Critical Swelling of Particle-Encapsulating Vesicles

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We consider a ubiquitous scenario where a fluctuating, semipermeable vesicle is embedded in solution while enclosing a fixed number of solute particles. The swelling with increasing number of particles or decreasing concentration of the outer solution exhibits a continuous phase transition from a fluctuating state to the maximum-volume configuration, whereupon appreciable pressure difference and surface tension build up. This criticality is unique to particle-encapsulating vesicles, whose volume and inner pressure both fluctuate. It implies a universal swelling behavior of such vesicles as they approach their limiting volume and osmotic lysis.

Membrane vesicles are fluctuating closed surfaces of 0.1–10 μm scale, made of a flexible bilayer of amphiphilic molecules in aqueous solution. Serving as a simple model of biological compartments (e.g., red blood cells), they have been one of the most extensively studied soft-matter systems [1, 2, 3]. Numerous works have addressed the elasticity and statistical mechanics of the membrane under various constraints, such as area and enclosed volume (e.g., [3, 4, 5]), or area and pressure difference across the membrane [6, 7, 8], yielding various shapes and shape transformations. Actual vesicles are always immersed in solution and thus contain both solvent (water) and solute. Such biomolecule-encapsulating vesicles are ubiquitous in cell functions such as signaling and transport into and out of (endocytosis) and out of (exocytosis) the cell [9]. They are also used in various applications as microreactors or delivery vehicles for cosmetics and drugs (liposomes) [10].

The hydrophobic core of the bilayer membrane hinders permeation of both water and solute molecules. Over sufficiently short time, therefore, the vesicle volume is fixed. Yet, while the activation barrier for water exchange is of the order of 20T (T being the thermal energy) [11], the barriers faced by the solute particles are typically much higher due to their size and/or charge, resulting in membrane permeabilities which are orders of magnitude lower [11]. Moreover, water exchange across the membrane can be tremendously enhanced (indeed, biologically controlled) via aquaporin channels, which lower the barrier to below 8T [12]. At sufficiently long times, therefore, most vesicles are found in a wide semipermeable regime, where water can be considered as exchanged between the interior and exterior, while the solute remains trapped inside. As a result, it has been assumed that the exterior solution concentration and number of encapsulated particles determine the vesicle volume in practice [3, 4, 5] — the mean volume adjusts through water permeation so as to annul the osmotic pressure difference across the membrane. This scenario has been experimentally verified [13] and utilized to measure membrane permeabilities of various solutes [11] and create osmotic motors [14]. Volume fluctuations around the osmotically determined mean value have been considered as well [15, 16]. However, at high swelling, as the vesicle approaches its maximum volume, this volume-adjustment description must break down, and appreciable pressure difference and surface tension will begin to build up. Further swelling eventually leads to pore formation and osmotic lysis [3, 17, 18]. The change in swelling behavior, in particular, whether it is a smooth crossover or a sharp transition, is the focus of the current Letter.

We describe the vesicle as a closed surface composed of N molecules and having maximum volume \( V_{\text{max}} \sim a^3 N^{3/2} \), a being a molecular length comparable to the membrane thickness. It is assumed that at \( V_{\text{max}} \) the vesicle has a nonextensive number of configurations. The vesicle encloses \( Q \) solute particles, which do not directly interact with the surface other than being trapped inside it. The vesicle is immersed in a solution of fixed concentration and temperature, which exerts an outer osmotic pressure \( p_o \) on the membrane. Since solvent molecules are exchanged between the interior and exterior, the vesicle volume is not specified and, hence, neither is its inner particle concentration and pressure. Thus, the partition function involves integration over all possible volumes,

\[
Z(T, p_o, Q, N) = \int dV Z_v(T, V, N) Z_s(T, V, Q) e^{-p_o V / T},
\]

(1)

where \( Z_v \) and \( Z_s \) are the canonical partition functions of the vesicle and solute particles, respectively, for a given volume \( V \). The thermal energy \( T \) is hereafter set to unity. For the solute we write

\[
Z_s = e^{-Q f(Q/V)},
\]

(2)

where \( f \) is the canonical free energy per solute molecule.

A key issue for the highly swollen vesicles studied here is how \( Z_v \) behaves as \( V \) approaches \( V_{\text{max}} \). It is shown below that, quite generally,

\[
Z_v(V \lesssim V_{\text{max}}) \sim (V_{\text{max}} - V)^{\alpha N},
\]

(3)

where \( \alpha \) is a coefficient of order unity. This result readily follows from the two requirements, that (i) the vesicle
free energy be extensive in $N$ for $V < V_{\text{max}}$, and (ii) the probability density function of volumes vanish at $V_{\text{max}}$. In more detail, $Z_c$ is found by integrating over all surface configurations the factor $e^{-H[R]}\delta(V-V[R])$, where $V[R]$ is the volume of configuration $R$, and $H[R]$ its energy (including, e.g., contributions from bending rigidity and surface interactions). For $V \approx V_{\text{max}}$ one can generally represent the configurations by the amplitudes $\{u_n\}$ of $N$ normal modes. (For example, in the case of nearly spherical vesicles these are spherical harmonics.) One then expands $Z[V]$ around $V_{\text{max}} = \sum C_n[u_n]^2$. Assuming that $H$ is nonsingular at $V_{\text{max}}$, and using the integral representation of the delta function, we get $Z_c \sim e^{-H(V_{\text{max}})} \int d[u_n]dp\exp[ip(V_{\text{max}}-V-\sum C_n[u_n]^2)]$. Integration over $\{u_n\}$ gives a factor of $p^{-1/2}$ per mode which, upon integration over $p$, yields Eq. (3) with $\alpha = 1/2$.

Substituting Eqs. (2) and (3) in Eq. (1) while specifying the solute free energy $f$, one can perform the integration over $V$ for given $Q$, $p_o$, and $N$. In Fig. 1 we present the resulting mean volume, $\langle V \rangle = -\partial \ln Z/\partial p_o$, as a function of $Q$ for an ideal solution, $f(Q/V) = \ln(\langle V \rangle / V)$. As $N$ is increased, $\langle V \rangle$ is seen to approach a discontinuous first derivative at $Q_c = p_o V_{\text{max}}$.

![Figure 1](image-url)

**FIG. 1:** (Color online) Order parameter as a function of control parameter for an ideal solution encapsulated in vesicles of various sizes. Solid curves show the mean-field results [Eq. (5)], while dotted curves are obtained from numerical integration of the partition function [Eq. (1)]. Datasets from top to bottom (top to bottom in the inset) correspond to $p_o = 1$, $N = 30$ (green); $p_o = 1$, $N = 10^3$ (blue); $p_o = 5$, $N = 10^3$ (indigo); $p_o = 1$, $N = 10^5$ (red); and the $N \to \infty$ limit [Eq. (3), black]. Only for the smallest vesicle size ($N = 30$) are the mean-field and numerical results distinguishable. Inset shows rescaled data according to Eq. (5), where the uppermost curve (solid black) is the theoretical scaling function. Values of $p_o$ are in units of $T/a^2$; in all data sets $\alpha = 1/2$ and $V_{\text{max}} = a^3 N^{3/2}$.

We now proceed to investigate this criticality analytically for a general (nonideal) solution. The partition function can be rewritten as $Z \sim \int dV e^{-F}$, with $F = -aN \ln(V_{\text{max}} - V) + Qf(Q/V) + p_o V$. Minimizing $F$ with respect to $V$ and applying a first-order saddle-point approximation (which is equivalent to a mean-field assumption), we obtain the following equation for $V$:

$$Q^2 f'(Q/V)/(V-V_0) = \alpha N (V)/(V_{\text{max}} - \langle V \rangle).$$

Expansion in $V_{\text{max}} - V$ yields for our order parameter,

$$M = \left[(\sqrt{|s-t(q)|^2 + 4sg(q)} - s-t(q))/(2g(q))\right].$$

Equations (3) and (6) describe a phase transition at $q = q_c$ which solves the equation $t(q_c) = 0$, i.e., for which, if the volume were equal to $V_{\text{max}}$, the inner pressure would just balance the outer one. The parameter $t(q)$ is related to the actual control parameter, $Q$ or $q$, via the solute equation of state. In the ideal-solution example, $f(Q/V) = \ln(\langle V \rangle / V)$, the critical point is at $q_c = p_o (Q_c = p_o V_{\text{max}})$, and we have $t = Q/Q_c - 1$ and $g = 1$. The transition occurs in the region $|t| \approx \Delta = (4sg)^{1/2} \sim a^{-3/2} p_o^{-1/2} N^{-1/2}$, along which $M$ crosses over from finite values to very small ones,

$$M = \begin{cases} 
-t/g \sim N^0 |t| & t \ll -\Delta \\
\Delta/(2g) \sim N^{-1/4} |t|^0 & |t| \ll \Delta \\
\Delta^2/(4gt) \sim N^{-1/2} t^{-1} & t \gg \Delta. 
\end{cases}$$

In the thermodynamic limit, $N \to \infty$, this crossover turns into a sharp corner [Eq. (6)], i.e., a discontinuity in $\partial M/\partial t$. From Eq. (5) we find that $M$ follows a scaling law within the transition region,

$$M/\Delta = g^{-1} M(t/\Delta), \quad M(x) = (\sqrt{x^2 + 1} - x)/2,$$

which is verified in Fig. 1 (inset). In addition, we calculate from Eq. (5) the compressibility, $\chi = \partial M/\partial p_o$,

$$\chi = (gp_o)^{-1} \chi(t/\Delta), \quad \chi(x) = (1-x/\sqrt{x^2 + 1})/2.$$
Equation (4) (upon division by \langle V \rangle) is just the Laplace law, balancing the pressure difference across the membrane (left-hand side) with a surface term (right-hand side). We therefore identify the pressure difference and surface tension as

\[
\Delta p = (\alpha N/V_{\text{max}})M^{-1} \sim a^{-3}N^{-1/2}M^{-1},
\]
\[
\sigma \sim R\Delta p \sim a^{-2}M^{-1},
\]

where \( R \sim aN^{1/2} \) is the vesicle radius. Thus, \( \Delta p \) and \( \sigma \) change from negligible values below the transition to appreciable ones above it. Specifically, \( a^3\Delta p \) is of order \( N^{-1/2}, N^{-1/4} \), and 1, while \( a^2\sigma \sim 1, N^{1/4}, \) and \( N^{1/2}, \) below, at, and above the critical point, respectively.

Note that Eqs. (1)–(3), which underly the entire analysis, contain no microscopic information. The model, therefore, is purely thermodynamic, in the sense that any specific microscopic model for the vesicle and encapsulated solution (so long as the vesicle has a state of maximum volume and negligible entropy) should lead to the same results. (For example, inclusion of bending rigidity will merely change the prefactor in Eq. (3).) The invariance to the choice of model implies also that the continuous transition does not necessarily involve a divergent correlation length [23]. We have checked these statements for the specific example of a nearly spherical envelope of \( N \) nodes and fixed total area \( 4\pi R_0^2 \), enclosing an ideal solution. The vesicle shape is defined in this case by \( R(\theta, \phi) \), the distance of the membrane from the center as a function of solid angle, whose deviation from \( R_0 \) can be decomposed into spherical harmonics, \( R(\theta, \phi) = R_0[1 + \sum_{l=0}^{\infty} \sum_{m=-l}^{l} u_{lm}Y_{lm}(\theta, \phi)] \), where \( (l_{\text{max}} + 1)^2 = N \). Integration of the resulting partition function over the amplitudes \( u_{lm} \) within a saddle-point approximation recovers Eqs. (3)–(9). The correlation function, \( \langle u_{lm}u_{(-l-m)} \rangle \sim (M/N)/(l(l + 1) - 1) \), exhibits a critical suppression of amplitude but no divergent correlation length. Expectedly, this fluctuation spectrum is identical to that of a spherical membrane with surface tension \( \sigma \sim M^{-1} \), in accord with Eq. (10).

Equations (8) and (10) characterize the sharpening of the transition with increasing system size. If we recast them in the conventional finite-size scaling form [22],

\[
M \sim R^{-\beta/\nu^*}M(R^{1/\nu^*}t) \quad \text{and} \quad \chi \sim R^{1/\nu^*}(R^{1/\nu^*}t),
\]

we readily extract \( \beta = 1, \gamma = 0, \) and \( \nu^* = 2 \). The values of \( \beta \) and \( \gamma \) are consistent with the linear increase of \( M \) below the transition [Eq. (6) and Fig. 1]. Notwithstanding the absence of a divergent correlation length, one can use \( \nu^* \) to define a length scale, \( \xi \sim a\xi^{-\nu^*} \), such that the system lies in the critical domain if \( R < \xi \). The divergent energy does not relate to correlations but to the competition between surface degrees of freedom (\( \sim N \)) and three-dimensional ones (\( \sim N^{3/2} \)). This competition determines the width of the transition, \( \Delta \sim [N/(p_0V_{\text{max}})]^{1/2} \), making it shrink with increasing \( N \). Repeating the analysis for a ring in two dimensions yields a similar mean-field transition with identical exponents. We are not aware of another transition whose mean-field limit has the exponents found above [25].

The phase transition just characterized is a unique feature of particle-encapsulating vesicles. It is a consequence of the effective inner pressure being dependent on the volume (through \( f(Q/V) \) for fixed \( Q \)), while the latter fluctuates. This leads to pressure difference and surface tension which are nonanalytic in \( Q \) [Eq. (10)] and a consequent breaking of the equivalence between the fixed-pressure (or fixed-tension) scenario and that of fixed \( Q \). Indeed, if the enclosed solution is replaced with a given inner pressure \( p_i \) (i.e., upon substituting in Eq. (1) \( Z_\alpha = e^{\alpha V/T} \)), it is straightforward to show that \( M = \alpha N[(p_i - p_0)V_{\text{max}}]^{-1} \), in agreement with Eq. (10) [20]. Therefore, in the case of a given pressure difference (or tension) the vesicle swells gradually with \( p_i \) (or \( \sigma \)) without criticality. Furthermore, replacing the particle-number constraint with a chemical potential [27] is equivalent (via the solute equation of state) to specifying the inner pressure. Hence, there is no criticality in the grand-canonical case either, and the two ensembles are manifestly not equivalent [25].

Another noteworthy limit is that of a pure solvent outside the vesicle, \( p_o \rightarrow 0 \), where the current analysis yields \( Q_o \rightarrow 0 \) and \( \Delta \rightarrow \infty \), i.e., the transition disappears. In this case the swelling of the vesicle toward its maximum volume occurs already for much smaller particle numbers \( Q \), scaling with the area \( N \) rather than the volume [28].

In summary, we have found that membrane vesicles, under rather general conditions, behave critically as the number of solute particles inside them is increased or, equivalently, the outer osmotic pressure is decreased. It should be possible to experimentally observe this phase transition, e.g., by creating vesicles and subsequently diluting the outer solution in a controlled manner, or by using isotonic solutions of molecules with different membrane permeabilities [18]. We mention three points relating to such experiments. First, they should cover such time scales that the vesicle could be considered permeable to water. This can be sensitively controlled if water (aquaporin) channels are incorporated in the membrane, yet common lipid vesicles are also found in this regime over readily accessible time scales (\( \sim 10 \) s) [13, 14, 18]. Next we examine the assumption of a sharply defined maximum volume. One definition of \( V_{\text{max}} \) would be the volume enclosed by an unstretchable vesicle of a given area once out-of-plane fluctuations have completely vanished [24]. This assumption should be relaxed when inplane (stretching) fluctuations become comparable to transverse ones. Since, for a tense membrane, the mean-square fluctuations of both modes have the same (quadratic) dependence on wavenumber, this crossover will occur simply when the surface tension, \( \sigma \sim (T/a^2)M^{-1} \) [Eq. (13)]}, becomes comparable to the membrane stretching modulus, which is typically of or-
nder $10^2$ dyne/cm. For $a \sim 1$ nm this happens when $M \lesssim 10^{-2}$, i.e., when the mean volume deviates from $V_{\text{max}}$ by less than 1%. Thus, we expect the transition from appreciable to small values of $M$, along with the corresponding scaling behavior, to be manifest well before stretching becomes important. Since lipid membranes can sustain inplane strains of only a few percent before rupturing [17], the crossover to stretching-dominated dynamics will be shortly followed by vesicle lysis [18]. Third, because of the weak dependence of the transition width on $N$, $\Delta \sim (a^3 p_0 / T)^{-1/2} N^{-1/4}$, the observed behavior will not be very sharp. A typical micron-sized vesicle has about $N \sim 10^8$ molecules in its membrane, leading, for a 0.1M solution, to $\Delta \sim 10^{-1}$ only. The criticality could be verified, nonetheless, by checking data collapse according to the scaling law, Eq. [5].

Thus, our assumptions concerning permeability, maximum volume, and number of molecules do not rule out an experiment aimed at the predicted critical swelling. (The suppression of small fluctuations near the transition, however, may be hard to resolve.) More generally, this study highlights the qualitative difference between semipermeable, particle-encapsulating vesicles and those having fixed volume or pressure. Since most natural and industrial vesicles belong to this class, their different behavior should be taken into account, particularly in cases of high swelling and osmotic lysis.

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