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Evaluation of the effects of hydrophilic probes on membrane permeability and stability

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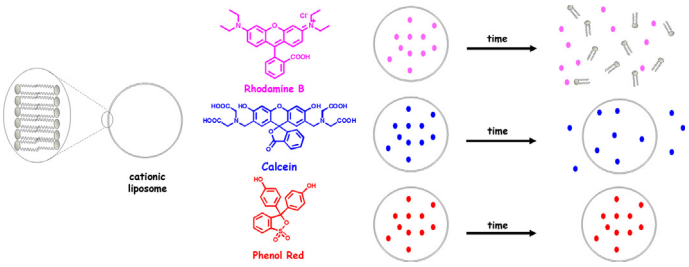
HIGHLIGHTS

- Physico-chemical properties of liposomes containing hydrophilic dyes were studied.
- Molecular structure of the probe influences liposomes dimensions and stability.
- Liposome composition affects their properties in the presence of the dye.

GRAPHICAL ABSTRACT



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ABSTRACT

The investigation of certain features of liposomes such as their lipid bilayer permeability or their propensity to fuse, quite often relies on the entrapment in the internal aqueous compartment of hydrophilic molecules whose optical properties change when they are released into the bulk. However, though defined as probes, these molecules bear functional groups that can interact with the liposome components thus having the potential of affecting the properties of the aggregate. To the best of our knowledge, this issue is scarcely hashed in the literature, despite its importance for an appropriate use of hydrophilic probes in liposome characterization.

The influence of calcein, rhodamine B and phenol red, three hydrophilic dyes generally employed in liposome characterization, on the features of some cationic liposomes was investigated by dynamic laser light scattering, fluorescence and absorption measurements. The presence of the hydrophilic dye in the internal aqueous compartment of liposomes strongly affects liposome stability, dimension and permeability depending on the molecular structure of probe and lipid components.

1. Introduction

Liposomes are vesicles formed by lipid bilayers that entrap an internal aqueous compartment whose content and pH condition might be different from the bulk. Many types of liposomes have been prepared using a wide array of natural and synthetic amphiphiles and have been employed as models of biological

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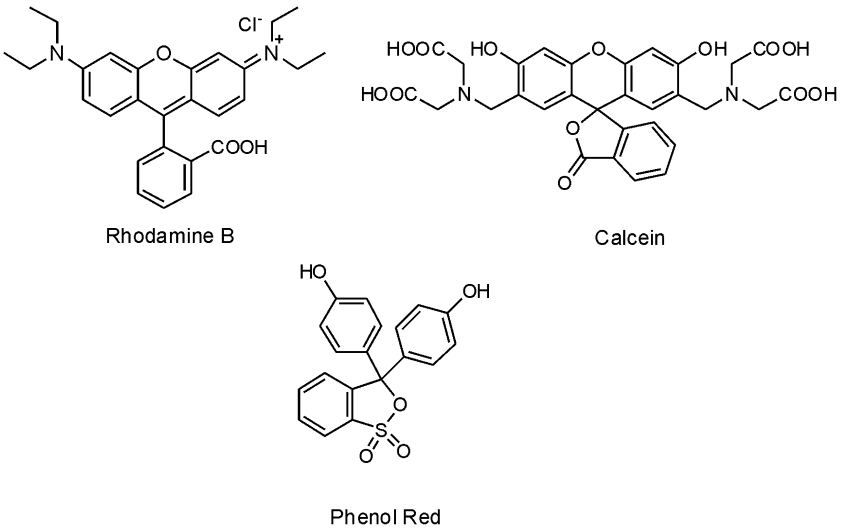

Fig. 1. Hydrophilic dyes.

membranes, as delivery systems in various pharmaceutical and cosmetic formulations, as sensor elements and in agro-food industry [1].

Loading of liposomes with a proper dye in the internal aqueous compartment has been largely exploited to evaluate the permeability of lipid bilayers [2], the leakage of liposomes content induced by the interaction with cells [3] and liposome-cell fusion [4]. However, although many of these hydrophilic dyes, defined as "probes" [5–7] are currently employed in liposome characterization, very little is known about their intrinsic influence on the investigated properties of liposomes. In fact, to best of our knowledge, only one paper reports on the destabilizing effect of multivalent anions on lipid bilayer of cationic liposomes [8]. On the other hand, calcein (Cal), one of the most used anionic fluorescent probe in liposome investigations, stabilizes negatively charged vesicles provoking a sealing effect that is complemented by the addition of cholesterol (Chol) exactly as observed in neutral and cationic vesicles [9].

Herein, we report on the investigation carried out to study the effect of three largely employed hydrophilic dyes, namely calcein, rhodamine B (RB) and phenol red (PR) (Fig. 1), on the physicochemical properties of liposomes composed of a natural phospholipid, either 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), in the presence and in the absence of Chol and/or of different amounts of the cationic saturated surfactant, **SS**, namely (1S,2S)-*N*-hexadecyl-*N*-methylprolinolinium bromide, or of the cationic unsaturated surfactant allyldimethylhexadecylammonium iodide, **US**, (Fig. 2). The cationic formulations used for this study were selected because under investigation in our laboratory as drug delivery systems. The two cationic surfactants differ for the presence/absence of an insaturation close to the differently sized polar head groups. Moreover, differently from phospholipids, they bear a single hydrophobic tail and are supposed to affect the organization of phospholipid bilayer. Thus they allowed us to investigate the influence on liposome permeability and stability both of the positive charge and of the molecular structure of liposome components.

Cal and RB are fluorescent when are in dilute conditions whereas their emission is self-quenched at high concentration [10,11]. These probes are characterized by water solubility and stability at

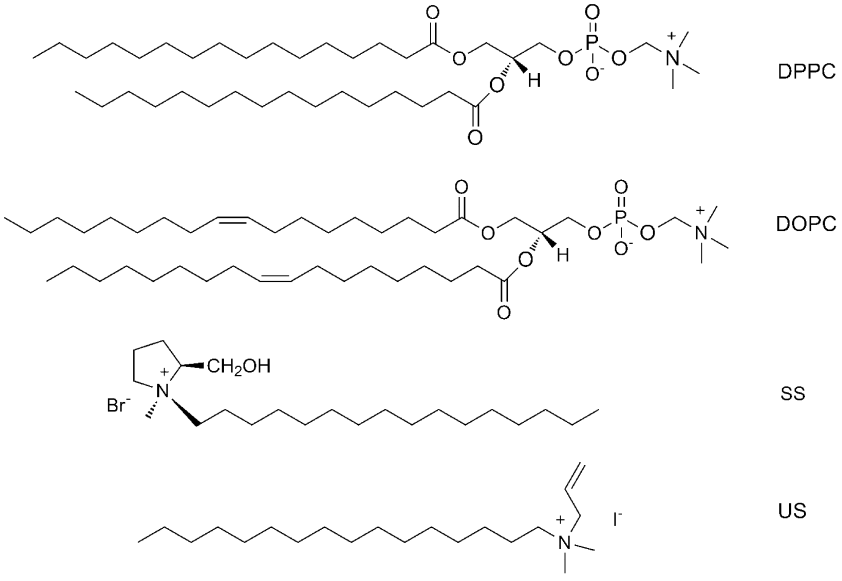

Fig. 2. Liposome component (DOPC and non DMPC, **SS** and **US**).