

Organization and Structural Properties of Langmuir Films Composed of Conjugated Polydiacetylene and Phospholipids

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Molecular assemblies containing phospholipids and conjugated polydiacetylene lipids exhibit unique biochromatic properties and have attracted increasing interest in recent years as potential bio- and chemosensors. We present a detailed study of the properties of mixed films formed at the air–water interface, which consist of phospholipid molecules and diacetylene lipids. The organization of the films has been characterized by surface pressure–area isotherms. Application of atomic force microscopy, polarized optical microscopy, and UV–vis spectroscopy provides further insight into the structures and interactions of the film components. The data indicate that the two constituents in the film are miscible at low surface pressure, while segregation of phospholipid and polymer domains occurs at higher surface pressures. The distribution and interactions between the diacetylene and phospholipid domains additionally depends on the molar fraction of phospholipid in the film. Characterization of the structural properties of the polydiacetylene domains in the films points to a formation of organized trilayer and multilayer phases at high surface pressures and high diacetylene concentrations.

Introduction

Polydiacetylene lipids have attracted considerable interest because of their unique chromatic properties.¹ Closely packed diacetylene lipid monomers can be topotactically polymerized by UV irradiation, yielding linear polydiacetylene assemblies, which appear intense blue to the eye. Polymerized diacetylenes have been investigated both in vesicle topographies² and in films at the air–water interface.³ Intriguingly, polydiacetylene assemblies undergo visible blue–red color transitions that can be induced by a variety of environmental perturbations, such as temperature,⁴ pH change,⁵ interfacial pressure,⁶ and specific chemical binding.⁷

Promising biological applications of polydiacetylenes have also been explored.^{2,3} We have recently demonstrated, for example, that various phospholipids, such as phosphatidylcholine (PC), can be incorporated within polydiacetylene matrices without disruption of the colorimetric properties of the conjugated polymer.⁸ Moreover, we have shown that mixed assemblies of polydiacetylene and phospholipids can be utilized as colorimetric biosensors for screening antibacterial membrane-peptides,⁹ detection of metal cations,¹⁰ and antibody-epitope recognition.¹¹ These studies have indicated that the phospholipid/

polydiacetylene surface essentially serves as a platform for interfacial membrane processes.

One of the most critical issues in studying phospholipid/polydiacetylene systems is the accurate assessment of the distribution and interactions of phospholipid molecules within the conjugated polymer matrix. In addition, a thorough understanding of the biochromatic properties of the lipid assemblies requires elucidation of the effects of the incorporated lipids upon the colorimetric properties of the polymer–lipid assembly. A fundamental question that has to be addressed as well is whether the mixed phospholipid/polydiacetylene vesicles are a good model system for studying actual membrane processes. In this work we examine the cooperative organization of mixed phospholipid/polydiacetylene films, to address the above issues. In particular, we investigate the microstructures, domain formation, and molecular distribution and interactions within Langmuir films composed of diacetylene and dimyristoylphosphatidylcholine (DMPC). Various surface techniques have been applied in order to probe the molecular and topographical properties of the films. Surface pressure–area isotherm analysis, polarized optical microscopy, and atomic force microscopy measurements provide a comprehensive description of the organization and molecular interactions of the lipid and polymer components. The results indicate the existence of segregated phospholipid domains within the polymerized lipid matrix, pointing to the utility of the system for membrane biosensor applications.

Experimental Section

Materials. 10,12-Tricosadiynoic acid (TRCDA) was purchased from GFS Chemicals (Powell, OH). The compound was purified by dissolving the solid in chloroform (CHCl₃) and filtration of the resulting solution through a 0.45 μ m Nylon filter. The purified solid was obtained by evaporation of the solvent. Dimyris-

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toylphosphatidylcholine (DMPC) [Avanti Polar Lipids, Alabaster, AL] and octadecyltrichlorosilane (OTS) at >98% purity [Aldrich] were used as received. Cyclohexane (C_6H_{12}), chloroform, sulfuric acid (H_2SO_4), and hydrogen peroxide (H_2O_2 30%) were HPLC grade (Frutarom Ltd.). The DMPC and TRCDA were dissolved in chloroform at a concentration of 2 mM. The various molar fractions were prepared by mixing the appropriate amounts of parent solutions of each compound. The water subphase used in the Langmuir trough was doubly purified by a Barnstead D7382 water purification system (Barnstead Thermolyne Corporation, Dubuque, IA), with 18.3 m Ω resistivity.

Surface Pressure–Area Isotherms. All surface pressure–area isotherms were measured with a computerized Langmuir trough (model 622/D1, Nima Technology Ltd, Coventry, U.K.). The surface pressure was monitored using a 1-cm-wide piece of filter paper as a Wilhelmy plate. For each isotherm experiment, 25 μ L of the desired lipid mixture in chloroform solution (2 mM concentration) was spread on the water subphase (pH = 6.3) and was allowed 15 min for solvent evaporation prior to compression. Compression was carried out at a constant barrier speed of 3.3 $\text{\AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$. The surface pressure measurements were repeated at least three times, using a fresh mixture in each experiment. The isotherms presented are the average of the three experimental runs, which were reproducible within the error area of $\pm 0.6 \text{ \AA}^2 \text{ molecule}^{-1}$.

Preparation of Substrates for Microscopy Experiments. Following compression, at a desired surface pressure, the films were UV-irradiated (254 nm) for 5 s to polymerize the TRCDA. The glass slides were dipped in a cleaning (piranha) solution consisting of 70 mL of H_2SO_4 and 30 mL of H_2O_2 for 30 min at 70 $^\circ\text{C}$, which was followed by sonication in the same solution for 10 min. Following the cleaning, the glass was rinsed thoroughly with pure water and dried at 70 $^\circ\text{C}$. To form the self-assembled monolayers on the surface, the glass was immersed in a solution of 300 μ L of OTS in 100 mL of cyclohexane for 12 h. Glass slides were then rinsed with cyclohexane to remove noncovalently bound OTS molecules.

UV Spectra. UV–vis measurements were carried out on a Hewlett-Packard 8452A diode-array spectrophotometer. The spectra were directly acquired using the DMPC/TRCDA films transferred onto the glass.

Polarized Optical Microscopy. Polarized optical microscopy images were obtained with an Axioplan 2 Universal Microscope (Zeiss, Jena, Germany). Images have been acquired using a Spot 2 digital camera.

Atomic Force Microscopy. AFM measurements were performed at ambient conditions using a Thermomicroscopes CP Research Instrument mounted on an active antivibration table. A 100 μ m scanner was used. Microfabricated Si oxide ultra-levers (Thermomicro) with integrated pyramidal tips were used. The 512 pixel \times 512 pixel images were taken in a noncontact mode with a scan size of up to 60 μ m, at a scan rate of 1 Hz.

Results and Discussion

Figure 1 shows surface pressure–area (π – A) isotherms of pure films of TRCDA, DMPC, and their mixtures on a pure water subphase. Isotherms of TRCDA, recorded at 5, 18, and 30 $^\circ\text{C}$, are shown in Figure 1A. TRCDA has been specifically selected in our studies since its chromatic response is significantly stronger than colorimetric transitions detected for 10,12-pentacosadiynoic acid (PDA, having two more carbons at the alkyl chain), which have been extensively studied previously.^{12–14}

At 5 $^\circ\text{C}$ (Figure 1A, long dash), the isotherm exhibits a condensed phase collapsing at around 13 mN/m. The limiting area per molecule of TRCDA at the condensed phase, denoted A_0 , is obtained by extrapolation of a line tangent to zero surface pressure, at the steep segment of the isotherm as the film is further compressed. The value

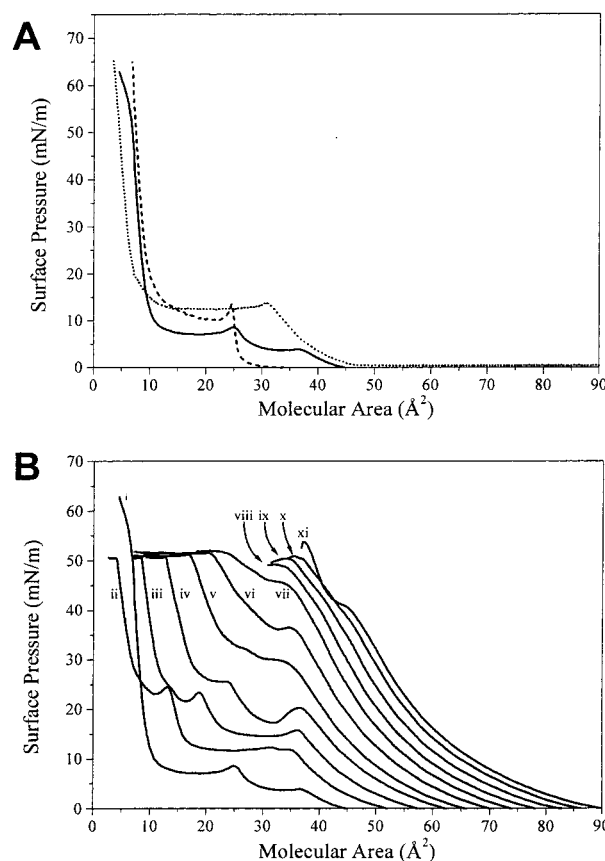


Figure 1. (A) Surface pressure–area isotherms of pure TRCDA, recorded at 5 $^\circ\text{C}$ (long dash); 18 $^\circ\text{C}$ (solid curve); 30 $^\circ\text{C}$ (short dash). (B) Surface pressure–area isotherms of DMPC/TRCDA films, containing different molar fractions of DMPC, spread on a pure water subphase at $T = 18 \text{ }^\circ\text{C}$. The molar fraction of DMPC in each film is (i) 0 [pure TRCDA]; (ii) 0.1; (iii) 0.2; (iv) 0.3; (v) 0.4; (vi) 0.5; (vii) 0.6; (viii) 0.7; (ix) 0.8; (x) 0.9; (xi) 1.0 [pure DMPC].

extracted for A_0 in the monolayer phase at 5 $^\circ\text{C}$ is approximately $26 \text{ \AA}^2 \text{ molecule}^{-1}$, which is similar to the limiting molecular area previously obtained for PDA films^{13,14} and is characteristic of single alkyl-chain compounds.

Intriguingly, the π – A isotherm of pure TRCDA at 18 $^\circ\text{C}$ (Figure 1A, solid curve) exhibits two phase transitions. As the molecular area is progressively reduced, the surface pressure starts to increase from around $45 \text{ \AA}^2 \text{ molecule}^{-1}$, most likely due to the formation of an expanded monolayer phase. Further compression leads to a transition into a plateau, at around 5 mN/m, corresponding to coexisting expanded and condensed phases,¹⁵ having a limiting molecular area of approximately 30 \AA^2 , similar to the value observed at 5 $^\circ\text{C}$. The TRCDA monolayer collapses at a surface pressure of 8.6 mN/m, and further compression leads to a reorganization of the film at a stable phase, with a limiting molecular area of around $10 \text{ \AA}^2 \text{ molecule}^{-1}$. This value suggests the formation of TRCDA trilayers, with an average molecular area of one-third of the corresponding area of 30 \AA^2 in the condensed monolayer. Previous studies conducted on PDA films at the air–water interface have similarly reported trilayer formation.^{13,16}

The shape of the TRCDA isotherm acquired at 30 $^\circ\text{C}$ (Figure 1A, dotted curve) supports the interpretation

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outlined above for TRCDA film organization. The isotherm recorded at 30 °C favors, as expected, an expanded phase up to a significantly higher surface pressure value, compared to the isotherms recorded at 5 and 30 °C, respectively. The isotherm further indicates a phase transition at around 15 mN/m, most likely corresponding to a transition between an expanded and condensed monolayer. The higher surface pressure for the expanded–condensed transition at 30 °C is most likely due to the higher Brownian motion induced by the increased temperature, which stabilizes the expanded phase of the monolayer. A broad plateau corresponding to a surface pressure of around 13 mN/m is observed following the transition. This plateau is similar to the one recorded at 18 °C; however, it covers a wider area range, which again is anticipated due to the stabilization effect of the expanded phase at a higher temperature.

The π – A isotherms of pure TRCDA shown in Figure 1A, and in particular the isotherm recorded at 18 °C, exhibit significant differences compared to the isotherms of pure PDA reported elsewhere.^{12–14} The TRCDA isotherms indicate the formation of a stable expanded phase for TRCDA. The expanded phase and the transition between the expanded and condensed phase have not been observed in films of pure PDA, which exhibit only condensed phases at various temperatures.^{12–14} The difference in assembly properties between TRCDA and PDA is probably related to the shorter alkyl chain of TRCDA (23 carbons versus 25 carbon atoms in PDA). The surface pressure leading to expanded-to-condensed phase transitions in single alkyl-chain lipids is correlated with the length of the hydrocarbon chain.¹⁵ As discussed above, shorter alkyl chains increase the surface pressure of the phase transition, because they essentially contribute to stabilization of the expanded phase.

Figure 1B features surface pressure–area isotherms of DMPC/PDA films having different ratios between the two components. The isotherm of pure DMPC, Figure 1B(xi), exhibits an expanded phase at lower surface pressures and a transition, at around 40 mN/m and a limiting molecular area of 50 Å²·molecule^{−1}, into a condensed phase. Similar appearances of surface–area isotherms have been previously reported for DMPC and for other phospholipids.^{17–19} The incorporation of DMPC molecules within the TRCDA monolayer modifies the compression properties of the film as shown in Figure 1B(ii–x). π – A isotherms of the mixed films reveal two phenomena. First, for DMPC molar fractions < 0.6, Figure 1B(ii–v), the isotherms exhibit two transitions. The surface pressures and limiting molecular areas of the transitions are modified upon increasing the phospholipid content in the film. In particular, higher surface pressures for the phase transition between the expanded phase and a condensed phase, at a mean molecular area of around 36 Å²·molecule^{−1}, are observed as the percentage of DMPC in the film is increased. This observation is most likely related to the increased stability of the expanded phase within mixed DMPC/TRCDA films, as discussed above.

A second transition is observed in curves ii–iv, at around 24 mN/m, which might correspond to multilayer formation. The relatively insignificant effect of DMPC percentage upon this phase transition might indicate segregation between DMPC and TRCDA domains within the mixed film. Such organization would diminish the effect of the incorporated phospholipid upon TRCDA compression.

Indeed, evidence for this hypothesis is described below. The phase transitions corresponding to multilayer formation are hardly detected in TRCDA films having DMPC molar fractions between 0.4 and 0.6 (Figure 1B(v–vii)). Significantly, the collapse surface pressures of all mixed DMPC/TRCDA films having DMPC fractions of between 0.1 and 0.6 are almost identical, at around 52 mN/m (Figure 1B(ii–vii)). This result, similar to that for constant collapse pressures previously recorded for other binary films,²⁰ will be discussed in detail below.

The surface–area isotherms recorded for TRCDA films containing DMPC molar fractions > 0.7 (Figure 1B(viii–x)) are distinctively different from the compression isotherms observed for the films with lower DMPC percentages. In particular, the isotherms depicted in Figure 1B(viii–x) exhibit a single phase beginning from zero surface pressure, up to the collapse pressure at around 53 mN/m. Similar isotherms have been previously recorded for Langmuir monolayers containing various phospholipids and phospholipid mixtures.^{20–22} Significantly, the isotherms in Figure 1B(viii–x) differ from the corresponding pressure–area curve of pure DMPC, shown in Figure 1B(xi). In particular, the isotherms shown in Figure 1B(viii–x) indicate that the presence of even a small percentage of TRCDA molecules in the DMPC film eliminates the condensed monolayer phase observed in the pure DMPC film (Figure 1B(xi)). As discussed above, the stabilization of the expanded phase in DMPC/TRCDA films most likely corresponds to the insertion of single-chain TRCDA lipids, which disrupt the close packing order of the condensed phase.

An important question addressed in this study is the extent of miscibility of TRCDA and DMPC in the films. Determination of the degree of miscibility of the film components is aided by application of the surface phase rule:²²

$$F = C^B + C^S - P^B - q + 1 \quad (1)$$

Equation 1 evaluates the number of degrees of freedom F , that is, the independent physical variables that can be measured, where C^B is the number of components in the bulk, C^S is the number of components confined to the surface, P^B is the number of bulk phases, and q is the number of surface phases in equilibrium with each other. For a two-component monolayer, at the interface between the two molecular species $C^B = 2$, $C^S = 2$, $P^B = 3$, and eq 1 becomes

$$F = 2 - q \quad (2)$$

Equation 2 essentially indicates that if there is one surface phase at the monolayer (i.e. the two components are miscible), there would be one degree of freedom; thus, for example, the surface pressure at a phase transition would vary as a function of the molecular area. Conversely, if there are two phases at the monolayer surface (the two components are *immiscible*), eq 2 indicates zero independent variables within the system, meaning that the surface pressure must remain constant during a phase transition.

Examination of Figure 1, and taking into account the surface phase rule, reveals that there are distinct arrangements within the mixtures of TRCDA and DMPC at

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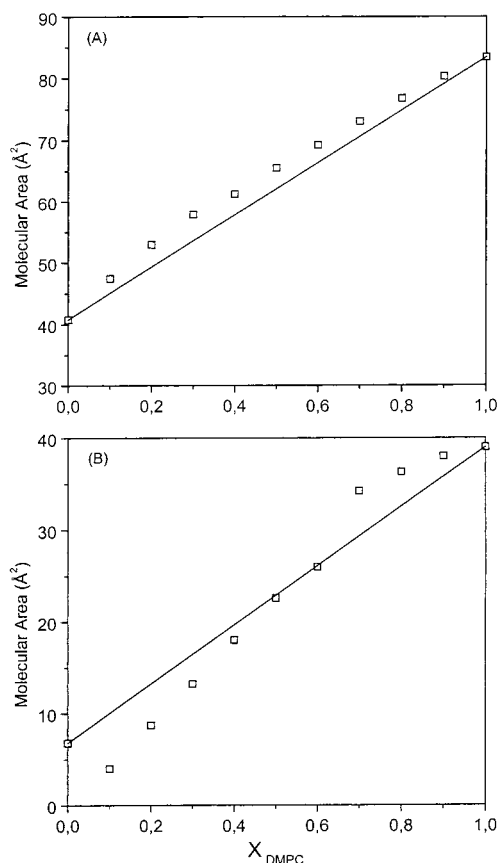


Figure 2. Average molecular area of mixed DMPC/TRCDA films as a function of the molar fraction of DMPC, measured at (A) the low surface pressure of 1.6 mN/m; (B) the high surface pressure of 48.5 mN/m. The solid lines represent the molecular areas determined by a statistical distribution of the components and are calculated according to the additivity rule (eq 3): (A) an ideal expanded phase of TRCDA and DMPC; (B) a statistical mixture of DMPC monolayer and TRCDA trilayer, respectively (see text). The height of the squares is the error in determination of the average molecular area ($0.6 \text{ Å}^2 \text{ molecule}^{-1}$).

the air–water interface. In films containing DMPC in a molar ratio < 0.7 (Figure 1B(ii–vii)) and at higher surface pressures, the collapse pressure hardly changes, at around 52 mN/m. Therefore, on the basis of eq 2, under those conditions the DMPC/TRCDA films exhibit distinct molecular domains of DMPC and TRCDA, respectively. However, in the Langmuir films having higher DMPC molar ratios (Figure 1B(viii–x)), the collapse pressure gradually increases upon increasing the percentage of DMPC in the film; in those compositions the phospholipid and diacetylene are miscible.

The observation that the miscibility of the constituents depends on their ratio in the film is significant, because it indicates that control over domain formation in the film is feasible. To further probe the organization and interactions between DMPC and TRCDA in the mixed films, an analysis of the limiting molecular areas (calculated from the isotherms) as a function of the molar ratio of DMPC in the film has been carried out (Figure 2). Figure 2A depicts the mean molecular areas for the DMPC/TRCDA films, plotted as a function of the mole fraction of DMPC, measured at the isotherms of Figure 1 at a very low surface pressure ($\pi = 1.6 \text{ mN/m}$). The solid line in Figure 2A has been calculated using the additivity rule:²³

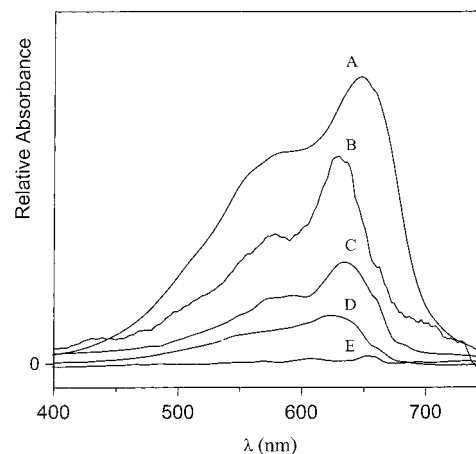


Figure 3. UV–vis spectra of mixed DMPC/TRCDA films deposited at a surface pressure of 30 mN/m ($T = 18 \text{ °C}$) for different molar fractions of DMPC in the film: (A) 0.0; (B) 0.1; (C) 0.2; (D) 0.3; (E) 0.5.

$$\hat{A}_{\text{mean}} = (1 - X_{\text{DMPC}})A_{\text{TRCDA}} + X_{\text{DMPC}}A_{\text{DMPC}} \quad (3)$$

where \hat{A}_{mean} is the average molecular area in the two-component film at a given surface pressure, X_{DMPC} is the molar fraction of DMPC, and A_{TRCDA} and A_{DMPC} are the corresponding molecular areas in the pure films of TRCDA and DMPC, respectively. The use of the additivity rule here is purely statistical; essentially, the additivity rule predicts the molecular area in an ideal binary mixture, assuming no chemical or physical interactions between the components in the mixture.¹⁹ In addition, eq 3 does not preclude the formation of segregated phases, in which the molecular components in a mixture do not interact.

The data presented in Figure 2A demonstrate that, at a low surface pressure in which all the films adopt expanded phases regardless of the molar fractions, essentially all the limiting molecular area values appear above the solid line. The positive deviations from the line describing ideal solutions are most likely due to physical interactions, such as electrostatic repulsion between the molecular components of the films. Such interactions will induce disruption of the closely packed TRCDA monolayers by the incorporated DMPC, as discussed above, giving rise to the observed positive deviations.

The relationship between the molecular areas and the mole fraction of DMPC was also statistically analyzed at a high surface pressure ($\pi = 48.5 \text{ mN/m}$), closer to the collapse pressure (Figure 2B). Probing the molecular arrangements at a high pressure is important because of the segregation between TRCDA and DMPC at those pressures (see discussion above). The analysis is also particularly significant because, at high surface pressures, the mixed films combine condensed monolayers (for pure DMPC at the surface pressure examined) and multilayers (for TRCDA).

The straight line depicted in Figure 2B corresponds to molecular areas calculated from purely statistical distributions of TRCDA trilayers and DMPC monolayers. Accordingly, the molecular areas at the two end points in Figure 2B are 8 and $40 \text{ Å}^2 \text{ mol}^{-1}$, respectively. These values were extracted from the isotherms for pure TRCDA in the trilayer region and for the pure DMPC monolayer, respectively. The experimental data points for the mixtures, shown in Figure 2B, indicate the presence of three film structures. At DMPC molar fractions > 0.6 , the limiting molecular areas exhibit strong *positive* deviations from the solid line. This result is expected, since the DMPC/

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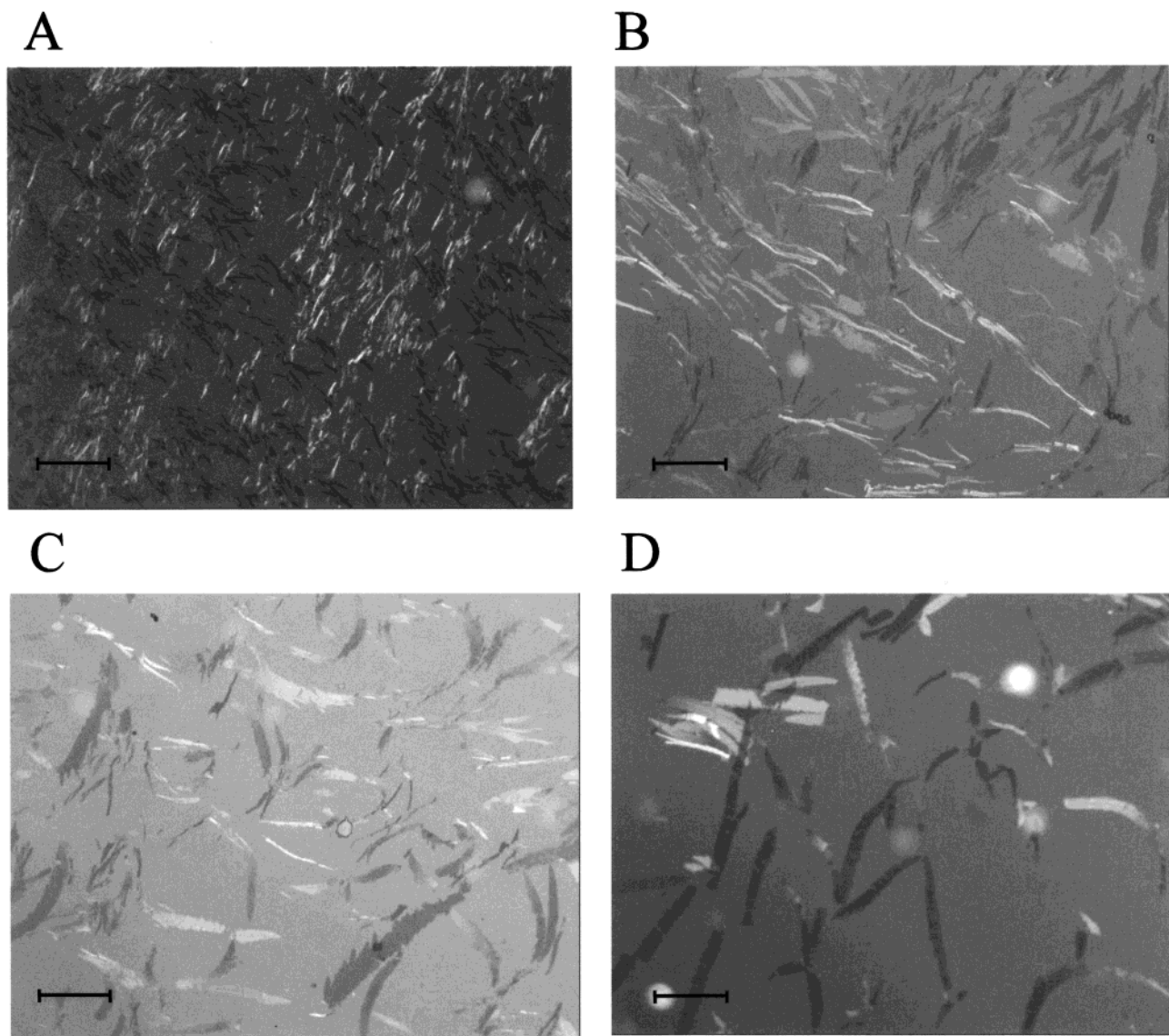


Figure 4. Polarized optical microscope images of mixed DMPC/TRCDA films deposited at a surface pressure of 40 mN/m for different molar fractions of DMPC: (A) 0.1; (B) 0.2; (C) 0.3; (D) 0.4. The size of the bar is 100 μm .

TRCDA mixed films having low molar fractions of DMPC exist in an expanded phase. In this situation, a *greater* average molecular area would be observed, since the TRCDA molecules do not organize in trilayer or multilayer structures but rather in an expanded monolayer. In films containing DMPC molar fractions between 0.4–0.6, on the other hand, the molecular area values are located close to the calculated line. Such films obey the statistical distribution exemplified in the additivity rule and can be described according to the structural model of TRCDA trilayers coexisting with DMPC monolayers.

An important result apparent in Figure 2B is the *negative* deviations from the additivity rule recorded for films containing between 0.1 and 0.3 mole fraction of DMPC. The lower molecular areas, compared with the statistical calculation, most likely originate from a formation of TRCDA multilayer structures. Stacking of the TRCDA layers beyond trilayer structures would reduce the average molecular structure measured in the pressure–area experiments. Support for this hypothesis is further provided by the AFM data, discussed below.

Additional structural information on the mixed DMPC/TRCDA films has been obtained from visible absorbance spectra of the polymerized films, shown in Figure 3.

Diacetylene films can be irradiated and polymerized at the air–water interface, and their chromatic properties are related to the organization of the monomers in the film.^{16,24} Figure 3 depicts UV–vis spectra recorded for mixed DMPC/TRCDA films at different DMPC molar ratios, following polymerization of the films at 18 °C, at a high surface pressure ($\pi = 30$ mN/m). The polymerization was carried out through irradiation of the monolayers at a wavelength of 280 nm for 5 s, close to the surface.

The data shown in Figure 3 confirm that polymerization of mixed DMPC/TRCDA films can be carried out for certain film compositions. The observation of the “blue phase” of the films indicates that the structure of the diacetylene domains within the mixed films facilitates polymerized structures within the TRCDA matrix.^{4–6} However, Figure 3 clearly demonstrates that the intensity of the blue color of the films (hence degree of polymerization) depends on the percentage of the phospholipid. The spectra shown in Figure 3 demonstrate that polymerization occurs only in mixed films with molar fractions of DMPC < 0.5 and which are transferred to the glass surface at pressures where the TRCDA is in trilayer or multilayer structures. This

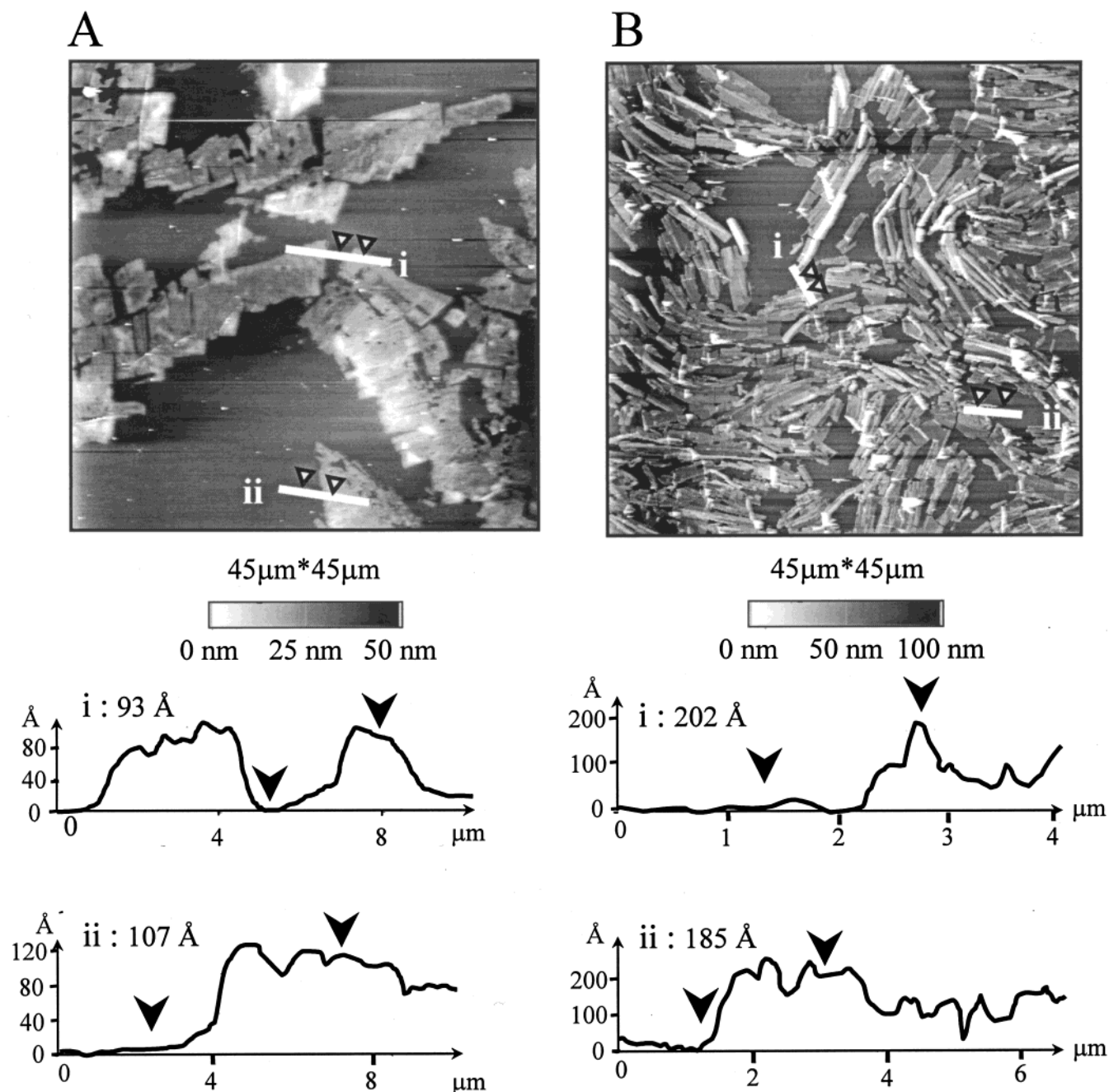


Figure 5. AFM images of DMPC/TRCDA films containing 10% DMPC deposited at (A) a surface pressure of 20 mN/m; (B) a surface pressure of 40 mN/m. The z-profiles describe height distributions along the lines shown within the AFM images.

result accounts for the fact that polymerization of the TRCDA film is feasible only when the concentration is above a "percolation threshold"—allowing a minimal distance between adjacent monomers.^{1,2} Importantly, the data depicted in Figure 3 are similar to previous observations in DMPC/TRCDA vesicle systems, in which polymerization is observed only when the mole fraction of DMPC in the vesicles is <0.5 ,^{8,9} which is similar to the situation revealed here for the films.

Polarized optical microscopy and atomic force microscopy (AFM) experiments, shown in Figures 4 and 5, complement the surface analysis of the films. Figure 4 depicts polarized optical microscopy images of DMPC/TRCDA films deposited at a surface pressure of 40 mN/m, having DMPC mole fractions of between 0.1 and 0.4. The compressed DMPC/TRCDA films were transferred onto an OTS-treated glass substrate horizontally from the Langmuir trough (Langmuir–Scafer technique).

Polarized optical microscopy is used for the examination of transparent, birefringent microscopic objects that reflect the polarized light.²⁵ This technique has been previously used for identification of topographical differences between thin films deposited on hydrophobic substrates.²⁶ Polarized optical microscopy has also been applied for studying oriented polydiacetylene, in which the observed birefringence patterns are associated with the oriented structure within the conjugated polymer network.²⁷ In contrast to the case of TRCDA, the phospholipid domains within the mixed films are not birefringent; thus, the DMPC domains appear transparent in the polarized optical microscopy images.

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The polarized optical microscopy pictures in Figure 4 show films which were transferred to the glass slides at a surface pressure of 40 mN/m, to probe the structural aspects of the DMPC/TRCDA films in the condensed phase (high pressure) region. The images clearly demonstrate that distinct polydiacetylene domains appear within the films; however, their distribution and sizes depend on the DMPC mole fraction. In particular, while the abundance of polymerized regions decreases as more phospholipid molecules are incorporated into the film, the sizes of the TRCDA domains evidently grow. This result can be explained by the formation of TRCDA multilayers at higher TRCDA ratios and higher pressures. Specifically, as the phospholipid content is increased, adjacent TRCDA trilayer domains merge into fewer, albeit larger, multilayer domains, such as the ones shown in the microscopy image in Figure 4D.

Atomic force microscopy (AFM) images shown in Figure 5 provide additional topographic information. Numerous studies have demonstrated the application of AFM for determination of the topography and *z*-profiles of lipid films assembled at the air–water interface.^{28–30} AFM has also been applied for providing structural information on polydiacetylene thin films.²⁴ Figure 5 shows two representative images and height profiles for DMPC/TRCDA films containing 10% (mole percent) DMPC. The films have been transferred from the water subphase at surface pressures of 20 and 40 mN/m, respectively, which correspond to the two condensed phases observed in the relevant isotherm (Figure 1Bii).

The AFM images displayed in Figure 5 show an abundance of bright domains, corresponding to elevated polydiacetylene,^{24,31} and darker areas, which account for both the hydrophobic silane film and the phospholipid monolayers.^{28,31} The brighter appearances of PDA films deposited onto silane-treated glass or mica surfaces have been confirmed in several reports^{24,32} and have been ascribed to multilayer formation.^{31,32}

The AFM images also indicate that the average thicknesses of the TRCDA films deposited at the two surface pressures are significantly different. While the TRCDA film compressed at 20 mN/m features expanded microstructures, approaching tens of microns (Figure 5A), the film transferred at 40 mN/m displays smaller micron-size domains (Figure 5B). These appearances are very similar to the polarized optical microscopy data discussed above. The factors contributing to the size difference between the TRCDA domains at the two pressures can be further probed by analyzing the thickness of the film fragments through application of the AFM experiments.

The *z*-traces extracted from the AFM images are shown below the respective images (Figure 5). Examination of

the height profiles indicates that the TRCDA domains deposited at 20 mN/m (Figure 5A) are significantly thinner, at around 100 Å, compared with the fragments of the film transferred at the higher surface pressure, which exhibit height values of around 200 Å (Figure 5B). In addition, the *z*-profiles in Figure 5 indicate that the heights of the TRCDA domains within the samples are relatively uniform. The differences in height profiles are consistent with the statistical analysis apparent in Figure 2B; see above. Specifically, the AFM data clearly point to the formation of TRCDA multilayers as the mixed DMPC/TRCDA film is compressed.

Conclusions

This work presents characterization of the molecular and structural properties of chromatic films assembled at the air–water interface from mixtures of diacetylene lipids and phospholipids. The data indicate that a significant difference exists between the compression properties of 10,12-tricosadiynoic acid (TRCDA) studied here and those of the more widely studied 10,12-pentacosadiynoic acid (PDA). The results suggest that the TRCDA monolayers adopt both expanded and condensed phases. Furthermore, at higher surface pressures TRCDA films exhibit formation of trilayers and multilayers.

The incorporation of phospholipid molecules within the TRCDA assembly strongly affects the compression properties of the films, in particular through stabilization of the expanded monolayer phase. The experiments indicate that the DMPC and TRCDA molecules are miscible in films containing low molar fractions of diacetylene, while segregation between the two components occurs in mixed films with low molar fractions of DMPC and under high surface pressure. Polarized optical microscopy and atomic force microscopy experiments indicate that the micron-size TRCDA domains adopt several phases, including condensed monolayers, trilayers, and multilayer structures.

The thermodynamic and structural data indicate that segregation between the DMPC and TRCDA domains is observed over a relatively wide range of DMPC compositions. The results further indicate that the chromatic properties of the mixed films are associated with formation of multilayer TRCDA structures. The observation of distinct domains of polydiacetylene and phospholipids within the mixed films is particularly important, because it confirms that biochemical membrane processes previously detected in DMPC/TRCDA assemblies occur in conjunction with the presence of the organized phospholipid domains. This result points to the possibilities for further use of mixed DMPC/TRCDA films and vesicles for screening and studying biological membrane phenomena. In conclusion, this work would further expand the scope and applicability of phospholipid/polymer systems as interfacial chemo- and biosensors.

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