Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 10, pp. 911–920, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Size distribution of liposomes by flow field-flow fractionation

MYEONG HEE MOON and J. CALVIN GIDDINGS*

Field-Flow Fractionation Research Center, Department of Chemistry, University of Utah, Salt Lake City, UT 84112, USA

Abstract: The applicability of field-flow fractionation (FFF) to the characterization of liposomes is discussed and theoretically described. Because of fundamental differences in their driving forces, sedimentation FFF and flow FFF measure different vesicle properties. Sedimentation FFF, although used previously to measure vesicle sizes and size distributions, is fundamentally a technique that measures the effective mass and mass distribution of particles. It is sensitive to small changes in the effective mass of either the biomembrane or its encapsulated load and thus is likely to be useful in characterizing such properties as drug loading, bimembrane volumes and areas, and distributions of these properties. Size characterization by sedimentation FFF can only be done by deducing size from effective mass. Flow FFF, by contrast, provides a direct measurement of vesicle size and size distribution. After demonstrating the high resolution and relative accuracy of size measurement of flow FFF by the separation of polystyrene latex standards, flow FFF was applied to two preparations of DSPC-DSPA liposomes that were sonicated under different temperature conditions. Fractograms and size distributions are reported as a function of sonication time. The rapid elimination of a large diameter tail to the distribution is shown to constitute a major mechanism for distribution narrowing. Finally, results are provided bearing on the reproducibility of size distribution measurements by flow FFF.

Keywords: *Flow field-flow fractionation; field-flow fractionation; size distribution of liposomes; FFF characterization of liposomes; liposome characterization.*

Introduction

Phospholipid liposomes have been extensively used as a material for drug delivery [1, 2] and as a basic model for the study of biological membrane structure and function [3, 4]. The size distribution of liposomes has an important bearing on their properties including vesicle stability, interaction with cells, entrapment efficiency and osmotic release, and ultimate drug delivery rates [5–12]. Accordingly, many size distribution studies have been carried out on liposomes using methods such as the freeze-fracture technique of electron microscopy [6, 9, 11–13], light scattering [6], and size exclusion chromatography [7]. While these approaches have been developed to a high level of sophistication and effectiveness, some important shortcomings remain. Thus the freeze-fracture method of electron microscopy consists of a rather lengthy experimental procedure whose results are complicated by the nonequatorial fracture of vesicles [12]. Light scattering techniques are much more convenient and they generally provide valid average diameters (see, however, ref. 12) but they cannot readily distinguish subpopulations of various sizes in a polydisperse mixture and thus these techniques cannot provide detailed size distribution curves. Size exclusion chromatography, while theoretically capable of resolving size subpopulations, suffers from particle-pore wall interactions and the difficulty of working with pore structures sufficiently large and with adequately high mass transport rates to handle larger particles or polymers [14].

In recent years field-flow fractionation (FFF) has been increasingly applied to the measurement of the size and mass distributions of particles ranging in size from protein molecules to biological cells [15–27]. These techniques are characterized by operational flexibility, high resolution, reasonably high speed, small sample requirements (a few microlitres), and the convenience typical of elution techniques such as chromatography. In addition, narrow fractions can be collected for further study. Despite such intrinsic advantages, the application of FFF to the characterization of liposomes has been very limited.

*Author to whom correspondence should be addressed.

FFF is a family of techniques. The primary FFF technique used so far for liposome characterization is sedimentation FFF, a member of the FFF family that utilizes a sedimentation field [16, 19]. Sedimentation FFF should be applicable to the characterization of a number of important properties of liposomes including membrane volume and area, density, drug loading, and size (see later). Most of these capabilities have not been exploited, with most previous studies focused on liposome size distributions. Caldwell *et al.* were able to show a bimodal size distribution with the larger mode having vesicles of mean diameter 0.86 μm; they also determined that these vesicles have a density of 0.9997 g ml^{-1} [28]. Kirkland *et al.* used sedimentation FFF to determine the size distribution of various liposome preparations with mean vesicle sizes ranging from 0.026 to 0.23 μm [29]. Further size distribution work was done by Dreyer *et al.* [30].

While sedimentation FFF is a versatile technique for particle characterization and has the potential to measure a variety of properties of liposomes of interest, it is not the most straightforward technique for measuring liposome size and size distributions. Another technique, flow FFF, provides a more direct measurement of the size and size distribution of colloidal materials [20, 31], but has not until now been applied to liposomes. Sedimentation FFF and flow FFF are complementary techniques that have the capability of providing a broad base of information on liposomes including not only size and size distribution but, as noted above, density and drug loading. The reasons for this complementarity are related to the fundamental nature of FFF, which is summarized below.

FFF is an elution technique in which particles and macromolecules are separated in a thin ribbonlike channel and then eluted into a detector and, if desired, a fraction collector [32, 33]. Separation is achieved by the application of a field in a direction perpendicular to the channel flow axis and thus perpendicular to the axis of separation. (The right-angle orientation of the field relative to the axis of separation distinguishes FFF from the more conventional techniques of sedimentation and electrophoresis.) The principles of FFF are illustrated in Fig. 1. Here an external field is used to drive vesicles (or other particles) toward one wall of the ribbonlike channel termed the accumulation wall. Generally the larger the particle, the stronger the force exerted on it by the field and the closer it is driven to the accumulation wall at equilibrium. The laminar flow in the channel assumes a parabolic flow profile between the major channel walls. This flow sweeps the vesicles downstream toward the channel exit and a detector. However, because of the parabolic flow profile, subpopulations of different sizes are driven downstream at different velocities according to their proximity to the accumulation wall. The smallest vesicles are driven at the highest mean velocity and thus elute first. Larger vesicles, which are driven closer to the accumulation wall by the field-based forces, are caught up in slower lamina and thus elute later. Because of the uniformity of the channel and the even application of the field, the retention times of particles of different size and mass can be calculated theoretically. More importantly for particle characterization work, size and mass can be deduced from measured retention times, making it possible to obtain size and mass distributions from the observed

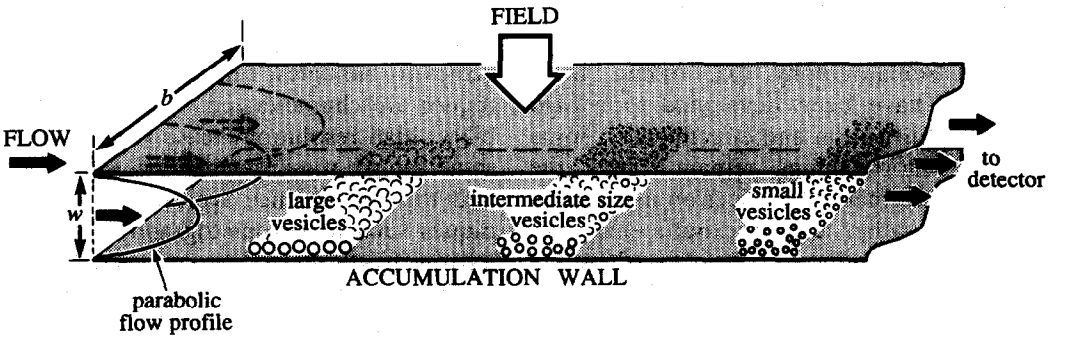

Figure 1
Section of FFF channel showing mechanism of separation. Channel thickness w, typically only a few hundred μm, is exaggerated here for purposes of illustration.

distribution in retention time, that is, the detector response versus time curve.

A number of different fields have been used in conjunction with FFF, giving a variety of techniques that include sedimentation FFF, flow FFF, thermal FFF, and electrical FFF [32, 33]. Each different field exerts a different kind of driving force on the vesicles, causing them to be differentially retained and separated on the basis of different properties. Sedimentation FFF achieves separation that depends on differences in the effective mass (true mass less buoyant mass) of particles. For particles consisting of homogeneous masses of solid or liquid material (the latter being emulsions), the effective mass is directly related to the particle size, in which case the detector response curve (fractogram) can provide a size distribution curve. However, for shell-like structures the relationship between mass and size is not always so straightforward as will be further discussed in the Theory section. (Because effective mass is density-dependent, particle densities can also be measured [34].)

In flow FFF the driving force on particles is generated by a cross flow of liquid directed perpendicular to the channel flow axis. This requires that a second stream of liquid be driven through the channel, but the second (cross flow) stream enters and exits across the major walls of the channel, which accordingly must be permeable. These walls are generally made up of membrane and frit elements as illustrated in Fig. 2.

The driving force in flow FFF is a Stokes force that depends only on the size of the particle being retained [31]. Thus as will be shown in the Theory section, the measurement of retention provides a measurement of size with no assumptions required concerning particle structure, homogeneity, or density. The elution profile yields the size distribution in a similarly straightforward manner.

The objectives of this paper are to (1) evaluate the general effectiveness of various FFF techniques, especially sedimentation FFF and flow FFF, in characterizing multiple properties and distributions of liposomes, (2) establish a distinction between the type of information gained from the application of sedimentation and flow FFF to liposomes, and (3) provide meaningful examples of the determination of size distribution curves for liposomes using flow FFF.

Theory

According to the standard model of FFF, the retention time t_r of any given particle subpopulation relative to the void time t^0 (the passage time of unretained material) is related to the retention parameter λ by the following expression [16, 33]

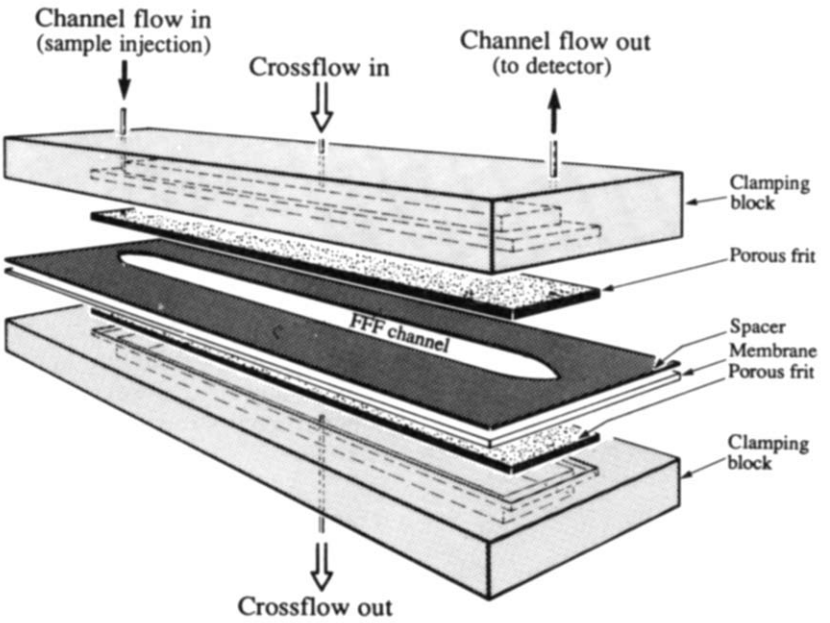

Figure 2
Diagram showing the layered construction of a flow FFF channel.

$$\frac{t_r}{t^0} = \frac{1}{6\lambda[\coth(1/2\lambda) - 2\lambda]} . \quad (1)$$

This equation has the unique property that when λ is small (i.e. $\lambda < 0.05$), t_r/t^0 can be well approximated by the limiting equation

$$\frac{t_r}{t^0} \cong \frac{1}{6\lambda} . \quad (2)$$

We note that while the more rigorous form of equation (1) is used for all size calculations in this paper, equation (2) presents a much clearer picture of the FFF measurement process and its interpretation.

In order to utilize equations (1) and (2), λ must be related to the particle size or to other characteristics of the particle subset. According to FFF theory, the retention parameter λ is related to the force F exerted by the field on a single particle of the homogeneous subset by

$$\lambda = \frac{kT}{Fw} , \quad (3)$$

where k is Boltzmann's constant, T is absolute temperature, and w is channel thickness.

For flow FFF, F is equal to the Stokes force exerted by the carrier fluid flowing around the particle at the cross flow velocity U

$$F = 3\pi\eta Ud, \quad (4)$$

where η is viscosity and d is the diameter of the particle (or the Stokes diameter if the particle is nonspherical). It is convenient to express U in terms of the experimentally realized cross flow rate $\dot{V}_c$ of carrier liquid permeating across the channel

$$U = \frac{\dot{V}_c}{bL} , \quad (5)$$

where b is the breadth of the channel and L is its effective (volume-based) length.

When equations (3), (4) and (5) are sequentially substituted into equation (2) and the product bLw replaced by the channel void volume V^0, we obtain

$$\frac{t_r}{t^0} \cong \frac{\pi\eta w^2}{2kT} \frac{\dot{V}_c}{V^0} d. \quad (6)$$

Equation (6) shows that the retention time t_r is approximately proportional to particle diameter d with a proportionality constant that can be readily varied by changes in the cross flow rate $\dot{V}_c$. Thus the sequence of particles eluted from flow FFF, proceeding from the smallest to the largest, represents a nearly linear particle diameter spectrum. The linear relationship between t_r and d ensures that small differences in d can be discerned and the relative amount of the close-lying subpopulations reported by the detector. Clearly, the particle diameter eluting at any given time t_r can be calculated from equation (6). The actual conversion of the fractogram into a particle size distribution curve is accomplished by laboratory software based on equation (1).

While the experimental results obtained here are based entirely on flow FFF, it is important to show how flow FFF compares with sedimentation FFF and to illustrate their possibility for playing complementary roles in liposome characterization. In sedimentation FFF, F is given by

$$F = m'G, \quad (7)$$

where m' is the effective mass (true mass less buoyant mass) of the particle and G is the field strength measured as acceleration. For a shelled structure such as a liposome, m' is the sum of the effective shell mass and the effective mass of the internal liquid enclosed by the shell

$$m' = m'(\text{shell}) + m'(\text{internal}). \quad (8)$$

The retention time is sensitive to both of these masses as seen by substituting equations (3), (7) and (8) into equation (2)

$$\frac{t_r}{t^0} = \frac{Gw}{6kT}[m'(\text{shell}) + m'(\text{internal})]. \quad (9)$$

This equation shows that if the entrained liquid is neutrally buoyant, t_r is proportional to m'(shell), which is in turn proportional to shell volume and area. Therefore for simple unilamellar shell structures, m'(shell) and t_r will increase with the square of liposome diameter. For multilamellar structures, t_r should provide a measure of the total membrane area. However, for this purpose m'(shell) must be sufficiently large to induce measurable liposome retention, which requires that $\lambda \leq 0.2$. (This treatment of vesicle retention is analogous to that dealing with adsorbed films on particles.

In some cases it has been shown that the properties of adsorbed films can be measured with great sensitivity using sedimentation FFF [35, 36].)

If the fluid within the liposome has a different density from the surrounding liquid, then m'(internal) [which will usually dominate over m'(shell)] will be proportional to the particle volume and t_r will therefore provide a measure of that volume. Subsequently the volume distribution can be obtained.

If drugs loaded into the liposome cause a proportionate change in internal liquid density, then m'(internal) and thus t_r will reflect the amount of drug loaded per vesicle. The fractogram will then represent the distribution of drug loading across the entire vesicle population. Thus while sedimentation FFF does not provide a direct measure of liposome size as does flow FFF, it is capable of probing other properties and distributions of importance in liposome science and technology. This capability has not been generally utilized nor even recognized.

We observe that another FFF technique, electrical FFF, is theoretically capable of measuring the charge distribution among vesicles, thus providing a further complement of information about liposome properties. However, at the present time electrical FFF is a less mature technology than either sedimentation or flow FFF.

Materials and Methods

Preparation of liposomes

The liposome samples used in this study were prepared in our laboratory by the solvent evaporation method [3, 10]. A mixture of 12 micromoles of DSPC (distearoyl phosphatidyl choline) and 0.5 micromoles of DSPA (distearoyl phosphatidic acid) was dissolved in 5 ml of $CHCl_3$ in a round-bottom flask. The phospholipids were purchased from Sigma (St Louis, MO, USA). The rotary evaporator and the flask containing the lipid solution were purged with N_2. The solvent was evaporated under vacuum by rotating the flask at high speed in a water bath at 70°C and a thin bilayer film of lipid was formed on the flask wall. The vacuum was maintained for 1 h to evaporate all traces of the solvent. Then 5 ml of doubly distilled and deionized water preheated to 70°C was added to the flask. The flask was rotated for 10 min while held at 70°C with no vacuum. Large multilamellar vesicles (MLV) were formed during this process, visible as a milky suspension. This vesicle mixture was transferred to a glass vial by using a prewarmed micropipette and subjected to sonication for the transformation of MLV into SUV (small unilamellar vesicles) utilizing a Model W-225R (microtip) Sonicator Cell Disruptor from Heat Systems-Ultrasonics (Plainview, NY, USA). The sonicator was operated at 50% duty cycle. During sonication, small amounts of the liposome suspension were removed for examination at 5, 15 and 35 min. As the sonication proceeded, the liposome suspension became a clear solution. After sonication each vial was kept in a drawer overnight for stabilization. All flow FFF measurements of size distribution were made within a week of preparation.

Two samples were prepared using the above steps but the procedure was varied slightly in the two cases. For preparation I the sonication vial was placed in a water bath held at 70°C. For preparation II no water bath was used and thus sonication began at 23°C.

Flow FFF

The flow FFF channel system used in this study is similar to that used in previous studies [18] and is nearly identical in structure to that employed in the F1000 Universal Fractionator from FFFractionation (Salt Lake City, UT, USA). As shown in Fig. 2, the channel system is constructed as a sandwich of multiple layers including the thin spacer from which the channel volume is cut, the membrane serving as the accumulation wall, and the symmetrical clamping blocks. Each block has a frit element incorporated in its structure to supply and receive the cross flowstream. The channel length L and breadth b of the channel are 26.5 and 2.0 cm, respectively. (The tip-to-tip length L_{tt} is 28.5 cm. This exceeds L because $L = V^0/bw$ is the effective length for a channel with the triangular endpieces squared off.) The thickness of the Teflon spacer used was 254 μm. The accumulation wall (shown in Fig. 2) consists of a sheet of YM-10 membrane from Amicon (Beverly, MA, USA) which is made of regenerated cellulose. Due to the compression of the membrane when clamped between the two channel blocks, the actual channel thickness is reduced to 206 μm. The modified channel thickness was calculated from the void volume (1.09 ml) which was measured by the rapid breakthrough method [37].

Experimental procedure

The flow FFF channel was orientated vertically to eliminate any possibility of sedimentation forces contributing to FFF retention. The flowing carrier liquid in the channel was doubly distilled and deionized water containing 0.05% SDS and 0.02% NaN_3 as a bactericide. This background liquid was delivered to the channel by two pulse-free syringe pumps constructed in-house. The polystyrene latex beads used to evaluate resolution were obtained from Duke Scientific (Palo Alto, CA, USA). Five to 10 μl volumes of each latex standard, diluted 100 times, were injected into the channel flowstream using a microsyringe and a septum injector. Following entry of the sample into the channel, the channel flow was halted by diverting the carrier stream to an outside bypass. The stopflow period, which was slightly longer than the time necessary to drive one void volume across the channel by the cross flowstream, was 60 s. Upon the completion of the stopflow procedure, the channel flow was resumed and the run started. The channel was operated at the ambient laboratory temperature of 23 ± 0.5°C.

The liposome samples were injected into the channel and subjected to the same stopflow procedure as used for the polystyrene latex beads. The injection volumes were 10–20 μl of the undiluted liposome suspension.

All eluted particles were monitored at 254 nm by an Altex Model 153 UV detector from Beckman Instrument (Fullerton, CA, USA), which acts as a turbidity detector. The resulting fractograms (signal response versus time curve) were converted to particle size distribution curves based on equation (1) and laboratory software. However, no light scattering correction was applied in obtaining the size distribution curves because existing software does not account properly for the shell-like vesicle structures. The absence of this correction will mean that the smaller vesicle diameters are under represented in the size distribution curve because of their weaker scattering signals [22, 38].

Results and Discussion

In order to examine both the retention time–diameter relationship and the resolving power of the flow FFF system, and thus to test its ability to discern and report small differences in particle diameter (a prerequisite for accurate sizing across the size distribution range), mixtures of monodisperse polystyrene latex beads were used as samples. Figure 3 shows the fractogram of a mixture of polystyrene beads of five different sizes (as specified in the figure) acquired at a channel flow rate of $\dot{V} = 5.30$ ml min^{-1} and a cross flow rate of $\dot{V}_c = 1.45$ ml min^{-1}. (The first sharp peak appearing in this and subsequent fractograms is a pressure transient caused by injection. Because of its early appearance, around time t^0, it does not interfere with size analysis.)

The fractogram shown in Fig. 3 demonstrates that flow FFF is capable of resolving broad size distributions into narrow subpopulations of different particle size. The retention time of each size of latex is reasonably well represented by equation (1) with λ expressed by equations (3), (4) and (5). This is shown by the comparison of experimental and theoretical retention times as shown in Table 1. The

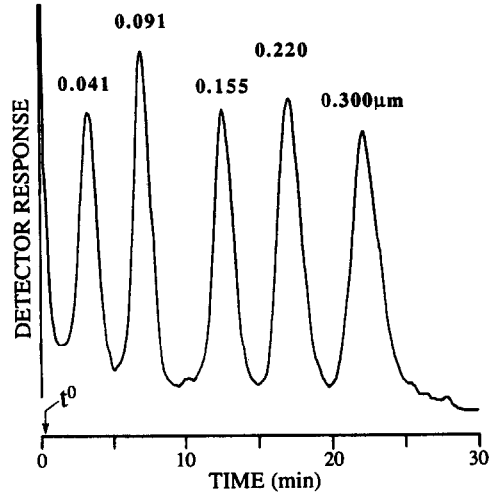

Figure 3
Separation of submicron polystyrene latex beads as an illustration of the resolving power of flow FFF. Flow conditions are $\dot{V} = 5.30$ and $\dot{V}_c = 1.45$ ml min^{-1}.

Table 1
Comparison of experimental and theoretical retention times of polystyrene latex standards

d (μm)	t_r (min) Exp.*	Theory
0.040	3.33	3.02
0.091	7.11	6.77
0.155	12.71	11.48
0.220	17.26	16.27
0.300	22.41	22.16

* Experimental t_r less 0.02 min allowing for dead time.

agreement is better than expected for the larger particles since their retention time should be reduced by the onset of steric effects [33]. It appears that the latter effects are offset by some other factor such as a channel thickness somewhat greater than that measured. Accordingly, the software was modified to remove steric effects from the diameter calculations.

Figure 4 shows three fractograms obtained for liposome preparation I subjected to different sonication times. The experimental conditions are identical to those reported for the separation of latex beads in Fig. 3 and thus particles eluting at the same time in the two cases should have virtually identical diameters. It is clear from the fractograms in Fig. 4 that an increase in sonication time shifts the liposome peak to the left (to smaller diameters) and causes a narrowing of the distribution. These changes are shown more explicitly in terms of vesicle diameter by the normalized size distribution curves derived from the three fractograms in Fig. 4 and presented in Fig. 5. The peak of the distribution shifts from 0.071 μm at 5 min sonication time to 0.060 μm at 15 min and 0.049 μm for 35 min of sonication. (Since these peaks are relatively sharp, this conclusion would not be significantly altered by applying a light scattering correction to the detector signal [38].)

The most important feature of Fig. 5 is that it provides a detailed picture of how the size distribution shifts with ongoing sonication. It is

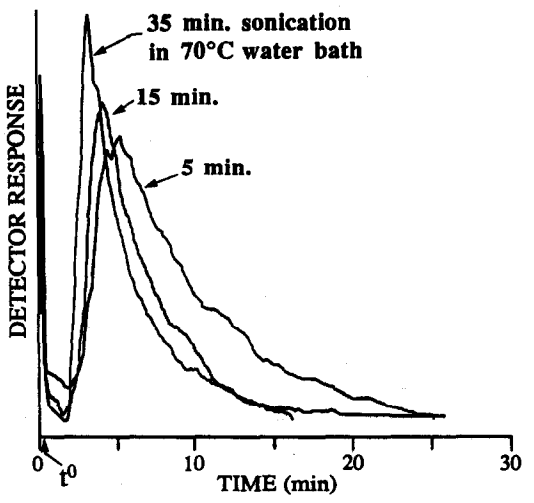

Figure 4
Fractograms of liposomes prepared by procedure 1 at different sonication times. Flow conditions are the same as reported for Fig. 3.

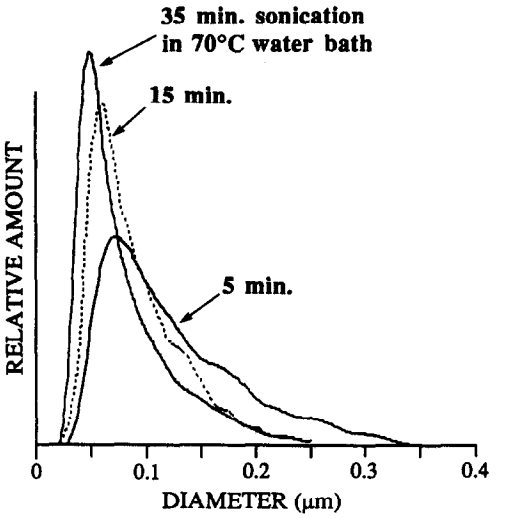

Figure 5
Size distribution curves obtained from the fractograms of the three liposome samples reported in Fig. 4.

clear that the larger vesicles (above ~0.2 μm diameter) are most rapidly decimated by sonication, being virtually eliminated after 15 min sonication time. As the sonication time extends to 35 min, the relative population of vesicles between 0.1 and 0.2 μm is somewhat decreased but not eliminated. Thus in the final 20 min sonication period (extending from 15 to 35 min), the distribution sharpens to only a modest degree and shifts slightly toward smaller diameters.

Similar results are found for liposome preparation II, which was sonicated without a water bath and thus subjected to a rising temperature (due to the energy input of the sonicator) starting at 23°C rather than a near-constant temperature of 70°C. Fractograms acquired at 5 and 35 min sonication times are shown in Fig. 6(a). The conditions are the same as those employed in obtaining the fractograms of Figs 3 and 4 and thus the diameters eluting at any given time are identical in the three cases. The corresponding size distributions are shown in Fig. 6(b). Also shown for comparison are the corresponding size distributions for preparation I as reported in Fig. 5. There are no major differences in the size distributions produced by the two methods of preparation, but some small differences are indicated. After 5 min sonication time, the liposome population of preparation I appears to have shifted to slightly smaller diameters than those of preparation II but the large diameter tail remains virtually identical in the two cases. The final

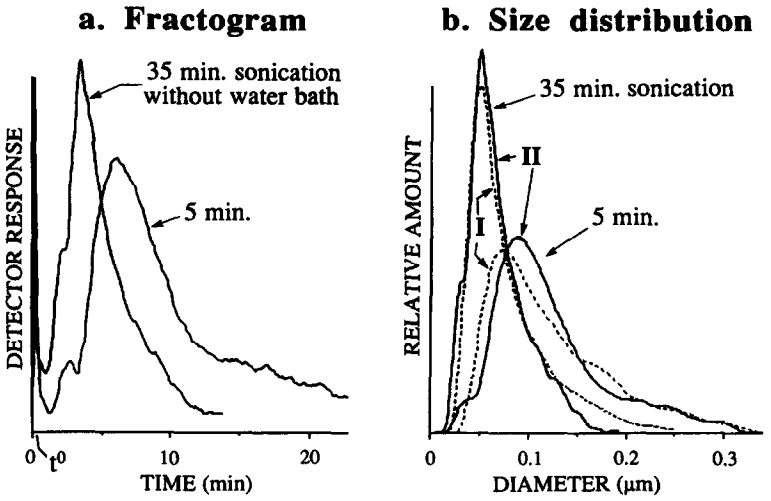

Figure 6
Flow FFF of liposome preparation II (without water bath). The size distribution curves for these samples (labelled as II) are compared to the size distribution curves of liposome preparation I reported in Fig. 5.

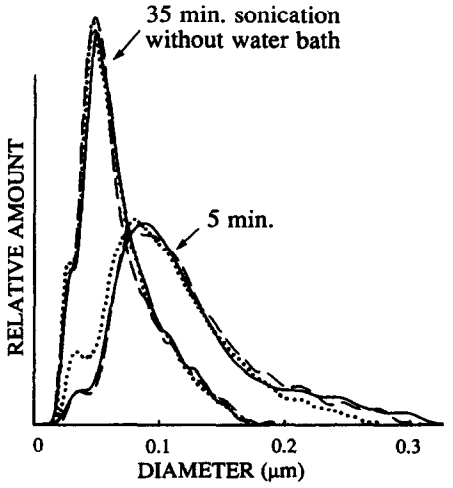

Figure 7
Reproducibility of size distribution curves obtained for different flow FFF runs on liposome preparation II.

distributions (after 35 min sonication time) are virtually identical except for a slightly elevated tail in the distribution for I between 0.15 and 0.25 μm diameter.

While we did not specifically pursue the question of the reality of the difference in the tails of the two distributions, we carried out further investigations on preparation II to examine the reproducibility of the size distribution curves. Figure 7 shows two sets of three curves, all based on the same conditions reported above. Two of the curves for the 35 min sonication time were obtained from sequential runs whereas the solid line was obtained 1 day later. There is little difference in the curves except for a barely perceptible shift of the solid curve to the right, which could conceivably represent sample aging but may simply represent the small variations observed from run to run. These curves are in agreement with that shown in Fig. 6(b) for preparation II sonicated 35 min in showing that virtually no vesicles remain above 0.2 μm diameter.

The agreement found between the three curves based on successive runs of samples subjected to 5 min sonication time display slightly larger variations than those corresponding to 35 min sonication. However, the agreement is still quite satisfactory.

All of the curves in Fig. 7 show a slight shoulder on the left hand side. This is probably an artifact of the stopflow injection procedure. It is anticipated that this artifact, as well as the initial pressure transient, can be eliminated using the newer technique of frit inlet injection [39].

Conclusions

A combination of theoretical and experimental evidence, some of which is presented here, suggests that flow FFF should be an effective technique for resolving liposome populations into close-lying subpopulations differing only in size. The size distribution curves obtained here for liposome samples subjected to different sonication times is consistent with that evidence and is particularly revealing on the rate of disappearance of the large diameter tail of the distribution with

increasing sonication time. Flow FFF would likely be equally informative concerning aging–stability properties of liposomes and association complexes that might be formed with other particles.

While flow FFF provides direct and rather unambiguous size distribution information, it is important to point out, as we have attempted here, that other FFF techniques are capable of yielding other forms of information about liposome preparations. In particular, sedimentation FFF provides effective mass distributions rather than size distributions. While effective mass can in some cases be converted to size, the measurement of this parameter and its distribution may be more useful in probing drug loading, bilayer volumes and areas, and their distributions. Sedimentation FFF also promises to be a useful tool for probing liposome complexes with other particulate matter.

Finally we note that the serial combination of flow and sedimentation FFF, where fractions are collected from one system and subjected to another, would multiply the information obtainable from either technique alone. Fractions collected from flow FFF would contain vesicles all of the same size. Any significant broadening of this fraction when subjected to sedimentation FFF would represent mass nonhomogeneity, perhaps due to variations in drug loading or in the concomitant existence of vesicles of the same size having unilamellar and multilamellar structures. A great deal more work is clearly needed to explore these possibilities and develop the appropriate methodology.

Acknowledgements — This work was supported by Public Health Service Grant GM10851-35 from the National Institutes of Health. The authors acknowledge the advice of Dr Yonghee Kim of the Department of Pharmaceutics at the University of Utah and Dr Sabrina Hoffstetter-Kuhn of Ciba–Geigy (Basel, Switzerland) on the preparation of liposomes.

References

[1] J. Weinstein, R. Blumenthal, S.O. Sharrow and P. Hankart, *Biochim. Biophys. Acta* **509**, 272–288 (1978).
[2] G. Gregoriadis (Ed.), *Liposome Technology, Vol. III, Targeted Drug Delivery and Biological Interaction*. CRC Press, Boca Raton (1984).
[3] A.D. Bangham, J. Gier and G.D. Greville, *Chem. Phys. Lipids* **1**, 225–246 (1967).
[4] D. Papahadjopoulos, S. Nir and S. Ohki, *Biochim. Biophys. Acta* **266**, 561–583 (1972).
[5] D. Chapman, in *Preparation of Liposomes, Vol. I, Preparation of Liposomes* (G. Gregoriadis, Ed.), Chapter 1. CRC Press, Boca Raton (1984).
[6] M. Wong and T.E. Thomson, *Biochemistry* **21**, 4139–4143 (1982).
[7] M. Wong, F.H. Anthony, T.W. Tillack and T.E. Thomson, *Biochemistry* **21**, 4126–4132 (1982).
[8] J.H. Prestegard and B. Fellmeth, *Biochemistry* **13**, 1122–1126 (1974).
[9] S.E. Schullery, C.F. Schmidt, P. Felgner, T.W. Tillack and T.E. Thomson, *Biochemistry* **19**, 3919–3923 (1980).
[10] A.L. Larrabee, *Biochemistry* **18**, 3321–3326 (1979).
[11] F. Szoka, F. Olson, T. Heath, W. Vail, E. Mayhew and D. Papahadjopoulos, *Biochim. Biophys. Acta* **601**, 559–571 (1980).
[12] P. Guiot and P. Bandhuin, in *Preparation of Liposomes, Vol. I, Preparation of Liposomes* (G. Gregoriadis, Ed.), Chapter 12. CRC Press, Boca Raton (1984).
[13] M.J. Hope, M.B. Bally, G. Webb and P.R. Cullis, *Biochim. Biophys. Acta* **812**, 55–65 (1985).
[14] J.C. Giddings, in *Advances in Chromatography*, Vol. 20 (J.C. Giddings, E. Grushka, J. Cazes and P.R. Brown, Eds), pp. 217–258. Marcel Dekker, New York (1982).
[15] J.C. Giddings, M.N. Myers and J.F. Moellmer, *J. Chromatogr.* **149**, 501–517 (1978).
[16] J.C. Giddings, G. Karaiskakis, K.D. Caldwell and M.N. Myers, *J. Colloid Interface Sci.* **92**, 66–80 (1983).
[17] F.-S. Yang, K.D. Caldwell, M.N. Myers and J.C. Giddings, *J. Colloid Interface Sci.* **93**, 115–125 (1983).
[18] S.K. Ratanathanawongs and J.C. Giddings, in *Particle Size Distribution II: Assessment and Characterization* (T. Provder, Ed.), pp. 229–246. ACS Symp. Ser. No. 472, American Chemical Society, Washington, DC (1991).
[19] J.C. Giddings, M.N. Myers, M.H. Moon and B.N. Barman, in *Particle Size Distribution: Assessment and Characterization* (T. Provder, Ed.), pp. 198–216. ACS Symp. Ser. No. 472, American Chemical Society, Washington, DC (1991).
[20] J.C. Giddings, M.A. Benincasa, M.-K. Liu and P. Li, *J. Liq. Chromatogr.* **15**, 1729–1747 (1992).
[21] J.C. Giddings and M.H. Moon, *Anal. Chem.* **63**, 2869–2877 (1991).
[22] J.J. Kirkland, S.W. Rementer and W.W. Yau, *Anal. Chem.* **53**, 1730–1736 (1981).
[23] R. Beckett, *Environ. Tech. Lett.* **8**, 339–354 (1987).
[24] M. Martin, in *Particle Size Analysis 1985* (P.J. Lloyd, Ed.), pp. 65–85. Wiley, New York (1987).
[25] A. Litzén and K.-G. Wahlund, *J. Chromatogr.* **476**, 413–421 (1989).
[26] K.D. Caldwell and J. Li, *J. Colloid Interface Sci.* **132**, 256–268 (1989).
[27] J.J. Kirkland, W. Liebald and K.K. Unger, *J. Chromatogr. Sci.* **28**, 374–378 (1990).
[28] K.D. Caldwell, G. Karaiskakis and J.C. Giddings, *Colloids Surfaces* **3**, 233–238 (1981).
[29] J.J. Kirkland, W.W. Yau and F.C. Szoka, *Science* **215**, 296–298 (1982).
[30] R. Dreyer, E. Hawrot, A.C. Sartorelli and P.P. Constantinides, *Anal. Biochem.* **175**, 433–441 (1988).
[31] S.K. Ratanathanawongs and J.C. Giddings, in *Chromatography of Polymers* (T. Provder, Ed.). ACS Symp. Series, American Chemical Society, Washington, DC, in press.
[32] J.C. Giddings, *Chem. Eng. News* (Oct. 10) **66**, 34–45 (1988).
[33] J.C. Giddings and K.D. Caldwell, in *Physical Methods of Chemistry* (B.W. Rossiter and J.F. Hamilton, Eds), Vol. 3B, pp. 867–938. Wiley, New York (1989).

[34] J.C. Giddings, G. Karaiskakis and K.D. Caldwell, *Sep. Sci. Technol.* **16**, 607–618 (1981).
[35] R. Beckett, J. Ho, Y. Jiang and J.C. Giddings, *Langmuir* **7**, 2040–2047 (1991).
[36] J.-T. Li and K.D. Caldwell, *Langmuir* **7**, 2034–2039 (1991).
[37] J.C. Giddings, P.S. Williams and M.A. Benincasa, *J. Chromatogr.* **627**, 23–35 (1992).
[38] F.-S. Yang, K.D. Caldwell and J.C. Giddings, *J. Colloid Interface Sci.* **92**, 81–91 (1983).
[39] M.-K. Liu, P.S. Williams, M.N. Myers and J.C. Giddings, *Anal. Chem.* **63**, 2115–2122 (1991).

[Received for review 26 February 1993; revised manuscript received 9 March 1993]

Appendix — Glossary

b	channel breadth
d	particle diameter
F	force exerted by field on a single particle
G	field strength (acceleration)
k	Boltzmann's constant
L	effective (volume-based) channel length
L_{tt}	channel length measured tip-to-tip
m'	effective particle mass
T	absolute temperature
t^0	void time
t_r	retention time
U	cross flow velocity
V^0	channel void volume
$\dot{V}$	channel flow rate
$\dot{V}_c$	cross flow rate
w	channel thickness
η	viscosity
λ	retention parameter