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Interactions of insoluble micro- and nanoparticles with the air-liquid interface of the model pulmonary fluids

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Abstract: The work discusses physicochemical phenomena related to interactions between the inhaled particles and the surface of pulmonary fluid which contains the lung surfactant. Dynamic surface phenomena which arise due to periodical variations of the interfacial area during breathing cycle are the extraordinary feature of this system and they are strictly related to the mechanics of ventilation and the pulmonary mass transfer processes. Presence of foreign material such as inhaled micro- and nanoparticles with different size, surface properties and morphology may alter these phenomena which may have some health consequences. This effect is discussed on two examples: mineral particles (CeO₂) and carbonaceous particles emitted from diesel engine running on two different fuels. Two experimental methods of research in this field are presented: the Langmuir balance and the oscillating pendant drop. The results show the sensitivity of dynamic surface properties of the lung surfactant on exogenous materials which may be introduced to the respiratory system by inhalation of dusty air. Some physicochemical interpretation of these results is presented.

Keywords: lung surfactant, pulmonary fluids, air-liquid interface, compressibility, nanoparticle

1. Introduction

Particles behavior on the air-liquid interface (ALI) is a problem of a great practical significance. For instance, the efficiency of flotation process is governed by particles interaction with ALI of air bubbles which depends e.g. on particle wettability, electrostatic interactions and the properties of surface-active compounds present in the slurry. The dynamics of particle behavior in the vicinity of ALI is an important step in understanding its further fate in the system (Niecikowska et al., 2012; Zawała et al., 2017). Another interesting example of practical importance of particles at ALI is their stabilizing effect on liquid foams (Maestro et al., 2015), but on the other hand it is known that some particles can be used as antifoaming entities as well (Denkov and Marinova, 2006). The mentioned processes are effective if particles can either stabilize (immobilize) or destabilize liquid films, which happens only if particles possess surface properties suitable in relation to adsorption of surface-active molecules and induction (or retardation) of liquid microflows due to Marangoni effects. Discussed examples show that the dynamic momentum and mass transfer processes always play the important role in the final outcome of particle-ALI interactions. In this work, we want to demonstrate the occurrence and significance of such interactions in the unique physicochemical system of the lung surfactant (LS) present in living organisms.

1.1 The basic characteristics of the lung surfactant

Lung surfactant (LS) is a mixture of lipids and proteins naturally produced by the pulmonary epithelial cells (Zuo et al., 2008; Chakraborty and Kotecha, 2013). It forms an extraordinary biological

system with vital functions in the respiratory tract. The basic one is related to the reduction of total work of breathing due to decreasing of the surface tension in the aqueous layer which covers the pulmonary (alveolar) epithelium. Due to a huge area of air-liquid interface (ALI) of this region - which can reach 100 m² in human - the work needed for surface extension have a very strong contribution in the total mechanical energy of lung inflation during the active, inspiratory phase of the breathing cycle. It is important to note that the surface tension in this system with periodic compression-expansion of the interface never attains the equilibrium, but instead it continuously follows the perturbations resulting from ALI deformations. Area expansion due to lung inflation (inspiration) leads to a local decrease of surfactant adsorption which, in turn, creates the driving force for the mass transfer of the LS molecules from the liquid subphase to the interface. As soon as the interface starts to shrink during air exhalation, the interface becomes oversaturated what leads to LS desorption. Assuming that the surface tension (γ) instantaneously follows changes in the surface concentration of the LS (Γ), γ is continuously varied according to surfactant adsorption. In fact, such system shows a kind of visco-elastic behavior which is manifested by the surface tension hysteresis schematically shown in Fig.1. The dissipative component of the dynamic response of the ALI to mechanical deformation is related to the rate of surfactant mass exchange between the ALI and the aqueous subphase. The hysteresis itself demonstrates the important feature of the LS system (Notter et al., 1982; Andreassen et al., 2010) and it is also related to the pressure - volume hysteresis recorded during pulmonological tests, considered as an indicator of the proper lung mechanics.

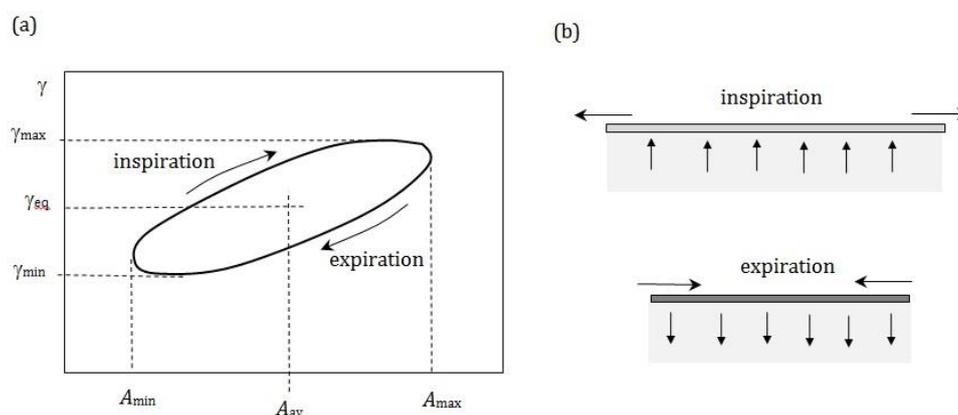


Fig. 1. Surface tension hysteresis (a) and the schematic picture of the surfactant mass transfer (b) during breathing cycle

From the above picture is evident that the kinetics of LS mass exchange between the ALI and the liquid sub-phase must be maintained within a certain regime, adjusted to the physiological rate of surface area changes during breathing cycle. Consequently, if either the mass transfer rate or the surface activity, i.e. the relationship between the surface tension and the surface concentration given by the equation of state: $\gamma(\Gamma)$, is altered by any reason (e.g. lung disease, surfactant inactivation by inhaled materials, etc.), the course of surface tension variations and the shape of the hysteresis will be changed. This, in turn, will have certain consequences for the mechanics of breathing. It is recognized that inactive or abundant LS in the lungs of premature babies leads to life-threatening pulmonary conditions where surfactant supplementation and mechanical ventilation is required to sustain the vital functions (Chakraborty and Kotecha, 2013).

Another, less obvious phenomenon, which is dependent on the surface tension dynamics in the LS system is also of physiological importance. Due to the spatial and temporal asymmetries of alveolar geometry during breathing cycle, variations of the local surface tension are responsible for creation of the Marangoni convection, Fig. 2. These superficial flows along the alveolar walls are responsible for the longitudinal mass transfer which is important for alveolar clearance i.e. the removal of deposited particles from the alveolated regions of lungs (Gradoń and Podgórski, 1989; Podgórski and Gradoń, 1993; Gradoń et al., 1996) but also maybe of significance for pulmonary gas-exchange (Sosnowski et al., 1998; Gradoń et al., 2000).

The importance of the dynamic surface tension in the lungs creates the basis for toxicology-related in vitro studies of the LS under influence of various factors/materials that may be present in air as gaseous or particulate (aerosol) contaminants. Presented physical picture of surface tension-driven processes in the lung alveoli requires that analysis of the interfacial phenomena in the LS system must be always done considering the dynamic conditions similar to those of breathing. This is why the surface tensiometers applied in experimental in vitro studies focused on the LS should allow dynamic variations of ALI. Typical devices employed are Langmuir balance, oscillating drop/bubble or growing bubble tensiometers (e.g. Sosnowski et al., 2017; Sosnowski et al. 2000; Kramek-Romanowska et al., 2015). All of them allow to trace the dynamic surface tension/pressure during mechanical deformation (shrinkage/growth) of the ALI with the surface-active material identical or similar to naturally found in the LS.

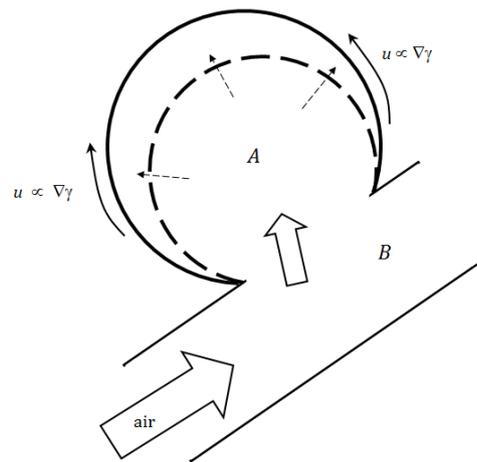


Fig. 2. Marangoni convection ($u \propto \nabla\gamma$) during inspiration i.e. expansion of the alveolus (A). Flow in the opposite direction occurs during expiration which is the longer phase of breathing, allowing the effective removal of deposits from the alveolus to the bronchiole (B)

1.2 Particle-lung surfactant interactions

Micrometer and nanometer-size particles which are present in the air constitute the aerosol and can be inhaled to the respiratory tract, where some of them remain on the lung surface. Deposition of such particles in the respiratory system depends on many factors including particle size, shape, density but also the dynamics of breathing cycle. An example of the relationship between particle size and deposition efficiency in various lung regions of human is presented in Fig. 3.

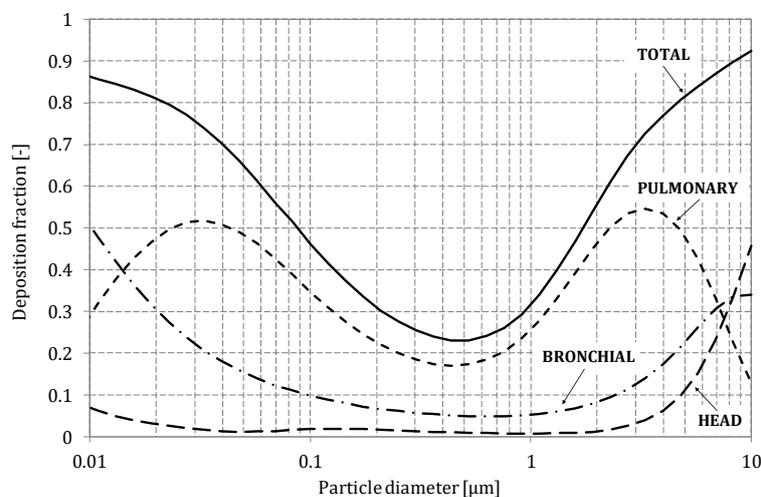


Fig. 3. Regional deposition of inhaled aerosol particles (based on MPPD, 2017)

It is seen that particles smaller than 10 μm are capable of penetration and deposition in the pulmonary (alveolar) region where they can interact with the LS after deposition. Low pulmonary deposition (below 20% in Fig. 3) is expected for particles in the size range 0.1 – 1 μm , but particles with diameter of 1.5-6 μm and 0.02-0.07 μm (nanoparticles) have a high probability of pulmonary deposition in the range of 40-55%. This information is important for the assessment of the inhaled dose of dusts, but also when aerosols are considered as drug carriers for inhalation therapy (Sosnowski, 2016).

Interactions of particles with the LS or its main constituents have been studied in several laboratories world-wide. A set of papers was published by Italian research group (e.g. Guzman et al. 2011; 2015) who studied hydrophobic and hydrophilic nanoparticles at ALI with DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine - the predominant LS phospholipid) and other LS-related lipids using the Langmuir trough. Their investigations were extended by Brewster Angle Microscopy (BAM) and Atomic Force Microscopy (AFM) analyses of the surface film, and the authors were able to demonstrate particles incorporation into the ALI. This process modifies monolayer architecture and mechanical (rheological) response to surface deformation. Particles' properties (hydrophobicity/hydrophilicity) and surface charge strongly influence these interactions. Negatively charged hydrophilic silica nanoparticles were shown to adsorb the phospholipid to form partially hydrophobic complexes which were assembled into ALI. These complexes influence both the compression isotherm and rheological properties of the interface. Hydrophobic nanoparticles (such as carbon black) are assembled with the ALI by the interactions with non-polar parts of lipid molecules, and they have less influence on the dynamics of air-liquid interface. Other researchers (Harishchandra et al., 2010; Tatur and Badia, 2012) demonstrated that different hydrophobic nanoparticles also cause structural changes of the ALI which contain lipids, and particle-lipid complexes are formed which alter the condensation processes during surface contraction (LE-LC transition in the lipid monolayer). Studies on interactions between hydrophilic sub-particles and DPPC monolayer at ALI were done also by Farnoud and Fiegel (2012), and they confirmed that negatively charged particles influence condensation in phospholipid monolayer during ALI compression. Works by Kondej and Sosnowski (2013; 2014; 2016a) were focused on nano-structured clays interacting with DPPC and the multicomponent LS on ALI with variable area. These authors demonstrated that all types of tested particles influence the dynamic surface tension in model LS systems, however there is the evident difference between the effect caused by hydrophilic particles (bentonite and halloysite) and hydrophobic, surface-modified montmorillonites. Some recent studies by various authors were focused also on the influence of particle size on alteration of the model LS on compressed ALI (Dwivedi et al., 2014; Kodama et al., 2014).

In this work, we present new results related to interactions of selected nanosize and nanostructured particles with the ALI in model LS systems. To demonstrate different possibilities of in vitro assessment of particle influence on the lung surfactant, two separate experimental approaches are shown in the studies of two types of insoluble particles which may contaminate the air we breathe.

2. Experimental evaluation of particle-lung surfactant interactions

2.1 Influence of micro- and nanoparticles of cerium oxide on mechanical properties on phospholipid monolayer as a model of the LS

Cerium oxide CeO_2 in powder form is used as a colorant in polymer industry, as an active additive in electronics (UV absorption, antioxidant agent) and as polishing material. Because of the mentioned industrial applications, CeO_2 aerosol can be found in several occupational settings creating a health hazard for workers. Moreover, sub-micrometric CeO_2 is used as a catalytic additive to diesel fuels, so it can be emitted to the atmosphere with diesel exhaust (Gantt et al., 2012). By inhalation, CeO_2 particles can penetrate to deep lungs and influence the properties of lung liquids after deposition. Due to a small sizes and large specific surface area, the impact of CeO_2 particles on the lung surfactant can be significant. Some preliminary data on the interactions of CeO_2 with a model LS have been reported recently, suggesting that such particles change the equilibrium surface tension (Kondej and Sosnowski, 2016b). Here we present new results which extend our knowledge on the influence of these particles on the dynamic properties of LS.

2.1.1 Materials and methods

Three types of CeO₂ particles were analyzed. CeO₂ powders denoted as C1, C2, and C3 were purchased from Sigma-Aldrich. According to the supplier's data, they were characterized by a different size distribution with the nominal particle sizes $d < 25$ nm (powder designation: C1), $d < 50$ nm (C2) and $d < 5$ μm (C3). The specific surface area SSA was measured by multipoint BET method (Gemini 2360 - Micromeritics, USA) and this data are shown in Fig. 4. As expected, powders with high content of nanoparticles (C1 and C2) have much higher SSA (≈ 30 m²/g) than the powder which contain larger, micro-size particles (C3, SSA ≈ 3 m²/g).

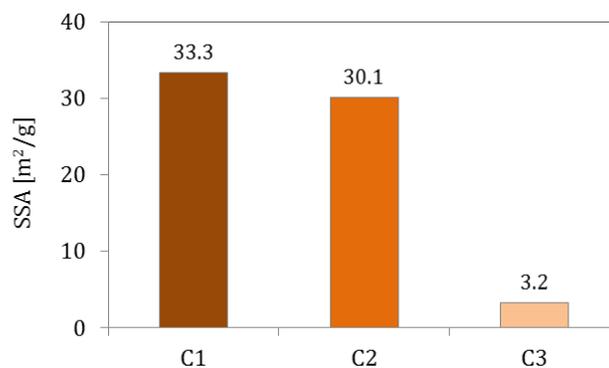


Fig. 4. Specific surface area SSA of three types of CeO₂ particles

The studies focused on particle-LS interactions were done at 37 ± 0.1 °C in a thermostated Langmuir-Willhelmy balance (model LB5000 - KSV, Finland) equipped with the minitrough (75×300 mm, maximum surface area: 242 cm²) and two symmetrical barriers needed to change the ALI area. In all experiments the ALI was compressed with the rate of 1.25 cm²/min. The LS was modeled by a monolayer of DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 99.9% pure, Sigma-Aldrich) formed directly at ALI from CHCl₃ solution (1 mg/ml). Water purified in filtration-reverse osmosis-adsorption system (Puricom, USA) was used as a subphase. The aqueous subphase was intentionally contaminated with CeO₂ (concentrations: 0.25, 0.5, 0.75 and 1 mg/cm³) to study the effect of these particles on the dynamic properties of the LS at the air/liquid interface. The applied concentrations correspond to the predicted cumulative pulmonary doses of deposited nano-powders during occupational inhalation exposure (Kondej and Sosnowski, 2013). Dispersions of CeO₂ in water was prepared by sonication (4,8 kJ/cm³, Sonic 14 - Polsonic, Poland) to reduce particles agglomeration in the aqueous environment. The results were obtained as compression curves: π - Ω , where π denotes the surface pressure [(mN)/m] and Ω - the surface area per DPPC molecule [\AA^2 /molecule], which were later recalculated to κ - π functions, where κ [m/N] denotes isothermal surface compressibility:

$$\kappa = - \left. \frac{1}{A} \frac{dA}{d\pi} \right|_{T=\text{const}} \quad (1)$$

2.1.2 Results and discussion

Figures 5 a-c show the compression isotherms obtained at different concentrations of each type of CeO₂ particles: C1, C2 and C3. The effect of CeO₂ on dynamic surface-active properties of DPPC is clearly visible. C1 particles even at the lowest concentration (0.25 mg/cm³) cause a shift of the compression isotherm towards lower molecular areas. The curves obtained at higher particle concentrations (0.5 and 0.75 mg/cm³) are very similar but a further shift is observed at C1 particles concentration equal to 1 mg/cm³. It suggests that C1 particles diffuse to the interface and lead to a partial decrease of surface-tension lowering properties of DPPC, most probably by binding (adsorbing) some of phospholipid molecules and forming partially hydrophobic aggregates which occupy some of the area in the surface layer. It may be noted, for instance, that LC-LE coexistence phase starts at approximately 70 \AA^2 /molecule in pure DPPC, but at approximately at 65 \AA^2 /molecule in the mixed monolayer when C1 particles are present in the subphase at the concentration of 1 mg/cm³.

C2 particles which are generally larger but have similar SSA as C1 show a similar behavior - the compression isotherm π - Ω is gradually shifted as particle concentration increases. C3 particles which have much lower SSA and are larger in size, induce no effect at low concentration (0.25 mg/cm³), however the isotherm is shifted to the left at higher particle contents. Observed results suggest that nanoparticles C1 and C2 induce interfacial effects already at lower concentration than microparticles C3, which can be attributed also to differences in the specific surface area available for adsorption of phospholipid molecules. Different effect of nanoparticles in comparison with larger particles is similar to observed by other authors (e.g. Dwivedi et al., 2014).

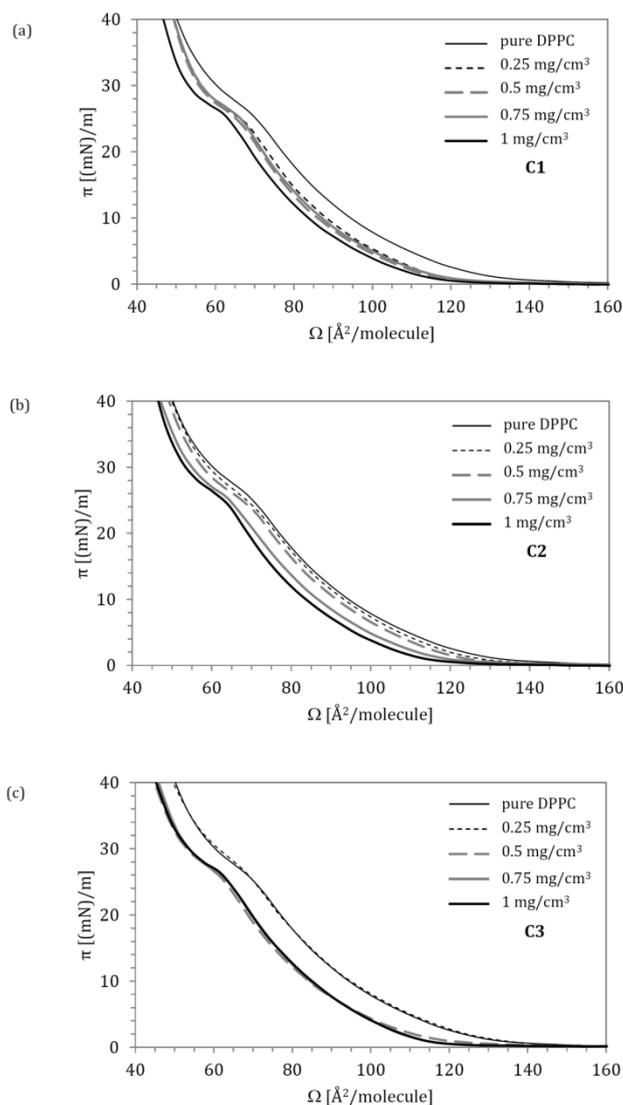


Fig. 5. Compression isotherms ($37 \pm 0.1^\circ\text{C}$) of DPPC in the presence of CeO_2 particles in the aqueous subphase: (a) C1, (b) C2 and (c) C3

The compressibility graphs presented in Fig. 6 a-c confirm the conclusion found from the isotherms. It can be seen that the maximum compressibility increases when CeO_2 particles are present in the system (from $\kappa \approx 36.5$ m/N for pure DPPC to $\kappa \approx 40$ -42.5 m/N for the highest particle concentration). Moreover, this maximum occurs at lower surface pressure (shift from $\pi \approx 29.5$ (mN)/m to $\pi \approx 28$ (mN)/m). It means that CeO_2 particles influence the mechanical properties and structure of the surface film, leading to a more compressible (i.e. less elastic) air-liquid interface at the certain range of π . These results may have some implications regarding the CeO_2 - LS interactions in the human lungs. If the amount of micro- and nanoparticles deposited in alveolar region is high enough,

such particles may collect some of the native surface-active material and reduce the local LS concentration in the lung fluid and at the pulmonary ALI. In such circumstances, the surfactant cannot properly fulfill its role related to periodic variations of the surface tension during breathing cycle, so both the mechanical and mass transport functions of the LS may be impaired.

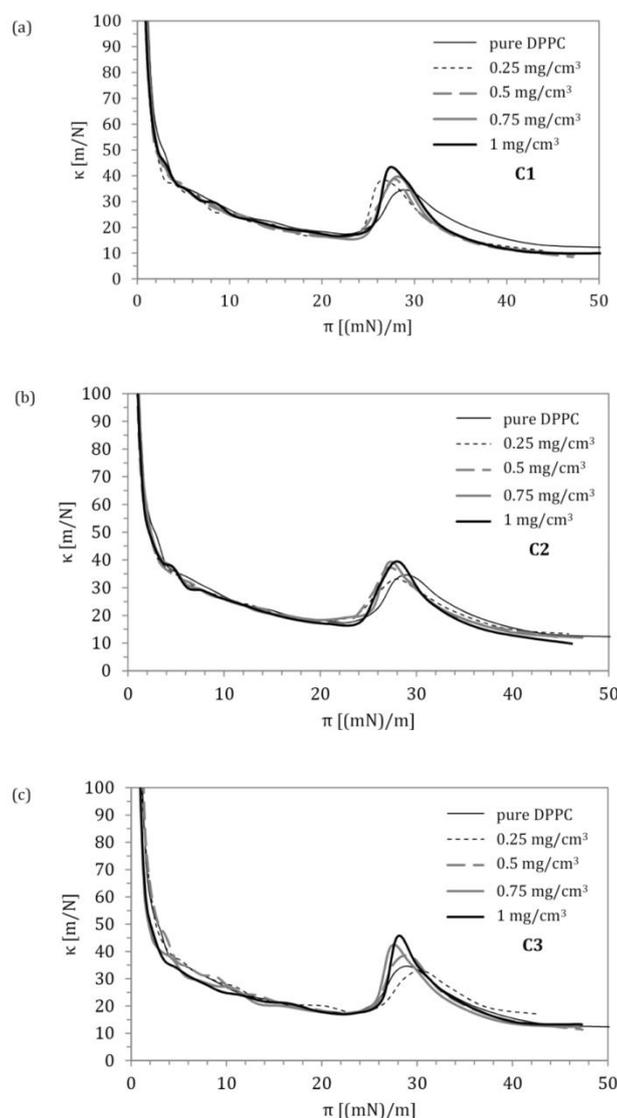


Fig. 6. Isothermal ($37 \pm 0.1^\circ\text{C}$) surface compressibility of DPPC monolayer in the presence of CeO₂ particles in the aqueous subphase: (a) C1, (b) C2 and (c) C3

2.2 Diesel fractal-like particulates interacting with multi-component model of the LS

Diesel particles (DPs), similarly to other combustion aerosols, form air contaminants which are dangerous for health (Pope, 2002). Their toxic potential is related not only to the small size and high surface area per mass, but also to the fact that DPs are usually carriers of volatile organic compounds (VOCs) formed during incomplete fuel combustion in the engine (Maricq, 2007). Many of VOCs are known to be carcinogenic (Boström et al., 2002). Due to the way of DPs formation, these particles typically have a form of nanostructured micrometer-size aggregates with a fractal-like geometry. Their SSA is up to 90 m²/g (Shaw et al. 2011).

2.2.1 Materials and methods

In this work, we analyzed DPs formed in the naturally-aspirated diesel engine (240D, Mercedes Benz - the laboratory stand) running on two types of diesel fuel: Verva ON and Eco-diesel (both from PKN

Orlen S.A., Poland). The choice of these two fuels was justified by the results of the previous studies (Penconek et al., 2013) which have shown that the size and morphology of particles emitted from the engine are different for each of these fuels, which also takes effect on their separation efficiency in filters. The particle size was determined by FAPES aerosol spectrometer (Grimm, Germany). Figure 7 shows SEM pictures indicating different particle structure and average size (ECMD denotes the equivalent count median diameter determined by FAPES device).

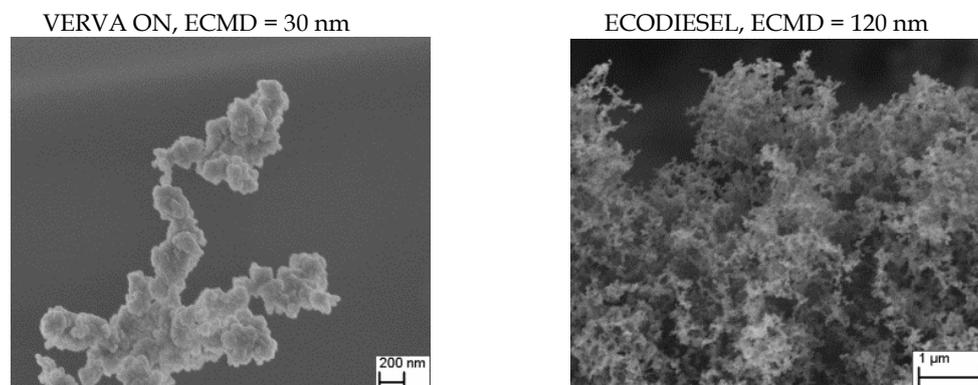


Fig. 7. SEM micrographs of aggregated diesel particles obtained from the engine running on different fuels. ECMD denotes the equivalent count median diameter

For the experiments focused on DPs interaction with the LS, the particles were collected in the laboratory impaction system and then recovered to separate vials. The influence of DPs on the model LS was studied with the oscillating drop method (ODP) using PAT-1M pendant drop tensiometer (Sinterface, Germany). Curosurf® (Chiesi, Italy) - a commercial standardized lung surfactant material obtained from pig lungs - was used as the LS model in these studies. When compared to the pure phospholipid (DPPC) model presented in the previous section, this material more realistically reproduces the composition of natural LS. On the other hand, due its limited availability for experimental in vitro studies (Curosurf delivered in 3 cm³ vials is the expensive intensive care medicine) it cannot be used for extensive studies in Langmuir balance. The ODP method has the advantage of a very low consumption of this material (microliters per single experiment). Curosurf diluted to the final phospholipid concentration of 8 mg/ml was mixed with DPs to obtain particle concentration 0.005 and 0.01 mg/cm³ (they correspond to doses received during short-term exposure to diesel exhaust). The samples were tested during sinusoidal drop oscillation at isothermal conditions (37 ± 0.2 °C) at frequencies $f = 0.1, 0.125, 0.25, 0.33$ and 0.5 Hz, which correspond to breathing cycle in human at various physical activity. The applied oscillation amplitude was set to 10% to assure that the measurements are done within the linear range of mechanical response to the interfacial deformation. Based on the measured dynamic surface tension data, the effective dilatational rheological parameters of the interface were determined (surface dilatational elasticity, ε [(mN)/m], and surface dilatational viscosity, μ [(mN s)/m]). Effective properties mean that they are dependent not only on intrinsic mechanical properties of the interface but also on other dynamic processes which take place during surface variation (e.g. mass exchange).

2.2.2 Results and discussion

Figures 8 and 9 show the dependence of surface elasticity and viscosity on deformation rate (frequency) and concentration of DPs produced from Verva ON and Ecodiesel fuels, respectively. It is seen that both types of particles have a certain influence on the dynamic response of the ALI to breathing-like deformations, but the effect is dependent on particle concentration and particle type. For all samples studied the elasticity increases and viscosity increases at higher deformation rates which confirms that dissipative effects (related e.g. to the mass exchange between the ALI and the subphase) become significant at longer time-scales. It is not surprising since all relaxation processes take effect after a certain period of time. Therefore, for faster cycles, the elastic response is predominant over the viscous response (e.g. for pure Curosurf at 0.5 Hz: $\varepsilon \sim 35.5$ (mN)/m, $\mu \sim 7$

(mN·s)/m) as compared to slower cycles (0.1 Hz: $\varepsilon \sim 20$ (mN)/m, $\mu \sim 20$ (mN·s)/m). Surface viscosity is related to the presence of surface tension hysteresis discussed in the Introduction.

DPs in the LS sample induce an increase of surface elasticity, however the effect is different for each particle type. For those obtained from Verva ON fuel, the elasticity increases at particles concentration 0.01 mg/cm^3 while no influence is observed for lower concentration (0.005 mg/cm^3 - Fig. 8a). For Ecodiesel fuel, the increase of elasticity is visible already at 0.005 mg/cm^3 and higher concentration does not change this relationship (Fig. 9a). Particle influence on viscosity is also different for each type of particles, where practically no effect is found for Verva ON fuel (Fig. 8b) and an increase of μ is observed already at 0.005 mg/cm^3 of particles obtained from Ecodiesel fuel (Fig 9b).

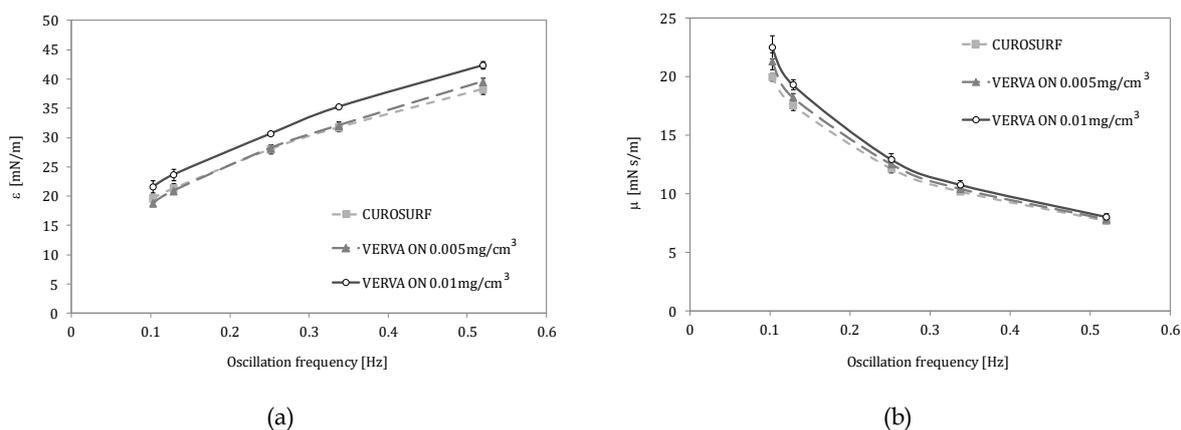


Fig. 8. Surface elasticity, ε , and surface viscosity, μ , of ALI with Curosurf in the presence of DPs emitted from the engine running on Verva ON fuel

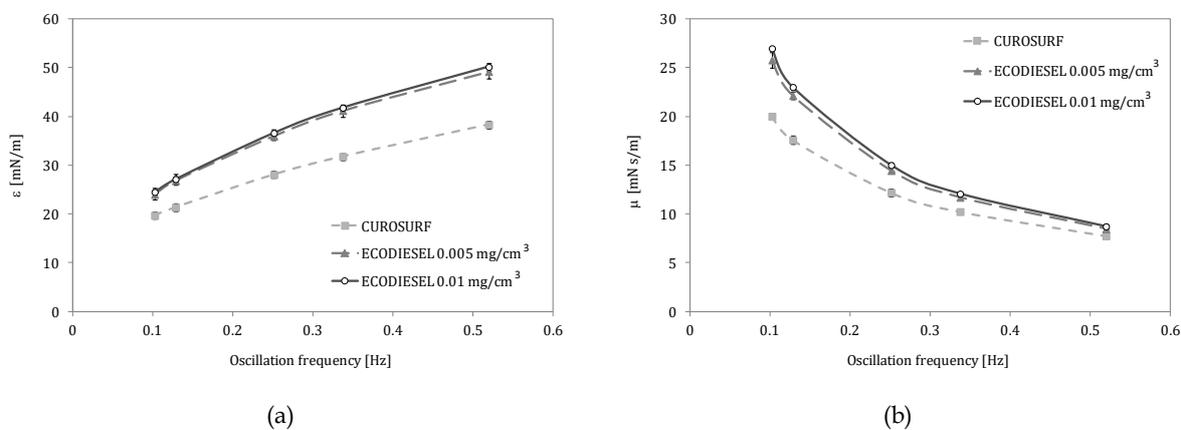


Fig. 9. Surface elasticity, ε , and surface viscosity, μ , of ALI with Curosurf the presence of DPs emitted from the engine running on Ecodiesel fuel

These effects show that tested DPs interact with the ALI at low particle concentrations, and differences in particle morphology have influence on these interactions. DPs are mostly hydrophobic and they may carry some additional organics (including poly-aromatics) adsorbed on their surface. Hydrophobic interactions between DPs surface and LS molecules may increase surface activity of the lung surfactant, but at the same time can influence the mass exchange rate during variations interfacial area. The more detailed analysis of these phenomena is not possible without additional studies, however, based on the already obtained results it can be confirmed that DPs influence properties of the LS. The outcome of this material- and dose-dependent interactions may have certain implications for natural functions of the lung surfactant in vivo. In fact, some human and animal studies suggest that lung functions, including those dependent on LS activity, are impaired after inhalation of diesel exhaust (Heinrich et al., 1986; Salvi et al., 1999; Ghiao et al., 2012)

3. Conclusions

The aim of this paper was to present the basic information regarding the technical requirements of the research in the field of particles interactions with the air-liquid interface containing the lung surfactant. The specific conditions of alveolar interface during breathing cycle in vivo requires that useful data on particle-LS interactions can be gathered only in the experiments which consider dynamic surface tension at the ALI with variable area. Two experimental approaches have been highlighted: Langmuir-Willhelmy balance and oscillating drop tensiometry, both allowing to obtain different but informative and quantitative data. The results obtained for CeO₂ nano- and microparticles and diesel exhaust particles indicate that each type of particulates is capable of altering the dynamic surface properties of the LS system, however the actual response is strongly dependent on particles concentration, size, surface properties and morphology. Presented physicochemical methods of the assessment of particle-lung interactions may be useful in preliminary analysis of a toxic potential of many different inhaled nano- and microparticles considered as air contaminants.

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