

# MEMBRANES AND VESICLES

## Morphologies of Vesicles Loaded with Aqueous Polymer Solution



When a water drop is placed on a surface, it can attain the shape of a spherical cap, or it can flatten and spread. The interaction between the surface and the drop can be characterized by the contact angle. Zero or nonzero contact angle correspond to complete or partial wetting, respectively. Varying parameters such as temperature or liquid composition, one can cause the system to undergo a

transition from complete to partial wetting. Examples for such transitions are not abundant. Some have been found for fluid-fluid interfaces in binary mixtures and for liquids at solid substrates. Liquid droplets at chemically patterned or topographically structured surfaces can also undergo morphological wetting transition, which reflects the freedom of the contact angles at pinned contact lines.

Recently, we discovered a complete to partial wetting transition occurring for an aqueous solution enclosed within a freely suspended lipid vesicle [1]. Here the substrate is a lipid membrane, permeable to water and with the thickness of a few nanometers.

Lipid vesicles have long been recognized as models for the cell membrane and have been widely used to study the properties of lipid membranes [2]. Recently, it has been found that giant unilamellar vesicles loaded with aqueous solutions of water-soluble polymers may exhibit several spatial compartments formed by phase separation within the vesicle interior. Thus, these artificial cell-like systems are a biomimetic setup for studying molecular crowding, fractionation and protein sorting in cells.

To study wetting transitions in vesicles, we encapsulated a homogeneous aqueous solution composed of poly(ethylene glycol) (PEG) and dextran in giant vesicles made of dioleoylphosphatidylcholine (96 mol %), and GM1 ganglioside (4 mol %). In order to obtain vesicles containing two phases, the vesicles were deflated osmotically by adding a hypertonic solution to the external medium in a stepwise manner.

### Partial-to-Complete Wetting Transition

The polymer solution loaded in the vesicles is in the one-phase state at room temperature (Fig. 1A). As the osmolarity of the external medium is increased, water is forced out of the vesicle in order to balance the resulting osmotic pressure. As a result, the polymer concentration inside the vesicle is raised and phase separation occurs (Fig. 1B). Since the dextran-rich phase is heavier than the PEG-rich phase, the newly formed spherical dextran-rich droplet is always located at the bottom of the vesicle (Fig. 1C). As the osmolarity of the external medium is further increased, the dextran-rich phase starts to wet the membrane (Fig. 1D). The contact area between the dextran-rich phase and the membrane grows with increasing osmolarity; see Fig. 1D-F. The morphology change of the dextran-rich droplet indicates a wetting transition from complete wetting of the PEG-rich phase or complete dewetting of the dextran-rich phase in Fig. 1B, C to partial wetting in Fig. 1D-F.

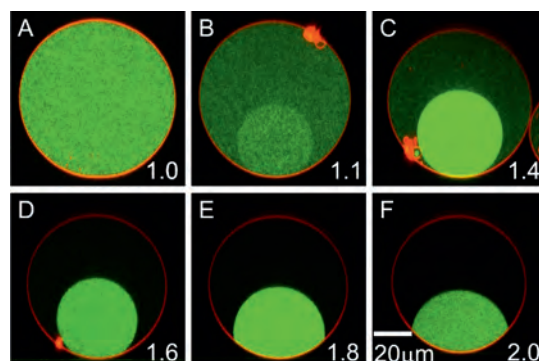


Fig. 1: Confocal micrographs of a vesicle (vertical cross sections), encapsulating polymer solution with of 4.05wt% PEG,  $M_w=8$  kg/mol, and 2.22wt% dextran,  $M_w=400-500$  kg/mol. 0.52wt % of the total dextran is labeled with fluorescein isothiocyanate (green). The membrane is labeled with 0.1 mol % dipalmitoylphosphatidylcholine-rhodamine (red). Initially, the polymer solution inside the vesicle is in the one-phase state (A). The vesicle is subjected to hypotonic solution and deflates inducing phase separation (B, C). Upon further deflation, the dextran-rich drop (green) undergoes wetting transition (D-F). The numbers on the snapshots indicate the osmolarity ratio between the external medium and the initial internal polymer solution. The system was left to equilibrate for at least 2 hours after each consecutive osmolarity change.

The overall vesicle shape seems to remain spherical during the deflation steps. The volume of the vesicle decreases with increasing osmolarity. The excess membrane area gained in this way forms a cluster of interconnected small vesicles and lipid aggregates partially visible in Fig. 1B, C, D.

Fitting the vesicle and the drop contours in the acquired images with spherical caps, allows us to obtain the vesicle volume under different osmolarity conditions. Because the membrane is not permeable to the polymers, the number of polymer molecules inside the vesicle is fixed and the decrease of vesicle volume is due to the loss of water. Thus, we can calculate the total polymer concentration in the vesicle at different osmolarities. In addition, the vesicle geometry allows us to measure the contact angle  $\Theta$ , between the dextran-rich phase and the membrane (see inset in Fig. 2). The cosine of the contact angle  $\Theta$ , defines the wettability via  $\cos(\Theta) \equiv (\Sigma_{pm} - \Sigma_{dm}) / \Sigma_{pd}$ , where  $\Sigma_{pm}$ ,  $\Sigma_{dm}$  and  $\Sigma_{pd}$  are the interfacial tensions at the interfaces between the PEG-rich phase and the membrane (pm), the dextran-rich phase and the membrane (dm), and the PEG-rich phase and the dextran-rich phase (pd). The wettability as a function of the total polymer concentration inside the vesicle is given in Fig. 2. A sharp change in the contact angle is observed for polymer concentration 8.5wt %, indicating a wetting transition.

After this transition point, the wettability of the dextran-rich phase increases with the polymer concentration as shown in Fig. 2.

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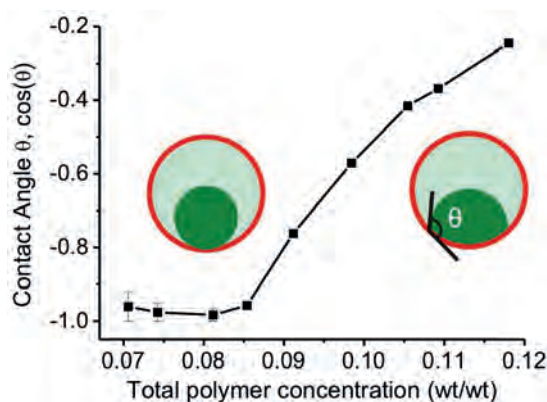


Fig. 2: The cosine of the contact angle  $\theta$  (see the right inset for definition) versus the total polymer concentration in the vesicle. The weight ratio between dextran and PEG is 0.55. The insets schematically illustrate the dewetted and wetted states.

We consider a possible mechanism involved in the observed wetting transition. When the polymer solution is close to the mixing point, the composition difference between the phases is very small, which leads to extremely low interfacial tension  $\Sigma_{pd}$ . When the latter is smaller than  $|\Sigma_{pm} - \Sigma_{dm}|$ , the membrane is fully wetted by the PEG-rich phase. Both  $\Sigma_{pd}$  and  $|\Sigma_{pm} - \Sigma_{dm}|$  increase with increasing polymer concentration, but  $\Sigma_{pd}$  increases faster than  $|\Sigma_{pm} - \Sigma_{dm}|$  because the compositions of both phases change, but the composition of the membrane does not. When  $\Sigma_{pd} = |\Sigma_{pm} - \Sigma_{dm}|$ , the wetting transition occurs, and the dextran-rich phase starts to wet the membrane. Vesicle simulations based on dissipative particle dynamics may offer a possible way to reveal the order of this wetting transition. Work in this direction is in progress.

### Wetting-Induced Budding

When both phases wet the membrane, the smaller one may bud out of the vesicle body upon further deflation. **Fig. 3** shows such an example. The vesicle with two liquid phases is approximately spherical at low osmolarity ratio between the external medium and the initial internal polymer solution; see **Fig. 3B**. When the vesicle is further dehydrated, the dextran-rich phase starts to form a bud away from the PEG-rich phase; see **Fig. 3C**. The excess area arising from dehydration is utilized by the vesicle to undergo morphological changes. In this way, the area of the liquid two-phase interface is decreased significantly. As the osmolarity of the medium is increased further, the dextran-rich phase may form a complete bud leading to a dumbbell-like vesicle where the area of the two-phase interface is almost zero.

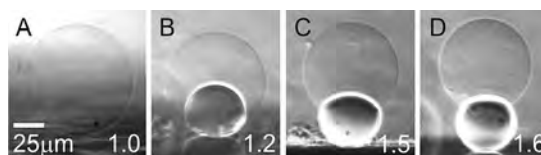


Fig. 3: Side-view phase contrast images of a vesicle sitting on a glass substrate. After phase separation (A, B), further deflation causes the dextran-rich phase to bud out (C, D). The dense part at the lower part of the vesicle is the dextran-rich phase. The light part is the PEG-rich phase. Note that the dextran was not fluorescently labeled in this vesicle as in Fig. 1. The numbers on the snapshots indicate the osmolarity ratio between the external medium and the initial internal polymer solution. The system was left to equilibrate for at least 2 hours after each consecutive osmolarity change.

The presence of the interfacial tension  $\Sigma_{pd}$  causes a pulling force on the membrane towards the vesicle interior. When  $\Sigma_{pd}$  is small, the membrane tension can easily balance this pulling in the normal direction. The excess area arising from dehydration can be stored in the form of lipid aggregates or tethers [3, 4], and the vesicle can remain spherical. As  $\Sigma_{pd}$  increases and the vesicle deflates further (creating more excess area), the membrane tension can no longer sustain the spherical vesicle shape. Because the membrane is very flexible it bends along the interface of the liquid phases and budding of the dextran-rich phase occurs as the vesicle is further deflated. The budding event significantly reduces the interfacial energy by decreasing the contact area between the liquid phases.

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