Onsager's irreversible thermodynamics of the dynamics of transient pores in spherical lipid vesicles

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Abstract Onsager's irreversible thermodynamics is used to perform a systematic deduction of the kinetic equations governing the opening and collapse of transient pores in spherical vesicles. We show that the edge tension has to be determined from the initial stage of the pore relaxation and that in the final state the vesicle membrane is not com-pletely relaxed, since the surface tension and the pressure difference are about 25 % of its initial value. We also show that the pore life-time is controlled by the solution viscos-ity and its opening is driven by the solution leak-out and the surface tension drop. The final collapse is due to a non-linear interplay between the edge and the surface tensions together with the pressure difference. We also discuss the connection with previous models.

Keywords Transient pore · Toroidal pore · Onsager irreversible thermodynamics · Spherical lipid vesicle · Membranes

Introduction

Biological pores are important entities in cells and play a crucial role in the spatial and temporal control of energy and matter fluxes. For instance, the modulation of membrane permeabilization mechanisms by the phys-icochemical properties of lipid bilayers is quite important for understanding antimicrobial mechanisms (Matsuzaki 1999; Zasloff 2002). When membrane organization is intact after transient pore formation, the permeabilization can be partially compensated by ion channels and pumps. When membrane organization becomes affected, this may eventually lead to the disruption of the membrane. In this case, the survival of the cell depends crucially on the speed with which the membrane is repaired (McNeil and Steinhardt 2003). In general terms, pores in biological membranes have different origins and their mechanisms of formation may depend upon proteins and peptides (Zasloff 2002; Papo and Shai 2003). In the literature, it is suggested that transient pores are more commonly formed after the interaction betwe en peptides and the lipid mem-branes (Matsuzaki 1999; Zasloff 2002). Nevertheless, pore formation has also been studied in prebiotic environ-ments, that is, without peptides and proteins, with the aim of understanding their role as a transport mechanism in protocells modeled by unilamellar vesicles (Sakuma and Imai 2015). Exocytosis and endocytosis involve processes of membrane fusion and fission giving rise to the forma-tion of a fusion pore, a channel through which secretions are released from the vesicle to the cell exterior (Vardjan et al. 2013; Suchmita et al. 1997; Doherty and McMa-hon 2009; Picco et al. 2015; Monck and Fernandez 1994; Palade 1975). The formation of the fusion pore occurs through the action of protein machinery (SNAREs) that has to apply mechanical forces in the range of 2–20 pN for the opening of the pore (Liu et al. 2015). Pores are also important for many technological applications such as nanomedicine, sensing, and nanoelectronics (Majd et al. 2010).

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Biological membranes are mainly composed of lipid molecules that form bilayers due to their amphiphilic structure. Lipid membranes are easily used in experiments to form uni- and multi-lamellar vesicles of different shapes and therefore they are very good models for investigating the main features of the formation and collapse of pores. In the simplest case, the pore formation and growth are gener-ated by physical forces and do not require the presence of proteins. This process can be modeled by liposome rupture due to swelling.

Recently, giant unilamellar vesicles immersed in an aqueous solution and composed of a single phospholipid bilayer and typical radius, $R \sim 10$–100 μm, have been used to study the formation and collapse of transient pores (Olivier et al. 1999; Erdem et al. 2003; Thomas and Dimova 2010; Riske and Dimova 2005). The rupture and a transient pore appear to be a response to mechanical stress over the membrane. Once the mechanical stresses along the membrane exceed the lysis tension, the vesicle ruptures and a transient pore may appear (Thomas and Dimova 2010). Application of intense optical illumination on vesicles containing fluorescent membrane probes induces a sudden increase of the membrane surface tension until the creation of a pore in the bilayer (Olivier et al. 1999; Erdem et al. 2003; Rodriguez et al. 2006). Another technique used for creating pores in vesicles is the adhesion of the vesicle on an attractive surface (Olivier et al. 1999). More recently, Dimova and coworkers (Thomas and Dimova 2010; Riske and Dimova 2005) have developed a different method of inducing pores in giant unilamellar vesicles by the applica-tion of electric pulses, the effect on the vesicles is similar to that produced in cells by the electroporation method, where the electric field may create a transient pore or it may turn the plasma membrane into a permeable one (Weaver 2000). The dynamics of the pore is then captured by means of a fast-imaging digital camera with a high temporal resolu-tion. This method avoids some disadvantages of previous techniques, such as the use of fluorescent probes and glyc-erol to increase the solution viscosity, and allows better resolution of pore dynamics. For instance, the initial pore size is not well documented in all cases except in Thomas and Dimova (2010), for which the initial pore size is about $r_{\min} = 0.75$ μm. However, for vesicles with similar diam-eters, all these techniques produce pores with maximum radii in the range $r_{\max} = 5$–11 μm.

In this work, we are interested in the theoretical descrip-tion of pore dynamics after its formation. This dynamics can be divided into three stages. The first stage is character-ized by rapid growth of the pore caused by the excess surface tension and the difference between the inner and the external pressures. The second stage is a relaxation process in which the slow shrinking of the pore is dominated by the edge tension. Finally, at long times, the rapid pore collapse is due to a non-linear interplay among the main forces par-ticipating in the dynamics, the edge and surface tensions together with the pressure difference.

The evolution of pores in membranes has been previ-ously described in Brochard-Wyart et al. (2000) by adopt-ing a model coming from the theory of viscous bare films in which the pore opening is governed by the transfer of surface energy to viscous losses, and edge forces are neglected (Debrégeas et al. 1995; Diederich et al. 1998). An important assumption of the mentioned description is that the solution viscosity is small and the membrane vis-cosity controls the closing process. However, this seems to be in contradiction to the experimental fact that in the case of membranes, the solution viscosity can be used to regu-late the pore closing velocity (Erdem et al. 2003; Thomas and Dimova 2010). An extension of this theory was for-mulated in Ryham et al. (2011) where a lateral stress on the pore arising from the tangential movement of viscous aqueous solution relative to the membrane was included. This correction introduces a dependence on solution vis-cosity that accounts for the dependence of the pore clos-ing velocity on the solution viscosity. As a consequence, the dependence of the pore collapse kinetics on the mem-brane viscosity becomes negligible in the initial stages. However, the final stage depends crucially on membrane viscosity. Other approaches taking into account the shape of the vesicle as a variable were proposed in Ryham et al. (2012).

In this article, we use Onsager's irreversible thermo-dynamics to deduce a model accounting for the kinetics of pore collapse in membranes. We begin by evaluating the free energy differential of an open vesicle, including the bending energy, the surface tension, the edge tension, and the coupling of the vesicle volume to its environment through a pressure difference term. Using the second law of thermodynamics, we are able to deduce the kinetic equa-tions that govern the time evolution of the pore and vesicle radii. By imposing consistent physical conditions on the resulting equations, we propose the correct dependence of the Onsager phenomenological coefficients on the pore and the vesicle radii. The obtained equations are numerically solved showing excellent comparison with experiments and a consistent behavior. In this way, we show that the clos-ing velocity is mainly controlled by the edge tension and the solution viscosity. We also determine the time evolu-tion of the surface tension and of the pressure difference. Our results question previous interpretations of the collapse dynamics. Previous models can be deduced from our gen-eral formalism depending on the election of the Onsager phenomenological coefficients. The corresponding incon-sistencies are discussed.

The article is organized as follows. Section 2 is devoted to discussing the expression for the free energy differential of an open vesicle using thermodynamics arguments. Then we use the free energy differential and Onsager's thermodynamics to derive the dynamical equations describing the process in Sect. 3. Finally, in Sect. 4, we discuss our results and present our conclusions.

Thermodynamics of a stressed vesicle

Consider a relaxed vesicle radius R_{eq} immersed in an aque-ous solution at constant temperature T and total volume $V = V_{in} + V_{out}$ with $V_{in} = (4/3)\pi R^3$ the volume inside the

vesicle and V_{out} the volume outside the vesicle, see Fig. 1c. The curved membrane has by definition a bending free energy per unit area given by κ_b/R^2, with $\kappa_b = 2\kappa + \bar{\kappa}$

being κ and $\bar{\kappa}$ the bending and the saddle-splay moduli, respectively (Helfrich 1973; Hernández-Zapata et al. 2009). Furthermore, due to the presence of the curved membrane, a finite pressure difference between the inside and outside of the vesicle exists, $\Delta P = P_{in} - P_{out} > 0$.

After an initial perturbation that increases the membrane surface tension to σ_0 and the vesicle radius to R_0, a pore radius $r(t)$ is produced that initiates a time-dependent pro-cess in which the radius of the vesicle becomes a function of time, $R(t)$, see Fig. 1b. Here, we will analyze the dynam-ical process of the pore growth and collapse in terms of $r(t)$ and $R(t)$.

The free energy change of the system has four contri-butions: $dF = dF_V + dF_\sigma + dF_B + dF_l$, coming from vol-ume ($F_V$), surface tension ($F_\sigma$), bending curvature ($F_B$) and edge tension ($F_l$). Thus, in the stressed state we have that the free energy has the following expression (Hernández-Zapata et al.2009)

$$dF = -\Delta P dV_{in} + \tilde{\sigma} dA + \gamma dl, \tag{1}$$

where at the right-hand side we used the relation $dV_{out} = -dV_{in}$ and $\tilde{\sigma}$ stands for the total effective surface

tension given by $\tilde{\sigma} = \sigma + \kappa_b/R^2$. The last term, responsi-ble for pore closure is the contour free energy with γ the edge tension and $l = 2\pi r$ the pore contour length. In this form, for small pore radius ($r(t)/R(t) \ll 1$) the open vesi-cle volume and area may be written as $V_{in} = 4\pi R^3/3$ and $A = 4\pi R^2 - \pi r^2$ respectively, thus allowing us to express the free energy differential in terms of radii r and R:

$$dF = 4\pi R(2\tilde{\sigma} - R\Delta P)dR + 2\pi(\gamma - \tilde{\sigma} r)dr. \tag{2}$$

Notice that before the initial perturbation that yields the appearance of a surface tension ($\sigma = 0$) in the membrane of the vesicle, the radius of the vesicle is R_{eq}. In that case, Eq. (2) reduces to: $\Delta P_{eq} = 2\kappa_B/R^3$.

The explicit expression of the surface tension can be deduced by noticing that the energy required to stretch a membrane by reducing the thermal fluctuations on the vesi-cle shape is given by the expression (Levin and Idiart 2004)

$$F_\sigma = \sigma_c \frac{(A - A_{eq})^2}{2A_{eq}}, \tag{3}$$

where σ_c is a characteristic surface tension, while A_{eq} and A are vesicle areas before and after increasing the mem-brane tension. Since the temperature and the volume of the solution are controlled, the change in surface energy $dF_{\bullet}$ with respect to the area of the membrane is (Kondepudi and Prigogine 2007; Levin and Idiart 2004)

$$\sigma = \left(\frac{\partial F_{\bullet}}{\partial A}\right)_{T,V} = \sigma_c\left(\frac{A}{A_{eq}} - 1\right). \tag{4}$$

Using the explicit expression of the area in terms of r and R the last expression takes the following form

$$\sigma = \sigma_c\left[\frac{R^2}{R_{eq}^2} - \frac{r^2}{4R_{eq}^2} - 1\right]. \tag{5}$$

Equation (5) allows one to calculate the evolution of the surface tension during the entire process of opening and collapse of the pore. A similar expression was used in Bro-chard-Wyart et al. (2000) to express the area occupied by the lipid when the membrane is stretched.

After the pore collapse, the final radius R_f of the vesi-cle may be, in general, slightly larger than R_{eq} (see Erdem et al. 2003 and Figure 2 below) and therefore the final value of the pressure difference contains an additional contribu-tion, with respect to ΔP_{eq}, coming from a residual surface tension

$$\Delta P_f = \frac{2\tilde{\sigma}_f}{R_f} = \frac{2\sigma_f}{R_f} + \frac{2\kappa_B}{R_f^3}. \tag{6}$$

This equation states that the Laplace relation gives the final pressure with a correction term due to the bending energy of the vesicle. For a lipid bilayer, the value of the surface tension just before the opening of the pore takes the value $\sigma_0 \sim 10^{-5}$ N/m whereas the bending modulus is about $\kappa_b \sim 10^{-20}$ J. This implies that the bending modulus is $\kappa \sim 10^{-20}$ J at 18 °C (Rawicz et al. 2000) whereas the saddle-splay modulus is approximately given by $\bar{\kappa} \sim -a\kappa$ with $a \sim 1$ or less (Le et al. 2000; Siegel and Kozlov 2004). If the vesicle size is smaller than a critical size, $R_{eq} < (\kappa_b/\sigma_{eq})^{-1/2} \sim 10^{-2}$ μm, then the correction term due to the bending energy of the ves-icle cannot be ignored. In the case of giant unilamellar vesi-cles, the bending energy effect on the final pressure is small.

The pressure difference ΔP decreases as the pore col-lapses and the radius of the vesicle decreases because a sol-vent outflow. Previous simulations of this system indicate that during the collapse the vesicle shape remains approxi-mately spherical (Ryham et al. 2012). Hence, an expression for the pressure difference can be obtained by calculating the mass outflow per unit time for a sphere with a pore which can be expressed in the general form

$$Q = \int \rho \mathbf{v} \cdot d\mathbf{s}, \tag{7}$$

where ρ is the solvent mass density, $\mathbf{v}$ is the flux velocity crossing the pore with differential surface area ds. Using the Navier-Stokes equation for stationary states and incom-pressible fluids, $\eta_s \nabla^2 \mathbf{v} - \nabla P = 0$, the approximate expres-sion for the velocity in this case is

$$v \simeq \frac{2r\Delta P}{\eta_s}. \tag{8}$$

Considering cylindrical coordinates and combining Eqs. (7) and (8), we obtain the following formula for the pressure difference in terms of the mass outflow per unit time

$$\Delta P = \frac{3\eta_s}{4\pi pr^3} Q(R). \tag{9}$$

An explicit relation in terms of the vesicle radius will be given in the next section.

Onsager's irreversible thermodynamics and the dynamic equations

The equations governing the dynamics for $r(t)$ and $R(t)$ can be deduced following Onsager's irreversible thermodynam-ics (Kondepudi and Prigogine 2007) that establishes phe-nomenological relations for the time evolution of the sys-tem thermodynamic variables in terms of the conjugated generalized forces (Kondepudi and Prigogine 2007). This procedure is systematic because it uses the second law of the thermodynamics through the calculation of the entropy production.

From thermodynamics, it can be shown the general rela-tion: $\mathrm{d}F = -T\mathrm{d}_i S < 0$, where $\mathrm{d}_i S$ is the entropy produced during an irreversible process occurring at constant volume and temperature (Kondepudi and Prigogine 2007). There-fore, the entropy produced per unit time is proportional to the time change of the system Helmholtz free energy

$$T\frac{\mathrm{d}_i S}{\mathrm{d}t} = -\frac{\mathrm{d}F}{\mathrm{d}t} = -\frac{\partial F}{\partial r}\frac{\mathrm{d}r}{\mathrm{d}t} - \frac{\partial F}{\partial R}\frac{\mathrm{d}R}{\mathrm{d}t} > 0 \tag{10}$$

In this expression, the time derivatives play the role of flows, $J_k = \mathrm{d}\zeta_k/\mathrm{d}t$, whereas the partial derivatives play the role of generalized forces $X_k = -\partial F/\partial\zeta_k$, (Kondepudi and Prigogine 2007). Following the rules of non-equilibrium thermodynamics, we may assume that the flows are linear functions of the forces $J_k = \sum_j L_{kj}X_j$, where the L_{kj} are the so-called Onsager's phenomenological coefficients. Addi-tionally, the elements L_{kj} also obey the Onsager reciprocal relations $L_{kj} = L_{jk}$. These reciprocal relations are associ-ated with cross effects (Onsager 1931). Therefore the linear laws imply

$$\begin{aligned} \frac{\mathrm{d}r}{\mathrm{d}t} &= -L_{rr}\frac{\partial F}{\partial r} - L_{rR}\frac{\partial F}{\partial R}, \\ \frac{\mathrm{d}R}{\mathrm{d}t} &= -L_{RR}\frac{\partial F}{\partial R} - L_{Rr}\frac{\partial F}{\partial r}. \end{aligned} \tag{11}$$

The Onsager's coefficients L_{ij} are mobilities characteriz-ing the change of the radii in time (flows) to the change of the energy as a function of the radii (forces). There-fore, the units of the Onsager coefficients are the inverse of Jules-second per square meters: $[L^{-1}] = \mathrm{J}\cdot\mathrm{s}/\mathrm{m}^2$. Fur thermore, the experimental results (Olivier et al. 1999; Erdem et al. 2003) suggest that the duration of the pore dynamics depends on the viscosity of the medium in such a way that the dynamics is slower when the solvent vis-cosity increases. Since the dimensions of the viscosity are s =J s m3, then we have that

$$L_{rr}^{-1} \propto \eta_s l_1, \quad L_{rR}^{-1} = L_{Rr}^{-1} \propto \eta_s l_2 \quad \text{and} \quad L_{RR}^{-1} \propto \eta_s l_3, \tag{12}$$

with l_i $(i = 1, 2, 3)$ a characteristic length of the system. In general, the Onsager coefficients may be functions of the

state variables r or R (Kondepudi and Prigogine 2007). The election of this dependence is crucial and a proper choice leads to a physically consistent description of the process. In the following we will show that pre-existing models can be obtained from our general theory by assuming constant coefficients, whereas a fully consistent system of equations need to consider that the coefficients should depend on the state variables.

The change with time of the free energy given by Eq. (2) is therefore

$$\frac{\mathrm{d}F}{\mathrm{d}t} = 4\pi R(2\tilde{\sigma} - R\Delta P)\frac{\mathrm{d}R}{\mathrm{d}t} + 2\pi(\gamma - \tilde{\sigma}r)\frac{\mathrm{d}r}{\mathrm{d}t}. \qquad (13)$$

In this form, following the general scheme already pre-sented we may write down the following set of dynamical equations for the time evolution of the pore and vesicle radii

$$\frac{\mathrm{d}r}{\mathrm{d}t} = 2\pi L_{rr}\{\tilde{\sigma}r - \gamma\} - 4\pi R L_{rR}\{2\tilde{\sigma} - R\Delta P\}, \qquad (14)$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = -4\pi R L_{RR}\{2\tilde{\sigma} - R\Delta P\} + 2\pi L_{Rr}\{\tilde{\sigma}r - \gamma\}. \qquad (15)$$

Equations (14) and (15) with a non-zero initial condition for the pore radius predict the dynamics of the transient pore in spherical vesicles. Both equations are coupled through the effective surface tension and the pressure difference terms.

Dynamic equations and Onsager coefficients

Dimensional analysis establishes that the Onsager coeffi-cients have to be inversely proportional to a viscosity and a characteristic length. Irreversible thermodynamics states that phenomenological coefficients can in general be func-tions of the state variables. Therefore, here we will propose an expression for the Onsager coefficients as functions of the system's state variables r and R, that yields a physically consistent model of pore dynamics.

First, we have to emphasize that Eqs. (14) and (15) are not physically consistent if the Onsager coefficients are constants, because in this case when the pore collapses ($r = 0$) the time derivatives $\mathrm{d}r/\mathrm{d}t$ and $\mathrm{d}R/\mathrm{d}t$ are propor-tional to $-\gamma$, which has been considered in the literature as a constant parameter (Olivier et al. 1999; Erdem et al. 2003; Thomas and Dimova 2010; Brochard-Wyart et al2000; Ryham et al2011). Hence, at$r = 0$ the dynamics does not vanishes.

The Onsager coefficients are the inverse relaxation times of the pore dynamics. As implemented in experi-ments (Olivier et al. 1999; Erdem et al. 2003; Thomas and Dimova 2010), changing the solution viscosity η_s allows slowing down of the pore dynamics. Thus, we may assume that the Onsager coefficients scale with the solution vis-cosity s. In view of this, a consistent choice of the coef-ficients that satisfies the conditions (12) does not intro-duce unknown free parameters and satisfies conditions dr dt = 0 and dR dt = 0 at r = 0, is given by the relations

$$L_{rr} = L_{RR} = \frac{1}{2\pi r_0 \eta_s}\frac{r}{R} \quad \text{and} \quad L_{rR} = \frac{1}{2\pi r_0 \eta_s}\frac{r^2}{R^2}, \qquad (16)$$

where $2\pi r_0$ is the initial length of the pore (perimeter) in which the initial radius r_0 can be estimated from Eq. (5). Note that both Onsager coefficients are well defined for all values of the radii. At the initial condition, they take the values: $L_{rr} = L_{RR} = 1/(2\pi\eta_s R_0)$ and $L_{rR} = r_0/(2\pi\eta_s R_0^2)$, whereas for the final state they vanish. A more general expression for the Onsager coefficients can be proposed by considering the effect of the lipid viscosity η_2 in the form: $L_{ii} \simeq (\eta_s r_0 + \eta_2 d)^{-1}(r/R)$, $L_{ij} \simeq (\eta_s r_0 + \eta_2 d)^{-1}(r/R)^2$ with d the thickness of the bilayer. This second term could be of importance in the case of low viscous environments, see Thomas and Dimova (2010).

Thus, Eqs. (14) and (15) take the form

$$r_0\eta_s\frac{dr}{dt} = \{\tilde{\sigma}r - \gamma\}\frac{r}{R} - 2\{2\tilde{\sigma} - R\Delta P\}\frac{r^2}{R}, \tag{17}$$

$$r_0\eta_s\frac{dR}{dt} = -2\{2\tilde{\sigma} - R\Delta P\}r + \{\tilde{\sigma}r - \gamma\}\frac{r^2}{R^2}. \tag{18}$$

This set of equations must be complemented with the expressions for the effective surface tension $\tilde{\sigma} = \sigma + \kappa_b/R^2$ with σ given by Eq. (5), and the pressure difference ΔP given by Eq. (9). The pressure difference ΔP is related with the time variation of the vesicle volume in the form

$$Q = -\rho\frac{dV_{in}}{dt} = -4\pi\rho R^2\dot{R}. \tag{19}$$

From Eqs. (9) and (19), we obtain

$$\Delta P = -\frac{3\eta_s R^2}{r^3}\dot{R}. \tag{20}$$

Equations (17) and (18) constitute a coupled set of two ordinary non-linear differential equations that have to be solved numerically along with (5) and (20). The results of the numerical integration of the radii $r(t)$ and $R(t)$ (solid lines) are shown in Figs. 2, 3, and 4 together with experi-mental data (symbols) taken from Erdem et al. (2003), Thomas and Dimova (2010), Brochard-Wyart et al. (2000). Our model reproduces very well the three stages of the dynamics of the pore radius (opening, decay, and collapse). In addition, our model allows determining the time dependence of the effective surface tension and of the pressure dif-ference as it is also shown in Figs. 2, 3, and 4.

The values of the solvent viscosity η_s, the equilibrium vesicle radius R_{eq}, the initial pore radius, r_0 and the initial vesicle radius, R_0 indicated in Table 1 and used in Figs. 2, 3, and 4, were taken from experimental reports from Erdem et al. (2003), Thomas and Dimova (2010), and Brochard-Wyart et al. (2000), respectively. According to Rawicz et al. (2000), we assumed that the bending modulus is equal to $\kappa_b = 29 \times 10^{-20}$ J in all cases. The characteris-tic surface tension was estimated by using the expression $\sigma_c = (48\pi\kappa^2)/(R_{eq}^2 K_B T)$, (Brochard-Wyart et al. 2000; Ryham et al. 2011). The last column in Table 1 reports the values of the edge tension obtained from the fits.

In order to conclude this section, we want to discuss the assumptions underlying a very well known model that has been widely used for estimating the value of the edge ten-sion γ from experiments (Brochard-Wyart et al. 2000). This model is based on the following equation for the pore radius

$$\frac{dr}{dt} = 2\pi L_{rr}\{\sigma r - \gamma\} \tag{21}$$

and on the following equation for the vesicle radius

$$\dot{R} = -\frac{2\sigma}{3\eta_s}\frac{r^3}{R^3}. \tag{22}$$

In writing this system of equations, one has to assume that the vesicle free energy does not depend on the vesicle radius R and that the total surface energy only depends on the surface tension σ [given by Eq. (5)], that is, the bending contribution κ_b is neglected. In addition, the equilibrium Laplace relation between the surface tension and the pres-sure difference is assumed to hold during the whole pro-cess, $\Delta P = 2\sigma/R$. As a consequence of these assumptions,

the model implicitly assumes that $L_{rR} = 0$ (no cross effects) and that $L_{rr}^{-1} = 4\pi\eta_2 d$, with η_2 the membrane viscosity (which is an unknown parameter) and d the bilayer width (Brochard-Wyart et al. 2000). Equations (21) and (22) reproduce the experimental results provided that the vesi-cle radius dynamics, $\mathrm{d}R/\mathrm{d}t$, is controlled by the solution viscosity η_s whereas the membrane viscosity, η_2, dictates the pore dynamics. This fact seems to be inconsistent with experimental evidence (Erdem et al. 2003; Thomas and Dimova 2010). In addition, Eq. (21) does not satisfy the condition that it must be a critical point, that is, $\mathrm{d}r/\mathrm{d}t = 0$ and $\mathrm{d}R/\mathrm{d}t = 0$ at $r = 0$ and $R = 0$. From this equation it is clear that $\mathrm{d}r/\mathrm{d}t|_{r=0} \neq 0$ yielding to negative pore radius.

Discussion and conclusion

In this work, we propose a theoretical model that accounts for all the features of the dynamics of transient pores in spherical lipid vesicles (see Figs. 2a, 3a, and 4a). Essen-tially, it allows determining the value of pore edge tension along with the time course of the vesicle radius, the effective surface tension, and the pressure difference from the measurements of the pore radius (Erdem et al. 2003; Thomas and Dimova 2010) (see Figs. 2b–d, 3b–d, and 4b–d). All the parameters included have a clear physical meaning and can be directly measured in experiments; for example, the viscosity of the solution, the bending modu-lus, or the initial surface tension (see Table 1).

The model was deduced by using Onsager's irreversible thermodynamics and it consists of two coupled first-order time differential equations for the pore and the vesicle radii, $r(t)$ and $R(t)$, respectively. The coupling emerges from the dependence of the surface tension and the pressure differ-ence on the state variables (r and R) and from cross effects coming from the linear laws of non-equilibrium thermo-dynamics. The latter introduce Onsager's phenomenologi-cal coefficients whose dependence on r and R is provided to have a consistent physical model. This condition is not fulfilled by previous models (Brochard-Wyart et al. 2000; Ryham et al. 2011). In agreement with experiments, the characteristic relaxation time of the pore dynamics is con-trolled by the solution viscosity, $\tau_{\mathrm{relax}} \propto \eta_s$, see Eqs. (17) and (18).

The continuous lines in 2b, 3b, and 4b show the evolu-tion of the radius of the vesicle, as predicted by the numeri-cal solution of Eqs. (17) and (18). This behavior is in agree-ment with the experimental results (Fig. 2b). In particular, in experiments with constant illumination, this evolution is typical, and the vesicle radius may be reduced by 40 % (Rodriguez et al. 2006). The panels (c) and (d) of Figs. 2, 3 and 4 show that after the pore formation, the surface tension and the pressure difference decrease drastically until the pore radius reaches its maximum value. After that, both increase slowly until a final equilibrium value of about 25 % of their initial value.

The previous results show that the membrane is not fully relaxed after the pore collapse. Furthermore, correlating the results shown in Figs. 2a, 3a, and 4a with their counterparts in (c) and (d), and with Eqs. (17) and (18), we see that it is more convenient to calculate the edge tension, γ, from the slope of the initial quasi-linear relaxation regime, because the change of the slope in the final collapse stage manifests clearly the non-linear nature of the process attributable to the interplay among the edge and the surface tensions together with the pressure difference. In Fig. 2a we show this through the average slopes of the two regimes, shown by the solid and dashed straight lines.

Near the initial quasi-linear relaxation regime, both $\tilde{\sigma}$ and ΔP nearly vanish (see Figs. 2a, 3, and 4a) and, there-fore, Eqs. (17) and (18) may be approximated to give the following relaxation equation for the pore radius

$$\frac{\mathrm{d}r}{\mathrm{d}t} = -\frac{\gamma}{r_0\eta_s R_0} r. \tag{23}$$

From this equation we can obtain a simple and useful expression for the edge tension as a function of the pore radius

$$\gamma \simeq -r_0\eta_s R_0 \frac{\Delta \ln |r|}{\Delta t}. \tag{24}$$

Using this expression, we can calculate the values of the edge tensions reported in the first column of Table 2. These results correlate well with those predicted by the whole model that incorporates all the mechanisms participating in the relaxation dynamics; that is, it includes the effects due to the surface tension and the pressure difference. If Eq. (24) is used in the final regime (a rough approximation), then the values obtained for the edge tension are about one order of magnitude larger than in the first regime. Notice, in addition, that these values almost duplicate the corresponding ones reported in previous works (Erdem et al. 2003; Thomas and Dimova 2010; Brochard-Wyart et al. 2000), see the third and fourth columns of Table 2. The rea-son is that previous estimations of the edge tension have been obtained using the model proposed in Brochard-Wyart

et al. (2000). As discussed after Eqs. (21) and (22), the elec-tion of the Onsager coefficients in that case corresponds to $L_{rr}^{-1} \sim 4\pi\eta_2 d$ whereas in our case should be $L_{rr}^{-1} \sim 2\pi r_0 \eta_s$, involving a factor of two.

The values of the parameters taken from experimental data reported in the literature are the solvent viscosity, the initial and final vesicle radius, the characteristic value of the surface tension, and the bending modulus (Erdem et al. 2003; Thomas and Dimova 2010; Brochard-Wyart et al. 2000; Rawicz et al. 2000; Lipowsky 1995). The magnitude of the initial pore radius can be obtained from the relation for the surface tension. Therefore, all fits were obtained by only adjusting the edge tension.

It is convenient to mention that pore formation in vesi-cles immersed in high-viscosity solutions (32 cP), Figs. 2 and 4, is about $\delta t_{\text{formation}} = 0.1$-$0.2$ s. In contrast, when the solution's viscosity is near that of the cytoplasm (between 1 and 3 cP, as in Fig. 3), then the formation time is much less, $\delta t_{\text{formation}} = 0.0075$ s. This second formation time scale is in the order of magnitude with what is expected for pore dynamics in living cells. It is interesting to notice that for the low-viscosity experiment, Fig. 3, all the values reported in Table 2 are consistent between them, in contrast with the data obtained in the other two cases.

To conclude, we may stress that Onsager's irrevers-ible thermodynamics is a powerful formalism that allows deducing simple and consistent physicochemical models for biological small systems and in particular in describing the dynamics of pores in biological membranes. The obtained equations are consistent with the second law of thermodynamics and it may easily be generalized to more complex situations like those in which noise or external forces affect the behavior of the system.

Acknowledgments We thank A. Ledesma-Durán and A. Arteaga-Jiménez for their useful discussions. This work was supported by UNAM DGAPA Grant No. IN113415 and CONACYT.

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