Surface energy and separation mechanics of droplet interface phospholipid bilayers

Y. Huang1,†, V. Chandran Suja1,†, J. Tajuelo1,2 and G. G. Fuller1

1Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA
2Departamento de Física Interdisciplinar, Universidad Nacional de Educación a Distancia UNED, Madrid 28040, Spain

Droplet interface bilayers are a convenient model system to study the physico-chemical properties of phospholipid bilayers, the major component of the cell membrane. The mechanical response of these bilayers to various external mechanical stimuli is an active area of research because of its implications for cellular viability and the development of artificial cells. In this article, we characterize the separation mechanics of droplet interface bilayers under step strain using a combination of experiments and numerical modelling. Initially, we show that the bilayer surface energy can be obtained using principles of energy conservation. Subsequently, we subject the system to a step strain by separating the drops in a step-wise manner, and track the evolution of the bilayer contact angle and radius. The relaxation time of the bilayer contact angle and radius along with the decay magnitude of the bilayer radius were observed to increase with each separation step. By analysing the forces acting on the bilayer and the rate of separation, we show that the bilayer separates primarily through the peeling process with the dominant resistance to separation coming from viscous dissipation associated with corner flows. Finally, we explain the intrinsic features of the observed bilayer separation by means of a mathematical model comprising the Young–Laplace equation and an evolution equation. We believe that the reported experimental and numerical results extend the scientific understanding of lipid bilayer mechanics, and that the developed experimental and numerical tools offer a convenient platform to study the mechanics of other types of bilayers.

1. Introduction

The phospholipid bilayer is an important component of cell membranes that regulates material transport in a wide range of conditions [1–3]. It was first extracted and identified by Gorter and Grendel in 1925 [4]. Subsequent research, notably by Singer & Nicolson [5] through their fluid mosaic model of the cell membrane, improved the physio-chemical understanding of the lipid bilayer. Currently, lipid bilayers are commonly used to study ion transport and protein interactions across cell membranes [6–8]. One important property of the bilayer that influences the above transport processes as well as other cellular functions such as membrane fusion, ion binding and integral protein activity is the bilayer surface energy [9–11]. Another important aspect of bilayers that influences cellular functioning are the interaction forces within the bilayer [12,13], which are explored in the present work through the separation of the two monolayers forming the bilayer. Convenient investigation of the above bilayer features requires in vitro lipid bilayer models.

Several techniques have been reported in the literature for fabricating in vitro bilayers. These include solid-supported lipid bilayers (SLBs), giant uni-lamellar vesicles (GUVs), black lipid membranes (BLMs) and droplet interface bilayers (DIBs) [14–16]. The DIB is an attractive technique for creating artificial bilayers and has been widely used in the characterization of multiple bilayer features [17,18]. One key advantage of this technique, when compared with SLBs and BLMs, is that DIBs can be conveniently imaged using cameras. The captured
drop shapes, prior to the formation of the bilayer, can be used to calculate the monolayer surface tension by employing the Young–Laplace equation [19,20]. After the formation of the bilayer, the captured drop profiles conveniently allow us to track the mechanical response of the drops and the bilayer to external forcing. Furthermore, DIBs can be easily integrated with electrodes for measuring electrical properties [21,22] and with arrangements such as atomic force microscopy cantilever beams for measuring forces within the bilayer [23]. Because of these advantages, DIBs have been commonly used to study the electrical properties [24,25], permeability [26,27], bilayer surface energy [28,29], interaction and adhesion forces [23,30,31] and bilayer separation mechanics in a variety of conditions [21,32,33].

Despite the vast literature on the mechanics of DIBs and vesicle separation, the separation mechanics (unzipping) of a single bilayer in response to step-strain deformations has not been previously considered. We address this knowledge gap in this article through a combination of experiments and mathematical modelling. The experiments are performed on a custom-built set-up called an interfacial drainage dilatational and stability stage (I-DDiaSS), where the relative positions of a sessile and pendant drop can be precisely manipulated. The experiments are supported by a mathematical model, which serves to physically explain the observed bilayer separation mechanics. The rest of the article is organized as follows. In §2, we describe in detail the experimental set-up, methodology and mathematical model. The key findings from this study are reported in §3, where we show (i) a new method to calculate the bilayer surface energy employing principles of energy conservation, (ii) experimental data highlighting the intrinsic features of DIB separation under step strain, and (iii) a quantitative comparison of the experimental data against the predictions from the mathematical model. Finally, we conclude the article by discussing key areas for future research.

2. Material and methods

2.1. Materials

DPhPC (1,2-diphytanoyl-sn-glycero-3-phosphocholine) was used as the model lipid for generating the phospholipid bilayers reported in this article. DPhPC is chemically synthesized and stored in chloroform for ease of transfer and storage. It also has the advantage of not having any phase transitions between −120°C and +120°C. Prior to the start of the experiments, DPhPC (catalogue no. 850356; Avanti Polar Lipids Inc., Alabaster, AL) was extracted from the suspending chloroform solution in two steps. Initially, the bulk of the chloroform was evaporated off by gently blowing a stream of nitrogen over it for 45 min. Subsequently, the residual lipid film was vacuum dried for another 60 min. The chloroform-free lipids were then dissolved in hexadecane to give a final concentration of 10 mM.

Agarose gel, used as a core to support the pendant drop (figure 1), was created using agarose powder purchased from Thermo Fisher Scientific (catalogue no. BP164100). To do this, 300 mg of the powder was mixed with 10 ml of distilled water at high temperature, and then cooled to make the agarose gel [34,35]. A 1 M KCl solution was used for preparing the aqueous sessile and pendant droplets. The agarose core size that was used was much smaller than the pendant drop size to avoid any undesired influence of the agarose core on the reported bilayer dynamics.

2.2. Experimental set-up

To create and separate the droplet interface phospholipid bilayers, we used the I-DDiaSS set-up [36–38]. As shown in figure 1a, the I-DDiaSS consists of a glass chamber to hold the hexadecane solution and a sessile drop. The sessile drop is pinned at the centre of the glass chamber bottom using a circular scratch (not visible in the figure), and remains immobilized on the glass substrate during the course of the experiment. The glass chamber sits atop a motorized stage run by a stepper motor with a rotary encoder (Newport TRA12PPD and SMC100PP), which enables us to accurately position the sessile drop relative to a pendant drop. The pendant drop is held in place by a blunt capillary needle (ID: 0.58 mm; OD: 0.81 mm) with agarose gel at its tip. The agarose gel in the core of the pendant drop anchors the drop [35] and prevents dripping. The drop profiles and the bilayer radius are obtained through a side camera (UI 3060CP; IDS) using principles of shadowgraphy. The formation of the bilayer (see electronic supplementary material, Lipid Bilayer Formation) is also confirmed via a bottom camera (Infinity3-3UR; Lumenex).

2.3. Experimental protocol

At the start of an experiment, a predetermined amount of KCl solution was pipetted onto the circular scratch on the bottom of the chamber to form a sessile drop, and 0.2 ml of the hexadecane solution with the DPhPC lipid was gently added into the chamber until it fully covered the sessile drop. Then, a capillary needle with agarose was placed above the sessile drop, and another drop of the KCl solution was pipetted onto the agarose to form a pendant drop of volume \( V_p = 1 \text{ ml} \). Then, two precision stages that control the position of the glass chamber along the horizontal axes were used to place the two droplets along the same vertical axis. To study the effects of drop size on the mechanics of DIBs, we varied the sessile drop volume and obtained three different sessile-to-pendant drop volume ratios, namely \( V_s/V_p = 0.5, 1, 1.5 \).

To form the bilayers, the motorized stage translated the pendant drop into the lipid oil phase. Both the pendant and sessile drops were then aged for 15 min in order to allow for the formation of the lipid monolayers at the oil–water interfaces [35]. After ageing, the sessile drop was slowly pushed against the pendant drop for approximately 0.35 mm and then held in place [22]. The thin liquid film between the pendant and sessile droplets drains until the lipid monolayers are close enough to form a bilayer. As the bilayer forms, an advancing front that crosses the entire contact area is observed between the droplets in a short time (figure 1c). This important event confirms the formation of a bilayer between the droplets. Further details confirming the formation of the bilayer are available in the electronic supplementary material, Lipid Bilayer Formation. For all the reported experiments, the initial bilayer radius was 0.25 mm.

To conduct the bilayer separation experiments, we again used the motorized stage to pull the sessile drop away from the pendant drop in a step-wise manner at a velocity of 0.05 mm s\(^{-1}\) for 1 s. The step size (d) has a constant value of 0.05 mm (see electronic supplementary material, Effects of Step Size for the effects of step size), resulting in a step strain of \( d/R_s = 0.067 \), where \( R_s = 0.75 \text{ mm} \) is the apex drop curvature of the pendant drop. After each separation step, we allowed the bilayer to relax for 2 min. This process was continued until the sessile and pendant drops separated completely. The entire process of the bilayer formation and separation was captured using both the side and bottom cameras, and was subsequently analysed using Matlab. All experiments described in this article were performed at room temperature. Note that we have ignored contributions from the mechanical compliance of the droplets, owing to relatively small strains acting on the droplets over the course of the experiment. A schematic diagram
of the above-mentioned bilayer formation and separation on the I-DDiaSS is shown in figure 1b.

2.4. Mathematical model for droplet interface bilayer separation

Here a mathematical model is developed based on the Young–Laplace equation for capturing the separation mechanics of DIBs. In this simplified axisymmetric model, as shown in figure 2a, the agarose attached to the needle is ignored. The drop profiles are further assumed to be symmetric with respect to the plane of the bilayer. Physically, this assumption holds at low Bond number for sessile and pendant drops of comparable sizes. Under these assumptions, the non-dimensional Young–Laplace equation governing the shapes of the drops can be written as

\[
\frac{d\phi}{ds} = 2 - Bo \sin \phi \frac{r}{r},
\]

\[
\frac{dp}{ds} = \cos \phi,
\]

and

\[
\frac{dz}{ds} = \sin \phi,
\]

where \(s\) is the arc length along the pendant drop, measured from the edge of the bilayer and non-dimensionalized by the apex drop curvature \(R_b\). \(\phi\) is the angle between the tangent to the pendant drop profile and the horizontal. \(r\) and \(z\) represent the non-dimensional cylindrical coordinates of the bilayer interface. Bo is the Bond number denoted by \(Bo = \Delta \rho g R_b^2 / \gamma_m\) where \(\gamma_m\) is the monolayer surface tension and \(\Delta \rho\) is the density difference between the aqueous and oil phases. For low Bond numbers, drops having similar sizes, the bilayer radius is set by the following force balance in the vertical direction (see electronic supplementary material, Simulation for details):

\[
- \gamma_b + \gamma_b \sin \theta_b = 0,
\]

where \(R_b\) is the normalized bilayer radius, \(\gamma_b\) is the external force acting on the drop non-dimensionalized by \(2\pi R_b \gamma_m\), \(\gamma_m\) is the monolayer surface tension and \(\theta_b\) is the contact angle between the monolayer and bilayer as illustrated in figure 2a. Physically, the first term is the non-dimensional Laplace pressure originating from the deformation of the drops along the plane of the bilayer, the second term is the external force pushing the drops against each other and the third term is the non-dimensional interfacial tension acting along the periphery of the bilayer.

Finally, the excess vertical interfacial tension acting on the bilayer, \(\gamma_{v} = \gamma_{v} - \gamma_{v,eq}\), is related to the bilayer radius, \(R_b\), and the separation velocity of the bilayer, \(v = -(dR_b/dt)\), where \(\gamma_{v} = \rho g \sin \theta_b\) and \(\gamma_{v,eq}\) is the value of \(\gamma_{v}\) when \(v = 0\). Expressing \(\gamma_{v}\) in terms of the variables in figure 2a closes the system of equations and gives the following non-dimensional evolution equation for \(\dot{v}\):

\[
\dot{v} = \left(\frac{R_b \sin \theta_b - \sin \theta_{b,eq}}{KR_b}\right),
\]
and \( v \). The rate of separation allows one to obtain the bilayer radius in the upcoming step, which is then used to generate a new droplet profile. The iterations proceed in steps of 0.05 s for a total duration of 120 s, which matches the duration of a single-step separation in our experiments. During this time, the bilayer continuously evolves until it reaches the equilibrium state where \( \theta_b \) equals \( \theta_{\text{eq}} \). To initiate a new step, \( \theta_b \) is altered to mimic the contact angle change following a pull-up in the actual experiment. \( F_{\text{ap}} \) is recalculated, and the above algorithm is repeated until the bilayer radius tends to zero, reflecting a complete separation of the bilayer. Further details regarding the model and its solution are available in the electronic supplementary material, Simulation.

3. Results and discussion

3.1. Bilayer surface energy

Bilayer surface energy is an important property that dictates the mechanics of bilayer separation by directly influencing the equilibrium bilayer contact angle. Here, we calculate the surface energy of our DPhPC DIBs using two independent techniques.

Firstly, we can exploit principles of energy conservation to calculate the bilayer surface energy. The total energy of the system (\( E_{\text{total}} \)) at equilibrium can be written as

\[
E_{\text{total}} = E_p + E_m^w + E_b^2, \tag{3.1}
\]

where \( E_p \) is the potential energy, \( E_m^w \) is the monolayer surface energy and \( E_b^2 \) is the bilayer surface energy as a function of time. We can relate each of these energies to measurable physical quantities as follows:

\[
E_p = \Delta \rho g V h, \tag{3.2}
\]

\[
E_m^w = \gamma_m s^w, \tag{3.3}
\]

and

\[
E_b^2 = \gamma_b s^2. \tag{3.4}
\]

Here, \( \gamma_b \) is the bilayer surface tension and \( \gamma_m \) is the monolayer surface tension, which we found to be equal to 1.62 mN m\(^{-1}\) using the pendant drop technique. \( s^w \) and \( s^2 \) are the monolayer and bilayer area, respectively, \( V \) is the combined volume of both the drops and \( h \) is the vertical co-ordinate of the centre of mass of the system. Substituting equations (3.2), (3.3) and (3.4) into equation (3.1), and rearranging, we obtain

\[
\Delta \rho g V h + \gamma_m s^w = -\gamma_b s^2 + E_{\text{total}}. \tag{3.5}
\]

The above equation produces a linear relaxation between \( \Delta \rho g V h + \gamma_m s^w \) and \( s^2 \). By measuring and plotting the LHS of equation (3.5) as a function of \( s^2 \) (see electronic supplementary material, Surface Energy Analysis), we can obtain \( \gamma_b \) from the slope of the best fit line to the data. \( \gamma_b \) obtained from the energy analysis is shown in figure 3c for three different drop size ratios. As expected, we find that the bilayer energy calculated from the above technique is independent of the size of the drops. Averaging across all measurements, we find \( \gamma_b = 2.90 \pm 0.16 \) mN m\(^{-1}\).

Secondly, a simple force balance along the contact line can also be used to calculate the bilayer surface energy [9]. Resolving the surface tensions along the tangent to the bilayer (figure 3b), we obtain the following expression for the bilayer energy:

\[
\gamma_b = 2 \gamma_m \cos \theta_b, \tag{3.6}
\]

where \( \theta_b \) is half the angle between the pendant and the sessile drops at equilibrium. Consistent with previous reports.
3.2. Mechanics of bilayer separation under step strain

The separation mechanics of DIBs have been previously investigated under constant droplet separation rates [23] and under a constant force [39]. Different from the separation of two adhered bilayers, here we report the separation mechanics (unzipping) of a single bilayer in response to a step-strain deformation. For this purpose, the droplets were separated in multiple steps by periodically displacing the stage supporting the sessile drop. During this process, we tracked the mechanical response of the bilayer to the applied step strain by measuring the contact angle \( \theta_b \) and the bilayer radius \( R_b \).

3.2.1. Contact angle and bilayer radius

The evolution of the bilayer contact angle and bilayer radius (see figure 4d for the definition) in response to the applied step strain for three different droplet size ratios is shown in figure 4b and figure 4c, respectively. The shaded error bar for the case where \( V_s/V_p = 1 \) indicates the standard deviation obtained from three independent measurements. The corresponding stage displacement profiles used in the experiment are shown using dashed lines.

These measurements reveal, at first sight, a couple of interesting features of bilayer separation mechanics under step strain. Firstly, following every step separation up to the last one, we observe a step increase of the contact angle, followed by a gradual relaxation of the contact angle to its equilibrium value. A caveat is in order here: notice in figure 4b that, for \( V_s/V_p \neq 1 \), there is a gradual decay in the equilibrium values of \( \theta_b \) across each step. The lipid bilayer is not expected to be flat for \( V_s/V_p \neq 1 \), which could induce a measurement error as we track the evolution of the contact angle by looking at the drop profiles a few pixels away from the contact point. In any case, the fact that \( \theta_b \) reaches a non-time-dependent value after each separation step allows us to safely assume that the geometry is such that \( \gamma_m \) and \( \gamma_b \) are balanced. Regarding the bilayer radius, we also see that it decays to a new equilibrium value following every step until complete bilayer separation is achieved. Interestingly, the magnitude of the bilayer radius decay, which we will refer to as \( \Delta R_b \), increases with each step. This is a characteristic of bilayer separation between curved surfaces and will be explained in more detail in §3.3. Secondly, we observe that, at the last step, there is a rapid change in the angle and radius close to the complete separation of the bilayer. It is also worth noting that, in the last step, the bilayers separated completely in all our experiments without the formation of thin strands of liquid, more commonly referred to as tethers in the literature [23,40].

All the above observations support that the mechanics of the separation of the DIBs in our experiments are primarily a result of the peeling process. During the peeling process, the thickness of the bilayer remains a constant as its radius decreases [23]. This is clearly the case up to the last step. Even in the last step, peeling is dominant, as seen by the smooth change in the bilayer radius over time. The pulling process, where the bilayer thickness changes with little change in bilayer radius, plays a minor role in the reported bilayer separation and possibly occurs only in the last few seconds prior to complete separation. Mathematically, we can show that it is energetically favourable for the bilayer to separate via peeling when \( R_b \gg b \) by comparing the hydrodynamic forces dictating the peeling and pulling mode of bilayer separation [23]. In fact, in §3.3, we will show that the observed variation in the bilayer radius (and contact angle) can be completely captured with a model that only considers peeling. Before we do so, let us examine the dynamics of bilayer separation by investigating the forces acting on the bilayer and the rates of separation.
3.2.2. Force and rate analysis

Recall that in §3.1 we mentioned that a simple force balance along the contact line is made to obtain the bilayer surface energy. Similarly, we can perform a force balance normal to the contact line to obtain the vertical component of $\gamma$ acting on the bilayer perimeter. This quantity is denoted as $\gamma_\perp$ and is obtained as

$$\gamma_\perp = \gamma_m \sin \theta_b,$$

where $\gamma$ drives the separation of the bilayer. When $\gamma_\perp$ exceeds the critical adhesive force per unit length, the bilayer starts to peel. The rate of peeling, which is denoted as $v$, can be obtained from the evolution of the bilayer radius as

$$v = -\frac{dR_b}{dt}.$$  \hspace{1cm} (3.8)

The magnitude of $v$ naturally depends on the excess tension acting on the bilayer. To explicitly identify this correlation,
we plot $\gamma_b / \gamma_{L,eq}$ as a function of $R_b$ vs for three different droplet size ratios in figure 4c. Here $\gamma_{L,eq}$ is the value of $\gamma_L$ at $v = 0$, and physically represents the critical surface tension above which the bilayer starts to separate. Note that the data in figure 4e were obtained from the relaxation mechanics observed in a single step, specifically from the second step in figure 4b,c. Plots of normalized surface tension versus $t$, $R_b$ vs $t$ for the first pull-up, and the lower and upper bound of the vertical forces are available in the electronic supplementary material, Separation Analysis under Step Strain.

From figure 4e, we can see that $\gamma_b / \gamma_{L,eq}$ is a linear function of $R_{b,eq}$ with the slope of the best fit line remaining independent of the size ratios. In agreement with prior studies [23,32,39], this correlation indicates that the peeling of non-specifically adhered phospholipid bilayers is retarded primarily by the viscous dissipation in the corners formed between the drops near the bilayer. Balancing the vertical surface tension with the viscous dissipation, we obtain the following expression [32]:

$$\frac{\gamma_b}{\gamma_{L,eq}} = 1 - \left( \frac{\mu}{2\eta \gamma_{L,eq}} \right) R_b \frac{dR_b}{dt},$$  

(3.9)

where $\mu$ is the viscosity in the bulk oil phase and $b$ is the thickness of the lipid bilayer. Performing an order of magnitude analysis by taking $\mu$ to be of the order of 1 cP, $b$ to be of the order of 1 nm and $\gamma_{L,eq}$ to be of the order of 1 mN m$^{-1}$, we see that the term in the bracket of the above equation has a value of $5 \times 10^{-4}$ s $\mu$m$^{-2}$. This value is comparable to the experimentally determined slope of the linear fit between $\gamma_b / \gamma_{L,eq}$ and $-R_b (dR_b/dt)$ (figure 4e). This agreement confirms that viscous dissipation is the dominant mechanism retarding the separation of the tested phospholipid drops. Finally, it is worth noting that this viscous resistance becomes negligible close to bilayer separation ($R_b \rightarrow 0$), and contributions from lubrication forces dominate as peeling goes way to the pulling mode of separation [23].

3.3. Simulation

Here we report the predictions from the mathematical model (see §2.4) obtained with the following values of the free variables: $Bo = 0.1$, $\theta_{b,eq} = 19.8^\circ$ and $\gamma_m = 1.62$ mN m$^{-1}$. Except for $Bo$, all the variables used in this model have values similar to those in the experiments, and $Bo$ can be considered as a fitting parameter used to compare the simulations with the experiments. This is done in order to accurately recreate the drop profile in the absence of the agarose core supporting the pendant drop.

From solving the bilayer separation model, we obtain the evolution of the droplet profiles (figure 5a), the contact angle (figure 5b) and the bilayer radius (figure 5c). In figure 5a, we see the droplet profiles at the start of the four steps (i–iv) and immediately before separation (v). Qualitatively, we observe a good agreement between the simulated and experimentally obtained profiles (figure 4a). Focusing on the contact angle plot, we observe that the simulation predicts a gradual decay within the first three steps similar to that in the experiments, while the minor decay of the contact angle across the steps observed solely in the experiments for
cases where $V_c/V_p \neq 1$ is likely to be a result of contact angle measurement error (see §3.2.1). Further, similar to the experiments, we also observe that the relaxation time of the contact angles increases with every separation step. Quantitatively, there is a 250% increase in the relaxation time between the first and the third step. The contact angle behaviour in the last step does not agree with the experiments, with the simulations predicting a drastic increase in the angle. It is likely that simulations are predicting the correct trend, as practical challenges, including optical artefacts, limit the accuracy of the contact angle measurements in the terminal seconds leading to the complete separation of the bilayer. Finally, the bilayer radius given by the simulation behaves similarly to those in the experiments (figure 5c). We also observe that, similar to the experiments, the simulation predicts an increasing decay in the magnitude of the radius and a longer relaxation time of the bilayer with each step separation.

The increase in the relaxation times of the contact angle and the radius as well as the increasing decay magnitude of the bilayer across subsequent separation steps are intrinsic features of DIB separation under step strain. The rationale for the increase in the relaxation time can be understood by combining equations (3.6) and (2.5), and expressing $F_{ap}$ in terms of the initial bilayer radius ($R_{b,ini}$) and the initial contact angle ($\theta_{b,ini}$) at the start of the step. This gives:

$$\bar{v} \propto R_{b,ini}^2 - R_{b,eq}^2 \sin \theta_{b,ini} - R_b^2 + R_b \sin \theta_{b,eq}. \quad (3.10)$$

Everything else remaining the same, the separation $\bar{v}$ decreases with decreasing initial radius, resulting in longer decay times with each separation step. Similarly, the rationale for the increasing magnitude of the radius decay ($\Delta R_b = R_{b,ini} - R_{b,eq}$) can be identified by noting that $R_b$ reaches its equilibrium value of $R_{b,eq}$ when $\bar{v} = 0$. Using this fact and simplifying using binomial series expansion, we obtain the following expression:

$$\Delta R_b = \frac{1}{8R_{b,ini}} (\sin^2 \theta_{b,ini} - \sin^2 \theta_{b,eq})$$

$$- \frac{1}{2} (\sin \theta_{b,eq} - \sin \theta_{b,ini}) + O\left(\frac{\sin^3 \theta_{b,ini}}{R_{b,ini}}\right). \quad (3.11)$$

Clearly, $\Delta R_b$ scales inversely with $R_{b,ini}$.

In addition to clarifying the different physical processes during bilayer separation, the simple mathematical model also serves the following purposes. Firstly, the model can be used to easily predict the separation behaviour of different lipids under various conditions by modifying the equilibrium angles, the monolayer surface tension and the bulk viscosity. Secondly, by modifying the force to rate relation, this model also gives a good framework to simulate the bilayer separation under a constant force or a constant rate. Thirdly, with simple modifications the governing equations can be adapted to study the bilayer separation between a pendant drop and a hard sphere (see electronic supplementary material, Simulation).

4. Conclusion

In this article, we report the separation of DIBs under step strain using a custom-built apparatus (I-DDiaSS). Initially, we showed that the bilayer energy can be determined using principles of energy conservation in addition to the well-established force balance method. Subsequently, we experimentally revealed the separation mechanics of the bilayer separation under step separation by tracking the evolution of the contact angle and the bilayer radius. Interestingly, the relaxation time of the contact angle and the bilayer radius as well as the decay magnitude of the bilayer radius were observed to increase with each separation step. Through analysis of the forces acting on the bilayer and the bilayer separation rates, we showed that the bilayer primarily separates through a peeling mechanism. Finally, we also developed a mathematical model to successfully simulate the bilayer separation process. From the governing equations, we also showed that the separation velocity scales with the square of the initial bilayer radius and that the bilayer decay magnitude scales inversely with the initial bilayer radius—the rationale for increasing relaxation times and bilayer decay magnitudes observed in the experiments. These results improve our understanding of bilayer separation mechanics under step strain and supplement the scientific efforts aimed at characterizing separation mechanics of bilayers under different separation modes [3,23,32,39].

There remain several opportunities for future work that would offer important extensions to the present work. First and foremost, the reported step-strain experiments are a valuable rheological tool for revealing relaxation time scales related to the unzipping of the bilayer. Future studies may use the reported protocols to study the relaxation time scale as a function of lipid chemistry, salt concentration and temperature. Secondly, it would be worthwhile studying the influence of bio-physically relevant molecules such as cholesterol on the separation mechanics of the lipid bilayers. Finally, understanding the distribution of stress on the monolayer during the separation process, possibly using membrane tension probes such as FliptR [41], is also a promising direction for future research.

Data accessibility. This article has no additional data.

Authors’ contributions. Y.H. conceived the study, designed and performed the experiments, developed the algorithms, analysed the data and wrote the manuscript. V.C.S developed the algorithms, analysed the data and wrote the manuscript. J.T. conceived the study and designed and performed the experiments. G.G.F. conceived and supervised the study, designed the experiments and critically reviewed the manuscript.

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