





## Article

# Interaction of Particles with Langmuir Monolayers of 1,2-Dipalmitoyl-Sn-Glycerol-3-Phosphocholine: A Matter of Chemistry?

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**Abstract:** Lipid layers are considered among the first protective barriers of the human body against pollutants, e.g., skin, lung surfactant, or tear film. This makes it necessary to explore the physico-chemical bases underlying the interaction of pollutants and lipid layers. This work evaluates using a pool of surface-sensitive techniques, the impact of carbon black and fumed silica particles on the behavior of Langmuir monolayers of 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC). The results show that the incorporation of particles into the lipid monolayers affects the surface pressure–area isotherm of the DPPC, modifying both the phase behavior and the collapse conditions. This is explained considering that particles occupy a part of the area available for lipid organization, which affects the lateral organization of the lipid molecules, and consequently the cohesion interactions within the monolayer. Furthermore, particles incorporation worsens the mechanical performance of lipid layers, which may impact negatively in different processes presenting biological relevance. The modification induced by the particles has been found to be dependent on their specific chemical nature. This work tries to shed light on some of the most fundamental physico-chemical bases governing the interaction of pollutants with lipid layers, which plays an essential role on the design of strategies for preventing the potential health hazards associated with pollution.

**Keywords:** lipids; pollutants; Langmuir monolayers; particles; rheology

## 1. Introduction

The continuous ejection of pollutants into the atmosphere as a result of the industrial activity and combustion processes has raised many questions related to the potential impact of pollution on human health [1–4]. This is even more important analyzing the World Health Organization (WHO) statistics which ascribe one third of the deaths caused by strokes, lung cancer, or cardiac diseases to the air pollution [5]. Therefore, the severity of this problem makes it necessary to deepen the study of the impact of pollutants on biological systems [6].

Lipid layers, e.g., skin, tear film, or lung surfactant, provide one of the first protective barriers of the human body against environmental pollution. Therefore, it should be expected that lipid layers should be considered among the most important biological structures where pollution may impact negatively [7–9]. This creates the careful examination of the impact of different chemical species,

such as nanoparticles, on the physico-chemical properties of lipid layers that may be used as a tool for a preliminary understanding of the most fundamental bases governing the alteration of their physiological response as a result of the incorporation of pollutants [9]. However, the direct *in vivo* evaluation of the impact of pollutants in the behavior of lipid films is difficult in most of the cases, which makes the use of model systems necessary [10]. The use of such models provides the bases for establishing preliminary assays for evaluating the alterations induced by pollutant species on the physico-chemical properties of lipid layers with potential biological relevance.

Langmuir monolayers of lipids at the water/vapor interface are probably among the most widespread models used as tools on the evaluation of the effects of different chemicals on the behavior of biologically relevant systems [11–16]. This is because Langmuir monolayers allow for performing physico-chemical studies on ordered lipid films, which are reminiscent of different biological layers, e.g., a single cellular membrane leaflet or the lung surfactant film [11,17,18]. However, the use of Langmuir monolayers only allows for mimicking some specific aspects of the physico-chemical behavior of biological relevant systems that are relatively complex. Thus, the use of Langmuir monolayers helps with the study of minimal systems, i.e., the model including a limited number of chemical species, which allows for exploring the potential role of each single species in the interaction with pollutants, and in the modification of the physico-chemical properties of the whole system. The most common lipid used as a model for studying the interaction of biological relevant layers and pollutants is the 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) [17,19–26]. This is because this lipid is one of the main components of many biological membranes and fluids, e.g., DPPC accounts for 40 wt % of the total weight of lung surfactant in mammals [27]. Despite the simplicity of the models based only on DPPC, they are useful tools for a preliminary evaluation of the worsening of the physico-chemical properties of lipid layers as a result of the incorporation of solid particles. However, the extrapolation of the results obtained from such studies to real biophysical situations studies may require some cautions and additional considerations [28,29]. This is especially important because the specific characteristic of the method used for evaluating the incorporation of particles into the lipid layer impacts the modifications of the interfacial behavior of DPPC layers strongly due to the incorporation of colloidal particle [9,30]. The effect of the methodology used for the incorporation of particles has been recently explored in relation to the interaction of ceria particles with DPPC at the water/vapor interface, using for such evaluation up to three different methodologies for preparing the monolayers containing both DPPC and particles: (i) particles deposited from dispersions in chloroform onto preformed DPPC monolayers, (ii) mixed monolayers prepared from simultaneous co-spreading of the particles and the DPPC at the interface, and (iii) aerosolized particles deposited onto the preformed DPPC monolayer [30]. The obtained results in such study provided evidence that the use of different delivery methods leads to different modifications of the surface tension behavior. Furthermore, special caution must be taken with the use of techniques based on the dispersion of the particles from dispersions because they can impact the agglomeration of the particles and the distribution of such agglomerated within the lipid layer, which may lead to a situation different to that occurring upon the *in vivo* interaction of pollutants with lipid layers.

The investigation of the impact of particles on lipid layers takes particular importance because several studies have provided evidence that the interaction of colloidal particles with surfactant layers modifies both the lateral organization and the mechanical of such surfactant layers [31–33]. Therefore, a strong modification of the physico-chemical behavior of biologically-relevant lipid layers as results of the particles incorporation may be expected [34,35]. Our previous study showed the different impact of particles with different hydrophobicity on the interfacial properties of DPPC monolayers, which is ascribed to the nature of the interactions involved in the incorporation of the particles [36]. Furthermore, previous studies have shown that hydrophobic particles present a strong impact on the lateral packing and mechanical properties of DPPC layers [21,23,37,38]. Despite this impact, there is not any systematic study comparing the impact of carbonaceous and silica particles on the interfacial properties of biologically-relevant lipid layers, with such comparative evaluation being of particular

interest due to the widespread of nanomaterials based in carbon and silica in different technologies and industries. This work tries to shed light on the potential effect of the above-mentioned particles on the behavior of DPPC Langmuir monolayers to perform a preliminary evaluation of the potential risks and hazards associated with their incorporation into biologically-relevant systems. This has been performed analyzing the modifications in the 2D lateral packing of the lipid molecules at the interface, and the cohesion interactions within the interfacial layers. It is expected that the picture obtained from this work may be useful as foundations for a broader study aimed to elucidate impact of pollutants on the physiological response of biological relevant layers.

## 2. Materials and Methods

### 2.1. Materials

1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), with a molecular weight of 734.1 g/mol, was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA) at 99.9% purity, and used as received. Hydrophobic fumed silica particles *Aerosil R972* (SiO<sub>2</sub>) purchased from Evonik-Degussa (Essen, Germany) and carbon black particles CB N110 (CB) supplied by Phillips Petroleum Co. (Bartlesville, OK, USA) were chosen as pollutant models. Table 1 summarizes some physico-chemical characteristic of the particles [21,23,39]. It is worth mentioning that, whereas SiO<sub>2</sub> particles lead to the formation of chain-like aggregates of primary particles, the primary particles of CB aggregates to form spherical-like agglomerates [21,39,40]. It is worth mentioning that, even though the densities and the average diameter (*d*) of the primary particles are similar for SiO<sub>2</sub> and CB, the different geometry of their aggregates helps may provide an explanation for the difference of the values of the BET surface area.

**Table 1.** Physico-chemical characteristics of the used particles.

Particles	BET Surface Area <sup>1</sup> (m <sup>2</sup> /g)	Density <sup>1</sup> (g/cm <sup>3</sup> )	<i>d</i> (nm)
SiO <sub>2</sub>	110 ± 20	2.2	16 ± 4 <sup>1</sup>
CB	51 ± 20	1.8	22 ± 7 <sup>2</sup>

<sup>1</sup> BET surface areas and densities values for both types of particles, as well as the diameter of the primary (*d*) particles for SiO<sub>2</sub>, were obtained from the suppliers manufacturers; <sup>2</sup> the diameter of the primary particles (*d*) of the primary particles of CB was obtained from Transmission Electron Microscopy image analysis in our previous publication [39].

Chloroform (CHROMASOLV™, for High Performance Liquid Chromatography, stabilized with ethanol) purchased from Sigma-Aldrich (Saint Louis, MO, USA) was used for preparing the spreading solutions of DPPC and the particles' dispersions.

Ultrapure deionized water for cleaning and experiments was obtained by a multi-cartridge purification system Elix + Milli-Q (Millipore, Burlington, MA, USA). This water presents a resistivity higher than 18 MΩ·cm, a total organic content lower than 6 ppm and a surface around 72 mN/m at 22 °C without evidence of surface tension kinetics were found for the used water over several hours. The pH of the water was around 6.5, and no salts were used for fixing the ionic strength.

### 2.2. Monolayers Preparation

The lipid monolayers were prepared at the water/vapor interface by dropping controlled volumes of DPPC from its solution in chloroform (concentration about 1 mg/mL or 1.36 mM) using a high-precision Hamilton syringe (Hamilton Company, Reno, NV, USA). This methodology ensures the control of the interfacial density of DPPC,  $\Gamma$ , upon solvent evaporation. The initial interfacial density of DPPC spread at the water/vapor interface  $\Gamma_0$  was fixed in all the experiments in a value of 1.7 μmol/m<sup>2</sup>, corresponding to an area per molecule of about 98 Å<sup>2</sup>.

The preparation of the mixed monolayers was done following a two-step approach: (i) a DPPC monolayer was obtained from the spreading of the lipid from its solution in chloroform (concentration

1 g/L) at the bare water/vapor interface, and (ii) a given amount of the particle dispersion (concentration 1 g/L) was spread onto the preformed DPPC monolayer, again using chloroform as the spreading solvent (Notice that particles dispersions were sonicated during 15 min using a laboratory ultrasound bath; this allows for minimizing particles aggregation before their spreading). This procedure allows for obtaining monolayers with specific DPPC: particles weight ratio at the interface. Once monolayers including particles and DPPC are obtained, it is necessary to wait during 1 h before starting the experiments to ensure the complete evaporation of the solvent and, in the case of mixed monolayer, the achievement of the equilibrium of the composite system which is driven by the nanoparticle–lipid interactions [21,23]. It is worth noting that the monolayers studied here cannot be a strictly considered mixed monolayer because they were not obtained after the spreading of a mixed dispersion containing the DPPC and the particles (first, the DPPC is spread at the pure water/vapor interface, and then the addition of the particles is made onto the preformed DPPC monolayer). However, for the sake of simplicity, the term mixed monolayers will be used for monolayers involving the DPPC and the particles.

The temperature was fixed  $22.0 \pm 0.1$  °C in all the experiments. Even though this temperature is far from the physiological one (37 °C), the main conclusions obtained in our study are extrapolated, at least from a semi-quantitative perspective, to the physiological conditions. This is because the phase transition of DPPC appears above the physiological temperature, thus only a shift of the phase behavior with the temperature is expected, without any significant impact on the main physico-chemical insights extracted from the experimental results [41].

It is worth mentioning that the properties of the mixed monolayers obtained upon chloroform evaporation will be affected by the conditions in which DPPC and particles are mixed [30]. This work has used a methodology for the preparation of the mixed layers where the interaction between particles and lipid layers occurs only at the water/vapor interface. This may be considered similar to that which happens during the interaction between environmental pollutants and lipid layers. However, this study does not consider two aspects that may have impact when in vivo conditions are concerned: (i) the presence of chloroform during particles addition may affect both the lateral packing of the DPPC molecules and the DPPC–particle interactions [30], and (ii) the interaction of particles and the lipid layers may be affected for specific mass transport boundary conditions which cannot be included in our studies using Langmuir monolayers [42,43].

### 2.3. Methods

The Langmuir monolayers were studied using a Langmuir trough KSV Nima model KN2002 (Biolin Scientific, Espoo, Finland), equipped with two Delrin barriers allowing for symmetric compression/expansion of the free liquid surface. The total surface area of the Teflon trough is 243 cm<sup>2</sup>. The surface tension,  $\gamma$ , was measured using a force balance fitted with a paper Wilhelmy plate (Whatman CHR1 chromatography paper, effective perimeter 20.6 mm, supplied by Sigma Aldrich, St. Louis, MO, USA), ensuring a zero contact angle. The surface pressure,  $\Pi$ , is obtained as the difference between the surface tension of the pure water/vapor interface  $\gamma_w$  and  $\gamma$ , i.e.,  $\Pi = \gamma_w - \gamma$ .

The quasi-equilibrium isotherms for the monolayers were obtained measuring the surface pressure as the interfacial area available for the monolayer,  $A$ , is reduced at a fixed compression velocity of 2 cm<sup>2</sup>/min, which is equivalent to a compression rate  $(\Delta A/A^0)/\Delta t$  of about  $10^{-5}$  s<sup>-1</sup>, with  $\Delta A/A^0$  being the amplitude of the deformation, represented as the ratio between the change of area  $\Delta A$  (amplitude of deformation) and the reference interfacial area  $A^0$  (generally the area in which the compression is started), and  $\Delta t$  the time needed for the deformation. This compression rate allows for avoiding an undesired non-equilibrium effects during the determination of the isotherms [44].

The Langmuir trough also enables the study of the effects of the incorporation of nanoparticles on the dilational rheology of the DPPC monolayers using the oscillatory barrier method. A detailed description of the foundations of this method can be found elsewhere [45–47]. The oscillatory barrier method allows for evaluating the modulus of the complex dilational viscoelasticity  $E = \Delta\gamma/(\Delta A/A)$ , i.e.,

the variation, of the surface tension  $\gamma$  as a result of a harmonic change at a controlled frequency  $\nu$  (in a range of frequencies from  $10^{-3}$  to 0.15 Hz) of the interfacial area which is written as follows:

$$A(t) = A^0 + \Delta A \sin(2\pi\nu t). \quad (1)$$

The harmonic change of the interfacial area (strain) results in a stress response  $\Delta\Pi = \Pi^0 - \Pi(t)$ , which is defined as the change of surface pressure between the reference state  $\Pi^0$  and the instantaneous value of the surface pressure  $\Pi(t)$ . When the deformation presents a small amplitude, i.e., deformation within the linear regime, the stress response also follows a sinusoidal profile with the same frequency than the deformation:

$$\Pi(t) = \Delta\Pi \sin(2\pi\nu t + \phi), \quad (2)$$

where  $\phi$  is a phase shift accounting for a possible delay of stress response (surface pressure change) in relation to the strain (area deformation). Considering the above-mentioned linear response, the stress can be considered proportional to the deformation  $u(t) = \Delta A/A^0$  (elastic term) and to the rate of deformation  $du(t)/dt$  (viscous term), which allows one to write the stress as:

$$\Pi(t) = \varepsilon u(t) + \eta(du(t)/dt), \quad (3)$$

with  $\varepsilon$  and  $\eta$  being the dilational elasticity and viscosity, respectively. Considering the definition given by Equation (3) and assuming a generic harmonic perturbation, it is possible to obtain a definition for the complex dilational viscoelasticity:

$$\varepsilon^* = \varepsilon + 2\pi\nu\eta i \quad (4)$$

where  $i = (-1)^{1/2}$ . The analysis of the curves corresponding to the strain and stress in terms of Equations (1) and (2) provide information about their amplitudes and the phase shift, enabling for the calculation of the dilational viscoelasticity. In the reported experiments, the amplitude of the dilational deformation  $u(t) = 0.01$  was adopted, which allows the response to remain within the linear regime. It is worth noting that the conditions considered in our work for the evaluation of the mechanical response of lipid layers, and how particles' incorporation impact such response, are far from those corresponding to the characteristic values of the dynamics processes involved in biologically relevant systems, e.g., respiratory cycle, where higher values for the frequency of the deformation and its amplitude are expected. In the particular case of the respiratory cycle, the frequency and the deformation amplitude assume values around 0.3 Hz and 0.30–0.40, respectively [48]. However, the evaluation of the dilational response within the linear regime provides helpful information for analyzing the impact of incorporation of particles on the relaxation mechanisms leading to equilibration of the lipid layers, which serve as a preliminary assay towards the understanding of more complex dynamics situations than those appearing in biologically relevant systems.

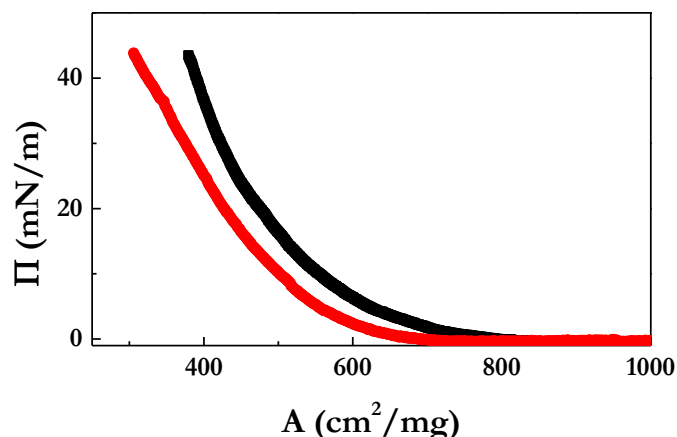
A Brewster Angle Microscope Multiskop from Optrel (Sinzing, Germany) fitted with a He-Ne laser ( $\lambda = 614$  nm) and coupled to the Langmuir trough was used to obtain information about the lateral organization of lipids and particles at the interface on the basis of Brewster Angle Microscopy (BAM) images of the interfacial textures.

### 3. Results

#### 3.1. Study of Particle Monolayers at the Bare Water/Vapor Interface

The hydrophobic nature of the particles suggests that they may be spread at the bare interface leading to the formation of a particle-laden interface. Therefore, the evaluation of the incorporation of the particles at the bare water/vapor interface, in terms of their surface pressure ( $\Pi$ )-area ( $A$ ) isotherms, can be useful as a preliminary step for understanding their incorporation into DPPC monolayers. Figure 1 shows the  $\Pi$ - $A$  isotherms for  $\text{SiO}_2$  and CB particles at the bare water/vapor interface.





**Figure 1.**  $\Pi$ - $A$  isotherms for CB (■) and SiO<sub>2</sub> (●) particles at the bare water/vapor interface.

The isotherms point out that neither CB particles nor SiO<sub>2</sub> ones affect the surface tension of the bare water/vapor significantly until the interfacial density of particle is large enough to form a highly packed film, which leads to the increase of the surface pressure of the monolayer.

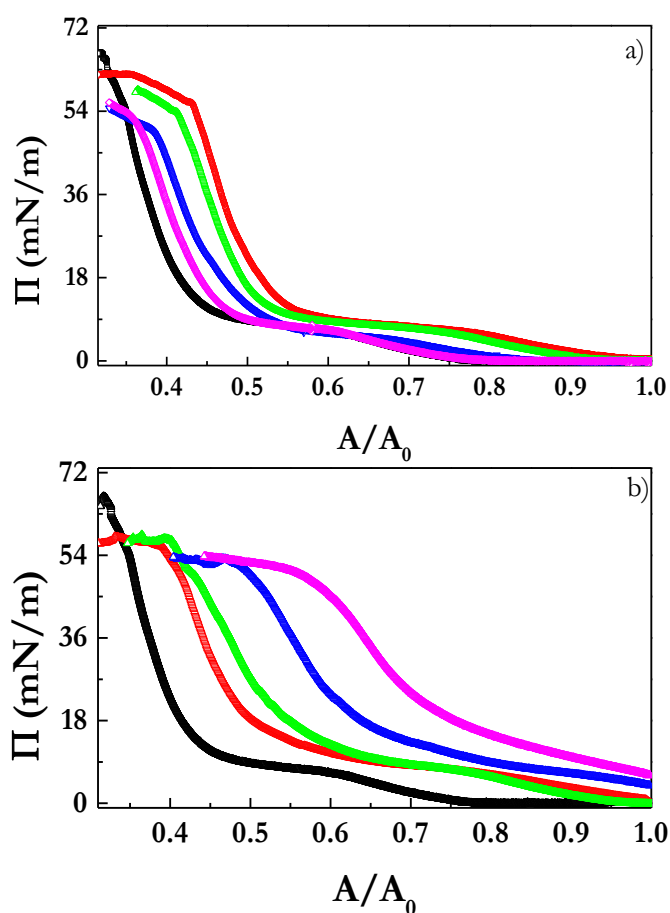
### 3.2. Evaluation of Particles Incorporation into DPPC Monolayers

The evaluation of the incorporation of particles into DPPC monolayers, and their potential impact on the lateral organization of the lipid layers, was done on the basis of the modifications associated with the presence of particles in the  $\Pi$ - $A$  isotherms of DPPC monolayers. This is important because the isotherms provide information related to the phase behavior of the mixed monolayers, and how the lateral packing of DPPC monolayers and their mechanical characteristics are modified due to the particles' incorporation, with such aspects being relevant on the biological function of the lipid layers. Figure 2 reports  $\Pi$  versus the area per DPPC molecule,  $A$ , normalized to its initial value,  $A_0$  (Note that this initial value  $A_0$  was fixed as a reference in all the experiments in 98 Å<sup>2</sup>/molecule), after the lipid spreading, corresponding to DPPC monolayers containing particles in a broad range of particles' weight fractions,  $x_p$ . Notice that the weight fractions of the particles spread onto the DPPC monolayers correspond to estimated doses in the 6–115 mg/mL range (assuming a realistic thickness for the interface of 10 nm), which are compatible with the values reported for the dose of deposited particles in the lung surfactant layer upon inhalation [49–51].

The isotherm for DPPC spread at the bare water/vapor interface presents the typical features reported for monolayers of this lipid [44,52–54]. At the highest value of the reduced area (gas and liquid expanded –LE– phases), a mild increase of the surface pressure with the increase of the interfacial density, i.e., with the  $A/A_0$  ratio decreases, was found. This proceeds up to a threshold value of the interfacial density, which defines the onset of the coexistence region between LE and liquid compressed (LC) phases (LE–LC coexistence is reached). This coexistence region is characterized by an almost vanishing change of the surface pressure (pseudo-plateau) as the interfacial density increases, which is associated with the disappearance of the LE phase as the re-orientation of the DPPC molecules occurs driving the system to a more ordered phase (LC). Once the LE–LC coexistence is overcome ( $A/A_0 \sim 0.45$ ), a sharp increase of the surface pressure with the increase of the packing density within the LE expanded and solid phases was found until the rupture of the monolayer occurs at the collapse surface pressure,  $\Pi_c$ .

The incorporation of particles into the DPPC monolayers does not modify substantially the shape of the isotherm in relation to that found for the pure lipid at the water/vapor interface, with this being almost independent from the chemical nature of the particles and the  $x_p$  value. However, the incorporation of particles into the DPPC monolayers leads to the emergence of two effects: (i) the shifting of the  $\Pi$ – $A/A_0$  isotherms to more expanded states, i.e., to higher values of the reduced area, and (ii) the modification of the collapse pressure of the monolayer, i.e., the maximum surface pressure

that a monolayer can reach before its rupture. The former aspect is evidenced from the earlier lifting-off of the LE phase in the mixed monolayers than in pure DPPC, which can be understood considering that particles take up a part of the area available for the reorganization of the lipid molecules [55]. This leads to a situation in which the monolayers upon particles incorporation behave in such a way which is reminiscent of a monolayer with a higher effective interfacial density of DPPC. Therefore, it is possible to assume that particles' incorporation induces excluded area effects in the DPPC monolayer, which are strongly dependent on both the chemical nature of the particles and the  $x_p$  value. Such dependences, and in particular that on the chemical nature of the particles, give an indication that the excluded area effects alone cannot account for the changes of the behavior of DPPC monolayers due to the incorporation of particles. This makes it necessary to analyze the role of the interactions between the different components forming the mixed layer (particle–particle, lipid–lipid and particle–lipid) to obtain a complete picture of the influence of particles in the behavior of DPPC monolayers. Such interactions affect the lipid lateral packing, and the aggregation and distribution of the particles at the interface, leading to a different behavior than that expected for systems where only the role of the area exclusion is considered.



**Figure 2.**  $\Pi$ - $A/A_0$  isotherms for DPPC Langmuir monolayers upon the incorporation of different weight fraction of particles at the interface ( $x_p$ ): CB (a) and SiO (b). Each curve corresponds to DPPC monolayers with a different weight fraction of particles at the interface ( $x_p$ ): ( $\Delta$ ) 0.00, ( $\Delta$ ) 0.10, ( $\Delta$ ) 0.33, ( $\Delta$ ) 0.75, and ( $\Delta$ ) 0.90.

The results show different dependences on the  $x_p$  value for DPPC monolayers upon the incorporation of particles with different chemical nature. The increase of the amount of SiO<sub>2</sub> particles incorporated into the DPPC monolayer shifts the isotherm to higher values of  $A/A_0$ , which is explained as result of enhanced importance of the excluded area effects with the increase of  $x_p$ .

However, the situation is different when CB particles are concerned. The incorporation of CB particles into DPPC monolayer leads to two different regimes of behavior as a function of  $x_p$ —on the impact of CB on the behavior of DPPC monolayers—were found: (i) for the smallest values of CB weight fraction, a strong shift of the isotherm to higher values of  $A/A_0$  than those corresponding to pure DPPC monolayer was found as result of the area exclusion effect, and (ii) for the highest CB amounts at the interface, even though particles' incorporation leads to excluded area effects, its importance is decreased as  $x_p$  increases.

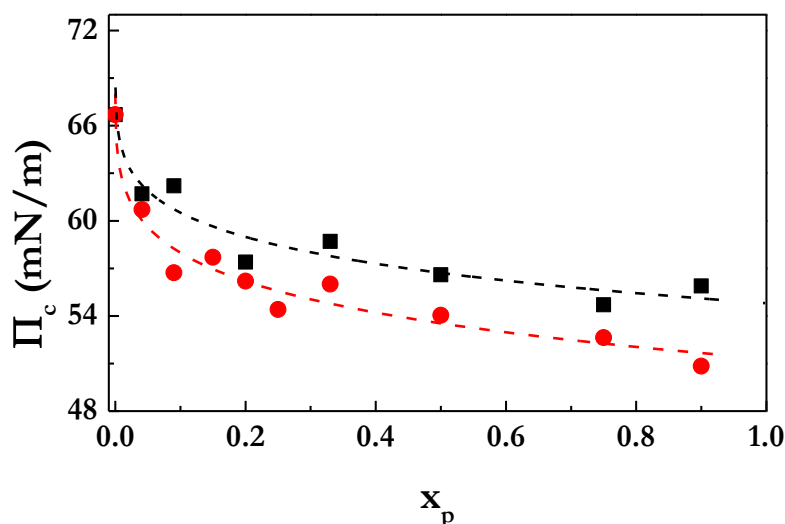
The above-mentioned differences suggest the existence of different distributions for  $\text{SiO}_2$  and CB particles upon incorporation into DPPC monolayers. Thus, whereas the  $\text{SiO}_2$  particles may be incorporated into the DPPC monolayer as pseudo-2D aggregates which tend to occupy the maximum area available at the interface, the incorporation of CB particles leads to the formation of 3D particle-stacking with the increase of  $x_p$ . Thus, the incorporation of CB particles in concentrations above a threshold value of  $x_p$  leads to their stacking onto the preformed mixed monolayer, which may result in the formation of out-of-plane structures, such as wrinkles, folds, or buckles. This leads to a situation in which the effective concentration of particles at the interface is lower than that expected from the complete spreading of the particles within the area available, and, as matter of fact, to a reduction of the importance of the excluded area effects with the increase of  $x_p$  [56–59]. The differences in the behavior of the DPPC monolayers upon the incorporation of  $\text{SiO}_2$  and CB particles are explained considering the different chemical nature of the particles. Thus, even though both types of particles are hydrophobic, the presence of dissociated silanol groups onto the surface of the  $\text{SiO}_2$  particles may introduce a repulsive electrostatic interaction between the particles, which facilitates its dispersion within the DPPC films. However, when CB particles are considered, a strong hydrophobic attraction should be expected, which favors their agglomeration with the increase of  $x_p$  [60].

The above discussion shows the strong impact of the chemical nature of the particles in their incorporation into DPPC monolayers, and its role in the excluded area effects. However, a complete picture of the impact of the particles on the DPPC monolayer behavior also needs a closer look at the the role of the interactions. For this purpose, a simple geometrical consideration may be useful. Assuming the incorporation of spherical particles which can cover a maximum area of the water/vapor interface defined as  $N\pi r^2$ , with  $r$  being the radius of a single particle and  $N$  the number of particles incorporated into the monolayer, it would be expected that the fraction of interfacial area occupied by particles oscillate between a value lower than 1% for the lowest value of  $x_p$  and a value around 10% for the highest one. However, the results show a higher impact than what was expected on the basis of the above simple geometrical considerations, with area expansions in the ranges 20–50% and 5–25% for  $\text{SiO}_2$  and CB particles, respectively. Thus, it is possible to assume that that the impact of particles in the lateral packing of DPPC monolayers results from a complex interplay between the excluded area effects, steric hindrance, and different types of interactions. The role of the interactions is clear from the analysis of the  $\Pi_c$  dependence on  $x_p$  shown in Figure 3. The results show that the incorporation of particles decreases the maximum surface pressure that the DPPC monolayers can reach before its rupture. This decrease of  $\Pi_c$  indicates an irreversible incorporation of particles into the DPPC monolayers, which results in a reduction of the ability of DPPC to form highly condensed phases. This is in contrast with what is found when the incorporation of hydrophilic particles in DPPC monolayers is concerned; in those cases, an effective refinement of the interfacial composition is generally found for the highest compression degree, with a partial squeezing-out of the particles from the interface [24,61].

The decrease of  $\Pi_c$  with the increase of  $x_p$  results from the impact of particles on the lateral cohesion interactions of the molecules within the interface. Thus, the incorporation of particles, independently of their chemical nature, reduces the strength of the cohesion interactions between the lipid molecules as a result of the emergence of heterogeneities on the lateral organization within the film. The higher decrease of  $\Pi_c$ , found when  $\text{SiO}_2$  particles are incorporated into the DPPC monolayer, in relation to those cases in which the incorporation of CB is considered, is explained considering



the differences of the steric hindrance associated with the presence of each type of particles at the interface [62]. Thus, whereas  $\text{SiO}_2$  particles tend to occupy the maximum area available at the interface, 3D stacking of particles are expected for CB particles, which results in a lower occupancy of the interface by CB particles. This leads to a situation in which  $\text{SiO}_2$  particles modify strongly the lateral organization of the lipid layer.



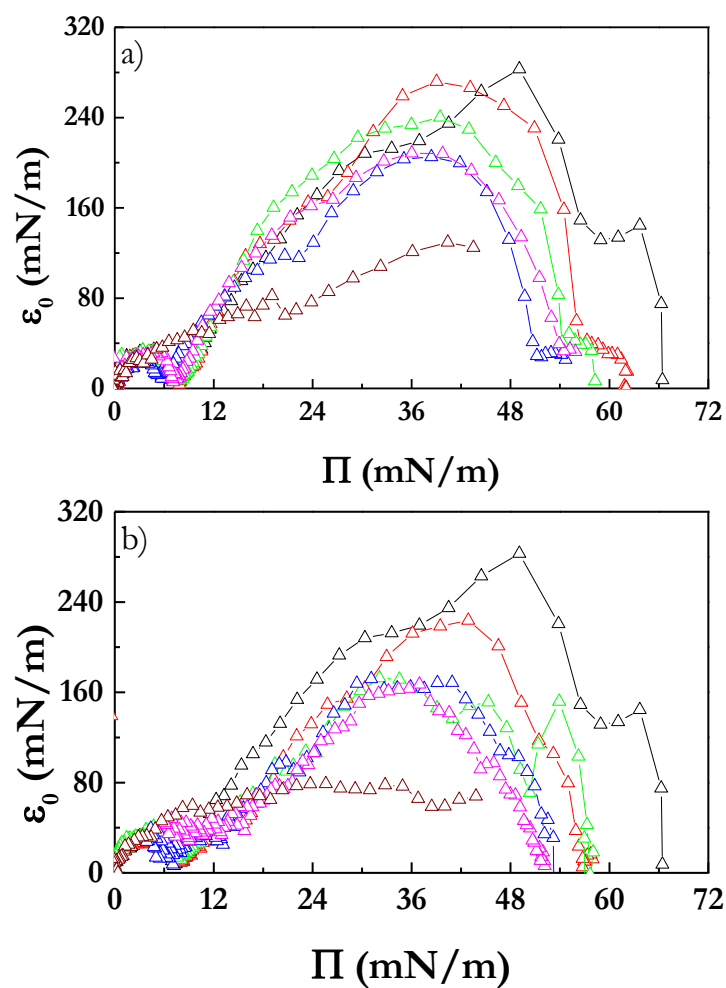
**Figure 3.** Dependence on  $x_p$  of the collapse pressure,  $\Pi_c$ , of DPPC monolayers upon the incorporation of CB (■) and  $\text{SiO}_2$  (●) particles. Note that the lines are guides for the eyes.

Additional information related to the incorporation of particles into the DPPC films are obtained from the changes of the quasi-static dilational elasticity  $\varepsilon_0$  obtained from the isotherm as

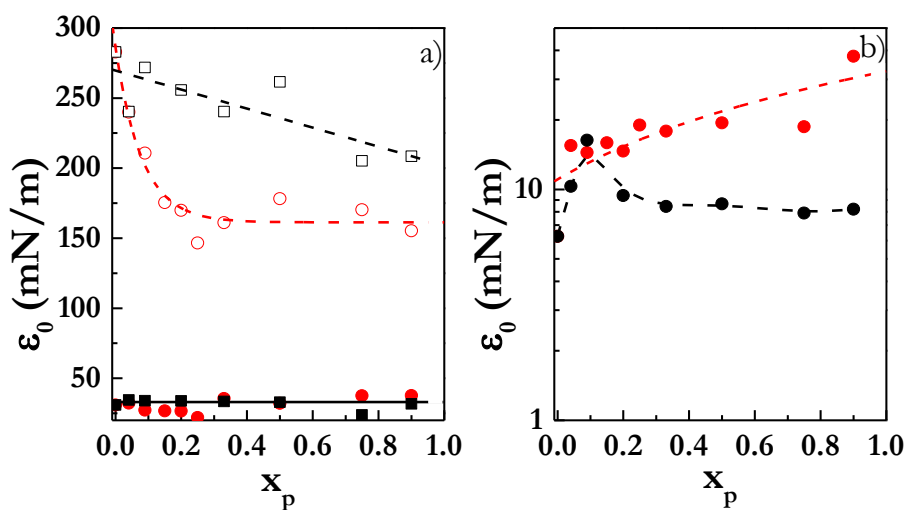
$$\varepsilon_0 = -A \left( \frac{\partial \Pi}{\partial A} \right)_T. \quad (5)$$

Figure 4 shows the surface pressure dependence of  $\varepsilon_0$  for DPPC monolayers after the incorporation of different weight fractions of particles (data for the incorporation of CB and  $\text{SiO}_2$  are shown in panels a and b, respectively). The results show three different features when the elasticity of monolayers of pure DPPC are considered: (i) an increase of the elasticity up to a first maximum associated with the formation of the disordered LE phase, which presents a weak lateral packing (for the lowest values of  $\Pi$ ), (ii) a drop of the elasticity, with the increase of  $\Pi$ , down to reach a quasi-null value for the LE–LC coexistence, and (iii) an increase of the elasticity within the LC phase up to reach its maximum value associated with an enhanced lateral packing of the monolayer, and then a drop of the elasticity as the monolayer approaches the collapse.

The incorporation of particles modifies dramatically the elasticity of the DPPC monolayers, with the average elasticity of the monolayer decreasing upon the incorporation of particles. This reduction of the monolayer rigidity is associated with a worsening of the lateral packing of the lipid molecules at the interface as a result of the weakening of the lateral cohesion interactions within the monolayer. A more detailed analysis of the impact of the particles' incorporation into the organization of the lipids within the interface is obtained from the changes of the quasi-static dilational elasticity corresponding to the maximum values of the elasticity for the LE and LC phases and to the LE–LC coexistence with  $x_p$  (see Figure 5).

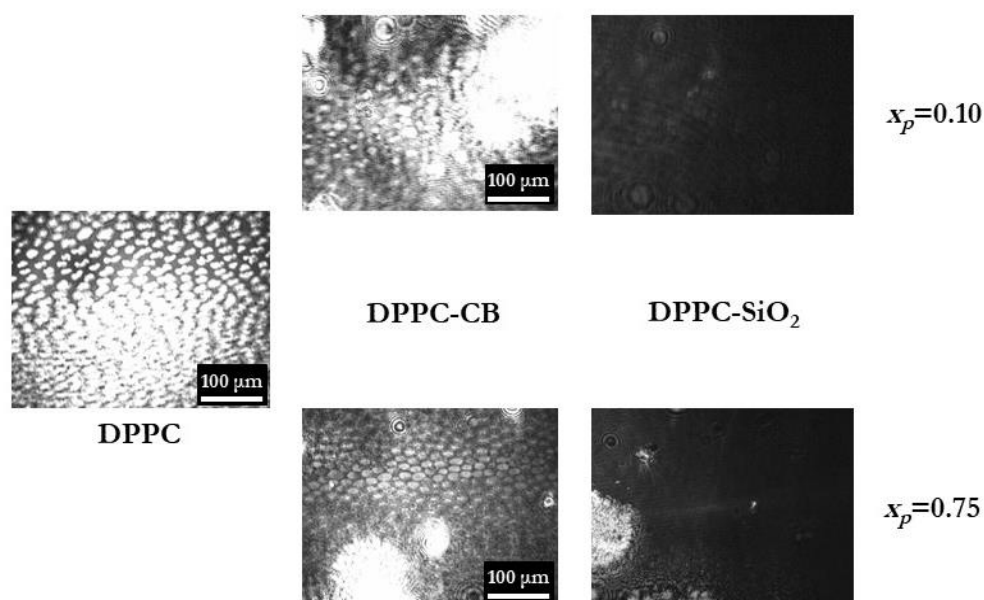


**Figure 4.** Quasi-static dilational elasticity  $\epsilon_0$  dependences on the surface pressure  $\Pi$  for DPPC Langmuir monolayer upon the incorporation of different weight fraction of particles at the interface ( $x_p$ ): CB (a) and SiO<sub>2</sub> (b). Each curve corresponds to DPPC monolayers with a different weight fraction of particles at the interface ( $x_p$ ): ( $\Delta$ ) 0.00, ( $\Delta$ ) 0.10, ( $\Delta$ ) 0.33, ( $\Delta$ ) 0.75, ( $\Delta$ ) 0.90 and ( $\Delta$ ) 1.00. Notice that the lines are guides for the eyes.



**Figure 5.** (a) Dependences on  $x_p$  of the maximum values of  $\epsilon_0$  for the LE (CB (■) and SiO<sub>2</sub> (●) particles) and LC (CB (□) and SiO<sub>2</sub> (○) particles) phases; (b) dependence on  $x_p$  of the maximum values of  $\epsilon_0$  for the phase coexistence region (CB (■) and SiO<sub>2</sub> (●) particles). Notice that the lines are guides for the eyes.

The absence of any noticeable change of the elasticity corresponding to the LE phases provide evidence that the lateral organization of the DPPC monolayer within this phase is not significantly modified, neither upon the incorporation of SiO<sub>2</sub> particles nor after the incorporation of CB ones. This may be understood considering that the LE phase is an intrinsically disordered phase in which the role of the lateral van der Waals interactions between the lipids molecules is almost negligible, thus it may be expected that a slight modification of such interactions due to the inclusion of the particles does not modify significantly the lateral packing of the lipids within the LE phase. However, a closer look at the elasticity dependences for the LE phases provides evidence of a slight increase of  $\varepsilon_0$  with  $x_p$  as result of the incorporation of SiO<sub>2</sub> particles, whereas the incorporation of CB results in an initial increase of  $\varepsilon_0$  with  $x_p$  up to a maximum for a  $x_p$  value about 0.10, which is followed by a decrease of  $\varepsilon_0$  down to a value slightly higher to that corresponding to pure DPPC. Thus, even though the impact of the particles is very limited in the LE phase, a certain degree of disorder is expected considering the experimental dependences, which is correlated to the differences in the particles' organization as function of their chemical nature. On the other side, the elasticities for the LE-LC coexistence and LC phases are strongly modified in relation to those corresponding to the DPPC monolayers. The incorporation of both types of particles reduces the maximum lateral packing of the monolayer, i.e., the quasi-static dilational elasticity for the LC phase decreases, independently on the nature of the particles. However, the impact of the incorporation of SiO<sub>2</sub> particles is again stronger than that found when the incorporation of CB ones is considered. The effect of particles in the  $\varepsilon_0$  value of the LE-LC coexistence results in being more intriguing, whereas the incorporation of CB particles into DPPC monolayers does not modify significantly the phase coexistence, and a strong increase of the elasticity of such region is found with the increase of  $x_p$  for SiO<sub>2</sub> particles. This allows one to assume that the impact of particles in the lateral packing of DPPC is driven by a complex balance involving different contributions, including the interactions involved in the mixed monolayers (hydrophobic vs. electrostatic), and the chemical nature and wettability of the particles (hydrophobicity vs. hydrophilicity of the particles). This leads to a hindering of the phase coexistence when SiO<sub>2</sub> particles are incorporated as evidenced in the BAM images shown in Figure 6.



**Figure 6.** BAM images of DPPC monolayers upon the incorporation of CB and SiO<sub>2</sub> particles at two different values of  $x_p$  for surface pressure about 7.5 mN/m, corresponding to the LE-LC phase coexistence.

The BAM images show that, whereas the DPPC monolayers upon the incorporation of CB particles presents ellipsoidal-like domains which are similar to those found for pure DPPC monolayers,

the incorporation of SiO<sub>2</sub> particles leads to the disappearance of such domains, i.e., the incorporation of SiO<sub>2</sub> drives to a hindering of the LC domains formation, which is compatible with the increase of the quasi-static dilational elasticity corresponding to such region. Furthermore, the BAM images also make clear the different distribution of the particles as a function of their chemical nature. Thus, a decrease of the size of the bright spots associated with particle agglomerates was found when CB particles are considered. However, no bright spots are found when the incorporation of SiO<sub>2</sub> is analyzed for the lowest value of  $x_p$ , which provides evidence that the distribution of the particles within the interface is better.

### 3.3. Interactions of Particles with DPPC at the Interface

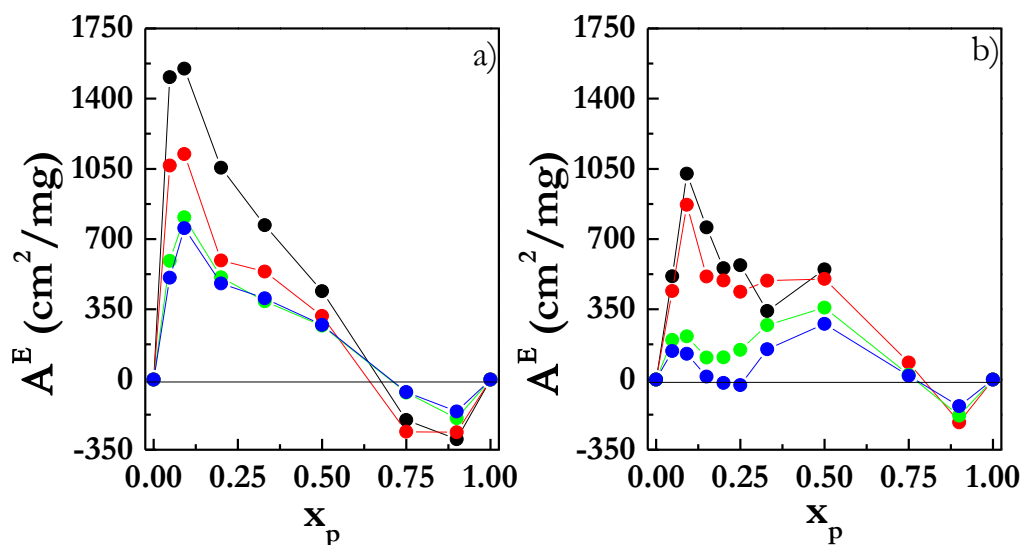
Additional insights on the impact of the particles incorporation into DPPC monolayers can be obtained using the concepts of the thermodynamics of interfacial mixtures [63,64]. This approach helps to understand how the interactions between DPPC and particles modify the behavior of the mixed monolayer in relation to what happens in those cases without DPPC–particles interactions, i.e., ideal mixture conditions. The interfacial area of an ideally mixed monolayer at a fixed value of the surface pressure is defined as follows:

$$A^{\text{id}} = x_{\text{DPPC}}A_{\text{DPPC}} + x_p A_p \quad (6)$$

where  $A_{\text{DPPC}}$  and  $A_p$  correspond to the areas per mass unit of DPPC and particles at the considered values of surface pressure for a monolayer of the pure compounds, respectively, and  $x_{\text{DPPC}}$  and  $x_p$  are referred to the weight fractions of DPPC and particles at the interface in the mixed monolayer, respectively. The differences associated with the mixing process at a fixed value of the surface pressure can be evaluated in terms of the excess area  $A^E$  defined as follows:

$$A^E = A_{12} - A^{\text{id}} \quad (7)$$

where  $A_{12}$  is referred to the area per mass unit corresponding to the mixed monolayer at a fixed value of the surface pressure. The  $A^E$  provides information related to the mutual miscibility between the compounds forming the monolayer, which is governed by the cohesion forces existing between them. Figure 7 shows the dependences of the  $A^E$  on the weight fraction of particles at the interface for different values of the surface pressure.



**Figure 7.** Dependences of the  $A^E$  on the particle weight fraction,  $x_p$ , for different values of the surface pressure  $\Pi$  for DPPC monolayers upon incorporation of CB (a) and SiO<sub>2</sub> (b): (—●—) 3 mN/m, (—●—) 7.5 mN/m, (—●—) 20 mN/m and (—●—) 40 mN/m. Notice that the lines are guides for the eyes.

The dependences of  $A^E$  on the surface pressure and the values of  $x_p$  are common to DPPC monolayers upon the incorporation of SiO<sub>2</sub> and CB particles. The results provide evidence of an enhanced interaction between the particles and the DPPC molecules at the interface with the increase of the packing of the film, i.e.,  $A^E$  decreases with the increase of the compression degree of the monolayers. This may be explained considering the existence of a forced cohesion of lipids and particles at the interface as a result of the reduction of the available interfacial area. On the other side, the increase of the weight fraction of particles at the interface leads to a decrease of  $A^E$ , which may be ascribed to the enhancing of the DPPC–particles cohesion interaction. However, it is worth mentioning that the average cohesion between the DPPC molecules is reduced with respect to what happens in monolayers of pure DPPC at the water/vapor interface. The experimental results show that the interactions between DPPC and particles are repulsive ( $A^E$  values  $> 0$ ) until  $x_p$  has reached high values when the excess area becomes negative. The repulsive interactions between particles and DPPC may be explained assuming the existence of a hindered lateral packing of the lipid due to the particles' incorporation. The enhanced miscibility appearing at the highest values of  $x_p$  is explained considering the emergence of many-bodies interactions favoring the lateral packing of the monolayer [65].

The differences of the  $A^E$  values found between monolayers after the incorporation of SiO<sub>2</sub> and CB particles may be again ascribed to the different organization of the particles within the DPPC monolayers as a function of their chemical nature. Therefore, the lowest values of  $A^E$  found upon the incorporation of SiO<sub>2</sub> are explained by the better distribution of these particles within the lipid layer, and the possible role of the electrostatic interactions between the silanol groups on the surface of the particles and the ammonium terminal group of the DPPC.

### 3.4. Effect of Particles' Incorporation in the Dilational Response of DPPC Langmuir Monolayers

The above discussion was focused on the impact of the particles' incorporation on the equilibrium properties of DPPC monolayers. However, biological systems are highly dynamic system, and hence the study of the effects associated with particles incorporation into DPPC monolayers in the response against mechanical deformations is a useful tool for a preliminary evaluation of the impact of the particles on the functionality of lipids layers. For this purpose, the influence of particles in the response of the DPPC to dilational deformations has been studied using the oscillatory barrier method at a fixed deformation amplitude within the linear response regime (1% of the initial area). These studies inform the modification of the relaxation mechanisms involved in the re-equilibration of the lipid layer as a result of the incorporation of particles [45,59]. For this purpose, the analysis of the frequency ( $\nu$ ) dependences of the viscoelastic modulus ( $|E|$ ) for the pure DPPC monolayers and upon the incorporation of the particles is performed. Figure 8 shows, for the sake of an example, some of the frequency dependences obtained for the interfacial dilational viscoelasticity at different surface pressures for the mixed monolayers containing different weight fractions of particles.

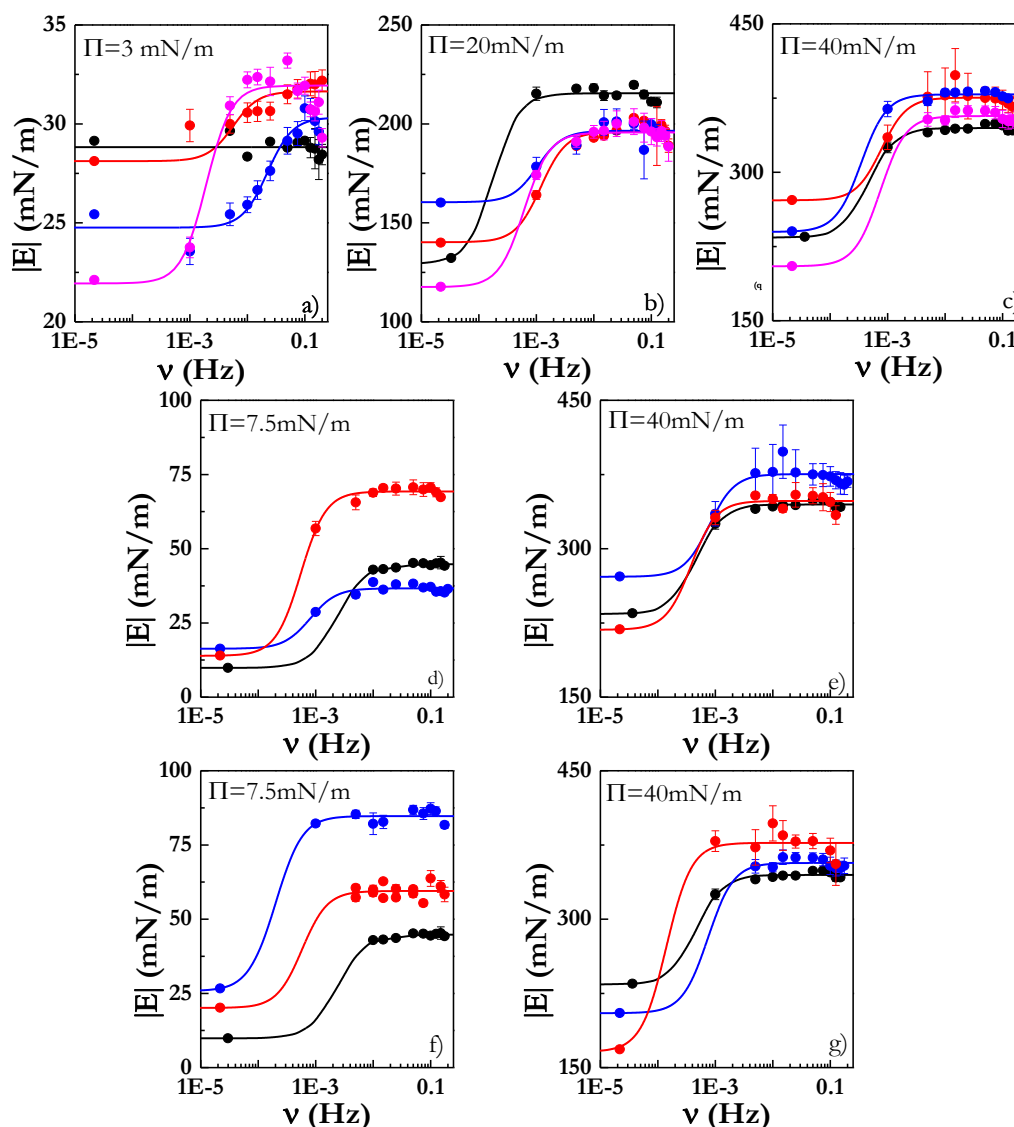
Most of the viscoelastic modulus–deformation frequency curves obtained show the existence of inflexion points which are associated with the characteristic frequency of the reorganization of the molecules and particles within the interface. The incorporation of particles, independently of their chemical nature, modifies the relaxation mechanism of the lipid monolayer, i.e., the characteristic relaxation frequencies, as is evidenced from the experimental curves. The characteristic relaxation frequencies can be estimated fitting the experimental data to the following theoretical expression which enables the description of interfacial relaxation occurring in insoluble adsorption layers [66]

$$|E| = [(E_1^2 + \lambda^2 E_0^2)/(1 + \lambda^2)]^{1/2}, \quad (8)$$

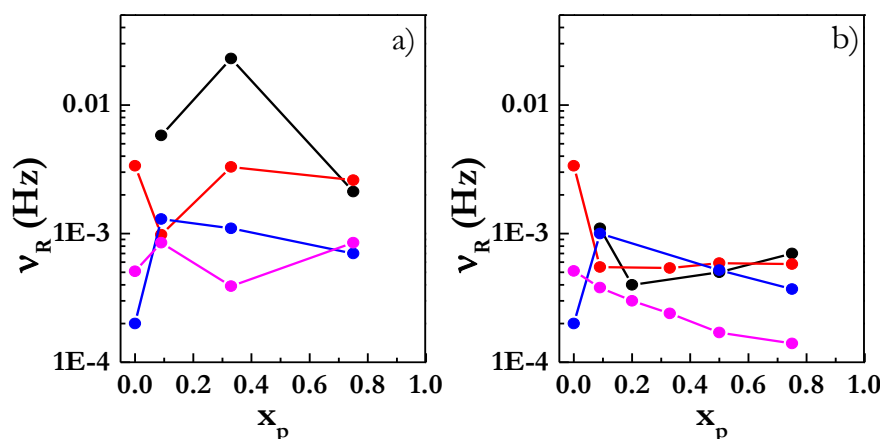
where  $\lambda = \nu_R/\nu$ , with  $\nu_R$  being the characteristic relaxation frequency, and  $E_0$  and  $E_1$  are the lower and upper limits of the elasticity within the considered frequency range. Note that, when insoluble monolayers are concerned,  $E_0$  coincides with the quasi-static dilational elasticity obtained from the isotherm. The theoretical curves obtained using the model defined by Equation (8) are shown in



Figure 8 together with the experimental data, and the values of the characteristic frequencies  $\nu_R$  of the dilational response of mixed monolayers are shown in Figure 9.



**Figure 8.** Experimental (symbols) and calculated using Equation (8) (lines) dependences of the interfacial dilational viscoelasticity modulus on frequency. (a–c) dependences of  $|E|$  on  $\nu$  for DPPC after the incorporation of different  $x_p$  values at fixed surface pressure values (data for CB): (●, —) 0, (●, —) 0.10, (●, —) 0.33 and (●, —) 0.75. (d,e) dependences of  $|E|$  on  $\nu$  for DPPC after the incorporation of particles with different chemical nature at fixed surface pressure values and  $x_p = 0.10$ : (●, —) pure DPPC, (●, —) DPPC upon incorporation of  $\text{SiO}_2$  particles and (●, —) DPPC upon incorporation of CB particles. (g,f) Dependences of  $|E|$  on  $\nu$  for DPPC after the incorporation of particles with different chemical nature at fixed surface pressure values and  $x_p = 0.75$ : (●, —) pure DPPC, (●, —) DPPC upon incorporation of  $\text{SiO}_2$  particles and (●, —) DPPC upon incorporation of CB particles. (Note that the value of the  $|E|$  for the lowest frequency value was assumed as the quasi-static dilational elasticity obtained from the isotherm, with the value of the frequency being the compression ratio which for the here experiments is about  $10^{-5}$  Hz).



**Figure 9.**  $\nu_R$  dependences, obtained using Equation (4), on the particle weight fraction,  $x_p$ , for different values of the surface pressure  $\Pi$  for DPPC monolayers upon incorporation of CB (a) and  $\text{SiO}_2$  (b): (—●—) 3 mN/m, (—●—) 7.5 mN/m, (—●—) 20 mN/m and (—●—) 40 mN/m. Notice that the lines are guides for the eyes.

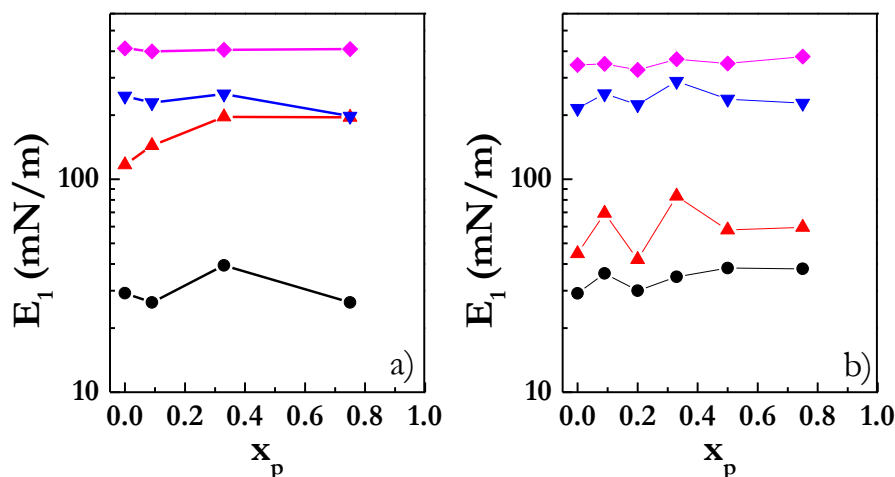
The analysis of the viscoelastic modulus-deformation frequency curves obtained for monolayers of pure DPPC shows the existence of an inflexion point, with the exception of that obtained for the lowest value of  $\Pi$ . The absence of such relaxation process for the lowest value of the surface pressure is explained assuming the low interfacial density, which allows the free reorganization of the DPPC molecules within the interface. This takes the relaxation process to time-scales below (higher frequencies) those tested by oscillatory barrier experiments.

The results show that the incorporation of particles modifies the relaxation mechanism of DPPC molecules at the water/vapor interface from the lowest values of surface pressure.  $\text{SiO}_2$  and CB particles' incorporation leads to the emergence of a relaxation process at  $\Pi$  values about 3 mN/m (LE phase for pure DPPC monolayers), with the time-scale for such relaxation process being faster when the incorporation of CB particles is concerned (0.001–0.01 Hz for CB particles incorporation vs. 0.0001–0.001 Hz for  $\text{SiO}_2$  ones). This results from the most important role of the steric hindrance associated with the incorporation of  $\text{SiO}_2$  particles, which makes it the lateral reorganization of the lipid molecules within the monolayer more difficult than when the incorporation of CB particles is considered. The origin of the emergence of a relaxation process may be explained considering the increase of the interfacial density associated with the presence of the particles, which reduces the time-scales involved in the reorganization of the molecules at the interface. The decrease of the time-scale involved in the reorganization process is stronger as the interfacial density of the particles increases. Therefore, it is possible to assume a slowing down of the velocity of this relaxation as a result of an increased role of the steric hindrance.

The approaching of the LE–LC coexistence phase provides evidence again of the differences on the effect of the incorporation of  $\text{SiO}_2$  and CB particles. The incorporation of CB particles does not modify significantly the relaxation mechanism, with a relaxation process presenting  $\nu_R \sim 10^{-3}$ – $10^{-4}$  Hz appearing independently of the considered monolayer, i.e., for pure DPPC monolayers and upon the incorporation of CB particles. This is explained considering that, within the phase coexistence region, the nucleation of the LC phase associated with the disappearance of the LE one is found in both cases. Thus, the relaxation process should be ascribed to the exchange of the lipid molecules between the LC and LE phase. However, the introduction of  $\text{SiO}_2$  particles leads to a slowdown of the relaxation process for almost one order of magnitude [31]. This may be explained considering that the incorporation of  $\text{SiO}_2$  particles hinders partially the phase coexistence as result of the stronger steric hindrance associated with the particles incorporation which modifies the lateral reorganization of the lipid molecules at the interface.

Once the phase coexistence is overcome, the incorporation of CB does not lead to any significant change of the relaxation mechanism, with the relaxation frequency remaining in values about  $10^{-4}$  Hz, independently of the considered state and the  $x_p$  value. This may be understood assuming that CB particles are simple obstacles that limit the average cohesion of the DPPC monolayers, shifting the phase behavior, without any significant change on the lipid lateral packing. However, the situation appears to be different when the incorporation of SiO<sub>2</sub> particles is concerned, and the relaxation mechanism was found to be dependent on the  $x_p$  value. The incorporation of SiO<sub>2</sub> particles increases the value of  $\nu_R$  with the  $x_p$  value up to reach a maximum value, and then a decrease of  $\nu_R$  with the increase of the  $x_p$  was observed with the enhancing of the lateral packing of the monolayer. This is explained assuming the complexity of the interactions balance involved in DPPC monolayers upon the incorporation of SiO<sub>2</sub> particles, which leads to the existence of coupled dynamics on the rheological response of the mixed monolayers against dilational deformations. It is worth mentioning that the increase of the interfacial density of the particles may induce a similar lateral packing of the monolayer for lower values of the surface pressure, and this may explain the characteristic features found for the  $x_p$  dependence of the relaxation frequencies. Notice that, for the highest values of the lateral packing, the characteristic relaxation frequency appears to be similar for pure DPPC monolayers and upon incorporation of SiO<sub>2</sub> that corresponds to the mixed layer appearing in larger time-scales. This subtle difference can be again ascribed to the role of the steric hindrance interactions, which makes it possible that the relaxation process may include complex rearrangements involving both the particles and the lipid molecules.

Figure 10 shows the dependences of  $E_1$  on the  $x_p$  value. The  $E_1$  values obtained prove clearly an increase with the  $x_p$  value when the incorporation of CB particles is considered; this may be understood considering that particles occupy partially the area available for the lipid organization, i.e., behave as obstacles, driving to a prior packing of the DPPC at the interface (packing occurs at higher values of the reduced area). About the dependences  $E_1$ , the impact of SiO<sub>2</sub> approximates the CB particle one. This aspect can be explained assuming the importance of the occupancy of the interfacial area by the incorporated particles in their impact, independently of their chemical nature.



**Figure 10.**  $E_1$  dependences, obtained using Equation (4), on the particle weight fraction,  $x_p$ , for different values of the surface pressure  $\Pi$  for DPPC monolayers upon incorporation of CB (a) and SiO<sub>2</sub> (b): (—●—) 3 mN/m, (—▲—) 7.5 mN/m, (—▼—) 20 mN/m and (—◆—) 40 mN/m. Notice that the lines are guides for the eyes.

#### 4. Conclusions

This study has shown the influence of the chemical nature of particles interacting with lipid layers. The results provided evidence that CB and SiO<sub>2</sub> particles alter the lateral packing of the DPPC monolayers, modifying the cohesion between the lipid molecules within the interface and worsening

the mechanical performance of the lipid film. However, the mechanisms driving such modifications are strongly dependent on the specific chemistry of the considered particles, and, in particular, on the interactions involved in their incorporation. This recalls a framework needing the consideration of the complex balance between the contribution of the hydrophobic and electrostatic interactions and the steric hindrance in the behavior of the lipid layer. Therefore, even though particles' most fundamental physical properties can appear to be similar, their specific chemistry, and possibly their geometry and aggregation at the interface, determine their impact on their incorporation into lipid layers, which may even result in the modification of the lipid ability to form ordered phases. This distortion of the lateral order of the lipid monolayers is also associated with the modification of the relaxation mechanism driving the re-equilibration of the interfacial layers after periodic dilational deformations, i.e., the reorganization of molecules within the interface. Despite the simplicity of the considered model, the results have shown that the study of the changes of the equilibrium properties and the rheological response of lipid layers due to their interaction can be used as a powerful for evaluating the impact of pollutants in the functioning of biological layers.

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