International Journal of Occupational Safety and Ergonomics
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tose20

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Published online: 08 Jan 2015.

To cite this article: Tomasz Sosnowski & Albert Podgórski (1999) Assessment of the Pulmonary Toxicity of Inhaled Gases and Particles With Physicochemical Methods, International Journal of Occupational Safety and Ergonomics, 5:3, 431-447

To link to this article: http://dx.doi.org/10.1080/10803548.1999.11076430

INTERNATIONAL JOURNAL OF
OCCUPATIONAL SAFETY AND ERGONOMICS 1999, VOL. 5, NO. 3, 431–447

Assessment of the Pulmonary Toxicity of Inhaled Gases and Particles With Physicochemical Methods

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Physicochemical techniques used for evaluating the pulmonary surfactant (PS) quality are discussed as methods useful in assessing toxicity of inhaled gases and particles. Two standard devices, Langmuir-Wilhelmy film balance and pulsating bubble apparatus, are presented in detail, and the measured results of interaction between sulfuric acid and 2 models of PS material are analyzed. The evident decrease in surface activity of the pulmonary surfactant after its contact with the acid at concentrations approaching 0.001 M may be considered as an indicator of the adverse effect, which can result in several health problems. The presented approach can be used as a method of assessing pulmonary toxicity of any substances present in the breathing air.

pulmonary surfactant surface activity pulsating bubble technique
Langmuir balance hysteresis

1. INTRODUCTION

Inhaled particles and gases may be responsible for a wide range of adverse health effects, which arise from the irritation of the respiratory tract (Lippmann, 1996; Rijcken & Britton, 1998). Beside the well recognized carcinogenic fibers (e.g., asbestos), particles carrying adsorbed

This work was supported by the State Committee for Scientific Research of Poland under the National Strategic Project SPR-1 ("Occupational Safety and Health Protection in the Working Environment"), project No. 04.10.16, co-ordinated by the Central Institute for Labor Protection (CIOP).

The authors appreciate the assistance of Prof. Goran Enhörning from the University of Toronto in supplying the INFASURF sample.

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chemicals (diesel aerosols), or choking gaseous compounds (which compete with oxygen, such as carbon monoxide), there exists a broad spectrum of airborne substances capable of causing moderate physiologic response after inhalation. Evident health effects are sometimes pronounced after a long exposure or in accidental episodes of high concentrations of irritants. Therefore, to characterize the potential hazard of the given substance present in the air, the concepts of acceptable daily intake (ADI) or maximum exposure limit (MEL) are widely used. Implementing adequate values of those parameters is especially important for workplaces, where exposures are long and repeated day by day, and more intense breathing during an effort additionally increases the amount of a toxicant taken in.

One of the mechanisms of toxicity caused by inhaled compounds is the impairment of clearance mechanisms in the lungs (McClellan, 1998). This effect leads to elevated accumulation of airborne particles in the alveoli, and consequently extends their retention time in the organism, so the chance of undesired action becomes higher. Clearance retardation may come from destabilization of the pulmonary surfactant (PS) system, which is essential not only for lung mechanics, but also for effective removal of particles deposited on the surface of the epithelium. As demonstrated by Podgórski and Gradoń (1993), interfacial activity of the pulmonary surfactant can promote the flow of the alveolar liquid layer towards ciliated airways, so PS can act as a carrier of deposited particles and alveolar macrophages. Other studies suggest that the surfactant also stimulates clearance activity of the alveolar macrophages (Gehr, Green, Geiser, Im Hof, Lee, & Schürch, 1997; Gradoń & Podgórski, 1995; Van Iwaarden, 1992). Thus, it seems reasonable to associate the activity of PS with the effectiveness of the alveolar clearance processes. We should also emphasize that inactivation of the surfactant (e.g., by inhaled toxins) deteriorates its basic physiological functions related to mechanical stability of the alveolar system (Goerke, 1992) and leads to severe respiratory problems such as Adult Respiratory Distress Syndrome (ARDS; Holm, 1992).

The facts presented suggest a new possibility of evaluation of the pulmonary toxicity of inhaled substances, which can be based on their ability to inactivate the surfactant. In this paper we would like to focus on experimental methods used for evaluation of PS activity, and propose ways of their application for the assessment of toxic potential of inhaled substances.

2. METHODS USED FOR STUDYING INTERFACIAL ACTIVITY OF PS IN VITRO

Several experimental methods are used for investigating pulmonary surfactant activity under physiological-like conditions in vitro. Since the earliest studies by Clements (1957) with a Langmuir-Wilhelmy balance, which had demonstrated the characteristic behavior of the surfactant during area oscillation, hundreds of experiments in various experimental systems were done to confirm and explain the unusual interfacial activity of PS. Most popular physicochemical experimental devices and methods used in the pulmonary surfactant studies follow:

- Langmuir-type Film Balance (LFB; e.g., Mendenhall, 1972; Notter, Taubold, & Mavis, 1982; Sosnowski, Gradoń, & Podgórski, 1997);
- Oscillating bubble apparatus (e.g., Egan, Notter, Kwong, & Shapiro, 1983; Enhörning, 1977; Sosnowski, Gradoń, & Podgórski, in press; Sosnowski, Gradoń, Podgórski, Wróbel, & Pirożyński, 1998);
- Captive bubble technique (e.g., Schürch, Bachofen, Goerke, & Green, 1992);
- Vertical surface balance (e.g., Boyle & Mautone, 1982);
- Spinning bubble tensiometry (e.g., Chung, Shanks, Hanneman, & Franses, 1990);
- Du Noy Ring Tensiometry (e.g., Barrow & Hills, 1979);
- Optical techniques (e.g., Belorgey, Tchoreloff, Benattar, & Proust, 1991; Pastrana-Rios, Taneva, Keough, Mautone, & Mendelsohn, 1995).

Detailed discussion of all the mentioned techniques is out of scope of the present paper, so we are providing a broad list of references. In the next parts of the paper we will present in detail only two methods, which are currently used in our group for evaluating pulmonary toxicity of gases and particles.

The majority of the PS in vitro studies published in the literature is focused on the identification of interfacial properties of the surfactant, comparison of behavior of various surfactant materials, and searching for PS substitutes. Consequently, the experiments are conducted with the use of the natural surfactant (usually obtained by BAL, bronchoalveolar lavage), or with the use of synthetic compounds identical to those found in PS. Both approaches give similar information, that is, static or dynamic values of the surface tension, considered as the essential indicators of PS activity in the organism. Dynamic changes of the area

of the gas/liquid interface, which simulate alveolar oscillation during the breathing cycle, result in the loop of surface tension hysteresis—the specific feature of the pulmonary surfactant (Figure 1). The hysteresis is believed to be of great importance for the lung mechanics, being the main contributor to pressure-volume hysteresis observed during the breathing cycle (Smith & Stamenovic, 1986; von Neergaard, 1929). Surface tension hysteresis recorded in studies of the PS material from pathological lungs (e.g., infant RDS) demonstrated significant deviation from the one observed for healthy lungs (Notter et al., 1982). It was proven that a decrease in the hysteresis area is related to inadequate quality of the surfactant. The size of the hysteresis can be characterized quantitatively by the value of the Normalized Hysteresis Area, HA_n:

$$HA_n = \frac{\left[\int_A \sigma dA\right]_{\text{expansion}} - \left[\int_A \sigma dA\right]_{\text{compression}}}{A_{\max} - A_{\min}}, \tag{1}$$

where σ denotes surface tension (N m^{-1}), and A—interfacial area (m^2).

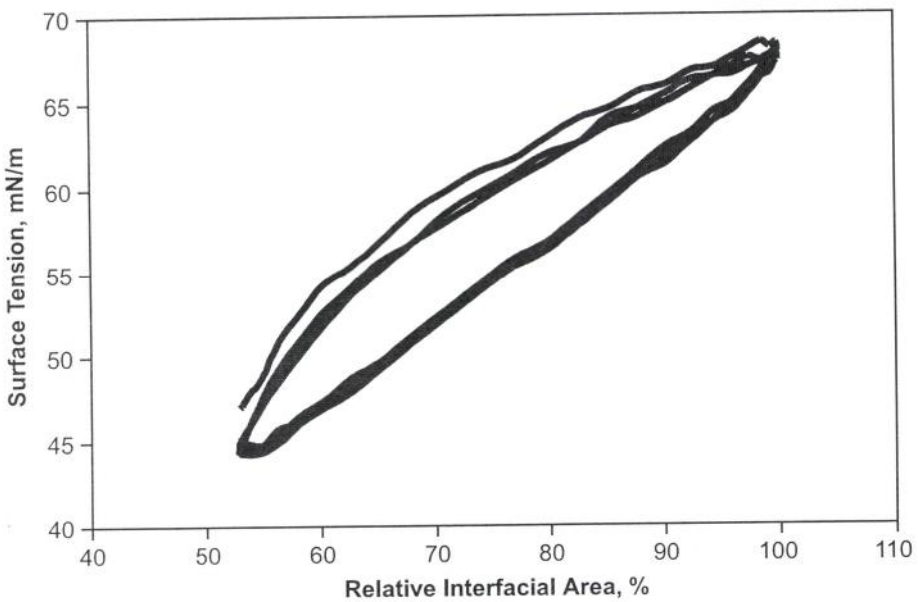

Figure 1. An example of surface tension hysteresis measured during dynamic compression and expansion of the air/water interface in the presence of PS.

Apart from the size of the hysteresis loop, also the minimum value of the surface tension (σ_{min}) and the variability of the surface tension recorded during oscillations of the interfacial area seem to be important from the physiological viewpoint (Goerke, 1992; Keough, 1992). The Stability Index, *SI*, was introduced by Clements, Hustead, and Johnson (1961) for quantification of the surface tension variability during area variation:

$$SI = 2\frac{\sigma_{max} - \sigma_{mix}}{\sigma_{max} + \sigma_{mix}}. \tag{2}$$

With the use of the aforementioned methods of interpretation of the experimental data it is possible to univocally analyze the influence of various factors on physical properties of the surfactant, and to formulate conclusions concerning possible alteration of PS physiological activity in the organism.

3. EVALUATION OF SURFACTANT PROPERTIES WITH THE PULSATING BUBBLE SURFACTOMETER

The pulsating (or oscillating) bubble technique was first introduced into the pulmonary surfactant studies by Slama, Schoedel, and Hansen (1973), and was developed by Enhörning (1977). Currently it is implemented in a commercial device called Pulsating Bubble Surfactometer (PBS; Electronetics Corp., USA), which is one of the standard devices used in the investigations of PS interfacial activity. The device design allows for simulations of the behavior of an alveolus (represented here as a bubble of air) during the breathing cycle. The idea of the PBS operation is depicted schematically in Figure 2a. The measuring principle relies on recording the pressure difference, ΔP (Pa), across the air/liquid interface of the bubble being pulsated in the surfactant suspension. The instantaneous surface tension, σ, is calculated from the Young-Laplace equation, assuming the spherical shape of the bubble:

$$\sigma = \frac{r\Delta P}{2}, \tag{3}$$

where r (m) denotes the bubble radius.

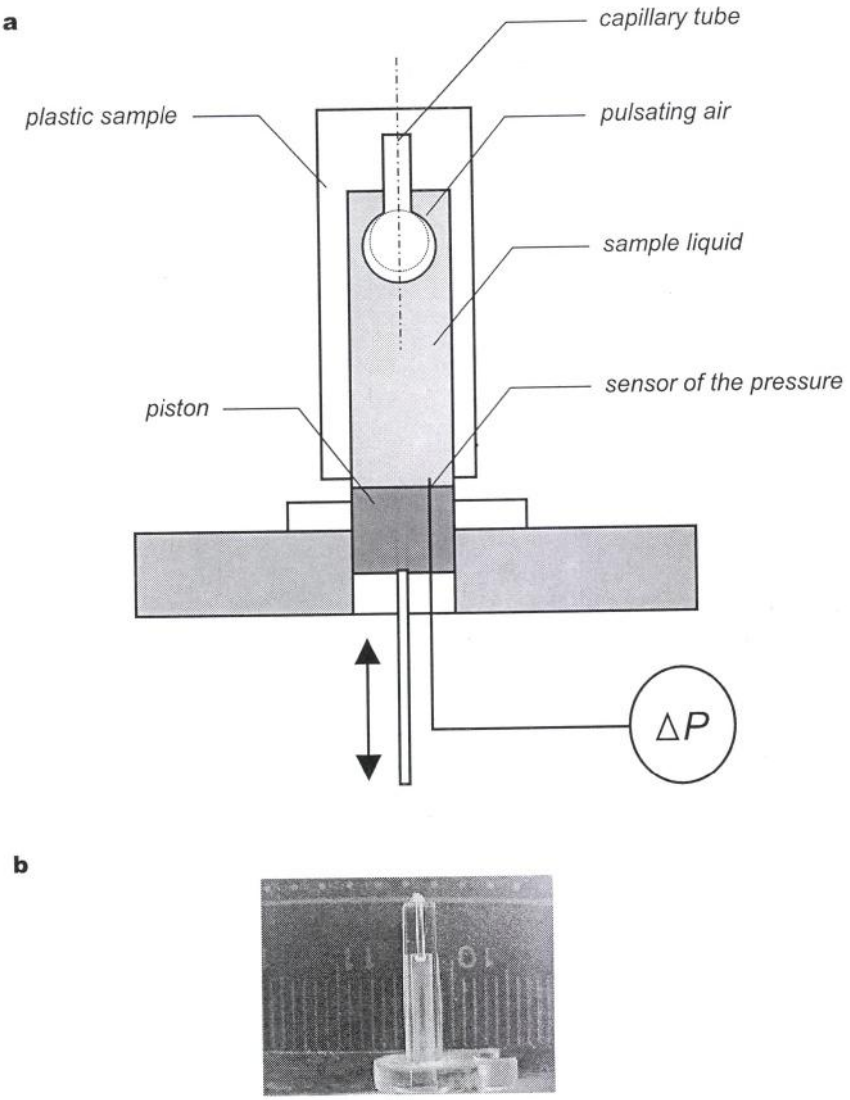

Figure 2. (a) The idea of Pulsating Bubble Surfactometer (PBS) operation. (b) Sample chamber for PBS studies (scale in mm in background).

To perform the experiment one needs as little as 30 μl of the surfactant-rich material, which has to be introduced into the sample chamber, made of transparent plastic (Figure 2b). The chamber is then fixed inside the thermostated vessel of the PBS and the small (r = 0.4–0.55 mm) air bubble can be observed during physiological-like pulsations through a microscope. The relationship between the dynamic surface tension and bubble size obtained as results of experiments can

be analyzed and compared using three criteria introduced earlier: HA_n, SI, and σ_{min}. Regardless of the ongoing discussion about some artifacts that may be included in the data from PBS (Hall et al., 1993), for the comparative studies the results directly indicate changes in physicochemical processes that can occur in the real PS system. Recently it was demonstrated (Sosnowski, 1999) that variation of the mentioned quantitative criteria can be associated with the well-defined phenomena in gas/liquid/surfactant systems. That analysis allows for more precise determination of the mechanisms responsible for changes of the PS properties.

4. EVALUATION OF SURFACTANT PROPERTIES WITH THE LANGMUIR-WILHELMY FILM BALANCE (LFB)

The idea of measurements conducted in the Langmuir-type balance is based on the analogy between cyclic changes of the interfacial area of a liquid in the LFB and the interfacial processes in the alveolar region of the lungs during breathing. A schematic view of a symmetric Langmuir-Wilhelmy film balance is presented in Figure 3. Measurement of the surface tension (or the surface pressure) is done by determining the sinking force acting at the 3-phase line contact on the immersed platinum Wilhelmy plate. Variation of surface concentration of the surfactant at the air/liquid interface is caused by a displacement of

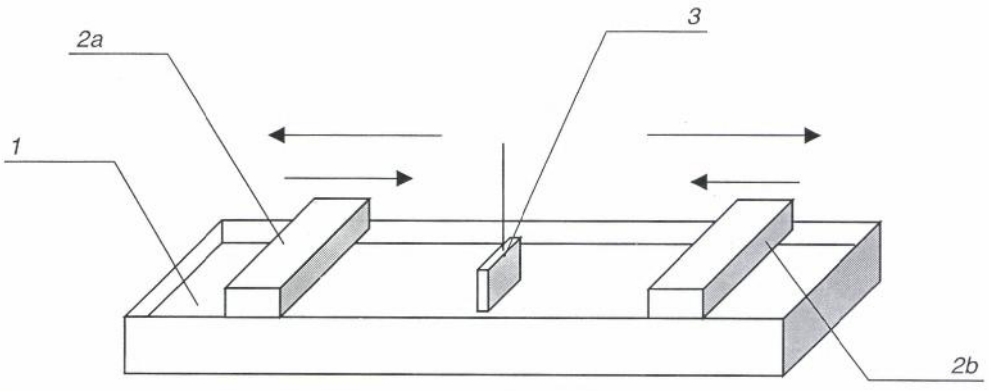

Figure 3. A schematic view of a Langmuir-Wilhelmy film balance (LFB): 1—Langmuir trough filled with liquid (hypophase); 2a, 2b—moving barriers; 3—Wilhelmy plate hung up to the force transducer.

moving barriers. In some devices a single barrier is used, and a different technique of surface pressure determination can be applied (Sosnowski, 1997). Generally there are two strategies of PS surface properties investigation with the LFB:

- Langmuir trough may be filled with the surfactant suspension, and PS adsorbs spontaneously from the hypophase at the air/liquid interface.
- Langmuir trough may be filled with a physiological salt solution or with water. The surfactant is then introduced directly at the air/liquid interface with a few droplets of an organic solution (e.g., with chloroform as a solvent).

The first method offers a more realistic approach but it requires large volumes of the surfactant samples, which are not often available. The second one is more convenient and reproducible, but the information gathered from experiments is not complete as it does not account for sorption processes. Planar geometry of a Langmuir trough instead of the spherical one of an alveolus is not a limitation of the results' validity, but the size of the vessel and the time scale of the experiments can introduce some uncertainties to the physiological conclusions from this kind of studies. Again, when used for comparative experiments, LFB seems to provide sufficient information for evaluating the influence of various factors on PS activity. The evident advantage of LFB studies in comparison to the PBS technique is the possibility of better identification of the interfacial processes. The air/liquid interface in the Langmuir trough is fully accessible for experiments in contrast to a small interface of the bubble confined inside the PBS sample chamber. For example, LFB studies allow to determine the compression isotherm, that is, the quasi-static relationship between increasing surface concentration of the surfactant (Γ, mol m^{-2}) and surface tension (σ, N m^{-1}). The comparison of such isotherms measured in the presence of various substances can clearly reveal the interaction between these substances and the surfactant monolayer. However, from the physiological viewpoint, determination of the surface tension hysteresis during interfacial area oscillations and its comparison in various experimental conditions seem more interesting. This kind of study can be confronted with the results from PBS experiments.

5. INTERACTION BETWEEN SULFURIC ACID AND THE PULMONARY SURFACTANT

In the current studies we investigated in both mentioned experimental systems the influence of sulfuric acid on the dynamic properties of the pulmonary surfactant. The presence of the acid in the respiratory tract can be associated with the inhalation of sulfuric dioxide or sulfuric acid fog, and is known to cause moderate to severe respiratory response (e.g., Balmes, Fine, Gordon, & Sheppard, 1989; Fujimaki, Katayama, & Wakamori, 1992; Utell, Bauer, Frampton, & Morrow, 1988). Only a few papers discussed a possible interaction between sulfuric acid and the PS system (Pawełek, Hanicka, & Sowińska, 1984; Sosnowski & Gradoń, 1997; Sosnowski & Podgórski, 1998), but no extensive investigations have been performed yet.

5.1. Materials and Methods

A fully automated Langmuir-Wilhelmy film balance depicted schematically in Figure 4 was manufactured by KSV Instruments Ltd. (Finland).

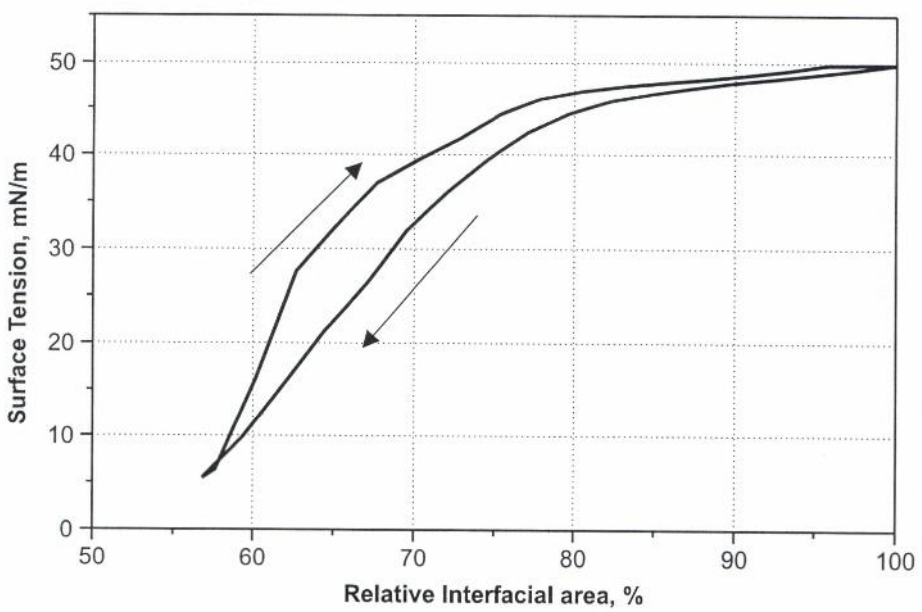

Figure 4. Surface tension hysteresis for 1,2-dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemicals, USA) on the hypophase of pure water, 36.6 ± 0.05 °C.

In LFB studies we used the predominant component of the natural pulmonary surfactant, 1,2-dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemicals, USA) as a model surfactant. The surfactant monolayer at the air/liquid interface was produced by introducing a few droplets of a DPPC solution in chloroform (concentration: 3.97 mg per ml). Experiments were done at 36.6 $\pm$ 0.05 °C, and the hystereses were measured in the surface tension range 5–50 mN/m. For investigations of the influence of H_2SO_4, acid solutions of given concentrations ($5 \cdot 10^{-4} - 5 \cdot 10^{-3}$ M) were prepared and introduced into the empty Langmuir trough. Then the DPPC monolayer was formed and the hysteresis was measured within a few minutes.

For studies with the Pulsating Bubble Surfactometer (Electronetics Corp., USA) we used the commercial pulmonary surfactant preparation INFASURF (ONY Inc., USA), made from bovine lung lavage material. INFASURF was diluted 1:10 with distilled water before experiments. Mixtures of the surfactant suspension with sulfuric acid were prepared directly prior the measurement in the PBS. Experiments were done at 37 $\pm$ 0.5 °C.

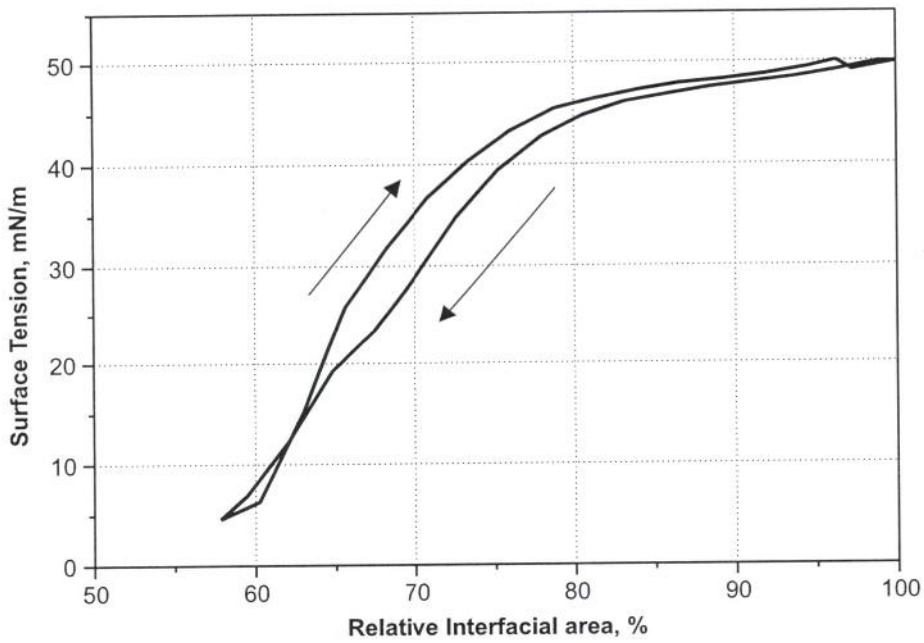

Figure 5. Surface tension hysteresis for 1,2-dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemicals, USA) on the hypophase of 0.001 M sulfuric acid, 36.6 $\pm$ 0.05 °C.

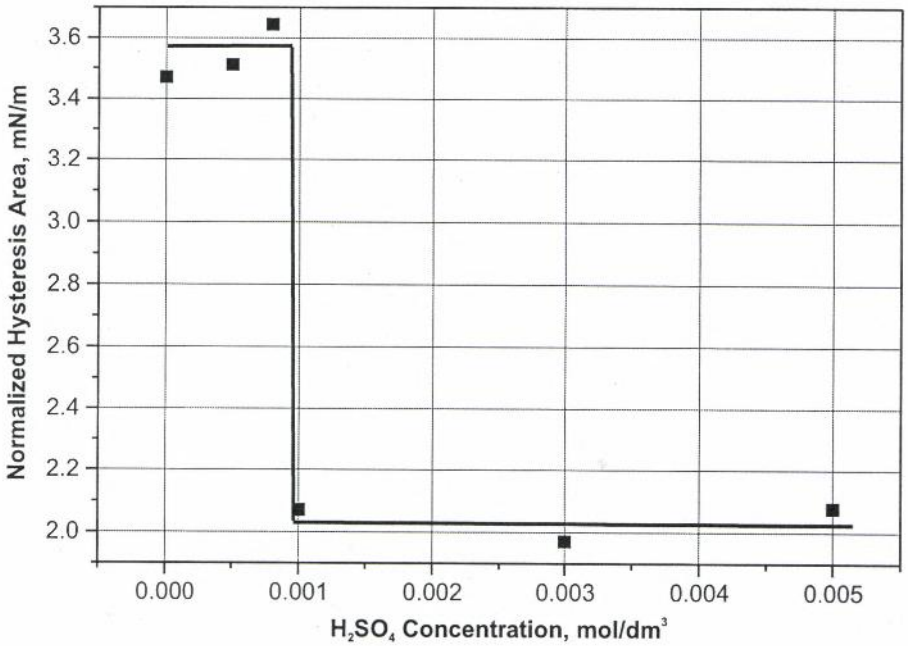

Figure 6. Normalized Hysteresis Area, HA_n, as a function of sulfuric acid concentration in the hypophase for 1,2-dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemicals, USA), 36.6 ± 0.05 °C.

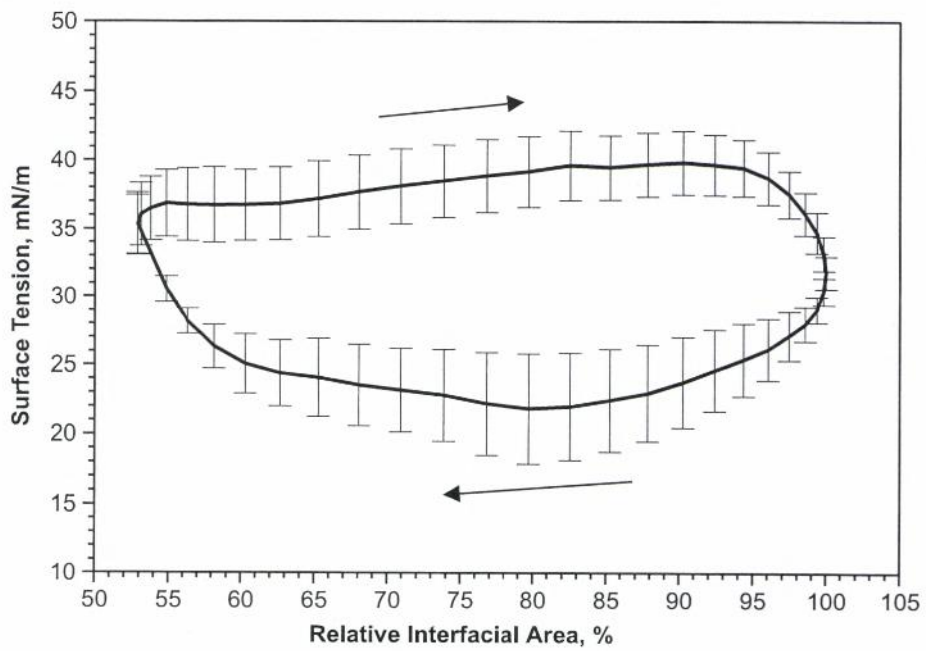

Figure 7. Surface tension hysteresis measured in PBS for INFASURF (1:10 aq.), 37 ± 0.5 °C.

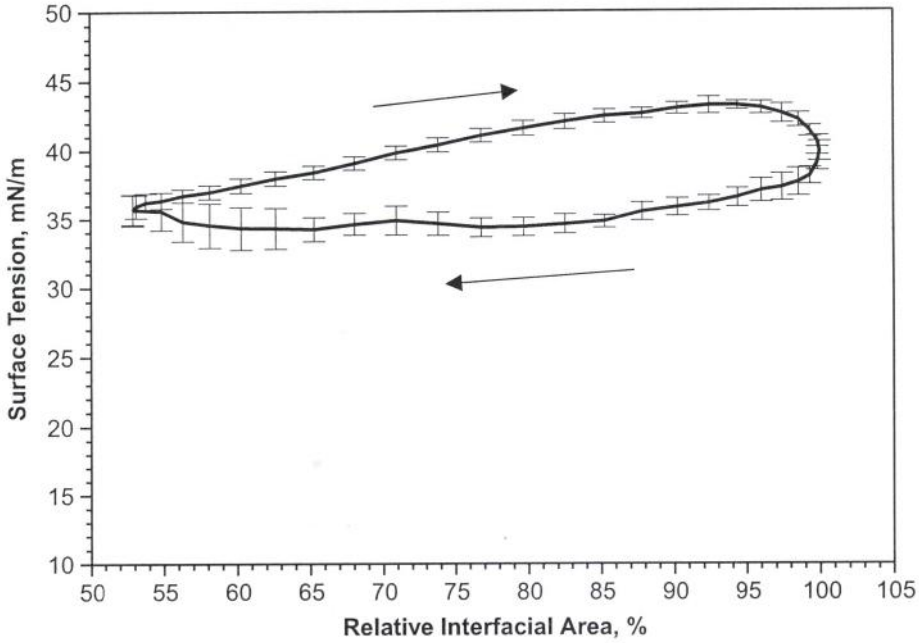

Figure 8. Surface tension hysteresis measured in PBS for INFASURF (1:10 aq.) mixed with sulfuric acid to final H_2SO_4 concentration 0.001 M, 37 ± 0.5 °C.

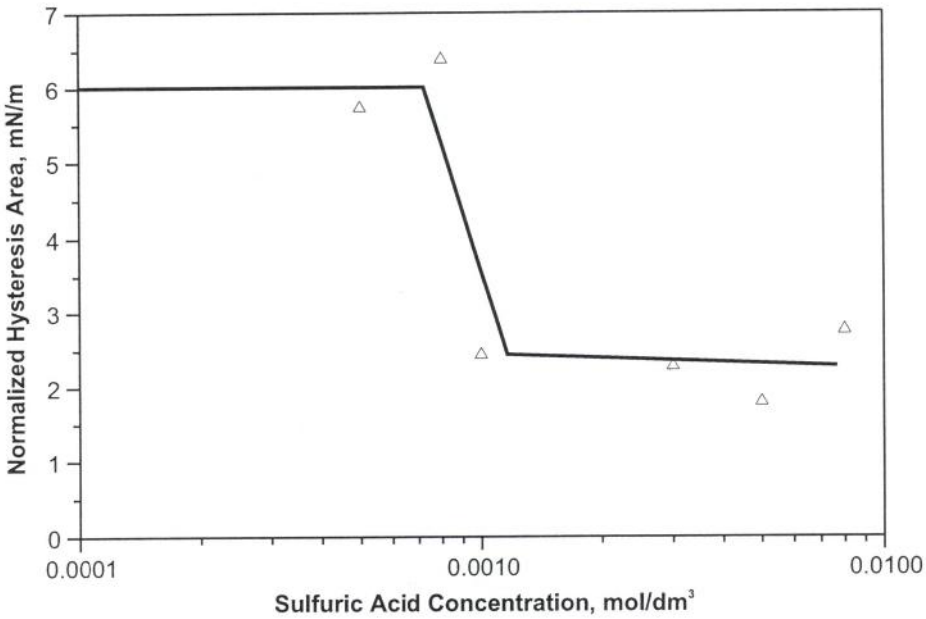

Figure 9. Normalized Hysteresis Area, HA_n, as a function of sulfuric acid concentration in the hypophase for INFASURF (1:10 aq.), 37 ± 0.5 °C.

5.2. Results and Discussion

Two examples of hystereses obtained from the LFB studies for DPPC monolayer on two different hypophases, pure water, and 0.001 M sulfuric acid are presented in Figures 4 and 5, respectively. Those curves are presented only to illustrate the pattern of alteration of interfacial activity of the model surfactant after change in the composition of the liquid. More comprehensive results are presented in Figure 6, which demonstrates the relationship between the Normalized Hysteresis Area, HA_n (Equation 1) and concentration of the sulfuric acid in the aqueous hypophase. The relationship presented in the last figure takes advantage of proper data analysis with the criteria introduced earlier over a limited and inconvenient method of qualitative comparison of experimental (σ-A) curves. It can be clearly seen that the physiologically significant surface activity of the model PS is reduced at sulfuric acid concentrations above 0.001 mol/dm^3. Those results suggest that there exists a threshold concentration of the toxicant. If H_2SO_4 concentration in the hypophase exceeds that value, then the surface tension hysteresis is significantly reduced, which suggests inadequate PS function. On the other hand, a further increase of acid dose does not cause more intense inactivation of the surfactant. The results from LFB may be compared to experimental data from PBS studies (Figures 7–9).

In spite of some differences in the shape of the surface tension hysteresis in comparison to the results of the LFB studies, the pattern of hysteresis disturbance due to the contact with sulfuric acid is quite similar, indicating the analogous threshold value of the acid concentration. Control hystereses obtained in PBS for another kind of PS-modeling material are generally larger that the loops observed for DPPC in the Lanmguir-Wilhelmy balance. It can be easily explained by different compositions of the surfactant materials and a different experimental approach. The pulmonary surfactant is modeled in the LFB studies by the single surface-active compound, which is believed to determine the activity of the whole PS. DPPC is present in that experimental system only at the air/liquid interface, hence its surface concentration is changed—in the definite range—due to movement of the barriers. On the contrary, a mixture of surface-active substances used in the PBS studies (i.e., INFASURF containing a large proportion of saturated phosphatidylcholines), exists as a suspension. The surfactant is present there both in the bulk phase and—due to spontaneous adsorption—at

the air/liquid interface. Surface concentration of the surfactant varies there as a result of predefined area oscillations and spontaneous sorption processes. As demonstrated here, different experimental models allowed to determine the surface tension hysteresis loop and for the observation of its disappearance after contact with sulfuric acid at the doses exceeding the limiting concentration 0.001 mol/dm^3. The proposed mechanism of destructive action of the sulfuric acid is related to modification of interaction between molecules of the surface-active substances and the ions present in the hypophase. Change in hydration of surfactant molecules can lead to the reduced interfacial stability and disturbed sorption processes. All those effects are pronounced in decreased surface tension hysteresis, which is considered as a significant disturbance of the natural physiological function of PS.

6. CONCLUSIONS

Several techniques of experimental evaluation of the pulmonary surfactant interfacial activity were presented. Two of them, pulsating bubble method and Langmuir-Wilhelmy method, were demonstrated to be suitable for in vitro analysis of the potential toxicity related to inactivation of the surfactant by inhaled substances. Based on the surface tension hystereses measurements, it was shown that surface activity of main PS components was significantly reduced for H_2SO_4 present in the hypophase at concentrations about 0.001 mol/dm^3. The unfavorable changes of surfactant properties were manifested by a reduction of the surface tension hysteresis loop, which is associated with the most important physiological functions of the pulmonary surfactant in the organism. Surfactant inactivation, which in vivo can result in severe health problems, in that case was a result of ionic interactions in the liquid hypophase. For different compounds, however, other mechanisms are also possible. The methodology proposed allows for analysis of the pulmonary surfactant inactivation by a variety of compounds present in the air, which may penetrate to the lungs during breathing. The presented study is the first in a series of comprehensive experiments, which are being conducted in our group in order to assess the potential pulmonary toxicity of airborne substances present in the workplace. The results of ongoing research will be published in the near future.

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