Interfacial Properties of Mixed DPPC–Hydrophobic Fumed Silica Nanoparticle Layers

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Published in J. Phys. Chem. C 2015, 119, 21024−21034

DOI: 10.1021/acs.jpcc.5b07258

Interaction of Hydrophobic Fumed Silica Nanoparticles with DPPC Langmuir Monolayers

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Abstract

The combination of interfacial thermodynamic, dilational rheology and Brewster Angle Microscopy (BAM) has allowed the evaluation of the effect of hydrophobic fumed silica nanoparticles on the interfacial properties of Langmuir monolayers of 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC). Fumed silica particles modify the surface pressure-area (Π-A) isotherm of DPPC, modifying both the phase behaviour and the collapse conditions. These modifications are strongly dependent on the relative quantity of DPPC and Nanoparticles initially spread at the air-water interface. The incorporation of nanoparticles at the fluid interface alters the balance of interactions within the monolayer. Thus, leading to the disruption of the interfacial structure and consequently of the lipid packing. Measurements of dilational visco-elastic modulus against the frequency have been carried out by the mean of the Oscillatory Barrier method at different degrees of compression of the monolayer. The dynamic response is also modified by the presence of increasingly amount of nanoparticles at the interface. Being DPPC a lipid generally used as classical model for the study of biological relevant systems, the results here obtained can be used to deepen on the understanding of the effects of nanoparticles on the interfacial properties of bio-systems.

1. Introduction

In the last years, the potential applications of nanoparticles (NPs) have driven a spectacular development of the research activities based on the use of particulate materials. This has lead to the design and fabrication of new materials with application in multiple industrial and technological fields.[1,2] In spite of the apparent benefits for the industrialized society associated with the use of NPs, some critical issues related to environmental and human health aspects associated with the injection of NPs to the atmosphere have been raised recently, opening a strong controversy related to the potential toxicity and hazards of the use of NPs.[3,4] In this context, the study of the interaction of NPs with biological relevant systems, e.g. biological fluids (lung surfactant) or membranes, plays a key role to propose strategies for the technological exploitation of the NPs properties in a responsible, efficient, safe and healthy way.[5]

The understanding of the potential adverse effects associated with the interaction of NPs and biological matter requires deepening on the physico-chemical bases that govern this interaction due to their recognized importance in the toxicological effects of NPs in cells and tissues, as well as in their applications as therapeutic vectors in nanomedicine.[6] Biological relevant systems are mainly formed by bilayers of fatty amphiphiles, being in many cases difficult to study. This leads to the necessity of design model systems to make easier the analysis of the physico-chemical feature of these important systems.[7]An elegant way to model this type of systems is by the use of Langmuir monolayers of these fatty molecules at the air-water interface.[8,9,10] These apparently simple models can be considered a first approach to the real systems due to their ability for mimicking some of the most relevant physico-chemical features of the real systems, being rather useful to analysis the interaction between NPs and bio-systems.

The topic of the interaction between nanoparticles of different nature and biological matters recurring to surface science approaches is a relevant topic on which some studies, both experimental and theoretical, can be found in the literature.[11,12,13,14,15,16] NPs modify both the thermodynamic[17] and mechanical behavior[18] as well as the structure[19,20] of interfacial layers of surfactant systems. Furthermore, the physico-chemical characteristics of the particles play a key role in the modification of the aforementioned aspects.

The most extended model of biological relevant systems is a saturated phospholipid called 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC). The interest on this system lies in its implication in many structures and processes of biological relevance,[8,10] being DPPC probably the most studied lipid in the literature.[21,22,23,24,25,26] This lipid takes special relevance as model lipid since its ability to form monolayers with very low surface tensions associated with the formation condensed phases which play a key role on the physiological function of many bio-systems.[27,28]

The goal of this work is to explore the effect of hydrophobic fumed silica NPs (SiO_2) on the structure and rheological properties of Langmuir monolayers of DPPC. For this purpose, we combine Langmuir trough experiments, equilibrium isotherms and oscillatory barriers experiments, with the information about interfacial texture obtained by Brewster Angle Microscopy (BAM). This makes possible to correlate the changes on the equilibrium and dynamics features, induced by SiO_2 NPs on DPPC monolayers, with a morphological scenario. The here presented study analyzes the effect of hydrophobic NPs on the behaviour of lipid monolayer, being useful to deepen on the understanding of the potential toxicological effects of NPs.

2. Materials and Methods

2.1.Materials

1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) was purchased from Sigma (Germany) at 99 % purity and used without further purification. The molecular weight of this lipid is 734.1 g/mol. Solutions of lipids for the spreading were prepared using chloroform for HPLC from Sigma (Germany).

Hydrophobic Fumed Silica Nanoparticles (SiO_2), Aerosil R972 from Evonik-Degussa (Germany), were chosen as model of hydrophobic NPs. This particles form ramified aggregates of primary particles with an average diameter of 16 nm. The SiO_2 density at 25°C is 50 g/cm^3 and the BET specific area is 110 m^2/g. Being SiO_2 nanoparticles hydrophobic, they can be spread on the water surface from dispersions in chloroform. The surface tension of the layer so obtained is not appreciably different from that of pure water unless the particle layer is highly packed.

Water for all the reported measurements was deionized and purified by a multi-cartridge system (Elix plus Milli-Q, Millipore) providing a resistivity greater than 18 MΩ·cm, and a surface tension of 72.5 mN/m without any appreciable kinetics over several hours.

2.2.Methods

All reported experiments have been performed using a Langmuir through (KSV minithrough, Finland) equipped with two barriers of Delrin® allowing for symmetric compression / expansion of the free liquid surface. The total surface area of the Teflon trough is 243 cm^2. The surface tension, γ, was measured through a paper Willhelmy plate (Whatman CHR1 chromatography paper, effective perimeter 20.6 mm, supplied by KSV),

ensuring a zero-angle contact angle. Surface pressure is then obtained as $\Pi = \gamma_w - \gamma$, where γ_w is the surface tension of pure water.

The lipid monolayers were spread on the aqueous subphase contained in the Langmuir trough, by dropping controlled volumes of their chloroform solutions with a precision syringe (Hamilton). From this volume and the solution concentration (typically 1g/L) it is then possible to control the number of molecules present on the surface after evaporation of the solvent. For the preparation of the mixed monolayers after the spreading of the lipid monolayers, the spreading of SiO_2 was made, adding the necessary amount of SiO_2 from a chloroform dispersion (concentration 1 g/L) till obtain the desired DPPC:SiO_2 ratio at the interface. The experiments were started after hour from the solution deposition. This time was checked to be long enough to ensure the complete evaporation of the solvent and, in case of nanoparticle dispersions, the achievement of the equilibrium of the composite system, driven by the nanoparticle-lipid interaction.

For a typical Π - A isotherm experiment, the surface pressure was measured during the compression of the free area of the monolayer, at a rate of 2 cm^2/min, which is equivalent to a compression rate R ≈ $3x10^{-5}$ s^{-1}. This compression velocity has been chosen to avoid undesired effect of non-equilibrium[29] in the obtained isotherms.

The Langmuir trough also allows us to carry out the rheological characterization of the interfacial layer at different reference surface states characterized by given values of equilibrium surface pressure. For this purpose, the oscillatory barrier method was used.[30] According to this method, it is possible to evaluate the modulus of the complex dilational viscoelasticity, which is defined as the variation of the surface tension γ due to the dilational deformation $u=\Delta A/A$, i.e. $E = \partial\gamma/\partial u$, against the frequency. The measurement is based on the acquisition of the surface pressure response to small amplitude sinusoidal

variation of the surface area. The measurements were here performed in a frequency range from 10^{-3} to 0.15 Hz with a fixed amplitude, u = 0.01, which, as checked by appropriate measurements of the surface pressure response, ensures for the systems here investigated the linearity of the layer response.

The Langmuir trough is coupled to a Brewster Angle Microscope (BAM) Multiskop, (Optrel, Germany) allowing for the in-situ acquisition of information on the layer texture with lateral resolution.[31]

For all the experiments the temperature was at a controlled value of 22.0 ± 0.1 °C.

3. Results and Discussion

The interaction between DPPC monolayer and SiO_2 has been investigated by adding different amounts of NPs from dispersions in chloroform onto the DPPC monolayers already spread on pure water. In this case the interaction between NPs and DPPC molecules is expected to be mainly controlled by the hydrophobic interaction between SiO_2 and the hydrophobic tails of DPPC molecules. Note that the systems containing SiO_2 cannot be considered strictly as mixed monolayers since the spreading of DPPC and SiO_2 is not made together from a premixed dispersion (first, the DPPC is spread at the pure air/water interface and then the spreading of the SiO_2 is made on the preformed DPPC monolayer). However, for simplicity, these monolayers are called mixed monolayers in the following. The conditions used to obtain the mixed layers ensures that the interaction between NPs and lipid molecules occurs only at the interface which provides a better simulation of the real effects of NPs on the properties of bio-systems than other works presented in literature where the mixed layers were obtained for the spreading of a premixed dispersion of nanoparticles and lipid.[¡Error! Marcador no definido.] Note that for all the

experiments the results will be discussed in terms of the weight fraction of nanoparticles in relation to the total mass of material added at the interface (particles + lipid molecules).

3.1. Direct Evidences of NPs incorporation to DPPC monolayers: An Equilibrium Study

The interaction of the NPs with DPPC monolayers can be evaluated by the equilibrium Surface Pressure – Area isotherms of the spread monolayers. Figure 1 reports the $\Pi - A/A_0$ isotherms for the DPPC + SiO_2 NPs mixed monolayer with different weight ratio between the lipid and the NPs. Note that A_0 is the area per molecule of a DPPC molecule after the spreading of the DPPC monolayer at the pure air-water interface, being the same for all the studied systems ($\Gamma = 0.12$ μg·cm^{-2}). The isotherms provide important information related to the phase behaviour and the function of the lipid layers. The isotherm of pure DPPC is also reported together to those of the mixed layers of DPPC + SiO_2, being in good agreement with those previously reported in literature.[32,33] Any further discussions referred to pure DPPC isotherms will be included here.

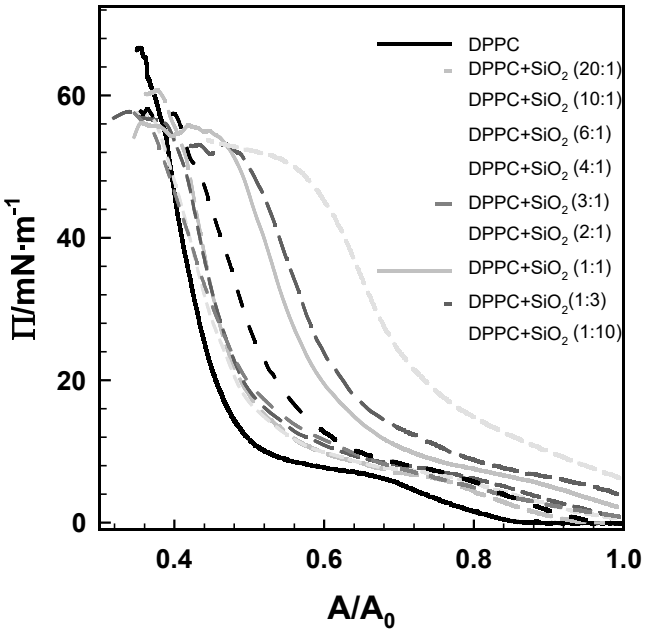

Figure 1. *Π-A/A0* isotherms for mixed DPPC-hydrophobic silica nanoparticles Langmuir monolayer. Each curve represents a different DPPC:SiO_2 weight ratio.

The inclusion of silica nanoparticles in the DPPC monolayers does not alter substantially the qualitative feature of the isotherms, being this independently on the SiO_2 weight fraction, x_{NP}. However, both the evolution of the surface pressure with the compression degree (A/A_0) of the monolayer and the collapse pressure depend strongly on the content of NPs at the interface. The incorporation of NPs shifts the isotherms to high compression degrees. Thus, a prior lifting-off of the LE phase of the isotherm than in the pure DPPC monolayers was observed. This can be rationalized considering that the NPs take up part of the area available for the molecules at the interface, leading to the appearance of excluded area effects with an important concentration dependent character.[34] In this context, the introduction of NPs reduces the area available for the distribution of the DPPC molecules at the interface, driving the prior packing of the monolayer, i.e. the monolayers behaves

similar than a system with a higher DPPC interfacial concentration. It is important to note that the excluded area effects are not the only driving force of the NPs induced modifications of the DPPC monolayers, being necessary to consider the role of the NPs-DPPC interactions, mainly hydrophobic one between the SiO_2 NPs and the hydrophobic tails of the DPPC molecules. These interactions can provoke changes in the tails ordering, contributing even more to the reduction of the area available per lipid molecule than the simple exclude area effects.[35] Furthermore, it cannot be ruled out the existence of secondary electrostatic interactions between non silanized silanol groups on the nanoparticles surface and the charged head-group of DPPC.

The influence of the interactions and ramification of the particles aggregates on the packing of the lipid molecules can be established by a simple geometrical consideration. Considering spherical NPs that can coat a maximum area at the air – water interface defined by $N\pi r^2$, being r the radio of a NP and N the number of spread NPs, it is possible to estimate the maximum possible area fraction occupied for the spread NPs at the interface. This simple calculation leads to values lower than 1 % of the total area for the lowest NPs weight ratios, reaching and a value around 10 % for the highest one. These values are well below of the changes in A/A_0 provoked for the introduction of NPs in the monolayer. Thus, allowing us to hypothesize that the interfacial packing of the mixed monolayers is governed by a complex interplay between excluded area effects, steric hindrance and different types of interactions.

Further insights on the interaction NPs-DPPC can be inferred by the analysis of the changes on the area occupied by a DPPC molecule under maximum packing condition, A_c (estimated by extrapolation of the steep, high pressure, linear part of the $\Pi - A/A_0$ curve to zero surface pressure) (Figure 2a). A_c increases with the initial density of spread NPs that allows hypothesising the existence of a non-effective squeezing-out of the NPs during the

compression. The absence of efficient refinement of the interface with the consequently expulsion of the NPs contrasts with the scenario found for DPPC monolayers in presence of adsorbed hydrophilic SiO_2 nanoparticles.[32] This differences can be ascribed to the role of the hydrophobic interaction between NPs and DPPC that improve the NPs-lipid cohesion at the monolayer, hindering the squeezing-out phenomena.

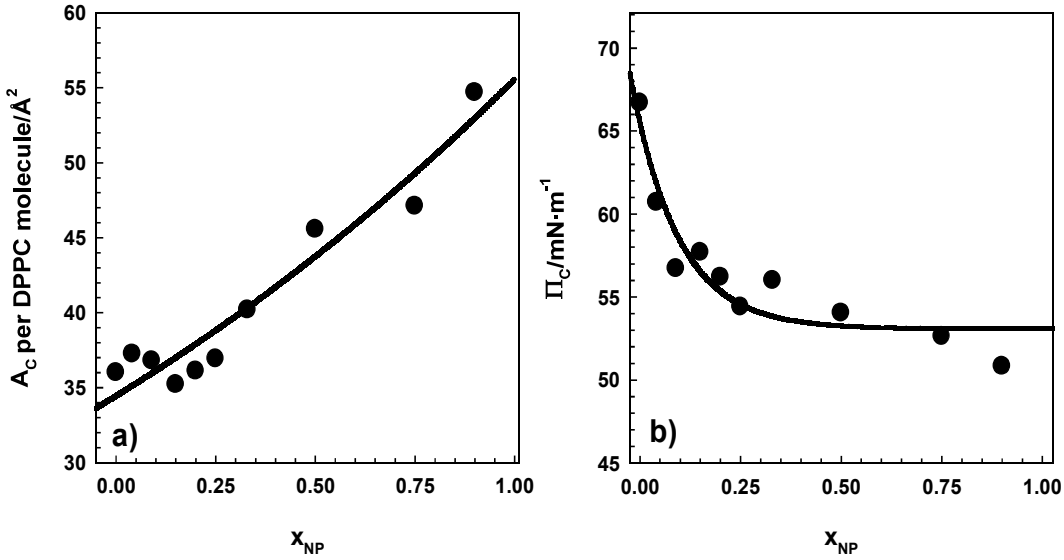

Figure 2. (a) Dependence of A_c per DPPC molecules on x_{NP}. (b) $\Pi_c$dependence on x_{NP}. The lines represent guides for the eyes.

Moreover, the dependence of the collapse pressure on the NPs weight fraction allows a better understanding on the interaction between NPs and the DPPC at the interface (Figure 2b). The collapse pressure, Π_c, decreases with the increase of the NPs weight fraction till reach a plateau value for x_{NP} around 0.25. This dependence of the Π_c on x_{NP} is ascribable to the hydrophobic interactions between DPPC tails and NPs as well as to the steric hindrance induced by the NPs. Both aspects lead to the reduction of DPPC packing at the interface and consequently to the decrease of the collapse pressure in relation to that of the pure

DPPC monolayers. This behaviour is associated with the hindering of the formation of highly condensed phases due to the formation of inhomogeneous layers with the NPs incorporated between the DPPC molecules within the monolayer. Furthermore, a irreversible incorporation of the NPs into the monolayer can be assumed from the dependences of Π_C and A_C on x_{NP}.[36]

A deeper understanding on the effect of the NPs can be obtained by the evaluation of the equilibrium dilational elasticity, ε_0. This physico-chemical parameter is a estimation of the elastic energy stored by the monolayer during a continuous compression of the interfacial area, providing information about the rigidity of the monolayers,[37,38] being defined as,

$$\varepsilon = -A\left(\frac{\partial \Pi}{\partial A}\right)_T \quad (1)$$

The equilibrium dilational elasticity for the different mixed monolayers is reported in Figure 3.

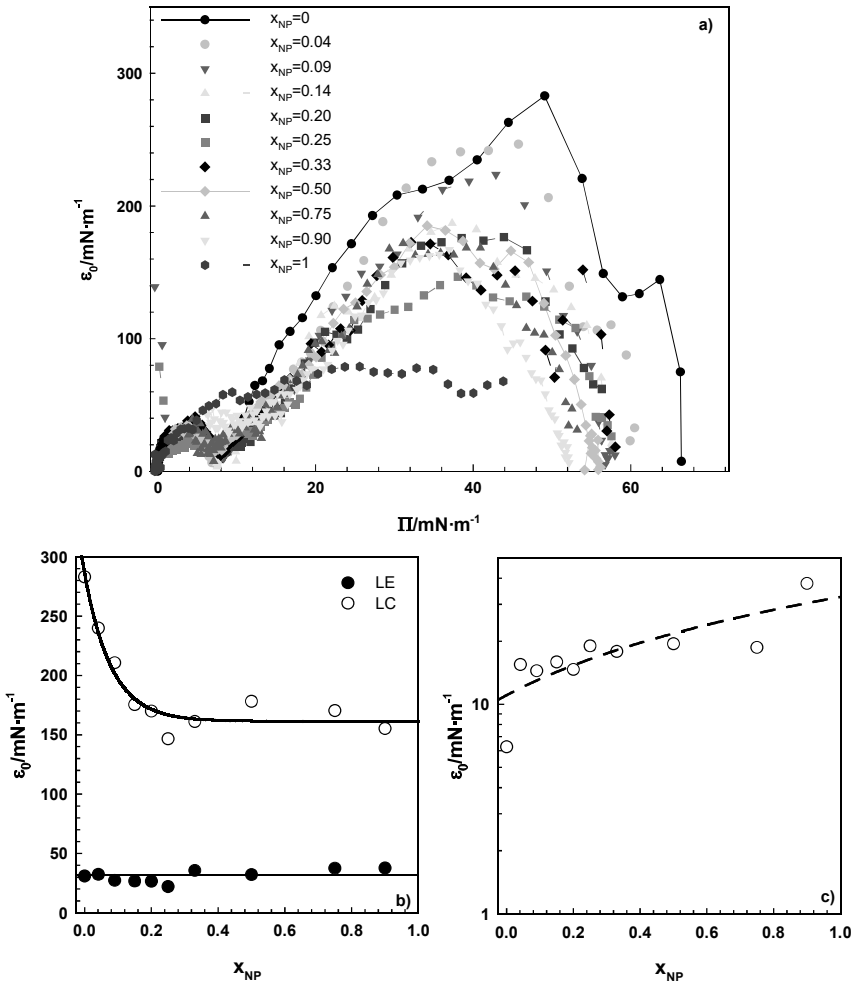

Figure 3. (a) Equilibrium dilational elasticity, ε0, calculated from *Π-A* isotherms for the mixed monolayers with different nanoparticles weight fraction, $x_{NP,}$ vs. *Π*. (b) Dependence on the particle weight fraction, $x_{NP,}$ of the limit values of ε0 for the liquid expanded phase, LE, and the liquid compressed phase, LC. (c) Dependence on the particle weight fraction, $x_{NP,}$ of ε0 for the phase coexistence region.

The equilibrium dilational elasticity of pure DPPC presents as main features two maximum than correspond to the ε_0 on the liquid expanded (LE) and the liquid compressed phase (LC) surface pressure. The maximum at the lowest surface pressure is ascribable to the LE phase with low values of elasticity due to its intrinsic disorder. The second maximum is observed at high surface pressures and presents values of elasticity higher than those of the

LE phase as consequence of the formation of highly packed LC phase. The coexistence region of DPPC monolayers presents a quasi-null value of elasticity as it is expected for the quasi-plateau found for the surface pressure (see Figure 1a) and the nucleation and growth of LC domains in a LE phase matrix as was found by BAM (see Figure 4).

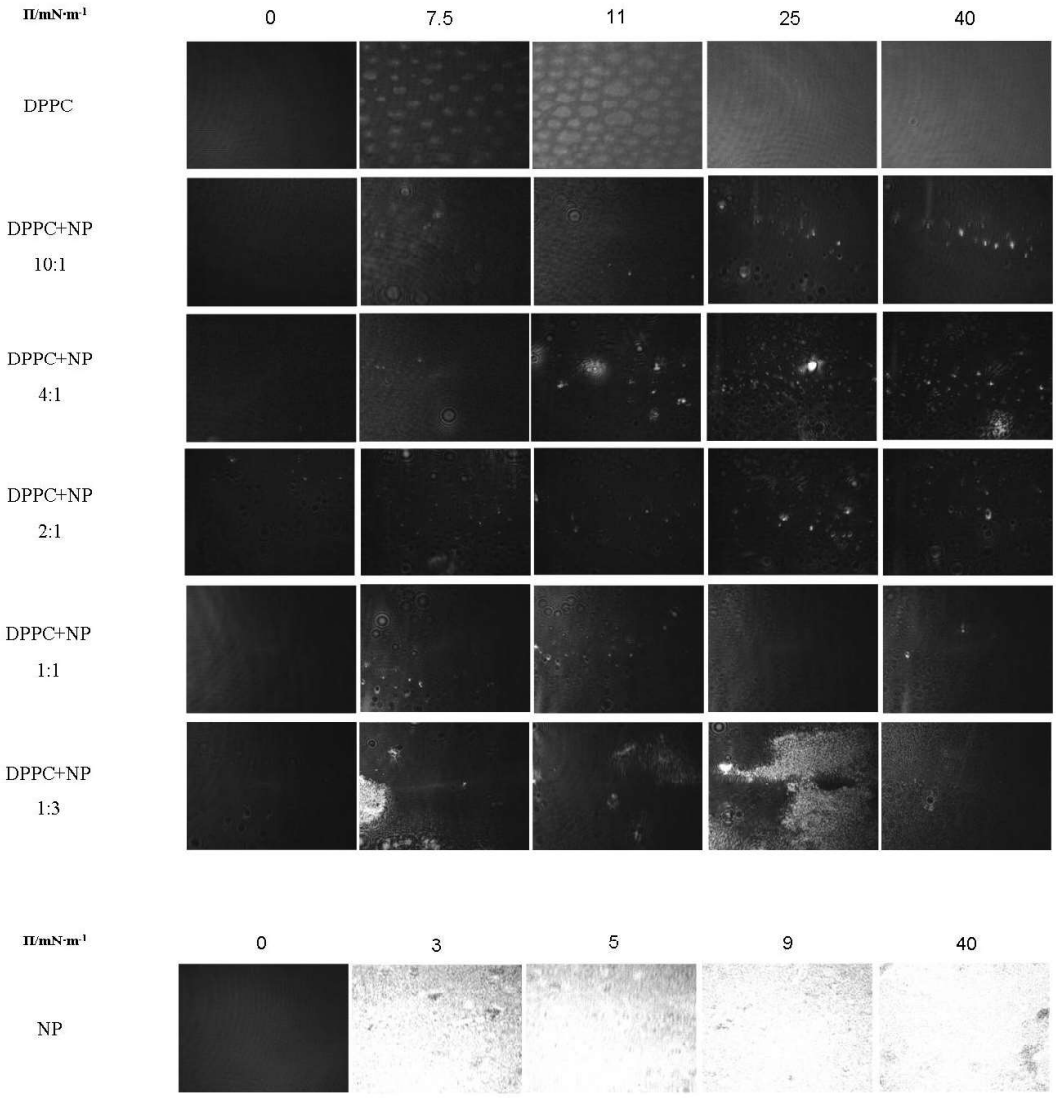

Figure 4. BAM images sequences (images size = 155 μm x 210 μm) of different *Π* states for mixed monolayers of DPPC and SiO_2 with different ratio DPPC:NP.

The incorporation of SiO_2 to the lipid monolayer does not modify the qualitative feature of the elasticities of the LE and LC phases of the pure DPPC. However, an important modification in the coexistence region ($\Pi \sim 7.5$ mN/m) is observed in presence of NPs (Figure 3c). The NPs increase the value of the elasticity of this coexistence region, changing from a value around 0 to reach values close to 40 mN/m for the highest values of x_{NP}. This is explained considering that the NPs provoked a faster packing of the monolayer and consequently the phase coexistence disappears, hindering the formation of domains as is evidenced from the BAM images (Figure 4). Furthermore, this phenomenon is strongly dependent on the NPs weight. Thus, it is possible to consider that the steric hindrance induced by the NPs is the main driving force governing the packing of the monolayers. The absence of domain growth does not allow claiming the existence of a true phase coexistence that contrasts with our previous results of mixed layers of DPPC and hydrophilic SiO_2 where NPs penetration allows the domain formation even their sizes were smaller that those observed for pure DPPC, acting the hydrophilic silica particles as a barrier for the formation of total condensed phases.[39,32] In the case of mixed layers of DPPC and Carbon Black NPs,[36] the presence of NPs did not alter the formation of domains even the expected interactions would be similar to those presented in mixed films of DPPC and fumed SiO_2. This allows us to propose a complex scenario where a complex balance of contributions (interactions hydrophobic vs. electrostatic, chemical nature, hydrophobicity vs. hydrophilicity, etc.) plays a key role in the effect induced by NPs in the lipid layers properties.

Moreover, the analysis of ε_0 for the LE and LC phases (Figure 3c) allows one to obtain additional information about the interactions between NPs and DPPC. The introduction of NPs does not alter the ε_0 of the LE that is ascribable to the intrinsic disorder of this phase with a reduced effect of the van der Waals cohesive interactions. However, the

introduction of NPs provokes a concentration dependence decrease of the elasticity of the condensed phase of DPPC monolayers. This is related to the formation of composite layers (DPPC + SiO_2) where the average cohesive van der Waals interactions between the lipid molecules are reduced. It is worth to mention that the increase on x_{NP} leads to an increasingly importance of the cohesive interaction between the NPs and between the NPs and the lipid molecules but the average cohesion at the interfacial layer is weaken. This latter is on the bases of the decrease of ε_0 with the increase of x_{NP}.

A detailed analysis of the BAM images (Figure 4) confirms the scenario above discussed The increase of x_{NP} hinders almost completely the nucleation of lipid domains which is only observed for the lowest values of x_{NP}. The presence of NPs along the monolayers is observed for all the values of x_{NP} with the NPs occupying big regions of the interface for the highest values of x_{NP} and for the highest compression degree. In these conditions, the interfacial texture is similar to those found for pure particles layers.

3.2. Effect of x_{NP} in the interfacial interaction between DPPC and SiO_2

The application of the thermodynamic of mixture have allowed us to obtain further insights on the interactions in mixed monolayers.[40,41] Considering an ideal mixture without interactions between the different components, it is possible to calculate the ideal area, A_{ideal}, for a given Π-state

$$A_{ideal} = x_{DPPC} A_{DPPC} + x_{NP} A_{NP} \quad (2)$$

where A_{DPPC} and A_{NP} are the areas occupied for DPPC and SiO_2 NPs in their pure monolayers at the considered Π state, being x_{DPPC} and x_{NP} the weight fractions of DPPC and SiO_2 in the mixed monolayers. The evaluation of the type of interactions in the mixed monolayer can be obtained from the deviations between the experimental isotherm of the

mixed system and those expected considering ideal mixing. These deviations from the ideal mixing behaviour are accounted by the excess area, A_{ex}, defined as,

$$A_{ex} = A_{12} - A_{ideal} \quad (3)$$

where A_{12} is the area in the mixed monolayer at a given value of Π. For an ideal mixed monolayer, A_{ex} is zero. Since the molecular weight of the nanoparticles is unknown, the evaluation of A_{ex} must be made considering a surface density of the components in unit of mg/m^2. Figure 5 shows the dependence on x_{NP} of A_{ex} for different Π-states of the mixed monolayers.

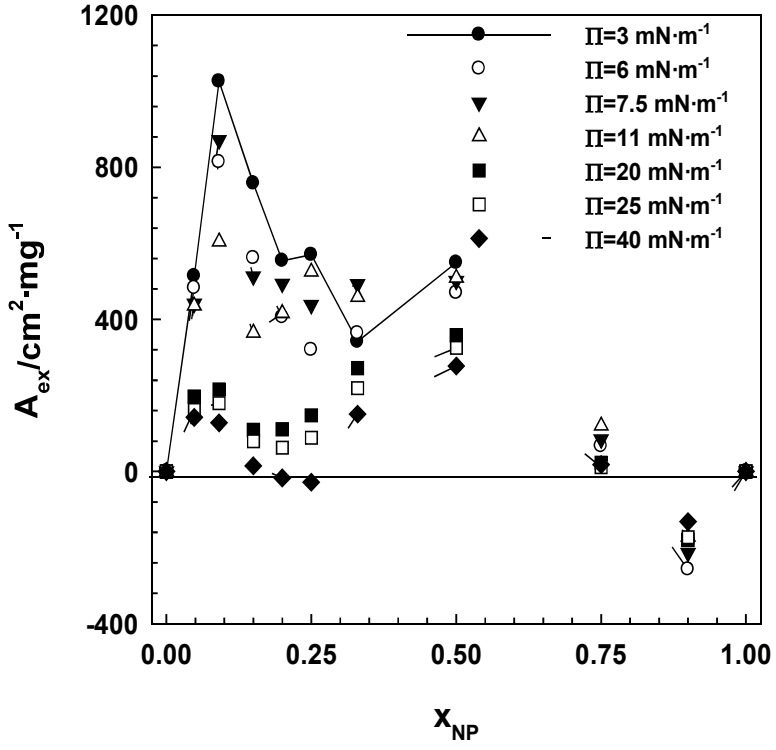

Figure 5. NPs weight fraction, x_{NP}, dependence of A_{ex} for different surface pressures.

A_{ex} decreases with the increase of the compression degree of the monolayer that is a clear evidence of a more favourable interaction between the NPs and lipid molecules at the interface with the increase of the packing of the film. This is due to a forced cohesion of the monolayer components due to the restriction of the area available. When the dependence of A_{ex} on the NPs weight fraction is considered, A_{ex} decreases with the increase of x_{NP}. This is explained considering that the increase of NPs amount at the interface lead to the increase of the importance of the hydrophobic interactions between NPs-lipid molecules, enhancing the monolayer cohesion, even the average cohesion of the monolayer is expected to be lower than that of pure DPPC monolayers as it was discussed above. However, it is necessary to consider that the interactions between DPPC and SiO_2 are repulsive till high values of x_{NP} when that A_{ex} becomes negative. The existence of repulsive interactions (positive values of A_{ex}) between NPs and DPPC is rationalized considering the hindered packing induced by the NPs. The existence of an enhanced miscibility for the monolayers at high NPs density can be rationalized considering many-bodies interactions that facilitate the packing.[42]

3.3. Effect of NPs in the low frequency dilational rheology of DPPC monolayers

In this section, the effect of the introduction of SiO_2 NPs in the dilational rheological properties of DPPC monolayers is investigated. The dilational viscoelasticity versus frequency has been measured by the mean of oscillatory barrier experiments at different degree of compression of the monolayers. Our previous works in the rheological characterization of composite layers of lipids and hydrophilic nanoparticles have evidenced that this technique is a powerful tool to evaluate the modification induced by the nanoparticles in the mechanical response of the lipid monolayers.[41,43]

The frequency dependences of the modulus of the dilational viscoelasticity, $|E|$, for DPPC-SiO_2 monolayers, with different SiO_2 content, are reported in Figures 5. For this low frequency rheological study, a frequency must be associated with the quasi-equilibrium viscoelasticity modulus above presented. A good estimation for this frequency is to assume that this frequency is defined by the rate of surface deformation under quasi-equilibrium compression, i.e. $du/dt= (dA/dt)/A$, which is of the order of 10^{-5} Hz. The relaxation processes with characteristic frequencies within the investigated frequency range, are evidenced by the presence of inflection points in the best fit curve. The results evidence, that the measured dilational viscoelasticities were well described by a theoretical expression corresponding to the case of one dynamic relaxation process occurring within the insoluble interfacial layer,[37,44]

$$|E| = \sqrt{\frac{E_1^2 + E_0^2 \lambda_1^2}{1 + \lambda_1^2}} \qquad (4)$$

where $\lambda_1= \nu_1/\nu$, and ν and ν_1 are the deformation frequency and the characteristic frequency of the relaxation process, respectively. The best fitting to Equation (4) for the different monolayers are reported in Figures 6.

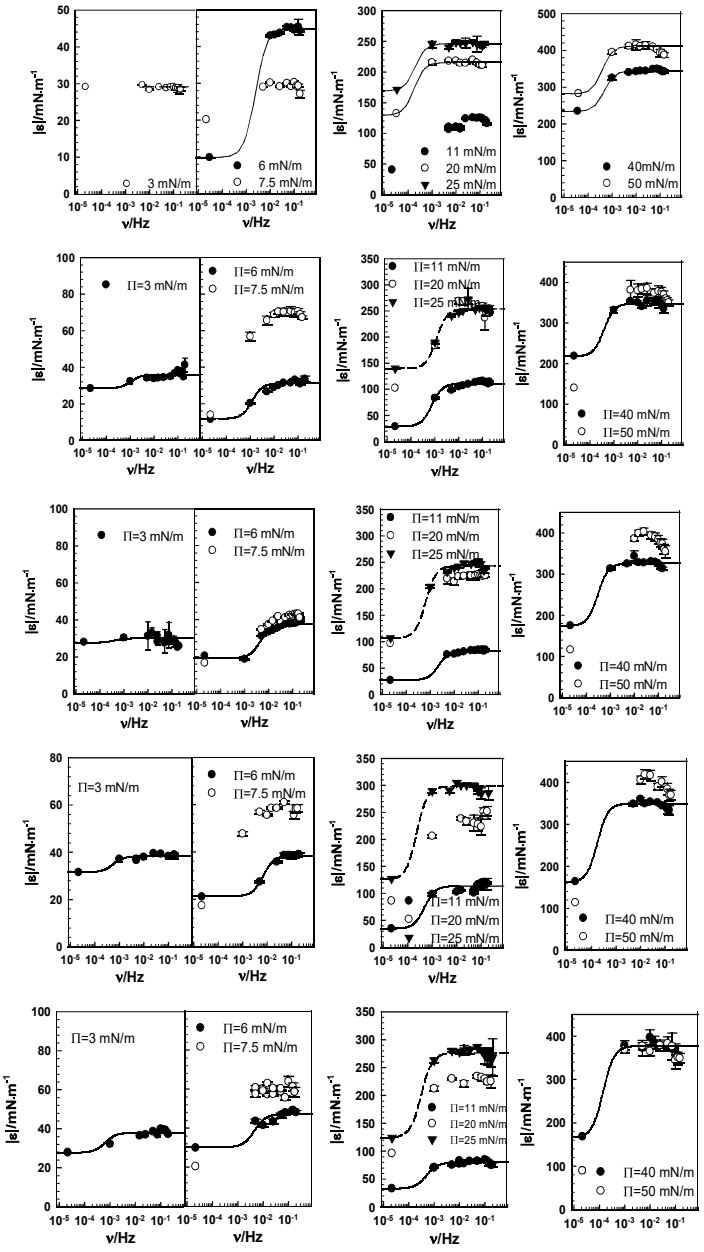

Figure 6. Modulus of the dilational viscoelasticity against frequency obtained by the mean of oscillatory barrier experiments for mixed monolayers of DPPC and different hydrophobic silica nanoparticles weight fraction at different values of surface pressure. Lines represent the best fit-curves obtained by eq. (4). From top to bottom: $x_{NP} = 0$, $x_{NP} = 0.09$, $x_{NP} = 0.20$, $x_{NP} = 0.50$ and $x_{NP} = 0.75$.

The incorporation of nanoparticles to the monolayer modifies both the limit elasticities and the characteristic frequency of the rheological response. However, no additional relaxation

processes are induced in the monolayer due to the NPs incorporation in the frequency range accessible in our experiments. Figure 7 shows the surface pressure dependence of the low frequency and high frequency elasticity limits obtained from the modelling of the experimental data with Eq. (4).

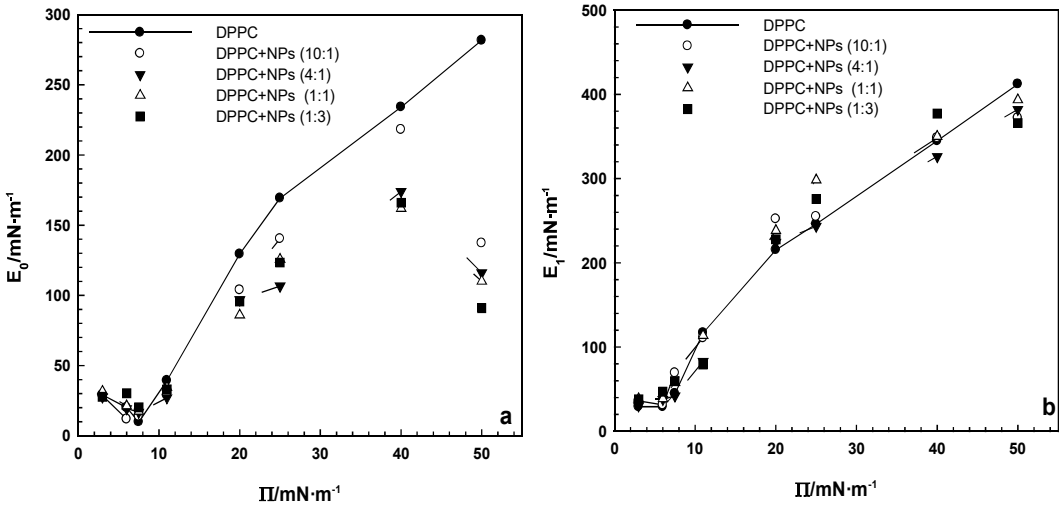

Figure 7. Surface pressure dependence of the limit elasticities, E_0 (left panel) and E_1 (right panel) for DPPC : NPs mixed monolayers with different values of x_{NP}.

The dependence on the surface pressure of the limit elasticities agrees well with the above discussion on the quasi-equilibrium elasticity. More interesting is the dependence on the weight fraction of x_{NP} E_0 and E_1. The dependence of E_0 on the x_{NP} shows a decrease with the increase of NPs concentration at the interface till reach a plateau value which can be ascribable to the formation of less ordered monolayers and the subsequent weakening of the interfacial interactions. In the case of E_1, the dependences on the NPs concentration are less clear but the obtained data seems to evidence a slight increase of E_1 with the NPs concentration. This makes difficult to perfom any discussion with physical sound on the dependence of E_1 on x_{NP}.

The dependence of the characteristic relaxation frequencies on x_{NP}, shown in Figure 8, presents more interest than the dependences found for the elasticities.

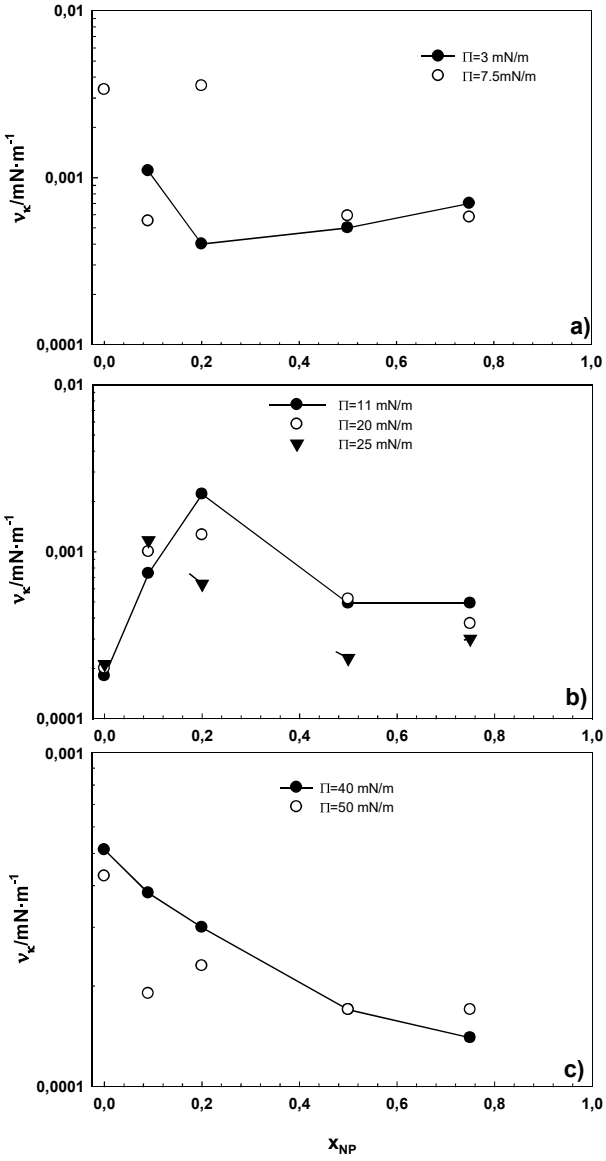

Figure 8. ν_1 dependence on x_{NP} for different mixed monolayers at different reference states.(a) ν_1 vs. x_{NP} in the LE (Π = 3 mN/m) and the LE-LC phase (Π = 7.5 mN/m). (b) ν_1 vs. x_{NP} in the LC phase. (c) ν_1 vs. x_{NP} in the solid phase.

The first effect associated with the incorporation of NPs to DPPC monolayers is the emergence of a relaxation process with $\nu_1 \sim 10^{-3}$ - 10^{-4} Hz from the lowest surface

pressures (Π ~ 3 mN/m, LE phase for the pure DPPC) which was not found for pure DPPC in similar state. This process can be ascribed to NPs induced decrease in the reorganization time of the lipid molecules at the interface, being this decrease more evident with the increase of the NPs interfacial density, indicating a slowing down of the velocity of this process due to the increasing steric hindrance. The scenario changes when the thermodynamic state of the monolayer approaches to the phase transition, under these conditions a relaxation process is observed independently of the presence of NPs at the interface. However, the presence of NPs affects to its characteristic frequency. The incorporation of NPs reduces the characteristic frequency of this process, ascribed for pure DPPC to the exchange of lipid molecules between the coexisting phases. SiO_2 makes slower, almost one order of magnitude, this process that can be explained considering the steric hindrance induced by the nanoparticles to the rearrangements of the lipid molecules at the interface.

Beyond the phase coexistence region, the incorporation of NPs leads to the increase in the characteristic frequency of the relaxation with the NPs interfacial density till reaching a maximum value, and then a decrease of this frequency with the increase of the NPs concentration was found. Furthermore, the maximum frequency is found for decreasing values of the NPs interfacial density as the packing degree of the monolayer increase. This is associated with the complex balance of interactions along the monolayer that can lead to the existence of several coupled dynamic in the rheological response of the system in this region. Furthermore, it cannot be ruled out that the increase of the value of the surface density of NPs provoked similar interfacial packing for lower values of the surface pressure, and consequently this can on the bases of the particular features found for the characteristic frequency dependence on x_{NP}.

Increasing Π till values characteristic for a total condensed phase on DPPC monolayer layers (solid region $\Pi > 35$), a relaxation process with $\nu_1 \sim 10^{-4}$ Hz is found, both for the pure DPPC and for the mixed monolayers. This relaxation may be related to general rearrangements of the DPPC molecules, being attributed in the mixed monolayers to more complexes rearrangements that imply the SiO_2 nanoparticles and the lipid molecules. This process is slightly slower for the mixed monolayers that can be explained considering the steric hindrance contribution of the nanoparticles to the reorganization of the lipid molecules at the interface. This effect is more important with the increase of the interfacial density of the NPs.

3.4. Non-linear rheology: Mimicking biological processes

In the previous section, the rheological characterization was focused so far on the study of the rheological response under linear conditions. However, most of the biological relevant processes present a non-linear character, implying high amplitude deformation, e.g. the lung surfactant is subjected to non-linear deformation of deformation in the range of 30 – 40 % of the total available area and frequencies spanned between 40 and 200 mHz.[45,46] In addition, it is worth mentioning that most of the biological relevant processes occur in highly condensed systems with surface pressure in the range 35 – 40 mN/m. In order to mimic this type of processes, we have study the rheological response of mixed monolayers with a surface pressure of 40 mN/m, using a fixed frequency of 50 mHz and different amplitude of deformation ranged between 1 and 40 % of the total available area. The stress response to the period deformation were analyzed by the mean of the Fast Fourier Transform (FFT),[30,47] the results obtained following this approach evidenced only the fundamental frequency under linear deformation conditions, whereas different overtones of the frequency fundamental where found when non-linear deformations were applied. Further details on these aspects can be found in our previous publications.[36,43]

An elegant way to evaluate the non-linearity of the response is by the mean of the total harmonic distortion (THD)[48] defined as follow,

$$THD = \frac{\sqrt{\sum_{k>1} \Delta\sigma_k^{\,2}}}{\Delta\sigma_1} \qquad (5)$$

where $\Delta\sigma_1$ is the amplitude of the fundamental frequency of the periodic signal and $\Delta\sigma_k$ makes reference to the frequency of the different overtones of the fundamental frequency. Linear systems assume vanishing values of THD, while larger values are found for systems with non-linear response. Thus, the THD value can be effectively used to discriminate and quantify the linearity of the monolayer response. Figure 9 shows the values of THD for different DPPC – NPs mixtures.

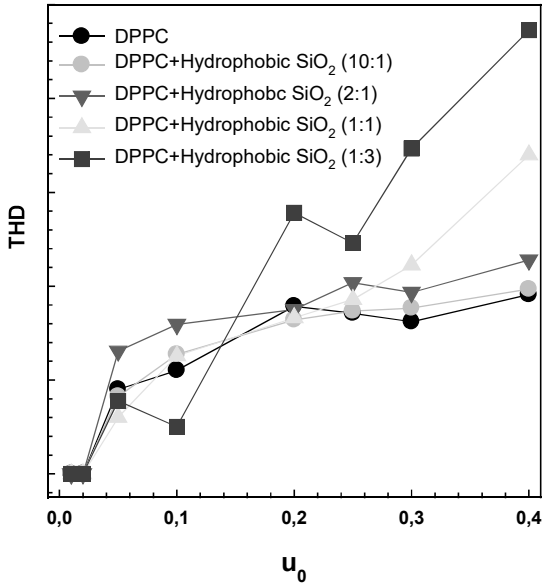

Figure 9. THD dependence on the deformation amplitude, u_0, for mixed monolayers with different values of the relative ratio DPPC:NPs.

The THD feature for the different studied systems points out the important non-linearity of the rheological response under biological relevant conditions. This emerging of the non-linearity is associated with the re-distribution of material along the monolayer. A hindered reorganization of the material usually is on the bases of the increase of the non-linear character of the rheological response.[49] The introduction of NPs enhances the non-linearity of the rheological response of the lipid monolayer in agreement with the found for other mixed monolayers.[36,43] This worsening of the linearity of the response is associated with the inhomogeneity of the mixed monolayers that induces an important hindrance to the material reorganization of the lipid molecules. This inhomogeneity also allows explaining the increase of the non-linearity with the particle weight fraction. Thus, on the basis of the obtained results it is possible to hypothesize that NPs worsen the physiological function of biological relevant systems.

4. Conclusions

The effect of hydrophobic fumed silica nanoparticles on the behaviour of DPPC monolayers has been investigated. This work was focused on the equilibrium and dynamic aspects of the monolayer behavior. In particular, the coupling of the Langmuir trough technique with the BAM characterization have resulted to be effective in the evaluation of the impact of NPs on the phase behavior, both from the thermodynamic and structural point of view, and the rheological properties of lipid monolayers.

From the results here obtained one can conclude that NPs influence strongly the interfacial behavior of DPPC monolayers. This is mainly driven by the interaction between the nanoparticles and the hydrophobic tails of the lipid molecules at the interface which

disrupts the interfacial structure and consequently alters the monolayer cohesion, modifying the structure and properties of the monolayers.

The NPs may have multiple effects. Besides an increase of the structural disorder of the monolayer NPs reduce the available free area water-air interface for the lipid distribution along the monolayer. These phenomena have important consequences on the system behavior, as it has been quantitatively determined by measuring the $\Pi - A$ isotherms. Furthermore, a strong effect was observed in the ability of DPPC to form condensed phase as it was reflected in the hindering of the domain formation, the reduction of the collapse pressure and equilibrium elasticity of the films. This is mainly ascribed to thestrong steric hindrance associated with the NPs incorporation. This scenario was confirmed by the frequency dependence of the viscoelastic modulus where the slowing down of the relaxation processes with the increase of NPs incorporation was found. In addition, the changes on the linearity of the rheological response due to the NPs incorporation allows hypothesizing that NPs induce potential modifications of the normal physiological function of biological relevant systems. All the modifications found are strongly dependent on the NPs dose.

In fact, even if the use of DPPC monolayers are rather rough models of biological relevant systems, some of the obtained results could allow for a better understanding of the mechanisms operating in the modifications occurring in real systems. In addition, the observed effects could be utilized to discriminate potential adverse effects of nanoparticles on biological structures and fluids.

Acknowledgements

This work was financially supported by IIT – Istituto Italiano di Tecnologia within the Project SEED 2009 "Nanoparticle Impact of Pulmonary Surfactant Interfacial Properties – NIPS", and was carry out in the framework of the European Science Foundation COST Actions CM-1101 "Colloidal Aspects of Nanoscience for Innovative Processes and Materials" and MP-1106 "Smart and Green Interfaces - from single bubbles and drops to industrial, environmental and biomedical applications".

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