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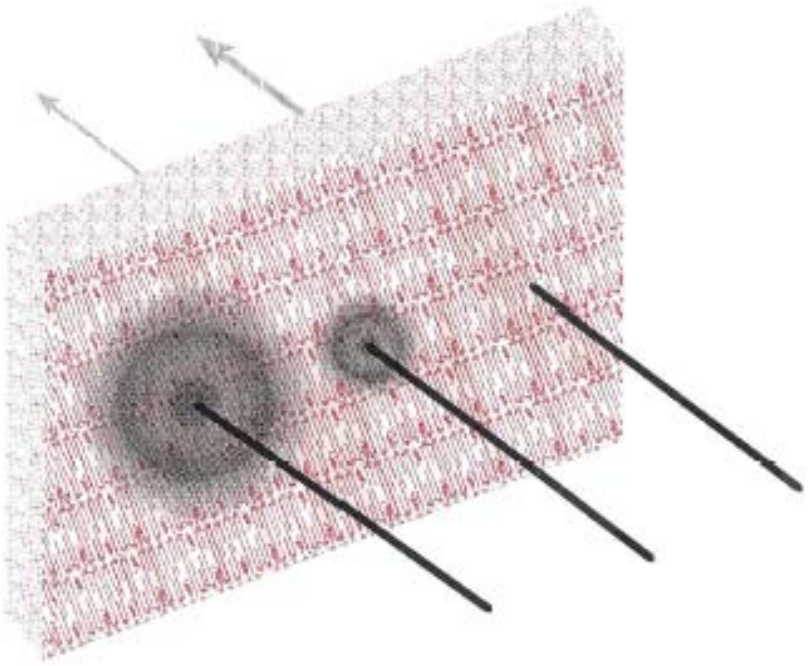

Edited by KR Brain and KA Walters

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INVERSION OF THE LIPIDS OF THE OUTER MONOLAYER OF THE LIPOSOMES AS STRATEGY OF ADAPTATION TO ENVIRONMENTS OF DIFFERENT POLARITY

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Use of liposomes in the field of dermatology and dermopharmacy has become more and more popular. From practical and theoretical viewpoints liposomes constitute suitable carriers that facilitate topical penetration[1]. However, a number of questions about the mechanism of action of these vesicular structures on the skin have to be still clarified.

Liposomes are constituted of membranes similar to the lipid layers of the intercellular spaces of the stratum corneum (SC)[2]. Their structure consists of two lipid monolayers in which the polar parts of the molecules are placed in contact with water and the apolar parts are located in the hydrophobic membrane core. Considering this arrangement, it may be predicted that variations in the polarity of the medium could lead to a spontaneous inversion of the lipid molecules of the liposome outer monolayer. In this work we studied this possible mechanism as a strategy of adaptation of the liposomes to environments of different polarity. Moreover, a method to form "a priori" this kind of vesicle, that might be called "*inverted liposomes"*, was investigated. Additionally, the stability and properties of these systems were evaluated.

Liposomes constituted of phospholipids were formed in an aqueous medium[3]. In order to induce inversion of the lipids of the outer monolayer the polarity of the medium was gradually changed by addition of different volumes of ethanol. To measure the degree of inversion of the lipids due to changes in the polarity of the medium, the electronic paramagnetic resonance (EPR) technique was used[4]. The size, shape and lamellarity of the vesicles in the different polar media were determined by dynamic light scattering (DLS)[3].

The results of the present study seemed to indicate that phospholipid liposomes show molecular mobility depending on the polarity of the medium. This molecular mobility due to the ethanol might induce spontaneous formation of liposomes in which lipids of the outer bilayer are inverted (inverted liposomes). Our results demonstrated the stability of this type of liposome in media containing < 30% ethanol.

Inverted liposomes could have a higher affinity to the less polar environments, such as the SC intercellular lipids. Moreover, these vesicles, that could cross more easily this potent barrier, might be of great interest in the development of new topical preparations.

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INTRODUCTION

Liposomes are constituted by membranes similar to the lipid layers of the intercellular spaces of the stratum corneum (SC). Their structure consists in two lipid monolayers in which the polar parts of the molecules are placed in contact with water and the apolar parts are located in the hydrophobic membrane core. Considering this arrangement, it may be predicted that *variations in the polarity of the medium could lead to the spontaneous inversion of the lipid molecules of the liposome outer monolayer.*

The *objective* of this work is to study this possible mechanism as a strategy of adaptation of the liposomes to environments of different polarity. The method to form "a priori" this kind of "adapted" vesicles, that might be called *"inverted liposomes"*, was investigated. Additionally, the stability and properties of these systems were evaluated.

METHODS

Liposomes constituted by phospholipids were formed by the evaporation-hidration method in an aqueous medium [1]. Once formed, the polarity of the medium was gradually changed by addition of different volumes of ethanol, obtaining *final ethanol percentages of 10%, 15%, 20%, 25%, 30%, 40% and 50%*. The final lipid concentration was 10mg/ml.

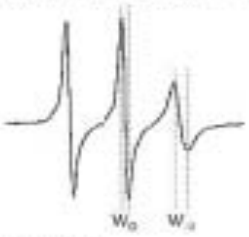
The size and shape of the vesicles in the different polar media were determined by dynamic light scattering (DLS) [1].

To measure the degree of inversion of the lipids due to changes in the polarity of the medium, the electronic paramagnetic resonance (EPR) technique was used [2]. The paramagnetic probe 16-doxil esteoric acid was included in the lipid phase of liposomes. The τ parameter (rotational correlation time) which describes the degree of freedom of the electronic spin movement was calculated from the EPR spectra according to the following equation:

$$\tau = 6.5 \times 10^{-10} W_0 [(W_1/W_0) - 1]$$

W_0 and W_{-1} are the width of the lines at medium and high fields of the first derivative of each absorption spectrum [2].

Figure 1: EPR spectrum of a liposome labeled with the 16-doxil esteoric acid.



RESULTS AND DISCUSSION

A. Formation and characterization of liposomes.

Figure 2: Change of size and polydispersity index of the liposomes when the proportion of ethanol in the medium is increased.

% ETHANOL	DIAMETER (nm)	POLYDISPERSITY INDEX
0	150	0.010
10	170	0.080
20	180	0.050
30	200	0.130
40	600	0.578
50	500	0.899

*Phospholipid liposomes in pure aqueous solution are populations of stable and spherical vesicles with an approximate diameter of 150 nm and a polydispersity index of 0.01.

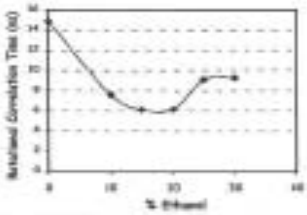
*Liposomes in media containing less than 30% ethanol were stable for at least one week.

*Ethanol percentages higher than 30% produced structural changes in the liposomes probably related with the first steps of vesicle solubilization.

Since the aim of the work is to study the inversion of the lipids of the outer bilayer without damage of the liposomes, it was considered that *an ethanol proportion of 30% is the maximum acceptable to ensure the liposome integrity.*

B. Effect of the medium polarity on the mobility of the lipids of the liposome outer monolayer

Figure 3: Rotational correlation time (τ) variation when the percentage of ethanol in the medium is increased. This parameter is inversely related with the mobility of the label and therefore with the mobility of the lipids in the bilayer. Thus, the decrease of this parameter when the proportion of ethanol in the medium is increased shows an increase of the molecular mobility.



*Solutions containing 10-20% of ethanol showed a maximum molecular mobility without changes of the physicochemical parameters of the liposomes (size and shape).

*Between 20-30% ethanol, an increase of τ was observed i.e., a decrease of the mobility of the lipids that could be due to an increase in the number of inverted lipid molecules. This fact could hinder the mobility of the probe.

Thus, EPR showed that *ethanol was able to induce molecular mobility in the lipids that form the liposomes.* This mobility might be *related with the process of inversion of the lipids* that constitute the outer monolayer of the liposomes forming the *"inverted liposomes"* (scheme A). The increase in the molecular mobility might cause *an increase in the flexibility of the bilayer* which might induce the deformation of the liposomes (scheme B).

The inclusion of ethanol in liposome formulation is not new. Touitou et al. have described a carrier for enhanced skin delivery, the ethosomal system, which are composed of phospholipid, ethanol and water [3]. Although the preparation method that these authors describe is different from that used in our systems, both structures could be similar. Our results seems to indicate an spontaneous inversion of the lipids of the outer monolayer of the liposomes depending on the polarity of the medium. This strategy might be considered as an adaptation of the liposomes to explain their pass through the lipid regions of the SC. This adaptation is based on two points: vesicles become more flexible and their outer surface becomes more lipophilic. Thus, the formation of "inverted liposomes" with a higher affinity for the lipid environment of the SC and, therefore, with more probability to cross this potent barrier could be of great interest in the development of new topical preparations.

CONCLUSIONS

- Phospholipid liposomes show certain molecular mobility depending on the polarity of the medium. This fact would constitute an strategy of adaptation to explain the pass of the liposomes through the lipid regions of the SC which have a specific polarity.
- This molecular mobility due to the ethanol could induce the formation of liposomes where the lipids of the outer bilayer are inverted: *"inverted liposomes"*.
- This kind of liposomes in media of certain polarity (less than 30% ethanol) are vesicles relatively stable. Thus, they might be of great interest in the development of new topical preparations.

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