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Size distributions of vesicles : the role of the effective rigidity of membranes

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Résumé. — On calcule la distribution des tailles de vésicules sphériques, et en particulier la manière dont la rigidité effective dépend de la taille des bicouches. Pour des vésicules sphériques on montre que cette rigidité décroît comme le logarithme de la taille. Pour les petites tailles, on construit une coupure de la distribution à partir des termes non hookéens de l'élasticité de courbure.

Abstract. — The size distribution of spherical vesicles is studied theoretically, in particular the influence of the dependence of the effective rigidity on the size of the bilayer. The logarithmic decrease of the effective rigidity with size is rederived for the sphere. Also, an abrupt cutoff of the distribution at small sizes is constructed in terms of non-Hookean bending elasticity.

1. Introduction.

The size distribution of small unilamellar vesicles has been an elusive problem, both experimentally and theoretically. The most frequently studied materials are phospholipids, such as lecithins in water. Sonication [1, 2] and injection of organic solutions [3] into water are standard methods of preparation. The most reliable procedure of measuring the distributions seems to be electron microscopy, despite the drawbacks of the necessary staining.

A collection of vesicles will in general be only metastable because of a high bending elastic energy associated with membrane curvature. However, if their number remains practically constant over a period long enough to permit the exchange of amphiphilic (and other) molecules one may obtain a final distribution. It can be regarded as a thermodynamic equilibrium under the constraint of fixed vesicle number. The experimental distribution functions are generally plotted *versus* the vesicle diameter. They are characterized by a lower cutoff near 200 Å followed by a steep rise to a maximum at variable positions and a gradual decay at larger diameters. The shape of the vesicles is spherical, which minimizes their bending energies.

The natural variable in the theoretical treatment of the distributions is the number of amphiphilic molecules making up a vesicle. Accordingly, we will deal with number distribution functions. Any theory has to start from the notion that the bending elastic energy of spherical vesicles is independent of their size, since the usual bending energy per unit area of a symmetric bilayer is a quadratic form in the curvatures. The scale invariance alone would lead to an exponential dependence of the number distribution function on the number of constituent molecules. Shape and width of such a distribution are related to existence and value of a chemical potential.

The exponential law must fail below a certain vesicle size. In order to develop a more complete theory we utilize two additional concepts. One is the recent proposition [4, 5] that the effective rigidity of a piece of membrane decreases logarithmically with its size, thus deviating from the local or « bare » rigidity. The decrease, so far studied only for planar geometry, is a consequence of the thermal undulations of the fluid layer. It should favour larger spheres over smaller ones, which is what we are looking for. The other concept is higher-order, i.e. non-Hookean, bending elasticity [6]. We consider energy terms quartic in the curvatures to derive a formula for rather abrupt cutoffs which has only one free parameter.

In systems with a freely variable number of vesicles, their mean number will be such as to minimize the free energy. The size distribution function is then practically the same as if this number were fixed. The distributions to be calculated may, therefore, be relevant to the recently discovered systems exhibiting spontaneous vesiculation [7, 8]. We will consider

here only fixed vesicle numbers. Moreover, we will regard the vesicles as an ideal gas, assuming rather dilute vesicle dispersions.

The plan of the paper is as follows : in section 2 we lay the foundation for treating the thermal shape fluctuations of a sphere. They correspond to the undulations of a planar membrane. The weakly deformed sphere is parametrized by the radial displacement of the surface from an ideal sphere. The bending elastic energy and other quantities are calculated in an approximation that includes all terms linear or quadratic in the displacement and its derivatives. Expansion of the displacement in spherical harmonics shows the fluctuation modes thus defined to be decoupled, except for the dependence of the effective radius of the sphere on the mean square amplitudes. The formula for their bending energies differs slightly from those reported recently by Safran [9] and Schneider *et al.* [10]. Also, it allows for a spontaneous membrane curvature (which may be induced by suitable solutes restricted, e.g., to the external aqueous medium).

Section 3 deals with the statistical mechanics of shape fluctuations. We first calculate the effective radius, which is the radius where the displacement of an individual mode starts on average when the sphere is fluctuating. The effective rigidity of the sphered membrane is deduced from the minute parts of the mean-square mode amplitudes that may be attributed to the spherical shape. The enhancements of the amplitudes give rise to a negative free energy which in turn can be expressed by a small decrease of the rigidity. The result agrees with our previous one [5], while the decrease obtained by Peliti and Leibler [4] was three times larger. The calculation of the effective rigidity poses some fundamental questions in the case of the planar membrane (about the measure of integration). It appears in a sense less problematical with the sphere where it can be done in a strictly quadratic rather than partly quartic approximation. The new proof, apart from being interesting in its own right, consolidates the basis on which to build the theory of size distributions. Although not needed in the rest of the paper, the effective spontaneous curvature is also calculated for the undulating sphere. The earlier result [5] is recovered. This may be viewed as another test of the validity of the formula for the effective rigidity.

Vesicle size distributions at vanishing spontaneous curvature are presented in section 4. We derive there the functional dependence of the cutoff at small vesicle sizes that is caused by higher-order elasticity, examining how it affects both the energy of the ideal sphere and the amplitudes of the undulations. Finally, the number distributions are transformed into functions of vesicle diameter and a few curves are plotted to facilitate comparison with experimental data. We also remark on the dramatic effect which spontaneous curvature may have on vesicle size distributions, wiping out the subtle influence of undulations.

2. Bending energies in terms of spherical harmonics.

The usual quadratic form for the curvature-elastic energy per unit area of a fluid layer may be written as

$$g = \tfrac{1}{2}\kappa(c_1 + c_2 - c_s)^2 + \bar{\kappa}c_1 c_2 . \qquad (1)$$

Here c_1 and c_2 are the principal curvatures, c_s is the spontaneous curvature, and the elastic moduli κ and $\bar{\kappa}$ are the bending rigidity and the modulus of Gaussian curvature, respectively. The second term of (1) is in general omitted as the integral of Gaussian curvature depends only on the genus of the surface over which it is taken. (It is $4\pi\bar{\kappa}$ for spheres, ideal or deformed.) Throughout this article the membranes will be assumed to be unstretchable and their finite thickness will mostly be disregarded.

In order to calculate the elastic energies of weakly deformed spherical surfaces we take the sphere radius r to be a function of the polar angle φ and the azimuthal angle θ, writing

$$r(\theta, \varphi) = r'_0 + u(\theta, \varphi) . \qquad (2)$$

Here r'_0 is an effective radius from which the displacement $u(\theta, \varphi)$ is thought to start. It is generally smaller than the radius r_0 of the ideal sphere (see below). The curvatures are easily obtained by introducing the layer normal or director $\mathbf{n}$. The field $\mathbf{n} = (n_r, n_\theta, n_\varphi)$ is related to the displacements by

$$\mathbf{n} = \frac{(1, -\partial u/r\,\partial\theta, -\partial u/r \sin\theta\,\partial\varphi)}{[1 + (\partial u/r\,\partial\theta)^2 + (\partial u/r \sin\theta\,\partial\varphi)^2]^{1/2}} \qquad (3)$$

if the director points outside the sphere.

Let us imagine, for a moment, the director field which depends only on θ and φ to fill all space. On the membrane, $\mathbf{n}$ automatically satisfies the surface constraint $\mathbf{n} \cdot \operatorname{curl} \mathbf{n} = 0$. It is well known that in a liquid crystalline director field layer curvature corresponds to splay, which permits the sum of curvatures to be written as an invariant

$$c_1 + c_2 = \operatorname{div} \mathbf{n} . \qquad (4)$$

The curvature of the sphere is positive in our notation. With spherical polar coordinates one has on the membrane

$$\operatorname{div} \mathbf{n} = \frac{1}{r^2}\frac{\partial}{\partial r}(r^2 n_r) + \frac{1}{r\sin\theta}\frac{\partial}{\partial\theta}(\sin\theta n_\theta) + \frac{1}{r\sin\theta}\frac{\partial n_\varphi}{\partial\varphi} \qquad (5)$$

where $\partial n_r/\partial r = 0$ in our special case. We are interested only in terms up to second order in u. Therefore, the denominator of (3) can be replaced by unity in

the last two terms of (5). Accordingly,

$$\operatorname{div} \mathbf{n} = \frac{2\,n_r}{r} - \frac{1}{r \sin\theta}\frac{\partial}{\partial\theta}\frac{\sin\theta}{r}\frac{\partial u}{\partial\theta} - \\ - \frac{1}{r\sin^2\theta}\frac{\partial}{\partial\varphi}\left(\frac{1}{r}\frac{\partial u}{\partial\varphi}\right) + 0(u^3). \quad (6)$$

Adopting the operators

$$\nabla_2 = \left(\frac{\partial}{\partial\theta}, \frac{1}{\sin\theta}\frac{\partial}{\partial\varphi}\right), \quad (7a)$$

$$\Delta_2 = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial}{\partial\theta}\right) + \frac{1}{\sin^2\theta}\frac{\partial^2}{\partial\varphi^2}, \quad (7b)$$

and the relative displacement

$$f(\theta, \varphi) = u(\theta, \varphi)/r_0' \quad (8)$$

will enable us to write some quantities in compact form. We begin with

$$\operatorname{div} \mathbf{n} = \frac{2}{r_0'}\left[1 - f - \frac{1}{2}\Delta_2 f + f^2 + f\,\Delta_2 f\right] \quad (9)$$

(where two terms $(\nabla_2 f)^2$ have cancelled out). The membrane area dA per solid angle $d\Omega = \sin\theta\, d\varphi\, d\theta$ is easily seen to be

$$dA = \frac{r^2}{n_r}\,d\Omega \quad (10)$$

as n_r is the cosine of the angle the director makes with the radius vector. In quadratic approximation it becomes

$$dA = r_0'^2\left[1 + 2f + f^2 + \frac{1}{2}(\nabla_2 f)^2\right]d\Omega. \quad (11)$$

In the same approximation one obtains

$$(\operatorname{div}\mathbf{n})^2 = \\ = \frac{4}{r_0'^2}\left[1 - 2f - \Delta_2 f + 3f^2 + \frac{1}{4}(\Delta_2 f)^2 + 3f\Delta_2 f\right], \quad (12)$$

$$(\operatorname{div}\mathbf{n})^2\,dA = \\ = 4\left[1 - \Delta_2 f + \frac{1}{4}(\Delta_2 f)^2 + f\Delta_2 f + \frac{1}{2}(\nabla_2 f)^2\right]d\Omega, \quad (13)$$

$$\operatorname{div}\mathbf{n}\,dA = 2r_0'\left[1 + f - \frac{1}{2}\Delta_2 f + \frac{1}{2}(\nabla_2 f)^2\right]d\Omega. \quad (14)$$

In a next step, the relative displacement is expanded in spherical harmonics,

$$f(\theta, \varphi) = \sum_{l,m} a_{lm}\, Y_{lm}, \quad (15)$$

where $a_{l,-m} = a_{l,m}^*$ since f is real. We recall three important properties of spherical harmonics : separability

$$Y_{lm} = P_l^m(\theta)\, e^{im\varphi} \quad (16)$$

where $P_l^m(\theta)$ is an (associated) Legendre polynomial (l and m are integers obeying $l \geqslant 0$, $|m| \leqslant l$), orthonormality

$$\oint Y_{lm}\, Y_{l'm'}^*\, d\Omega = \delta_{ll'}\,\delta_{mm'}, \quad (17)$$

and the eigenvalue equation

$$\Delta_2 Y_{lm} = -\,l(l+1)\,Y_{lm}. \quad (18)$$

When calculating integrals over the closed surface we will frequently use the transformation

$$\oint (\nabla_2 f)^2\, d\Omega = -\oint f\,\Delta_2 f\, d\Omega \quad (19)$$

which is readily proved by partial integration. The total curvature-elastic energy of the deformed sphere is

$$E = \frac{1}{2}\kappa\oint(\operatorname{div}\mathbf{n})^2\,dA - \kappa c_s\oint\operatorname{div}\mathbf{n}\,dA + \\ + \frac{1}{2}\kappa c_s^2\oint dA. \quad (20)$$

Considering first the special case of vanishing spontaneous curvature ($c_s = 0$) one finds from (13) and with the expansion (15)

$$E = 8\pi\kappa + \sum_{l,m}\frac{1}{2}\kappa[l^2(l+1)^2 - 2l(l+1)]\,|a_{lm}|^2. \quad (21)$$

Note that the result is scale invariant. The square bracket vanishes for $l = 0$ and $l = 1$. This may have been anticipated as $l = 0$ corresponds to a change in sphere size and $l = 1$ to the three orthogonal translations of the sphere (and to a change in sphere size, see below). All four operations must leave E unchanged. Very similar formulae were obtained earlier by Safran [9] and by Schneider *et al.* [10], who took most but not all of the quadratic terms into account in their calculations.

There is no scale invariance in the presence of spontaneous curvature ($c_s \neq 0$). It is then advantageous to use r_0, the radius of the ideal sphere, rather than the effective radius r_0'. The two radii are related through the conservation of area which reads

$$4\pi r_0^2 = \int dA = 4\pi r_0'^2 + \\ + r_0'^2\sum_{l,m}{}'\left[1 + \frac{1}{2}l(l+1)\right]|a_{lm}|^2 \quad (22)$$

in the quadratic approximation. The $l = 0$ mode is now excluded from the summation, which is indicated

by the prime ($\sum'$). Equation (22) shows that the $l = 1$ modes are not only translations, but also increase the sphere size. Another representation of (22) is

$$r'_0 = r_0 \left\{ 1 - \frac{1}{8\pi} \sum_{l,m}' \left[1 + \frac{1}{2} l(l+1) \right] | a_{lm} |^2 \right\}. \tag{23}$$

Integration of (14) yields

$$\int \operatorname{div} \mathbf{n} \, dA = 8\pi r'_0 + r'_0 \sum_{l,m}' l(l+1) | a_{lm} |^2 . \tag{24}$$

Inserting this and replacing r'_0 by r_0 in (20), one finally obtains instead of (21)

$$E = 8\pi - 8\pi\kappa c_s r_0 + 2\pi\kappa c_s^2 r_0^2 + \\ + \frac{1}{2}\kappa \sum_{l,m}'' \left\{ [l^2(l+1)^2 - 2l(l+1)] \right. \\ \left. + 2 c_s r_0 \left[1 - \frac{1}{2} l(l+1) \right] \right\} | a_{lm} |^2 \tag{25}$$

in the same quadratic approximation. The sum represents the total elastic energy of deformation. The coefficient in front of the squared amplitudes vanishes for $l = 1$, in accordance with the fact that these modes are pure translations if the membrane area is conserved. Therefore, the summation starts only at $l = 2$, as indicated by the double prime. Note that the $l^2(l+1)^2$ term tends to be the leading one and always dominates for large enough l. For zero or negative spontaneous curvature all modes require energy to be excited. However, the energy E may be decreased by a deformation if there is a sufficient positive spontaneous curvature. The lowest modes ($l = 2$) are the first to become unstable. The limit of instability can be calculated by equating the coefficient to zero for $l = 2$. The result, $c_s r_0 - 6 = 0$, has long been known [11] to characterize the limit of stability or metastability of a vesicular sphere with respect to ellipsoidal deformations.

3. Shape fluctuations and their effects.

The shape fluctuation modes ($l \geqslant 2$) are decoupled in the quadratic approximation (25) so that the equipartition theorem applies to them. Accordingly, the mean square amplitudes obey

$$\langle | a_{lm} |^2 \rangle = \\ = \frac{kT}{\kappa \{ l^2(l+1)^2 - 2l(l+1) + 2 c_s r_0 [1 - \frac{1}{2} l(l+1)] \}} \tag{26}$$

where T is absolute temperature and k Boltzmann's constant. If not otherwise stated we assume vanishing spontaneous curvature. In this particular case the equipartition theorem takes the form

$$\langle | a_{lm} |^2 \rangle = \frac{kT}{\kappa [l^2(l+1)^2 - 2l(l+1)]} \tag{27}$$

which is given here for easy reference. Starting from these equations we can calculate a number of interesting quantities.

Let us begin with the difference

$$A - A' = 4\pi r_0^2 - 4\pi \langle r_0'^2 \rangle \tag{28}$$

between true and effective membrane area, A and A', respectively. Rewriting (22) gives

$$A - A' = r_0^2 \sum_{l,m}' \left[1 + \frac{1}{2} l(l+1) \right] \langle | a_{lm} |^2 \rangle \tag{29}$$

which because of (27) becomes

$$A - A' = r_0^2 \frac{kT}{\kappa} \sum_{l,m} \frac{1 + \frac{1}{2} l(l+1)}{l^2(l+1)^2 - 2l(l+1)} . \tag{30}$$

Replacing the sum by an integral,

$$\sum_{l,m} = \sum_l (2l+1) \approx \int_0^{l_{max}} dl(2l+1) , \tag{31}$$

and retaining only the highest powers of l in nominator and denominator results in

$$A - A' = r_0^2 \frac{kT}{\kappa} \ln l_{max} . \tag{32}$$

The upper limit l_{max} is readily seen to be related to the total number M of modes by

$$M = \sum_{l=0}^{l_{max}} (2l+1) = (l_{max} + 1)^2 . \tag{33}$$

M may be put equal to half of the number of amphiphilic molecules in the bilayer. Insertion in (32) yields, because of $A = 4\pi r_0^2$,

$$\frac{A - A'}{A} = \frac{kT}{8\pi\kappa} \ln M . \tag{34}$$

Exactly the same relative change in area was calculated earlier for the planar membrane with periodic boundary conditions [12, 13]. Here as there, the formula is valid only for small relative changes ($\ll 1$). The casual treatment of its lower cutoff does no harm because of the logarithm.

It follows from equation (21) and the equipartition theorem that the bending energy of the fluctuating sphere is that of the ideal sphere plus (1/2) kT for each deformation mode. However, it appears reasonable to argue that the deformation reduces the energy of sphering. Because of (6) the contribution of the spherical shape to the local curvature div $\mathbf{n}$ is $2 n_r/r$. The two other contributions are like those of undulations on a planar membrane, at least in a small neighbourhood where substitutions $r \, d\theta = dx$,

$r \sin\theta \, d\varphi = dy$ are permissible and for $l \gg 1$ [1]. Note that as previously [5] we are dealing with curvatures. Representing the physical strain, curvature seems to be the natural choice for the measure of integration in calculating entropy and free energy. Multiplying $2\, n_r/r$ squared with $1/n_r$ to allow for the increase in area due to the angle made by director and radius vector gives the energy differential $(1/2)\, \kappa(4/r^2)\, n_r\, r^2\, d\Omega$. Integration leads to

$$E_{\text{sphere}} - E'_{\text{sphere}} = \kappa \int (\nabla_2 f)^2 \, d\Omega = \kappa \sum_{l,m} l(l+1) \, | a_{lm} |^2 . \tag{35}$$

where $E_{\text{sphere}} = 8\pi\kappa$ and E'_{sphere} are the energies of sphering for the ideal and deformed sphere, respectively. We have used the approximation $n_r = 1 - (1/2)\, (\nabla_2 f)^2$ and, in the last expression, expanded f in spherical harmonics.

The energy released by the sphere goes into the deformation modes so that their mean elastic energies are (slightly) larger than $(1/2)\, kT$. The mean square curvatures associated with a mode must rise by the same factor. Obviously, the factor equals the ratio R of the coefficients of $| a_{lm} |^2$ in the equation obtained from (21) by substituting (35) for $8\pi\kappa = E_{\text{sphere}}$ and in (21) itself. Inspection yields at once

$$R_l = \frac{l^2(l+1)^2}{l^2(l+1)^2 - 2\, l(l+1)} . \tag{36}$$

Therefore, the entropy of a single mode increases by

$$\Delta S_{lm} = \frac{k}{2} \ln R_l = k \frac{1}{l(l+1)} \tag{37}$$

where the logarithm has been expanded to first order. Summation over all modes as in (31) and (33) leads to the total entropy change

$$\Delta S = k \ln M . \tag{38}$$

Defining now an effective rigidity κ' by the identity

$$8\pi(\kappa - \kappa') = \Delta F \tag{39}$$

where $\Delta F = T\, \Delta S$, with ΔS taken from (38), results in

$$\kappa' - \kappa = - \frac{kT}{8\pi} \ln M . \tag{40}$$

(1) A more precise way of distinguishing deformational and spherical curvature is to assign to the former the terms of r div $\mathbf{n}$ that are linear in f. There is only one such term, namely $r'_0\, \Delta_2 f$. The quadratic terms of $n_r^{-1/2}\, r$ div $\mathbf{n}$ interact with the curvature of the ideal sphere, thus lowering its energy. One arrives again at (35). It would be appropriate to expand curvature, the measure of integration, rather than displacement in spherical harmonics. However, the more tractable and experimentally useful displacement modes are satisfactory as their bending elastic energies are uncoupled in the quadratic approximation.

The final formula could also have been obtained in a more direct but, perhaps, less transparent fashion from (35), the equipartition theorem (27), and (39). It agrees exactly with our prediction for the planar membrane [5].

For completeness and as a check on consistency we also examine the effect of nonvanishing spontaneous curvature. c_s is assumed to be negative or, if positive, small enough to avoid destabilization of the spherical shape. We proceed as in the last paragraph. Comparison of (26) and (27) indicates that the spontaneous curvature affects the mean square mode amplitudes. The concomitant change of entropy per mode is readily found to be, for $l \gg 1$,

$$\Delta S_{lm} = \frac{k}{2} c_s r_0 \frac{1}{l(l+1)} . \tag{41}$$

The total change is

$$\Delta S = \frac{k}{2} c_s r_0 \ln M . \tag{42}$$

Consideration of (25) suggests the ansatz

$$8\pi\kappa'\, c'_s\, r'_0 = 8\pi\kappa c_s\, r_0 + \frac{kT}{2} c_s\, r_0 \ln M \tag{43}$$

which may be rewritten as

$$\frac{\kappa' - \kappa}{\kappa} + \frac{c'_s - c_s}{c_s} + \frac{r'_0 - r_0}{r_0} = \frac{kT}{16\pi\kappa} \ln M . \tag{44}$$

The first term is supplied by (40). The third one is obtained by transforming (34) into

$$\frac{r_0 - r'_0}{r_0} = \frac{kT}{16\pi\kappa} \ln M . \tag{45}$$

We do not distinguish here between r'_0 and $(\langle r'^2_0 \rangle)^{1/2}$. Evidently, the effective spontaneous curvature c'_s satisfies

$$\frac{c'_s - c_s}{c_s} = \frac{kT}{4\pi\kappa} \ln M . \tag{46}$$

This is again identical to the earlier result [5] and correct only for $(c'_s - c_s)/c_s \ll 1$.

Inspection of (26) reveals that the enhancement of the curvature amplitudes due to the spherical geometry may be counter-balanced by the spontaneous curvature $c_s = -2/r_0$ which is equal but opposite to the curvature of the sphere. In other words, this particular spontaneous curvature permits simulating the hypothetical case of no energy exchange between undulations and spherical curvature ($R_l = 1$).

4. Size distributions of vesicles.

In considering size distributions of vesicles we limit ourselves to systems with a fixed number of vesicles.

It is assumed that there is a single species of amphiphilic molecules, that the exchange of all molecules, including water and solutes, is fast enough between and within the vesicles to ensure thermal equilibrium and that the fraction of amphiphilic molecules dissolved in water is negligible. The spontaneous curvature is zero by definition under the given symmetry. If in addition we postulate $\kappa > 0$, which should in general be true, the shape of lowest energy of such vesicles, as obtained on the basis of (1), is spherical. The bending energy of the ideal sphere is

$$E = 8\,\pi\kappa + 4\,\pi\bar{\kappa}\,, \tag{47}$$

regardless of sphere size. Shape fluctuations are supposed to be weak so that the theory of the last section is applicable.

If κ and thus E were indeed independent of vesicle size one would expect an exponential distribution

$$w(N) = (1/\bar{N})\exp(-\,N/\bar{N})\,, \tag{48}$$

N being the number of amphiphilic molecules in the vesicle and $\bar{N}$ its mean value. The preliminary formula is normalized to unity for large $\bar{N}$ where

$$\sum_N w(N) = \int_0^\infty w(N)\,\mathrm{d}N = 1\,. \tag{49}$$

The exponential dependence is easily proved : if a sufficient number of other vesicles acts as a reservoir, making the chemical potential μ of the amphiphile relative to its free energy in the bilayer independent of N, one immediately obtains (48) through

$$w(N) \sim \exp\frac{\mu N - E}{kT} \tag{50}$$

where necessarily $\mu < 0$.

In a next step we take into account that the effective rigidity and thus E are functions of vesicles size. It follows from (40) and with $M = N/2$ that

$$\kappa' = \kappa - \frac{kT}{8\,\pi}\ln\frac{N}{2}\,. \tag{51}$$

The contributions of the lowest modes need not be correctly rendered by $\ln M$, and M need not exactly equal half the number of molecules making up the bilayer (e.g. for amphiphiles with two hydrocarbon chains). Such corrections will be small and can be lumped in with κ. Inserting (51) in (47) yields

$$E = 8\,\pi\kappa + 4\,\pi\bar{\kappa} - kT\ln\frac{N}{2} \tag{52}$$

and, *via* (50),

$$w(N) \sim N\exp(\mu N/kT)\,. \tag{53}$$

Equations (52) and (53) are of remarkable simplicity due to the cancellations of $8\,\pi$ and kT. The chemical potential can be related to the mean number of molecules per vesicle. Normalization to unity (by integrating from 0 to ∞) leads to

$$w(N) = \left(\frac{2}{\bar{N}}\right)^2 N\exp(-\,2\,N/\bar{N})\,. \tag{54}$$

The function belongs to the class of Schulz distributions which have recently been used in the analysis of quasi-elastic light scattering from various types of dispersions [14, 15].

Although the Schulz distribution (54) is a consequence of curvature elasticity, it does not depend on the rigidity, sharing this feature with vesicle shapes. The Schulz distribution assigns less weight than the exponential function (48) to the smallest vesicle sizes. Therefore, it is more likely to describe size distributions realistically. The improved distribution must still fail at very small N as the geometry of vesicles rules out vesicles below a certain size. Whether or not the failure is significant depends, among other things, on the mean number of molecules per vesicle.

A more stringent cutoff may be obtained by invoking non-Hookean curvature elasticity. Equation (1) contains first and second powers of curvatures. For symmetric bilayers only the second-order terms survive as $c_s = 0$. The next non-vanishing order in this case is the fourth. The associated elastic energy density per unit area has been given by Mitov [6] :

$$g^{(4)} = \kappa_1^{(4)}(c_1 + c_2)^4 + \kappa_2^{(4)}(c_1 + c_2)^2\,c_1\,c_2 + \kappa_3^{(4)}(c_1\,c_2)^2 \tag{55}$$

with three new elastic moduli. (We omit here second powers of derivatives of the curvatures as a strong curvature of the sphere, which gives rise to higher-order corrections, is itself uniform.) In order to find a formula for the cutoff we examine how fourth-order elasticity affects both the energy of the ideal sphere and the amplitudes of shape fluctuations.

With the compound modulus

$$K_1^{(4)} = \kappa_1^{(4)} + \frac{1}{4}\kappa_2^{(4)} + \frac{1}{16}\kappa_3^{(4)} \tag{56}$$

the fourth-order bending elastic energy of the sphere becomes

$$E^{(4)} = K_1^{(4)}\left(\frac{2}{r_0}\right)^4 4\,\pi r_0^2\,. \tag{57}$$

Using

$$N = 8\,\pi r_0^2/A_0\,, \tag{58}$$

where A_0 is the molecular cross section in the bilayer, we find

$$E^{(4)} = \frac{512\,\pi^2\,K_1^{(4)}}{A_0\,N}\,. \tag{59}$$

Note that $E^{(4)}$ is inversely proportional to N.

The effect of fourth-order elasticity on the undulations may be treated in terms of an effective second-order elastic modulus κ_{eff} which is the second derivative of the elastic energy density per unit area with respect to the sum of curvatures. Using the abbreviation $c = c_1 + c_2$, one can write

$$\kappa_{\text{eff}} = \kappa + \frac{\partial^2 g^{(4)}}{\partial c^2} . \tag{60}$$

With the additional compound modulus

$$K_2^{(4)} = 12\,\kappa_1^{(4)} + \frac{1}{2}\kappa_2^{(4)} \tag{61}$$

one obtains

$$\kappa_{\text{eff}} = \kappa + K_2^{(4)}\left(\frac{2}{r_0}\right)^2 \tag{62}$$

and finally, by means of (58),

$$\kappa_{\text{eff}} = \kappa + \frac{32\,K_2^{(4)}}{A_0\,N} . \tag{63}$$

The mean square fluctuation amplitudes are inversely proportional to the rigidity, so the replacement of κ by κ_{eff} changes the entropy of a mode by

$$\Delta S_{lm}^{(4)} = \frac{k}{2}\ln\frac{\kappa}{\kappa_{\text{eff}}} , \tag{64}$$

With $M = N/2$ modes, the ensuing change in the free energy of the vesicle is

$$-\,T\,\Delta S^{(4)} = \frac{1}{2}NkT\ln\frac{\kappa_{\text{eff}}}{\kappa} . \tag{65}$$

Expanding the logarithm in $(\kappa_{\text{eff}} - \kappa)/\kappa$ leads to

$$-T\,\Delta S^{(4)} = \frac{1}{2}kT\left[\frac{32\,\pi K_2^{(4)}}{A_0\,\kappa} - \frac{1}{2}\left(\frac{32\,\pi K_2^{(4)}}{A_0\,\kappa}\right)^2\frac{1}{N} + 0\left(\frac{1}{N^2}\right)\right] . \tag{66}$$

The first term of (66), being independent of N, is not very interesting. Combining the second term and (59), which both vary as $1/N$, results in the fourth-order free energy

$$\Delta F^{(4)} = \frac{128\,\pi^2}{N}\left[4\frac{K_1^{(4)}}{A_0} - kT\left(\frac{K_2^{(4)}}{A_0\,\kappa}\right)^2\right] . \tag{67}$$

Neither the sign nor the magnitude of any fourth-order elastic modulus are known to date. It is therefore impossible to predict whether $\Delta F^{(4)}$ is positive or negative. Only a positive $\Delta F^{(4)}$ can provide the desired cutoff at small vesicle sizes. In fact, a negative $\Delta F^{(4)}$ would favour the smallest sizes. One could then try to construct a cutoff from sixth-order elasticity and from the third term of the expansion of the logarithm of (65), i.e. from free-energy contributions proportional to $1/N^2$.

Let us assume here that $\Delta F^{(4)}$ is positive, i.e.

$$\Delta F^{(4)} = kTB/N , \quad \text{where} \quad B > 0 , \tag{68}$$

and neglect all terms of higher order in $1/N$. The size distribution (54) has then to be complemented by another Boltzmann factor so that

$$w(N) \sim \mathrm{e}^{-B/N}\,N\,\mathrm{e}^{-AN} . \tag{69}$$

The constant $A > 0$ can be determined by normalizing $w(N)$ to unity. (We do not try here to perform the necessary integration).

It seems worthwhile to draw the three size distributions (48), (55), and (69) for comparison with experiment. Measured distributions being generally plotted *versus* vesicle diameter Φ, we first transform the theoretical functions accordingly. Using $N = 2\,\pi\Phi^2/A_0$ and $w(\Phi) = w(N)\,\mathrm{d}N/\mathrm{d}\Phi$, one finds, in the same order,

$$w_1(\varphi) = 2(\Phi/\overline{\Phi^2})\exp(-\,\Phi^2/\overline{\Phi^2}) , \tag{70}$$

$$w_2(\varphi) = 8(\Phi^3/(\overline{\Phi^2})^2)\exp(-\,2\,\Phi^2/\overline{\Phi^2}) , \tag{71}$$

$$w_3(\Phi) \sim \Phi^3\exp(-\,B'/\Phi^2 - A'\,\Phi^2) . \tag{72}$$

where the first two distributions are normalized to unity and $A' > 0$, $B' > 0$. Only the last distribution contains a freely adjustable parameter, the « cutoff » parameter B'. Obviously, the distribution is shifted to larger diameters and turns sharper as B' increases. Examples of the three distributions are shown in figure 1. It should be noted that our diameter Φ refers to the middle of the bilayer rather than to the surface of the vesicle which is observed. Experimental data are still scarce and not very accurate. It appears an open question whether or not they represent thermal

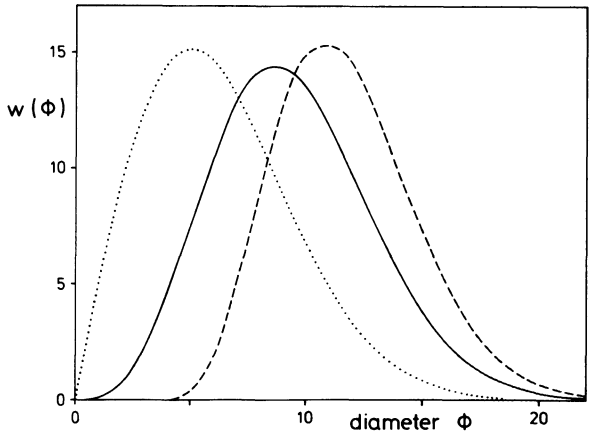

Fig. 1. — Theoretical size distribution functions in arbitrary units. Independent variable is the vesicle diameter Φ. Solid line : $w \sim \Phi^3\exp(-\,A\Phi^2)$; dashed line : $w \sim \Phi^3\exp(-\,B/\Phi^2 - A\Phi^2)$; dotted line (unrealistic case) : $w(\Phi) \sim \Phi\exp(-\,A\Phi^2)$. The curves represent equations (71), (72), and (70), respectively. The constants are $A = 0.02$, $B = 100$. B depends on material, A is governed by mean number of molecules per vesicle.

equilibrium at fixed vesicle numbers. The data of Larrabee [1] and Holzwarth and Groll [3] which were measured on two different synthetic lecithins are fairly well described by (72) and (71), respectively, while those of Duckwitz-Peterlein *et al.* [2] which were obtained with natural lipids lie between (71) and (70).

As pointed out at the end of section 2, a positive spontaneous curvature $c_s > 6/r_0$ destabilizes the sphere which at smaller c_s is in stable or metastable equilibrium. Even stronger positive spontaneous curvatures will convert the spheres into elongated shapes and finally cylinders [16], provided the spontaneous curvature, if chemically induced, is not associated with an osmotic pressure difference between outside and inside water. The consequences of a negative spontaneous curvature are different. The second term of the total elastic energy (25) of the sphere, that is $-8\pi\kappa c_s r_0$, will now increase with r_0. With (58) the term can be written in the form

$$-(8\pi A_0)^{1/2}\kappa c_s N^{1/2}. \qquad (73)$$

Since it rises as the square root of N its second derivative with respect to N is negative for $c_s < 0$. This implies a phase separation into smaller and larger vesicles. Ideally, just one large vesicle should coexist with small vesicles. We do not go into the details of this interesting effect, but note that the square root of N grows much larger than $\ln N$ which governs the effective rigidity. Therefore, even a very small negative c_s may induce phase separation while the remaining distribution of vesicles of reduced size may still obey (71) or (72). The present paper was devoted to the simplest and probably the most important case of vanishing spontaneous curvature.

5. Conclusion.

In the present paper we have exploited the logarithmic decrease of the effective rigidity with membrane area to deal with the problem of vesicle size distributions. As mentioned at the beginning, the concept of an effective rigidity is very recent and was treated only for planar membranes, with different results [4, 5]. The independent treatment given here has the advantage of being strictly in the framework of a quadratic approximation with respect to deformational amplitudes. This results from the fact that the spherical curvature is inherent in our system, the closed vesicle. The use of displacement modes in the statistical mechanics is conventional and convenient. However, local curvature being the strain, any « absolute » mode entropies should be expressed in terms of curvatures. On the other hand, there was no need for us to use mean square curvatures in deriving the entropy increase per mode due to energy taken from the sphere since in this case only the ratio of some mean square amplitudes is of interest.

We hope that a theory of size distributions will be useful in several respects. It should now be possible to distinguish equilibrium distributions from others which can be narrower (e.g. after chromatography) or wider. The theoretical curves suggest that it could be difficult to achieve or maintain very narrow distributions under conditions of thermal equilibrium. Future accurate measurements may furnish data on non-Hookean bending elasticity, although of a rather complex type. The theory could also be applicable to monolayer vesicles in certain microemulsions whenever spontaneous curvature is low. In fact, comparison between experiment and theory could be used as a test in this regard.

Being a quadratic approximation, the present theory presupposes membranes stiff enough to prevent dramatic deformations of the sphere. We do not know the limit of its applicability, but it is safe to say that the effective rigidity κ' should be distinctly larger than $kT/8\pi$ so that the bending elastic energy of the sphere is much larger than kT. As this very low rigidity is approached one may no longer expect vesicles to possess well-defined shapes.

Acknowledgments.

The study of vesicle size distributions was initiated by discussions with R. Pecora during a stay at Stanford University in 1982.

Note added in proof. — Safran's [9] deformation energies of the sphere do not differ from those given here. His final formulae do because of the additional constraint of fixed total enclosed volume. I am grateful to him for pointing this out to me.

In the mean time a number of experimental size distributions of unilamellar vesicles [17-21] came to my attention. Various materials, techniques of preparation, and methods of observation were used, the average vesicle diameters ranging up to 10 μm. The majority of plots agree fairly well with the parameter-free distribution (71). Marked deviations [18, 20] may be due in one case [18] to very extended electrostatic double layers, too wide to treat their effect as a contribution to elasticity.

A different theory of vesicle size distributions was proposed some time ago by Israelachvili *et al.* [22].

References

[1] Larrabee, A. L., *Biochemistry* **18** (1979) 3321.

[2] Duckwitz-Peterlein, G., Eilenberger, G. and Overath, P., *Biochim. Biophys. Acta* **469** (1977) 311.

[3] Grunewald, B., Frisch, W. and Holzwarth, J. F., *Biochim. Biophys. Acta* **469** (1981) 311 (for the method);

HOLZWARTH, J. F. and GROLL, R., unpublished (for the distribution).

[4] PELITI, L. and LEIBLER, S., *Phys. Rev. Lett.* **54** (1985) 1960.

[5] HELFRICH, W., *J. Physique* **46** (1985) 1263.

[6] MITOV, M. D., *C.R. Acad. Bulgare Sci.* **31** (1978) 513.

[7] HAUSER, H. and GAINS, N., *Proc. Natl. Acad. Sci. USA* **79** (1982) 1683;
HAUSER, H., GAINS, N. and MÜLLER, M., *Biochemistry* **22** (1983) 4775.

[8] TALMON, Y., EVANS, D. F. and NINHAM, B. W., *Science* **221** (1983) 1047;
NINHAM, B. W., EVANS, D. F. and WEI, G. J., *J. Phys. Chem.* **87** (1983) 5020.

[9] SAFRAN, S. A., *J. Chem. Phys.* **78** (1983) 2073.

[10] SCHNEIDER, M. B., JENKINS, J. T. and WEBB, W. W., *J. Physique* **45** (1984) 1457.

[11] HELFRICH, W., *Z. Naturforsch.* **28c** (1973) 693.

[12] HELFRICH, W., *Z. Naturforsch.* **30c** (1975) 841.

[13] HELFRICH, W. and SERVUSS, R. M., *Nuovo Cimento* **3D** (1984) 137.

[14] PECORA, R., in *Measurements of Suspended Particles by Quasi-Elastic Light Scattering*, ed. by Dahneke, B. (John Wiley & Son Inc. New York), 1983.

[15] BERTERO, M., BRIANZI, P., PIKE, E. R., DE VILLIERS, G., LAN, K. H. and OSTROWSKY, N., *J. Chem. Phys.* **82** (1985) 1551.

[16] DEULING, H. J. and HELFRICH, W., *J. Physique* **37** (1976) 1335.

[17] KIM, S. and MARTIN, G. M., *Biochim. Biophys. Acta* **646** (1981) 1.

[18] HAMMOND, K., REBOIRAS, M. D., LYLE, I. G. and JONES, M. N., *Biochim. Biophys. Acta* **774** (1984) 19.

[19] PARENTE, R. A. and LENTZ, B. R., *Biochemistry* **23** (1984) 2353.

[20] AURORA, T. S., LI, W., CUMMINS, H. Z. and HAINES, T. H., *Biochim. Biophys. Acta* **820** (1985) 250.

[21] PEREVUCNIK, G., SCHURTENBERGER, P., LASIC, D. D. and HAUSER, H., *Biochim. Biophys. Acta* **821** (1985) 169.

[22] ISRAELACHVILI, J. N., MITCHELL, D. J. and NINHAM, B. W., *J. Chem. Soc., Faraday Trans. II* **72** (1976) 1525.