# EXTRUSION OF VESICLES THROUGH CALIBRATED PORES

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Bilayers made of natural or synthetic lipids are known to be fluid and flexible. The shape fluctuations of giant vesicles or biological cells can be observed directly under an optical microscope but it not easy to study the deformations of these membranes at a more microscopic scale ranging from 1 to a few hundred of nanometers. In order to fill this gap, we have undertaken a series of studies where we probe the mechanical properties of small unilamellar vesicles, whose size varies between 30 and 150 nm, by extruding them through porous membranes with well-defined uniform straight parallel pores. These membranes, known since a long time under the name "Nuclepore", are made by chemically etching the latent tracks produced after irradiation of polycarbonate foils by heavy ions (figure 1). They are already used to produce monodisperse vesicles by extruding lipid lamellar phases [1] and a few experimental [2] and theoretical studies [3] of this process have already started.

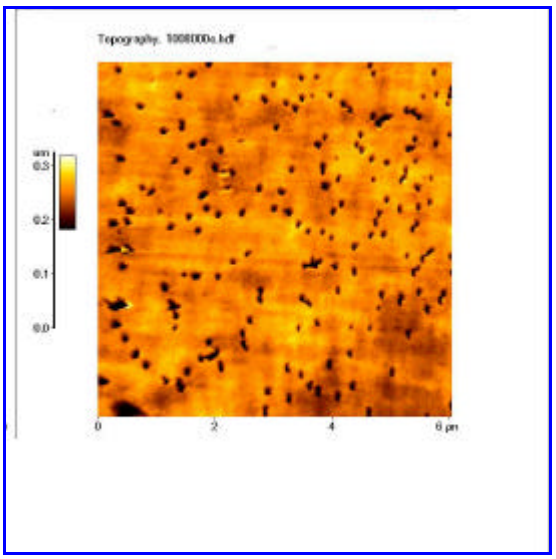

Figure 1. Nuclepore membrane observed by AFM (pore diameter 100 nm).

The studies are made at two levels. At a microscopic level we study the relationship between the flow rate and the pressure that we must apply to extrude the vesicles (figure 2). At a microscopic level, we compare by light and neutron scattering experiments the structure of the vesicles before and after extrusion (Figure 4).

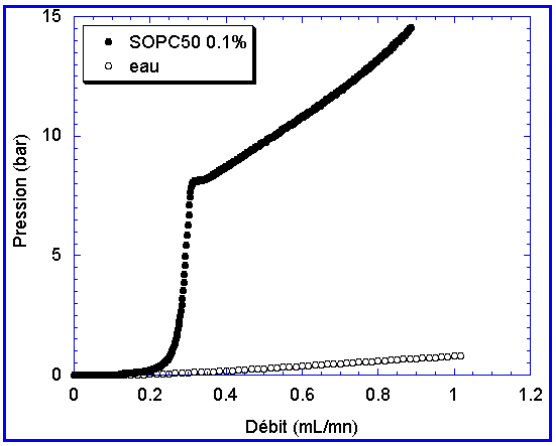


Figure 2. Pressure-flow-rate relationship measured on a porous membrane with a pore radius $R_p$ = 25nm, in presence of pure water (empty symbol) and SOPC vesicles (radius 34 nm) (full symbol).

We have focused our studies on the situation where the radius of the vesicles is larger than the radius of the pores. It appears then necessary to vary the applied pressure and the flow rate in a large range. To improve the precision of the measurements we choose to impose the flow rate and measure the pressure with a miniature piezo-resistive pressure sensor. We use either a syringe pump (for low pressure measurements below 1 bar) or a HPLC chromatography pump, enabling us to vary the flow rate between 0.01 and 10 ml/min with a possible pressure range between 0 and 300 bars.

Our main result is visible on figure 2. The vesicles are extruded only if the applied pressure excess a certain threshold, which depends on the vesicles size, pore diameter and lipid composition (figure 3). We expect theoretically two limiting behaviours :

1) for large pore radius $R_P$, one may possibly deform the vesicles without rupture, increasing only the vesicle curvature energy; this leads to a threshold pressure varying as $K/R^3_P$ where $K$ is the membrane rigidity;

2) for small pores, the vesicles must be stretched in order to enter the pores and break; Laplace's law then lead to a threshold pressure varying as $\sigma_l/R_P$ where $\sigma_l$ is the lysis tension of the bilayer. This behaviour has already been observed [2].

As shown on figure 3, these two laws interpret rather well the experimental observations,

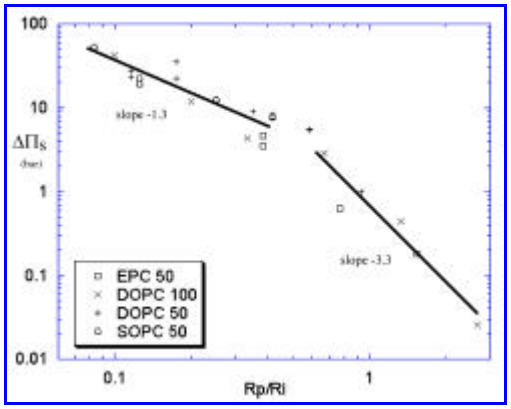


Figure 3. Threshold pressure as a function of the pore size for different vesicles ($R_p/R_i$ is the ratio of the pore to vesicle radius)

We observe on figure 4 showing the neutron and light scattering spectra of surfactant vesicles before and after their passage through different membranes the two main microscopic effects of the extrusion : 1) the diminution of the vesicle radius which shifts the spectra towards the largest q and 2) the vesicles retention which diminishes the value of the scattered intensity in the asymptotic regime. Basically, as shown on figure 5, the pores impose their size.

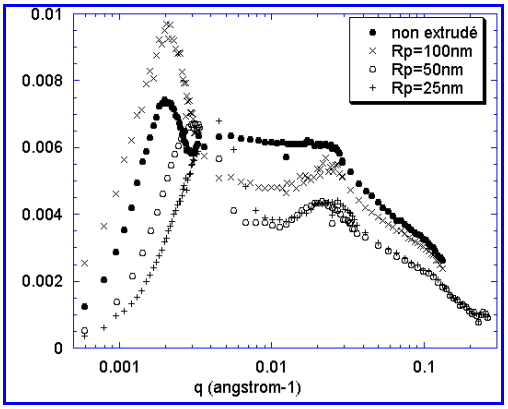


Figure 4. Neutron and light scattering intensity in the representation $q^2I(q)$ versus $q$ for surfactant vesicles (initial radius 107 nm) extruded through different pores.

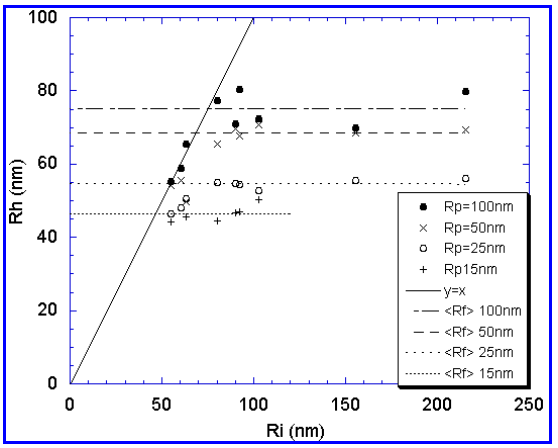


Figure 5. Final radius of surfactant vesicles as a fonction of their initial radius for different pore size.

The size diminution cannot be explained without assuming the rupture of the vesicles, this is confirmed by a direct observation of the liberation of encapsulated fluorescent labels during the extrusion. These fluorescence experiments also confirm the phenomena of vesicles retention and enable us to quantify it.

Finally it was interesting to compare directly the behaviour of small and large vesicles. We thus have observed directly the passage of giant vesicles through micrometric pores made by PDMS moulding at Institut Curie (figure 6). The deformations are very strong even at low flow rate, the vesicle rupture has been observed once.

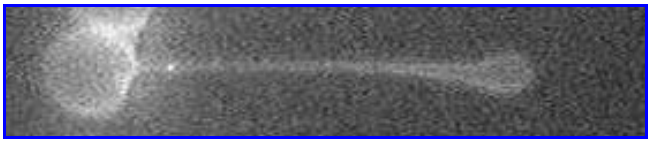

Figure 6. Giant fluorescent vesicles entering a PDMS channel under flow.

## References

[1] M.J. Hope, M.B. Bally, G. Webb, P.R. Cullis, *B.B.A.* **812** (1985) 5-65
[2] B.J. Frisken, C. Asmanpatty *Langmuir* **16** (2000) 928-933
[3] R. Bruinsma *Physica A* **234** (1996) 249-270