Studies of Vesicle Extrusion

B. J. Frisken,* C. Asman, and P. J. Patty

Department of Physics, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Received April 27, 1999. In Final Form: September 28, 1999

This paper reports studies of vesicles made by the extrusion of lipid suspensions through the pores of polycarbonate membranes as a function of the concentration of the lipid suspension and of the average pore size of the membranes. Experiments clearly show that the applied pressure, rather than the flow rate achieved, determines the size of vesicle produced. Vesicle size decreases as the applied pressure increases for all pore sizes measured. However, the size of vesicles produced in larger pores reduces more quickly as the pressure is increased as compared to those produced in smaller pores. While the size of vesicle produced and the polydispersity of the vesicle population is only weakly dependent on the concentration of the lipid suspension, the minimum pressure required for extrusion increases dramatically for higher lipid concentrations. A method for estimating lysis tension of the membrane using the minimum extrusion pressure is discussed; for POPC (1-palmitoyl-2-oleoyl-*sn*-phosphatidylcholine) we measure a lysis tension of 7.7 ± 0.3 mN/m.

1. Introduction

Vesicles are quasi-spherical shells composed of lipid bilayers that encapsulate an aqueous space. They are used in the research laboratory as model membranes and in the pharmaceutical and cosmetic industries as nanoscale containers for drugs and other substances. They are produced in the laboratory by several methods including detergent dialysis and extrusion.[1] Extrusion methods[2] are a popular means of production; they involve pushing a lipid suspension through pores with diameters of the order of 100 nm. They have the advantage that they produce a relatively monodisperse, predictable vesicle size without the addition of contaminants. However the mechanism by which the multilamellar, polydisperse lipid structures are converted into unilamellar vesicles of a controlled size is not well understood. Characteristics of the extrusion procedure (applied pressure, flow rate, pore size) as well as physical properties of the lipids and the lipid suspension (surface tension, bending modulus, concentration) may all play a role in the size and polydispersity of the vesicles produced. Understanding the role of these various factors will contribute to the reproducibility achieved and should lead to production of more ideal vesicle solutions.

Previous studies have shown that the size and polydispersity of vesicles produced by extrusion depend on the number of times the solution is extruded and the pore size,[3] lipid properties,[4] and pressure.[5] However, the studies were not extensive enough to lead to models of vesicle formation by extrusion. In a previous paper[6] some of us studied the effect of lipid, temperature, and pressure on the vesicles produced. It was observed that the vesicle size decreased as the pressure increased and that no vesicles were produced if less than a certain minimum pressure was applied. This minimum pressure was lipid dependent and was used to estimate the lysis or rupture tension of the lipid bilayer. The average flow rate was observed to stabilize after several passes of the solution through the extruder, and this appeared to be a good indicator that the number of extrusions was sufficient to produce a reproducible vesicle size. However the size of vesicles produced at the same pressure did not appear to depend significantly on the lipid used, and the polydispersity of the vesicle solution appeared completely independent of any parameter which was varied. These studies also indicated that rupture of the bilayer is an important feature of vesicle extrusion, a feature which has not yet been incorporated into theories involving vesicles in pores,[7,8] under shear,[9] or under axial tension.[10] Furthermore, many questions about the extrusion process remain. Do the large multilamellar vesicles rupture as they enter the pores, in the pores, or as they leave the pores? Why does the vesicle radius decrease as the pressure increases? Is pressure the crucial extrusion feature, or is it the flow rate (and consequent shear rate) resulting from the applied pressure difference which primarily affects the characteristics of the extruded vesicles?

In this paper we report the results of several experiments designed to address these questions. The lipid concentration of the extruded solution was varied from 1 to 100 mg/mL in order to test theories of vesicles in pores[8] and to study the effect of flow rate on vesicle size. Even lower concentrations were used to determine the lysis tension of the lipid vesicles being extruded. The membrane pore size was varied from 47 to 161 nm in order to understand why the vesicle size decreases as the pressure is increased. Using results of these studies, we describe possible mechanisms for vesicle extrusion.

2. Materials and Methods

2.1. Flow of Vesicles in a Pore. The flow rate of a fluid through a channel depends on the pressure difference

(1) New, R. R. C. In *Liposomes as Tools in Basic Research and Industry*; Philippot, J. R., Schuber, F., Eds.; CRC Press: Boca Raton, 1995; p 19.
(2) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55.
(3) Mayer, L. D.; Hope, M. J.; Cullis, P. R. *Biochim. Biophys. Acta* **1986**, *858*, 161.
(4) Nayer, R.; Hope, M. J.; Cullis, P. R. *Biochim. Biophys. Acta* **1989**, *986*, 200.
(5) Kölchens, S.; Ramaswami, V.; Birgenheier, J.; Nett, L.; O'Brien, D. F. *Chem. Phys. Lipids* **1993**, *65*, 1.
(6) Hunter, D. G.; Frisken, B. J. *Biophys. J.* **1998**, *74*, 2996.
(7) Gompper G.; Kroll, D. M. *Phys. Rev. E.* **1995**, *52*, 4198.
(8) Bruinsma, R. *Physica A* **1996**, *234*, 249.
(9) Kraus, M.; Wintz, W.; Seifert, U.; Lipowsky, R. *Phys. Rev. Lett.* **1996**, *77*, 3685.
(10) Bukman, D. J.; Yao, J. H.; Wortis, M. *Phys. Rev. E* **1996**, *54*, 5463.

10.1021/la9905113 CCC: $19.00
Published on Web 11/27/1999

applied across the channel and on the channel's length. For laminar flow of a viscous fluid, the relationship between these quantities is given by Darcy's law

$$\eta Q = K\left(\frac{\Delta P}{\Delta L}\right) \quad (1)$$

where η is the viscosity of the fluid, Q is the volume flow rate, ΔP is the applied pressure difference, and ΔL is the length of the channel. For the flow of a homogeneous fluid through a membrane consisting of cylindrical pores, the permeability K is a purely geometrical factor given by

$$K = N\pi R_p^{\,4}/8 \quad (2)$$

where N is the number of pores and R_p is the pore radius. When there are vesicles in the fluid, viscous dissipation occurs as vesicles are pushed through the pores. This must be overcome by applying an excess pressure drop across each vesicle. Bruinsma[8] argues that this leads to an effective permeability

$$K_{\text{eff}} = \frac{N\pi R_p^{\,4}}{8 + 0.233(nL^*)(L^*/R)^2} \quad (3)$$

This theory assumes that the vesicle travels through the pore as a spherocylinder of length L^* and radius $R = R_p - h^*(v)$, where $h^*(v)$ is the thickness of the (velocity-dependent) lubrication layer around the vesicle as it flows through the pore and v is the average velocity of fluid in the pore. The effective permeability depends on the number of vesicles per unit length n inside the pore and is no longer purely geometrical, but the only nongeometrical parameter it will depend on should be the lipid concentration. Bruinsma also shows that, as the vesicle flows through the pore, viscous stress induces a variation in the surface tension along the vesicle given by

$$\gamma(z) = \gamma(z_o) - \frac{\eta v}{h^*(v)}z \quad (4)$$

The z-coordinate is along the vesicle axis and $\gamma(z_o)$ is the tension at the frontal endcap of the spherocylinder.

2.2. Preparation of Phospholipid Vesicles. The phospholipids used were 1,2-dimyristol- *sn*-phosphatidylcholine (DMPC) and 1-palmitoyl-2-oleoyl- *sn*-phosphatidylcholine (POPC) (Northern Lipids, Vancouver BC); the lipids were obtained in powder form. For each sample, a single variety of phospholipid was hydrated using purified water from a Milli-Q Plus water purification system (Millipore, Bedford MA), in ratios of 1−200 mg of phospholipid per milliliter of water. Water from a Milli-Q Plus filtration system was used to keep the concentration of contaminants in the vesicle solution negligible. This ensures that the vesicles will swell to spherical shapes after extrusion.[11] The mixture of water and phospholipid was taken through a freeze−thaw procedure five times. This procedure involved freezing the solution by immersion into liquid nitrogen, followed by thawing by immersion into 50 °C water, followed by thorough vortexing. The procedure has been shown to result in more unilamellar samples.[3]

After the freeze−thaw process, the vesicle suspension was cleaned and regularized by extruding it once through two polycarbonate membrane filters (Osmonics Inc., Livermore, CA) with pore diameter 400 nm at 50 psi. This process, which we call pre-extrusion, was found to improve the reproducibility of the light scattering and extrusion flow rate measurements. After this step, light scattering measurements revealed very polydisperse samples with average radii of ∼200 nm.

Table 1. Properties of Polycarbonate Membranes as Reported by the Manufacturer

batch	lipid	nominal pore radius (nm)	av pore radius (nm)	av pore density (pores/cm^2)
AE86AQ12B	POPC	25	23.5	6.1×10^8
AE84AH11C	DMPC	50	47.0	3.8×10^8
AE84AG11D	POPC	50	49.3	3.9×10^8
AG83CC11A	POPC	100	80.5	2.6×10^8

Prior to extrusion through the final filter size, the pre-extruded vesicle suspension was diluted with purified water to the required concentration. The pre-extruded vesicle suspension was then extruded through two polycarbonate membranes with nominal pore diameters of 50, 100, or 200 nm using an extruder (Lipex Biomembranes, Vancouver, BC) by applying a pressure gradient as provided by prepurified, compressed N_2 gas. Batch number, average pore size, and average pore density as reported by the manufacturer are shown in Table 1. The area of the membrane available for extrusion was 4.15 cm^2. Water from an external water bath was circulated through the extruder in order to control the extrusion temperature. DMPC vesicles were extruded at 40 °C and POPC vesicles were extruded at 25 °C so that both lipids were at temperatures well above their gel-transition temperatures. The vesicle suspension was re-extruded a minimum of 10 times or until the average flow rate became constant on consecutive extrusions. The average flow rate of the extruded suspension was measured either with a stopwatch and a graduated cylinder or by observing a video recording with on-screen time stamp of the flow into a graduated cylinder.

2.3. Vesicle Characterization by DLS. Vesicle size and polydispersity were characterized primarily by dynamic light scattering (DLS) measurements as described previously.[6] Prior to size and size distribution analysis of the sample by DLS, the vesicle suspension was diluted in Milli-Q water to approximately 0.1 mg/mL and placed in a 10 mL cylindrical glass vial. The apparatus used for the light scattering experiments was an ALV DLS/SLS-5000 spectrometer/goniometer (ALV−Laser GmbH, Langen, Germany). The sample was placed in a toluene bath maintained at the temperature at which the vesicles were extruded in order to minimize thermal expansion and/or contraction of the vesicles. The light source for the experiments was a helium−neon laser of wavelength 633 nm (SpectraPhysics 127-35, Mountain View, CA). Light from the laser passed through the sample and light scattered by the sample was detected at angles of 45, 60, 75, 90, 115, and 150° from the transmitted beam for DLS measurements by a photomultiplier tube (model 9130, EMI Hayes, England).

Dynamic light scattering measures the time correlation of the intensity of the scattered light which is simply related to the time correlation of the field in most cases.[12] The field−field time-correlation function decays exponentially with a decay rate Γ, which depends on the size of the particles. Polydisperse samples are characterized by a distribution of decay rates $G(\Gamma)$.

Light scattering data for polydisperse vesicle solutions were analyzed primarily using the method of cumulants[13]

(11) Mui, B.; Cullis, P. R.; Evans, E. A.; Madden, T. D. *Biophys. J.* **1993**, *64*, 443.
(12) Berne, B. J.; Pecora, R. *Dynamic Light Scattering*; Robert E. Krieger Publishing Co.: Melbourne, FL, 1990.
(13) Koppel, D. E. *J. Chem. Phys.* **1972**, *57*, 4814.

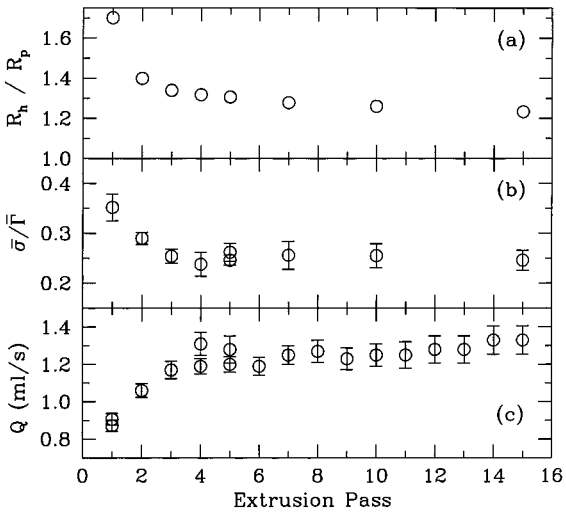

Figure 1. Results for (a) hydrodynamic radius, (b) relative standard deviation of the distribution of decay rates, and (c) flow rate after each extrusion pass through polycarbonate membrane filters with an average pore radius of 47 nm for DMPC in water at a concentration of 1 mg/mL and temperature of 40 °C. The applied pressure was 125 psi. The hydrodynamic radius, relative standard deviation, and flow rate equilibrate within about four extrusions. The error bars designate the standard deviation of the mean of the measurements.

to obtain the mean decay rate Γ and the standard deviation σ of the distribution of decay rates. The hydrodynamic radius R_h was calculated from Γ using the Stokes−Einstein relation. Data taken at angles spanning the range of the instrument were consistent with results expected for polydisperse samples. The measurements reported here were taken at 90° where the effect of reflection is minimized. The intensity correlation function was measured five times for each sample; each data set was analyzed to find R_h and σ. Mean values for the hydrodynamic radius $\bar{R}_h$ and the standard deviation of the decay rate distribution $\bar{\sigma}$ were calculated from these values. The experimental uncertainty in these values was estimated from the standard deviation of the mean.

3. Results and Discussion

Multiple extrusions are necessary to reduce the polydispersity of the extruded vesicles and to reach a reproducible radius for a given extrusion pressure. In general we find that the radius and polydispersity cease to change significantly in about the same number of extrusions required for the flow rate to be the same on consecutive passes of the vesicle solution through the extruder. For example, Figure 1 shows average vesicle radius $\bar{R}_h$ relative to the average pore size R_p, the relative standard deviation, and the average flow rate for 1 mg/mL DMPC solution extruded at 125 psi (where 1 psi = 6895 Pa or 6.895×10^4 dyn/cm^2) and 40 °C. In the first four passes there are significant decreases in $\bar{R}_h$ and $\bar{\sigma}$ and increases in the flow rate through the membrane. Changes in these quantities become smaller as the number of passes is increased and values eventually equilibrate. In practice, we extrude the vesicle solution at least 10 times or until the flow rate becomes constant between consecutive extrusions.

A simple experiment was designed to investigate the effect of flow rate and pressure on vesicle radius. Vesicles were made from 1 mg/mL POPC solution using the same extrusion pressure but using either one or two polycarbonate membranes. Because the flow rate should be inversely proportional to the length of tube over which the pressure difference is applied, this results in the flow rate changing by a factor of 2 while the pressure applied across the sample is kept constant. The pressure was chosen to be in a range where the vesicle size was strongly pressure-dependent. The flow rate did indeed double when the lipid suspension was extruded with one rather than two membranes, but the average final radii of the vesicles produced were essentially the same. Results are tabulated in Table 2. The flow rate does not seem to affect the final vesicle size.

Table 2. Flow through One or Two Membranes for 1 mg/mL DMPC and POPC Solutions

batch	ΔP (psi)	no. of filters	av final flow rate (mL/s)	av final radius (nm)
AE84AH11C	62 ± 2	2	0.378	67.2
AE84AH11C	62 ± 2	1	0.858	67.1
AE84AG11D	60 ± 2	2	0.465	68.4
AE84AG11D	60 ± 2	1	1.00	68.9

This observation implies that the vesicle breaks up at the pore entrance. This follows from a consideration of possible scenarios for vesicle breakup. The vesicle fragments when the surface tension becomes larger than the lysis tension of the bilayer. This surface tension increase can appear either due to a buildup of pressure across the vesicle as given by the Laplace relation or in response to viscous stress. A buildup of pressure will occur at the pore entrance as large vesicles are pushed into the pores. Viscous stress mechanisms will dominate when the vesicle is flowing through the pore or exiting from the pore and should depend on the flow rate of the vesicle through the pore as given by eq 4. Since vesicle size is independent of flow rate, the mechanism of small vesicle formation should involve a quasi-static buildup of pressure across the vesicle at the pore entrance.

To study the effect of concentration on the vesicles produced, experiments were done at 10 and 100 mg/mL DMPC in water to complement previous measurements at 1 mg/mL.[6] The flow rate, final vesicle size, and polydispersity were measured for vesicles produced by extrusion through membranes with an average pore diameter of 94 nm.

Results for the flow rate measured during the final extrusion pass for 1, 10, and 100 mg/mL DMPC solutions as well as pure water are shown in Figure 2. In general the flow rate increases as the pressure increases. Below a certain minimum pressure, the flow rate is minimal and few vesicles are produced. It is evident that the flow rate at a given pressure, compared to that for the 1 mg/mL solution, is smaller for the 10 mg/mL solution and significantly smaller for the 100 mg/mL solution. At low pressure and low concentration, the flow rate is proportional to the applied pressure difference. At high pressure the flow rate is not proportional to the applied pressure; the behavior is no longer consistent with Darcy's law. It is also apparent that the minimum pressure required to achieve extrusion increases significantly at higher concentrations.

The dashed and dotted lines shown in Figure 2 are calculated using eq 3 after an additional term representing the minimum pressure for extrusion was added. The number of vesicles per unit length n in the pore is calculated from the lipid concentration of the solution. The length of the spherocylinder is estimated by assuming that the spherocylinder has the same area as the final vesicle and by assuming that the vesicles are formed from cylindrical fragments of length $2\pi R$,[14] where R is the

(14) Clerc, S. G.; Thompson, T. E. *Biophys. J.* **1994**, *67*, 475.

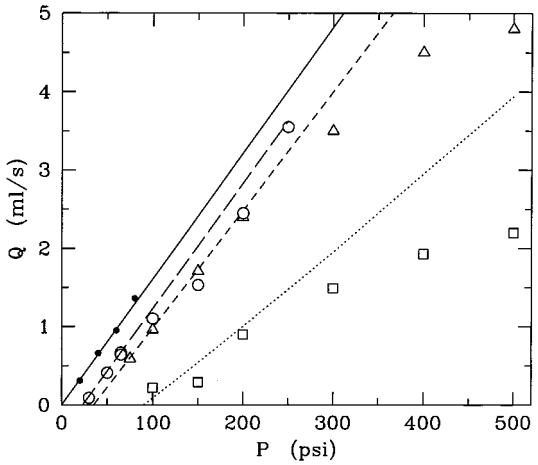

Figure 2. Final flow rate of (□) 100 mg/mL, (△) 10 mg/mL, and (○) 1 mg/mL DMPC solutions through membrane filters with an average pore radius of 47 nm as a function of pressure. The flow rate of water is shown as solid circles for comparison. The flow rate of water is typically somewhat higher than that of the vesicle solutions at the same extrusion pressure. The solid line is calculated from previous measurements (Hunter and Frisken, 1998). The dashed lines are calculated using eq 3.

radius of the spherocylinder. The term representing the minimum extrusion pressure was chosen to be consistent with the data. The change in vesicle size with pressure has very little effect on the slope of the calculated lines; only a slight increase in slope at the highest pressures for the highest concentration is noticeable. In previous studies[6] with low-concentration vesicle solutions (less than one vesicle per pore), K_{eff} values consistent with that measured for water were measured, independent of the lipid used. With an increase of the concentration of lipid in solution, a decrease in the flow rate at the same applied pressure is observed. At low pressure, K_{eff} is consistent with eq 3. Flow rate of the high concentration samples at high pressure falls below predicted values; however the theory only takes the pressure drop due to viscous dissipation in the pore into account and does not account for crowding at the pore entrance.

The size and polydispersity of vesicles produced from mixtures of different concentration do not vary as significantly. Figure 3a shows the radius relative to the average pore size and Figure 3b shows the relative standard deviation of the distribution of decay rates. The vesicles made from the 100 mg/mL mixture are slightly larger than those made at lower concentration, but the standard deviation still remains essentially constant at all pressures within the uncertainty of the measurements.

In previous work[6] it was found that the minimum extrusion pressure was consistent with the pressure required to burst or lyse the membrane, and thus it was possible to estimate the lysis tension of the lipid bilayer. Thus we were a little worried about the apparent change in the minimum pressure for extrusion with concentration as shown in Figure 2 and subsequently made further measurements. Figure 4 shows the flow rate during the first pass through the extruder for DMPC samples of various concentrations. Extrusion in the first pass will certainly be dominated by the breakup of large vesicles as they enter the pores. Figure 4a shows the behavior over the entire pressure range studied and Figure 4b shows detail at low pressure. Data for concentrations ranging from 0.2 to 100 mg/mL as well as pure water are shown. For high concentration samples the flow rate is low even at high pressure and approaches zero flow rate asymptotically. As the concentration is decreased, the behavior becomes more and more linear.

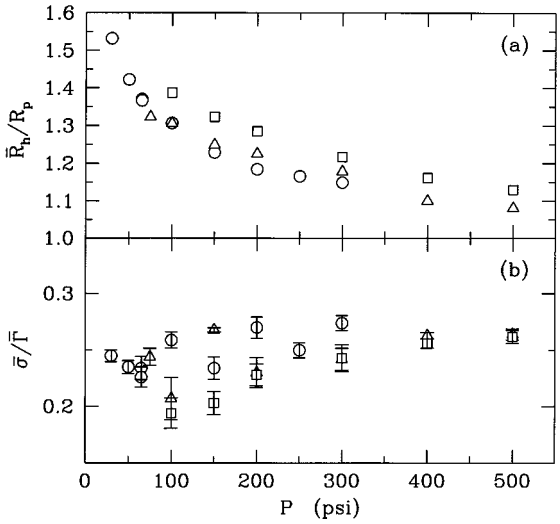

Figure 3. Results for vesicles extruded through pores of average radius 47 nm at different extrusion pressures of (a) hydrodynamic radius relative to the pore radius and (b) relative standard deviation. The symbols represent DMPC concentrations of (□) 100 mg/mL, (△) 10 mg/mL, and (○) 1 mg/mL. The hydrodynamic radius decreases as the applied pressure increases. The relative standard deviation is independent of extrusion pressure within experimental uncertainty. Vesicles extruded at 100 mg/mL are somewhat larger than vesicles extruded at smaller concentrations.

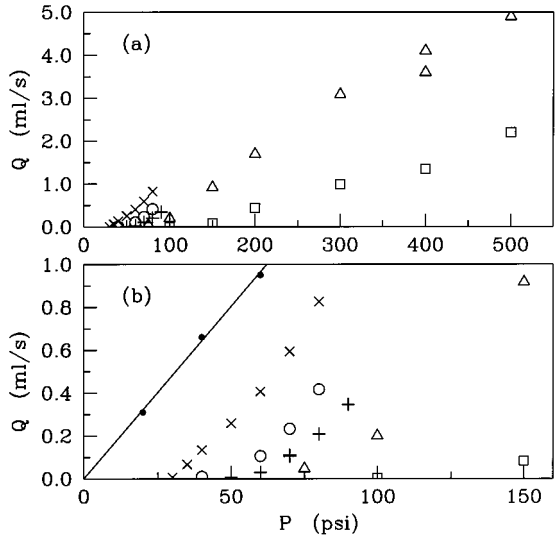

Figure 4. The flow rate as measured on the first pass of the lipid solution through the extruder for samples of various concentrations of DMPC ((□) 100 mg/mL, (△) 10 mg/mL, (+) 5 mg/mL, (○) 1 mg/mL, and (×) 0.2 mg/mL) using membrane filters with an average pore radius of 47 nm. Part b shows detail of part a at low pressure. The solid circles and line are the data and fit for pure water though polycarbonate membranes from the same batch.

Similar results were obtained using with various concentrations of POPC as shown in Figure 5. Again it is evident that the flow rate approaches zero asymptotically for higher concentration samples but becomes more linear as the concentration is decreased. However, for the lowest concentration sample (0.01 mg/mL) the behavior changes again and the flow rate does not approach zero as quickly as expected.

In general, the flow rate for a given applied pressure will depend on the pressure drop across vesicles blocking

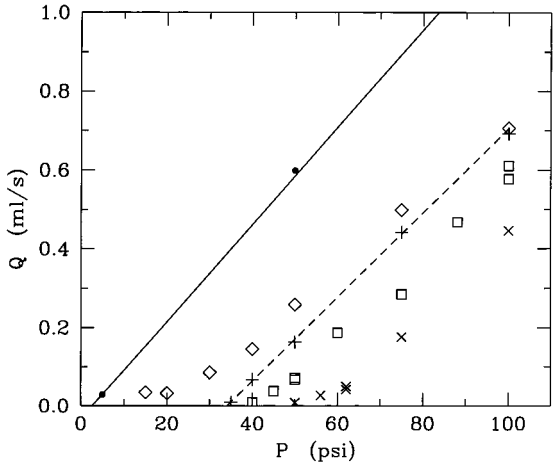

Figure 5. The flow rate as measured on the first pass of the lipid mixture through the extruder for samples of various concentrations of POPC ((×) 1.0 mg/mL, (□) 0.2 mg/mL, (+) 0.05 mg/mL, and (◇) 0.01 mg/mL) using filters with an average pore radius of 49.3 nm. The solid circles and line are the data and fit for pure water though polycarbonate membranes from the same batch. At the lowest concentration measured, the flow rate does not go to zero as the pressure is decreased. At high concentrations, the flow rate approaches zero asymptotically as the pressure is decreased. At a concentration of 0.05 mg/mL, the flow rate is linear in pressure and has a slope close to that of the pure water data.

Table 3. Linear Fits to Low Concentration Flowrate Data

sample	R_p (nm)	K (mL/(s·psi))	P_{min} (psi)
water	49.3	0.0123 ± 0.0004	2.67 ± 0.20
POPC (0.05 mg/mL)	49.3	0.0107 ± 0.0003	34.14 ± 0.05

the pore as well as the pressure drop required to force the vesicles through the pore. We believe that the first pass is the pass where flow rate will be limited by $P_{min} \sim 2\gamma_l/R_p$, where γ_l is the lysis or rupture tension. In the first pass through the extruder, there will certainly be large polydisperse multilamellar vesicles blocking the pores. As pressure is applied across a vesicle, part of it will be pulled into the pore. This induces a surface tension γ. If the applied pressure P is large enough, the vesicle will lyse and a vesicle fragment will be pulled into the pore. Similar events have been observed in experiments with red blod cells.[15] Pressure in excess of P_{min} will cause flow of the vesicle fragment through the pore. In the limit where only one vesicle blocks each pore and where the pressure drop across the vesicle is approximately P_{min}, the flow rate observed should be proportional to $P - P_{min}$. In fact, at low concentration, the proportionality constant should be the same as is observed for pure water because the proportionality constant depends only on geometrical factors in this limit. If there is an average of more than one vesicle blocking each pore, then the pressure drop across the tube will be even smaller and the flow through the pore will be further reduced. If there is an average of less than one vesicle blocking each pore, then some water will leak through the unblocked pores and the flow rate will not go to zero at P_{min}.

The data shown in Figure 5 are consistent with this model. The flow rate is linear in P at a concentration of 0.05 mg/mL. The slope of the curve is also comparable to that observed for pure water. The two curves have been fit to the form $Q = K(P - P_{min})$, results are summarized

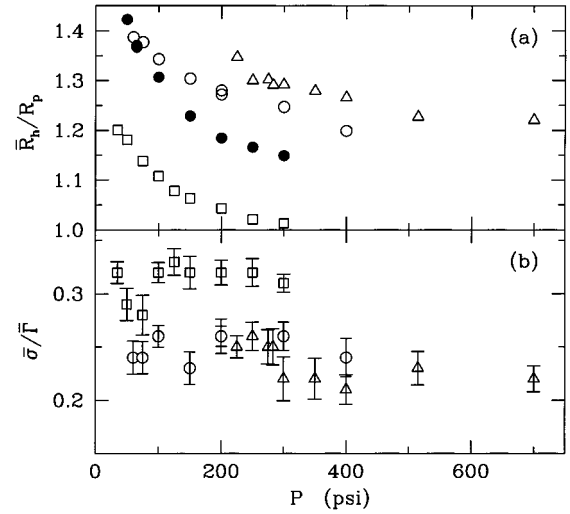

Figure 6. Results for vesicles extruded at least 10 times as a function of extrusion pressure. Part a shows the hydrodynamic radius relative to the pore radius for 1 mg/mL POPC (open symbols) and 1 mg/mL DMPC (closed symbols) solutions and (b) shows relative standard deviation from 1 mg/mL POPC solutions. The symbols represent average membrane pore radii of (□) 80.5 nm, (○) 49.3 nm, and (△) 23.5 nm. The hydrodynamic radius decreases as the applied pressure increases. The relative standard deviation is independent of extrusion pressure for a given pore size but increases with pore size.

in Table 3. At higher concentration the flow rate is lower, presumably because there is more than one vesicle blocking each pore and because the density of vesicles in the pore is higher. At the lowest concentration measured (0.01 mg/mL), the flow rate does not go to zero as the pressure is decreased, consistent with the idea that there is less than one vesicle blocking each pore and that water is leaking through the unblocked pores. Best conditions for measurement of P_{min} and thus the lysis tension should be indicated by a linear dependence of the flow rate on pressure with a slope which is the same as that of pure water.

By use of the results for P_{min} from Table 3 and the following equation relating the lysis tension γ_l and P_{min},[6]

$$P_{min} = 2\gamma_l\left[\frac{1}{R_p} - \frac{1}{R_o}\right] \tag{5}$$

where R_p is the pore size and R_o is the average size of the vesicles before extrusion, the lysis tension for POPC can be calculated. We find that the lysis tension is 7.7 ± 0.3 mN/m, in good agreement with results from Rawicz and Evans[16] who found $\gamma_l = 8.3 \pm 0.5$ mN/m by pipet aspiration techniques.[17]

To understand why the size changes with pressure, extrusion through polycarbonate membranes with pores of different radius was studied. The results are shown in Figure 6 for extrusion of 1 mg/mL POPC solution through 161, 97.5, and 47 nm pores. This graph shows that the size of the vesicles always decreases with pressure and highlights differences between pore sizes. The vesicles "size down" better for larger pore sizes the vesicles made in 161 nm pores have radii only slightly larger than R_p when extruded at high pressure. In contrast, vesicles extruded through 47 nm pores are always significantly larger than the average pore size, even for vesicles extruded at 700 psi. We have also plotted data for 1.0

(15) Meier, W.; Lerche, D.; Wendt, M.-O.; Paulitschke, M. *Biorheology* **1987**, *24*, 287.

(16) Rawicz, W.; Evans, E. Unpublished.

(17) Evans, E.; Rawicz, W. *Phys. Rev. Lett.* **1990**, *64*, 2094.

mg/mL DMPC solutions extruded at 40 °C through 94 nm pores. It is interesting to note that the POPC vesicles extruded through comparable pores do not size down as well as was observed for DMPC vesicles.

We believe that the final size of the vesicle depends on the applied pressure because the radius of the piece of the vesicle that is pulled into the pore will depend on the force applied. It has been shown[10] that the radius of a cylindrical tube or tether R_o pulled from a large vesicle is related to the applied force F_o and the bending modulus κ such that $R_o \sim 2\pi\kappa/F_o$. This theory does not take into account the fact that bilayers cannot be bent to infinite curvature or that there should be some influence of the pore radius. But these simple concepts are consistent with results for DMPC and POPC which show DMPC vesicles closer to the size of the pore at lower pressures; DMPC has a smaller bending modulus than POPC ($\kappa_{DMPC} = 1.16 \pm 0.17 \times 10^{-19}$ J, $\kappa_{POPC} = 1.43 \pm 0.22 \times 10^{-19}$ J).[18] It should be possible to measure κ from the pressure dependence of the radius with a little further understanding of the extrusion process. However, we have not yet been able to collapse our data with simple scaling factors.

The results of measurements of the polydispersity of the vesicle solutions are plotted in Figure 6b. It is obvious that the breakup of large multilamellar vesicles into smaller vesicles approximately the size of the pore does not occur with a great deal of uniformity as indicated by the large polydispersity of the distribution of decay rates. The polydispersity is much larger than the polydispersity of the pores as quoted by the manufacturer (≈3−5%) for all of the membranes used here. The large polydispersity is consistent with the vesicles undergoing some sort of fragmentation or pinch-off process as they are pulled into the pore. Fragmentation processes are known to create broad and asymmetric size distributions, and other methods are perhaps better suited to measurement of the breadth and shape of vesicle size distributions produced under different extrusion conditions (e.g., ref 19). An interesting feature of the data is that they show the relative polydispersity decreasing with pore size.

4. Conclusions

Lipid extrusion can be used to determine physical properties of the lipid bilayer. In particular, the lysis tension of a lipid bilayer can be measured by detecting the minimum pressure for extrusion of a low concentration lipid suspension through a polycarbonate membrane. Using this method, the lysis tension of POPC was measured to be 7.7 ± 0.3 mN/m, in good agreement with results from Rawicz and Evans (1998) who found $\gamma_l = 8.3 \pm 0.5$ mN/m by pipet aspiration techniques.

These experiments have revealed further characteristics of extruded vesicles that should shed light on the extrusion process. The applied pressure determines the average vesicle size rather than any flow rate induced. For all pore sizes measured, extruded vesicles are generally larger than the pore size, but the average size as measured by DLS, decreases as the extrusion pressure increases. For nominally 100 nm pores, there is a clear difference in the size of vesicle produced using different lipids at the same pressure. This variation may be due to differences in the bending modulus of the bilayer. The average size decreases more quickly with pressure for larger pore membranes than for smaller pore membranes. The polydispersity of the vesicle suspension decreases as the pore size decreases. The pore size is the only factor that we have found to influence the polydispersity of vesicles produced by this method.

On the basis of studies of the properties of vesicles extruded under different conditions we propose the following model of vesicle extrusion.

During the initial passes through the extruder, large, multilamellar vesicles block the pores of the polycarbonate membrane. Parts of these vesicles are pulled into the pore due to the applied pressure P. This induces a surface tension in the vesicles. If P is greater than $P_{min} \sim 2\gamma_l/R_p$, where γ_l is the lysis or rupture tension, then the vesicle lyses near the pore entrance and part of the vesicle is pulled away and continues to flow through the pore.

The flow rate observed will generally be smaller than expected for water flowing through pores because part of the pressure drop occurs across vesicles which block the pore and part occurs across vesicles in the tube. In the limit where each pore is blocked by only one large vesicle and where the pressure drop across the vesicle is approximately P_{min}, the initial flow rate observed should be proportional to $P - P_{min}$. On subsequent passes, the flow rate of vesicle solution through the extruder increases and eventually stabilizes as fewer vesicles are being broken up on each pass and all of the pressure drop is applied across the pore.

The decrease in vesicle size with increasing pressure may be related to results linking the radius of a cylindrical tether pulled from a vesicle to the force applied and to the bending modulus.[10] In this scenario, the radius of the vesicle tube pulled into the pore decreases and subsequent fragments are smaller as the applied pressure is increased. The observation that vesicles made in larger pores size down better than those made in smaller pores may be related to the radius of curvature which the bilayer can be forced into for a given pressure or, equivalently, to a curvature dependence of the bending elasticity of the bilayer.

Acknowledgment. The authors gratefully acknowledge helpful conversations with Mike Wortis and Robijn Bruinsma. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

LA9905113

(18) Häckl, W.; Seifert, U.; Sackmann, E. *J. Phys. II* **1997**, *7*, 1141.
(19) Korgel, B. A.; van Zanten, J. H.; Monbouquette, H. G. *Biophys. J.* **1998**, *74*, 3264.