Effect of Diesel-Emitted Nanostructured Particles on the Apparent Surface Rheology of Model Lung Surfactant

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Abstract* – *Diesel exhaust contain nanostructured particles which may be inhaled and may interact with the lung surfactant (LS) present in the respiratory system. This work reports the experimental analysis of surface tension variations in model LS system which contain diesel exhaust particles (DEPs). Experiments of oscillatory deformations of air/liquid interface under physiological-like conditions allowed to determine the changes in apparent surface rheological parameters, which indicated evident alteration of mechanical properties of LS caused by DEPs. These results allow to speculate on possible adverse effects of inhaled DEPs on LS in vivo.

Key words – nanostructured aerosol particles, diesel exhaust, lung surfactant, surface rheology, health effects.

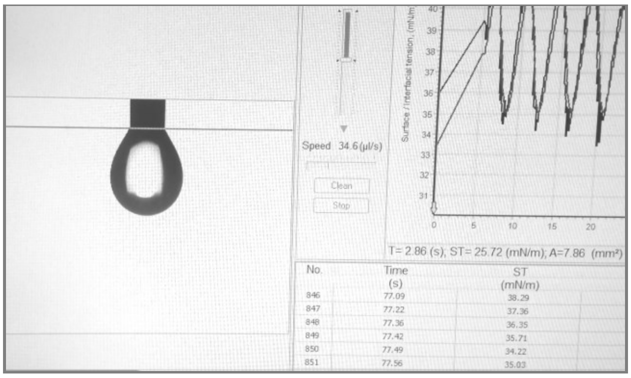
I. Introduction

Aerosols emitted from diesel engines contain submicron or micrometer-sized aggregates of primary nanoparticles [1,2]. Typical shape of these aggregates is fractal-like, however, for a given engine, it may depend on type of diesel fuel used [1]. Air pollution due to diesel exhaust particles (DEPs) is known to contribute to pulmonary effects of people who are exposed to them [3]. Although modern cars are equipped with Diesel Particulate Filters (DPFs) which eliminate the aerosol emission, older vehicles as well as many field machines and devices driven by diesel engines still remain the sources of inhalable DEPs.

Inhaled particles which are deposited in the lungs, meet the first barrier formed by, so called, lung surfactant (LS). This specialized structure is composed of biosurfactants produced by type II alveolar cells and it plays essential role in lung mechanics and pulmonary mass transfer [4,5]. Previous studies done in our laboratory demonstrated that analysis of the surface tension of LS measured with a variety of in vitro techniques allow to evaluate the influence of airborne particles on integrity and functionality of this biological structure [5-7]. The current work is focused on the measurements of interfacial effects caused by DEPs contacted with a model lung surfactant.

II. Methods

Curosurf (Chiesi, Italy) was used as a LS model. DEPs were collected from the emission of Mercedes Benz 240D engine used in our laboratory [1]. The engine was supplied with petrol diesel fuel (Verva - Orlen, Poland). Particle size of the fresh aerosol emitted from the engine was analyzed using FAPES spectrometer (Grimm, Germany) as described elsewhere [2,8]. Matured aerosol particles (aggregates) were collected on the fibrous filter and analyzed by scanning electron microscopy (SEM). Dynamic surface tension, σ [mN/m], of pure LS and LS mixed with DEPs (various particle concentration: 0.5, 1 and 2.5 mg/ml) was measured under simulated physiological conditions, i.e. oscillations of air/liquid interface (36.6 ± 0.2°C) with drop shape analysis tensiometer PAT-1M (Sinterface, Germany) - Fig. 1.



T= 2.86 (s); ST= 25.72 (mN/m); A=7.86 (mm²)

No.	Time (s)	ST (mN/m)
846	77.09	38.29
847	77.22	37.36
848	77.36	36.35
849	77.42	35.71
850	77.49	34.22
851	77.56	35.03

Fig. 1. Screen snapshot showing the principle
of surface tension measurement by drop shape analysis

Drop oscillations were done for frequencies, *f* [Hz], which corresponded to the typical rates of alveolar surface variation during breathing (2, 4 or 8 s for a full cycle of inhalation and exhalation). At such conditions the air/liquid interface of LS system demonstrates a visco-elastic response, which is reflected by the surface tension-surface area hysteresis [4,5]. Based on results obtained during sinusoidal oscillations of the liquid drop, apparent rheological parameters of the interface were determined (surface dilatational elasticity, ε [mN/m], and surface dilatational viscosity, μ [mN·s/m]). It should be stressed, that discussed apparent rheological properties of the surface are related only to dialatational deformation (surface expansion/contraction), not to surface shear.

III. Results and discussion

Fig. 2 presents the SEM picture of DEPs obtained during earlier studies [8]. Size distribution of primary particles emitted from the engine is depicted in Figure 3.

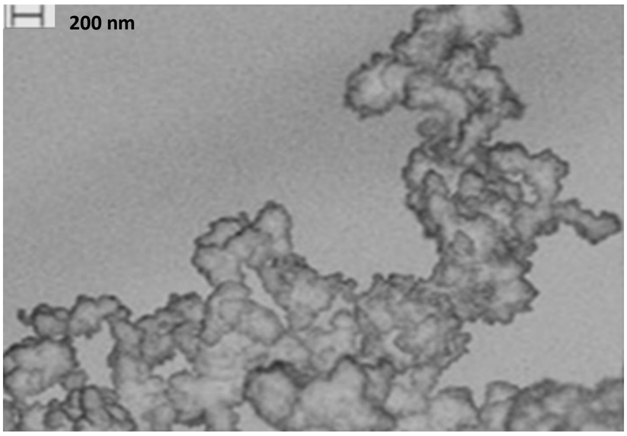


Fig. 2. SEM picture of DEP nano-aggregate

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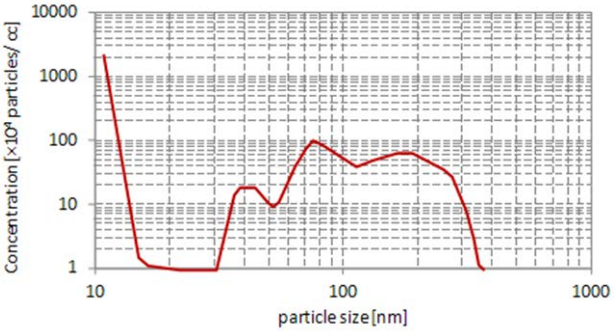

Fig .3. Size distribution of primary diesel exhaust particles

It can be seen that the finally formed nanostructured particles are dendrite-like aggregates. They are composed of primary particles with 10-350 nm in size. Coagulation of these primary particles takes place during exhaust mixing with ambient air and aerosol maturation [8]. As a result, the aggregated structures are formed. Their size is still within the respirable range (may be above 1 µm), which allow them to reach the alveolar region of the human lungs during inhalation.

A sample result of surface tension variations during oscillation of LS droplet in PAT-1M device is presented in Figure 4. Analogous type of results was obtained for all tested frequencies of droplet oscillations and for LS-DEPs mixtures at all studied concentrations. Each type of such experiment was triplicated.

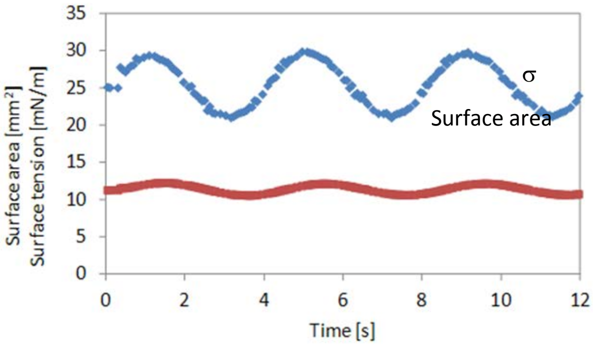

Fig. 4. Time variations of air/liquid interfacial area (red) and the surface tension (blue)

Based on obtained results, fast Fourier transform (FFT) was performed (the algorithm was available in the software of PAT-1M instrument), which allowed to calculate ε and µ as a function of oscillation frequency. These rheological parameters of air/liquid surface for the lung surfactant in presence of DEPs at different concentrations are shown in Figures 5a-d.

Figure 5a demonstrates that higher frequency of surface oscillation in the LS system leads to the increase of surface elasticity and the decrease of surface viscosity. It is not surprising since the interface always becomes more solid-like at high-frequency deformations. High viscosity found at low *f* means that surface tension hysteresis loop during surface oscillation is broad, however, since ε is low, the amplitude of surface tension variation remains small. Decrease of µ at higher oscillation frequencies means that the hysteresis becomes smaller.

It should be stressed that surface tension hysteresis measured in the LS system is often considered as a marker of surfactant quality and an indicator of its proper physiological functionality related to gas exchange and self-defense mechanisms in the lungs [4,5]. Therefore we focus on the hysteresis during analysis of the influence of DEPs on the LS.

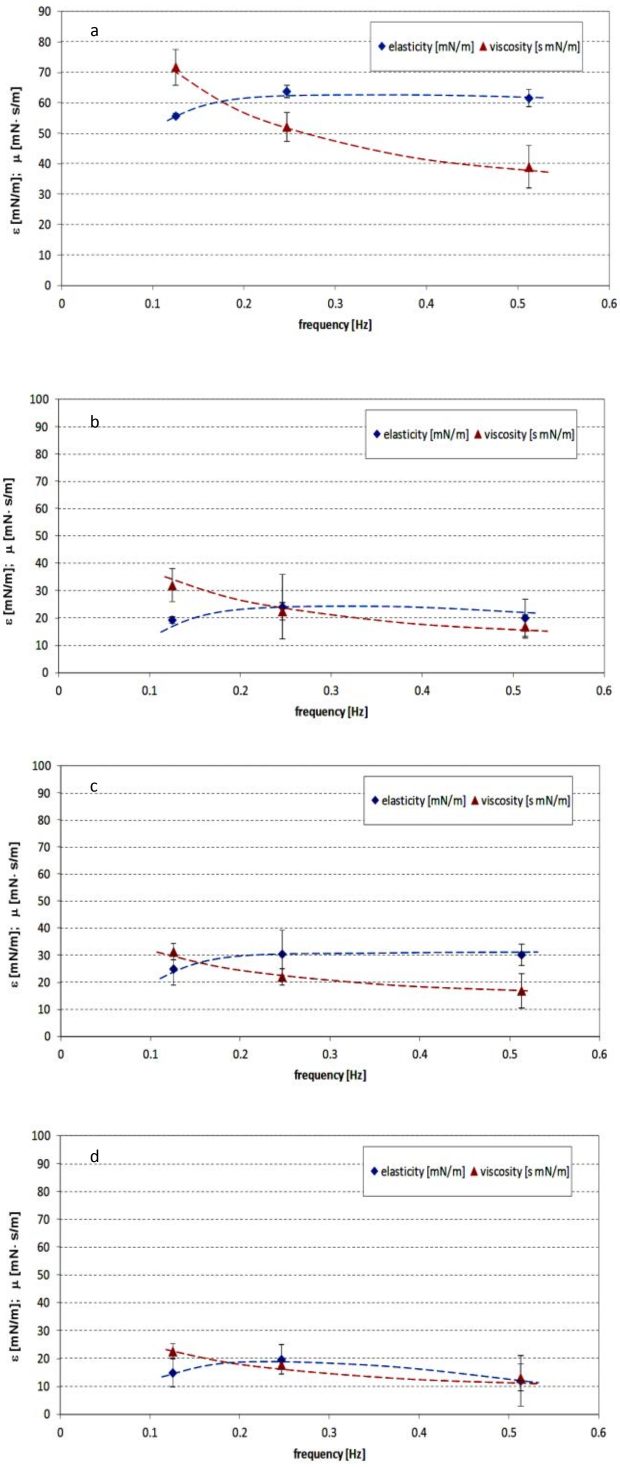

Fig. 5. Dilatational surface elasticity and dilatational surface viscosity in (a) LS system (Curosurf 4 mg/ml); (b) mixed LS-DEPs (0.5 mg/ml); (c) mixed LS-DEPs (1.0 mg/ml); (d) mixed LS-DEPs (2.5 mg/ml). All measurements at 36.6±0.2°C

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Presence of DEPs significantly reduces both the surface elasticity and surface viscosity of LS system in a concentration-dependent manner. For the highest DEPs concentration (2.5 mg/ml), ε is less than 20 mN/m for all frequencies studied which means that surface tension variations during successive compressions and expansions of air/liquid interface gets smaller. Strong reduction of μ in that case indicates that surface tension hysteresis becomes very minute. However, even low DEPs concentrations (0.5 mg/ml - Fig. 5b) significantly reduce ε and μ as compared to the values found for pure LS (Fig. 5a). These results confirm that mechanical properties of air-liquid interface are notably disturbed by nanostructured DEPs. It should be noted that rheological parameters measured in the system have their origin both in intrinsic surface properties (i.e. intermolecular forces of components adsorbed at the interface) but also in the mass transfer phenomena near and on the oscillating air/liquid interface [10]. Therefore, both parameters (ε and μ) should be considered as apparent ones.

One possible explanation of decreased ε and μ in presence of DEPs is that nanostructured particles, which obviously have very high interfacial area, offer the space for surfactant adsorption. If LS adsorption on these particles takes place, the amount of free surfactant in the liquid is reduced, so the rate of mass transfer and surfactant adsorption at the air/liquid interface must be lower. Such phenomena should be reflected by alterations in air/liquid surface response to deformations.

It is also possible that DEPs are co-adsorbed with LS at the air/liquid interface, so they directly influence intermolecular interactions in the interfacial region. By this mechanism surface may become less elastic, however surface viscosity is also reduced. In effect, surface tension hysteresis is disturbed (decreased), what suggest that adverse health effect may be expected in vivo.

Conclusions

Presented experimental analysis indicate that nanostructured particles present in diesel exhaust are capable of distorting the dynamic surface activity of the lung surfactant. These effects can be evaluated by comparing the values of apparent rheological parameters of air/liquid interface (dilatational surface elasticity and viscosity). According to the obtained results, both surface elasticity and viscosity are reduced when lung surfactant system becomes contaminated with diesel exhaust particles. Reduction of these parameters suggest that surface tension hysteresis - known as the sensitive marker of physiological functionality of the lung surfactant - is also decreased. This allows to speculate on adverse effects of inhaled diesel nano-aggregates in vivo and to suggest possible pathways of their pulmonary toxicity.

Symbols and abbreviations

f	oscillation frequency, Hz
ε	dilatational surface elasticity (apparent), mN/m
μ	dilatational surface viscosity (apparent), mN·s/m
σ	surface tension, mN/m
DEPs	diesel exhaust particles
DPF	diesel particulate filter
FFT	fast Fourier transform
LS	lung surfactant

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