Colloids and Surfaces A: Physicochem. Eng. Aspects 482 (2015) 662–669

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Zwitterionic betaine-cholesterol system: Effects of sonication duration and aging on vesicles stability

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HIGHLIGHTS

- Vesicles (42–179 nm) formed from zwitterionic betaine surfactant/cholesterol system.
- At C_{tot} = 65 mM with 3:1 mol% of betaine/cholesterol the vesicles are stable for 8 months.
- 20–25 mol% mixing ratio of cholesterol minimized the vesicles size diameter.
- Long sonication with aging effects reduces vesicles size within a period of 6 weeks.

GRAPHICAL ABSTRACT

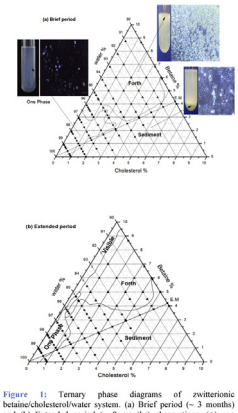

Figure 1: Ternary phase diagrams of zwitterionic betaine/cholesterol/water system. (a) Brief period (~ 3 months) and (b) Extended period (~ 8 months) observations. (♦) one istropic-phase, (▲) froth seeparation, (■) sediment separation, and (*) visible isotropic-phase. Insets repersent birefringent visual representations of light microscopy.

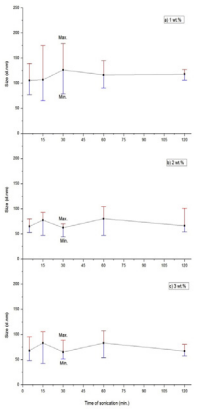

Figure 2: The mean diameter of vesicles formed at 3:1 mol % zwitterionic betaine/cholesterol and at different total concentration as a function of sonication time, with size fluctuations over a 6- week period indicated.

ARTICLE INFO

Article history:
Received 8 May 2015
Received in revised form 1 July 2015
Accepted 3 July 2015
Available online 11 July 2015

Keywords:
Vesicles
Zwitterionic surfactant
Cholesterol
Ternary phase diagram
Dynamic light scattering
Negative-staining TEM

ABSTRACT

Nanovesicle structures are an important class of nanoparticle self-assembly offering potential drug delivery applications depending on vesicle shape, size, and stability. The influence of the sonication time and aging effects on size distribution and stability of the vesicles formed in aqueous mixtures of the Zwitterionic surfactant betaine and cholesterol was studied using ternary phase diagrams, negative-staining transmission electron microscopy, and dynamic light scattering. The vast majority of our results reveal assembly of vesicles displaying spherical morphology with diameters varying between 42 and 179 nm during the 42 days of aging period at room temperature. Furthermore, analysis of vesicle structure and conformation following sonication indicate conservation of structural stability without using any stabilizing additives.

1. Introduction

In the last decade, vesicles have attracted considerable attention due to their wide range of applications, including biomimetics, targeted drug delivery, and other nanomedical applications [1–4]. Vesicles are closed bilayers with unilamellar or multilamellar

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http://dx.doi.org/10.1016/j.colsurfa.2015.07.009
0927-7757/

structures enclosing a core of aqueous solution [5]. Similarities in structural composition between vesicles and cellular membranes afford vesicles the ability to encapsulate and transport both hydrophobic and hydrophilic molecules [6].

Liposomes are artificially-created vesicles consisting of bilayers that may contain combinations of phospholipids and sterols, such as cholesterol [7]. Effective utilization in roles including drug delivery and tumor therapy depend on vesicle size and stability [8]. Liposomes with diameters of 100–300 nm are suitable for targeted drug delivery, capable of targeting specific tissue types and exhibiting prolonged retention in the blood stream [9,10]. The metastable structure of liposomes [11,12] increases susceptibility to aggregation, fusion, or content leakage during storage in aqueous suspensions [13,14]. Of increasing interest are methods allowing creation of non-lipid vesicles in order to enhance liposomal stability [1,15]. Combinations of surfactant mixtures [16–20] have been used to initiate assembly and increase the thermodynamic stability of vesicles under different environmental conditions, including aging time, temperature, pH, and pressure [20–23].

Non-lipid vesicle bilayers formation can occur by employing aqueous mixtures of ionic, non-ionic and mostly zwitterionic surfactants with cholesterol [24–29]. Zwitterionic surfactants have unique properties as neutral head group and charge orientation, allowing them to mimic phospholipids in biological membranes (mimetic chemistry) [1,19]. Cholesterol is among the most important lipid species found in animal cell membranes, exhibiting concentrations ranging from 20 to 50 mol% in red blood cells [30], and regulating rigidity and fluidity of membranes phase behavior [31,32]. Although cholesterol alone cannot initiate bilayers formation, interaction with surfactants containing a hydrophobic moiety of a single alkyl chain can result in the assembly of vesicles exhibiting structural characteristics similar to those observed in liposomes [27]. In general cholesterol have a complex behavior and incorporation of cholesterol into small unilamellar liposomes, increases the dispersion stability with respect to aggregation and fusion [33,34]. Vesicle stability and the relatively low cost of associated materials make them desirable for nanomedical applications [35].

Vesicles formation initiated from interactions between single tail zwitterionic surfactants with cholesterol have not been studied to the same extent as other classes of surfactants. Furthermore, the interaction due to the effect of process factors such as sonication duration in the presence of cholesterol is still obscure. Here we report data associated with vesicle assembly in an aqueous mixture consisting of different ratios of the zwitterionic surfactant betaine and cholesterol. Furthermore, we investigated variations in vesicle size and stability following sonication and aging effects through the use of ternary phase diagrams, negative-staining transmission electron microscopy (TEM), and dynamic light scattering (DLS). The vast majority of our results reveal assembly of vesicles displaying spherical morphology and capable of retaining structural stability in the absence of stabilizing additives.

2. Experimental

2.1. Materials

N-(Alkyl C_{10}-C_{16})-N,N-dimethylglycine betaine (EMPIGEN® BB detergent) with 35% active substance in H_2O and 5-cholesten-3β-ol (Cholesterol, purity ≥99%) were purchased from Sigma–Aldrich, USA and used as received without any further purification. All experiments were conducted with pure deionized water that was passed through a Milli-Q Plus purification system with a resistivity of 18.2 MΩ cm.

2.2. Vesicles preparation

Vesicles were prepared by mixing stock micellar solution of zwitterionic betaine surfactant (35% active substance in H_2O) with cholesterol at desired total concentration and molar ratios with deionized water. The appropriate amounts were weighed directly into glass tubes and water was then added. The mixtures were heated for 45 min at 60 °C prior to vortex mixing for 2 min, followed by overnight storage at room temperature. The mixtures then underwent sonication at 60 °C for 30 min to produce vesicle dispersion by using continuous bath sonication (Wiseclean Ultrasonic Cleaner WUC-A03H, 40 kHz and 124 W). The homogenous dispersions were then centrifuged at 4000 rpm for 30 min to detect phase separation by using rotating centrifuge mode (Universal 320). Before analysis, the dispersed samples were not subject to mechanical agitation and were equilibrated at room temperature (~25 °C).

2.3. Phase diagrams

Sealed samples containing the desired total concentrations and molar ratios of zwitterionic betaine/cholesterol were prepared (as mentioned above) and stored in a thermostat cabinet at 25 °C for equilibration. Phases were identified periodically by visual observation with and without cross polarizer's. Birefringent lamellar textures were characterized by a polarizing microscopy (Leica DM750-ICC50 HD).

2.4. Negative staining TEM

Vesicles were visualized using negative-staining TEM. A drop of vesicles dispersion was adsorbed onto a 200-mesh copper grid coated with a formvar film and allowed to adhere following removal of excess liquid. After 5 min, a few drops of 3% uranyl acetate solution was added to grid and left for 10 s prior to removal of excess solution The grid was then washed three times using distilled water, the sample air dried, and then visualized using a transmission electron microscope operated at 120 kV (Tecnai Biotwin, Netherland).

2.5. Dynamic light scatting (DLS) measurements

The size distributions of each sample were measured using a Zetasizer instrument (Nano-ZS, Malvern instruments, UK) operated with a 4 mW He–Ne ion laser. Zetasizer Software version 7.03 was used to analyze the data as follows: the intensity fluctuation and relaxation rate, Γ, were analyzed by the cumulant method with multiple narrow modes to obtain the mean translational diffusion coefficient, D, ($D = \Gamma/q^2$), where q is the scattering vector written as,

$$q = \left(\frac{4\pi\eta_s}{\lambda}\right) \sin\left(\frac{\theta}{2}\right) \quad (1)$$

where η_s is the refractive index of the dispersed samples previously measured for each sample by using (Pocket refractometer PAL-1), λ is the wavelength of radiation in vacuum (λ = 633 nm), and θ is the scattering angle ($\theta = 173^\circ$). The Stokes–Einstein equation adequately describes the relationship between the average hydrodynamic radius, R_h, of the particles and the diffusion coefficient, D, written as

$$q = \left(\frac{4\pi\eta_s}{\lambda}\right) \sin\left(\frac{\theta}{2}\right) \quad D = K_B T/(6\pi\eta R_h) \quad (2)$$