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# Effect of ions on the interactions of glass nanoparticles with monolayer lung surfactant: applications for the study of inhaled airborne wear particles

B. Munteanu<sup>a\*</sup>, Y. Berthier<sup>a</sup>, J.-P. Rieu<sup>b</sup> and A.-M. Trunfio Sfarighiu<sup>a</sup>

<sup>a</sup>Université de Lyon, CNRS INSA de Lyon, LaMCoS, UMR 5259, F-69621 Villeurbanne, France; <sup>b</sup>Université de Lyon, CNRS – UCBL – ILM, UMR 5306, F-69622 Villeurbanne, France

## 1. Introduction

From a tribological point of view, wear always translates to mass loss accompanied by emission of particles. Although this mass loss is combated for its negative effects, as in the case of mechanical components or prostheses, in some applications such as tribological security the wear can be useful. For example, security of systems transportations is provided largely by the detachment of wear particles of:

- brake pads for efficient braking,
- tyres for the regeneration of the adherence tyre/road, and
- rails to produce a wear that ‘spreads’ faster than microcracks in order to ‘erase’ them.

Every year in France, from the wear of the brake pads of small cars, 9000 tons of airborne particles smaller than 2.5  $\mu\text{m}$  are released into the environment which react with the human body, as shown in a few studies on the increasing number of lung diseases caused by airborne wear particles (Gasser et al. 2009).

These inhaled particles deposit along the lung airways where they are retained by lung surfactant (LS) (Oberdörster et al. 1994), which implies that the structure and function of the LS may be greatly affected by these particles.

LS is a complex structure (Pérez-Gil 2008) (Figure 1) constituting of phospholipids (90%) and protein (10% SP-A, SP-B, SP-C and SP-D) having two major roles: to reduce the surface tension by avoiding pulmonary–alveoli collapse and to act as a barrier against inhaled particles and pathogens. Its dysfunctions lead to severe pathologies such as acute respiratory distress syndrome, often fatal. Recent studies have shown a high influence of nanoparticles (NPs) on pressure response of the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayer (Guzman et al. 2011).

In this context, our study aims to better understand the interaction mechanisms of size-controlled spherical glass NPs with a complex lipid mixture of LS.

## 2. Methods

Our model of LS is composed of DPPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) 1,2-dihexadecanoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DPPS) and 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (DPPE) in a molar ratio of 12:4:3:1 (Pérez-Gil 2008), which provide a closer composition to real conditions with respect to other studies (Guzman et al. 2011). The lipid powders were purchased from Avanti Polar Lipids (Avanti Polar Lipids, Inc., Alabaster, USA) and dissolved in a chloroform:ethanol (9:1 v/v) solvent. All the experiments were done using a Nima Langmuir Trough (Nima KSV Instruments, Espoo, Finland) with a total area of 266  $\text{cm}^2$  and equipped with a Wilhelmy balance for surface pressure measurements and two movable barriers for lateral compression. Thirty microlitres of 1 mM solution of LS were spread on the ultrapure water (18.2  $\text{M}\Omega\text{cm}$ ) sub-phase containing 0.006% spherical ( $\varnothing = 0.5 \mu\text{m}$ ) borosilicate glass NPs. After solvent evaporation ( $\sim 15$  min), the formed monolayer was compressed at a constant compression rate of 20  $\text{cm}^2/\text{min}$  while measuring the surface pressure. From surface pressure  $V_s$  area curves, the equilibrium elasticity modulus  $E$  of the monolayer can be calculated for different surface pressures using the Landau Theory:

$$E = -A \times \frac{dP}{dA} \times \frac{1}{H},$$

where  $A$  is the area between the two barriers,  $P$  is the surface pressure and  $H$  is the monolayer thickness.

At higher surface pressures ( $\sim 35$  mN/m simulating the surface pressure of pulmonary alveoli), the monolayer was subjected to dynamic solicitations using oscillatory barrier experiment simulating breathing cycles described elsewhere (Lucero Caro et al. 2008). This technique allows the measurement of the viscoelasticity modulus  $E'$  as the response of the surface pressure to harmonic variation of the area between barriers  $A$  at controlled amplitude  $\Delta A$  and

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\*Corresponding author. Email: bogdan.munteanu@insa-lyon.fr

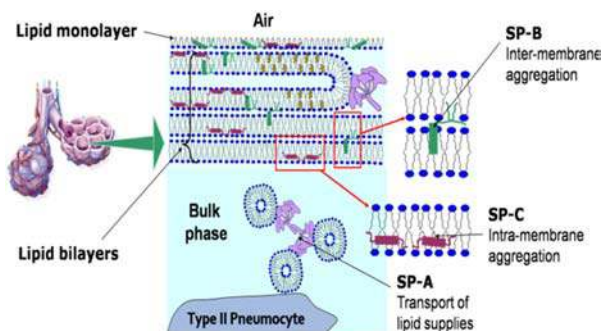


Figure 1. Structure of LS.

frequency  $f$ , using the following relation:

$$E'(f) = \frac{dP}{d \ln A}.$$

For control, measurement was also done without NPs in the subphase.

### 3. Results and discussion

We measured the equilibrium elasticity modulus  $E$  under constant compression rate and viscoelasticity modulus  $E'$  under dynamic solicitations (compression–expansion cycles simulating breath cycles) of LS in the presence of NPs. The equilibrium elasticity of the LS monolayer on ultrapure water measured in the absence of NPs is about 80 kPa (results not shown). By adding approximately 0.006% of 0.5  $\mu\text{m}$  NPs in the subphase,  $E$  decreases to  $\sim 40\text{ kPa}$  (Figure 2). As discussed elsewhere (Guzman et al. 2011), the hydrophilic NPs from the subphase driven by attractive electrostatic forces are incorporated into the monolayer, decreasing its elasticity. The attractive electrostatic forces are established between ammonium groups from positively charged phospholipids hydrophilic heads (+) and negatively charged borosilicate

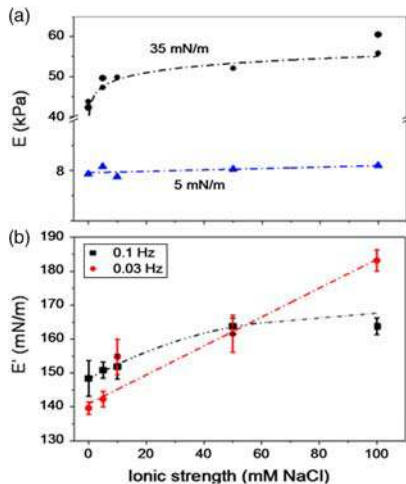


Figure 2. Variation of the equilibrium elasticity  $E$  (a) and viscoelasticity modulus  $E'$  (b) of the monolayer deposited on the glass NPs dispersion as a function of ionic strength and for different surface pressures or frequencies.

glass NPs' surface (-). As can be seen in Figure 2(a), by adding monovalent ions in the subphase for higher surface pressures, the equilibrium elasticity increases.

This phenomenon is based on the strength of electrostatic interactions between NPs and phospholipid heads. In ultrapure water, these interactions are strong and even at high-lateral pressure, the NPs will 'stick' on the monolayer. The presence of monovalent ions in the subphase will screen out the electric field, weakening the electrostatic interactions by binding to free bonds from the NP surface and lipid head. Thus, the NPs being weakly attached to the phospholipid heads are squeezed out from the LS monolayer, increasing its elasticity. The squeeze-out phenomenon is more dominant at high-surface pressures.

The squeeze-out phenomenon is also observable during compression under dynamic solicitations. Thus, the  $E'$  progressively increases from 140–150 to 160–190 mN/m by increasing the ionic strength of the subphase. As observed in Figure 2(b), the variation of  $E'$  is different for different solicitation frequencies, suggesting that the squeeze out of the NPs is a slow process and its rate decreases at higher frequencies.

### 4. Conclusions

We studied the effect of NPs on the mechanical properties in constant compression rate and dynamic compression–expansion cycles of LS.

- A common trend is that the mechanical properties (equilibrium elasticity and viscoelasticity modulus) of LS decrease in the presence of NPs, which can affect the normal function of the lungs. Both  $E$  and  $E'$  are important parameters related to the rigidity of the LS, so efficient LS should have a high  $E$  and  $E'$ .
- Interactions between NPs and LS are mostly electrostatic and their strength decreases by adding monovalent ions in the subphase. The ions will screen out the electric field of NPs and phospholipid heads, respectively, decreasing the Debye length and weakening the interactions between them.

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