Quatsomes: Vesicles Formed by Self-Assembly of Sterols and Quaternary Ammonium Surfactants

L. Ferrer-Tasies,[†,‡,ς] E. Moreno-Calvo,[†,‡,ς] E. Elizondo, [†,‡,ς] M. Cano-Sarabia,[†,‡] M. Aguilella-Arzo,[§] A. Angelova,[¥] S. Lesieur,[¥] S. Ricart,[†] J. Faraudo,*[,†] N. Ventosa,*[,†,‡] J. Veciana[†,‡]

[†]Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Esfera UAB; Campus UAB s/n; E-08193 Cerdanyola del Vallès, Spain.; ventosa@icmab.es; jfaraudo@icmab.es
[‡]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)
[§]Biophysics Group, Department of Physics, Universitat Jaume I, E-12080 Castelló, Spain
[¥]Equipe Physico-chimie de Systèmes Polyphasés, UMR CNRS 8612, Univ Paris-Sud, 92296 Châtenay-Malabry, France

ABSTRACT

There is a large interest in finding non-lipid building-blocks or tectons, which self-assemble into stable vesicles, and which satisfy the quality standards required in pharmaceutical formulations. Here we show the ability of quaternary ammonium surfactants and sterols to self-assemble forming stable amphiphilic bimolecular building-blocks with the appropriate structural characteristics to form, in aqueous phases, closed bilayers, which we named Quatsomes. When prepared by using compressed fluids, these colloidal structures are stable for periods as long as several years and their morphology do not change upon rising temperature or dilution. Many functionalities can be implemented simultaneously in Quatsomes, either by covalent attachment to sterol like molecules, by electrostatic interaction with the cationic head of surfactant units or by hydrophobic interaction with the bilayer. These possibilities open a broad range of applications in pharmacy, cosmetics and materials synthesis.

Keywords: cholesterol, sterols, cationic surfactants, vesicles, drug nanocarriers

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1 INTRODUCTION

Liposomes made with phospholipids, are among the most studied self-assembled nanoobjects since their serendipitous discovery in 1964.[1] They are described as vesicles composed of one or more concentric lipidic bilayers, which separate a small enclosed liquid compartment from its surroundings. Their unique structure enables them to trap hydrophobic molecules within their bilayers and hydrophilic molecules within their lumen making liposomes excellent candidates to be used as nanocarriers for the protection and delivery of active ingredients in pharmaceutical and cosmetic formulations.[2] Despite their versatility, the translation to the clinic of liposomal formulations is often being limited by the tendency of these lipid self-assemblies to aggregate and by their low degree of structural homogeneity, which are critical quality attributes with a major impact on the in vivo pharmacokinetic and pharmacodynamic properties.

There is a large interest in finding non-lipid building-blocks or tectons, which self-assemble into stable vesicles, and which satisfy the quality standards required in pharmaceutical formulations.[3,4] Using CO_2-expanded solvents we have been able to prepare stable vesicular structures composed by quaternary amonium surfactants and sterols, which we named Quatsomes, with structural characteristcs not achievable by commonly used vesicle formation techniques, such as film-hydration.[5]

2 ONE-STEP PREPARATION OF QUATSOMES

Quatsomes, composed by sterols and quaternary ammonium surfactants, were prepared using compressed CO_2 following the DELOS-susp procedure schematically represented in Figure 1.[5] Briefly, the method consists in loading a solution of the desired sterol in an organic solvent (e.g. ethanol) into a high-pressure autoclave and pressurizing it with a large amount of compressed CO_2.

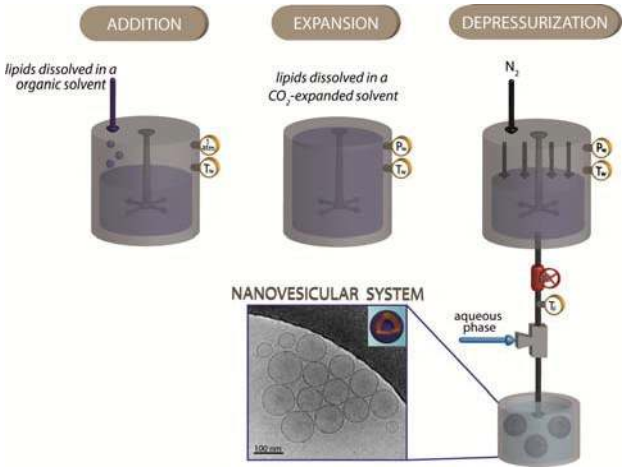

Figure 1. Scheme of the DELOS process for the preparation of vesicles.

Nanoscopic vesicular structures are straightforward formed, by depressurizing the resulting CO_2 expanded

solution over an aqueous phase, which contain the water soluble surfactant. The CO_2 here acts as a co-solvent and its evaporation from the organic expanded solution during the depressurization stage produces a fast, large and homogeneous cooling responsible of the high vesicle-to-vesicle structural homogeneity in comparison to the one reached by conventional thin-film hydration.

It should be pointed out, that lipids, such as sterols, have a great sensitivity to solvent media variations.[6] Therefore, homogeneous vesicle formation paths are required to guarantee a high degree of structural homogeneity. In Figure 2, are shown the cryo-TEM images of some representative Quatsomes prepared by this methodology.

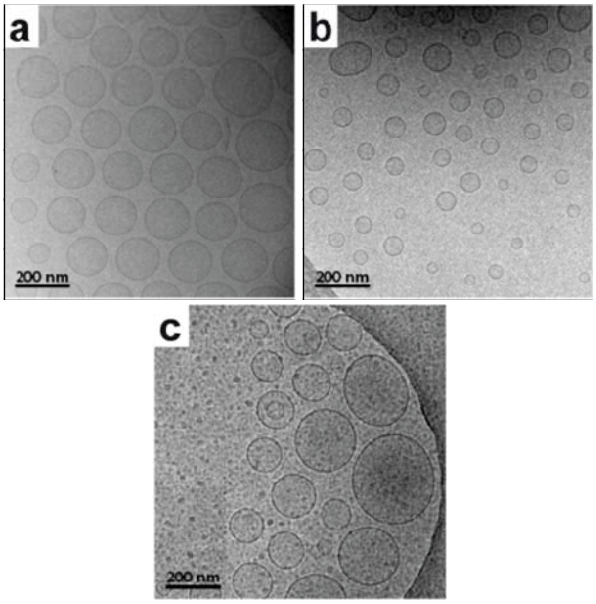

Figure 2. Representative cryo-TEM images of quatsomes prepared by DELOS-susp with composition: (a) Cholesterol/CTAB, (b) Cholesterol/MTAB and (c) β-sitosterol/CTAB. The scale bar represents 200 nm.

These vesicular systems are stable for periods as long as several years, their morphology do not change upon rising temperature up to 343 K or dilution and show outstanding vesicle to vesicle homogeneity regarding size, lamelarity and membrane supramolecular organization. These quatsomes have a great potential in the development of new nanomedicines and they have already shown to be effective nanostructures to enhance specific bioactivity of proteins and to protect them against premature degradation in topical pharmaceutical formulations.[7]

3 IMPACT OF THE PREPARATION ROUTE ON THE SELF-ASSEMBLY OF STEROLS AND QUATS

Recently, technical advances have allowed the monitoring of single vesicles within a population[8,9] and have provided unique information on heterogeneous properties that were otherwise obviated due to ensemble averaging. Particularly, a recent confocal fluorescence microscopy study on the lipid composition of single vesicles has revealed intrasample compositional variations.[10] Using this methodology, we have studied the impact of the preparation route on the supramolecular organization of Quatsomes, composed of sterols and ammonium quaternary surfactants, and on the self-assembling of lipids in classical Liposomes.[11] We prepared vesicles with two lipid compositions by the CF-based one-step procedure, described in last section, or a multistep conventional film hydration technique. The first composition consisted of 1/1 mol % of cholesterol (Chol) and cationic surfactant CTAB, which can suffer phase separation, and the second one was a fully miscible mixture consisting of 2/8 mol % of Chol and DOPC. The degree of membrane inhomogeneity (DI) between individual vesicles within each sample and the supramolecular arrangement of the lipids was then characterized and compared. Our findings indicate a strong preparation route dependence on the compositional inhomogeneity and supramolecular organization for mixtures that phase separate.

As observed in Figure 3, the DI found for the Chol/CTAB system prepared by the multistep hydration method was more than double than that of the samples obtained by the one step CF-based method (DIs of 1.08 ± 0.12 and 0.46 ± 0.04 for hydration method and DELOS-susp, respectively). According to these values, DELOS-susp provides a more homogeneous path for the assembling of the lipids, leading to less disperse vesicular systems. This highlights the impact that the preparation route exerts not only on the particle size distribution and morphological uniformity of a vesicular formulation (cryoTEM images of Figure 2) but also in the vesicle to vesicle homogeneity regarding the supramolecular organization of the lipids in their membrane. The greater heterogeneity encountered for vesicles prepared by hydration could be explained by lipid demixing during film formation. As reported by Buboltz et al.,[12] those preparation methods involving a solvent-free state, such as a lipid film, may favor demixing of membrane components and therefore a heterogeneous formation of the individual vesicles in an ensemble. In the case of Chol/CTAB (1/1 molar %) mixtures lipid demixing and formation of cholesterol-rich domains are probably promoted during film formation, explaining the large degree of compositional inhomogenity (*DI* = 1.08) achieved by the conventional hydration preparation route. In contrast, vesicles of the same composition prepared by DELOS-susp, which avoids any intermediary solvent-free state, showed higher compositional homogeneity. To confirm this hypothesis, a fully miscible lipid mixture, Chol/DOPC (2/8 molar %), was chosen to prepare vesicles by hydration and DELOS-susp procedures. Using the same dyes as molecular probes, the compositional homogeneity of samples, prepared by both methods, was analyzed following the same methodology. Identical *DI* values were encountered

for both preparation routes (*DIs* of 0.30 ± 0.04 and 0.30 ± 0.01 for DELOS-susp and hydration respectively). Whereas by DELOS-susp similar *DI* values were recorded for both lipid mixtures, a dramatic decrease (1.08 in Chol/CTAB vs 0.30 in Chol/DOPC) was found for the hydration method. Though more experiments may be required to fully elucidate the mechanistic explanation of these results, our findings generally support that the compositional heterogeneity for lipid compositions that can suffer phase separation may be amplified by preparation routes involving an intermediate solvent-free state such as in the case of film hydration methods. Since many lipid mixtures may suffer from demixing, methods like DELOS-susp that maintains lipids in solution during vesicle preparation would be a safer choice for achieving systems with superior homogeneity.

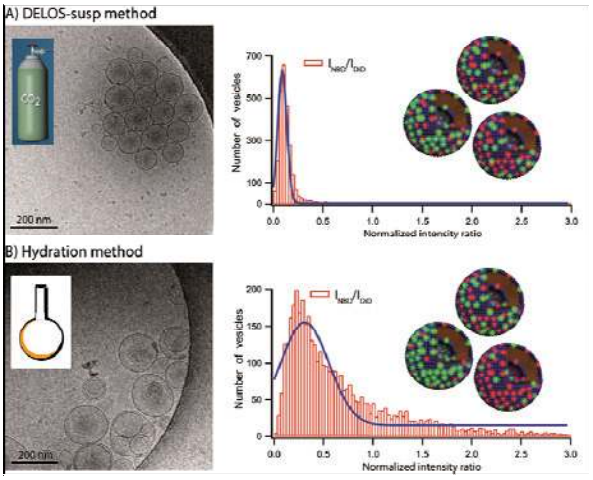

Figure 3. Cryo-TEM images and I_{NBD}/I_{DiD} histograms of Chol/CTAB (1/1 molar %) vesicular samples prepared by DELOS-susp (A) and the hydration method (B). A schematic representation of vesicles with different heterogeneities is also depicted.

3 CONCLUSION

The results reported reinforced the idea of membrane heterogeneity between individual vesicles within the same ensemble, demonstrated the influence of the preparation route on the assembly of lipids as vesicles, and showed the potential of CF-based methods for providing more homogeneous non-crystalline ordered materials, which is primordial in the field of drug delivery and nanomedicine.[13, 14]

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