Vesicles as osmotically stressed capsules

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Vesicular capsules are used to carry biochemicals in biology and liposome technology. Being water-permeable with differing interior and exterior compositions, they are necessarily under osmotic stress. Recent studies have underlined the different thermodynamic behavior of osmotically stressed vesicles in comparison to vesicles subjected to a hydrostatic pressure as studied earlier. Through their different behavior one gains access to the parameters affecting the osmotic swelling of vesicles, such as the membrane-permeability coefficients of solute molecules.

INTRODUCTION

Bilayer vesicles serve as capsules for delivery of various molecules as part of the biological cell function and in pharmaceutical and cosmetic applications. This utility relies on the potential barrier posed by the hydrophobic core of the bilayer for the penetration of water-soluble molecules. The sensitivity of molecular transport to the barrier height makes the membrane-permeability coefficients of different solutes span many orders of magnitude — from values of order $10^{-2}$ m/s for water down to a mere $10^{-8}$ m/s for potassium ions, for example. Thus, the membrane of a vesicle of micron size or smaller, over time scales longer than about $10^{-2}$ s, behaves as a semi-permeable partition, allowing solvent to be exchanged between the interior and exterior while keeping certain solute molecules either enclosed inside or locked outside. This, in turn, allows for the buildup of an osmotic pressure difference across the membrane.

We open with a very basic question: What are the thermodynamic constraints imposed in practice on such a vesicular capsule? The surrounding solution dictates the temperature $T$, external pressure $p_{\text{out}}$, and chemical potential $\mu_{\text{w}}$ of the solvent (water). Over the time scales under consideration the numbers $\{Q_i\}$ of the encapsulated solute molecules are fixed as well. On the other hand, since solvent is exchanged across the membrane, the values of neither the encapsulated volume $V$ nor the inner pressure $p_{\text{in}}$ are a priori set. In addition, there are surface constraints associated with the membrane, such as the number $N_s$ of amphiphilic molecules making the bilayer (or, alternatively, their chemical potential $\mu_s$). These two-dimensional variables raise various subtle issues, which nonetheless do not concern us here; we simply represent them all by a single symbol, $\sigma$. The set of thermodynamic constraints imposed on the capsule, therefore, is $(T, p_{\text{out}}, \mu_{\text{w}}, \{Q_i\}, \sigma)$.

Theoretically, vesicles have been studied over the years under different sets of constraints, such as $(T, V, \sigma)$ or $(T, \Delta p = p_{\text{in}} - p_{\text{out}}, \sigma)$. The former will hold in practice when the solvent does not have sufficient time to be exchanged across the membrane, i.e., over sufficiently short time scales (shorter than $\sim 10^{-2}$ s). The latter corresponds, for example, to micropipette aspiration experiments, where the hydrostatic pressure difference across the membrane is controlled.

The key point that we wish to highlight here is that the behaviors of vesicles under these different sets of thermodynamic constraints are not necessarily equivalent. This has been demonstrated in a series of recent studies. We are used to the fact that the distinction between different sets of thermodynamic constraints (equivalently, different statistical ensembles) is not important for large systems at equilibrium. Why should it matter much whether a certain pressure difference is externally imposed by a pump or self-attained as an average equilibrium value due to osmosis? This broadly valid statement assumes, however, that surface effects and fluctuations are negligible. For fluid vesicles, which often strongly fluctuate and may introduce strong surface effects, the validity of this assumption is not self-evident. It should be stressed that the distinction between sets of constraints is not merely a theoretical curiosity. The explicit treatment of the encapsulated solution has in certain cases important and useful implications. We choose to postpone the discussion of these to the end of this Highlight piece and discuss first two more artificial cases, which nevertheless serve well to demonstrate the point.

OSMOTIC SWELLING OF MODEL CAPSULES

Arguably the simplest model for a fluctuating closed envelope is a two-dimensional (2D) ring made of a fixed number $N_s$ of freely-jointed segments at temperature $T$. (Such systems are actually realizable experimentally.) When we control and increase the 2D pressure difference $\Delta p$ between the inner and outer regions, the mean volume enclosed by the envelope (i.e., the area of the ring) increases. For self-intersecting rings this swelling encounters a criticality — at a critical pressure, $\Delta p = \Delta p_c \propto N_s^{-1}$, the mean volume either diverges (if the segments are taken to be extensible harmonic springs) or exhibits a second-order transition between crumpled and smooth states (if the segments are...
made inextensible. However, when we let the ring swell due to an increasing number \( Q \) of enclosed particles rather than impose a pressure difference, the criticality disappears and the mean volume increases gradually with \( Q \).

A similar clear-cut example of a qualitatively different behavior of particle-encapsulating envelopes is found in a discrete model of three-dimensional (3D) fluid vesicles. In this model, due to Gompper and Kroll, the vesicle is represented by a closed triangulated network of \( N_s \) self-avoiding nodes whose connectivity is random and variable. When the pressure difference \( \Delta p \) is controlled and increased, the vesicle undergoes a first-order transition at a certain critical pressure, \( \Delta p_c \propto N_s^{-1/2} \), between crumpled and smooth states. However, if the vesicle is inflated instead by an increasing number \( Q \) of enclosed particles, the discontinuous transition disappears and is replaced by gradual swelling with \( Q \).

The way in which the criticality is removed in these two examples is quite unusual. When we control \( Q \), neither \( \Delta p \) nor \( V \) is fixed. The large flexibility of those two model capsules allows them to adjust their mean volume such that the mean pressure difference, determined by the mean particle concentration \( Q/V \) and \( T \), never hits \( \Delta p_c \) for any value of \( Q \), thus avoiding the transition. This is demonstrated in Fig. 1. Upon decreasing the number of enclosed particles the pressure inside the 3D vesicle first decreases but eventually stops changing with \( Q \) and never reaches arbitrarily low values. Thus, the two sets of constraints, \((T, \Delta p, N)\) and \((T, p_{\text{out}}, Q, N)\), are manifestly not equivalent in these examples — there are macrostates in the former that become inaccessible in the latter. (For a unified description of the swelling of random manifolds by either imposed pressure or enclosed particles, and the related issue of equivalence, see ref 10.)

![FIG. 1.](image)

**OSMOTIC SWELLING OF MEMBRANE VESICLES**

The two examples given in the preceding section pertain to random envelopes whose size is much larger than their bending persistence length. Actual bilayer vesicles belong to the opposite smooth limit, where the bending persistence length is larger than the vesicle. When real vesicles swell, they first undergo an “ironing” stage, where their volume-to-area ratio increases as they become increasingly more spherical. This is followed by a stretching stage, in which the membrane area \( A \) increases while the shape remains essentially spherical. When the surface strain exceeds a few percent, the membrane ruptures. In the case of osmotic swelling, the rupture (osmotic lysis) may be followed by a sequence of additional swelling–rupture cycles.

We focus here on the crossover between the ironing and stretching stages. We define an order parameter,

\[
M = 1 - 6\sqrt{\pi V/A^{3/2}},
\]

which measures how far the vesicle is from a sphere. Way before the crossover, when the vesicle is not swollen, \( M \) is appreciable, whereas well into the stretching stage, when the vesicle is nearly spherical, \( M \) approaches zero. It
was found out that, in the case of osmotic swelling, this crossover can be represented as a rounded continuous phase transition.\textsuperscript{11,12} Specifically, if a control parameter \( q \), dependent on the number of encapsulated particles, is defined as

\[ q = Q/Q_c - 1, \quad Q_c = p_{\text{out}} V_0/(k_B T), \quad V_0 = A_0^{3/2}/(6\sqrt{\pi}), \]

\( A_0 \) being the relaxed area of the vesicle, one finds the following critical scaling in the vicinity of the transition and beyond it:

\[ M = \Delta M(q/\Delta). \]

In eqn (3) \( \Delta \) is the width of the transition, and \( \Delta M(x) = (\sqrt{1 + x^2} - x)/2 \) is a scaling function. (The expressions given above for \( Q_c \) and \( M \) assume that the solution inside the vesicle is ideal; yet, the results can be generalized to an arbitrary equation of state for the encapsulated solution.\textsuperscript{14}

In the limit \( \Delta \to 0 \) the swelling curve \( M(q) \) has a singular corner at the point \( q = 0, M = 0 \). If membrane stretching is neglected (the area being fixed at \( A = A_0 \)), then \( \Delta \propto N_c^{-1/4} \), i.e., the rounding of the transition arises, as in any phase transition, from the finite size of the system.\textsuperscript{14} In this approximation there is no stretching stage, and the vesicle approaches its maximum volume \( V_0 \), the volume of a sphere of area \( A_0 \). When a finite stretching modulus \( K \) is included, the rounding of the transition arises from both finite size and finite stretchability (with \( \Delta \propto K^{-1/2} \) if the latter dominates).\textsuperscript{12}

The critical properties of the crossover disappear when the swelling is hydrostatic rather than osmotic, i.e., when the pressure difference is constrained instead of the number of enclosed solute molecules. Unlike the examples in the preceding section, in this case the same macrostates are encountered when sweeping through the values of either \( \Delta p > 0 \) or \( Q \); it is the sharpness of the corresponding changes in the order parameter that differs between the two sets of constraints. When the vesicle swells by osmosis, both the mean volume and mean pressure behave critically as a function of \( Q \), and we find \( \Delta p(q) \propto 1/M(q)\textsuperscript{12,13} \), the sharp approach to a spherical shape is accompanied by a similarly sharp increase in pressure difference and surface tension. Consequently, once transformed into pressure dependence, the order parameter decreases slowly with increasing pressure, \( M(\Delta p) \propto 1/\Delta p \).

The critical scaling has had two beneficial implications. One is theoretical — eqn (3) constitutes a law of corresponding states for the osmotic swelling of vesicles.\textsuperscript{14} It implies that the osmotic swelling curves of various vesicles under various conditions (e.g., due to the permeation of various solutes at various concentrations) can be collapsed in the vicinity of the transition (and above it) onto a single master curve, thus achieving a simple, unified theoretical description.

Osmotic swelling has been experimentally studied in detail using dynamic light scattering\textsuperscript{14} or optical tracking\textsuperscript{18} depending on vesicle size. In a typical procedure vesicles are formed in a solution of a non-permeating solute, in which they are free of osmotic stress. Subsequently, the exterior is replaced by a solution of equal concentration but containing a permeating solute. As the outer solute permeates inward, the vesicles swell through osmosis. Changes in the shape and size of the vesicle as it progresses from a stress-free state toward a strongly stretched sphere and the ultimate lysis are related to the corresponding decrease in the order parameter \( M \), eqn (1). The control parameter \( q \), eqn (2), is linearly related to the time axis via the membrane permeability coefficient, \( P \), of the permeating solute.

Thus, high-resolution optical tracking can be used to obtain swelling curves, \( M(q) \), and the associated data collapse.\textsuperscript{12,13} This is demonstrated in Fig. 3.

The other beneficial implication of the critical scaling has been the introduction of a new method to measure membrane-permeability coefficients of various solutes.\textsuperscript{12,13} Data collapse such as the one shown in Fig. 3 involves two fitting parameters: the transition width \( \Delta \), separately fitted for each curve, and the permeability coefficient \( P \), globally fitted for all curves and corresponding to a mere scaling of the horizontal (time) axis. This yields a sensitive and reliable measurement of \( P \). For example, from the data shown in Fig. 3 one finds for the permeation of urea through a POPC membrane \( P = 0.013 \pm 0.001 \mu m/s \). A similar procedure has yielded a significant concentration dependence of \( P \) for polyols (glycerol and ethylene glycol), which was not recognized before.\textsuperscript{12,13}

\section*{CONCLUSION}

The examples given above demonstrate how useful vesicular capsules may be for investigating fundamental issues of the thermodynamics of small systems, such as strong surface effects, fluctuations, and ensemble equivalence. The different behavior of osmotically stressed vesicles, as highlighted here, makes it necessary in certain cases to explicitly consider the properties of the encapsulated solution rather than just specify its mean volume or pressure. At the same time the different behavior allows access to features of the osmotic swelling process that would otherwise be difficult to extract, such as the membrane-permeability coefficients. One may be able to utilize it further and come up with more detailed predictions — for example, regarding the average time between the onset of osmotic stress and membrane lysis, which may be important for drug delivery and release.
FIG. 2. (a) Osmotic swelling of POPC giant unilamellar vesicles due to the permeation of urea. Each curve shows the optically tracked principal radius of a spheroidal vesicle as a function of time. The decreasing part corresponds to the ironing stage, and the increasing part to the stretching stage, after which the vesicle ruptures. The curves are flattened because of the large polydispersity of vesicles. (b) Collapse of the swelling curves onto the master curve, \( f(x) = (\sqrt{1 + x^2} - x)^{1/2} \) (represented by a gray dashed line). For more details, including the precise rescaling scheme, see ref 12.

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