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The calorimetric properties of liposomes determine the morphology of dried droplets

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ABSTRACT

The evaporation of liquid droplets deposited on a substrate is a very complex phenomenon. Driven by capillary and Marangoni flows, particle–particle and particle–substrate interactions, the deposits they leave are vestiges of such complexity. We study the formation of patterns during the evaporation of liposome suspension droplets deposited on a hydrophobic substrate at different temperatures. We observed that as we change the temperature of the substrate, a morphological phase transition occurs at a given temperature T_m. This temperature corresponds to the gel-fluid lipid melting transition of the liposome suspension. Optical microscopy and atomic force microscopy are used to study the morphology of the patterns. Based on the radial density profiles we found that all structures can be classified into two groups: patterns composed by nearly uniform deposition (below T_m) and prominent structures containing randomly distributed voids (above T_m).

1. Introduction

Understanding how a droplet containing colloidal particles dries and deposits onto a surface is relevant in processes like inkjet printing [1–4], medical tests [5–9], DNA/RNA recognition [10–14], among many others. During the evaporation of a droplet of this kind, there is an intricate competition between capillary flows (driven by continuity) and Marangoni flows (driven by surface tension gradients) [15]. Capillary flows emerge when the evaporation occurs at the base edge of the droplet; the fluid moves radially outwards to compensate the evaporated mass. Moreover, the droplet remains with a constant diameter and the contact angle decreases due to the deposition of particles at the contact line. This generates the commonly seen "coffee ring effect"; a ring pattern formed at the edges of the droplet [16–18]. Marangoni flows, on the other hand, produced by surfactants and temperature gradients, can be considered as the opposite of capillary flows. They force the fluid to circulate inwards, and this halts the coffee ring effect. Indeed, when surfactants are present surface tension gradients promote the uniform deposition on the surface contact due to the formation of eddies, vortexes, recirculating flows and radially inward flows [18–21]. In general, surface tension decreases with the concentration of surfactant.

Marangoni flows could be also modified by the surface temperature distribution present in the liquid droplets, but there are two different points of view on how this may occur. One suggests that the edge of the droplet is colder than its apex due to greater evaporation in this region [22]. The other point of view proposes that the top of the droplet is at a lower temperature due to the distance from the substrate, which involves greater conduction [15,23]. Xuefeng Xu and Jianbin Luo [24] reported a stagnation point where the surface tension, the surface temperature gradient, and the surface flow change no-monotonically along the droplet surface [24].

The phenomenon described above is also affected by particle–particle [25] and particle–substrate interactions [26,27]. These interactions play a crucial role in biological fluids due to the fact they contain electrolytes, amino acids, proteins, or even bacterial populations which affect transport mechanisms and aggregation phenomena. Although such biofluids show a complex behavior, several works claim that the patterns they form are similar from the ones observed during the evaporation of simple colloidal suspensions [5–9,28]. Hence, the study of the evaporation of simple biofluids is a good starting point to fully understand how complex biological fluids dry.

Since lipid membranes are essential for the function of a cell, hundreds of studies have been carried out to understand how lipids self-assemble. This self-organization depends, among other things, on temperature, composition of lipids, and external agents. A key element to characterize lipid vesicles is the phase transition temperature T_m. This is the temperature at which the gel and the fluid

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phase coexist. Within the context above described, the only work that studies how a liposome suspension dries is, as far as we know, the one carried out by Dana et al. [29]. With the aim of exploring anhydrobiotic preservation, they studied pattern formations during the evaporation of a droplet containing liposomes (1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC) and trehalose. The authors found that the surface and liposome concentration are two factors that strongly influence crack formation, external ring, and central liposome accumulation.

In this paper, we report how droplets made of liposome suspensions dry at different temperatures. Optical microscopy and atomic force microscopy (AFM) are used to study the morphology of the deposits. Interestingly, we found a morphological transition. Below and above this transition, the structures can be classified into two groups: patterns composed of a nearly uniform deposition and prominent structures containing randomly distributed voids. This morphological transition correlates with the calorimetric gel-fluid phase transition of the liposome suspension.

2. Materials and methods

2.1. Liposomes preparation

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) sodium salt (DPPG) were obtained from Avanti Polar Lipids and used without further purification. Suspensions of multilamellar vesicles (MLV) of DPPC at 4 mM were prepared by hydration of powder lipid above their melting transition (55 °C) with deionised water (18.2 MΩ). The dispersion was stirred at 1000 rpm for 30 min at 55 °C using a Degassing Station (TA Instruments). To prepare suspensions of lipid mixtures of DPPC:DPPG (4 mM, 2:1 molar ratio), each lipid was dissolved in methanol:chloroform 1:1 (v/v). The organic solutions were mixed, softly vortexed and homogenized. The solvents were evaporated by rotatory evaporation and the lipid film was kept in a vacuum chamber for 3 h to eliminate solvent traces. Finally, the lipid film was hydrated with deionised water at 55 °C, stirring at 1000 rpm for 30 min in the Degassing Station.

2.2. Drop evaporation

Ten small droplets of each suspension were deposited on a hydrophobic substrate of polydimethylsiloxane (PDMS) under controlled temperature and humidity conditions (30–47 °C and 40%, respectively). This number of drops allows us to check for reproducibility. The volume of the deposited droplets is (2.5 μl). Once the drying process ends, pictures of the final structures are taken using a digital camera with a resolution of 1200 × 600 pixels (on an area of 22.3 mm × 14.9 mm) coupled to an optical microscope. These are saved in a computer disk for later analysis. Details for the above mentioned conditions are the following: (1) A hydrophobic surface was used to improve drop deposition and reduce wetting. (2) Since our aim was to study the morphology of the patterns left by the droplets through the phase transitions of the liposomes, temperature was the only parameter to change. Using calcium chloride in the evaporation cell we maintained a constant humidity of 40%. According to the literature, in a complete dried environment the evaporation is much faster [30]. Since the humidity in our experiments was intermediate between 0% and 100%, we consider that as long as the temperature crosses the transition the patterns would be similar below/above 40%. (3) Normally, the droplets take less than 300 s for drying, but we wait another 600 s to assuring a complete dehydration.

2.3. Image analysis

We use the radial density profile $I(r)$ to carry out the structural analysis of the patterns. This quantity has the ability to describe a profile of integrated intensities produced by concentric circles as a function of radial distance. For 2D objects this quantity is given by the following expression:

$$I(r) = \frac{1}{2\pi}\int_0^{2\pi} i(r,\theta)d\theta, \quad (1)$$

where $i(r, \theta)$ is the local light intensity contained in a circle of radius r. Each value of $I(r)$ represents the sum of the pixel intensities around a circle with radius r.

2.4. Calorimetric measurements

Profiles of molar heat capacity (C_p) as a function of temperature were measured in a microcalorimeter NanoDSC (TA Instruments). The calorimetric samples were degassed at low pressure (635 mmHg) for 10 min at 25 °C and equilibrated for 5 min in the DSC cell at the same temperature. Each sample was scanned in a heating mode three times at a constant pressure (3 atm) and constant scan rate of 1 °C/min. Due to the high reproducibility, the measurements were repeated only two times. The acquisition and analysis of data were done using the software provided with the equipment.

2.5. Atomic force microscopy

Atomic force microscopy images were obtained with a microscope (Innova Bruker, Santa Barbara, CA) equipped with a large area scanner. Imaging was performed in tapping mode with settings of 1024 pixels/line, and scanning rate of 0.5 Hz using antimony doped Si cantilevers (Bruker, Santa Barbara, CA) with nominal tip radius of curvature of 2 nm, nominal spring constant of 40 N/m and a nominal resonance frequency of 300 Hz. Experiments were recorded in air at room temperature (24 °C). The force applied to the sample was adjusted to the lowest possible value. All the AFM data were converted to PNG files using the Gwyddion 2.38 software package. Two different areas were analyzed for each sample.

3. Results and discussion

3.1. Morphology vs temperature

We show in Fig. 1a the remnants of the DPPC suspension droplets once the drying process ends. It is clear that the morphology of the dried droplets presents a transition at a temperature (T_m) between 42 and 43 °C. When the droplets are deposited below T_m, the patterns are quite similar. Let us classify them in group 1. Conversely, above T_m, the patterns suddenly change and thus we classified them into group 2. The order parameter of such transition is the radius r of the patterns. Fig. 1b shows the relative radius $R = r/r_m$ as a function of temperature; where r_m is the radius at T_m. The data follow a sigmoid curve. The derivative of this curve gives us more precise information about the transition. Moreover, note the similarity between this derivative and the calorimetric profile of the suspended DPPC liposomes in the droplet. The transition peaks are at 42.7 and 41.9 °C, respectively (see Fig. 1c). This implies that the thermotropic properties of the suspended vesicles, with a melting transition occurring at T_m, are conserved during the droplet evaporation, determining the final morphologies.

The above results were obtained with liposomes made by only one type of lipid, DPPC. Now, we combine two: DPPC and DPPG. Fig. 2a shows the corresponding sequence of patterns obtained with