Pure & Appl. Chem., Vol. 64, No. 11, pp. 1745–1750, 1992.
Printed in Great Britain.

Pulmonary surfactant: Surface properties and function of alveolar and airway surfactant

Samuel Schürch[1,2], Martin Lee[1] and Peter Gehr[2]

[1]Respiratory Research Group, Faculty of Medicine, The University of Calgary, Alberta, Canada.
[2]Department of Anatomy, University of Berne, Switzerland.

Abstract

The pulmonary surfactant film at the alveolar air-liquid interface reduces the surface tension to a value below 1 mN/m on lung deflation. In addition to a low and stable surface tension, interdependence provided by the fibrous continuum enables the lung to maintain a large alveolar surface area, necessary for an efficient gas exchange. Surface tension-area relations from lung surfactant extracts are obtained with a new apparatus that contains a captive bubble of controllable size. Upon 3-4 compression-expansion cycles, surfactant films exhibit the low surface tensions, collapse rates and compressibilities characteristic of alveolar surfaces.

Not only the alveoli but also the bronchi are covered by a surfactant film. Inhaled particles, when deposited in the airways or alveoli, are displaced by surface forces towards the epithelium where they are retained. Experiments in the Langmuir-Wilhelmy balance showed that pulmonary surfactant promotes the displacement of spherical latex particles from air to the aqueous phase. The extent of particle immersion depends on the surface tension of the surface active film. The lower the surface tension, the greater is the immersion of the particles into the aqueous subphase. Electron microscopy demonstrates that particles in peripheral airways and alveoli are found below the surfactant film and submerged in the subphase. This may promote clearance by macrophages.

FUNCTION AND COMPOSITION

The pulmonary surfactant film at the alveolar air-liquid interface reduces the surface tension to less than 1mN/m on lung deflation. In addition to a low and stable surface tension, interdependence provided by the fibrous continuum enables the lung to maintain a large alveolar surface area, necessary for an efficient gas exchange.

Pulmonary surfactant of mammalian lungs consists of a variety of macromelecular complexes comprised of lipids and specific proteins. Surfactant is synthesized by the alveolar type II cell, in which it is stored in lamellar bodies (1,2). These lamellar bodies, when secreted into the alveoli form tubular myelin (3), which appears to be the principal precursor of the surface film that lowers the surface tension. Lamellar bodies and tubular myelin, both contain lipid and protein components of surfactant. However, the compressed surface film is thought to consist primarily of dipalmitoylphosphatidylcholine (DPPC) (4). Surfactant obtained through bronchiolar lavage contains approximately 90% lipid, 10% protein and small amounts of carbohydrate. DPPC, which accounts for approximately half the lipid in surfactant, is primarily responsible for the surface tension reducing property of the surfactant complex. The synthesis, secretion and metabolism of DPPC and other surfactant lipids has been the subject of recent reviews (5). The proteins associated with surfactant have been designated SP-A, SP-B and SP-C by Possmayer and his associates (6). SP-A is a variably glycosylated protein with a molecular mass of 28 to 36 kDa (reduced). SP-A is relatively water-soluble but SP-B and SP-C remain with the lipids extracted with organic solvents. SP-B has a molecular mass of 15 kDa (non-reduced) while SP-C has a molecular mass at 3-5 kDa in the non-reduced or reduced states.

Surfactant proteins directly affect the interfacial properties of surfactant lipids. Recent studies imply that surfactant-associated protein A and the recently identified SP-D have other important roles in the lung, including immunologic defence (7). Rapid adsorption of surfactant phospholipids to the air-liquid interface is thought to be critical for maintaining the morphological integrity of the gas exchange region of the lung (8). Purified SP-B (9), SP-C or mixtures of the two proteins (10) substantially enhanced the rate of formation of a surface film at an air-liquid interface in vitro; this action was further enhanced by the addition of SP-A (11). Preparations of phospholipids containing SP-B alone were more effective than similar preparations containing SP-C in reducing surface tension in a pulsating bubble surfactometer (10). Preparations of surfactant lipids containing mixtures of SP-B and SP-C were shown to increase lung compliance and preserve the morphological integrity in the distal airways in prematurely delivered ventilated fetal rabbits (8). Similar lipid extract surfactants have been widely tested in clinical trials and shown to improve oxygenation and decrease the need for respiratory support in infants suffering from the respiratory distress syndrome (12). However, the precise assignment of specific roles for SP-A, SP-B and SP-C in the surface activity of surfactant lipids has not yet been clarified (7). Ongoing research on the interactions between the purified surfactant proteins and individual surfactant lipid components will substantially enhance our knowledge about surfactant surface activity in the near future.

A thorough understanding of the mechanical effects of surfactant in the lung has been hindered by several problems: the behaviour of films of lung surfactant in vitro, e.g., in the Langmuir-Wilhelmy balance or in the pulsating bubble surfactometer, is different from that of the alveolar lining layer in situ (4). Although minimum surface tensions

below 5 mN/m in the balance or below 1 mN/m in the bubble surfactometer, can be obtained at a relatively high speed of film compression, these low surface tensions have much less stability than the alveolar film in situ. The alveolar film itself has, as calculated from pressure-volume studies on lungs, of intact animals or on excised lungs, a far greater stability (13). In the lungs held at 40% total lung capacity (TLC), the surface tension increased only 1 to 2 mN/m in twenty minutes. These results are in line with the surface tension stability data obtained by direct measurement of alveolar surface tension by the microdroplet spreading technique (14). According to the pressure-volume studies and the results obtained by the microdroplet spreading technique surface tension below 1 mN/m are obtained under quasi-static conditions. Goerke and his coworkers (4) have repeatedly drawn attention to the fact of the leaky nature of surface balances and open bubbles which permit a substantial escape of film material around the Teflon barrier and restraining walls of the surface balance and up the capillary tube in the pulsating bubble surfactometer. Since film material is spread over a large surface area of approximately 140 m^2 in the adult human lung (15) and this material has a very small escape route up the major airways, the lung provides an almost leak proof system for the alveolar surface film. In order to mimic the situation in the lung in vitro, we have recently developed a captive bubble technique (16,17) in which the bubble does not communicate with the atmosphere and where the surface film is completely surrounded by aqueous media (Figs. 1, 2).

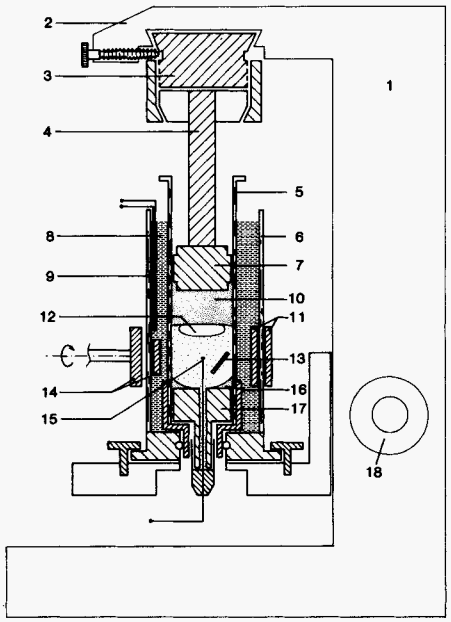

Figure 1

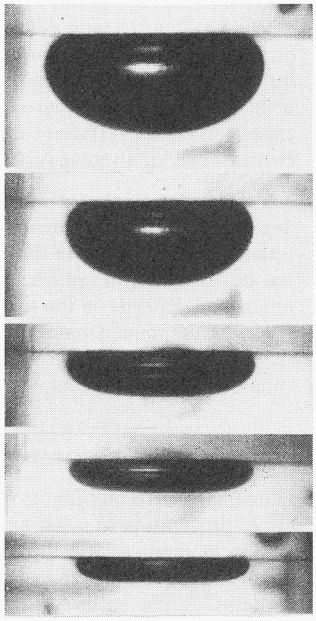

Figure 2

Figure 1: The captive bubble surface tensiometer. 1.2 Microscope stand. 3. Chuck for clamping syringe plunger onto microscope stand. 4. Plunger. 5. Glass cylinder (gastight syringe). 6. Plastic chamber with glass windows. 7. Teflon piston. 8. Water. 9. Heating element. 10. 1% agarose gel. 11. Magnets, position adjustable. 12. Air bubble. 13. Stir bar. 14. Magnetic stir bars. 15. Temperature probe. 16. 1% agarose meniscus or stainless steel funnel to prevent sticking of air bubbles. 17. Teflon plug. 18. Focusing knob. Figure 1 from Schürch et al. (17) with permission.

Procedure: A bubble of atmospheric air, 2mm in diameter is formed below a slightly concave 1% agarose ceiling. The chamber, a 5ml syringe (Unimetrics, gastight, Shorewood, IL) is closed pressure-tight and mounted between the stage and nosepiece holder of a microscope stand. The focusing mechanism of the microscope stand is used to drive the syringe piston. The pressure in the chamber is reduced to expand the bubble to maximum size, 6-8 mm in diameter. The bubble area and therefore the surfactant film area decreases when pressure to the chamber is applied which decreases the bubble size.

Figure 2: Bubble shape at five different surface tensions, from top to bottom: 25, 16, 4, 2, 1 mN/m. The surfactant was purified rat pulmonary surfactant. Figure 2 from Schürch et al. (17) with permission.

SURFACE TENSION–AREA RELATIONS

a) Adsorption, hysteresis and stability of films from purified rat pulmonary surfactant at phospholipid concentrations of 50, 200 and 400 μg/ml were studied in the captive bubble apparatus. At the highest concentration, adsorption was rapid, reaching surface tensions below 30 mN/m within 1 sec, while at the lowest concentration 3 min were required. Upon a first quasi-static (2-3 min) or dynamic (1 sec) compression stable surface tensions below 1 mN/m could be obtained by a film area reduction of about 50%. After 3-4 cycles the surface tension-area relations became stationary, and the tension fell from 25-30 to 1 mN/m for a film area reduction of less than 20% (Fig 3). Once the minimum tension was attained, hysteresis became negligible (Fig. 3), provided the films were not collapsed at minimum surface tension.

Under these conditions, the films could be cycled between 24 and 1 mN/m for more than 400 cycles without any noticeable loss of surface activity. After repeated cycling and collapse at minimum surface tension (collapse plateau) the results indicated that film material is displaced from the film, and the collapsed material does not participate in film formation upon bubble expansion. This observation might be relevant for replacement therapies with artificial surfactant in cases of the the respiratory distress syndrome of the newborn. Maintaining a certain level of positive end expiratory pressure might be beneficial for film stability because with this procedure film collapse at minimum surface tension is avoided. It was also observed that when the bubble "clicks" the surface tension suddenly increased, and the bubble shape changed from flat to more spherical (16). The associated isovolumetric decrease in surface area prevents the surface tension from rising as much as it would have in a constant area situation. This feedback mechanism could also have a favourable effect in stabilizing alveolar surface tension at low lung volumes, since Bachofen et al. (18) observed that alveolar septa tend to retract, decreasing alveolar surface area, when the surface tension is raised.

b) Pulmonary Surfactant Associated Protein A (SP-A) enhances adsorption and surface refinement of films from lipid extract surfactant. The effect of surfactant concentration and supplementation with SP-A on the surface activity of lipid extract surfactant (LES) was examined using the captive bubble technique. Adsorption of LES is strongly dependent on the phospholipid concentration. At 50 μg/ml adsorption is slow, taking more than 30 min to reach values below 40 mN/m. At 100-200 μg/ml adsorption to 25-26 mN/m was faster, taking between 20 and 30 sec, while at and above 800 μg/ml adsorption to 25-26 mN/m occurred within one sec. Addition of SP-A (1.0-4.0%) to LES at low concentrations (200 μg/ml) dramatically increases the rate of adsorption (1 sec to 25-26 mN/m in the presence of calcium). In quasi-static cycling experiments samples of relatively low phospholipid concentration (200 μg/ml) but with 1% SP-A added, require 20-30% less film area reduction than without SP-A to achieve minimum surface tensions of about 1 mN/m. The calculated film compressibilities at 15 mN/m imply that SP-A alters the surfactant film such that within 4 cycles, film compressibility is indistinguishable from that of a pure DPPC film.

In addition, SP-A reduces the incidence of "clicking", indicating a stabilization of the film at low surface tensions. In stationary surface tension-area hysteresis loops, produced by dynamic cycling, SP-A reduces the compression of the film area required to achieve low surface tension (1 mN/m) and eliminates the relatively flat compression part (squeeze-out plateau) at about 20 (mN/m) (Fig 4). During dynamic hysteresis loops of LES films, the surface tension remains nearly constant as the film area starts to increase. This behaviour is not compatible with that of a monolayer. The results imply that the surfactant film might change into a multilayer configuration which can maintain very low surface tensions during initial area expansion.

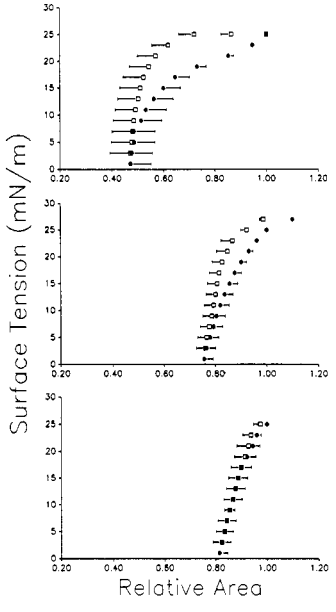

Figure 3

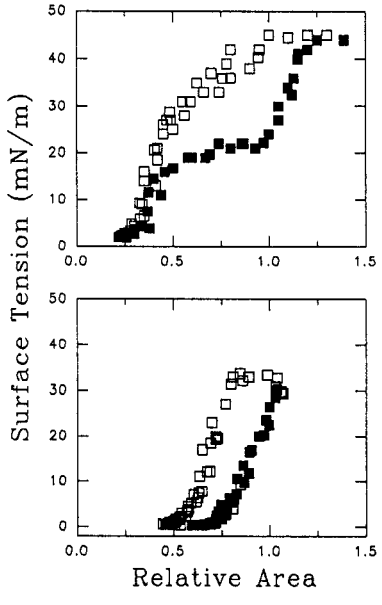

Figure 4

Figure 3: Quasi-static surface tension-area relation obtained from rat pulmonary surfactant. Collapse at minimum surface tension was avoided by expanding the bubble after reaching a surface tension of 1-2 mN/m. Top, first cycle middle second cycle, bottom fourth cycle. Note: decreasing area reduction necessary to reach near zero tension upon consecutive cycling, and reduced hysteresis. Filled circles, compression. Mean ± SEM, 8 independent experiments.

Figure 4: The effect of concentration and SP-A on dynamic hysteresis loops of Lipid Extract Surfactant. Surface tension (mN/m) is plotted versus relative area. The plots are the summaries of four consecutive dynamic cycles centering on the 20th cycle. Filled symbols represent measurements made during compression, open symbols represent those made during expansion. Sample are suspended in 0.9% NaCl, 1.5 mN $CaCl_2$. Top: LES at 200 μg/mL. Bottom: LES at 200 μg/mL supplemented with 4.0% SP-A.

PARTICLE DISPLACEMENT AND AIRWAY SURFACTANT

a) Introduction: Currently our knowledge of pulmonary surfactant is limited to the alveolar compartment and to large (accessible) airways. In both locations, the surface tension has been estimated by observing the spreading behaviour of oil droplets. In alveoli droplets were placed onto the surface film by micropipettes e.g.(14), while in large airways a bronchoscope was used for droplet placement and observation. By using this method, we have demonstrated the existence of a surfactant film lining the trachea and bronchi (19,20). This film has a surface tension of 30-32 mN/m.

In contrast to the extensive knowledge about alveolar surfactant, little is known with regard to surfactant in airways. Widdicombe (1) recently reviewed the possible sources and potential biological role of tracheal surfactants. He concluded that the trachea contains a complex mixture of lipids, including surface active phospholipids. Since <1% of the alveolar phospholipid is carried up into the trachea, and the composition of other lipids in the trachea is not characteristic of alveolar surfactant, Widdicombe deducted that the lipids in the trachea must be derived from the tracheal epithelium and/or tracheo-bronchial glands.
Gebhart et al., (21) suggested that cycling surface tension gradients provide a transport mechanism for clearance of inhaled particles. It seems likely that the peripheral airway zones are important in stabilizing the surfactant film and provide a transport mechanism for particles from the peripheral airspaces to the mucocilliary escalator.

b) Particle-Surfactant Interactions: Experiments conducted in the Langmuir-Wilhelmy balance and in vivo experiments using aerosols of latex beads showed that the airway surfactant film promotes the displacement of particles from air into the aqueous phase and that the extent of particle immersion depends on the surface tension of the film (20), Fig. 5 shows particles placed onto a surfactant film at differing surface tensions.

An examination of electron micrographs with particles in peripheral airways or alveoli demonstrated that these particles are coated with an osmiophilic film (Fig.6). This film extends over the whole surface of particles totally immersed in the aqueous phase as well as over the surface partially protruding into the airspace. It appears that our description of particle displacement has not been complete. We have dealt with the immersion process showing that the lower the film surface tension the greater is the extent of particle immersion because of more extensive wetting or film spreading over the particle surface. Regardless of the particle material, in the alveoli and likely in the peripheral airways, particles will be completely wetted by the film whose surface tension falls substantially (<1mN/m) below that of the particle on expiration (14). Remarkable, even the central airways we found the particles submerged in the aqueous phase, and the epithelium was frequently deformed indicating that a force acting on the particle had been transmitted to the underlying cell layer.

In order to completely describe particle-surface film interactions, we have to refer to an additional force whose action is noticeable only for small particles of diameter less than 10 μm. This force is called "line tension", a well-defined thermodynamic quantity, whose action is characterized in the literature dealing with capillarity and nucleation in surface and colloid chemistry. In analogy to the surface tension defined for a two-dimensional surface, line tension is defined

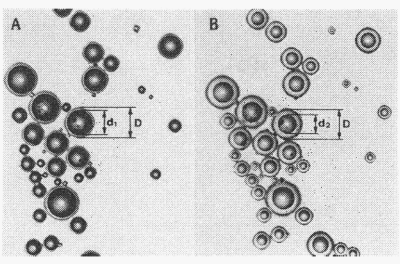

Figure 5

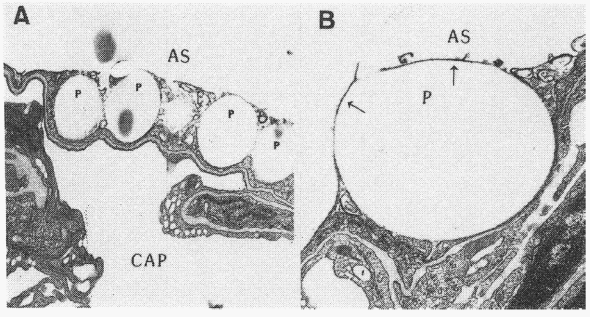

Figure 6

Figure 5: Polymethylmethacrylate (PMMA) beads on a DPPC monolayer supported by an aqueous subphase of a density (1.20 $g \cdot cm^{-3}$) at decreasing film surface tensions, A: 50 mN/m, B: 35 mN/m. Note: Label D indicates the total particle diameter, 80 μm, d is the diameter of the segment exposed to air.

Figure 6: (A) TEM of alveolar wall showing 1 μm diameter polystyrene beads in an alveolar space (AS). The particles have been displaced toward the epithelium and are completely covered by an osmiophilic layer (surfactant). Surface tension forces have caused deformation of the underlying capillary (CAP) by the particles. (B) Higher magnification of same general area showing osmiophilic (lipid) coating of a 3 μm particle (arrows). Figure 6 from Schürch et al. (20) with permission.

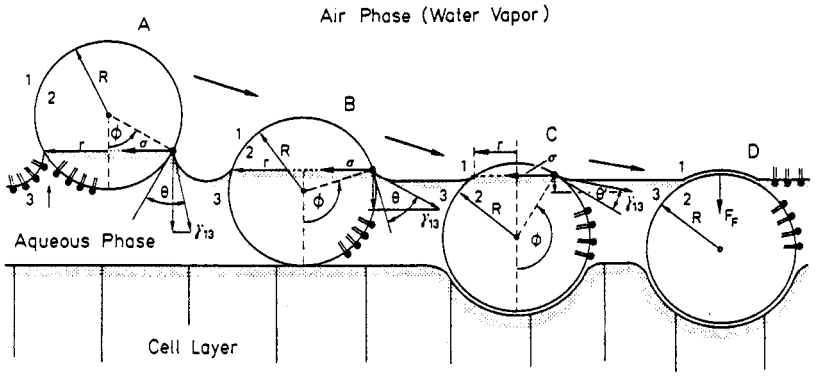

Figure 7: A: Situation immediately after deposition during immersion: The contact angle, θ, is characteristic for the particle surface tension (free energy, not shown) and the film surface tension, γ_{13}, at the air (1), particle (2) and water (3) contact line. The lower γ_{13} for a given particle surface material, the lower is θ. ϕ indicates the position at the three-phase line, σ is the line tension, it is directed towards the centre of the three-phase contact line (a circle here). In this situation the particle equator is above the water level, σ tends to prevent wetting. However, the vertical component of γ_{13} is dominant here. B: The particle is further displaced and contact withe cell layer is established. The line tension promotes wetting as the particle equator is below the water level. C: The surface tension γ_{13} and the line tension σ promote further particle displacement, the cell layer is deformed by the particle. θ is substantially lower here than the original θ because of the line tension contribution: σ promotes further particle wetting. D: The particle is situated below the surfactant film which may be considered as an elastic film. The vertical force F_F proportional to the film surface tension acts in vertical direction. Final deformation of the cell layer has occurred. There is no longer an air-particle-water three-phase line and, thus, no line tension.

as the force operating in the one-dimensional three-phase line, or alternatively, as the free energy per unit length of the three-phase line (22). An examination of the modified Young equation (22) which governs the equilibrium of a line element or edge when the three-phases (particle, liquid, air) join, shows that the contact angle varies with the drop size because of the line tension. For a particle, immersed more than 50% in the aqueous phase, the line tension promotes surface wetting: The excess free energy residing in the three-phase line is minimized by minimizing the length of this contact line. Therefore, the three-phase line is moved closer to the particle apex than it would have been without line tension.

In our work on line tension we have used a system that can be applied to the polystyrene particles at the air-liquid interface modified by a surfactant film (23). Instead of a solid particle we used an oil droplet whose surface tension was close to that of polystyrene, 32-35 mN/m. The drop was sitting on a surfactant layer of dipalmitoylphosphatidyl choline (DPPC). Our value for the line tension, approximately $2x10^{-8}$ J/m, was in close agreement with that obtained by Torza and Mason (24). We have estimated the contribution of the line tension to the position of the three-phase contact line, (Fig 7) for particles of a diameter less than 5 μm. This contribution was of the same order of magnitude as the surface tension forces.

In a recent pilot experiment we placed small microspheres, average radius 4 μm, and larger microspheres, average radius 25 μm, on a DPPC film at 25 mN/m. The smaller particles were displaced significantly more into the aqueous phase than the larger particles. In these experiments everything was equal except for the particle size. Electron micrographs showed very smooth particle surfaces with protrusions of less than 10 nm. Thus we attributed the above effect to the line tension, not to contact angle hysteresis.

In conclusion, line tension effects have to be considered for the interaction of micrometer sized particles of different sizes, shapes and surface characteristics with interfacial films and most likely for particle-membrane interactions. A summary of the forces involved in particle displacement at the airway surface are shown in Fig 7.

CONCLUSIONS

1. The studies on alveolar surfactant should enhance our understanding of the mechanics involved in film formation, in film structure and film refinement. These should help to optimize the surfactant properties of exogenous surfactant to be used in the respiratory distress syndrome of the newborn and adult.
2. The studies on airway surfactant are focused on the ways in which particles interact with the site of initial deposition of inhaled particles, i.e., the mucous surface. This should provide new information on the structure and function of airway surfactant, and particle kinetics. By comparing the interactions of particles with the mucous blanket under normal circumstances with their behaviour under conditions of airway injury, new insights into the pathogenesis of airway disease can be obtained.

Acknowledgement

This work was supported by the M.R.C., Canada, Mt-6435, the Alberta Heritage Foundation for Medical Research and the Swiss N.S.F., Grant 3.909-0.85. ML is a recipient of the Alberta Lung Association Studentship.

REFERENCES

1. L.G. Dobbs, J.R. Wright, S. Hawgood, L. Gonzales, J. Ventstrom and J. Nellenbogen, Proc. Natl. Acad. Sci., 84, 1010-1014, (1987).
2. R.J. King, J. Appl. Physiol., 53, 1-8, (1982).
3. M.C. Williams, J. Cell Biol., 72, 260-277, (1987).
4. J. Goerke and J.A. Clements, Handbook of Physiology, Section 3, Vol. III, Part 1, 247-261, (1986).
5. M. Post and L.M.G. van Golde, Biochim. Biophys. Acta 947, 249-286, (1988).
6. F. Possmayer, Am. Rev. Respir. Dis., 138, 990-998, (1988).
7. T.E. Weaver and J.A. Whitsett, Biochim. J., 273, 249-264, (1991).
8. G. Grossmann, R. Nilsson and B. Robertson, Eur. J. Pediatr., 145, 361-367, (1986).
9. F. Possmayer and S.-H. Yu, Biochim. Biophys. Acta, 1046, 233-241, (1990).
10. S.-H. Yu and F. Possmayer, Biochim. Biophys. Acta, 961, 337-350, (1988).
11. A.M. Cockshutt, J. Weitz and F. Possmayer, Biochemistry, 29, 8424-8429, (1990).
12. A. Jobe and M. Ikegami, Am. Rev. Respir. Dis., 136, 1256-1275, (1987).
13. J. Mead and C. Collier, J. Appl. Physiol., 14, 669-678, (1959).
14. S. Schürch, Respir. Physiol, 48, 339-355, (1982).
15. E.R. Weibel, The pathway for oxygen, Harvard University Press, 1-425, (1984).
16. S. Schürch, H. Bachofen, J. Goerke and F. Possmayer, J. Appl. Physiol., 67, 2389-2396, (1989).
17. S. Schürch, H. Bachofen, J. Goerke and F. Green, Biochim. Biophys. Acta, in press, (1991).
18. H. Bachofen, S. Schürch, M. Urbinelli and E.R. Weibel, J. Appl. Physiol., 62, 1878-1887, (1987).
19. P. Gehr, Y. Berthiaume, V. Im Hof and M. Geiser, J. Aerosol Med., 3, 27-43, (1990).
20. S. Schürch, P. Gehr, U. Im Hof, M. Geiser and F. Green, Respir. Physiol., 80, 17-32, (1990).
21. K.F. Gebhardt, H. Rensch and H. von Seefeld, Progr. Respir. Res., 67, 2234-2243, (1984).
22. D. Li and A.W. Neumann, Colloids and Surfaces, 43, 307-314, (1990).
23. J.A. Wallace and S. Schürch, J. Colloid and Interface Sci., 124, 452-461 (1988).
24. S. Torza and S. G. Mason, Kolloid-Z.u.A., Polymere, 246, 593-599 (1971).