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Influence of Silica Nanoparticles on Thermodynamic and Structural Properties of DPPC – Palmitic Acid Langmuir Monolayers

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Abstract

The effect of silica nanoparticles on the thermodynamic and structural properties of monolayers of 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), Palmitic acid (PA) and a mixture of them, has been investigated using a combination of Langmuir trough technique and Brewster Angle Microscopy (BAM). This study shows that the presence of nanoparticles in the aqueous sub-phase affects the surface pressure-area (*Π-A*) isotherm, leading to a different phase behavior of the monolayer. The observed effects are explained assuming the incorporation of nanoparticles into the monolayer driven by the adsorption of lipid molecules onto the silica which makes the nanoparticles more hydrophobic. This process hinders the ordering and affects the composition of the investigated lipid monolayers significantly modifying also their quasi–equilibrium dilational elasticity

The reported results are useful in the framework of a wider study aimed at elucidate the impact of nanoparticles on the physico-chemical properties of pulmonary surfactant, being DPPC its major component and PA utilized as an important component in the formulation of therapeutic substitutes.

Keywords: DPPC; Palmitic acid; nanoparticles; silica; monolayer; pulmonary surfactant; surface pressure isotherms; BAM; Brewster Angle

1. Introduction

Monolayers of fatty amphiphiles have been extensively investigated using Langmuir trough technique [1,2,3, 4] which is an effective tool to access the phase behavior of this kind of systems. In fact, from the analysis of the area - surface pressure isotherms it is possible to gain information on the structural changes of the monolayer induced by molecular lateral packing [5, 6]. More information about the structure of the monolayers can be obtained by X-ray diffraction technique, which is the most appropriate to study the lateral packing in the monolayer [7], and by other in situ optical techniques like Fluorescence Microscopy [8], Laser Light Scattering [9] or Brewster Angle Microscopy [10, 11].

It is moreover of increasing interest to understand how the introduction of additional components, such as solid nanoparticles, changes the equilibrium and dynamic properties and the structure of these insoluble monolayers.

For example, the interaction of nanoparticles with lung surfactant and their impact on its behavior and structure is a relevant topic on which some results can be already found in literature [12,13]. The increasing interest on this topic is due to the wide spreading of nanosystems in various technological fields [14], which rises the need of evaluating their potential toxicity and hazards. Among them, most relevant is the effect on respiratory functionality, lungs being the major entry point for microscopic particulates [15, 16].

The lung surfactant is a complex mixture containing 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), as main component, unsaturated phosphatidylglycerols (PG) and phosphatidylcholines (PC), cholesterol and the surfactant-specific proteins B and C [17.18, 19]. Additionally it is possible to find in lower proportion other surfactant-proteins (A and D) and other components such as Palmitic Acid (PA) which is also used as a component in lung surfactant substitute therapies [20, 21]

It is also important to mention that the effect of the nanoparticle on the physico-chemical properties of liquid interfaces has been investigated in several works [22, 23, 24, 25, 26]. It has been shown that their surface segregation affects the thermodynamic and kinetic behavior of surfactant systems influencing the interfacial tension and the dilational rheology of the systems [24, 25].

In the present work the interaction of silica nanoparticles with lipid monolayers is investigated from the thermodynamic and structural point of view, by using a Langmuir trough technique coupled with Brewster Angle Microscopy diagnostics. The investigated systems were, in particular, monolayer composed by DPPC and PA, spread on silica nanoparticle dispersions.

The reason of this choice is related to the importance of DPPC as essential component of the lung surfactant [27], able to form monolayers with very low surface tensions, near zero, corresponding to a surface pressure of about 70 $mN \cdot m^{-1}$ [28].

The DPPC-PA mixture with a 9:1 ratio in weight is also investigated as representative of substitutive formulations for the pulmonary surfactant, as reported by other authors [29]. In fact, being the effect of PA related to the kinetics of surface tension lowering [30 ,31] this type of mixtures has been used in the field of synthetic preparation of lung surfactant [32].

The present experimental study wants to be a starting point for more complex investigations on the effect of nanoparticles on the lung surfactant behavior. The results here obtained can contribute to improve the understanding, on fundamental bases, of the potential toxicological effects.

2. Materials and Methods

2.1 Materials

1,2-Dipalmitoyl-sn-glycerol-3- phosphocholine (DPPC), and Palmitic acid (PA), were purchased from Sigma (Germany) at 99 % purity and used without further purification. The molecular weights of these lipids are 734.1 g/mol and 256.4 g/mol for DPPC and PA respectively. Solutions of lipids for the spreading were prepared using chloroform for HPLC from Sigma (Germany).

Aqueous dispersions containing 1 wt % of silica nanoparticles were obtained by diluting a commercial colloidal dispersion at 30 wt% of spherical silica particles with specific surface area of 200 m^2/g. This product is characterized by a high stability, caused by the large negative surface charge. The dispersions therefore do not contain any stabilizing agent which could false the data interpretation. The dimensions of the particles, as well as the dispersion stability, have been characterized by Dynamic Light Scattering and ζ-potential measurements which provided an hydrodynamic radius of 15 ± 2 nm, ζ=-42±1 mV for the colloidal dispersion and a pH close to 9 [33].

Water for producing the dispersion and for all reported measurements was deionized and purified by a multi-cartridge, Elix plus Milli-Q (Millipore), system, providing a resistivity greater than 18 MΩ·cm. Purity of water was checked by surface tension measurements which provided a value of 72.5 mN/m without any appreciable kinetics over several hours. The above silica dispersions present the same constant value of

the surface tension as pure water [24]. This proves that the bare particle dispersion does not contain any surfactant impurity and nanoparticles are not surface active. In fact being these silica nanoparticles highly hydrophilic, they do not segregate at the water / interface.

2.2. Methods

All reported experiments were performed by using a Langmuir trough (KSV Minitrough, Finland) equipped with two Delrin® barriers allowing for symmetric compression / expansion of the free liquid surface. Delrin® is a hydrophilic material that prevents the typical leakage of lipids monolayers beneath the barriers. The total surface area of the Teflon trough is 243 cm2. The set up allows the surface tension, γ, to be measured by the Willhelmy plate method, using a paper probe (Whatman CHR1 chromatography paper, effective perimeter 20.6 mm, supplied by KSV) in order to ensure a zero contact angle. Surface pressure is then obtained as $\Pi = \gamma_w - \gamma$, where γ_w is the surface tension of pure water. For the determination of the Surface Pressure – Area (Π-A) isotherm, the surface pressure is typically acquired during the compression of the free area of the monolayer, at a rate of 2 cm2/ min which corresponds to a rate of area deformation, $d(\Delta A/A0)/dt$, of about 3×10^{-5} s-1.

The spreading of lipids as Langmuir monolayer was made using a Hamilton syringe to drop a controlled volume of lipid solution on the aqueous sub-phase (pure water or nanoparticle dispersion) contained in the Langmuir trough. 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.

The compression for the *Π-A* isotherm experiment was started after 1 hour from the monolayer deposition. This time was checked to be long enough to ensure the complete evaporation of the solvent and, in the case of nanoparticle dispersions, the achievement of the equilibrium of the composite system, driven by the nanoparticle-lipid interaction.

The Langmuir trough was coupled with a Brewster Angle Microscope, BAM, (Multiskop, Optrel, Germany) in order to obtain information on the layer morphology during the area compression [10, 11]. In particular this techniques allowed visualizing and monitoring the microdomains forming during the compression of the monolayer evidencing, in particular, the structural modification related to the presence of nanoparticles.

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

3. Results and Discussion

3.1. Effect of nanoparticles on the Π-A isotherm

The phase behavior of the lipid monolayers spread on pure water were firstly investigated for sake of comparison with the previous literature data and in order to adequately establish the properties of the investigated lipid monolayers before the interaction with silica nanoparticles.

The *Π -A* isotherms, obtained for DPPC, PA and the 9:1 in weight DPPC-PA mixture are reported in Figure 1. The measurements were executed several times and the results showed a very good repeatability, however, for sake of simplicity, only one curve was reported for each system.

The isotherms here obtained are in good agreement with the previous ones reported in literature, especially for the widely investigated DPPC [[28], [34], [35]] and PA [[36, 37, 38]] pure monolayers.

In particular, for pure DPPC monolayers it was found the typical feature of the *Π-A* isotherm. At high areas per molecule, the surface pressure which initially presents a low quasi constant value, starts to increase smoothly with the compression indicating an increasing packing of the homogeneous liquid-expanded (LE) phase. Advancing with the compression, at area per molecule of about 66 $Å^2$, the *Π-A* isotherm presents the typical quasi-flat plateau corresponding to the coexistence of a LE fluid phase and a highly ordered liquid-condensed (LC) phase characterized by a lower fluidity [[39]].

When the layer is further compressed below a value of the area per molecule of abut 50 $Å^2$, the surface pressure increases with a larger slope, indicating that only the LC phases is present. Further compression leads to a new change in the slope of the isotherm corresponding to the appearance of the solid like state followed by a collapse of the monolayer occurring at the surface pressure of about 70 $mN \cdot m^{-1}$.

The feature of the *Π-A* isotherm found for PA monolayers, in agreement with what reported refs. [36, 37, 38], is coherent with the assumption that, for large surface area, gaseous and tilted-condensed (TC) phases coexist in the monolayer providing $\Pi \approx 0$. For area per molecule lower than about 30 $Å^2$, the surface pressure starts to increase indicating the presence of the single TC phase and its compression. Upon further compression, at a surface pressure ~ 24 $mN \cdot m^{-1}$, the slope of the isotherm changes again indicating that the TC phase has been transformed into an untilted condensed (UC) phase. Further area

compression induces the surface pressure to pass through a maximum, of about 36 mN/m, related to the collapse of the monolayer with the formation of a three-dimensional phase.

Also the *Π-A* isotherm obtained for the DPPC-PA mixture is in agreement with the previously reported in the literature for similar systems [29, 40]. Comparing this isotherm with that obtained for pure DPPC, it is apparent the disappearance of the plateau corresponding to the LE-LC phase coexistence. The presence of PA seems to lead the mixed monolayer to pass from an expanded to a condensed phase without any appreciable change in the isotherm slope.

To explain that, one has to consider that the nucleation of the LC phase responsible of the plateau in the DPPC isotherm is related to the particular order in which DPPC molecules position in the monolayer. As fatty acids do not mix well with zwitter-ionic lipids [41], like DPPC, the introduction of PA hinders, as a matter of fact, this molecular ordering and delays the formation of the condensed phase. Moreover, due to this chemical characteristic, it is also possible that two kinds of domains coexist in the monolayer, when it starts to condense, mostly rich in DPPC and in PA, respectively.

In order to investigate the effect of silica nanoparticles, the same measurements were executed on the same lipid systems spread on a dispersion of silica nanoparticles (1wt %).

Silica nanoparticles being completely hydrophilic, do not adsorb at the pure water/air interface [24, 25]. However, when a lipid layer is deposited on the surface of the dispersion, the particles, colliding with it, strongly interact with the lipid molecules modifying, in some cases, the layer composition and forming partially hydrophobic complexes which can be incorporated in the monolayer. Such effect induces appreciable changes in the thermodynamic and kinetic properties of the layer. This is the general mechanism assumed to explain the observed effects. Here in the following the *Π-A* isotherms obtained for the different layer compositions are analyzed in details and compared with the BAM images obtained, with and without nanoparticles, when systems passed through the most significant states of the monolayers.

The *Π-A* compression isotherm of DPPC monolayer spread on the 1 wt % silica nanoparticle dispersion is reported in Figure 2. The comparison with that obtained for DPPC spread on pure water, also reported in Figure 2, shows that in presence of nanoparticles, the surface pressure starts to increase already at the beginning of the compression, i. e. at A/A_0 very close to 1. This means that in the expanded region the isotherm appears shifted toward larger areas, while in the region of condensed phase the difference between the two systems is reduced.

The second evident effect of nanoparticles concerns the typical plateau of the DPPC isotherm, corresponding to the coexistence region between LE and LC phases, which appears in this case less horizontal. This means that the monolayer presents an increasing resistance against the compression [28] which can be attributed to a delay of the LE-LC transition. Coherently, the BAM images reported in Figure 3 show, for pure water sub-phase, the typical bean-shaped LC domains, according to what observed by other authors [1, 3, 4, 28] while, in presence of nanoparticles, at the same degree of monolayer compression, the formation of LC domains is hindered and the existing domains present smaller size. Moreover the complete coverage by the condensed phase obtained for pure DPPC at a monolayer compression of about 0.5, is not observed in this case.

To explain the observed changes in the monolayer behavior the following scenario is proposed. Nanoparticles colliding with the surface where DPPC is deposited, form nanoparticle-DPPC complexes and consequently are incorporated in the monolayer. The essential reason for the formation of these complexes is the electrostatic interaction between the ammonium group of the DPPC hydrophilic head, oriented towards the aqueous phase, and the dissociated silanol groups of the silica nanoparticles, with negative charge [42].

The incorporation of nanoparticles results in a partial coverage of the liquid surface and a consequent reduction of the available area for DPPC molecule. This effect can explain the shift toward higher relative areas in the expanded region, observed in presence of nanoparticles. In practice the Π increase, corresponding to the compression of the LE phase, occurs at surface area higher than for pure DPPC monolayer.

The different feature of the coexistence LE-LC zone may be also related to the attachment of nanoparticles at the monolayer. This acts as limiting factor for the nucleation and growth of the condensed domains. In fact, as already discussed in [35] where the effect of insoluble impurity was investigated, the nanoparticles at the interface induce a looser packing of the phospholipid hydrophobic tails and, consequently, a weakening of van der Waals cohesive interactions. Notice that the packing of the hydrophobic tails in monolayers of lipids is the main driving force for the formation of ordered phases. Thus, as for saturated lipids like DPPC, this packing is controlled by the reorientation of the electrical dipole of the molecule, electrostatic perturbations modify the rearrangement of the chain at the interface, affecting the interfacial order. In this case, the negative charge of the silica nanoparticles may be an important disrupting agent of the ordering of the DPPC monolayers.

During the compression of the condensed phase, for a surface pressure around 50 $mN \cdot m^{-1}$ the surface pressure increases with a smaller slope, crossing the surface isotherm of pure DPPC, as it can be observed in Figure 2. To explain this behavior one can assume

that at such large surface pressure some of the DPPC-nanoparticles complexes may be squeezed out from the interface under the effect of the compression. This squeezing out of material into the sub-phase, similarly to what observed for DPPC – protein complexes in [43], allows the monolayer to reach compression states higher than in the case of pure lipid. In this case the effect of silica nanoparticles may be the reduction of the real surface concentration of lipids in the monolayer.

The behavior observed for the DPPC monolayer spread on the silica dispersion is in qualitative accordance with the results obtained by Harischandra et al. [12] where the presence of hydrophobic nanoparticles deposited on a lipid monolayer reduces and delays the LE-LC phase transition.

The effect of the silica nanoparticles on the phase behavior of PA monolayer is shown in Figure 4. By the comparison of the two surface pressure responses to area compression, it is evident that the presence of nanoparticles in the sub-phase has a dramatic effect on the PA monolayer. In this case in fact the surface pressure, during compression, keeps almost constant at a zero value.

The BAM images reported in Figure 5 show that the typical condensed domains, growing during the compression of the pure PA monolayer until the complete coverage of the surface and the consequent monolayer collapse, completely disappear in presence of nanoparticles. The interaction with the silica particles seems therefore to completely remove the PA molecules from the liquid interface. Such behavior can be explained assuming the adsorption of the PA molecules on the silica particles. Such adsorption is justified by the existence of an attractive interaction, , between the carboxylic group of the palmitic acid molecules and the non-dissociated silanol group of the silica nanoparticles. However, for the monolayer surface density here investigated, i.e. Γ^0 = 19 μmol/m^2, the PA adsorption on nanoparticles is not enough to confer them the degree of hydrophobisation necessary for their irreversible attachment at the liquid interface. In practice, under these conditions, the PA – silica particle complexes formed at the liquid interface, due to their practically unchanged hydrophilic character, transfer back into the aqueous phase impoverishing the PA monolayer.

Further compression isotherm experiments were performed increasing the initial amount of PA spread on the silica dispersion. The results of this study, reported in the insert of Figure 4, confirm the above interpretation. In fact, after the spreading of an amount of PA corresponding to Γ^0 = 100 μmol/m^2, an increase of the surface pressure is obtained by compressing the monolayer. Increasing further Γ^0, the pressure increases with different slopes and initial values.

This behavior proves that larger values of the initial PA amount, providing larger PA adsorption onto the silica particles, increasingly favors their incorporation in the

interfacial layer. Thus, monolayers obtained by spreading different amounts of PA are characterized by different PA-nanoparticles compositions and present different phase behaviors.

In Figure 6 the compression isotherm of the 9:1 DPPC-PA mixture spread on the silica particle dispersion is reported together with that previously obtained with pure water sub-phase.

As we found for the single lipids, the presence of silica nanoparticles significantly affects the phase behavior of this mixed monolayer. The feature of the isotherm reported in Figure 6 is coherent with the assumption, also for this mixed system, that PA molecules are removed from the interface by the silica nanoparticles. The isotherm is in fact rather similar to the one obtained for DPPC monolayer spread on silica dispersion, reported in Figure 6 as well. In particular, these two isotherms are almost matching for $A/A_0 > 0.4$, corresponding to Π around 50 mN/m and lower.

The BAM images reported in Figure 7 show the presence of microdomains with a non-homogeneous size distribution, for DPPC–PA monolayer on pure water, coherently with what assumed by the analysis of the Π-A isotherm, while, in presence of nanoparticles, the observed domains have smaller size, more similar to those found for DPPC monolayer in presence of nanoparticles (see Figure 3).

Compressing the condensed phase to $A/A_0 < 0.4$, the two isotherms, with and without PA, start to diverge with a faster increase of the surface pressure in the case of DPPC-PA monolayer. This is possibly due to the presence of adsorbed PA on the silica nanoparticles which may have, especially for condensed monolayers, a likely different interaction with DPPC molecules, and consequently a different phase behavior.

The other important effect of the presence of nanoparticles in the DPPC-PA monolayer is the reduction of the collapse pressure. As previously discussed for DPPC in presence of nanoparticles, this can be considered related to the squeezing out of materials as lipid-nanoparticles complexes similar to what reported in ref. [43]. The reason for a collapse pressure lower than in case of DPPC-nanoparticles is probably the presence at the interface of different species (PA-lipid complexes and residual PA) that can lead to a prior disruption of the structure of DPPC-PA monolayers.

To complete this thermodynamic study, the effect of silica nanoparticles has been investigated also by quasi – static compression – expansion cycles of the surface area. This kind of experiments allows some characteristics of the lipid monolayers, such as internal reorganization, formation of three-dimensional aggregates and re-spreading processes to be investigate in more details through the analysis of the hysteresis of the surface pressure

response to the area cycles. These properties are of great importance in the physiological response of the pulmonary surfactant during the breathing cycle [44].

The compression-expansion cycles obtained for DPPC and DPPC-PA lipid systems are shown in Figure 8. As expected, these systems present a clear hysteresis related to the non-efficient re-spreading of material into the interface [45]. In fact, being the character of these molecules strongly hydrophobic, they are expected to squeeze out from the monolayer, during the compression, forming three-dimensional aggregates. During the expansion of the surface area the aggregated lipids tend to re-spread on the water surface with a slower dynamics characterized by a different surface pressure.

In presence of nanoparticle, the hysteresis for DPPC monolayers is even more important. This is possibly related to the squeezing out also of DPPC-nanoparticles complexes during compression. In fact, during the expansion, the DPPC molecules bound to the nanoparticles return to the interface in time-scales presumably longer than in the case of free DPPC molecules.

For DPPC-PA monolayers, the hysteresis in presence of nanoparticles, is slightly reduced with respect to lipids alone, even if it is still present. This can be related to the already discussed PA removing from the interface by nanoparticles which modifies the structure of the interface already before the compression. Consequently the dynamic of the re-spreading is in this case less influenced.

Thus it is possible to conclude that the presence of nanoparticles affects, not only the phase behavior of the lipid monolayers, but also the surface pressure response to compression-expansion cycles and in particular the hysteresis, being this latter strictly related to the dynamics of re-spreading of material which in turns is conditioned by the formation of lipid – nanoparticles complexes.

3.2. The Quasi-Equilibrium Dilational Elasticity

The Π - A isotherms presented and discussed in previous section allow the determination of the dilational elasticity, under quasi-static compression of the surface area. This is an important characteristics related to the elastic energy stored by the monolayer when it is continuously compressed (constant rate of deformation) and provides information about the rigidity of the monolayer upon quasi-static uniaxial dilation [28]. Notice, in fact, that for very low rate of deformation, the dilational viscosity can be assumed as vanishing.

For isothermal compression in the Langmuir trough, ε_0, can be written as

$$\varepsilon_0 = -A\left(\frac{\partial \Pi}{\partial A}\right)_T \qquad (1)$$

and consequently can be evaluated by the numerical derivative of the *Π-A* isotherms.

Figure 9 shows the ε_0 calculated for DPPC and the mixture DPPC-PA, spread on water and silica dispersions.

For DPPC monolayers on water two maxima are observed corresponding to the LE phase, at the larger area, and to the LC phase. At large relative areas, where the LE phase is highly compressible the quasi-equilibrium elasticity, is close to zero and the same happens in the coexistence region. On the other side, the LC phase is characterized by comparatively higher values of the dilational elasticity as expected for a condensed ordered phase [28].

The introduction of PA into the DPPC monolayer does not affect the value of the quasi-equilibrium dilational elasticity for the LC phase while the maximum observed at small surface pressure for DPPC (corresponding to the LE phase) disappears. This last effect, together with the continuous increase of the elasticity with the surface pressure, observed for the expanded phases, is coherent with the disappearance of the LC-LE coexistence region.

It is evident from the data reported in Figure 9 that, coherently with what observed for the *Π-A* isotherms, the presence of nanoparticles in the subphase affects also the dilational elasticity. For the LE phase of DPPC monolayers, the essential difference is the shift to higher relative areas while in the coexistence region a value clearly different from zero is found with nanoparticles. This is related to the increasing monolayer resistance to compression, discussed in previous section. For the LC phase, moreover, lower values of dilational elasticity are found in presence of nanoparticles. This means that the increase of the disorder in the monolayer, induced by the incorporation of nanoparticles, leads also to a decrease of the monolayer rigidity.

The second plot in Figure 9b shows that the presence of nanoparticles for the mixed DPPC –PA systems, makes the feature of the dilational elasticity similar to that found for pure DPPC. This was expected, considering the already discussed PA removal by the silica nanoparticles.

In Figure 10, the same quasi-equilibrium elasticity values are plotted against the surface pressure. It is interesting to notice that the presence of nanoparticles increases ε_0 when approaching the most condensed phase. For higher surface pressure this effect is inverted. In fact the layer resistance to compression is reduced due to the likely formation of three-dimensional structures.

This is an important effect, for the application of these results in the field of pulmonary surfactants. In fact, these rheological characteristics are related to the rigidity of the monolayer and to the response to expansion-contraction of the surface area.

4. Conclusions

The aim of the present study was the analysis of the impact of silica nanoparticles on the thermodynamic characteristics of lipid monolayers relevant in the field of lung surfactant.

To this purpose, the properties of DPPC, PA and mixed DPPC-PA monolayers, spread on pure water and silica nanoparticle dispersions, have been systematically investigated using the Langmuir trough techniques to evaluate the Π-A compression-expansion isotherms and the quasi-equilibrium dilational elasticity. The implementation of a BAM diagnostics coupled with the Langmuir trough provided an important support for the interpretation of the results obtained, especially for what concerns the phase transitions and the structure of the lipid monolayers and the changes of them induced by the presence of the silica nanoparticles.

From the results here obtained one can conclude that a significant effect of nanoparticles exists on the phase behavior of these monolayers. This is essentially driven by the formation of nanoparticle-lipid complexes which can induce a modification of the lipid content of the monolayer and/or the incorporation of nanoparticles into it, being their degree of hydrophobicity modified by the lipid adsorption.

The principal effects evidenced by this study are:

- the hindering of the emergence of condensed phases due to the decrease of the ordering of lipids within the monolayer

- a strong change of the monolayer composition induced by the removing of PA from the interface due to adsorption on the silica particles,

- the lowering of quasi equilibrium elasticity of the compressed monolayer and the modification of the surface pressure response to compression/expansion cycles of the surface area.

This last point is due to the influence of the presence of lipid-nanoparticle complexes on the collapse conditions and on the formation of three dimensional lipid aggregates and for that is a relevant aspect for the application of lung surfactant functionality.

Concluding, even if the DPPC-PA mixture here used is a rather rough model of the pulmonary surfactant, the results obtained provide information applicable to realistic systems. These are, for example, the change in the composition and formation of condensed phases induced by silica nanoparticles as well as the general lowering of the dilational elasticity.

For a better understanding of these effects it is necessary to complement this study with other investigations on the dynamic characteristics of the same systems. To this aim another work is under preparation, focused on the dilational rheology of these model systems to better assess their dynamic properties and the effects that silica particles may have on them.

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References

[1] H. Mohwald, Phospholipid and Phospholipid-Protein Monolayers at the Air/Water Interface, *Ann. Rev. Phys. Chem.* 41 (1990) 441-476.

[2] N. Nandi, D. Vollhardt, Effect of Molecular Chirality on the Morphology of Biomimetic Langmuir Monolayer, *Chem. Rev.* 103 (2003) 4033-4076.

[3] H.M. McConnell, Structures and Transitions in Lipid Monolayers at the Air-Water Interface, *Ann. Rev. Phys. Chem.* 42 (1991) 171-195.

[4] C.M. Knobler, R.C. Desai, Phase Transitions in Monolayers, *Ann. Rev. Phys. Chem.* 43 (1992) 207-236.

[5] D. Dervichian, Changes of Phase and Transformations of Higher Order in Monolayers, *J. Chem. Phys.* 7 (1938) 931-948.

[6] S. Stallberg-Stenhagen, E. Stenhagen, N. Sheppard, G. B. B. M. Sutherland, A. Walsh, Phase Transitions in Condensed Monolayers of Normal Chain Carboxylic Acids, *Nature* 156 (1945) 239-240.

[7] K.Y. Lee, A. Gopal, A. von Nahmen, J.A. Zadsadzinski, J. Majewski, G.S. Smith, P.B. Howes, K. Kjaer, *J. Chem. Phys.* 16 (2002) 775-783.

[8] C.M. Knobler, Seeing Phenomena in Flatland: Studies of Monolayers by Fluorescence Microscopy, *Science* 249 (1990) 870-874.

[9] W. Lu, C. M. Knobler, R. F. Bruinsma, M. Twardos, M. Dennin, Folding Langmuir Monolayers, *Phys. Rev. Lett.* 89 (2002) 146107.

[10] S. Sundaram; J.K. Ferri; D. Vollhardt; K.J. Stebe, *Langmuir* 14 (1998) 1208-1218.

[11] J. Zhao, D. Vollhardt, G. Brezesinski, S. Siegel, J. Wu, J. B. Li, R. Miller, *Colloids Surf. A* 171 (2000) 175-184.

[12] Wang, Z.; Li, X.; Yang, S. *Langmuir* **2009**, 25, 12968-12973

[13] R. K. Harishchandra, M. Saleem, H.-J. Galla, *J. Royal Soc. Interface* 7 (2010) S15-S27.

[14] L. Mazzola, Commercializing nanotechnology, *Nature Biotech.* 21 (2003) 1137-1143.

[15] P. H. M. Hoet, I. Brüske-Hohlfeld, O.V. Salata, *J. Nanobiotech.* 2 (2004) doi: 10.1186/1477-3155-2-12.

[16] D. Kondej, T.R. Sosnowski, *Chem. Eng. Transact.* 19 (2010) 315-320.

[17] R. Wüstneck, J. Perez-Gil, N. Wüstneck, A. Cruz, V. B. Fainerman, U. Pison, *Adv. Colloid Interfaces Sci.* 117 (2005) 33 – 58.

[18] J. Johansson,T. Curstedt, B. Robertson, *Eur. Resp. J.* 7 (1994) 372-391.

[19] S. Hawgood, M. Derrick, F. Poulain, *Biochim. Biophys. Acta* 1408 (1998) 150-160.

[20] J. Johansson, M. Gustafsson, M. Palmblad, S. Zaltash, B. Robertson, T. Curstedt, Synthetic Surfactant Protein Analogues, *Biol. Neonate* 74 (1998) 9-14.

[21] R. Veldhuizen, K. Nag, S. Orgeig, F. Possmayer, *Biochim. Biophys. Acta* 1408 (1998) 90-108.

[22] B.P. Binks, *Curr. Opin. Colloid Interfaces Sci.* 7 (2002) 21-41.

[23] R.J. Pugh, *Adv. Colloid Interfaces Sci.* 64 (1996) 67-142.

[24] F. Ravera, E. Santini, G. Loglio, M. Ferrari, L. Liggieri, *J. Phys. Chem. B* 2006, 110, 19543-19551.

[25] F. Ravera, M. Ferrari, L. Liggieri, G. Loglio, E. Santini, A. Zanobini, *Colloids Surf. A* 2008, 323, 99-108.

[26] L. Liggieri, E. Santini, E. Guzmán, A. Maestro, F. Ravera, *Soft Matter* 7 (2011) 7699-7709.

[27] Y. Y. Zuo, R. A. W. Veldhuizen, A. W. Neumann, N. O. Petersen, F. Possmayer, *Biochim. Biophys. Acta* 1778 (2008) 1947–1977.

[28] L. R. Arriaga, I. López-Montero, J. Ignés-Mullol, F. Monroy, *J. Phys. Chem. B* 114 (2010) 4509-4520.

[29] H. Nakahara, A. Dudek, Y. Nakamura, S. Lee, C.-H. Chang, O. Shibata, *Colloids Surf. B* 68 (2009) 61-67.

[30] U. Pison, R. Herold, S. Schürch, *Colloids Surf. A* 114 (1996) 165-184.

[31] B. Robertson, H. L. Halliday, *Biochim. Biophys. Acta* 1408 (1998) 346-361.

[32] Y. Tanaka, T. Takei, T. Aiba, K. Masuda, A. Kiuchi, T. Fujiwara, *J. Lipid Res.* 27 (1986) 475-485.

[33] A. Maestro, E. Guzmán, E. Santini, F. Ravera, L. Liggieri, F. Ortega, R.G. Rubio, *submitted to Soft Matter* (2011).

[34] M. C. Phillips; D. Chapman, Monolayer characteristics of saturated 1,2-diacyl phosphatidylcholines (lecithins) and phosphatidylethanolamines at the air-water interface, *Biochim. Biophys. Acta* 163 (1968) 301-313.

[35] K. J. Klopfer, T. K. Vanderlick, sotherms of Dipalmitoylphosphatidylcholine (DPPC) Monolayers: Features Revealed and Features Obscured, *J. Colloid Interface Sci.* 182 (1996) 220-229.

[36] M. M. Lipp, K. Y. C. Lee, A. J. Waring, J. A. Zasadzinski, Fluorescence, Polarized Fluorescence, and Brewster Angle Microscopy of Palmitic Acid and Lung Surfactant Protein B Monolayers, *Biophys. J.* 72 (1997) 2783-2804.

[37] J. Ding, D. Y. Takamoto, A. von Nahmen, M. M. Lipp, K. Y. C. Lee, A. J. Waring, J. A. Zasadzinski, Effects of Lung Surfactant Proteins, SP-B and SP-C, and Palmitic Acid on Monolayer Stability, *Biophys. J.* 80 (2001) 2262–2272.

[38] C.Y. Tang, H.C. Allen, Ionic Binding of K+ and Na+ to the Carboxylic Acid Head Group of Palmitic Acid in Monolayers using Vibrational Sum Frequency Spectroscopy, *J. Phys. Chem. A* 113 (2009) 7383–7393.

[39] V. M. Kaganer, H. Mohwald, P. Dutta, Structure and phase transitions in Langmuir monolayers, *Rev. Mod. Phys.* 71 (1999) 779-819.

[40] A. Gopal, K. Y. C. Lee, *J. Phys. Chem. A* 110 (2006) 22079-22087.

[41] F. Bringezu, J. Ding, G. Brezesinski, J. A. Zasadzinski, *Langmuir* 2001, 17, 4641-4648.

[42] C.E. McNamme, M. Kappl, H.-J. Butt, K. Higashitani, K. Graf, *Langmuir* 2010, 26, 14574-14581.

[43] B. Pastrana-Rios, C. R. Flach, J. W. Brauner, A. J. Mautone, R. Mendelsohn, *Biochemistry* 33 (1994) 5121-5127.

[44] G. Ma, H.C. Allen, *Langmuir* 22 (2006) 11267-11274.

[45] L. Gómez-Gil, D. Schürch, E. Goormaghtigh, J. Pérez-Gil, *Biophys. J.* 97 (2009) 2736-2745.

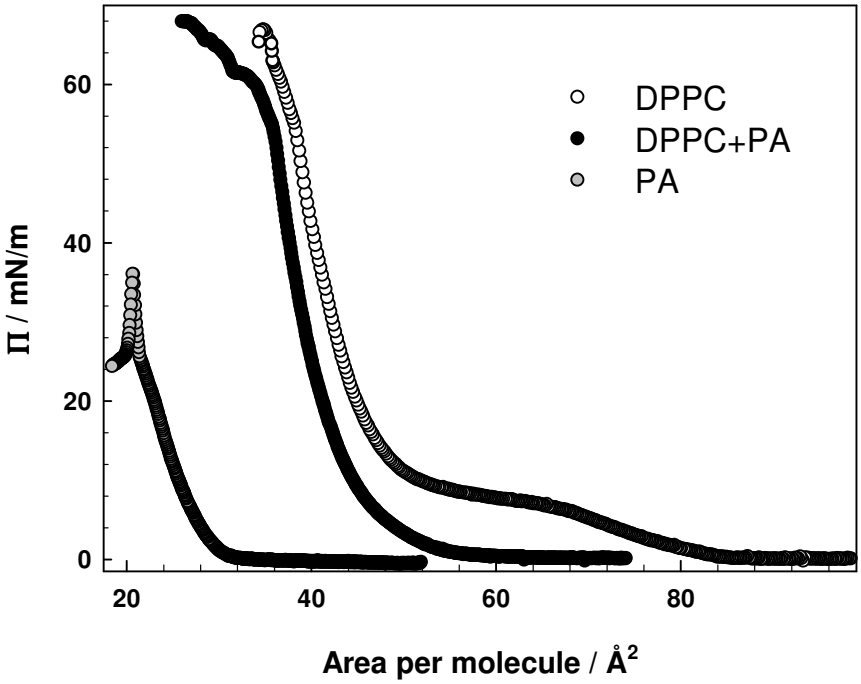

Figure1. *Π-A* isotherms of DPPC (top), PA (bottom) and DPPC+PA (mid) with a ratio 9:1 in weight, Langmuir monolayer on water at 22° C.

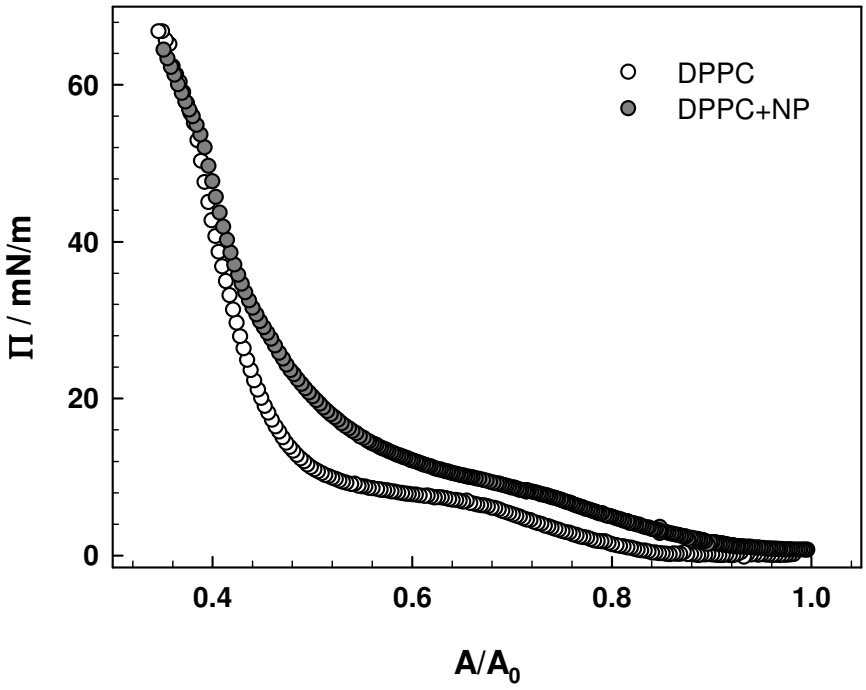

Figure 2. Compression *Π-A* isotherms of DPPC monolayers spread on water and on 1wt% aqueous silica nanoparticle dispersions for initial surface concentration Γ^0= 10 μ mol/m^2.

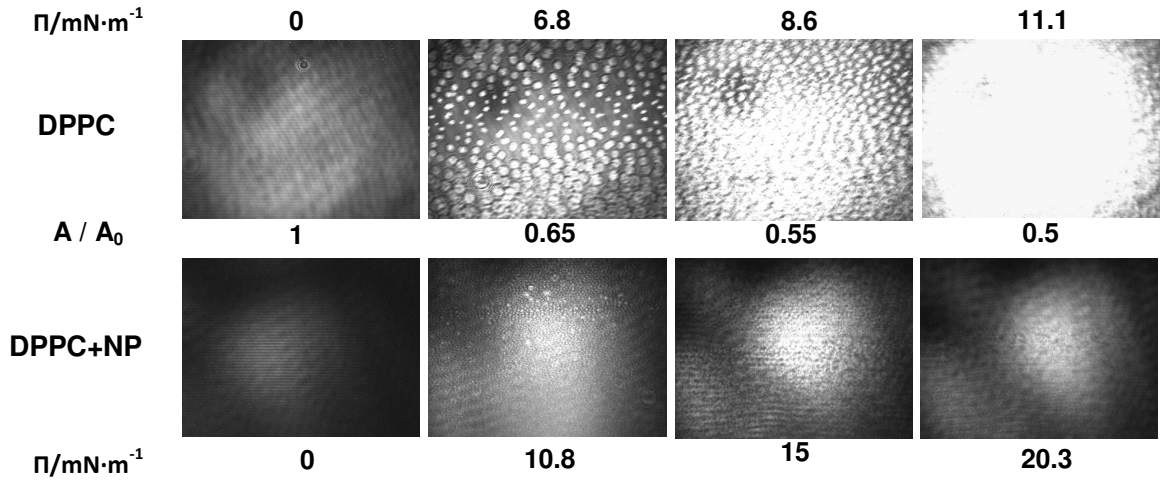

Figure 3. BAM image sequences of DPPC monolayers spread on water (upper) and on 1wt% aqueous silica nanoparticle dispersions (below), corresponding to difference compression degrees. The images size is 311 μm x 418 μm.

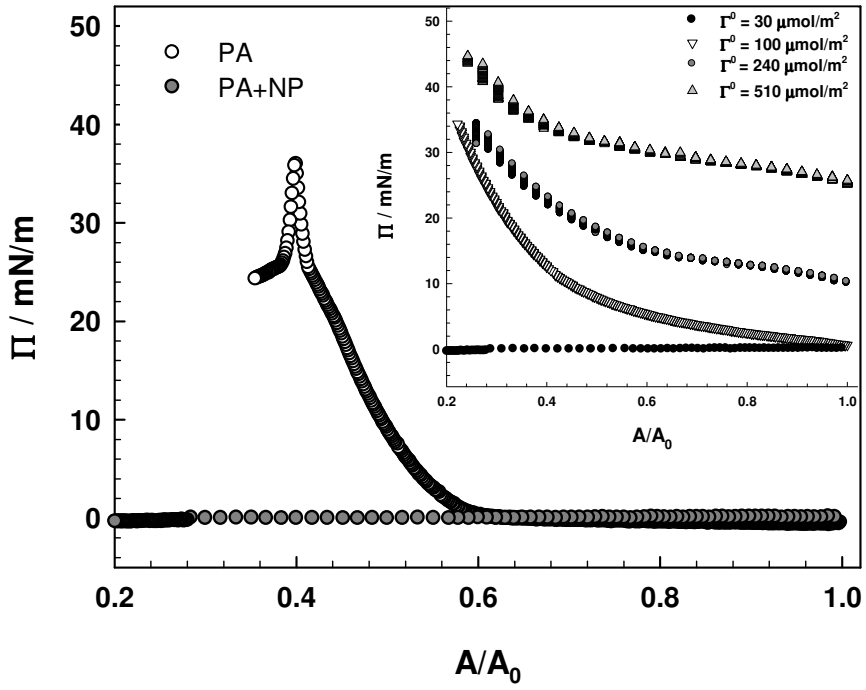

Figure 4. Compression *Π-A* isotherms of PA monolayers on water and on 1wt% aqueous silica nanoparticle dispersions for initial surface concentration Γ^0= 19 µmol/m^2. In the insert compression *Π-A* isotherms of PA monolayer on 1wt% aqueous silica nanoparticle dispersions, for different values of Γ^0.

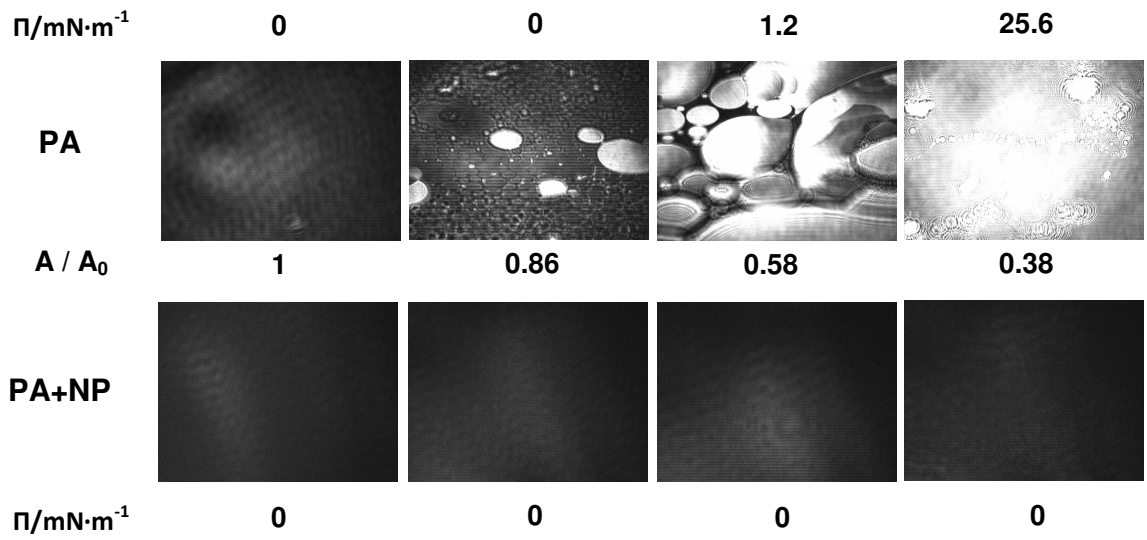

Figure 5. BAM image sequences of PA monolayers spread on water (upper) and on 1wt% aqueous silica nanoparticle dispersions (below), corresponding to difference compression degrees. The images size is 311 μm x 418 μm.

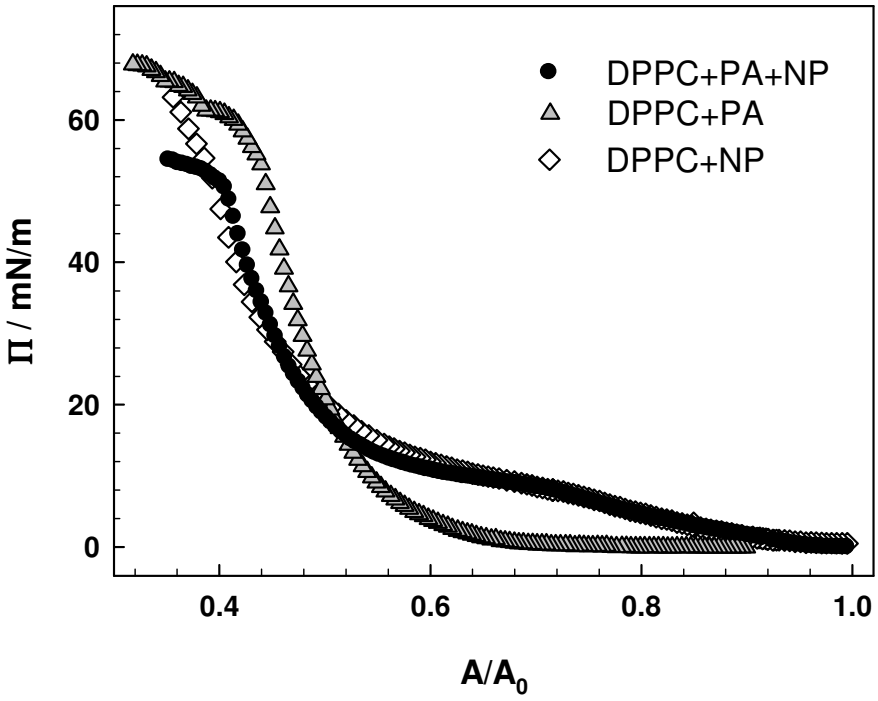

Figure 6. Compression *Π-A* isotherms of DPPC+PA Langmuir monolayer spread on water and of DPPC+PA and DPPC monolayers spread on 1wt% aqueous silica nanoparticle dispersions. The total initial surface concentration is Γ^0= 10 μM/m^2.

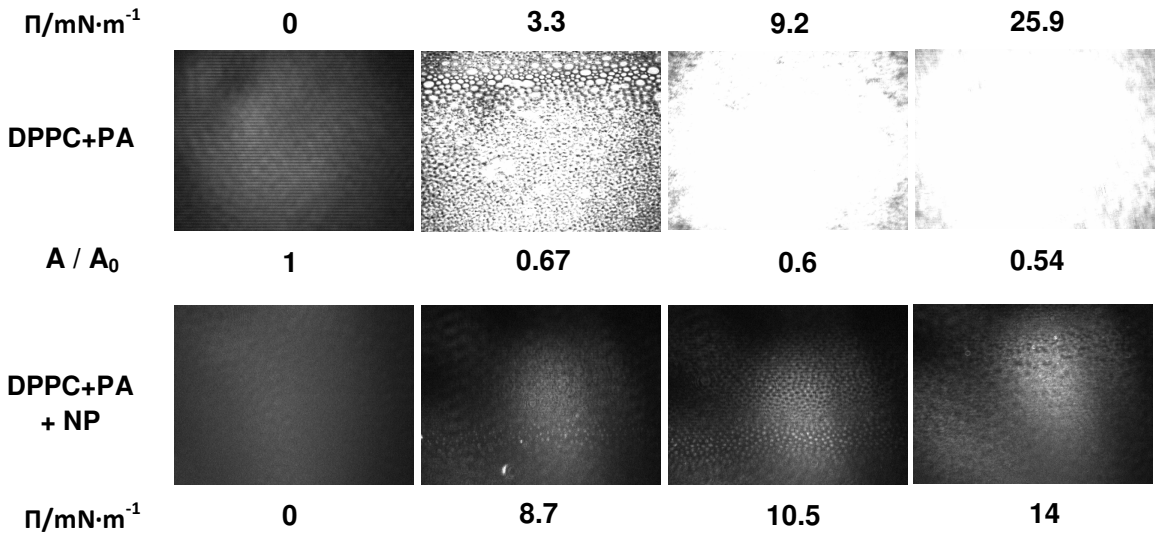

Figure 7. BAM image sequences of DPPC+PA (9:1) monolayers spread on water (upper) and on 1wt% aqueous silica nanoparticle dispersions (below), corresponding to difference compression degrees. The images size is 311 μm x 418 μm.

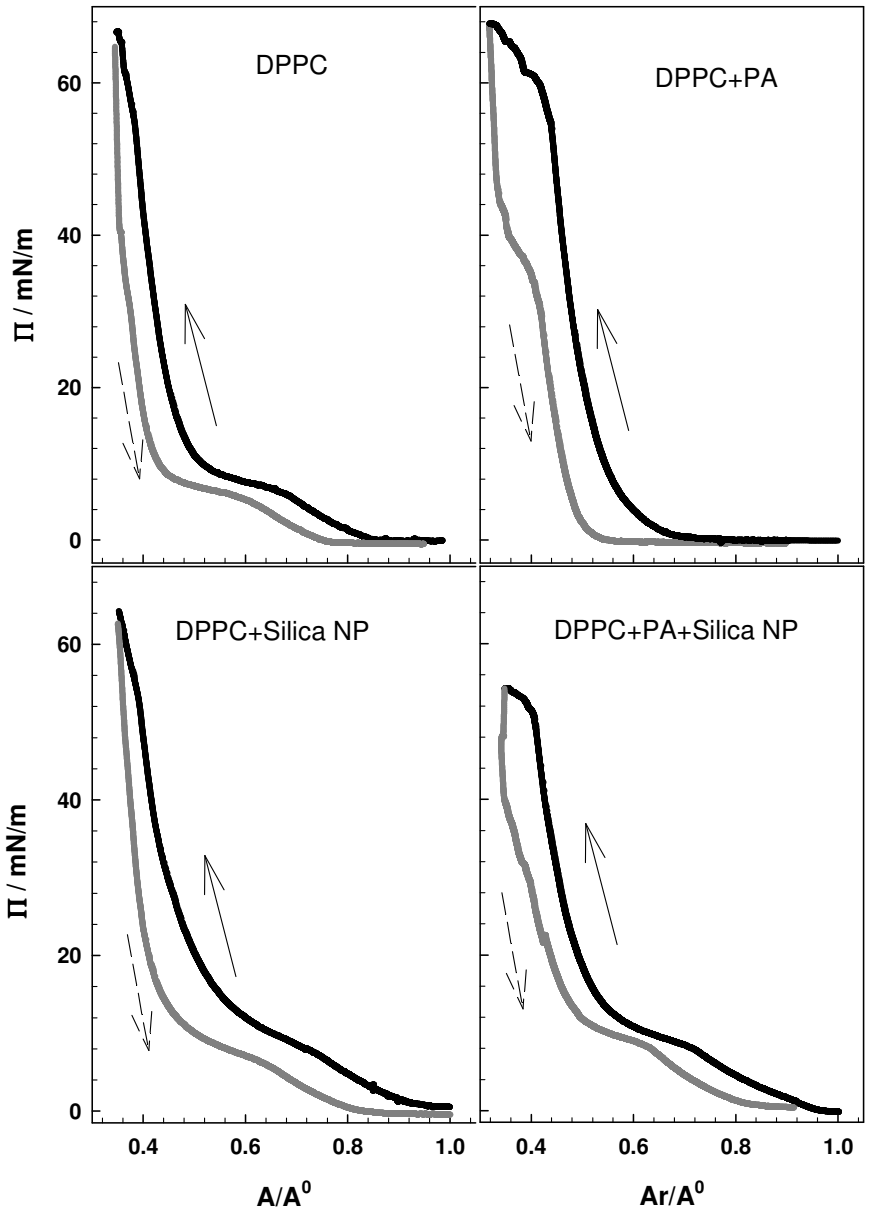

Figure 8. *Π-A* cycles for DPPC and DPPC+PA monolayers, spread on water and on 1wt% aqueous silica nanoparticle dispersions. The arrows show the direction of the area change.

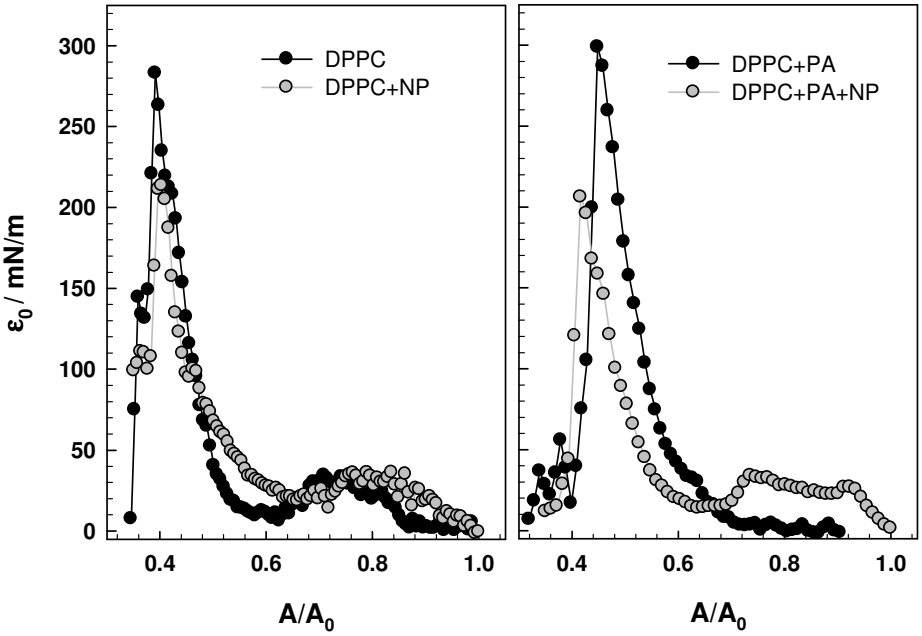

Figure 9. Quasi Static Dilational Elasticity for DPPC (a) and DPPC+PA (b) monolayers spread on water and on 1wt% aqueous silica nanoparticle dispersions.

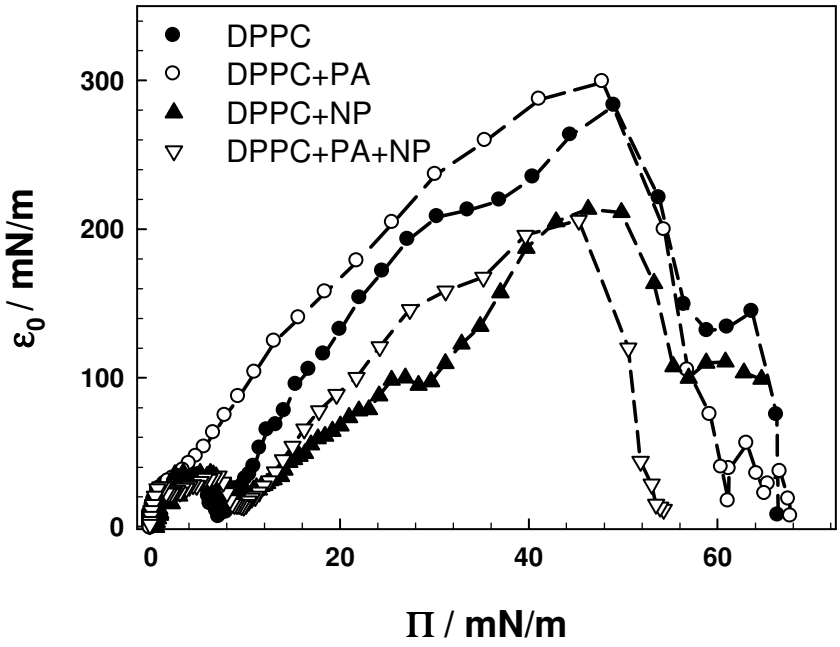

Figure 10. Same data as Figure 9, plotted against the surface pressure.