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A probability concept about size distributions of sonicated lipid vesicles

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(1) The sonication procedure of preparation of small unilamellar vesicles is modelled as a process of uniform random fragmentation of the lipid aggregates. The vesicle size distribution evolving in this process is shown to be identical with the Weibull extremal probability distribution. (2) Size histograms of sonicated small vesicles of various phospholipid composition were obtained by using electron microscopy (negative staining). Their successful simulation with Weibull curves shows that theory agrees with experiment. A similarly good agreement is found also with size histograms obtained by freeze-fracture of phosphatidylcholine-cholesterol vesicles (Van Venetië, R., Leunissen-Bijvelt, J., Verkleij, A.J. and Ververgaert, P.H.J.T. (1980) J. Microsc. 118, 401–408). This analysis allows a refinement of some earlier conclusions about the effect of cholesterol on the size of the sonicated vesicles. (3) It follows from the theoretical model that the only intrinsic characteristic of the sonicated vesicles is the lower limit of their size. The other characteristics of the size distribution such as expectancy, dispersion, position and height of the maximum depend on the intensity of fragmentation. (4) It is concluded that the size distribution of sonicated small vesicles is completely determined by the procedure of their preparation and, therefore, the condition of thermodynamic equilibrium between aggregated and monomeric lipid is irrelevant in this case.

Introduction

The sizing of the phospholipid vesicles is important at least for two reasons. First, it is a methodological requisite allowing a more precise quantitative interpretation of the experimental results. This is especially important in optical and electrooptical studies, for determination of curvature effects in small unilamellar vesicles, etc. Second, but probably not less important, is the circumstance that the equilibrium size distribution of vesicles is closely related to the process of self-assembly and stability of lipid bilayers. This second point becomes obvious by noting the close similarity between formation of micelles and vesicles from amphiphilic molecules [1,2].

There can be two principally different approaches for analysis of the shape of the vesicle size distribution. The first one consistently realised by Israelachvili et al. [1–4] is confined to equilibrium size distributions as it starts from the thermodynamic equilibrium between aggregated and monomeric lipid. This approach can be equally applied both to vesicles and micelles. Although the physical foundations of this model seem to be invulnerable from a general point of view, its specific realisation might be assessed by comparison of its main predictions with experimentally obtained characteristics of the vesicle populations. In particular, this model predicts extremely narrow vesicle size distributions. Their dispersion of few Å is clearly at variance with the dispersions of tens and hundreds of Å of vesicles prepared by

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

sonication [5,6,19–23]. In contradiction to experiment appears to be also the predicted extremely strong dependence of the vesicle size on the length of the lipid molecules [7]. Although a detailed analysis of these discrepancies has not been made, it seems that there are good reasons to reexamine the extent of validity of the thermodynamic approach and to look for some other starting point in the explanation of the experimentally observed vesicle size distributions. This paper presents an alternative approach based on the obvious argument that the vesicle sizes depend on the procedure of preparation. In its frame, the condition of thermodynamic equilibrium between aggregated and monomeric lipid is irrelevant, since the observed size distributions are regarded as resulting from the process of vesicle preparation and not necessarily being in thermodynamic equilibrium. In order to substantiate this second approach we analyse the frequently used sonication procedure for preparation of small unilamellar vesicles and show that the resulting size distributions should be described by the Weibull extremal probability distribution. The theoretical conclusions are confirmed by a comparison with experimental size histograms of sonicated vesicles. This analysis allows reinterpretation and refinement of some conclusions about the sizes of sonicated small unilamellar vesicles.

Theory of the Weibull extremal probability distribution

The purpose of this section is to introduce the Weibull extremal probability distribution and to show its applicability for describing the sonication procedure of vesicle preparation.

Definition of the Weibull extremal distribution

The Weibull distribution [9–11] is not as well-known and widely used as the Gauss, Poisson and some other probability distributions, so it is pertinent to outline here its definition and main properties.

Let us consider a random variable d limited from below ($d_0 \geqslant 0$ is its lower limit) and obeying to some arbitrary probability distribution. Let us denote by $(d_1^k, d_2^k, \ldots, d_m^k;\ k = 1, \ldots, n)$ a number of sets of experimentally obtained independent values of d. These values are put in the order of their increase so that d_1^k is the smallest value in each set. The values d_1^k $(k = 1, \ldots, n)$ constitute a set of minimal terms and the problem is what is the probability distribution describing this set. It follows from the theorem of Gnedenko [14] that only one probability distribution exists which describes the defined above set of minimal terms. For sufficiently great m and n, this distribution is given by Eqn. 1:

$$P(d_{\min} \leqslant d) = 1 - \left(1 - C(d - d_0)^{\delta}\right)^n \tag{1}$$

Here C and δ are positive constants. After some transformations of Eqn. 1 (details not shown here) we obtain Eqn. 2 which shows explicitly the shape of the distribution of the minimal terms:

$$P(d_{\min} \leqslant d) = 1 - \exp\left[-\left(\frac{d - d_0}{\eta}\right)^{\delta}\right] \tag{2}$$

where $\eta^{\delta} = 1/(c \cdot n)$. Eqn. 2 is known also as the integral form of the Weibull extremal distribution. Eqn. 3 shows the density of the distribution:

$$f(d) = \frac{\delta}{\eta} \cdot \left(\frac{d - d_0}{\eta}\right)^{\delta - 1} \cdot \exp\left[-\left(\frac{d - d_0}{\eta}\right)^{\delta}\right] \tag{3}$$

The shape of the Weibull distribution (Eqn. 3) is shown in Fig. 1. The peak values $d_{\max}$, $f_{\max}$, and the lower limit d_0 are related to δ and η as shown in Eqn. 4a–c:

$$d_{\max} = d_0 + \eta\left(\frac{\delta - 1}{\delta}\right)^{1/\delta} \tag{4a}$$

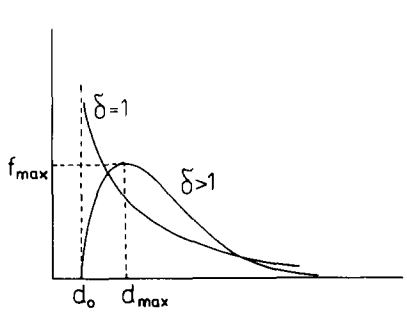

Fig. 1. The shape of the Weibull extremal probability distribution (Eqn. 3). A partial case ($\delta = 1$) is the well-known exponential distribution.

$$f_{max} = \frac{\delta}{\eta} \cdot \left(\frac{\delta - 1}{\delta}\right)^{(\delta-1)/\delta} \cdot \exp\left(-\frac{\delta - 1}{\delta}\right) \quad (4b)$$

$$f_{max} \cdot (d_{max} - d_0) = (\delta - 1) \cdot \exp\left(-\frac{\delta - 1}{\delta}\right) \quad (4c)$$

Eqn. 5a and b show the expectancy and dispersion of the Weibull distribution, respectively:

$$E = d_0 + \eta \Gamma\left(\frac{1}{\delta} + 1\right) \quad (5a)$$

$$D^2 = \eta^2 \left(\Gamma\left(\frac{2}{\delta} + 1\right) - \Gamma^2\left(\frac{1}{\delta} + 1\right)\right) \quad (5b)$$

where Γ is the gamma-function.

An important partial case of the Weibull distribution is the well-known exponential distribution following from Eqn. 3 at $\delta = 1$.

The sonication procedure modelled as uniform random fragmentation

The ultrasonic irradiation of a lipid suspension disrupts the larger vesicles and tends to concentrate the newly formed smaller vesicles in a small vicinity above the lower size limit. The vesicle size distributions obtained in this way are asymmetric due to the tail of larger vesicles and limited from below. As the same two geometric features are characteristic also for the Weibull distribution, it might be expected that Eqn. 3 presents a satisfactory empirical description of the sizes of the sonicated vesicles. However, it is desirable to carry the analysis further and to find out if, besides the empirical arguments, there is any real theoretical justification to expect that the sonication procedure leads to the distribution of the minimal terms. With this purpose in mind, we consider here an idealised model in which the disruption of the lipid particles by ultrasound is treated as a process of uniform random fragmentation. The simplest possible model is the unit segment shown in Fig. 2 which is split into smaller segments by n random numbers. We assume that the density of these random numbers is constant, i.e., their probability p_0 is linear with x. This kind of random fragmentation we call uniform random fragmentation. The probability distribution of the fragment sizes is given by Eqn. 6. It can be obtained by analyzing the kinetics of the uniform random fragmentation [24] and, independently,

Fig. 2. One-dimensional model of uniform random fragmentation. A unit segment ($ab = 1$) is split into fragments by n uniformly distributed random numbers. The distribution of the fragment sizes is given by Eqn. 6.

from theorem 3.1.2 in Ref. 12:

$$P(x) = 1 - (1 - x)^n \quad (6)$$

With increase of the number of fragmentations, Eqn. 6 transforms into the exponential distribution which is a partial case of the Weibull distribution.

The one-dimensional model clearly shows that a uniform random fragmentation leads to an extremal probability distribution of the fragment sizes, but it can be applied directly only to very few cases, for example, to the processes of fragmentation of linear polymers. When considering the random fragmentation of two- or three-dimensional particles, the model must be extended in order to account for the circumstance that the quantity which is randomly split by the external force (volume, surface) might differ from the observable quantity (diameter, area of cross-section). In that case, it becomes necessary to introduce the relation between these two quantities into Eqn. 6. In principle, this relation can be found by analyzing the specific properties of the fragmentation mechanism. However, the disruption of lipid particles by ultrasound seems to be a very complex process which is not clear enough as to allow a straightforward, unambiguous description revealing the relation between split and measured quantities. However, a simple mathematical argument allows to circumvent this difficulty and to substantiate directly the applicability of the Weibull distribution. Let us denote by $x = F(d)$ the relation between the quantity x which is uniformly split and the observable quantity d (the vesicle diameter). As the sonication concentrates the vesicles towards their lower size limit d_0, one can expand this relation in terms of powers of $d - d_0$ and retain only the first term $x \approx C \cdot (d - d_0)^{\delta}$. Substituting it in Eqn. 6, we obtain Eqn. 1 which transforms precisely into the Weibull distribution

(Eqns. 2 and 3). The conditions for validity of this argument are formulated in Ref. 24. These considerations reveal the physical meaning of the parameter δ. For example, $\delta = 1$ corresponds to equivalence between randomly split and measured quantities (one-dimensional case), $\delta = 2$ and $\delta = 3$ reflect relations of surface-diameter and volume-diameter types, respectively. Since for vesicles disrupted by ultrasound this relation is not known, the parameter δ is treated further as a free, adjustable parameter.

The vesicle sizes are limited from below since their diameter cannot become less than two-times the thickness of the lipid bilayer. More precise estimates of the lower limit can be obtained by taking into account curvature elasticity, packing restrictions, hydration shells, etc. [8,17]. However, it is conceivable that particles smaller than the lower limit may appear during sonication. These particles are necessarily short-living as they cannot close into a vesicular configuration in order to avoid the unfavorable contacts of the lipid acyl chains with the aqueous medium. They must quickly disappear by means of fusion with other aggregates after termination of the sonication. An important question is whether the size distribution in the final vesicle preparation would be drawn away from the Weibull distribution by this process. In order to answer it let us consider again the one-dimensional model shown in Fig. 2 and introduce into it an additional condition requiring that segments smaller than a fixed value d_0 (which is much smaller than the length of the initial segment) must 'fuse' randomly with the other segments so that in the final distribution only segments greater than d_0 will exist. If the fusion probability is independent of the segment sizes, it is clear that the total effect of the fusion process will reduce to a shape-preserving shift of the exponent along the horizontal axis at a distance d_0. This simple assumption about equal probabilities of fusion seems reasonable enough for the fusion of lipid aggregates which are similar in shape and size. In addition, other, more sophisticated assumptions about the vesicle fusion might be formulated which would also preserve the shape of the size distribution.

All these considerations show the reasons to expect that the size distribution of sonicated small unilamellar vesicles prepared by sonication must be well approximated by the Weibull extremal distribution.

Experimental

In order to check the theoretical conclusion stated in the previous section we obtained a number of size histograms of sonicated vesicles by means of electron microscopy (negative staining).

Preparation of small unilamellar vesicles

Egg L-α-phosphatidylcholine (Koch Light) and L-β,γ-dipalmitoyl-α-phosphatidylethanolamine (Fluka) were used, chromatographically pure. Phosphatidylserine ex brain (Serva) was purified on a column with silikon acid using chloroform/methanol (3 : 2, v/v) as an eluent.

Lipids were mixed as chloroform/methanol (9 : 1, v/v) solutions and the organic solvent was removed by rotary evaporation under nitrogen. Vesicles were prepared in Tricine buffer (pH 8.15) by sonication under nitrogen, 20 min for pure PC vesicles, 30 min for mixed vesicles. The phospholipid concentration was 0.5 mg/ml. The buffer concentration was varied between 1 and 100 mM. During the sonication, the sample was maintained at a temperature above the phase-transition temperature of the lipids used. The sample was then centrifuged at 100 000 $\times g$ for 30 min. An estimate of the vesicle sedimentation shows that the size histograms in Figs. 3–5 are not influenced by the

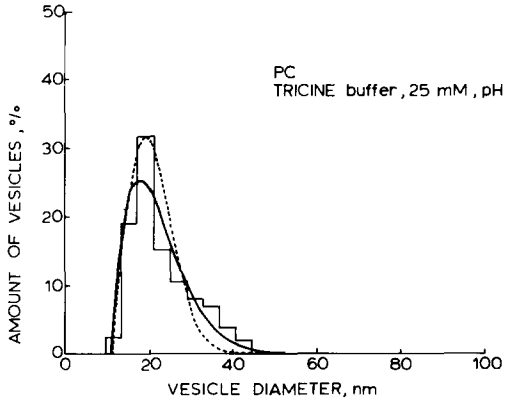

Fig. 3. Size histogram of sonicated PC vesicles. The dashed and dense lines are the initial and best-fit Weibull curves, respectively. For details see text and Tables I–III.

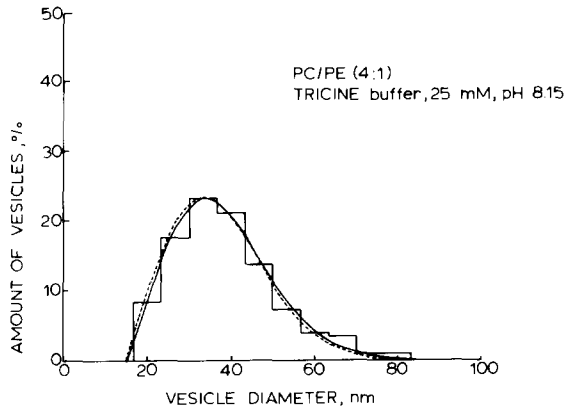

Fig. 4. Size histogram of sonicated PC/PE (4:1) vesicles, Designation as in Fig. 3.

centrifugation step. Pure PC and mixed PC/PE and PC/PS vesicles were prepared (Table III).

Negative staining

Negatively stained samples were prepared immediately after the centrifugation of the vesicles in order to avoid possible time-dependent changes in the suspension. A drop of the vesicle suspension was applied onto a formwar-covered copper grid (150 mesh) and in 3–4 min the excess was drawn off. A drop of 1% (w/v) uranyl acetate aqueous solution (filtered through a 0.25 μm Millipore filter) was then applied to the grid. A technical difficulty in obtaining good negative stains of liposomes is the bad spreading of the vesicles on the grid. Treating the grids with bacitracin [15], or

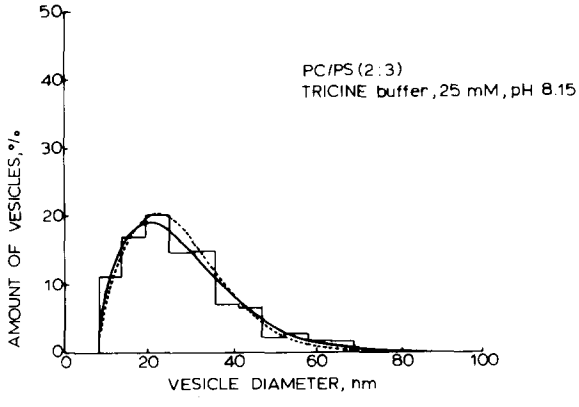

Fig. 5. Size histogram of sonicated PC/PS (2:3) vesicles. Designation as in Fig. 3.

with silica [6] usually permits a satisfactory spreading of liposomes on the support film. In our case, we used 0.015% octadecanol solution in hexane. A drop of 1 μl was applied on the surface of the uranyl acetate drop. In 1–2 min the excess of the lipid was drawn-off with a piece of filter paper and the grid allowed to dry. The micrographs were taken on a JEOL 100B electron microscope.

Size histograms of small unilamellar vesicles

This size histograms were obtained by manual measurements of the small unilamellar vesicle diameters on properly magnified micrographs. The diameter of the elongated vesicles was determined as an average value between maximal and minimal size. The resolution of this method is about 3 nm so the columns in the histograms are 3–6 nm wide. Each histogram contains between 800 and 1200 measurements of vesicle profiles. Size histograms were obtained for 15 samples: PC in 1,25, and 100 mM; PC/PE (19:1) in 10 and 25 mM; PC/PE (4:1) in 10,25, and 100 mM; PC/PE (3:2) in 1,10,25, and 100 mM; PC/PS (2:3) in 1, 10, and 100 mM.

Comparison of theory and experiment

Simulation of the experimental size histograms

The size histograms of small unilamellar vesicles were compared with the Weibull distribution by using a best-fit computer program. The quality of the fit was evaluated by the least-squares method. The initial fit introduced as an initial condition into the program was obtained from an estimate of the lower limit d_0, position d_{max}, and value f_{max} of the maximum in the experimental histograms. The values of δ and η were obtained from Eqn. 4a–c by using an iterative procedure and were used as initial fit values. The best-fit Weibull curves were found by adjusting of three parameters – δ, η, and d_0. Here are shown three of the 15 cases analyzed by this method. They include the worst and best fits (Figs. 3 and 4, respectively) and a relatively good fit (Fig. 5). The parameters of the Weibull curves are shown in Tables I and II. The range of discrepancy between the best fits and experiment in all 15 cases is illustrated by the values of the sums of the least-squares which are given in Table III. As is seen from Table III, the

TABLE I

PARAMETERS OF THE INITIAL WEIBULL CURVES FITTING THE EXPERIMENTAL SIZE HISTOGRAMS OF SONICATED VESICLES (DASHED LINES IN FIGS. 3–5)

Lipid composition	Buffer concn. (mM)	d_0 (nm)	d_{max} (nm)	f_{max} (1/nm)
PC	25	11.5	19.3	0.081
PC/PE (4:1)	25	15.0	33.3	0.035
PC/PS (2:3)	1	8.0	22.0	0.036

worst fit observed is probably not very representative as it is somewhat apart from the other cases

Drawbacks of the negative-staining technique

The negative-staining technique makes visible some contours traced out by the staining material around the outer vesicle surface. In fact, the histograms in Figs. 3–5 show the sizes of these contours and it is an open question to what degree they correspond to the real vesicle diameters. Although frequently discussed (see, e.g., Refs. 16, 18 and the papers cited there), this matter still remains controversial. Larrabee et al. [6] have found that artefacts such as shrinkage or flattening of the vesicles during drying are not important in vesicle size determinations. On the other side, it is often pointed out by other workers that the negative staining is not appropriate for a precise determination of the vesicle sizes [19–21]. Fortunately, this objection is not of primary importance in the present work, as our main objective is analysis of the shape of the size distribution. Although negative staining may not show the true absolute dimensions of the vesicles, there are hardly any serious reasons to believe that it significantly deviates the shape of the size distribution. In order to lend support to this statement, let us examine the distortions caused by some of the artefacts inherent to negative staining. A most important artefact is thought to be the vesicle collapse on the support surface in the course of drying [18,20,21]. However, simple geometrical considerations show that the size of a collapsed vesicle (flat or concave) is related to the radius of the corresponding spherical vesicle by a linear dependence of the type $R_{disk} = a \cdot R_{sphere} + b$. An experimental confirmation of this conclusion can be deduced from

TABLE III

MINIMAL VALUES OF THE LEAST-SQUARES SUMS $\theta = \Sigma(f_{exp} - f_W)^2$ * FOR 15 VESICLE SIZE HISTOGRAMS SIMULATED WITH WEIBULL CURVES

Lipid composition	Buffer concn. (mM)	θ ($\cdot 10^{-5}$ nm^{-2})
PC	1	7.34
PC **	25	68.38
PC	100	5.38
PC/PE (19:1)	10	7.96
PC/PE (19:1)	25	3.49
PC/PE (4:1)	10	4.53
PC/PE (4:1) **	25	1.95
PC/PE (4:1)	100	33.28
PC/PE (3:2)	1	16.66
PC/PE (3:2)	10	3.09
PC/PE (3:2)	25	12.63
PC/PE (3:2)	100	15.36
PC/PS (2:3) **	1	6.31
PC/PS (2:3)	10	20.69
PC/PS (2:3)	100	22.49

* f_{exp} are the heights of the columns; f_W are the values of the Weibull curves in the middles of the columns.

** The size histograms shown in Figs. 3–5.

TABLE II

PARAMETERS OF THE WEIBULL CURVES GIVING THE BEST FIT TO THE EXPERIMENTAL SIZE HISTOGRAMS OF SONICATED VESICLES (DENSE LINES IN FIGS. 3–5)

Lipid composition	Buffer concn. (mM)	d_0 (nm)	d_{max} (nm)	f_{max} (1/nm)	δ	η	E (nm)	D (nm)
PC	25	11.5	17.0	0.063	1.61	11.9	21.1	4.9
PC/PE (4:1)	25	15.3	33.3	0.035	2.08	25.6	37.3	11.2
PC/PS (2:3)	1	8.1	20.0	0.034	1.61	22.1	27.5	11.2

studies on the vesicle collapse during negative staining [21]. Another possible error might arise due to the lack of coincidence between visible contours and the edges of the flattened vesicles. If this effect is an 'edge' effect, as seems to be the case, and does not depend perceptibly on the vesicle size, then the size distribution will be merely shifted along the horizontal axis. On the whole, it seems likely that the difference between visible contours and real sizes of small unilamellar vesicles can be presented, to a good approximation, as a sum of a constant shift and a linear term. However, it is evident from Eqns. 2 and 3 that a distortion of this type modifies the values of the parameters η and d_0 but leaves unaffected the shape of the Weibull distribution. (Distortions of some other types can also be accomodated by changing the parameters but not the Weibull distribution itself.) On this basis we conclude that if a small unilamellar vesicle size histogram obtained by negative staining is described by the Weibull distribution then the real size distribution will be also described by a Weibull curve, but with somewhat different values of its parameters.

Simulation of size distributions obtained by freeze-fracture of PC-cholesterol vesicles

In order to test further the theoretical model, it is imperative to try to simulate with Weibull curves size distributions of sonicated small unilamellar vesicles obtained by using other methods free from the limitations of the negative staining. For this purpose, we use here the freeze-fracture data of Van Venetië et al. [19]. These authors took special precautions, such as very rapid freezing and counting only of spherical vesicles which are equatorially fractured, in order to avoid distortions specific for the freeze-fracture method. Their data fitted with Weibull curves are shown in Fig. 6. The parameters of the Weibull curves are summarized in Table IV.

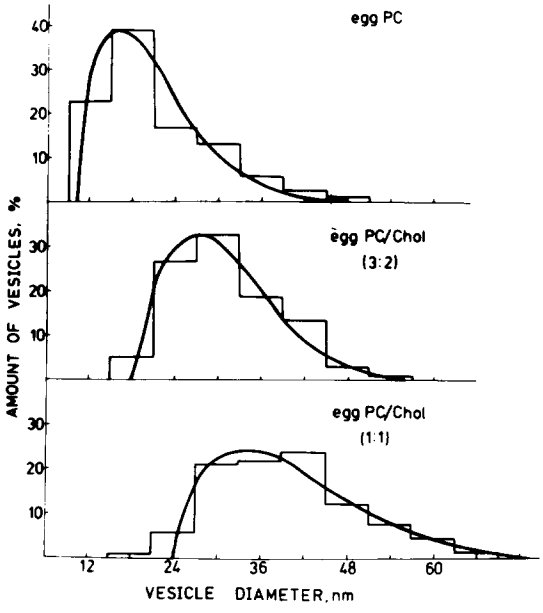

Fig. 6. Size histograms obtained by freeze-fracture of PC-cholesterol vesicles [19] fitted with Weibull curves. The parameters of the Weibull curves are given in Table IV.

Discussion

The main rigorous theoretical result briefly outlined in this paper is that the presentation of the sonication procedure as a process of uniform random fragmentation of the lipid aggregates leads to a Weibull distribution of the vesicle sizes. This treatment is tested by simulating experimental size histograms of sonicated small unilamellar vesicles with Weibull curves (Figs. 3–6). The very good fits

TABLE IV

PARAMETERS OF THE WEIBULL CURVES FITTING THE SIZE HISTOGRAMS OF PC-CHOLESTEROL VESICLES SHOWN IN FIG. 6

Lipid composition	d_0 (nm)	d_{max} (nm)	f_{max} (1/nm)	δ	η	E (nm)	D (nm)
PC	10	16	0.064	1.55	11.70	20.5	6.9
PC/cholesterol (3:2)	18	27	0.054	1.75	14.66	31.0	7.8
PC/cholesterol (1:1)	24	34	0.040	1.58	18.86	40.9	11.0

observed show that the theoretical model agrees very well with experiment. A similarly good agreement can be found also with the size histograms of sonicated small unilamellar vesicles published by Larrabee [22], Guiot et al. [5] and Forge et al. [20]. Of course, the experimental size histograms are not always smooth enough [20,23] and the roughness and scattering sometimes present in them may deteriorate the quality of the fit. Nevertheless, on the whole it seems indisputable that the Weibull curves quite satisfactorily describe the size distributions of sonicated small unilamellar vesicles. Although not being a definitive proof, this is a fairly strong indication that, at least within the accuracy limits of determination of small unilamellar vesicle size histograms, the vesicle fragmentation by ultrasound can be reasonably well modelled as a uniform random fragmentation. It is possible, however, that a more precise experimental measurement may reveal some systematic deviation from this conclusion. In that case, the Weibull distribution might serve as a standard for uniform fragmentation.

It is conceivable also that, besides the Weibull distribution, some other asymmetric profiles such as the Poisson distribution or the χ^2 distribution, can also be used for a simulation of small unilamellar vesicle size histograms. However, due to the lack of a theory relating them to the processes of random fragmentation, their supposedly successful application must be attributed to a close similarity (within the error limits) of their shape to the shape of the Weibull distribution. Another matter of interest is a comparison of the Weibull distribution with the log-normal distribution as the latter distribution also has theoretical justification [13]. However, especially in the case of ultrasonic irradiation the condition for its application seems to be far from reality. A discussion on this subject is presented elsewhere [24].

On the basis of the theoretical model some new conclusions about the main characteristics of the size distributions of sonicated small unilamellar vesicles can be formulated. The Weibull distribution is a three-parametric curve with d_0, δ, and η as parameters. Among them, only the lower limit d_0 of the vesicle size is an intrinsic vesicle characteristic which is determined by the membrane properties such as lipid packing and membrane elasticity and which is not influenced by the procedure of vesicle preparation. According to their interpretation given in the theoretical part of this paper the other two parameters, δ and η, reflect specific features of the fragmentation procedure. For this reason, all characteristics of the size distribution depending on them such as mean size and dispersion (Eqn. 5a,b), position and height of the maximum (Eqn. 4a–c) must be considered as reflecting not only the membrane properties but also the mechanism and intensity of fragmentation. A direct conclusion following from here is that the results of different studies cannot be compared on the basis of parameters such as the mean vesicle size because the differences encountered might be due to some unrecognized difference in the sonication protocols. The only parameter suitable for a comparative study is the lower limit d_0 so that its correct determination appears to be quite an important task.

This reasoning is relevant also in the investigations of the effect of lipid admixtures on the vesicle size. As an example, let us consider the data in Fig. 6 and Table IV which illustrate the effect of cholesterol on the size of egg PC vesicles. The addition of increasing amounts of cholesterol results in a shift towards greater values of d_0, d_{max} and E and flattening (decrease of f_{max} and increase of D) of the size distribution. The flattening of the distribution is a consequence of the increase of the parameter η. According to the definition of η, its increase reflects a less intense fragmentation of the particles. As the same sonication protocol has been used in all three cases shown in Fig. 6 [19], it remains to assume that membranes of higher content of cholesterol are less susceptible to fragmentation by ultrasound. This assumption is in accordance with the well-known increase of the rigidity of egg PC bilayers upon addition of cholesterol. Due to the flattening of the size distribution, the absolute increases of d_{max} and E are greater than the increase of d_0 (Table IV). In fact, they are sums of two effects – an increase of the intrinsic vesicle size enhanced by a less intense fragmentation presumably due to an increased rigidity of the membrane. Again, the only precise measure of the size of sonicated small unilamellar vesicles appears to be their lower size limit.

Although the data summarized in Tables II and

IV and the unshown data are not ample enough as to allow a sound statistical evaluation, they are sufficient for some preliminary estimates of the typical parametric values of the Weibull curves simulating the experimental distribution. It follows from Tables II and IV that the values of δ are usually between 1.5 and 2 (about 1.6 on the average). Such values indicate a rather complex geometrical nature of the vesicle parameter which is uniformly split. Its intermediate dimension certainly requires a separate investigation in order to be properly explained. With respect to the lower limit d_0, it is worth mentioning that very often its values determined from the Weibull curves are quite near to the doubled thickness of a lipid bilayer (Figs. 3, 5, and 6 top panel). However, the accuracy of determination of d_0 from the present histograms is not satisfactory. It cannot be easily improved as it cannot become less than the column width (3–6 nm). A trivial, but yet noteworthy consequence of the asymmetric shape of the Weibull distribution is that the mean size E is always greater than the most frequent vesicle size d_{max}. The difference between them is proportional to η and therefore depends on the intensity of fragmentation.

It is clear that the sonicated small unilamellar vesicles are not in thermodynamic equilibrium because, first, they are not stable with time and convert gradually into larger structures [22], and, second, they cannot form spontaneously. The present theoretical model is compatible with these observations as it treats the size distribution of the sonicated small unilamellar vesicles as being completely determined by the procedure of their preparation and totally discards the condition of thermodynamic equilibrium between aggregated and monomeric lipid.

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