CHEMICAL ENGINEERING TRANSACTIONS
VOL. 26, 2012
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Guest Editors: Valerio Cozzani, Eddy De Rademaeker

ISBN 978-88-95608-17-4; **ISSN** 1974-9791
DOI: 10.3303/CET1226089

Changes in the Activity of the Pulmonary Surfactant after Contact with Bentonite Nanoclay Particles

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Nanoclays are used as fillers in the production of polymer nanocomposites. They contribute to improving the physical, heat and resistance properties of nanocomposites. Nanomaterials used to manufacture of modern plastics may have the harmful impact on the workers' health. Effect of inhalation of these dopants on the important structures of breathing system remains still unrecognized. Pulmonary surfactant plays the important role in proper functioning of the respiratory system by lowering the surface tension in the alveoli. This phenomenon prevents them from collapsing in the final phase of exhalation, increases their stability and prevents pulmonary edema. It also takes part in the self-cleaning of the alveoli from inhaled and deposited aerosol particles.
The aim of this study was to evaluate the influence of bentonite nanoparticles on the surface activity of the pulmonary surfactant.
Pharmaceutical preparation SURVANTA (Abbott, France) was used as the substance representing physicochemical properties of natural surfactant. The preparation was diluted to concentration of 1.25 mg of phospholipids/ml with sterile physiological salt solution containing the known mass of bentonite particles. The study of surface activity during oscillations (15 min^{-1}, 37 ºC) of air bubble created in the solution of the model surfactant was conducted with the dynamic tensiometric method using Pulsating Bubble Surfactometer (Electronetics Corp., USA).
It was found that the quantitative criteria which describe the dynamic surface properties of the pulmonary surfactant (i.e., the minimum surface tension σ_{min}, the normalized hysteresis area HA_n and the stability index SI) were changed after surfactant contact with the nanoparticles. The results indicate strong influence of bentonite nanoclay on pulmonary surfactant activity. These findings correspond to the observations from studies related to the impact of other nanoparticles (e.g., of gold, diesel exhaust components, etc) on the pulmonary surfactant. It is speculated that observed effects may be associated with the development of occupational respiratory diseases.

1. Introduction

Workers can be exposed to the inhalation of a number of harmful agents in their working environment. The development of nanotechnologies and the related increasing use of nanomaterials are associated with new risks. According to the opinion of experts of the European Agency for Safety and Health at Work expressed in their reports (EU OSHA, 2009a; EU OSHA, 2009b), nanoparticles (and other nanoobjects) unintentionally released during manufacture and processing of products will constitute one of the main risks to workers' health in the nearest future.

Please cite this article as: Kondej D. and Sosnowski T., 2012, Changes in the activity of the pulmonary surfactant after contact with bentonite nanoclay particles, Chemical Engineering Transactions, 26, 531-536 DOI: 10.3303/CET1226089

Nanomaterials constitute the basis for the manufacture of many products, including polymer nanocomposites. Nanocomposites are considered the materials of the twenty-first century and the scope of their use is expected to dynamically increase in the years to come. Currently, nanocomposites are used mainly in automotive, packaging, aviation and electrotechnical industries.
Very good resistance and physicochemical properties of polymer nanocomposites are obtained by adding nanofillers such as nanoclay particles to conventional polymers. The addition of nanofillers results in a marked increase of the elasticity module, higher thermal stability, enhanced barrier properties, resistance to organic solvents, better optic properties, and a lower linear expansion coefficient.
One of nanoclays most commonly used as polymer composite filler is bentonite (PGV). Montmorillonite (MMT) is the basic ingredient of PGV. MMT is hydrated aluminium hydrosilicate with a formula $Al_2[(OH)_2Si_4O_{10}]\cdot nH_2O$ which may also contain iron, calcium or magnesium atoms. The chemical composition of montmorillonites is highly diversified because the silicon atom may be partially replaced by aluminium and aluminium can be replaced in turn by magnesium, iron, zinc, nickel and copper ions. The MMT structure consists of three combined layers: two external layers made of tetrahedral silicon dioxide crystals and one internal layer formed by octahedral aluminium oxide or magnesium oxide crystals. MMT is a highly reactive layered silicate which reacts with various organic molecules through electrostatic interactions (e.g. ion exchange), secondary bonding (e.g. adsorption of neutral particles) or covalent bonding (e.g. inoculation).
The use of nanomaterials contributes undeniable benefits to the economy but the knowledge on their effect on workers' health is as yet incomplete. The health aspect of the effect of nanoclay particles used in the production of polymer nanocomposites on such an essential element of the respiratory system as pulmonary surfactant still remains to be elucidated.

1.1 Pulmonary Surfactant System

Pulmonary surfactant constitutes the first barrier separating the inhaled air present in pulmonary alveoli from the lung tissue with pulmonary capillaries. According to the Clements's theory, alveolar epithelium is covered with a thin (ca. 0.1 μm thick) layer of liquid called hypophase (Clements et al. 1961; Notter and Finkelstein, 1984; Sosnowski et al., 2008). Surface-active substances secreted by specialised cells called type II pneumocytes are adsorbed on the hypophase surface, creating pulmonary surfactant. Through adsorption on the liquid-gas interface surface of the alveolar lining, pulmonary surfactant influences the surface tension in the alveoli during the respiratory cycle.
Pulmonary surfactant is attributed important physiological and defensive functions which are important for the normal functioning of the body. When adsorbed on the liquid-gas interface surface of alveolar lining, the surfactant performs the role of a surface tension regulator — it contributes to lowering of the surface tension in the alveoli, which prevents their collapse in the final phase of expiration, and to the increase in alveolar stability; it also prevents pulmonary edema (Notter and Finkelstein, 1984). Furthermore, pulmonary surfactant performs a very important defensive function which involves, for example, enabling the movement of deposited particles (e.g. dusts in the working environment) to higher levels of the bronchial tree. This process progresses owing to the Marangoni effect — the flows of liquid layers induced by surface tension gradients created in the pulmonary surfactant system with a specific dynamic surface activity (Gradoń and Podgórski, 1989; Gradoń et al, 1996).
Pulmonary surfactant activity is defined as its ability to lower surface tension in dynamic conditions induced by breathing (a cyclic change of the liquid-gas surface covering the alveolar epithelium), and it can be studied in vitro. The presence of marked lowering of the surface tension during compression of the liquid-gas surface (expiration) and the presence of surface tension hysteresis as a function of the interface area in the expansion-compression (inhalation-exhalation) cycle are the characteristic features of effective pulmonary surfactant system. Abnormalities of surface activity of pulmonary surfactant caused by pathological conditions (e.g. acute lung injury, ALI, or acute respiratory distress syndrome, ARDS) or external factors (e.g. particle deposition on alveolar surface) are accompanied by a change of the shape and position of the hysteresis loop of surface tension in the inhalation-exhalation cycle (Notter et al, 1982).

The following numerical criteria are used for the quantitative description of these changes and evaluation of surface activity of pulmonary surfactant during the oscillation of the liquid-gas interface surface (detailed methods of their calculations were given elsewhere (Kondej and Sosnowski, 2010a):

- Minimum value of surface tension in the compression-expansion cycle of the surface (σ_{min})
- Normalized hysteresis area (HA_n) in a cycle — an index proposed by Notter et al. (1982) to describe the hysteresis area value determined for the compression and expansion cycle, in relation to the unit surface change
- Stability index (SI) — an index introduced by Clements et al. (1961) to describe the difference between the maximum and the minimum surface tension values in the cycle in relation to their arithmetic mean. SI defines the ability to reduce surface tension in dynamic conditions of surface oscillation.

The aim of this study was to evaluate the influence of bentonite nanoclay particles on the surface activity of the pulmonary surfactant.

2. Materials and Methods

2.1 Tested Nanofillers

Natural nanoclay with the lamellar structure, representing the montmorillonite group, was selected for testing. This nanomaterial was purchased in the form of nanopowder in Sigma-Aldrich.

2.2 Microscopic Analysis

Observations of morphology of the tested particles were conducted with the use of a scanning electron microscope, Zeiss model 1530, upgraded by the use of the Supra optic elements. The test specimens were prepared by the dry method by placing the sample on a conductive tape and by the suspension method from 0.1 mg/mL and 1 mg/mL aqueous suspensions. Thus prepared test specimens were sprayed with carbon in an SCD 005 (BalTec AG) sputter-coater with a carbon attachment. During microscopic analysis, an accelerating voltage of 2 kV and successive image magnifications of 10,000 x, 25,000 x, 50,000 x, 100,000 x and 250,000 x were used.

2.3 Measurement of Specific Surface Area

Specific surface area measurements were performed by the analysis of nitrogen vapour adsorption isotherms at 77 K with the use of the multi-point method of Brunauer, Emmett and Teller (BET). A 1 cm^3 sample was weighed and then placed in the Flow Sorb 060 (Micrometics) desorption station for 3 h. Desorption was conducted by heating at 150 °C in helium flow. Specific surface measurements were performed with the use of a Gemini 2360 (Micromeritics) surface analyser. The tests were conducted within a range of relative pressures p/p_0 between 0.1 and 0.3.

2.4 Particle Size Analysis

Particle size analysis was conducted by NTA (Nanoparticle Tracking Analysis) method with the use of the NS500 analyser (Nanosight, UK). The NTA method consists of a direct visualisation and analysis of the movement of separate particles in suspension. During the Brownian motions, nanoparticles diffuse laser light which is registered by a CCD camera. Analysis of the recordings allows to identify all particles and track their movements. Based on the distance travelled by a particle, the diffusion coefficient is determined and then, with the known sample temperature T and solvent viscosity η, hydrodynamic particle diameter d_h is calculated. NTA particle size measurement of the nanoclay was conducted in aqueous suspension at 37 °C.

2.5 Pulmonary Surfactant Activity

Pharmaceutical preparation SURVANTA (Abbott, France) was used as the substance representing physicochemical properties of natural surfactant. The preparation was diluted to concentration of phospholipids 1.25 mg/mL with sterile physiological salt solution containing the known mass of bentonite particles. The study of surface activity during oscillations (15 min^{-1}, 37 °C) of air bubble created in the solution of the model surfactant was conducted with the dynamic tensiometric method using Pulsating Bubble Surfactometer (Electronetics Corp., USA), (Enhörning, 2001). Method of testing

of the surface activity of the model pulmonary surfactant are described in more details elsewhere (Kondej and Sosnowski, 2010a; Kondej and Sosnowski, 2010b).

3. Results and discussion

Microscopic analysis confirmed the lamellar nature of PGV. Figure 1 presents an image visible under a scanning electron microscope at magnifications of 100,000 x. The image of the particles of the PGV nanopowder was slightly distorted by the layer of carbon sprayed during the preparation of the microscope test specimen. Nevertheless, a clearly outlined lamellar structure with very well developed surface is visible. The PGV nanopowder was characterised by the large specific surface (67.31 m^2/g).

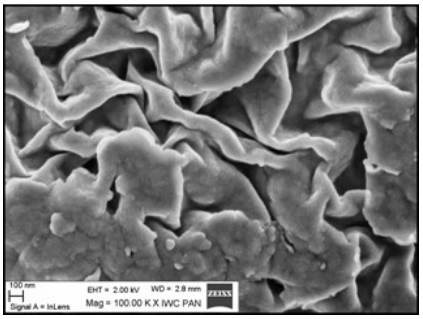

Figure 1: The image of the particles of the PGV nanopowder visible under a scanning electron microscope at magnifications of 100,000 x

Size distribution of PGV particles in 1 mg/ml aqueous suspension thermostated at 37°C and sonicated for 5 min is shown in Figure 2. The mean number concentration of PGV nanoparticles in the suspension was $2.7 \cdot 10^8$ particles/mL. Most of the particles (ca. $6.5 \cdot 10^7$ of partciles/mL) had a diameter of ca. 150 nm. Hydrodynamic diameters of 90 % of all particles were smaller than 820 nm. The mean hydrodynamic diameter of particles was 238 nm, and the median hydrodynamic diameter was 153 nm.

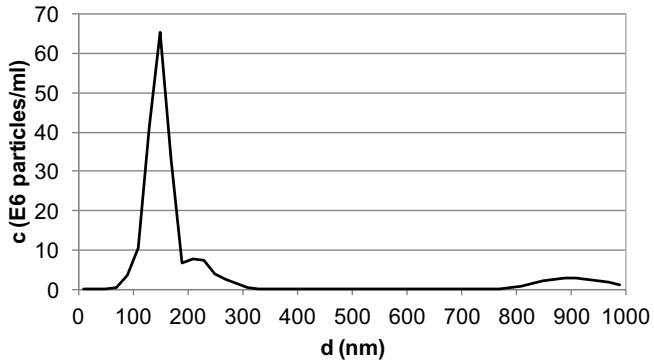

Figure 2: Size distribution of PGV particles in aqueous suspension determined by NTA

Results obtained by surface tension analysis are shown in Figure 3 and they indicate changes of the quantitative criteria related to surface properties of pulmonary surfactant caused by the presence of nanoclay particles. The subsequent graphs show minimum surface tension σ_{min}, normalized hysteresis area HA_n and stability index SI as a function of nanoparticle concentration (up to 1 mg/mL) in the pulmonary surfactant solution. Decrease of the mean normalized hysteresis area by 16.9 mN/m and of the stability index by 0.6 were found. The mean minimum surface tension increased by 55.7 mN/m.

These effects are probably caused by adsorption of the surfactant molecules on nanoparticles. Taking into account the specific surface area of the particles it can be calculated that the solid-liquid surface area in the suspensions is more a several orders of magnitude times greater than the air-liquid (bubble) surface area in the system.

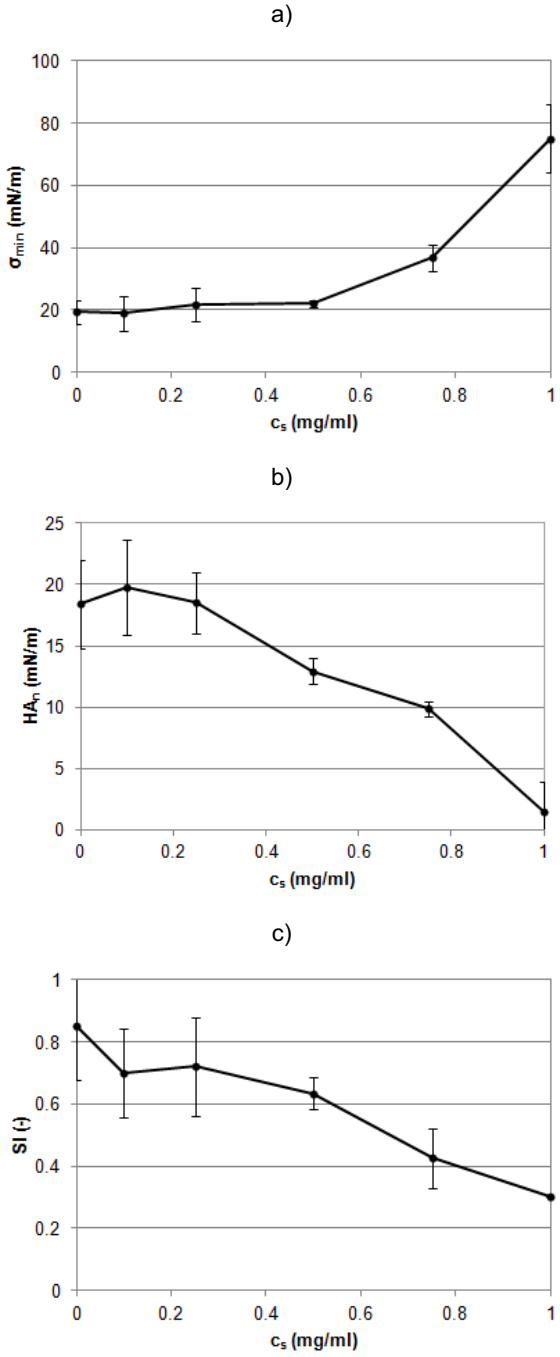

Figure 3: Changes of: a) minimum surface tension (σ_{min}), b) normalized hysteresis area (HA_n), c) stability index (SI) as a function of nanoclay concentration (c_s) in solution of the model pulmonary surfactant

4. Conclusions

Results of this research indicate that the quantitative criteria which characterize the dynamic surface properties of the pulmonary surfactant (i.e., the minimum surface tension σ_{min}, the normalized hysteresis area HA_n and the stability index SI) were unfavourably changed in a dose-dependent manner after surfactant contact with the nanoparticles. These findings correspond to the observations from studies of the impact of other nanoparticles (e.g., of gold, diesel exhaust components, etc) on the pulmonary surfactant (Bakshi et al., 2008; Wallace et al., 2007; Sosnowski et al, 2011). It is speculated that observed effects may be associated with adverse health effects and development of occupational respiratory diseases.

Acknowledgements

This paper has been prepared on the basis of the results of research task No. I.B.10 carried out within the National Programme "Improvement of safety and working conditions" partly supported in 2011-2013 within the scope of research and development by the Ministry of Science and Higher Education. CIOP-PIB has been the Programme main coordinator.

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