



Molecular determinants for the line tension of coexisting liquid phases in monolayers

Andrea Alejandra Bischof, Natalia Wilke*

Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Dpto. de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Pabellón Argentina, Ciudad Universitaria, X5000HUA Córdoba, Argentina

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ABSTRACT

The line tension (λ) in biphasic membranes has been determined in monolayers and bilayers using a variety of techniques. In this work we present a novel approach to the determination of λ in monolayers with liquid/liquid phase coexistence, overcoming several of the drawbacks of current techniques. Using our method, we determined the line tension of liquid/liquid phases in binary mixtures of different lipids and a molecule similar to cholesterol but less oxidizable. We analyzed the effect of the hydrocarbon chain length and the polar head-group of the non-sterol lipid and found the latter to exert much more influence than the former. The presence of PE led to high λ values, PG to low values and PS and PC to intermediate values. The line tension showed a strong correlation with the critical packing parameter of the phospholipid. The spontaneous curvature displayed by the phases constituted by a particular lipid appears to be an important parameter for determining the line tension in mixed films.

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1. Introduction

When two phases coexist in a membrane, a 1D-border between each phase is generated and creates an energy penalty. This energy can be measured through a parameter called “line tension”. Analogous to surface tension, line tension (λ) is a consequence of the different environments of the molecules at the border of a 2D domain compared to the molecules in the bulk of each phase. The role of line tension becomes obvious when a domain is driven out of a more stable shape, since λ induces the restoration to its original shape. The different intermolecular interactions in each coexisting phase have a direct impact on phase stability (distance to critical point (Honerkamp-Smith et al., 2008)), domain shape in biphasic regions of the phase diagram (McConnell, 1991), the nucleation rate and size of stable nuclei (Blanchette et al., 2007), and the domain growth rate (Garcia-Saez et al., 2007). Line tension is therefore an important parameter in defining the size of a stable nucleus, the nucleation kinetics and the capture zone of each domain. In turn, the capture zone determines domain size, which influences domain shape. Thus, knowing the line tension and the molecular parameters involved in defining its value can help in understanding and predicting domain size, shape and distribution in membranes (membrane texture). A very complete review on line tension in

monolayers and bilayers of surfactants was recently published by Sriram and Schwartz (2012).

Line tension has been determined in monolayers and in bilayers using a variety of techniques. The research group of McConnell performed a pioneering work by measuring the temporary evolution of the shape relaxation of domains in monolayers with liquid-liquid phase coexistence, previously elongating the domain using the convection of the subphase (Benvegnu and McConnell, 1992). Line tensions of 0.1 and 1.6 pN were reported, depending on the surface pressure. Mann et al. also explored this technique using a more controlled subphase motion and determined the line tension of monolayers of polymers ($\lambda = 1.1$ pN) (Mann et al., 1995) and multilayers of 4'-8-alkyl[1,1'-biphenyl]-4-carbonitrile ($\lambda = 200$ –600 pN) (Wintersmith et al., 2007). In both types of domain deformation there is residual subphase motion in the first frames (very deformed domains) which may lead to out-of-focus frames and, more important, to additional stress on the domain, unaccounted for in the theory. Fischer et al. deformed gas domains in the LE phase ($\lambda = 4$ –7 pN) and in liquid-condensed (LC) domains ($\lambda = 29$ pN) in the LE phase by trapping micrometer-sized beads attached to the domain border with optical tweezers and also by localized heating (Wurlitzer et al., 2000). In this method, the most difficult task is modeling the effect of the monolayer and the subphase friction on the bead. Lee et al. (2011) estimated the line tension of monolayers with liquid-liquid coexistence composed of a complex lipid mixture. They derived an equation for domain size distribution that assumes it to be an equilibrium property that depends on λ , the difference in dipole moment densities and the entropy of distributing

* Corresponding author. Tel.: +54 351 4334171; fax: +54 351 4334171.

E-mail addresses: wilke@mail.fcq.unc.edu.ar, wilke@fcq.unc.edu.ar (N. Wilke).

the molecules between different domains. By fitting the experimental domain size histogram with the derived equation, λ values were found in the order of fN, and it was proposed that domains in complex mixtures exhibit a non-homogeneous composition at their borders, thus explaining the rather low λ values. However, non-equilibrium domain textures are also possible, particularly for domains generated during the spreading stage. The nuclei density (and thus future domain size) can be modulated by regulating the lateral pressure reached at the spreading stage (Vega Mercado et al., 2012). Moreover, it has been shown that domain size equilibrations are in the order of days or weeks (McConnell, 1996). Line tension is a determining factor for the generation of nuclei, and the domain nucleation rate has been used for determining the value of λ in supported bilayers in LC/LE coexisting phases (Blanchette et al., 2007), with values of 2–4 pN being reported for different lipid mixtures. The measurement of domain shape undulation has also been explored in monolayers (Seul and Sammon, 1990; Stottrup et al., 2007) and in bilayers (Honerkamp-Smith et al., 2008), with values in the order of pN being reported in both systems. The drawback of this technique is that only mixtures close to the critical point can be analyzed. Line tension in bilayers has also been determined using micropipette aspiration of giant unilamellar vesicles (Tian et al., 2007).

Several models for the calculation of λ have also been reported. These models take into account that in order to minimize the hydrophobic mismatch (δ) caused by the thickness difference between the coexisting phases, molecules at the domain border undergo deformation. In their model for bilayers, Kuzmin et al. evaluated the elastic splay modulus, the tilt modulus, the spontaneous curvature, the average phase height (h_0) and the hydrophobic mismatch, and developed an equation that predicts the linear dependence of λ with δ^2/h_0^2 . Similar approaches were also used by Lee et al. (2011) for monolayers and by Towles and Dan (2008) and Akimov et al. (2004) for bilayers. However, few experiments comparing the values of line tension calculated from theoretical models or elucidating the influence of the chemical characteristics of the molecules forming the membrane have been reported (Blanchette et al., 2008a,b).

In this work, we report the experimental determination of λ in monolayers with a liquid–liquid phase coexistence using a technique that overcomes some of the problems of the above-mentioned methods. We promoted the deformation and subsequent rupture of domains using a non-homogeneous electric field, and followed the domain relaxation in the absence of the field, once the domains had broken. Since this approach does not require beads for the process of deformation, it circumvents the drawback of bead motion hydrodynamics. Furthermore, there is no residual motion of the subphase, since the domain deformation is promoted by the electric field and not by subphase motion.

We demonstrate that in the experimental approach described below, the monolayer viscosity and the electrostatic repulsion need not be taken into account for the systems analyzed here since they are not determining factors for domain shape relaxation. We determine line tension values for binary mixtures of different lipids and a molecule similar to cholesterol but less oxidizable. The effect of hydrocarbon length and polar head-group is analyzed and the correlations among line tension, monolayer height, monolayer texture and mixing pressure are discussed.

The results indicate that an increase from 12 to 16 carbons in the hydrocarbon chain does not significantly affect the value of λ , whereas the nature of the polar head-group does. The presence of PE led to high λ values, of PG to low values and of PS and PC to intermediate values, which could be explained in terms of the spontaneous curvature of each lipid. As expected, a strong correlation between the critical pressure of mixing and line tension was found (Honerkamp-Smith et al., 2008).

2. Materials and methods

2.1. Materials

Dilauroyl phosphatidylcholine (DLPC), Dilauroyl phosphatidylserine (DLPS), dilauroyl phosphatidylethanolamine (DLPE), dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylserine (DPPS), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidylglycerol (DPPG) and the lipophilic fluorescent probe 1- α -phosphatidylethanolamine-*N*-(lissamine rhodamine B sulfonyl) ammonium salt (chicken egg-transphosphatidylated) were purchased from Avanti polar lipids (Alabaster, AL, USA). Dihydrocholesterol (dchol, a less-hydrolyzable cholesterol analog (Rosetti et al., 2010)) was obtained from Sigma. Solvents and chemicals were of the highest commercial purity available. The water used for the subphase was from a Milli-Q system (Millipore), 18 M Ω cm and lipid monolayers were prepared and characterized on subphases of 150 mM NaCl at 21 ± 1 °C.

2.2. Monolayer observation

The monolayer was observed while compressed using Brewster Angle Microscopy (BAM) or Fluorescence Microscopy (FM) and simultaneously registering the lateral pressure and mean molecular area of the film. For the BAM experiments, we used an EP3 Imaging ellipsometer (Accurion, Goettingen, Germany) with a 20 \times objective, and the monolayer was spread on a Langmuir film balance (KSV minitrough, KSV Instruments, Ltd., Helsinki, Finland). Since the liquid expanded (LE) phase is thinner than the dchol-rich phase (D) phase, it can be observed as the darker regions in the micrographs. For the FM experiments, the fluorescent probe was incorporated in the lipid solution before spreading. The Langmuir film balance (microthrough, Kibron, Helsinki, Finland) was placed on the stage of an inverted fluorescence microscope (Axiovert 200, Carl Zeiss, Oberkochen, Germany) with a 20 \times objective and images were registered by a CCD video camera (IXON). The fluorescent probe concentration in the D phase is lower, and thus this phase appears darker in the micrographs.

2.3. Application of an inhomogeneous electric field to the monolayer

The experimental setup for applying an electrostatic field to the lipid monolayer was carried out as described previously (Wilke et al., 2006) and is similar to the setup first reported by Miller and Möhwald (1986). Briefly, a metal wire was held at 200 μ m above the subphase. A second electrode was then placed in the subphase and a potential difference was applied between the electrodes. The upper electrode was charged by applying potentials of up to 300 V with respect to the subphase electrode. If the dipole density of the domains is different from that of the continuous phase, a non-homogeneous electric field will generate a net force on the domains and cause their migration from or to the region under the upper electrode and subsequently lead to their deformation.

2.4. Monolayer thickness calculation

The optical thickness of each phase (h) was calculated from the BAM images taken after calibration of the BAM equipment. We evaluated the gray level of at least 10 different regions from each photo for each phase. The gray level was evaluated using imageJ, analyzing circular regions of 1–2 μ m diameter in the center of the domains for the D phase and far from them for the LE phase. At least 10 micrographs were analyzed for each system for at least 3 independent experiments (different monolayers). The gray level of each section

of the micrograph was then converted to reflected light intensity (R_p), and h was calculated assuming a smooth but thin interface in which the refractive index varies along the normal of the interface on a distance h , much smaller than the incident light wavelength λ (Vega Mercado et al., 2011), giving:

$$h = \frac{\sqrt{R_p}}{\sin(2\theta_B - 90)} \left(\frac{\pi \sqrt{n_1^2 + n_2^2} (n_1^2 - n^2) (n_2^2 - n^2)}{\lambda (n_1^2 - n_2^2) n^2} \right)^{-1} \quad (1)$$

In Eq. (1), n , n_1 and n_2 are the film, air and subphase refractive index, respectively and θ_B is the Brewster angle. The refractive indexes used were 1.00 for air, 1.33 for the subphase, 1.42 for the LE phases (Ducharme et al., 1990). Since no reported values were found for the denser D phase, we calculated the monolayer optical thicknesses using 1.45 (index for liquid condensed phases (Petrov et al., 1999)).

3. Results

3.1. Experimental approach for the determination of line tension

It has been previously shown that non-homogeneous electrostatic fields applied to biphasic monolayers can lead to domain migration in the interface due to the difference of dipole density between the phases in coexistence (Miller and Möhwald, 1986; Klinger and McConnell, 1993; Wilke and Maggio, 2006, 2009). In the case of fluid and deformable domains (domains in the LE/D coexistence region), domains fuse together and change shape as they migrate. A movie of this process is shown in Supporting Material (Movie S1). Some very long domain strips are generated that break at random and if the field is turned off at this stage, the relaxation of the strip can be followed over time. Fig. 1 shows a typical experiment and a corresponding movie can be found in Supporting Material (Movie S2).

As previously stated by Wurlitzer et al. (2000), the driving force for strip relaxation can be thought of as a spring whose strength depends on the line tension and that moves the head of the strip closer to the domain (see scheme in Fig. 1). If the head of the strip moves at a constant velocity, the forces acting on the system are balanced during this process (Wurlitzer et al., 2000).

The above-mentioned forces are: line tension (F_λ), which promotes the decrease in strip length, friction (F_η) and electrostatic repulsion due to the difference in dipole density of each phase (F_{el}), which opposes the motion of the strip head. The electrostatic term varies with the logarithm of the length of the strip; $F_\eta = f a \eta (dL/dt)$ where η is the viscosity of the subphase f , is a dimensionless coefficient that depends on the rheological properties of the monolayer and the subphase (Wurlitzer et al., 2000) and is equal to 8 if the subphase viscosity dominates the dissipation (low monolayer viscosity) (Hughes et al., 1981); and a is the radius of the head of the strip. Since the head is not a perfect circle, we approximate a as the radius of a circle with the same area of the ellipse shown in the scheme of Fig. 1 ($a = \sqrt{a_1 \times a_2}$), and since the size of the head changes slightly with time, especially at the beginning and at the end of the experiment, only the intermediate times were considered, with a_1 and a_2 changing up to 1%. In order to determine the value of f , we calculated the diffusion coefficient of domains of different sizes in the range of the typical values of a (2–9 μm) for mixed monolayers composed of dchol with DLPC (20:80). The diffusion coefficient was determined from the Brownian motion of the domains (Wilke and Maggio, 2009; Wilke et al., 2010). The determined values for the diffusion coefficients are the same (within errors) as those calculated without taking into account monolayer viscosity and considering that the Brownian motion is determined by the subphase viscosity. For example, for 6 domains of 5 μm

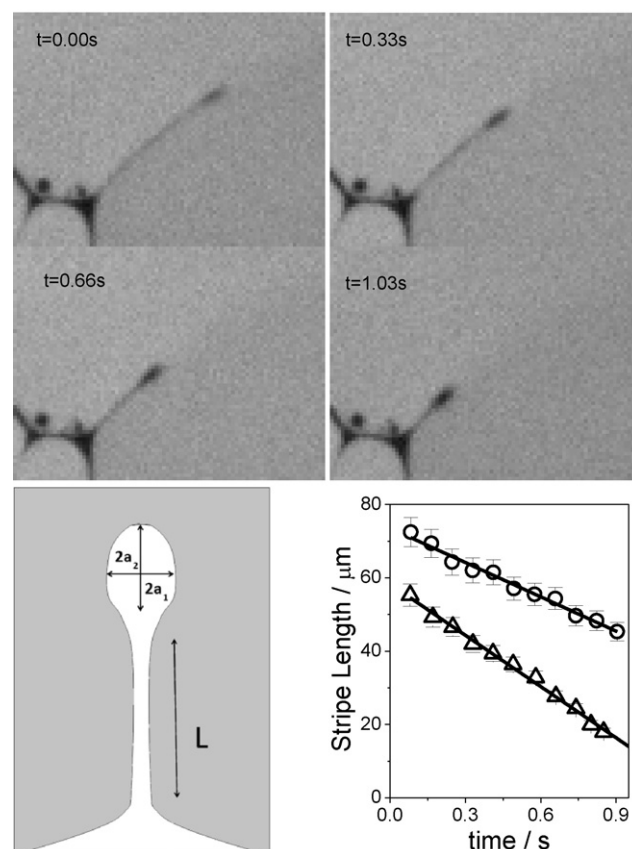


Fig. 1. Up: representative experiment showing the time evolution of the strips generated with the electric field. The field was turned off after the rupture (before the photos that are shown in this figure). Monolayer composition: DLPC/dchol (80:20). Sizes: 100 $\mu\text{m} \times 132 \mu\text{m}$. Bottom left: scheme showing the parameters involved in the calculation of the line tension. Bottom right: time evolution of the length of the strip shown in the photos (triangles) and in the Movie S2 (circles, Supplementary Material, monolayer composition: DPPS/dchol, 80:20) and the corresponding linear fits to the data (line). The errors correspond to the standard deviation of 5 independent determinations of the length at each time. A typical error of 5–6% was found. The values for the slopes are $-46 \pm 1 \mu\text{m/s}$ with $R^2 = 0.996$ (DLPC/dchol monolayer) and $-31.2 \pm 0.3 \mu\text{m/s}$ with $R^2 = 0.993$ (DPPS/dchol monolayer). The corresponding α and λ values are $2.8 \pm 0.5 \mu\text{m}$ and $0.5 \pm 0.1 \text{ pN}$ for the DLPC/dchol monolayer and $4 \pm 1 \mu\text{m}$ and $0.5 \pm 0.1 \text{ pN}$ for the DPPS/dchol monolayer.

diameter, the diffusion coefficient value was $0.24 \pm 0.05 \mu\text{m}^2/\text{s}$, compared with a theoretical value of $D = kT/8\eta_W a = 0.21 \mu\text{m}^2/\text{s}$.

We determined the value of L through time for each experiment. Since the strips were not usually straight, we segmented the strip in each frame and determined the total length as the sum of the segments. For all the analyzed systems, L decreased linearly with time. Fig. 1 shows two representative examples with $R^2 = 0.992$ and 0.996 . The obtained values of R^2 were within 0.98 and 0.9996. The linearity indicates that the electrostatic interactions can be neglected (Wurlitzer et al., 2000). Thus, by eliminating monolayer viscosity and electrostatic interactions and considering $\eta = 1 \times 10^{-2} \text{ Pa s}$, the line tension can be calculated as:

$$F_\lambda = F_\eta \quad (2)$$

$$F_\lambda = 2\lambda \quad (3)$$

$$F_\eta = f a \eta \frac{dL}{dt} \quad (4)$$

$$\lambda = 4 \times 10^{-2} a \frac{dL}{dt} (\text{Pa s}) \quad (5)$$

Using this approach, we found the same line tension value for experiments with different values of a . Furthermore, the same value

of λ was determined for strips composed of lipids in the D phase surrounded by lipids in LE phase as in the reverse case. The constancy of the line tension with the a value and with the nature of the phase that forms the strip demonstrates the validity of neglecting monolayer viscosity and electrostatic interactions, and thus, the validity of the experimental approach.

3.2. Line tension values

We determined the line tension values for binary mixtures of dchol and different lipids as described in the preceding section. The line tension values are the average of at least 3 independent measurements, with the uncertainties given by the standard deviation. Dchol was used instead of cholesterol because it shows a similar interfacial packing behavior and phase diagrams (Rosetti et al., 2010; McConnell and Radhakrishnan, 2003) and does not undergo detectable oxidation during experiments. The lipids were selected with different polar head-groups and hydrocarbon chain lengths in order to analyze the effect of each moiety on the value of the line tension. The lipids studied were DLPS, DPPS, DLPC, DPPC, DLPE and DPPE. All the systems were tested at 4 mN/m since all the mixtures show phase coexistence at this pressure (see Fig. 3A) and, their surface tension can thus be compared under similar conditions. DPPG was subsequently added to the analysis, and since the binary mixtures of this lipid with dchol are homogeneous at pressures higher than 3 mN/m, this mixture was studied at 2 mN/m.

For all the mixtures, monolayers with 20–30% dchol were studied (alpha region in the phase diagram (Okonogi and McConnell, 2004), see Fig. 3A). Since the experiments were performed in the same coexistence region of the phase diagram, a change in the dchol proportion implies a change in the amount of each phase in coexistence according to the lever rule. The line tension value obtained using our approach would vary with the percentage area of each phase only if the electrostatic interactions were not negligible. However, since the electrostatic interactions were in fact negligible, the value of λ was independent of the amount of dchol in the analyzed composition region.

Fig. 2A shows the determined λ values, indicating a variation in the line tension for different mixtures, with higher values for films containing PE lipids and lower values for those with PG lipids. The variation of the magnitude of the line tension was in the order: DPPG < DLPS ~ DLPC ~ DPPS < DPPC < DLPE ~ DPPE.

3.3. Phase height

Using BAM, the optical thickness of each phase was determined at 4 mN/m (2 mN/m for DPPG) for monolayers in the alpha region of the phase diagram (10–30 mole% of dchol, see (Okonogi and McConnell, 2004) and Fig. 3A). Since the calculated thicknesses depend on the refractive indexes used in the calculations, we also show the reflectivity of the different phases in each system (Fig. 2B). Assuming the same refractive index for all the mixtures in the D or LE phase (see Section 2.4), the obtained optical thicknesses vary in the range 1.2–2.2 nm for the LE phases and 1.3–2.3 nm for the D phases (see Fig. 2C) whereas the difference in optical thicknesses ranges from 0.1 to 0.7 nm. Comparison of Fig. 2A with 2C shows there is no correlation between the line tension values and optical thickness of each phase or their differences. Furthermore, Fig. 2D also shows a lack of correlation between the ratio of the difference in thicknesses and the average thickness for each mixture (δ/h_0), a fundamental parameter in terms of the influence of the hydrophobic mismatch on line tension (Kuzmin et al., 2005; Lee et al., 2011). The trend line tension values shown in Fig. 2A will therefore not be correlated with the phase optical thicknesses, the difference in thicknesses or the value for δ/h_0 .

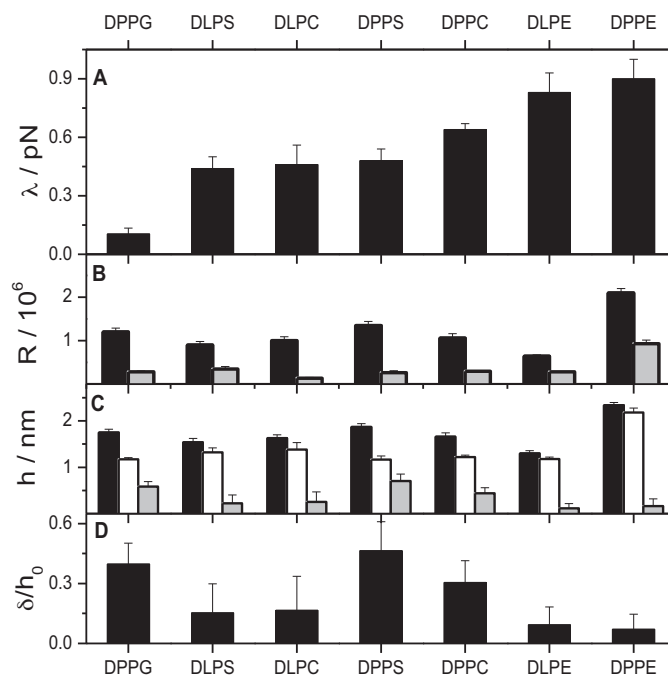


Fig. 2. (A) Values determined for the line tension of coexisting liquid/liquid phases. (B) Reflectivity of D phases (black) and LE phases (gray) determined using BAM. (C) Optical thicknesses of the D (black) and LE phases (white) obtained from the data in figure B as explained in Section 2. The gray bars show the differences in optical thickness between each phase. (D) Values for the ratio of the differences in thicknesses between each phase to the average thicknesses. In all plots the monolayers are composed of dchol (15–25 mole%) and the indicated lipid at 4 mN/m (except for the mixtures with DPPG, which were determined at 2 mN/m).

3.4. Monolayer texture

Line tension is the energy cost per unit length for creating an interface, and arises from differences between the interactions of the molecules in each phase. When two phases coexist in equilibrium, the electrochemical potential of the molecules in each phase is the same. In mixed monolayers with cholesterol, the phases become more similar with increasing surface pressure, and the monolayer becomes homogeneous at the mixing pressure (π_M). The critical pressure (π_C) is expected to be lower for systems with similar phases, and thus low π_C values should correlate with low values of line tension.

We determined the π_M values for each mixture at 10, 20 and 30 mole% of dchol (see Fig. 3A), all mixtures showing a maximum π_M value close to 20%. Since the critical point composition in most of the monolayers formed by binary mixtures with dchol in the alpha region of the phase diagrams is close to 20 mole% dchol (Okonogi and McConnell, 2004), we compared π_M values with line tension values for mixtures with this proportion of dchol. The mixture with DPPG was excluded from this analysis since in this case λ was determined at a different surface pressure. Fig. 3B shows that there is an apparent linear relationship between these two parameters, in agreement with the previous analysis and with other reports that analyzed the melting temperature in bilayers (Garcia-Saez et al., 2007; Honerkamp-Smith et al., 2008). This expected correlation validates the determined line tension values and thus the proposed technique.

According to the classic theory of nucleation, the interfacial line tension between a crystallizing phase and the medium that surrounds it controls the rate of nucleation in supercooled solutions. The activation barrier for nucleating a 2D disk from the melt depends on the line tension, enthalpy difference and the distance to the transition point (Blanchette et al., 2007). In other words, in

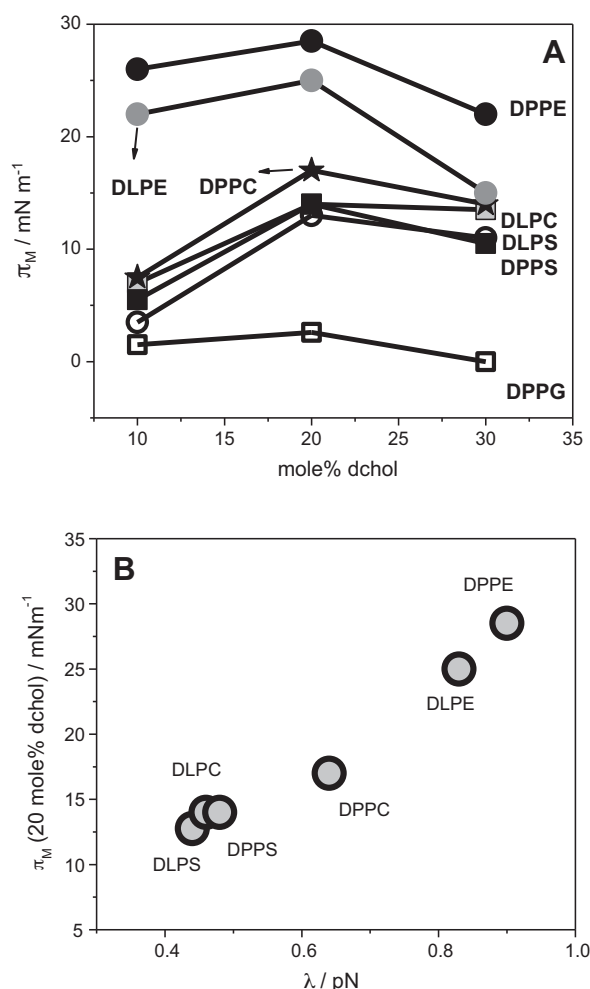


Fig. 3. (A) Lateral pressure where the D and LE phases fuse as a function of the dchol proportion. The symbols correspond to mixtures of dchol with DPPE (black circles), DLPE (gray circles), DPPC (stars), DLPC (grey squares), DLPS (white circles), DPPS (black squares) and DPPG (white squares). (B) Lateral pressure where the D and LE phases fuse (for monolayers with 20 mole% of dchol) as a function of the line tension for the indicated mixtures.

systems with lower line tensions the nucleation process is faster and the minimum size for a nucleus to be stable is lower. Therefore, the number of nuclei should increase as the line tension decreases, leading to a higher number but smaller domains. Lee et al. (2011) proposed a method to calculate the line tension from equilibrium size distribution of domains in lipid monolayers. Garcia-Saez et al. (2007) showed that domain size distribution shifts to higher values when the height mismatch between phases (and thus the line tension) increases in bilayers composed of ternary mixtures with cholesterol, sphingomyelin and PCs. Taking these results into account, we explored the distribution of domain sizes for the mixtures at 4 mN/m (2 mN/m for DPPG). In Fig. 4, we show the results for the systems analyzed and Table 1 provides a summary of the values obtained for the fitting parameters. Although the equations derived by Lee et al. (2011) show a good fit to our data, the λ values obtained from this fit were two or three orders of magnitude smaller than those found through the deformation of the domains.

The reason for the difference between the values of line tension obtained by the two methods probably resides in the fact that the domains had no equilibrium size distribution. Although the results shown in Fig. 4 were obtained with monolayers that were compressed very slowly ($0.02 \text{ nm}^2/\text{molecule s}$), starting from average molecular areas of about double the lift-off, the domains

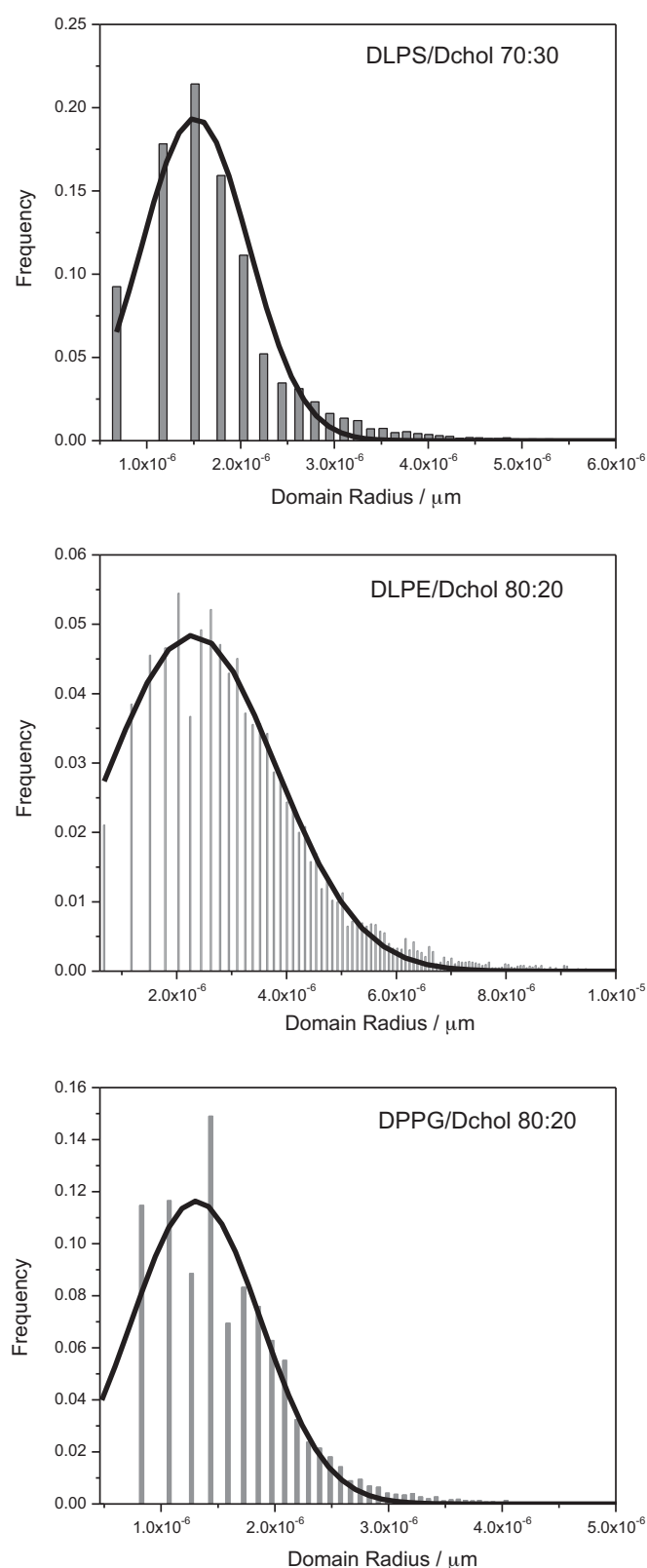


Fig. 4. Domain size distribution for monolayers composed of mixtures of DPPS/dchol (70:30), DLPE/dchol (80:20) and DPPG/dchol (80:20) (bars) and the corresponding fit according to Lee et al. (2011) (lines). Table 1 summarizes the values of the fitting parameters.

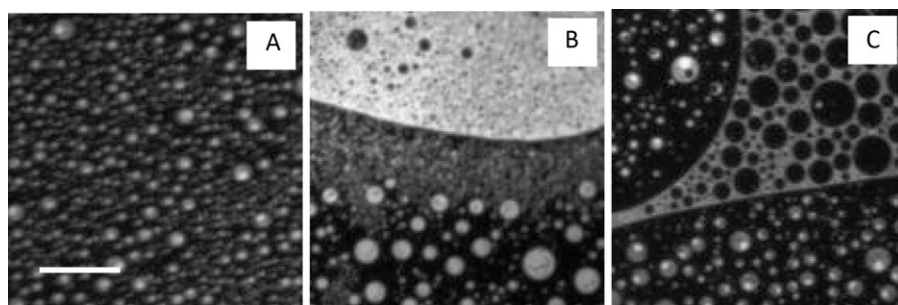


Fig. 5. Representative micrographs for monolayers composed of DLPS/dchol (80:20) at 4 mN/m taken using FM. (A) Domains are formed by expanding the monolayer from lateral pressures higher than the mixing pressure. (B) Monolayer is spread up to 3 mN/m and quickly compressed. (C) Monolayer is spread to twice the lift-off area and slowly compressed. Bar: 50 μm .

were formed during the seeding stage and were thus most probably generated in an out-of-equilibrium manner (Vega Mercado et al., 2012). To investigate this possibility, we prepared monolayers of the same composition in 3 different ways: as previously described (spreading to twice the lift-off molecular area and slowly compressing up to 4 mN/m), spreading to 3 mN/m and quickly compressing to 4 mN/m, and expanding the monolayer from a pressure higher than the mixing pressure. If the texture of the monolayer were an equilibrium property, the domain distribution would be the same for these three types of experiments, or at least for the first and last one. Fig. 5 shows representative micrographs of monolayers composed of DLPS/dchol (80:20) obtained through the three different methods at 4 mN/m; further micrographs are presented in Fig. S1 of the Supporting Material. The three photos show different textures, indicating that as the domain distribution in our case depended on the manner in which the monolayer was prepared, it is not an equilibrium property. Furthermore, different textures were observed in different regions of the same monolayer. Hence, the system was not at equilibrium in terms of domain size distribution and it is thus not possible to apply the analysis proposed by Lee et al. to our systems. However, it is possible that such analysis would be appropriate in the case of domain size distribution obtained by expansion of a homogeneous monolayer. In these experiments, the barriers can be moved slowly, thus nucleation and growth of domains are slow, and an equilibrium distribution of domain sizes is more likely to be attained. Unfortunately, the domains generated by expansion in our experiments were very small (some of them were about 1 px2 or lower, see Fig. S1.A), thus the histogram would carry a great deal of error. However, during the experiments we frequently observed large circular domains (at least 40 μm of radius, see Fig. S1.B as an example). According to the “equivalent dipole model” proposed by McConnell (1991), the presence of circular domains of 40 μm radius correlates with an equilibrium radius larger than 40 μm so that equilibrium distribution of domain sizes should not present the maxima around 1 μm or less. Therefore, even in the case of slow nucleation and growth (as slow as possible), the distribution of domain sizes did not correlate with the measured line tension values and the model proposed by Lee et al.

Table 1

Summary of the average of the best-fitting parameters for the size distribution shown in Fig. 5 and other lipid proportions using the equations derived in Lee et al. (2011). λ : line tension, Δm : dipole density difference, and R_0 : domain radius of minimum energy. Analyzed lipid proportions for DLPS/dchol: 90:10, 80:20 and 70:30; for DPPG/dchol: 90:10 and 80:20 and for DLPE/dchol: 80:20 and 70:30.

	DLPS	DLPE	DPPG
λ (pN)	0.02	0.005	0.02
Δm (pCm $^{-1}$)	5	2	5
R_0 (μm)	2	2	8

Finally, we would like to emphasize that in order to successfully apply the approach proposed by Lee et al. it would be necessary to prove that the observed texture is in equilibrium in the particular system and conditions under study.

4. Discussion

Our results show a variation in the line tension value that did not correlate with the hydrophobic mismatch or hydrocarbon chain length. According to Kuzmin et al. (2005) there is a linear relationship between δ^2/h_0^2 and λ in bilayers if the energy of lipid splay and tilt and spontaneous curvature remain constant. In our experiments the λ variation did not correlate with the value of δ/h_0 (compare Fig. 2A with 2D). It seems clear that for monolayers at least, other factors (different from δ^2/h_0^2) play a more important role. The line tension values were clearly higher for the mixtures containing DPPG and DLPE lipids and lower for those with DPPG lipids, varying in the order: DPPG < DLPS ~ DLPC ~ DPPS < DPPC < DLPE ~ DPPE. The noticeably high values for the mixtures with PE lipids suggest that the intrinsic curvature of the non-sterol lipid may have been a determining factor in the line tension value, since it is known that PE tends to have a negative spontaneous curvature (Daghastanli et al., 2004). In this connection, all the proposed theoretical models for both monolayers and bilayers emphasize the importance of the spontaneous curvature of the lipid phases in coexistence, which in turn influences the out-of-plane angle (Lee et al., 2011; Kuzmin et al., 2005; Towles and Dan, 2008).

Next, we determined the critical packing parameter $P_c = v/\alpha_0 l_c$ (α_0 is the cross-section of the molecule, v and l_c are the volume and the length of the hydrophobic tail, respectively) (Israelachvili et al., 1976). The value for α_0 was considered to be the average molecular area at high packing (40 mN/m) of the compression isotherms of monolayers of pure non-sterol lipids on NaCl 0.15 M at 21 °C, with v and l_c calculated as in (Israelachvili et al., 1976). It has been previously shown that P_c calculated using this approach correlates with the spontaneous 3D organization of single molecules or lipid mixtures in solution (Maggio, 1985; Maggio et al., 1988; Carrer and Maggio, 2001; Caboi and Monduzzi, 1996; Kakehashi et al., 2005; Li and Yamazaki, 2004; Lee et al., 1993; Janes, 1996). The line tension of the mixtures was plotted as a function of the critical packing parameter of pure monolayers of non-sterol lipids (Fig. 6), showing a strong correlation between these parameters. It should be kept in mind that the system with DPPG was studied at a surface pressure 2 mN/m lower than that in the other systems. However, there is agreement in the literature that line tension increases as surface pressure decreases (Heinrich et al., 2008; Benvegna and McConnell, 1992; Lee et al., 2011; Discher et al., 2002; Stottrup et al., 2007). The line tension of the system with DPPG should therefore be taken as an upper limit of the line tensions of this system at 4 mN/m, so that

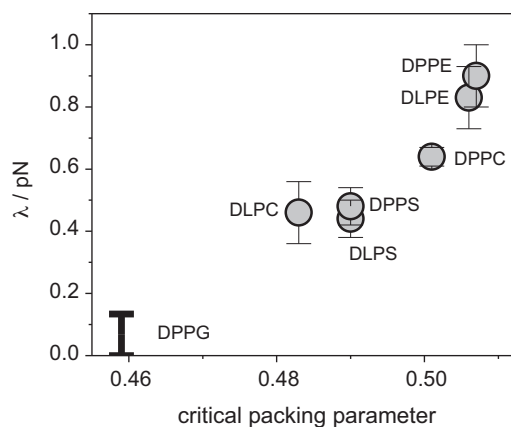


Fig. 6. Line tension at 4 mN/m of mixed monolayers with dchol and the indicated lipids as a function of the critical packing parameter of the pure non-sterol lipids. For DPPG we plotted the line tension value at 2 mN/m, and thus it should be taken as an upper limit (see text).

the difference between mixtures with DPPG and the other mixtures would be higher. Fig. 6 shows a correlation between λ and P_c , indicating that line tension depends on the head-group and the critical packing parameter of the non-sterol lipid.

5. Conclusion

In this work we have presented a technique for determining the line tension in monolayers with liquid–liquid phase coexistence. This experimental approach appears to be a reliable method for comparing the line tension of different mixtures under similar experimental conditions, enabling us to compare the line tension of binary mixtures composed of a lipid and dchol. We found that the mixtures with DLPE and DPPE exhibit high λ values, whereas the mixtures with DPPG show low λ values.

The changes in λ values did not correlate with the changes in monolayer thickness and the head-group was found to exert a stronger influence than chain length. We propose that the spontaneous curvature of the pure lipid constituting the mixed surface perturbed the spontaneous curvature of each coexisting phase when the lipid was mixed with dchol, and as a consequence, line tension showed a strong correlation with the critical packing parameter.

It would be interesting to compare the results reported here with a similar set of systematic line tension measurements on bilayers to test for any differences between monolayer and bilayer systems in the relative importance of hydrophobic mismatch and other effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemphyslip.2012.08.002>.

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