An investigation of pulmonary surfactant physicochemical behavior under airway reopening conditions

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Ghadiali, Samir N., and Donald P. Gaver III. An investigation of pulmonary surfactant physicochemical behavior under airway reopening conditions. J. Appl. Physiol. 88: 493–506, 2000.—Airway reopening mechanics depend on surfactant physicochemical properties. During reopening, the progression of a finger of air down an airway creates an interface that is continually expanding into the bulk fluid. Conventional surfactometers are not capable of evaluating physicochemical behavior under these conditions. To study these aspects, we investigated the pressure required to push a semi-infinite bubble of air down a fluid-filled cylindrical capillary of radius R. The ionic surfactant SDS and pulmonary surfactant analogs L-α-dipalmitoylphosphatidylcholine and Infasurf were investigated. We found that the nonequilibrium adsorption of surfactant can create a large nonequilibrium normal stress and a surface shear stress (Marangoni stress) that increase the bubble pressure. The nonphysiological surfactant SDS is capable of eliminating the normal stress and partially reducing the Marangoni stress. The main component of pulmonary surfactant, L-α-dipalmitoylphosphatidylcholine, is not capable of reducing either stress, demonstrating slow adsorption properties. The clinically relevant surfactant Infasurf is shown to have intermediate adsorption properties, such that the nonequilibrium normal stress is reduced but the Marangoni stress remains large. Infasurf’s behavior suggests that an optimal surfactant solution will have sorption properties that are fast enough to reduce the reopening pressure that may damage airway wall epithelial cells but slow enough to maintain the Marangoni stress that enhances airway stability.

airway closure; dynamic surface tension; L-α-dipalmitoylphosphatidylcholine; Infasurf; Marangoni stress

PULMONARY AIRWAY CLOSURE can occur when the lung reaches very low volumes (20). Healthy individuals reopen these airways on the next inspiratory effort. However, these airways remain closed in premature infants suffering from respiratory distress syndrome (RDS) and individuals with asthma, emphysema, or cystic fibrosis. These conditions can result in atelectasis and/or local hypoventilation. Pulmonary surfactant insufficiency, loss of parenchymal tethering, and an increase in fluid viscosity have been implicated as possible mechanisms by which airways remain closed (2, 19).

Avery and Mead (2) were the first to observe a lack of pulmonary surfactant production in premature neonates suffering from RDS. The production of fetal surfactant begins during the 4th mo of gestation. However, this surfactant system (consisting of phospholipids and proteins) may not mature until the 7th mo (20). A mature surfactant system decreases lung inflation pressures by reducing the surface tension forces that oppose airway opening. Therefore, an immature surfactant system results in large lung inflation pressures, mechanical instability, and subsequent atelectasis.

One possible method to treat RDS involves mechanical ventilation. However, large ventilation pressures can result in airway wall damage. Specifically, mechanical stresses at the wall can peel apart epithelial cells (22). As a result, the epithelium becomes highly permeable to proteins that can inactivate surfactant (32). This inactivation further elevates reopening pressures and establishes a positive-feedback cycle in which significant airway wall damage can occur. To minimize this damage, neonates with RDS have been treated with positive end-expiratory pressure ventilation, high-frequency ventilation, and/or several surfactant replacement therapies (14). The advent of surfactant replacement therapy has resulted in a 60% reduction in the infant mortality rate for RDS since 1989 (13). However, RDS was still the fourth leading cause of infant death, with 1,368 fatalities in 1996 (13).

Three distinct types of surfactant replacements, natural, modified natural, and synthetic, have been developed. One concern with natural surfactant is that sensitive patients may develop an allergic reaction to the surfactant-associated proteins (33). However, because of an immature immune system, a severe allergic response in neonates is rare (43). Synthetic surfactants frequently do not perform as well as the natural surfactant system (14). This is primarily due to a synthetic surfactant’s inability to mimic the chemical complexity of a natural surfactant.

We hypothesize that the effectiveness of a surfactant replacement depends on its dynamic surface tension properties. Most researchers have focused on the fact that an ideal surfactant system should promote alveolar stability by reducing the surface tension on compression (3, 12, 25) or stabilize the airway lining fluid by preventing Rayleigh-Taylor instabilities (15, 17). However, to limit epithelial cell damage during airway reopening, the surfactant must also minimize reopening pressures and airway wall stresses. The ability of a
surfactant to perform adequately during interfacial expansion depends on its transport characteristics (e.g., diffusivity and interfacial adsorption/desorption rates) and surface activity. The interplay between these mechanisms determines the efficacy with which a surfactant can reduce airway reopening stresses. The goals of the present study are to 1) develop a method for determining the capability of a surfactant to reduce airway reopening pressures and 2) demonstrate this method by determining the efficacy of several surfactants (physiological and nonphysiological) to reduce the dynamic surface tension.

**BACKGROUND**

Airway closure occurs when a liquid occlusion spans the airway and blocks airflow. This occlusion can be extensive (as occurs before an infant’s 1st breath or in atelectasis) or consist of a short meniscus (e.g., a mucous plug). The research described here relates to reopening an extended region of closure, wherein a semi-infinite air bubble penetrates the liquid occlusion and separates the airway walls as it displaces the lining fluid. This scenario has been described previously (9, 10, 16, 27, 28) and results in two-phase interfacial flow between the bubble and the lining fluid. Suki and colleagues (38, 39) investigated the avalanche-type behavior that occurs when multiple generations of airways open sequentially. Recent studies have used “stochastic resonance” to take advantage of this type of behavior and, thus, open airways with reduced average pressures (37).

A classic problem in the hydrodynamic literature related to pulmonary airway reopening involves semi-infinite bubble progression in a rigid capillary tube. This system was originally studied by Bretherton (4), Fairbrother and Stubbs (7), and Taylor (40). These studies formulated a relationship between the dimensionless interfacial pressure drop, \( P/\gamma/R \), and the capillary number, \( C_a = \mu U/\gamma \), where \( P \) is the dimensionless pressure drop across the air-liquid interface in the hemispherical bubble cap region, \( \gamma \) is the surface tension, \( R \) is the radius of the tube, \( \mu \) is the fluid viscosity, and \( U \) is the bubble speed. \( C_a \) is a dimensionless velocity that represents the relationship of viscous to surface tension stresses. These studies assumed a constant \( \gamma \) and, therefore, neglected the influence of surfactant. However, surfactant modifies the flow field by altering the local surface tension and mechanical stress balance at the interface. Several investigators (11, 31, 35, 36) have studied the interaction between surfactant physicochemistry and fluid mechanics. In rigid systems, these interactions modify the interfacial shape and pressure drop and, therefore, affect airway reopening characteristics. Recently, Yap and Gaver (45) demonstrated the importance of surfactant physicochemistry in flexible-walled systems intended to emulate collapsible airways and predicted that surfactant uptake could significantly influence airway reopening.

In coupling surfactant physicochemistry and fluid mechanics, it is important to understand the mechanisms of surfactant action and transport. Surfactant can reside in two phases: a bulk phase (\( C, \text{ mol/vol} \)) and a surface phase (\( I', \text{ mol/interfacial area} \)). The surface phase directly modifies the interfacial surface tension by \( \gamma = f(I') \); this relationship is referred to as the surfactant equation of state. In general, increasing \( I' \) reduces \( \gamma \); however, regions exist where \( d\gamma/dI' \) is nearly zero (41). In a static system, an equilibrium relationship between \( C \) and \( I' \) exists, with an associated equilibrium surface tension (\( \gamma_{eq} \)). The interface becomes saturated with surfactant as \( C \) increases, resulting in a maximum equilibrium surface concentration (\( I'_{sat} \)), which determines the minimum equilibrium surface tension in a static system (\( \gamma_{sat} \)). In a dynamic system, \( I' \) and \( \gamma \) can vary as a function of interfacial position. In addition, \( I' \) can exceed \( I'_{sat} \) under dynamic conditions resulting from surface compression (as occurs in the alveoli in vivo or experimentally in a Langmuir trough), which can reduce \( \gamma \) significantly.

Dynamic surfactant transport is modeled as a multistep process between two phases: the bulk and the interface. In the bulk phase, surfactants are transported by bulk convection and/or diffusion, resulting in \( C(x,t) \), where \( x \) defines the general spatial coordinate system. The bulk phase concentration in contact with the interface is the subphase concentration \( C_s(s,t) \), where \( s \) is a spatial coordinate along the interface. The rate of transport between the subphase and interface is modeled by an adsorption isotherm, where the rate of adsorption/desorption depends on the deviation from equilibrium between \( I' \) and \( C_s \). This step is balanced by diffusion in the bulk. Finally, interfacial convection and diffusion along the interface can influence the local \( I' \). These transport interactions determine the distribution of surfactant in the bulk and along the interface. This distribution establishes a stress balance along the air-liquid interface through the equation of state and thus impacts airway reopening pressures and stresses.

To conceptualize this interaction, consider the flow field surrounding a bubble flowing down a liquid-filled tube (Fig. 1A). Surfactant exists in the bulk (\( C \)) and on the interface (\( I' \)). Streamlines are drawn in a bubble-fixed reference frame in which flow enters from the right and exits to the left in the thin film. A recirculating region near the bubble tip occurs at low velocities. As a result, the rate of interfacial expansion/compression will vary with interfacial position (Fig. 1B). As shown in Fig. 1, the interface can be divided into three regions: a nearly hemispherical bubble cap region, a thin film, and a transition region between the bubble cap and thin film. A small portion of the bubble cap region near the tip will experience interfacial compression, whereas the majority of interfacial expansion takes place in upstream portions of the bubble cap and transition regions. In addition, this recirculating flow field creates a converging stagnation point at the bubble tip and a diverging stagnation ring near the thin film (42). Surface convection increases \( I' \) (reducing \( \gamma \)) at the converging stagnation point and reduces \( I' \) (increasing \( \gamma \)) at the diverging stagnation points. Variation of \( \gamma \) from \( \gamma_{eq} \) can alter the bubble pressure through two distinct, yet interrelated, mechanisms.
A

Diverging Stagnation Ring

Converging Stagnation Point

Γ(s,t)
g

Thin Film
Transition Region
Bubble Cap Region

B

Fig. 1. A: flow field surrounding a bubble flowing down a liquid-filled tube. Streamlines are drawn for a reference frame fixed with bubble. Γ, Direction of Marangoni stress. B: example profile of interfacial stretching as a function of interfacial position in a surfactant-free system. Negative stretching indicates interfacial compression; positive stretching indicates interfacial expansion. Capillary number (Ca) = 0.12.

One mechanism is related to the normal-stress boundary condition (law of Laplace), which states that the pressure difference across an interface is \( ΔP = γκ \), where \( γ \) is the local surface tension and \( κ \) is the curvature. Under dynamic interfacial expansion, the effective dynamic surface tension in the bubble cap region can exceed the equilibrium surface tension (\( γ > γ_{eq} \)). Therefore, the reopening pressure can exceed the pressure that would exist if \( γ = γ_{eq} \) uniformly. We refer to this deviation as the nonequilibrium normal stress.

A second mechanism that increases the bubble pressure is related to the development of a surface tension gradient, \( dγ/ds \). This gradient allows the interface to support a shear stress (Marangoni stress, \( τ_M \)) that is directed from regions of low \( γ \) to regions of high \( γ \) and opposes the surfactant-free flow field shown in Fig. 1A. Interfaces without a Marangoni stress are mobile, because they do not support a shear stress. Mobile interfaces occur when \( γ \) is spatially constant, which can happen if \( Γ = 0 \) (Dean interface), \( Γ \) is uniform, or \( Γ \) is nonuniform and \( dγ/ds \) is small. Because the Marangoni stress opposes the basic flow field, the interface acts more like a rigid boundary, resulting in a larger bubble pressure.

Several theoretical models have explained the importance of surfactant transport properties on bubble motion in rigid tubes. Ratulowski and Chang (31) analytically modeled trace surfactant transport and evaluated several different scenarios related to hindered transport from the bulk to the interface. Fundamentally, two processes can hinder surfactant transport: diffusion limitation in the bulk and kinetic adsorption/desorption barriers from the subphase to the interface. The relative relationship between diffusion and convection in the bulk is governed by the Péclet number, \( Pe = U(Λ/R)/D \), where \( D \) is the surfactant molecular diffusivity and the adsorption depth \( Λ = Γ/C \) is a length scale related to the fluid thickness that contains sufficient surfactant molecules to bring the interfacial concentration to \( Γ_{eq} \). The relationship between surfactant adsorption and surface convection is governed by the Stanton number, \( St = k_sCR/U \), where \( k_s \) is the adsorption rate constant.

The interface remains near equilibrium in the limit of \( Pe \rightarrow 0 \) and \( St \rightarrow \infty \). The diffusion barrier can be reduced (small \( Pe \)) by diminishing \( Λ \) through an increase in \( C \), a scenario analyzed by Stebe and Barthès-Biesel (35). Under these large \( C \) conditions, the adsorption barrier will be the only limitation to surfactant transport. Therefore, equilibrium behavior at large \( C \) indicates fast adsorption properties.

In summary, nonequilibrium normal and Marangoni stresses can significantly elevate reopening pressures when the transfer of surfactant to the interface is limited by bulk or sorptive transport processes. If bulk and sorptive transport processes are rapid, any surfactant deficiency on the interface is quickly replaced with surfactants from the bulk, resulting in a uniform \( γ \). Under these conditions, \( γ \rightarrow γ_{eq} \) and the normal stress approaches the equilibrium value. Furthermore, gradients in \( γ \) (and, therefore, the Marangoni stress) are eliminated, and the interface is said to be “remobilized.” Therefore, if adsorption is quick, the bubble motion is no longer hindered by the two mechanisms outlined above under large \( C \) conditions.

The main objective of the present study is to use these concepts to investigate how various pulmonary surfactant analogs affect the mechanics of a continually expanding interface, as occurs during airway reopening. We hypothesize that different surfactant systems will exhibit different capabilities for reducing reopening pressures and stresses owing to differences in physicochemical properties. Identifying these properties may be useful in recognizing and developing suitable surfactant replacements.

**EXPERIMENTAL METHODS**

Several methods are available for measuring dynamic surface tension. Schürr et al. (34) used a captive bubble apparatus to measure surface tension as a function of interfacial concentration. Enhörning (6) developed a pulsating bubble surfactometer to mimic alveolar dynamics. In both systems, volume oscillations produce an oscillating surface area. Al-
though these oscillatory techniques may model alveolar dynamicas, they are not as useful in understanding airway reopening. The key aspect that differentiates airway reopening from alveolar dynamics is the continual expansion of an air-liquid interface into the bulk solution. In this situation, tangential flows create nonuniform interfacial concentrations, and a Marangoni stress is generated. To identify the importance of nonequilibrium normal and Marangoni stresses during airway reopening, we developed a simple experimental apparatus that mimics the continual interfacial expansion aspects of airway reopening.

Model Description

The apparatus (Fig. 2) consists of a 1-m-long precision-bored glass capillary tube (Friedrich & Dimmock, Millville, NJ) with an internal diameter of 1 ± 0.005 mm. The tube is mounted on an aluminum bar (not shown) for accurate leveling. The tube is connected on one end to a constant-head reservoir and on the other end to a Gastight glass syringe (Hamilton, Reno, NV). The constant-head reservoir and capillary tube are filled with a surfactant solution and embedded in a Plexiglas jacket. A constant-temperature bath provides 37°C water that circulates through the Plexiglas jacket. A glass syringe is placed in a gear-controlled precision syringe pump (Cole-Parmer, Niles, IL), which generates flow rates of 0.01–10.0 ml/min ± 1%. The syringe pump pushes an air bubble forward while a very-low-range (≤14 cm H₂O), high-accuracy pressure transducer (model DP103-22, Validyne, Northridge, CA) is used to record the gas pressure. Two infrared emitter-detector pairs are placed such that the passage of the bubble tip produces a triggering signal. The detector signals and pressure signals are recorded as a function of time by the LabView data acquisition routine (National Instruments, Austin, TX). All tubing connections are made of Teflon polytetrafluoroethylene to avoid plasticizer contamination.

Surfactant Preparation Methods

Purified water with a resistivity of 18.2 MΩ·cm (Alpha-Q, Millipore, Bedford, MA) was used to make all solutions. Calcium-buffered saline (150 mM NaCl and 1 mM CaCl₂) was prepared using purified water, 99% pure NaCl, and CaCl₂. All glassware was cleaned in a 2% Micro solution (International Products, Burlington, NJ) for 24 h, rinsed with purified water, and dried in a drying oven (Blue M, Blue Island, IL). The glass capillary tube was dried by flushing it with filtered nitrogen for 5 min. Because of differences in solubilities, each surfactant solution was prepared with a unique method.

SDS (>99% purity; Sigma Chemical, St. Louis, MO) was used without further purification. Because SDS is ionic, solubilization was achieved by simply adding a known quantity to the volume of pure water in the end tank and stirring for 5 min.

L-α-Dipalmitoylphosphatidylcholine (DPPC) in powdered form (>99% purity; Sigma Chemical) was also used without further purification. DPPC dispersions were prepared using the method of Chung et al. (5). Batch solutions of DPPC at high concentrations (>0.3 mg/ml) were created by adding a known quantity of DPPC to a given volume of calcium-buffered saline. The suspension was stirred at temperatures above the DPPC transition temperature (43°C) to liquify DPPC crystals. This milky-white solution was then sonicated in 5-min intervals in an ultrasonic cleaner (model 1210, Branson) at 50°C until the solution was clear. The number of 5-min sonication intervals required to achieve the desired clarity was not constant. Dilution of the batch DPPC solution was performed in the end tank and stirred for 5 min. The batch solutions of DPPC were stored at 5°C. Before addition to the end tank, a batch solution was reheated and sonicated until the solution became clear. Because of the hydrolysis of DPPC that occurs with age (18), fresh batch solutions were made every 2 days.

The exogenous replacement surfactant Infasurf was obtained from ONY (Buffalo, NY). Infasurf is a natural lung extract that contains DPPC, fatty acids, and low-molecular-weight surfactant-associated proteins SP-B and SP-C. Infasurf is prepared by a saline rinse lavage of a calf lung and subsequent hydrophobic extraction and, therefore, contains all natural compounds. Infasurf was obtained as a milky-white liquid suspension. The phospholipid content of these
batch solutions is 35 mg/ml. Dilution of this solution was performed in the end tank with buffered saline and stirred for 5 min.

With these preparatory techniques, a wide range of bulk concentrations could be investigated. We determine the surfactant’s surface activity by investigating equilibrium interfacial properties. We also determine a surfactant’s ability to minimize the nonequilibrium normal and Marangoni stresses that oppose reopening by investigating dynamic properties.

**Equilibrium Measurements**

The \( \gamma_{eq} \) as a function of \( C \) was determined by measuring the pressure drop across a static bubble (\( \Delta P \)) and by using Laplace’s law (\( \Delta P = 2 \gamma_{eq}/R \)). The capillary tube was filled with a surfactant solution under the influence of the hydrodynamic head. Before any measurements, this surfactant solution was allowed to equilibrate with the interface for 30–40 min. After this equilibration time, the bubble was pushed forward at a very low speed (\( \sim 0.01 \) cm/s). This ensured that the film thickness left behind was very small compared with the tube radius (\( R \)) and that a zero contact angle was present. The bubble was then stopped, and surfactant was allowed to adsorb to the interface until equilibrium. For DPPC, this process occurred over a period of \( \sim 100 \) s. Under these conditions, the interface can be assumed to be a hemispherical cap of radius \( R \) (4), as required by Laplace’s law. Small errors due to a nonzero contact angle may cause an underestimate of \( \gamma_{eq} \).

**Dynamic Measurements**

Dynamic measurements involve calculating the pressure jump across the interface in the bubble cap region (\( P_{cap} \)) required to clear the tube of liquid with a bubble speed \( U \) and a bulk surfactant concentration \( C \). After an equilibrium surface tension measurement was performed, the syringe pump was used to force a bubble down the tube at a constant rate, while the pressure in the air phase (\( P_{bub} \)) was monitored continuously. In addition, the bubble tip velocity was measured via two infrared emitter-detector pairs placed a known distance apart. A typical trace of these measurements is shown in Fig. 3. During start-up, the pressure increases while the bubble moves forward at a nonconstant velocity. However, once the bubble attains a steady-state velocity, the pressure drops linearly because of a Poiseuille pressure drop that exists downstream of the meniscus. The trigger signals from the infrared detectors provide timing information from which the bubble tip velocity (\( U \)), as well as the dimensional bubble cap pressure jump (\( P_{cap} \)), can be calculated, as shown below.

Before any surfactant experiments, baseline equilibrium and dynamic measurements of the surfactant-free system (calcium-buffered saline solution) were obtained daily. The surfactant was then added to the end tank to achieve a desired concentration. An equilibrium measurement was made first, and then a set of dynamic measurements was obtained at nine different velocities. This procedure could be repeated for several concentrations by addition of aliquots of surfactant to the end reservoir. At the end of each set of experimental trials, the entire system was cleaned with a 2% Micro solution.

**Determination of \( P_{cap} \)**

To understand how the pressure drop across the bubble cap (\( P_{cap} \)) can be extracted from the dynamic measurements, we consider the pressures that exist during steady-state bubble progression. Figure 4 illustrates the pressure components that contribute to \( P_{bub} \). \( L_{np} \) is the length of the region in front of the bubble that has non-Poiseuille flow. The distances between the detectors (\( D_{detector} \)) and between the last detector and the outlet point (\( D_{end} \)) are known. \( L_{p}(t) \) is the length over which Poiseuille flow exists. The distances between the detectors (\( D_{detector} \)) and between the last detector and the outlet point (\( D_{end} \)) are known. \( L_{p}(t) \) is the length over which Poiseuille flow exists. \( L_{p}(t) \) decreases linearly as the bubble progresses down the tube at a constant velocity. We define \( t_{1} \) by the time at which the bubble crosses the first detector, \( t_{2} \) by the time at which the bubble crosses the second detector, and \( t_{end} \) by the time the bubble reaches the outlet. \( P_{bub} \) is the bubble pressure, and the hydrostatic pressure in the end tank is used as the reference pressure, assumed to be constant because of the large cross-sectional area of the end.

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**Fig. 3.** Bubble pressure (\( P_{bub} \)) and infrared detector signals acquired as a function of time.

**Fig. 4.** Pressures that exist at steady state. \( P_{1} \), fluid pressure at bubble tip; \( P_{2} \), fluid pressure where Poiseuille flow begins; \( P_{3} \), fluid pressure at end of tube. Hydrostatic head pressure in end tank is used as reference pressure. \( D_{detector} \) and \( D_{end} \) are known distances.
tanks. We express $P_{bub}$ in terms of several pressure losses in the system

$$P_{bub} = P_{cap} + P_{np} + P_p + P_{end}$$

(1)

where $P_{cap} = (P_{bub} - P_1)$ is the bubble cap pressure jump, $P_{np} = (P_1 - P_2)$ is a pressure loss due to non-Poiseuille flow in front of the bubble, $P_p = (P_2 - P_3)$ is the Poiseuille pressure loss, and $P_{end} = (P_3 - 0)$ is the pressure loss due to expansion flow into an infinite reservoir.

To extract $P_{cap}$, the following assumptions are made. First, under low capillary number ($Ca_{eq}$ = $\mu U/\gamma_{eq}$, a dimensionless velocity) conditions, the error associated with neglecting $P_{np}$ is insignificant. We use the results from a computational model, which indicates that $P_{np}$ is <1% of $P_{cap}$ for $Ca < 10^{-2}$ (see APPENDIX A) to justify this assumption. To determine whether pressure losses associated with exit flow into the end tank ($P_{end}$) are significant, we used laminar head-loss coefficients determined by Rao (29), which show that $P_{end} < 1%$ of $P_{cap}$. Finally, $P_p(t) = -\theta \times L_p(t)$, where $\theta$ is a Poiseuille flow proportionality constant. Because $L_{np}$ is small (APPENDIX A), $L_p(t)$ is the distance from the bubble tip to the outlet and $L_\theta(t) = U(t - t_{end})$. Therefore, from Eq. 1

$$P_{cap} = P_{bub}(t) - \theta \times U \times (t - t_{end})$$

(2)

Therefore, $P_{cap}$ can be calculated once the bubble tip velocity [$U = D_{detector}/(t_2 - t_1)$], the slope ($\theta$) of the $P_{bub}$ vs. $t$ curve in the steady-state region, and the time at which the bubble exits the capillary tube [$t_{end} = (D_{end} + D_{detector})/U$] are determined.

Identification of Noninertial Component ($P_{cap}\text{Stokes}$)

The above technique allows us to identify the $P_{cap}$ vs. $U$ relationships for aqueous surfactant solutions ($\mu = 1 \times 10^{-3}$ kg·m$^{-1}$·s$^{-1}$). In Data Analysis, we use models described previously (31, 35) to estimate nonequilibrium normal and Marangoni stresses. However, these studies and the theory on which they are based (4, 30) assume noninertial conditions, where Stokes flow prevails. Under constant surface tension conditions over $10^{-5} < Ca < 3 \times 10^{-3}$, Ratulowski's calculations (30) can be correlated with ($\Pi_{cap}\text{Stokes} = (P_{cap}\text{Stokes})/\gamma/\gamma_{eq}$) = $2 + 5.72Ca^3$, where ($\Pi_{cap}\text{Stokes}$) is a dimensionless Stokes (noninertial) pressure based on the Laplace pressure drop across a static bubble of radius $R$ and surface tension $\gamma$. When surfactant is incorporated, deviation from this relationship will provide estimates of the nonequilibrium normal and Marangoni stresses. To utilize these models, we must first identify the inertial and noninertial pressure components of $P_{cap}$.

Dimensional analysis indicates that $\Pi_{cap} = f(Ca, Wb)$, where $Wb$ (Weber number) = $\mu U^2/\gamma/\gamma_{eq}$ relates inertial to surface tension effects. As shown in APPENDIX B, surfactant-free experiments conducted with ethylene glycol ($\mu = 2.1 \times 10^{-2}$ kg·m$^{-1}$·s$^{-1}$, $\gamma = 48$ mN/m) reproduce the Ca$^3$ behavior over $10^{-5} \leq Wb < 10^{-4}$, validating the data analysis approach described above. However, surfactant experiments were conducted in an aqueous solution in which inertial is potentially significant ($10^{-3} \leq Wb \leq 10^{-1}$). By comparing the Stokes flow behavior of the ethylene glycol system with the moderate Wb behavior of the aqueous system (see APPENDIX B), we can estimate the inertial and Stokes components of $P_{cap}$

$$P_{cap} = (P_{cap}\text{Stokes}) + P_{inertial}$$

(3)

where

$$(P_{cap}\text{Stokes}) = 2\gamma R^2 + \gamma R^2 \beta Ca^{2/3}, P_{inertial} = \gamma R^2 A(Wb)^8$$

The inertial pressure ($P_{inertial}$) is estimated by comparing aqueous surfactant-free measurements with ethylene glycol measurements by use of a least squares regression of both data sets to determine coefficients $A$ and $B$ (see APPENDIX B). Eight different determinations of $A$ and $B$ (based on 8 different surfactant-free data sets) varied by <7% ($A = 1.24 \pm 0.03$, $B = 0.565 \pm 0.03$). Errors associated with this approximation are most significant at higher velocities and are insignificant at the lowest velocities studied.

For a surfactant-free system, $\gamma_{eq}$ in Eq. 3 is the surface tension. However, when surfactant is incorporated, $\gamma$ is a representative dynamic surface tension ($\gamma_{eq}$), which we will show is not necessarily equal to $\gamma_{eq}$ (see Data Analysis). The $\gamma_{eq}$ will be estimated from regression analysis presented below, thus allowing us to estimate $P_{inertial}$ and thus $P_{cap}\text{Stokes}$. From this, the dimensionless Stokes pressure is calculated based on $\gamma_{eq}$: ($\Pi_{cap}\text{Stokes} = (P_{cap}\text{Stokes})/\gamma_{eq}/R$).

RESULTS

Figure 5 shows the results of $\gamma_{eq}$ measurements for the three different surfactant systems under investigation. As described in BACKGROUND, increasing $C$ increases $\gamma_{eq}$ until the interface is saturated. Because $\gamma_{eq}$ is inversely related to $\gamma$, this results in $\gamma_{eq}$ decreasing with increasing $C$ until a critical bulk concentration ($C_{CBC}$) is reached. For $C > C_{CBC}$, the interface is saturated ($\gamma_{eq} = \gamma_{sat}$), and $\gamma_{eq} \rightarrow \gamma_{sat}$. Large $C$ behavior indicates that Infasurf is the most surface active of the three surfactants studied, with $\gamma_{sat} \sim 27$ mN/m.

To demonstrate how the nonlinear pressure, ($\Pi_{cap}\text{Stokes}$), helps identify the magnitude of the nonequilibrium normal and Marangoni stresses, we present dimensional and nondimensional pressure vs. velocity data for each surfactant in Figs. 6 and 7, respectively. For each $C$, ($P_{cap}\text{Stokes}$) is determined over a range of $U$ (Fig. 6). For a given $C$, ($P_{cap}\text{Stokes}$) increases slightly with $U$. However, as $C$ is increased, ($P_{cap}\text{Stokes}$) vs. $U$ curves shift downward. This downward shift is directly related to the decrease in $\gamma_{eq}$ that occurs with increasing $C$ (Fig. 5).

Fig. 5. Equilibrium surface tension as a function of bulk surfactant concentration for SDS (■), 1,12-dipalmitylophosphatidylcholine (DPPC, ○), and Infasurf (■). $C_{CBC}$, critical bulk concentration. Error bars are based on SD of 3 measurements.
In contrast, consider the dimensionless relationship \( (\Pi)_{\text{Stokes}} \) vs. \( C_{\text{eq}} \) for SDS in Fig. 7A. The solid and dashed curves represent the data regression described in Data Analysis. This representation normalizes \( (\Pi)_{\text{Stokes}} \) based on \( g_{\text{eq}} \). Therefore, if the interface approaches equilibrium, the \( (\Pi)_{\text{Stokes}} \) vs. \( C_{\text{eq}} \) curve for a given C will resemble the \( C = 0 \) data (solid circles in Fig. 7A), because the nonequilibrium normal and Marangoni stresses will be insignificant. At small C, the data in Fig. 7A initially shift upward with increasing C. This indicates that the interface is not in equilibrium and that the magnitudes of the nonequilibrium normal and Marangoni stresses are increasing with increasing C. However, as C increases further, the curves begin to shift downward toward \( C = 0 \). This downward shift indicates that the SDS system can approach (but not completely obtain) equilibrium at high C, which reduces the nonequilibrium normal stress and significantly remobilizes the interface.

Figure 7, B and C, demonstrates \( (\Pi)_{\text{Stokes}} \) relationships for different pulmonary surfactant analogs. In the DPPC system (Fig. 7B), \( (\Pi)_{\text{Stokes}} \) is larger than the SDS values over all ranges of C. At low C, \( (\Pi)_{\text{Stokes}} \) increases dramatically to \( (\Pi)_{\text{Stokes}} \sim 3 \), even at the smallest velocities. As C is increased beyond 0.416 mg/ml, a minimal downward shift of \( (\Pi)_{\text{Stokes}} \) is observed. However, increasing C does not return the system to the equilibrium behavior, even at the largest C (3.84 mg/ml). At these large concentrations the interface would be saturated in equilibrium. The large magnitude of \( (\Pi)_{\text{Stokes}} \) indicates that nonequilibrium normal and Marangoni stresses are large in this system, whereas the minimal downward shift indicates that DPPC is not capable of significant nonequilibrium normal stress reduction or interfacial remobilization under the velocities studied.

Large \( (\Pi)_{\text{Stokes}} \) values are also observed in the Infasurf system (Fig. 7C) for small C. These data show that at small C the combined effect of nonequilibrium normal and Marangoni stresses can also be significant in the Infasurf system. However, for \( C > 0.07 \) mg/ml, a significant downward shift of \( (\Pi)_{\text{Stokes}} \) is observed, indicating that Infasurf contains constituents other than DPPC that allow this surfactant system to approach equilibrium under dynamic conditions.

Data Analysis

Our goal is to develop a theoretically based regression model that will allow us to estimate the nonequilibrium normal and Marangoni stress magnitudes from the pressure-velocity measurements described above. This analysis is based on concepts developed by Stebe and Barthès-Biesel (35), who performed a theoretical investigation of the influence of Marangoni stresses on bubble progression. Their study followed Bretherton's analysis (4), except surfactant effects were included. Stebe and Barthès-Biesel demonstrated that the inertia-free \( C_{\text{eq}}^{1/3} \) power law relationship of Eq. 3 could be used to identify Marangoni effects

\[
(\Pi)_{\text{Stokes}} = 2 + \beta C_{\text{eq}}^{2/3}
\]  

(4)
where $\beta$ is related to the magnitude of viscous stresses and $(\Pi_{\text{cap}})_{\text{Stokes}}$ and $C_{\text{aeq}}$ are based on $\gamma_{eq}$. Ratulowski and Chang (31) predict $\beta = 5.72$ under surfactant-free conditions. The Marangoni stress that results from surfactant adsorption elevates the viscous contribution and thus results in $\beta > 5.72$. The analysis used by Stebe and Barthès-Biesel assumed that the surface tension in the bubble cap region (hydrodynamically assumed to be static) was constant and equal to $\gamma_{eq}$.

Clearly, when $\gamma_{eq}$ is used as the surface tension scale, Eq. 4 cannot adequately describe the data in Fig. 7. This inadequacy is a direct result of the constant $\gamma_{eq}$ bubble cap surface tension assumption. In reality, surface convection coupled with transport limitations can create a distribution of surfactant along the interface in the bubble cap region, as illustrated in Fig. 1A, resulting in an effective bubble cap surface tension $\tilde{\gamma} = \gamma_{eq}$ in the dynamic system. Only if diffusive barriers are removed and the adsorption rate is fast ($St = k_a R/C U \to \infty$) will $\tilde{\gamma} = \gamma_{eq}$. This limit is observed only during our equilibrium measurements, where $U$ is identically zero.

However, because the time to reach equilibrium can be $\sim 100$ s for DPPC at $C = C_{\text{CBC}}$, it is not surprising that the dynamic surface tension deviates from $\gamma_{eq}$. To estimate the representative dynamic surface tension, we simplistically assume that $\tilde{\gamma}$ depends only on the given bulk concentration for a given surfactant in the dynamic system and is independent of velocity over the range of velocities investigated (static system excluded). This assumption will be explained below. We define the surface tension ratio

$$\tilde{\gamma} = \frac{\gamma}{\gamma_{eq}} \quad (5)$$

where $\tilde{\gamma}$ is a measure of the relative surface tension deviation from equilibrium and thus is a measure of the nonequilibrium normal stress. From Eq. 3 with $\tilde{\gamma}$ as the dynamic surface tension scale

$$\Pi_{\text{cap}} = \frac{P_{\text{cap}}}{\gamma_{eq} R} = (\Pi_{\text{cap}})_{\text{Stokes}} + \Pi_{\text{inertial}} \quad (6)$$

where

$$(\Pi_{\text{cap}})_{\text{Stokes}} = \frac{(P_{\text{cap}})_{\text{Stokes}}}{\gamma_{eq} R} = 2\tilde{\gamma} + \tilde{\gamma}^{1/3} \beta C_{\text{aeq}}^{2/3},$$

$$\Pi_{\text{inertial}} = \frac{P_{\text{inertial}}}{\gamma_{eq} R} = \tilde{\gamma}^{(1-\beta)} A W_{\text{eq}}^{\beta}$$

To estimate the magnitudes of nonequilibrium normal and Marangoni stresses, for each experiment, we calculate $\Pi_{\text{cap}}$, $C_{\text{aeq}}$, and $W_{\text{eq}}$. A least squares regres-

Fig. 7. A: dimensionless Stokes bubble cap pressure jump as a function of equilibrium capillary number for SDS. B: dimensionless Stokes bubble cap pressure jump as a function of equilibrium capillary number for DPPC. C: dimensionless Stokes bubble cap pressure jump as a function of equilibrium capillary number for Infasurf. See Fig. 6 legend for concentrations of SDS, DPPC, and Infasurf.
sion is performed with Eq. 6, with $\Pi_{\text{cap}}$ as the dependent variable and $C_{\text{eq}}$ and $W_{\text{beq}}$ as the independent variables at each bulk concentration. During each regression analysis, the concentration is constant, and, therefore, variations in $C_{\text{eq}}$ and $W_{\text{beq}}$ are governed only by variations in $U$. The best-fit relationships faithfully reproduce the experimental behavior at each concentration, as shown in Fig. 7, with $R^2 > 0.98$. As a result, $\tilde{\gamma}$ and $\beta$ are determined for each surfactant at each concentration (A and B are known from Appendix B). Once these parameters are known for a given concentration, $P_{\text{inertial}}$ can be calculated, and Eq. 3 can be used to convert the $P_{\text{cap}}$ data to $(P_{\text{cap}})_{\text{Stokes}}$ data. Because we are primarily concerned with noninertial or capillary effects, we have presented only $(P_{\text{cap}})_{\text{Stokes}}$ and $(\Pi_{\text{cap}})_{\text{Stokes}}$ data in Figs. 6 and 7, respectively.

During dynamic conditions, $\tilde{\gamma}$ reflects the magnitude of the nonequilibrium normal stress near the bubble cap region. In Fig. 8A, $\tilde{\gamma}$ vs. $C/C_{\text{CBC}}$ is presented. The $\tilde{\gamma} \sim 1$ for SDS, indicating that the nonequilibrium normal stress in the cap region is miniscule. In contrast, DPPC and Infasurf exhibit much larger nonequilibrium normal stresses, with $\tilde{\gamma} \sim 1.5$, even when $C \sim C_{\text{CBC}}$. As discussed in Background, increasing $C$ decreases the adsorption depth ($\lambda$); therefore, the major transport limitation that remains is due to interfacial adsorption. Therefore, the magnitude of $\tilde{\gamma}$ at large $C$ is directly related to the surfactant’s adsorption properties and equation of state. The data in Fig. 8A suggest that SDS adsorption rates are rapid enough for $\tilde{\gamma} \rightarrow \gamma_{\text{eq}}$ uniformly at high concentrations. In contrast, the slight reduction of $\tilde{\gamma}$ at large $C$ in the DPPC system indicates that an adsorption limitation exists for this surfactant. The pulmonary replacement surfactant Infasurf apparently adsorbs more rapidly than DPPC and thus suffers only modest adsorption limitation. Evidently, constituents of Infasurf other than DPPC (the primary component) improve adsorption to the moving interface.

The term $\beta \tilde{\gamma}^2 C_{\text{eq}}^{2/3}$ represents viscous contributions to $(\Pi_{\text{cap}})_{\text{Stokes}}$. In the absence of Marangoni stress, $\beta = 5.72$ (ideally; see Appendix B). The presence of a Marangoni stress in the region between the bubble cap and thin film (transition region) can increase $\beta$. In Fig. 8B the values of $\beta$ are plotted vs. $C/C_{\text{CBC}}$. The experimentally determined constant surface tension value for $\beta$ (5.25) is shown as a horizontal line (see Appendix B). The values of $\beta$ in the SDS system initially increase to a maximum of $\beta \approx 20$ and then decrease slightly after $C_{\text{CBC}}$. The magnitude of $\beta$ in the DPPC and Infasurf systems increases with $C$ and does not show any remobilization trends. This indicates that the Marangoni stress in the transition region remains large in DPPC and Infasurf systems, even at high bulk concentrations.

Justification of regression model. In the regression analysis, $\tilde{\gamma}$ is implicitly assumed to be constant for each concentration over the range of velocities investigated. This violates the fact that at $U = 0$, $\tilde{\gamma} = 1$, and thus the relationships obviously cannot extend to a static system ($C_{\text{eq}}$ identically equal to 0). Several questions naturally arise: For a given concentration, is it likely that $\tilde{\gamma}$ is independent of velocity? Is it justifiable to assume that nonequilibrium behavior exists, even at the exceedingly small velocities spanned in the present study?

Dimensional analysis indicates that $\tilde{\gamma}$ will depend on several dimensionless groups

$$\tilde{\gamma} = \frac{\tilde{\gamma}}{\gamma_{\text{eq}}} = f \left( \frac{\Lambda}{R}, \text{StPe}, C_{\text{eq}} \right)$$

where $\text{St} = k_a C R / U$ relates adsorption to interfacial creation, $\Lambda / R = I / (R C)$ is the dimensionless adsorption depth, and $\text{StPe} = k_a (1/2) (D C)$ relates adsorptive to diffusant transport rates. The $C_{\text{eq}}$ dependence in Eq. 7 is related to the interfacial convection pattern.

In the limit $U \rightarrow \infty$ ($C_{\text{eq}} \rightarrow \infty$, $\text{St} \rightarrow 0$), obviously $\tilde{\gamma} \rightarrow \gamma_{\text{clean}}$, since adsorption to the ever-increasing interface would be slow compared with interfacial production.
Thus one might expect that $\gamma_{\text{clean}} \geq \tilde{\gamma} \geq \gamma_{\text{eq}}$. However, this range is far too large for the conditions studied. If $C_{a\text{eq}} < 0.5$, the basic flow field has a recirculating region, as shown in Fig. 1A. This creates a diverging stagnation ring around the bubble and a converging stagnation point at the bubble tip. This flow exists in the present study, since $U < 5$ cm/s ($C_{aeq} < 10^{-3}$), whereas $U > 1$ cm/s is necessary to remove the recirculating flow pattern. If $St \ll 1$ and $C_{aeq} \ll 1$, we hypothesize an insensitivity to $C_{aeq}$ because the recirculating flow field always exists. Thus

$$\tilde{\gamma} = \frac{\gamma_{\text{eq}}}{\gamma_{\text{eq}} = f(St, \frac{A}{R}, StPe)} \quad \text{if } C_{aeq} \ll 1 \quad (8)$$

As $St$ decreases with increasing $U$ while retaining recirculation, our conjecture is that $\tilde{\gamma} \rightarrow \gamma_{0}$, where $\gamma_{0} < \gamma_{\text{clean}}$. To understand $\gamma_{0}$, consider an equilibrated interface that is pushed forward with $St \ll 1$ and $C_{aeq} \ll 1$. The recirculating flow pattern will sequester surfactant at the bubble tip ($\Gamma', \tilde{\gamma}$) and deplete surfactant from upstream portions of the bubble cap ($\Gamma', \tilde{\gamma}$). Therefore, a small portion of the interface near the tip, which experiences interfacial compression (Fig. 1B), will have a low surface tension, while the remainder of the bubble cap will have an increased surface tension, as predicted by Yap and Gaver (45). Therefore, if $St \ll 1$, we expect $\tilde{\gamma} \rightarrow \gamma_{0}$, where $\gamma_{\text{clean}} > \gamma_{0} > \gamma_{\text{eq}}$. Thus, for a given $C$, if $St \ll 1$, we expect that the recirculation field will hold the surfactant to a relatively fixed effective dynamic concentration in the tip region. Of course, the concentration-dependent parameters $\Lambda/R$ and $StPe$ are instrumental in determining the magnitude of $\gamma_{0}$, since they determine whether a diffusion limitation exists.

To approximate $St$, $k_{a}$ was estimated from surface tension relaxation curves following Lin et al. (21). These measurements were made at a large concentration ($C = C_{CBC}$), such that diffusion limitation could be neglected. For DPPC, we found $k_{a} = 0.029 \text{cm}^{3} \cdot \text{mg}^{-1} \cdot \text{s}^{-1}$, consistent with a value reported elsewhere (18). For Infasurf, we found $k_{a} = 0.70 \text{cm}^{3} \cdot \text{mg}^{-1} \cdot \text{s}^{-1}$, comparable to $k_{a} \sim 1.7 \text{cm}^{3} \cdot \text{mg}^{-1} \cdot \text{s}^{-1}$ for natural surfactant (Survanta) (26). For SDS, we found $k_{a} = 0.10 \text{cm}^{3} \cdot \text{mg}^{-1} \cdot \text{s}^{-1}$. Although $k_{a}(\text{SDS}) < k_{a}(\text{Infasurf})$, the adsorption time constant $\tau \sim (k_{a} * C_{CBC})^{-1}$ for SDS will be smaller than that for Infasurf because $C_{CBC}(\text{SDS}) \gg C_{CBC}(\text{Infasurf})$. From these estimates of $k_{a}$, the range of $St$ at $C = C_{CBC}$ for the velocities investigated ($4.7 \text{ cm/s} > U > 0.22 \text{ cm/s}$) are

- **SDS**: $2 \times 10^{-3} \leq St \leq 5 \times 10^{-2}$
- **DPPC**: $2 \times 10^{-4} \leq St \leq 3 \times 10^{-3}$
- **Infasurf**: $7 \times 10^{-4} \leq St \leq 2 \times 10^{-2}$

Therefore, in all experiments, $St \ll 1$, and it is justifiable to assume that $\tilde{\gamma} \sim \gamma_{0}$ over the range of velocities inspected. Obviously, a true validation of this assumption must await theoretical investigations of the transport issues related to this situation.

**DISCUSSION**

The data presented above demonstrate that SDS, DPPC, and Infasurf significantly modify the interfacial pressure drop associated with bubble motion through a capillary tube, which is intended to model interfacial flows similar to those that exist during pulmonary airflow reopening. We will attempt to further interpret these data to more completely distinguish the relative contributions of nonequilibrium normal and Marangoni stresses on the system. The complete identification of the magnitudes of these contributions must await the full analysis of convection, diffusion, and adsorption dynamics in this system, which is beyond the scope of this study.

**Physical Interpretation**

To understand how surfactant adsorption properties are responsible for the coefficient trends in Fig. 8, one must consider the interfacial expansion and compression dynamics associated with bubble progression (Fig. 1B). In regions where the expansion rate is large (primarily the transition region), equilibrium will be achieved only if adsorption rates are large. In contrast, stationary and compression regions will achieve equilibrium at lower adsorption rates. Therefore, for a given adsorption rate, portions of the interface in the bubble cap region will be closer to equilibrium than portions in the transition region.

The magnitude of the nonequilibrium normal stress ($\tilde{\gamma}$) is related to the effective bubble cap surface tension and is thus a function of adsorption dynamics in this region. The magnitude of the Marangoni stress ($\beta$) is primarily a function of adsorption dynamics in the transition region, where interfacial stretching is large. A reduction of $\tilde{\gamma}$ indicates that the bubble cap region is in equilibrium, whereas a reduction in $\beta$ would indicate that the transition region is in equilibrium. Therefore, as the adsorption rate is increased, we expect to observe a reduction in $\tilde{\gamma}$ before a reduction in $\beta$.

The SDS system is capable of reducing $\tilde{\gamma}$ values to that of an equilibrated system at large $C$, whereas only a modest reduction in $\beta$ is observed. Apparently, adsorption rates are large enough to equilibrate the bubble cap region. In addition, SDS’s adsorption rates also appear fast enough to achieve a modest reduction in Marangoni stress, but full equilibrium in the transition region is not obtained. In contrast, $\tilde{\gamma}$ and $\beta$ remain large in the DPPC system, even when diffusion limitations are reduced by increasing $C$. This indicates that DPPC’s adsorption properties are slow, and nonequilibrium surface tension exists in the bubble cap and transition regions. Finally, Infasurf is capable of partially reducing $\tilde{\gamma}$ but is not capable of reducing $\beta$ at large $C$. This suggests that Infasurf’s adsorption properties are fast enough to nearly equilibrate the bubble cap but not fast enough to equilibrate the transition region.
Physiological Significance

The present study demonstrates that SDS, DPPC, and Infasurf exhibit markedly different behavior due to differences in adsorption properties. SDS exhibits near-equilibrium properties due to fast adsorption rates. DPPC exhibits nonequilibrium properties due to a slow adsorption rate. Infasurf, the clinically relevant (natural) surfactant system, exhibits partial-equilibrium properties due to a moderate adsorption rate. To understand why nature has selected a surfactant that possesses only moderate adsorption properties, we must consider the multiple influences of surfactant on airway mechanics.

From an airway reopening standpoint, the most important influence of surfactant is the ability to lower the dimensional reopening pressures. The data in Fig. 6 indicate that, at high concentrations, $(P_{eq})_{Stokes}$ in the Infasurf system (Fig. 6C) is lower than $(P_{eq})_{Stokes}$ in the DPPC system (Fig. 6B). Because DPPC and Infasurf have similar Marangoni stress values (Fig. 8B), we conclude that Infasurf’s ability to reduce the nonequilibrium normal stress near the bubble cap (Fig. 8A) is responsible for its ability to lower the dimensional reopening pressures.

However, surfactants are also responsible for preventing airway closure by maintaining airway stability at low lung volumes. Several investigators (15, 27) have predicted that surfactant-induced Marangoni stresses can retard closure. The creation of a Marangoni stress requires that adsorption rates be slow, as in the DPPC system. However, for DPPC alone, these slow adsorption properties can also create a large nonequilibrium normal stress that increases reopening pressures and may result in airway wall damage. The $\gamma$ and $\beta$ data presented above suggest that Infasurf’s sorption properties are optimized to reduce the nonequilibrium normal stress near the bubble cap and still maintain the Marangoni stress. This behavior may be critical in maintaining airway stability while protecting the lung from large inflation pressures.

Limitations

It is important to consider the physical and physiological limitations of the present system. The main physical limitation of the present system involves the fact that the surfactant phospholipid components may adhere to the glass wall. This adsorption occurs via an electrostatic effect, in which the positively charged hydrophilic heads of various phospholipids, including DPPC, are attracted to the negative surface charge on the glass surface (23). The adsorbed surfactant layer may influence static surface tension measurements, since the interface would equilibrate with surfactants in the bulk as well as a concentrated layer of surfactants on the glass wall. Under these conditions, we would expect a lower $\gamma_{sat}$ than that measured by systems in which surfactants can only equilibrate with the bulk concentration. However, the SDS measurement ($\gamma_{sat} = 40 \text{ mN}$) is within 5% of the value obtained using purified SDS solutions and the maximum bubble pressure method (24). In addition, the Infasurf measurement ($\gamma_{sat} = 27 \text{ mN}$) deviates by only $\sim 10\%$ from the value obtained using a calf lung surfactant extract solution and a pulsating bubble surfactometer (PBS) under static conditions (44). In addition, Infasurf measurements in our laboratory with a PBS were also within $10\%$ of measurements made with the present technique. To determine whether the adsorbed layer could influence DPPC measurements, we coated the walls of the capillary tube with an albumin blocking solution typically used in ELISA (1). Under these conditions, albumin strongly adhered to the glass wall, and DPPC can only exist in the bulk solution. The DPPC measurement ($\gamma_{sat} = 33 \text{ mN}$) in a non-albumin-coated tube was within $6\%$ of the value obtained in an albumin-coated tube. Therefore, the electrostatic adsorption of surfactant appears to have little influence on $\gamma_{sat}$ measurements.

The adsorbed surfactant layer may also influence the dynamic surface tension by providing a reservoir of surfactant that is available to expanding regions of the interface. If this were true, dynamic measurements would be influenced by surfactants in the bulk as well as surfactants adsorbed on the wall. However, a discernible event, namely, a change in slope, occurs at $C_{BC}$ in static ($\gamma_{eq}$ data, Fig. 5) and dynamic ($\gamma$ data, Fig. 8A) measurements. This indicates that the static and dynamic systems are influenced by the same bulk concentration. Because we have demonstrated that static measurements are not influenced by the adsorbed layer, it follows that electrostatic adsorption also has little influence on dynamic measurements.

A minor limitation related to the regression model involves the inertial term. Recall that the magnitude of inertial to surface tension effects in the aqueous system was identified with the $AWb^B$ term, where $A$ and $B$ were determined using constant surface tension fluids (see APPENDIX B). In the regression of the surfactant data, this term is modified by $\gamma^1 - B^A(W_{beq}^B)$ to account for changes in the effective surface tension $\gamma$. Because $\gamma$ can vary with concentration, the term $\gamma^1 - B^A$ is modified as a result of variations in $\gamma$. However, it is conceivable that $A$ varies on its own because of surface tension gradients. Because this aspect has not been addressed by theoretical models and to avoid overspecification, it was assumed that the magnitude of coefficient $A$ is not a function of concentration.

Although the present system allows us to evaluate the physicochemical properties of surfactants under continual interfacial expansion conditions, it contains certain physiological limitations in its application to the pulmonary system. First, the airways in which closure takes place are most certainly flexible. Previous investigators (9) have demonstrated that parenchymal tethering forces and airway compliance can greatly influence reopening pressures. Nevertheless, the airway reopening process in these flexible airways involves the continual creation of an air-liquid interface. The stresses investigated in this study, nonequilibrium normal and Marangoni, will combine with the stresses
that arise in a flexible system to determine the physiological airway reopening pressure.

Another limitation involves the fact that dynamic measurements were taken under steady-state conditions. Because of the length of pulmonary airways in which closure takes place, steady-state reopening may never occur in vivo. The interfacial stresses investigated in this study (nonequilibrium normal and Marangoni) will exist whenever new interfacial area is created, as occurs during unsteady airway reopening. We have demonstrated that these stresses are reduced when surfactant adsorption properties are fast with respect to the interfacial creation rate. The interfacial creation rate during unsteady reopening, which can occur as "short miniature explosions" (8), will be larger than the steady-state interfacial creation rate. Therefore, we would expect that, during rapid unsteady reopening, the nonequilibrium behavior would exceed the magnitudes observed in this study. Although the present system does not exactly reproduce the physiological dynamics of airway reopening, it allows us to investigate how surfactant physicochemistry, namely, adsorption rates, influences the continual interfacial expansion aspects of airway reopening.

Conclusions

We have investigated the influence of several surfactants in a continual expanding interfacial system. This system has been designed to mimic the interfacial flows that exist during pulmonary airway reopening. The interfacial stresses that oppose airway reopening and elevate reopening pressures have been identified. Specifically, a large nonequilibrium normal stress can exist in the bubble cap region. In addition, a Marangoni or interfacial shear stress that rigidifies the interface is present in the transition region when surfactants do not adsorb uniformly. The magnitudes of these stresses have been estimated by analyzing the dimensionless \( \left( \frac{P_{\text{cap}}}{\mu U' \gamma} \right)_{\text{Stokes}} \) vs. \( \text{Ca}_{\text{eq}} \) relationship. To elucidate surfactant adsorption properties, the bulk transport limitation was eliminated by increasing the bulk concentration, \( C \). The nonphysiological surfactant SDS exhibited a minimal nonequilibrium normal stress and a relatively small Marangoni stress. The main phospholipid component of pulmonary surfactant, DPPC, exhibited large nonequilibrium normal and Marangoni stresses and, therefore, has slow adsorption properties. The pulmonary replacement surfactant Infasurf was found to have moderate adsorption properties, since the nonequilibrium normal stress was reduced while the Marangoni stress remained large. The most significant difference between Infasurf and DPPC is the presence of low-molecular-weight, hydrophobic surfactant-associated proteins SP-B and SP-C. Wang et al. (44) demonstrated the importance of hydrophobic proteins in enhancing phospholipid adsorption in an air-water interface. Therefore, we believe that these proteins are
directly responsible for Infasurf’s moderate adsorption properties. Infasurf was also found to be more effective than DPPC in reducing the clinically relevant dimensional reopening pressure. This result suggests that airway reopening pressures are primarily reduced when adsorption properties are fast enough to reduce the nonequilibrium normal stress in the bubble cap region. Furthermore, our observation that Infasurf supports a Marangoni stress suggests that this property may be related to Infasurf’s ability to maintain airway stability. We therefore hypothesize that Infasurf’s adsorptive properties may be optimized to balance low reopening pressures with the maintenance of stable airways. Future studies will quantify the advantages of Infasurf by correlating the present experimental data with a computational model. With this correlation, we will attempt to quantify the physicochemical properties (namely, adsorption and desorption rates) of pulmonary surfactant that may be used to optimally protect the lung from airway closure.

APPENDIX A
Calculation of error associated with neglecting non-Poiseuille region. To assess the relative magnitude of the non-Poiseuille pressure component \( P_{np} \) associated with the flow of a bubble down a capillary tube (Eq. 1), we developed a model using the boundary element method that computationally solves the governing Stokes flow equations. Figure 9A demonstrates how the dimensionless centerline fluid pressure \( P_{cm}(y/R) \) is a function of the dimensionless distance from the bubble tip \( (y/R) \). Downstream, the flow is Poiseuille, and \( P_{np} \) decreases linearly with \( y \), which in turn results in lower \( \Gamma \), i.e., \( O(10^{-8}) \leq \Gamma < O(10^{-3}) \). Therefore, the ethylene glycol system should correlate to the Stokes behavior shown in Eq. 9. In Fig. 10, \( \Pi_{cap} = \frac{P_{cap}(y/R)}{\gamma/R} \) is plotted as a function of Ca for purified water and ethylene glycol. The clean water and ethylene glycol data could be fit with the following expression

\[
\Pi_{cap} = \frac{P_{cap}(y/R)}{\gamma/R} = 2 + \beta Ca^\alpha + A (Wb)^B \tag{10}
\]

Before each set of surfactant experiments, data from the initial surfactant-free experiment were obtained and compared with the ethylene glycol results. The average values of the coefficients from eight different determinations are \( \beta = 5.25, \gamma = 0.679, A = 1.24, \) and \( B = 0.565 \). In the ethylene glycol system, where \( Wb = 10^{-4} \), the 1.24(\( Wb \))^0.565 term is negligible, and we can identify the Stokes flow pressure as

\[
\Pi_{cap}^{\text{Stokes}} = \frac{P_{cap}(y/R)}{\gamma/R} = 2 + 5.25 Ca^0.662 \tag{average coefficient values},
\]

which agrees with the Stokes flow prediction (Eq. 9). However, when water is used, the \( Wb \) term can be significant at large \( U \). Under these conditions, the progression of the bubble is opposed by the presence of inertial forces. From the regression, we see that this inertial effect can be accounted for by augmenting the Stokes flow expression with an inertial term, \( \Pi_{\text{inertial}} = 1.24(Wb)^{0.565} \), such that the total bubble cap pressure is \( \Pi_{cap} = \Pi_{cap}^{\text{Stokes}} + \Pi_{\text{inertial}} \). To calculate the Stokes flow pressure drop when the working fluid has a viscosity similar to that of water, the inertial term must be subtracted from the total bubble cap pressure: \( \Pi_{cap}^{\text{Stokes}} = \Pi_{cap} - \Pi_{\text{inertial}} \).

We are grateful for Dr. David Halpern’s valuable assistance and insight into the analysis of the results. We thank Dr. Melissa Krueger for help in surfactant preparation and for providing the PBS equilibrium Infasurf measurements. This work was supported by National Heart, Lung, and Blood Institute Grant HL-51334, National Science Foundation Grants BCS-9358207 and DMS-9709754, and Louisiana Board of Regents Grant LEQSF-GF-22.

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Received 13 July 1998; accepted in final form 28 August 1999.

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\[
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\]

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\]

\[
\Pi_{cap} = \Pi_{cap}^{\text{Stokes}} + \Pi_{\text{inertial}}
\]

\[
\Pi_{cap}^{\text{Stokes}} = \Pi_{cap} - \Pi_{\text{inertial}}
\]