Prosimy cytować jako: Inż. Ap. Chem. 2014, 53, 4, 263-264

Katarzyna KRAMEK-ROMANOWSKA, Tomasz R. SOSNOWSKI

e-mail: t.sosnowski@ichip.pw.edu.pl

Katedra Inżynierii Procesów Zintegrowanych, Wydział Inżynierii Chemicznej i Procesowej, Politechnika Warszawska

Application of maximum bubble pressure tensiometry in the studies of pulmonary surfactant activity

Introduction

The entire alveolar surface of human lungs is lined with a thin fluid continuum, called the alveolar lining layer. This lining consists of an aqueous hypophase covered by a film of pulmonary surfactant – a complicated mixture of approximately 90% lipids and 10% proteins. During the breathing cycle its surface active components (mainly phospholipids) adsorb at the air-water interface and lower the alveolar surface tension. As a consequence, the energy required to inflate the lungs is reduced and the likelihood of alveolar collapse during expiration is minimized [*Zuo et al. 2008*]. Inhaled environmental particles and aerosol drugs deposited on the surface of alveolar lining layer may interact with pulmonary surfactant and reduce its physiological functions. Hence, it is justified to dedicate experimental investigations to examine the effect of various aerosols on the dynamic surface activity of lung surfactant.

In the present work, maximum bubble pressure (MBP) tensiometry was employed to evaluate the physicochemical impact of selected mineral nanoparticles and components of novel multifunctional composite powders, intended for drug delivery by inhalation, on the dynamic properties of pulmonary surfactant at the air-water interface. Among all known methods applied in the dynamic tensiometry, the MBP method is established as the most appropriate one for medical applications involving biological liquids [*Trukhin et al., 2001*].

Materials and Methods

In the present study, the therapeutic formulation *Survanta* (*Abbott Laboratories*, France), being an animal-derived pulmonary surfactant preparation intended for treatment of *Respiratory Distress Syndrome* in newborn premature infants, was used as a model of pulmonary surfactant. As it was concluded in work by *Notter et al.* [*2002*], *Survanta* possesses surface activity similar to natural human surfactant in dynamic conditions. All experiments were run in such a way that the concentration of phospholipids in all investigated surfactant formulations was constant (0.75 mg/ml), regardless of the contents of additives. For dilution purposes the sterile isotonic saline (0.9 % w/w NaCl aq.) was applied (*Gilbert Laboratories*, France).

As a representation of environmental particles interacting with model pulmonary surfactant, three types of aluminosilicate nanoparticles were chosen: halloysite (HN), bentonite (PGV) and surface-modified montmorillonite (MM2): 70÷75% montmorillonite, 25÷30% octadecylamine), all purchased from *Sigma Aldrich*. These mineral nanoparticels are widely used as enhancers in the production of polymer composites, increasing thermal and mechanical properties and lowering flammability of ready-made materials. In the second part of the study,focused on interactions of aerosol drugs with lung surfactant, two components of multifunctional composite powders intended for medical inhalation were selected – mannitol (*POCh SA*, Poland) and disodium cromoglycate (*GlaxoSmithKlein Pharmaceuticals*). Mannitol is a mucolytic agent that promotes the transport of therapeutic substances to the receptors situated under the mucus lining in the respiratory tract, while disodium cromoglycate (DSCG) is an antiasthmatic drug. Characterization of investigated particles are discussed in detail in earlier works [*Kramek-Romanowska et al., 2011; Kondej and Sosnowski, 2013*], for nanoparticles and therapeutic powders respectively. For this study, three concentrations of each additive in the tested surfactant formulations were investigated: 0.1, 0.5 and 1.0 mg/ml. The choice of powders' concentrations were done according to work by *Kondej and Sosnowski* [*2013*] in which the approximated concentration of model particles in pulmonary surfactant sample, after inhalation in specified conditions, were calculated.

Dynamic surface activity of the model pulmonary surfactant components was studied with the aid of bubble tensiometer BP2 (*Krüss*, Germany). The experimental device enabled measurements of surface tension changes during the formation of a fresh air-water interface which is represented by growing air bubble in the tested solution. The time range of surface formation extended from 10 ms to 20÷30 s in every single experiment. All experiments were run in triplicate in constant phy-siological temperature of 37°C assured by the external thermostat.

Results and Discussion

The results of experiments with model pulmonary surfactant and mineral nanoparticles are summarized in Figs. 1, 2, 3 for halloysite (H), bentonite (B) and surface modified montmorillonite (MM2), respectively. On each graph there is a comparison of dynamic surface tension profiles of physiological salt, *Survanta* solution with phospholipid concentration of 0.75 mg/ml and *Survanta* solution with different particles contents: 0.1, 0.5 and 1 mg/ml. Curves are expressed as means with average standard deviation in all cases around ±1.0 mN/m (standard deviation not presented on the graphs). Isotonic saline exhibits no surface activity as reflected by a horizontal line in all plots, while *Survanta* solution – even though at relatively low concentration – shows noticeable surface activity illustrated by a falling surface tension curve. Considering the impact of mineral particles on the physicochemical properties of the surfactant solution, it is seen that all investigated substances changed the *Survanta* tensiograms. Halloysite (Fig. 1) and bentonite (Fig. 2) nanoparticles increase surface tension of surfactant solution in

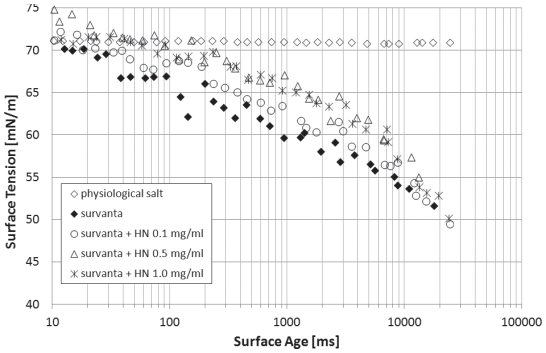

Fig. 1. Dynamic tensiograms of physiological salt, *Survanta* solution and *Survanta* with various concentration of halloysite (HN) nanoparticles at 37°C

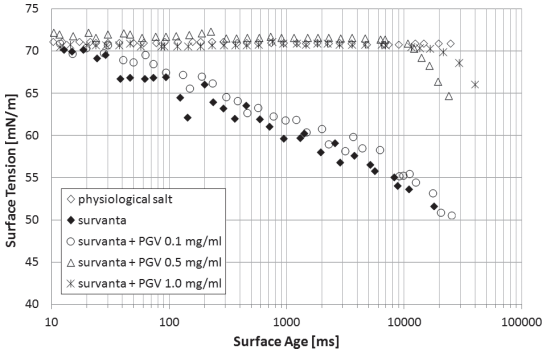

Fig. 2. Dynamic tensiograms of physiological salt, *Survanta* solution and *Survanta* with various concentrations of bentonite (PGV) nanoparticles at 37°C

Prosimy cytować jako: Inż. Ap. Chem. 2014, 53, 4, 263-264

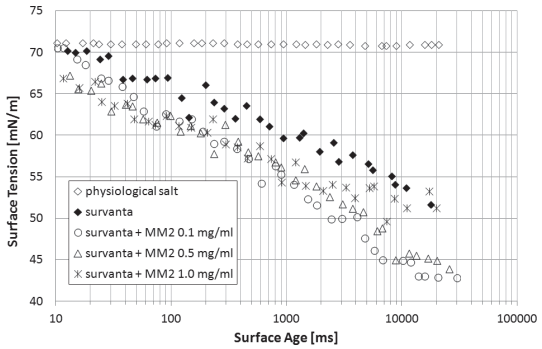

Fig. 3. Dynamic tensiograms of physiological salt, *Survanta* solution and *Survanta* with various concentrations of surface-modified montmorillonite (MM2) at 37°C

a concentration-dependent manner and first effects can be observed already for the lowest contents.

What is interesting, influence of bentonite nanoparticles is much stronger than of halloysite, and starting from bentonite concentration of 0.5 mg/ml, practically no surface activity of *Survanta* solution is detected. The result can be explained by the adsorption of surfactant components on nanoparticles surface, which was also implied in other works on interactions of PGV and HN particles with pulmonary surfactant [*Kondej and Sosnowski, 2012; 2013*]. Authors suggest that the attachment of surfactant molecules on nanoparticles is responsible for their depletion from the bulk solution. As a consequence less surface active components can adsorb at the air-water interface and the measured surface tension increases. The difference between results for HN and PGV particles clearly corresponds to the considerable difference in their intrinsic surface area (25 and 67 m^2/g, respectively [*Kondej and Sosnowski, 2013*]). The opposite effect than for HN and PGV particles was observed for surface modified montmorillonite (Fig. 3). These nanoparticles decreased the surface tension of *Survanta* solution and hardly any dependence on their concentration could be found. It can be explained by a kind of synergy of surface activity caused by M nanoparticles or by surfactants wash-out from their surface after their contact with the aqueous phase [*Kondej and Sosnowski, 2013*].

The results of the experiments focused on drug interactions with lung surfactant are depicted on Figs. 4 and 5 for mannitol and disodium cromoglycate (DSCG), respectively.

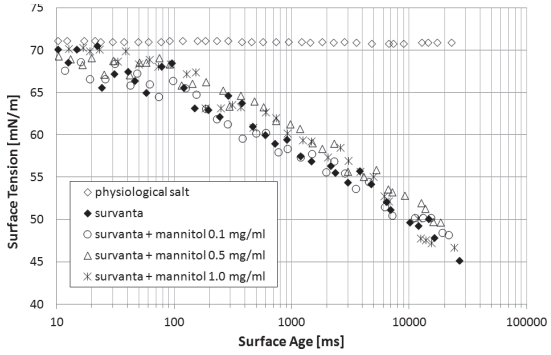

Fig. 4. Dynamic tensiograms of physiological salt, *Survanta* solution and *Survanta* with various concentrations of mannitol at 37°C

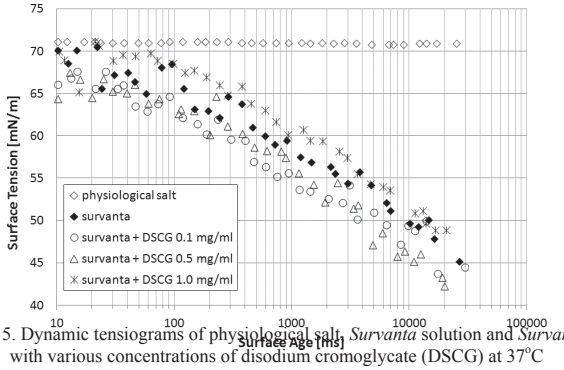

Fig. 5. Dynamic tensiograms of physiological salt, *Survanta* solution and *Survanta* with various concentrations of disodium cromoglycate (DSCG) at 37°C

Both graphs show a comparison of dynamic surface tension profiles of saline, *Survanta* solution (0.75 mg/ml) and *Survanta* solution with different contents of drugs: 0.1, 0.5 and 1 mg/ml. The *Survanta* solution tensiogram differs a bit from the ones presented in Figs. 1–3 as the relevant measurements were performed with various samples of the therapeutic formulation. Dynamic surface tension profiles presented in Fig. 4 reflect a low impact of mannitol on the surface activity of *Survanta*, independently on drug concentration. This fact can be explained by a good solubility of mannitol and the lack of amphiphilic properties. By contrast, the tensiograms shown in Fig. 5 indicate a decrease of the surface tension for DSCG at concentrations of 0.1 and 0.5 mg/ml, but the opposite effect at DSCG concentration of 1.0 mg/ml. Both types of interactions, positive and negative, seem not to be as significant as for mineral nanoparticles discussed earlier, but still indicate a certain deviation from the original physicochemical properties of pulmonary surfactant. Similarly to mannitol, DSCG is also characterized by good water solubility and has no surface activity, so the explanation of the obtained results requires taking into account probable interactions between drug molecules and phospholipids or other components of *Survanta* (lung surfactant). However, the outcome of this study is not sufficient to make further speculations on that subject.

Conclusions

The impact of three types of aluminosilicate nanoparticles, as model environmental particles, and two components of multifunctional composite powders intended for medical inhalation, on the physicochemical properties of model pulmonary surfactant was investigated by dynamic bubble tensiometry. For halloysite and bentonite nanoparticles a concentration- and intrinsic surface-area dependent negative influence on biophysical activity of *Survanta* solutions was demonstrated, while the opposite result was observed for the surface modified montmorillonite. However, it has to be stated that artificially induced increase as well as decrease of surfactant activity may be responsible for undesired physiological effects in vivo. In case of drugs interacting with lung surfactant, hardly any influence of mannitol was detected, while the impact of DSCG on pulmonary surfactant properties, though not so significant as for mineral nanoparticles, cannot be totally omitted.

The dynamic surface tensiometry, applied in this work, proved to be useful for such kind of research as it enabled the analysis of the surface phenomena with relation to the time scales characteristic for the alveolar area changes during breathing. The fact is crucial for the physiological relevance of the obtained results.

REFERENCES

Kondej D., Sosnowski T.R., 2013. Alteration of biophysical activity of pulmonary surfactant by aluminosilicate nanoparticles. *Inhal. Toxicol.*, **25**, 77-83. DOI: 10.3109/08958378.2012.756087

Kramek-Romanowska K., Odziomek M., Sosnowski T.R., Gradoń L., 2011. Effects of process variables on the properties of spray-dried mannitol and mannitol/disodium cromoglycate powders suitable for drug delivery by inhalation. *Ind. Eng. Chem. Res.*, **50**, 13922-13931. DOI: 10.1021/ ie2006998

Trukhin D.V., Sinyachenko O.V., Kazakov V.N., Lylyk. S.V., Beokon A.M., Pison U., 2001. Dynamic surface tension and surface rheology of biological liquids. *Coll. Surf. B.*, **21**, 231-238. DOI: 10.1016/S0927-7765(01)00175-8

Notter R.H., Wang Z., Egan E.A., Holm B.A., 2002. Component-specific surface and physiological activity in bovine-derived lung surfactants. *Chem. Phys. Lip.*, **114**, 21-34. DOI: 10.1016/S0009-3084(01)00197-9

Zuo Y.Y., Veldhuizen R.A.W., Neumann A.W., Petersen N.O., Possmayer F., 2008. Current perspectives in pulmonary surfactnat – Inhibition, enhancement and evaluation. *Biochim. Biophys. Acta*, **1778**, 1947-1977. DOI:10.1016/ j.bbamem.2008.03.021

The study is related to the activity of the European network action COST MP1106 "Smart and green interfaces – from single bubbles and drops to industrial, environmental and biomedical applications". Publication has been co-financed with the European Union funds by the European Social Fund. The authors thank Mrs Dorota Kondej for providing samples of the nanoclays.