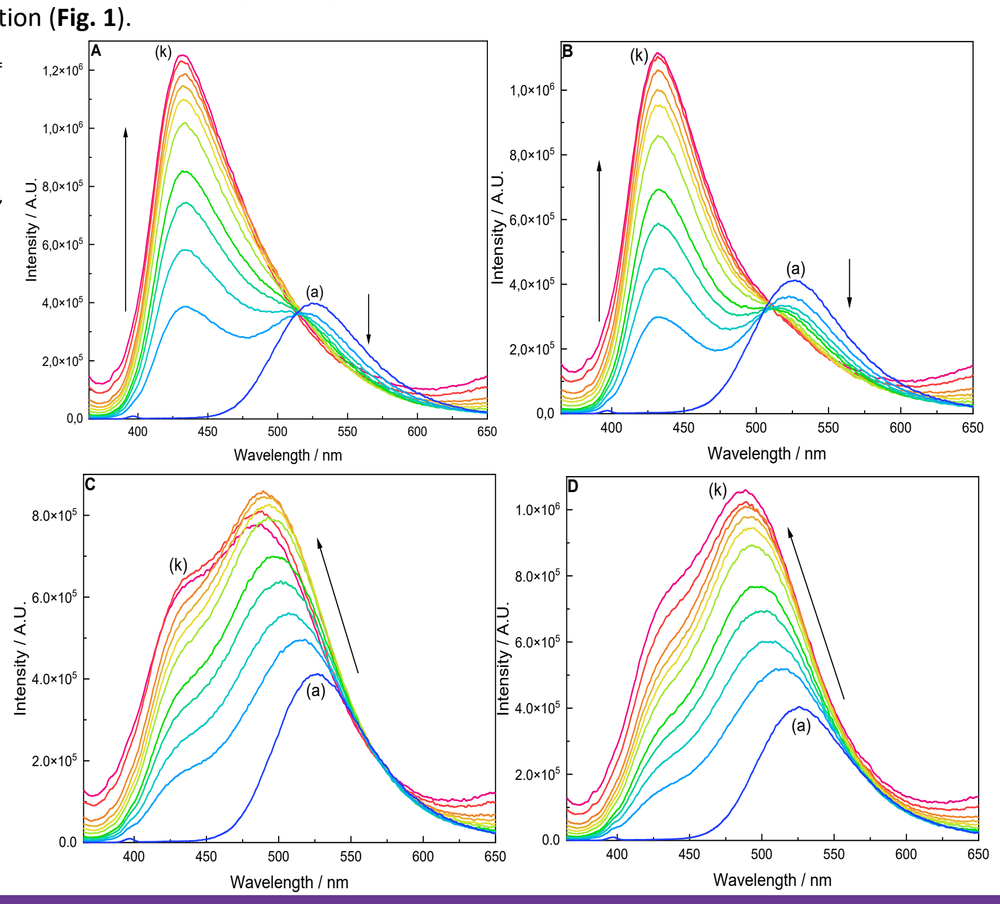


Fig. 1. Emission spectra (λexc= 370 nm) of PRODAN in LUVs of (A) Lec:DPPC 1:2, (B) Lec:Cho 1:1.6 (C) Lec:DOPC 1:1 and Lec:DOPC 1:2; as a function of the surfactant concentration in mg/mL: (a) 0, (b) 0.05, (c) 0.1, (d) 0.15, (d) 0.22, (e) 0.4, (f) 0.57, (g) 0.75, (h) 1, (i) 1.5 and (k) 2. [PRODAN] = 2 x10^{-6} M.

PRODAN fluorescence spectra revealed higher bilayer fluidity for Lec:DPPC 1:2 and Lec:Cho 1:1.6 mixtures. The rapid hypsochromic shift from 525 nm (in water) to 430 nm indicated a fast transition to a more hydrophobic environment.

In contrast, Lec:DOPC mixtures showed lower bilayer fluidity, especially Lec:DOPC 1:2. Although a hypsochromic shift was also observed, the maximum emission appeared at 488 nm, with the 430 nm band only as a shoulder. This behavior agrees with previous reports showing that DOPC reduces lecithin bilayer fluidity.[1]

Bilayer Permeability

Permeability was evaluated through the electrochemical response of $K_4[Fe(CN)_6]$ (**Fig. 2A**). Since electroactive molecules confined within the vesicle core cannot interact with the electrode, the appearance of a signal indicates that the analyte has crossed the bilayer and reached the bulk solution.

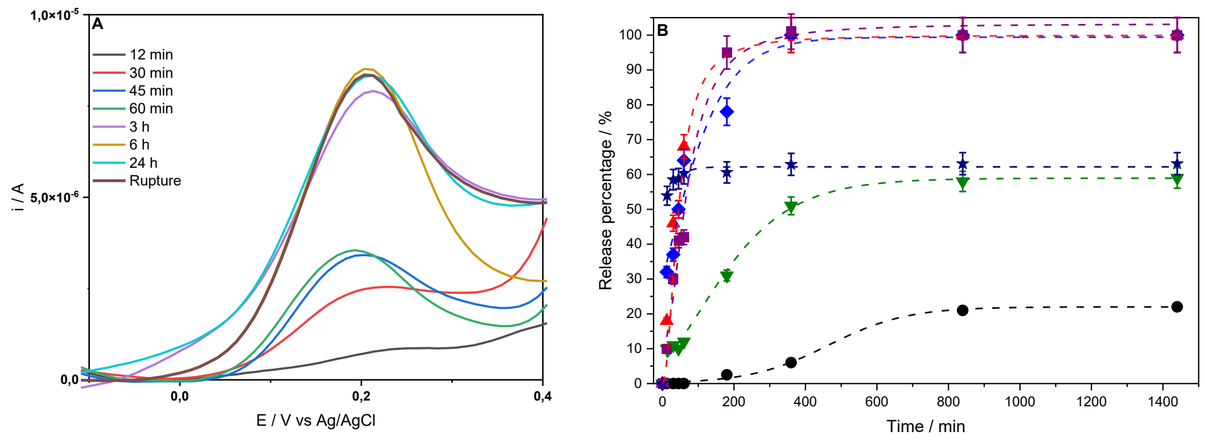


Fig. 2. (A) Voltammograms obtained through time for the Lec:Cho1:1,6 mixture. (B) Release profiles obtained from the mixtures: (●) DOPC, [2] (▲) Lec:DOPC 1:1, (▼) Lec:DOPC 1:2, (♦) Lec:DPPC 1:2, (■) Lec:Cho 1:1.6 and ★ lecithin.

Release studies

The electrochemical analysis of $K_4[Fe(CN)_6]$ release showed that the system's permeability depended on bilayer composition. Representative voltammograms for Lec:Cho (1:1.6) (**Figure 2A**) and the corresponding release profile for different systems (**Figure 2B**) confirmed that vesicle structure influences the diffusion of ionic species across the bilayer.

Finally, release rate was determined from the release profile's slopes. The results are gathered in **Table 1**.

Table 1. Release percentage and release rate of $K_4[Fe(CN)_6]$ obtained for all the mixtures studied.

Vesicles	*Release rate (%/min)*	*Release percentage (%)*
Lecithin	0.79	63
Lec:Cho 1:1.6	0.27	100
Lec:DPPC 1:2	0.22	100
Lec:DOPC 1:1	0.19	100
Lec:DOPC 1:2	0.13	59
DOPC	0.03	22

From the results shown in **Table 1**, it is evident that both the release percentage and the release rate vary among the systems studied. In particular, the lecithin system exhibits the highest release rate but reaches only 63% total release, indicating that these two parameters are not necessarily correlated.

Additionally, the behavior of the Lec:DOPC (1:1) mixture is not intermediate between its pure components, emphasizing the importance of analyzing each system individually, as the properties of mixed systems cannot always be predicted from those of their constituents. Furthermore, the Lec:DPPC (1:2) and Lec:Cho (1:1.6) systems display remarkably similar characteristics, suggesting that comparable behaviors can be achieved through different compositional modifications. This highlights the versatility and tunability of these nanocarriers.

Interestingly, the results show that as the bilayer fluidity decreases, the release rate also diminishes. This indicates a direct correlation between bilayer fluidity and membrane permeability, particularly in systems encapsulating hydrophilic molecules.

CONCLUSION

These results demonstrate that simple lipid mixtures can produce nanocarriers with distinct bilayer fluidity and permeability profiles, broadening their potential for application in nanomedicine, where carrier properties can be precisely tailored. Notably, the release rate and the total release percentage are not necessarily correlated, as observed for the lecithin system. The Lec:DOPC (1:1) mixture does not display an average behavior relative to its pure components, underscoring the importance of individually characterizing mixed systems. In contrast, the Lec:DPPC (1:2) and Lec:Cho (1:1.6) systems exhibit highly similar characteristics, demonstrating that comparable performance can be achieved through different compositional modifications. Overall, bilayer fluidity was found to directly influence membrane permeability for hydrophilic molecules, confirming the strong interdependence between these two properties.

FUTURE WORK / REFERENCES

Having characterized the permeability of these systems using an electrochemical probe, future work will focus on a more specific approach involving the encapsulation of a hydrophilic drug within the vesicles. This strategy will allow the design and evaluation of nanocarriers with potential applications in nanomedicine, tailored for the controlled delivery of that specific compound.

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[2] Farías, M. E.; Alejandra Luna, M.; Niebylski, A. M.; Mariano Correa, N.; Molina, P. G. Characterization of a Label System Formed by Large Unilamellar Vesicles for Its Potential Use in the Design of Electrochemical Biosensors. *Microchemical Journal* **2018**, *140*, 105–113. https://doi.org/10.1016/j.microc.2018.04.013.