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

L. Martínez-Balbuena1 · E. Hernández-Zapata2 · I. Santamaría-Holek3

Received: 29 January 2015 / Revised: 31 March 2015 / Accepted: 2 June 2015 / Published online: 21 June 2015
© European Biophysical Societies’ Association 2015

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 completely 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 viscosity 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 physicochemical 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 between peptides and the lipid membranes (Matsuzaki 1999; Zasloff 2002). Nevertheless, pore formation has also been studied in prebiotic environments, 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 formation 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 McMahon 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).
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 generated 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, $R \sim 10–100 \mu$m, 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 application 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 resolution. This method avoids some disadvantages of previous techniques, such as the use of fluorescent probes and glycerol 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 $r_{\text{min}} = 0.75 \mu$m. However, for vesicles with similar diameters, all these techniques produce pores with maximum radii in the range $r_{\text{max}} = 5–11 \mu$m.

In this work, we are interested in the theoretical description of pore dynamics after its formation. This dynamics can be divided into three stages. The first stage is characterized 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 participating in the dynamics, the edge and surface tensions together with the pressure difference.

The evolution of pores in membranes has been previously described in Brochard-Wyart et al. (2000) by adopting 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 (Degréas et al. 1995; Diederich et al. 1998). An important assumption of the mentioned description is that the solution viscosity is small and the membrane viscosity 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 regulate the pore closing velocity (Erdem et al. 2003; Thomas and Dimova 2010). An extension of this theory was formulated 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 viscosity that accounts for the dependence of the pore closing velocity on the solution viscosity. As a consequence, the dependence of the pore collapse kinetics on the membrane 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 thermodynamics 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 equations 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 closing velocity is mainly controlled by the edge tension and the solution viscosity. We also determine the time evolution 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 general formalism depending on the election of the Onsager phenomenological coefficients. The corresponding inconsistencies 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 aqueous solution at constant temperature $T$ and total volume $V = V_{\text{in}} + V_{\text{out}}$ with $V_{\text{in}} = (4/3)\pi R^{3}$ the volume inside the vesicle and $V_{\text{out}}$ the volume outside the vesicle, see Fig. 1c. The curved membrane has by definition a bending free energy per unit area given by $\kappa_{b}/R_{eq}^{2}$, with $\kappa_{b} = 2\kappa + \kappa_{b}$ being $\kappa$ and $\kappa_{b}$ 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, $\Delta P = P_{\text{in}} - P_{\text{out}} > 0$.

After an initial perturbation that increases the membrane surface tension to $\sigma_{0}$ and the vesicle radius to $R_{0}$, a pore radius $r(t)$ is produced that initiates a time-dependent process in which the radius of the vesicle becomes a function of time, $R(t)$, see Fig. 1b. Here, we will analyze the dynamical process of the pore growth and collapse in terms of $r(t)$ and $R(t)$.

The free energy change of the system has four contributions: $dF = dF_{V} + dF_{\sigma} + dF_{B} + dF_{l}$, coming from volume ($dF_{V}$), surface tension ($dF_{\sigma}$), bending curvature ($dF_{B}$) and edge tension ($dF_{l}$). Thus, in the stressed state we have that the free energy has the following expression (Hernández-Zapata et al. 2009)

$$dF = -\Delta PdV_{\text{in}} + \tilde{\sigma}dA + \gamma dl,$$

where at the right-hand side we used the relation $dV_{\text{out}} = -dV_{\text{in}}$ and $\tilde{\sigma}$ stands for the total effective surface tension given by $\tilde{\sigma} = \sigma + \kappa_{b}/R^{2}$. The last term, responsible for pore closure is the contour free energy with $\gamma$ 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 vesicle volume and area may be written as $V_{\text{in}} = 4\pi R_{\text{eq}}^{3}/3$ 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 $R$:

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

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: $\Delta P_{eq} = 2\kappa_{B}/R_{eq}^{3}$.

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 vesicle shape is given by the expression (Levin and Idiart 2004)

$$F_{\sigma} = \sigma_{c} \frac{(A - A_{eq})^{2}}{2A_{eq}}, \quad (3)$$

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

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

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

$$\sigma = \sigma_{c} \left[ \frac{R^{2}}{R_{eq}^{2}} - \frac{r^{2}}{4R_{eq}^{2}} - 1 \right]. \quad (5)$$

Equation (5) allows one to calculate the evolution of the surface tension during the entire process of opening and

![Fig. 1](image-url)  
**Fig. 1** a Schematic representation of a vesicle with pore, b stressed vesicle, and formation of a transient pore. c Relaxed vesicle
collapse of the pore. A similar expression was used in Brochard-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 vesicle 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 contribution, with respect to \( \Delta P_{eq} \), coming from a residual surface tension

\[
\Delta P_f = \frac{2\sigma_f}{R_f} = \frac{2\sigma_f}{R_f} + \frac{2\kappa_B}{R_f^3}.
\]

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 \( \kappa = a \sim 1 \) or less (Le et al. 2000; Siegel and Kozlov 2004). If the vesicle size is smaller than a critical size, \( R_{eq} \) \( \sim \) \( (\kappa_B/\sigma_{eq})^{-1/2} \) \( \sim 10^{-2} \) \( \mu \)m, then the correction term due to the bending energy of the vesicle cannot be ignored. In the case of giant unilamellar vesicles, the bending energy effect on the final pressure is small.

The pressure difference \( \Delta P \) decreases as the pore collapses and the radius of the vesicle decreases because a solvent outflow. Previous simulations of this system indicate that during the collapse the vesicle shape remains approximately 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

\[
Q = \int \rho v \cdot ds,
\]

where \( \rho \) is the solvent mass density, \( v \) is the flux velocity crossing the pore with differential surface area \( ds \). Using the Navier-Stokes equation for stationary states and incompressible fluids, \( \eta_s \nabla^2 v - \nabla P = 0 \), the approximate expression for the velocity in this case is

\[
v \simeq \frac{2r \Delta P}{\eta_s}.
\]

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

\[
\Delta P = \frac{3\eta_s}{4\pi \rho r^2} Q(R).
\]

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 thermodynamics (Kondepudi and Prigogine 2007) that establishes phenomenological relations for the time evolution of the system 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 relation:

\[
\frac{d}{dt} \left( -T \frac{dS}{d\tau} \right) < 0,
\]

where \( dS \) is the entropy produced during an irreversible process occurring at constant volume and temperature (Kondepudi and Prigogine 2007). Therefore, the entropy produced per unit time is proportional to the time change of the system Helmholtz free energy

\[
\frac{d}{dt} \frac{dS}{d\tau} = -\frac{dF}{d\tau} < 0.
\]

In this expression, the time derivatives play the role of flows, \( J_k = d\xi_k/dt \), whereas the partial derivatives play the role of forces \( \xi_k = -\partial F/\partial \xi_k \) (Kondepudi and Prigogine 2007). Following the rules of non-equilibrium thermodynamics, we may assume that the flows are linear functions of the forces \( J_k = \sum_j L_{kj} \xi_j \), where the \( L_{kj} \) are the so-called Onsager’s phenomenological coefficients. Additionally, the elements \( L_{kj} \) also obey the Onsager reciprocal relations \( L_{kj} = L_{jk} \). These reciprocal relations are associated with cross effects (Onsager 1931). Therefore the linear laws imply

\[
\begin{align*}
\frac{dr}{dt} & = -L_{rr} \frac{\partial F}{\partial r} - L_{rR} \frac{\partial F}{\partial R}, \\
\frac{dR}{dr} & = -L_{RR} \frac{\partial F}{\partial R} - L_{Rr} \frac{\partial F}{\partial r}.
\end{align*}
\]

The Onsager’s coefficients \( L_{ij} \) are mobilities characterizing the change of the radii in time (flows) to the change of the energy as a function of the radii (forces). Therefore, the units of the Onsager coefficients are the inverse of Jules-second per square meter: \( [L_{ij}^{-1}] = J \cdot s/m^2 \). Furthermore, 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 viscosity increases. Since the dimensions of the viscosity are \( [\eta] = J \cdot s/m^3 \), then we have that

\[
L_{rr}^{-1} \propto \eta_1, \quad L_{rR}^{-1} = L_{Rr}^{-1} \propto \eta_2 \quad \text{and} \quad L_{RR}^{-1} \propto \eta_3.
\]

with \( \eta_i \) (\( i = 1, 2, 3 \)) 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

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

(13)

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

\[
\frac{dr}{dt} = 2\pi L_{rr} [\tilde{\sigma} r - \gamma] - 4\pi RL_{rr} (2\tilde{\sigma} - R\Delta P),
\]  

(14)

\[
\frac{dR}{dt} = -4\pi RL_{RR} (2\tilde{\sigma} - R\Delta P) + 2\pi L_{Rr} [\tilde{\sigma} r - \gamma].
\]  

(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 coefficients have to be inversely proportional to a viscosity and a characteristic length. Irreversible thermodynamics states that phenomenological coefficients can in general be functions 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 \( dr/dt \) and \( dR/dt \) are proportional 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 al. 2000; Ryham et al. 2011). Hence, at \( r = 0 \) the dynamics does not vanishes.

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

\[
L_{rr} = L_{RR} = \frac{1}{2\pi r_0 \eta_s}, \quad L_{rr} = \frac{1}{2\pi r_0 \eta_s} \frac{r^2}{R^2},
\]  

(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 \( \eta_2 \) in the form:

\[
L_{ii} \simeq (\eta_s r_0 + \eta_2 d)^{-1} (r/R), \quad L_{ij} \simeq (\eta_s r_0 + \eta_2 d)^{-1} (r/R)^2
\]  

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

\[
r_0 \eta_s \frac{dr}{dt} = (\tilde{\sigma} r - \gamma) \frac{r}{R} - 2(2\tilde{\sigma} - R\Delta P) \frac{r^2}{R^2}.
\]  

(17)

\[
r_0 \eta_s \frac{dR}{dt} = -2(2\tilde{\sigma} - R\Delta P) r + (\tilde{\sigma} r - \gamma) \frac{r^2}{R^2}.
\]  

(18)

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

\[
Q = -\rho \frac{dV_{in}}{dt} = -4\pi \rho R^2 \dot{\dot{R}}.
\]  

(19)

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

\[
\Delta P = -\frac{3\eta_s R^2}{r^3} \dot{\dot{R}}.
\]  

(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 experimental 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 difference as it is also shown in Figs. 2, 3, and 4.

The values of the solvent viscosity, \( \eta_s \), 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 characteristic surface tension was estimated by using the expression $\sigma_c = \frac{48\pi \kappa^2}{(R_0^2 K_B T)}$, (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 tension $\gamma$ from experiments (Brochard-Wyart et al. 2000). This model is based on the following equation for the pore radius

$$\frac{dr}{dt} = 2\pi L_{tr}(\sigma r - \gamma)$$

(21)

and on the following equation for the vesicle radius

$$\dot{R} = -\frac{2\sigma}{3\eta s} \frac{r^3}{R^3}.$$  

(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 $\sigma$ [given by Eq. (5)], that is, the bending contribution $\kappa_b$ is neglected. In addition, the equilibrium Laplace relation between the surface tension and the pressure difference is assumed to hold during the whole process, $\Delta P = 2\sigma / R$. As a consequence of these assumptions,
the model implicitly assumes that \( L_{rR} = 0 \) (no cross effects) and that \( L_{rr} = 4\pi \eta_2 d \), with \( \eta_2 \) 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 vesicle radius dynamics, \( dr/dt \), is controlled by the solution viscosity \( \eta_s \) whereas the membrane viscosity, \( \eta_2 \), 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, \( dr/dt = 0 \) and \( dR/dt = 0 \) at \( r = 0 \) and \( R = 0 \). From this equation it is clear that \( dr/dt|_{r=0} \neq 0 \) 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). Essentially, 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 modulus, 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 difference on the state variables \( r \) and \( R \) and from cross effects coming from the linear laws of non-equilibrium thermodynamics. The latter introduce Onsager’s phenomenological 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 controlled by the solution viscosity, \( \gamma_{\text{relax}} \propto \eta_s \), see Eqs. (17) and (18).

The continuous lines in 2b, 3b, and 4b show the evolution of the radius of the vesicle, as predicted by the numerical solution of Eqs. (17) and (18). This behavior is in agreement 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 %

| \( \eta_s \) (cP) | \( R_{eq} \) (\( \mu \)m) | \( r_0 \) (\( \mu \)m) | \( R_0 \) (\( \mu \)m) | \( \kappa_b \) (10\(^{-20}\) J) | \( \sigma_c \) (10\(^{-5}\) Nm\(^{-1}\)) | \( \gamma \) (pN) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 32              | 19.6            | 1.10            | 20.5            | 29              | 2.9             | 0.92            |
| 1.13            | 19.7            | 1.15            | 20.4            | 29              | 3.0             | 1.21            |
| 32              | 14.5            | 1.15            | 14.7            | 29              | 3.1             | 0.57            |

Fig. 4 Comparison of the numerical solutions (solid lines) of Eqs. (17) and (18), along with experimental data (symbols) taken from Brochard-Wyart et al. (2000): a pore radius as a function of time, b vesicle radius as a function of time, c surface tension, and d pressure difference. The parameters taken from the experimental measurements are \( R_{eq} = 14.5 \) \( \mu \)m, \( R_0 = 14.7 \) \( \mu \)m, \( r_0 = 1.15 \) \( \mu \)m, \( \sigma_c = 3.1 \times 10^{-5} \) N m\(^{-1}\), \( \eta_s = 32 \) cP, and \( \kappa_b = 29 \times 10^{-20} \) J. The edge tension was considered as a fitting parameter and was found to be equal to \( \gamma = 0.57 \) pN.
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, $\gamma$, 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 $\Delta P$ nearly vanish (see Figs. 2a, 3, and 4a) and, therefore, Eqs. (17) and (18) may be approximated to give the following relaxation equation for the pore radius

$$\frac{dr}{dt} = -\frac{\gamma}{r_0 \eta_s R_0} r. \quad (23)$$

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

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

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 reason 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 election 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 vesicles 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 irreversible 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.

References

Brochard-Wyart F, de Gennes PG, Sandre O (2000) Transient pores in stretched vesicles: role of leak-out. Physica A 278:32–51
Cooper RA, Shattil SJ (1971) Mechanisms of hemolysis—the minimal red-cell defect. N Engl J Med 285(27):1514–1520
Debrégeas G, Martin P, Brochard-Wyart F (1995) Viscous bursting of suspended films. Phys Rev Lett 75:3886–3889
Diederich A, Bähr G, Winterhalter M (1998) Influence of polylysine on the rupture of negatively charged membranes. Langmuir 14:4597–4605

Doherty GJ, McMahon HT (2009) Mechanisms of endocytosis. Annu Rev Biochem 78:857–902

Helfrich W (1973) Elastic properties of lipid bilayers—theory and possible experiments. Z Naturforsch C C28:693–703

Hernández-Zapata E, Matínez-Balbuena L, Santamaria-Holek I (2009) Thermodynamics and dynamics of the formation of spherical lipid vesicles. J Biol Phys 35:297–308

Isambert H (1998) Understanding the electroporation of cells and artificial bilayer membranes. Phys Rev Lett 80:3404–3407

Karatekin E, Sandre O, Guiouni H, Borghi N, Puech P-H, Brochard-Wyart Françoise (2003) Cascades of transient pores in giant vesicles: line tension and transport. Biophys J 84:1734–1749

Kondepudi D, Prigogine I (2007) Modern thermodynamics. Wiley, England

Koslov MM, Markin VS (1984) A theory of osmotic lysis of lipid vesicles. J Theor Biol 109:17–39

Le TD, Olsson U, Mortensen K (2000) Topological transformation of a surfactant bilayer. Physica B 276–278:379–380

Levin Y, Idiart MA (2004) Pore dynamics of osmotically stressed vesicles. Physica A 331:571–578

Lipowsky R (1995) Generic interactions of flexible membranes. In: Lipowsky BR, Sackmann E (eds) Structure and dynamics of membranes: generic and specific interactions. Handbook of biological physics, V. 1. Elsevier Science, B.V. Amsterdam, The Netherlands, pp 521–602

Liu T, Singh P, Jenkins JT, Jagota A, Bykhovskaia M, Hui C-Y (2015) A continuum model of docking of synaptic vesicle to plasma membrane. J R Soc Interf 12:20141119

Majd S, Yusko EC, Billeh YN, Macrea MX, Yang J, Mayer M (2010) Applications of biological pores in nanomedicine, sensing, and nanoelectronics. Curr Opin Biotechnol 21:439–476

Matsuzaki K (1999) Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim Biophys Acta 1462:1–10

McNeil PL, Steinhardt RA (2003) Plasma membrane disruption: repair, prevention, adaptation. Annu Rev Cell Dev Biol 19:697–731

Monck JR, Fernandez JM (1994) The exocytotic fusion pore and neurotransmitter release. Neuron 12:707–716

Mukherjee S, Ghosh RN, Maxfield FR (1997) Endocytosis. Physiol Rev 77:759–803

Onsager L (1931) Reciprocal relations in irreversible processes I. Phys Rev 37:405–426

Palade G (1975) Intracellular aspects of the process of protein synthesis. Science 189:347–358

Papo N, Shai Y (2003) Exploring peptide membrane interaction using surface plasmon resonance: differentiation between pore formation versus membrane disruption by lytic peptides. Biochemistry 42:458–466

Pavlín M, Kotník T, Miklavčič D, Kramar P, Lebar AM (2008) Electroporation of planar lipid bilayers and membranes. Adv Planar Lipid Bilayers Liposomes 6:165–226

Picco A, Mund M, Ries J, Nédélec F, Kaksonen M (2015) Visualizing the functional architecture of the endocytotic machinery. eLife. doi:10.7554/eLife.04553

Portet T, Dimova R (2010) A new method for measuring edge tensions and stability of lipid bilayers: effect of membrane composition. Biophys J 99:3264–3273

Prigogine I (1968) Introduction to thermodynamics of irreversible processes. Wiley, New York

Rawicz W, Olbrich KC, McIntosh T, Needham D, Evans E (2000) Effect of chain length and unsaturation on elasticity of lipid bilayers. Biophys J 79:328–339

Riske KA, Dimova R (2005) Electro-deformation and poration of giant vesicles viewed with high temporal resolution. Biophys J 88:1143–1155

Rodriguez N, Cribier S, Pincet F (2006) Transformation from long- to short-lived transient pores in giant vesicles in an aqueous medium. Phys Rev E 74:061902–061912

Ryham R, Berezovik I, Cohen FS (2011) Aqueous viscosity is the primary source of friction in lipidic pore dynamics. Biophys J 101:2929–2938

Ryham R, Cohen FS, Eisenberg R (2012) A dynamic model of open vesicles in fluids. Commun Math Sci 10(4):1273–1285

Sakuma Y, Imai M (2015) From vesicles to protocells: the roles of amphiphilic molecules. Life 5:651–675. doi:10.3390/life5010651

Sandre O, Moreaux L, Brochard-Wyart F (1999) Dynamics of transient pores in stretched vesicles. Proc Natl Acad Sci USA 96:10591–10596

Siegel DP, Kozlov MM (2004) The Gaussian curvature elastic modulus of N-monomethylated dioleoylphosphatidylethanolamine: relevance to membrane fusion and lipid phase behavior. Biophys J 87:366–374

Vardjan N, Jorgacevski J, Zorec R (2013) Fusion pores, SNAREs, and exocytosis. Neuroscientist 19:160–174

Weaver JC (2000) Electroporation of cells and tissues. IEEE Trans Plasma Sci 28:24–33

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415:389–395