Equilibrium and Dynamic Interfacial Tension Measurements at Microscopic Interfaces Using a Micropipet Technique. 1. A New Method for Determination of Interfacial Tension

Sunghee Lee, Dennis H. Kim, and David Needham*

Department of Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina 27708-0300

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A new micropipet technique has been developed to measure the equilibrium and dynamic interfacial tensions of microscopic liquid−gas and liquid−liquid interfaces. In this technique, a liquid−gas or liquid−liquid interface with a radius of curvature ranging from 1 to 100 μm can be created inside a tapered micropipet. On the basis of the Laplace equation (a work balance between tension and applied pressure for the curved interface), the equilibrium interfacial tension between the two phases (clean or surfactant adsorbed) can be determined by measuring the radius of curvature of the interface for a series of pressure changes. With an additional surfactant-delivering micropipet, we show how this technique also offers an effective way to study adsorption/desorption dynamics upon exposure and washout for various surfactants and provides the whole history of surfactant exchange for microscopic interfaces. Here, we verify that the results of this technique are consistent with the interfacial tension values previously obtained by other methods typically conducted on macroscopic interfaces. The technique has been used to study the adsorption of PEG-40-Stearate as a monolayer at the liquid−gas interface. From a plot of the measured surface tension as a function of PEG-40-Stearate concentration, the critical micelle concentration and area per molecule have been determined to be 40 ± 2 μM and 1.19 nm^2, respectively. In a companion paper, we report additional new data on a series of phospholipid monolayers.

1. Introduction

It is well-known that the adsorption of surface-active agents at interfaces is a key factor in the formation and stability of colloidal systems such as gas microbubbles and liquid-phase emulsions. The study of how surfactants and lipids spread from aggregates suspended in the aqueous phase, such as micelles and liposomes, to liquid−gas and liquid−liquid interfaces can lead to a better understanding of monolayer formation in these colloidal systems. One direct measure of this transfer is the change in the interfacial (liquid−gas and liquid−liquid) tension. Such changes reflect the rate of adsorption and desorption of various surfactant molecules at the interface. To date, many techniques have been developed for measuring equilibrium and dynamic interfacial tension, and detailed descriptions of available methods can be found in the literature for relatively large interfaces (interfacial areas of square millimeters or larger).[1−5] Briefly, common methods of measuring interfacial tension for macroscopic interfaces include (1) *force methods* that derive the interfacial tension from the weight of the entrained meniscus at the perimeter of a device, such as a Wilhelmy plate or Du Noüy ring, impinging upon the interface, (2) *pressure methods* that determine the interfacial tension from the maximum pressure required to blow a bubble, and (3) *shape methods* that analyze the distortion of a droplet surface as a function of interfacial tension (e.g., spinning drop, pendant drop, and sessile drop methods). These various methods differ with respect to principle of operation, the range of time scales of measurement, convenience, accuracy, and suitability for liquid−gas and/or liquid−liquid interfaces. For all of these techniques, special care is required to keep the environment pure and minimize exposure to contaminants. The macroscopic sizes of these interfaces require relatively large amounts of materials for the preparation of films and can also require long monolayer equilibration times. In light of these limitations, it would be advantageous to have a method of measuring equilibrium and dynamic interfacial tensions in which the clean interface is rapidly replenished and equilibration times are short (seconds to minutes). To achieve this goal, a new micropipet method for the measurement of interfacial tension has been developed in which the characteristic surface area of the interface is square micrometers instead of square millimeters or larger. In this technique, a fresh, curved interface with a radius of curvature ranging from 1 to 100 μm can be repeatedly formed in a tapered micropipet, that is, curvatures in the range of colloidal particles of emulsions and microbubbles. These interfaces can then be rapidly exposed to a solution of desired surfactant concentration, and the interfacial tension can be determined by measuring the radius of curvature of the interface in the pipet for a given pipet pressure. This technique is applicable to both liquid−gas and liquid−liquid interfaces over a limited but useful range of temperatures (∼15−60 °C) by using a custom-made temperature-controlled microchamber in which only a small amount of solution (≤400 μL) is needed. The accuracy of this method has been verified by making equilibrium interfacial tension measurements of various systems whose interfacial tension is already reported in

* Corresponding author.

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the literature, for example, for pure liquids, and for surfactant adsorbed interfaces. Dynamic changes in tension can also be measured in the same system following the delivery of surfactant to the interface via a flow pipet or desorption of accumulated material by washout. This convenient and reliable new micropipet technique provides many advantages, including ease of operation and maintenance, cleanliness, and reproducibility. This method has been applied to measure the surface tensions of PEG-40-Stearate (a molecule of special interest to our laboratory as one of the stabilizers in formulations of microbubbles[6,7]) in order to study the formation of its monolayers at liquid−gas interfaces. In a companion paper, we use the technique to fully characterize the dynamic and equilibrium properties of a series of lipid monolayers at the water−air interface.[8]

2. Methodology for Interfacial Tension Measurements Using the Micropipet Technique

The micropipet method for measuring interfacial tension is based on the equation, attributed to Young (1804) and Laplace (1805), that relates the pressure across a curved interface, ΔP (Pa), to the interfacial tension, γ (mN/m), and radius of curvature, R_c (μm):[9−11]

$$\Delta P = 2\gamma/R_c \qquad (1)$$

The interface is characterized by a single radius of curvature that is equivalent to the pipet radius only if the interface is a hemispherical cap in the pipet with zero contact angle at the pipet wall (see later). For a nonzero contact angle, the radius of curvature is greater than the pipet radius. The relationship follows from a force balance on the cap; a derivation has been given by Fung.[12]

Thus, the difference in pressure is inversely proportional to the radius of curvature of the interface. Then, for a given pressure, if the radius of curvature can be measured, the interfacial tension can be determined from eq 1 by plotting ΔP versus $1/R_c$ for changing pressures acting on an interface in a tapered pipet. When an air-filled micropipet (or liquid-filled micropipet for liquid−liquid interface study) is inserted into an aqueous solution, a liquid−air (or a liquid−liquid) interface is created in the pipet. Figure 1A,B shows videomicrographs of such an interface inside a pipet where the water−air interface and the water−liquid (in this case, the liquid is decane) interfaces are curved to the pipet radius. The wetting of the glass by air is replaced by water (Figure 1A), and the contact angle is close to zero. The diffraction at the walls of the pipet and the curvature of the glass itself, however, introduce potential errors in measurements of the curvature of the interface. To calculate the radius of curvature of the interface, a segment cap of the curved interface is used, where dimensions can be more accurately determined. As shown in Figure 1A, the radius of curvature (R_c) of the interface can be defined by the vertical (Y) and the horizontal distance (X) that correspond, respectively,

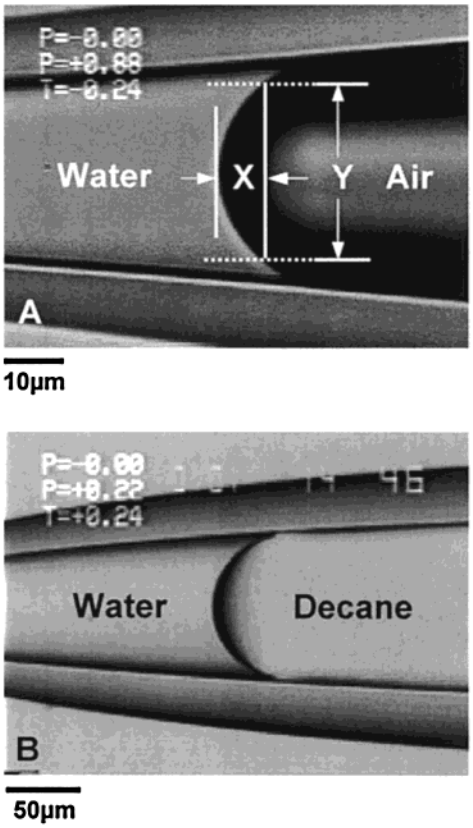

Figure 1. Videomicrograph of interface inside a micropipet with measured horizontal (X) and vertical (Y) distances shown for (A) water−air and (B) water−decane interfaces.

to the chord between the ends of an arc spanning the cap and the height of the cap. From geometry, the following relation is obtained:

$$R_c = \frac{(Y/2)^2 + X^2}{2X} \qquad (2)$$

Substituting eq 2 into eq 1 gives the interfacial tension for these microscopic interfaces from a direct measurement of the micropipet pressure and the interfacial radius. Glass micropipets provide a unique way of monitoring and controlling parameters such as pressure and concentration gradients across these liquid−gas and liquid−liquid interfaces. The pressure can be measured and controlled (with an accuracy down to 0.1 Pa), and the radius of curvature can be measured microscopically and is set by the pipet dimension. It is important to optimize the optical video image to minimize the optical diffraction at the interface by using a high numerical aperture objective and monochromatic illumination. But even with such optimization, the absolute value of the R_c is still limited by the level of the optical resolution (~0.3 μm). However, with the high-resolution video digitization system, it is possible to measure displacements of the interface far better than the level of the optical resolution, as previously addressed by Evans.[13] For this reason, the interfacial tension is determined by measuring the radius of curvature (ΔR_c) before and after the pressure change and plotting the data so that we can determine the surface tension from the slope (i.e., $dP/d(1/R_c)$), rather than using a single absolute value of the R_c at one pressure. The resulting slope (that represents differences in R_c) is a more

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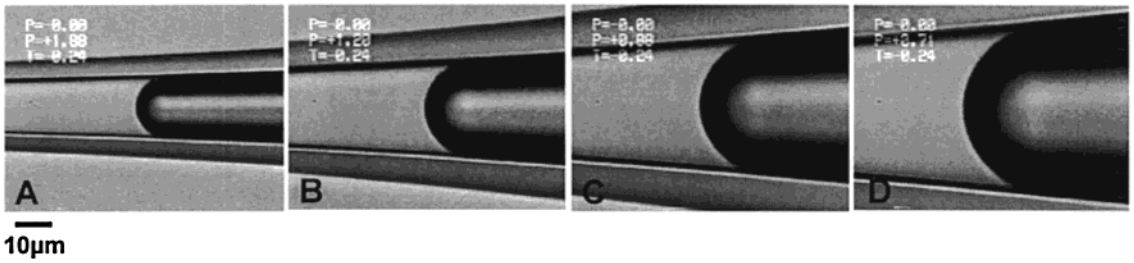

Figure 2. Videomicrographs showing the change in the position and therefore curvature of the interface (water−air) inside a tapered pipet as a function of the applied pressure: (A) $\Delta P = 18\,800$ Pa, (B) $\Delta P = 12\,000$ Pa, (C) $\Delta P = 8800$ Pa, and (D) $\Delta P = 7100$ Pa.

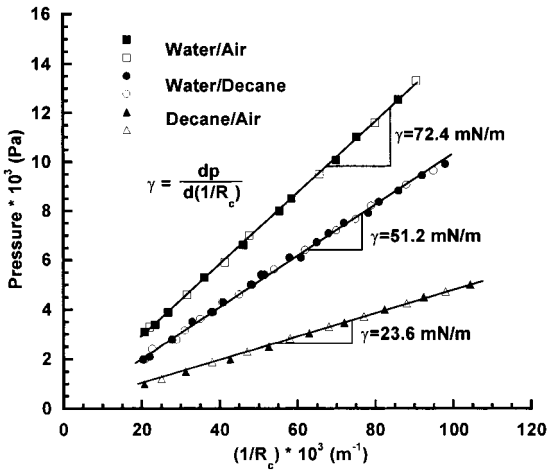

Figure 3. Determination by the micropipet technique of water−air (□,■), water−decane (○,●), and decane−air (△,▲) interfacial tensions. When the interface approaches the pipet tip with increasing pressures (open symbol), the procedure is repeated in reverse with decreasing pressures (filled symbol) to calculate advancing and receding interface radii to check for hysteresis due to adhesional wetting of the glass by the aqueous solution and any changes due to the possible presence of impurities. The interfacial tension (γ) is determined from the slope ($dP/d(1/R_c)$), γ = slope/2, which is found to be 72.4 ± 0.6, 51.2 ± 0.4, and 23.6 ± 0.5 mN/m for water−air, water−decane, and decane−air, respectively.

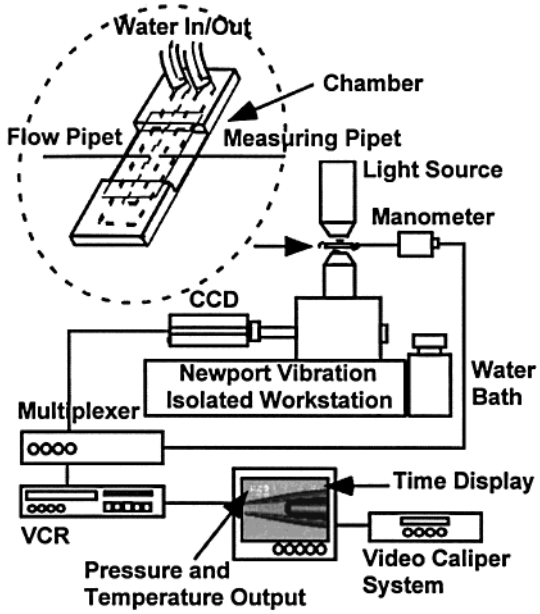

Figure 4. Schematic illustration of a micromanipulation system with temperature-controlled chamber and micropipet arrangement. The length and width of the chamber base are 7.6 and 2.5 cm, respectively. The volume of a full chamber is approximately 400 μL.

accurate representation of the interfacial tension regardless of an ability to accurately measure the absolute value of the R_c, as long as the operator is consistent throughout the measurement (in terms of positioning the cursor in the same position relative to the diffraction pattern of the interface). For a straight pipet with one radius, there can only be one equilibrium point. However, and this is a key feature of this technique, in a tapered pipet a continuum of equilibrium points can be obtained simply by changing the applied pressure and moving the interface to a new position in the taper as shown in Figure 2. The reciprocal of the radius of curvature ($1/R_c$) is calculated and plotted against the corresponding micropipet pressure as shown in Figure 3 for pure water−air, water−decane, and decane−air interfaces. The interfacial tension is then determined from the slope (interfacial tension (γ) = slope/2 from eq 1) of the linear fit in each system. Different slopes, $dP/d(1/R_c)$, reflect the characteristic interfacial tension values for each system, which were found to be 72.4 ± 0.6 mN/m for water−air, 51.2 ± 0.4 mN/m for water−decane, and 23.6 ± 0.5 mN/m for decane−air interfaces at 23 °C. The interfacial tension values are represented as mean ± standard deviation for a repeated number of measurements. These values are consistent with the interfacial tension values reported in the literature measured by other techniques for macroscopic interfaces.[14−16]

3. Description of the Micropipet Technique for Interfacial Tension Measurements

3.1. Micromanipulation System. Much of the pioneering work employing the micromanipulation technique was performed on red blood cells by Evans and Hochmuth[13,17] and on giant lipid vesicles by Evans[18,19] and Needham and co-workers.[20,21] In terms of the experimental setup, essentially the same micropipet technique and microscopic methods were employed here for the interfacial tension measurement with minimal modifications. Briefly, the micromanipulation system (as shown in Figure 4) is centered around an inverted microscope (Nikon DIAPHOT-TMD) with a modified stage to accommodate micromanipulators (up to

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four micropipets, mounted along the periphery of the microscope stage) and a custom-made temperature-controlled microchamber. Pipets and interfaces are viewed by bright field optics with magnification capabilities of 10× and 40× (in air) and 60× and 100× (oil immersion) and differential interference contrast (Nomarski DIC) if needed. The microscope is placed on a Newport vibration isolated workstation to minimize positional oscillations generated by vibration from external sources. During these experiments, the pressures are applied by manometer and/or syringes, and pressures are detected by in-line pressure transducers (Validyne Engineering Corp.). The experiments are recorded on videotape using a charge-coupled device (CCD) camera (Hamamatsu) to obtain video images. The elapsed time and pressure transducer outputs are displayed directly on videotape by a video multiplexing system, which is composed of a video time code generator (Instrumentation for Physiology and Medicine, Inc.), digital display generator (Colorado Video, Inc.), a sine wave carrier demodulator (Validyne Engineering Corp.), a videocassette recorder, and a video monitor. The recordings are then analyzed using a video caliper system (Vista Electronics) to measure the height (X, in Figure 1A) and diameter (Y, in Figure 1A) of the cap of the air (or liquid) column in the micropipet.

3.2. Interfacial Tension Measurement. As shown in Figure 4, the temperature-controlled microchamber was constructed from stainless steel and Gold Seal glass slides (Highland Park, IL) and glued together with Duro Crystal Clear ultraviolet-curing glass adhesive (Loctite Corp.). Clean microscope cover glass (Corning cover glass no. 1, 24 × 60 mm) was cut to form the top of the chamber and mounted on the base with a minimum amount of Dow Corning vacuum grease, an inert silicone lubricant, that was not allowed to contact the solution in the chambers. The temperature of the microchamber was maintained at a fixed temperature by circulating water through a water channel built into the microchamber. The system is equipped with a dual water bath arrangement to control temperature (±0.1 °C) and to change temperature in the microchamber rapidly (~20 °C/min). Once positioned on the microscope stage, solution was delivered to the chambers by Pasteur pipet. In general, one micropipet with a sharp taper (designated the *measuring pipet*) was used for the interfacial tension measurement. For dynamic measurements, a second pipet (designated the *flow pipet*) of smaller internal diameter relative to that of the measuring pipet was used to deliver a surfactant solution directly to the clean interface in the measuring pipet. All pipets were fashioned from standard glass capillary tubing (6 in. long, 0.75 mm outer diameter, 0.4 mm inner diameter). The capillary was heated and pulled to create a suitable taper using a vertical pipet puller (David Kopf Instruments). By control of the heat setting and pulling speed applied by the pipet puller, tapered pipets with relatively steep conical slopes can be created. A flat tip was produced using a custom-made microforge, a device whose central components include a pipet holder and a heatable glass bead (both mounted coaxially on sliders), a light source, and a long working distance dissecting microscope, 12× objective. The measuring pipet with desired taper (tip diameter is typically 7−8 μm) was then connected to the manometer system, mounted in a micromanipulator, and then introduced gently into the chamber. For liquid−air systems, the micropipet was simply left air filled, and for other gases it can be prefilled with the desired gas, for example, fluorocarbon. For liquid−liquid systems, the interfacial tension measuring pipet was backfilled with the nonaqueous test liquid using a cleaned MicroFil nonmetallic syringe needle (World Precision Instruments, Inc.). During this initial process, the pipet pressure system was open to the atmosphere, and the negative pressure due to capillary pressure causes aqueous solution to immediately be drawn into the pipet tip. By closing the manometer taps and isolating the pipet from atmospheric pressure, an overpressure can be applied (e.g., on the order of 10 000 Pa) to oppose the capillary pressure and bring the interface into view close to the pipet tip. After further pressurization to release a few gas bubbles (or oil droplets, in the case of, say, liquid alkanes), a new and clean interface is readily created. The pressure is rapidly adjusted so that the interface is positioned at a desired, relatively large radius in the pipet, several tens of microns back from the pipet tip. This procedure ensures that a fresh interface is produced, and this can be done repeatedly to easily create clean microscopic interfaces for a series of measurements. The micropipet pressure is then changed in steps of 500 Pa, and at each pressure step, once equilibrium is reached, the position and geometry of the interface is recorded on videotape for subsequent measurement of the corresponding interfacial radius of curvature as depicted in Figure 1. When the interface approaches the pipet tip, the procedure is repeated in reverse with decreasing pressures until the original position is attained. In this way, both receding and advancing interface radii are calculated to check for hysteresis due to adhesional wetting of the glass by the aqueous solution and any changes due to the presence of possible impurities. No hysteresis was found for simple surfactant and lipid systems, nor for pure interfaces, which are the most sensitive to impurity adsorption. Following the interfacial tension measurement, a new clean interface was produced again by blowing another bubble (or liquid droplet) and drawing in solution to repeat the procedure. During the course of an experiment and between any surfactant system measurements, the cleanliness of the solutions and calibration of the interfacial tension measurement system were routinely checked with a newly formed clean interface of pure liquid for which the interfacial tension is well-known.

4. Materials and Other Methods

4.1. Materials. The organic liquids used in this study were research grade with a purity of greater than 99%. Sodium dodecyl sulfate (SDS) (Sigma, 99%) and PEG-40-Stearate (Sigma) were used as received. Clean water from a Milli-Q water (18.2 MΩ cm) purification system was used in preparing all sample solutions. All glassware was cleaned using Nochromix (Godax Laboratory) solution and oven dried before use.

4.2. Preparation of Surfactant Solutions. Aqueous solutions of SDS of known concentration were prepared fresh for each experiment either in pure water or in NaCl (0.1 M) solution. For determination of the critical micelle concentration (cmc) of PEG-40-Stearate, the poly(ethylene glycol) fatty acid ester was weighed as a dry solid in a borosilicate scintillation vial and dissolved in pure water at various concentrations (μM to mM). The interfacial tension measurements were performed at 23 °C unless otherwise specified.

4.3. Fluorimetric Determination of PEG-40-Stearate CMC. A fluorimetric method developed by Chattopadhyay and London[22] was used to determine the cmc of PEG-40-Stearate. Briefly, a neutral fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH, Aldrich Chemical Co., Inc.), was codissolved with PEG-40-Stearate in a volatile solvent mixture (tetrahydrofuran and methanol) in a series of culture tubes in which the amount of DPH was fixed and the PEG-40-Stearate concentration varied between tubes. The solvent was removed by evaporation, and the samples were rehydrated with water to a total volume of 2 mL. Tubes were covered, vortexed slightly to mix the contents, and then incubated in the dark at room temperature for 30 min. Fluorescence intensity measurements of the samples were made at 23 °C in an AMINCO-Bowman Series 2 luminescence spectrofluorophotometer with excitation and emission wavelengths at 358 and 430 nm, respectively. A change in the slope of fluorescence intensity versus concentration signifies the formation of micelles and an increased amount of DPH solubilized in a hydrophobic environment within the micelles.

5. Results and Discussion

The presentation of results and their discussion is divided into three subsections. Subsections 5.1 and 5.2 demonstrate the feasibility and accuracy of the new micropipet method using pure liquids and the well-studied surfactant SDS for which values of interfacial tensions have already been reported. These experiments provide an important check of the new technique and demonstrate its utility for both equilibrium and dynamic tension measurements that are readily applicable to other soluble surfactant systems. In subsection 5.3, we report for the

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Table 1. Interfacial Tensions of Pure Systems at Their Liquid−Air and Liquid−Liquid interfaces at 23 °C[a]

system	interfacial tensions (mN/m) micropipet technique	other methods
Liquid−Air Interface		
water	72.4 ± 0.6	72−73 (ref 14)
n-butanol	24 ± 0.6	24.6 (ref 14)
NaCl solution (1.9 M)	76.2 ± 0.7	76.05 (ref 25)
NaCl solution (5.7 M)	82.5 ± 0.7	82.55 (ref 25)
decane	23.6 ± 0.5	23.9 (ref 10)
hexadecane	27.9 ± 0.5	27.6 (ref 14)
Liquid−Liquid Interface		
water−decane	51.2 ± 0.4	51.2 (ref 15), 51.9 (ref 16)
water−hexadecane	52.5 ± 0.4	52.1 (ref 32), 53.3 (ref 16)
water−chloroform	32 ± 1.0	32.7 (ref 32)

[a] The interfacial tension values are represented as mean ± standard deviation for a repeated number of measurements.

first time the relationship between surface tension and concentration for PEG-40-Stearate and compare this with an independent measure of the cmc for this soluble polymer surfactant.

5.1. Feasibility and Accuracy of the Method. 5.1.1. Application to Pure Systems at the Liquid−Air and Liquid−Liquid Interfaces. To determine the accuracy and reproducibility of this method, we have made equilibrium interfacial tension measurements on pure systems at their liquid−air and liquid−liquid interfaces. These results compared with the reported values available from the literature are given in Table 1. Excellent agreement with results already obtained by other methods for these systems on macroscopic interfaces indicates that the micropipet technique can be used to provide reliable interfacial tension measurements, at both liquid−gas and liquid−liquid microscopic interfaces.

5.1.2. Application to Surfactant Systems at the Liquid−Air Interface. In dilute solutions, surface-active agents adsorb to and thereby lower the interfacial tension of the liquid−air and liquid−liquid interface. The well-known Gibbs adsorption equation that relates interfacial tension to the bulk solution concentration of a surfactant can be used to calculate the surface excess concentration, and therefore area per molecule, of the surfactant at the interface. At a well-defined concentration, an abrupt change in several properties, including the interfacial tension, occurs and is indicative of the formation of micelles, that is, cmc. The presence of electrolytes in the solution reduces the repulsion between the charged groups at the surface of the micelle by the screening action of the added ions, and as a result, the critical micelle concentration is lowered. This effect was observed with the new micropipet surface tension technique for SDS in pure water and in the presence of 0.1 M sodium chloride. In addition, the surface tension for the SDS solution at its cmc also fell from 39 mN/m in pure water to a value of 34 mN/m in 0.1 M NaCl. A plot of the measured surface tension at the water−air interface as a function of SDS concentration is shown in Figure 5 along with values given in the literature.[15,23,24] The reported cmc value for SDS is ∼8 mM in the absence of any electrolytes, and our results agree very well with this value. The cmc value of SDS reduces to 1.5 mM in 0.1 M NaCl solution at 25 °C.[24−27] The slope of the linear section of the surface tension versus log concentration plot gives the surface excess concentration. From this slope, the area per molecule of SDS was

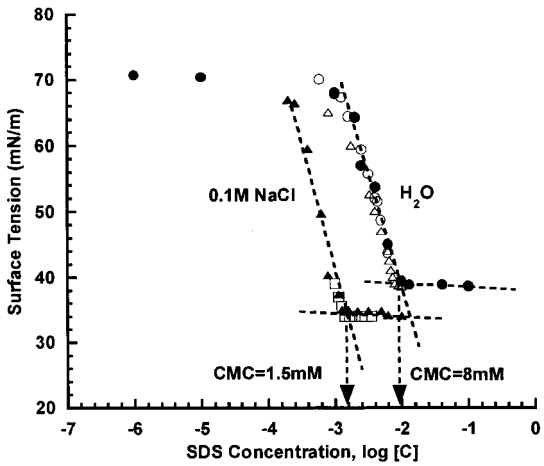

Figure 5. Surface tension of SDS in water and in aqueous solution of NaCl at the water−air interface as a function of logarithm of SDS concentration, determined from the micropipet technique (at 23 °C). From the graph, it can be seen that the cmc value of SDS is 8 mM in pure water (●) and 1.5 mM in 0.1 M NaCl solution (▲). The area per molecule is calculated to be 0.50 and 0.41 nm^2 for water and NaCl solution, respectively. Literature values are plotted together for comparison (△, ref 14; ○, ref 23; □, ref 24).

calculated to be 0.50 nm^2 in pure water and 0.41 nm^2 in salt solution, consistent with the screening effect of salt solution, and agrees well with values reported in the literature.[26,28−30]

5.2. Application to Dynamic Interfacial Tension Measurement. The time-dependent changes in interfacial tension (dynamic interfacial tensions) are as important as equilibrium tension in understanding the whole history of monolayer formation and provide insight into the mechanism of surfactant adsorption at, and transport across, an interface. To measure the dynamic interfacial tension with the single micropipet configuration, the pressure in a tapered pipet and the temperature of the chamber are fixed and the time necessary for interfaces (liquid−gas and liquid−liquid) to reach an equilibrium position is monitored. Starting from the moment when the fresh interface is formed by blowing out a bubble of gas (or liquid), this method can be used to monitor the change of interfacial tension over a wide range of time scales, from ∼0.1 s to hours. Furthermore, the introduction of a second flow pipet provides one of the main advantages of this new technique, namely, the ability to monitor in situ the change in interfacial tension as a result of the introduction of a surfactant or change of local concentration. A new solution can be delivered by inserting the flow pipet into the measuring pipet and blowing the new solution into the pipet mouth (Figure 6B−D). If the tension of the exposed interface changes, for example, due to the adsorption and spreading of a surface-active material at the interface, these changes in tension can be easily measured by tracking the position of the interface in the tapered pipet with time for a constant ΔP. To demonstrate this aspect of the technique for exchangeable and soluble surfactants, the SDS system has been studied at the

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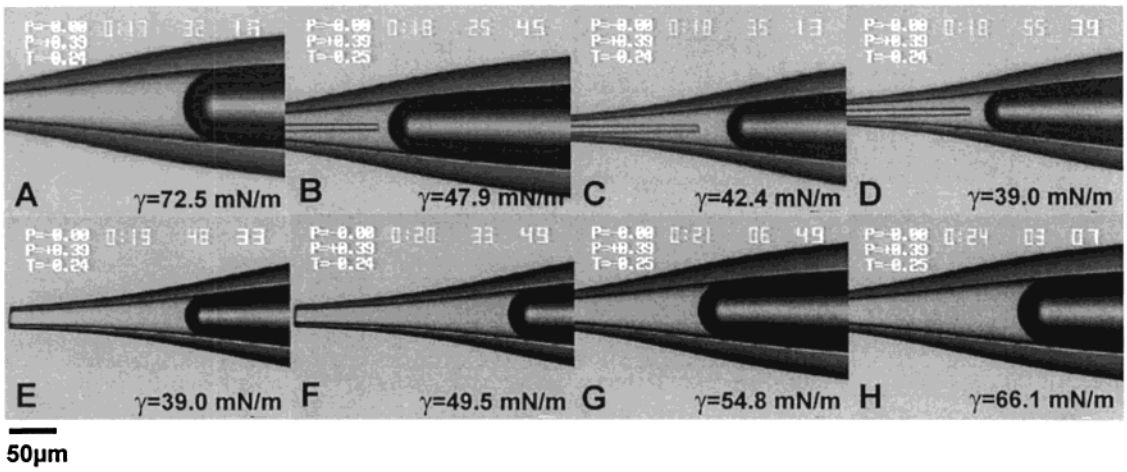

Figure 6. Videomicrograph sequence of the dynamic surface tension experiment showing the change in the position of the interface: (A) clean water−air interface, $\gamma = 72.5$ mN/m; (B) insertion of the delivering pipet containing SDS solution (10 mM in water) into the surface tension measuring pipet where the equilibrated clean water−air interface is formed, $t = 16$ s, $\gamma = 47.9$ mN/m; (C) $t = 26$ s, $\gamma = 42.4$ mN/m; (D) $t = 46$ s, $\gamma = 39.0$ mN/m, the interface moves toward the narrow end of the pipet with time as a result of surface tension reduction caused by adsorption of the SDS molecules at the water−air interface; (E) withdrawing the delivering pipet from the surface tension measuring pipet, $t = 99$ s, $\gamma = 39.0$ mN/m; (F) $t = 144$ s, $\gamma = 49.5$ mN/m; (G) $t = 177$ s, $\gamma = 54.8$ mN/m; (H) $t = 354$ s, $\gamma = 66.1$ mN/m, the interface has returned almost to its original position, indicating a desorption of SDS molecule at the water−air interface.

water−air interface. Figure 6A−D shows a sequence of videomicrographs monitoring the change in the position of the interface in the tapered pipet with time as the SDS solution (above its cmc, 10 mM) is delivered to the water−air interface. The flow pipet was then removed in order to slowly replace the SDS solution with SDS-free bathing solution. Figure 6E−H shows how simple diffusive exchange between the surrounding bathing medium and the solution in the pipet mouth allows washout of the SDS and a return to higher tensions. During this dynamic surface tension measurement, the pressure in the pipet and temperature of the chamber were held constant and the movement of the interface was therefore a consequence solely of the surfactant adsorption/desorption at the water−air interface. For dynamic surface tension measurements (Figure 6), the use of the low magnification objective (10×) is necessary because the experiment requires a larger field of view in order to manipulate the second pipet that needs to be inserted into the measuring pipet and to follow the large displacements of the interface. A change in the position of the interface inside the pipet and the corresponding surface tension were observed as a function of time, as plotted in Figure 7. As the SDS flow pipet reached the vicinity of the water−air interface and exchanged the solution in the pipet with SDS solution, the surface tension was reduced rapidly (within seconds), and the interface moved to smaller radii at constant pipet pressure of 3900 Pa. The equilibrium value of 39 mN/m (as measured in Figure 5) was reached in ∼50 s. Removing the SDS flow pipet from the measuring pipet resulted in the movement of the interface back up the pipet and an increase in the radius of curvature almost back to the original position, as shown in Figure 6E−H and plotted in Figure 7. The resulting surface tension of 66 mN/m indicated that the SDS largely washed off the previously saturated interface. However, in the 400 s of washout, the value of the surface tension did not reach the original pure interface value showing that some residual SDS was still left on the interface. A longer equilibration time and convective washout with a second flow pipet might be expected to approach the clean interface condition. In general, molecules that are relatively water soluble, with a cmc greater than a few micromolar, exhibit this fast

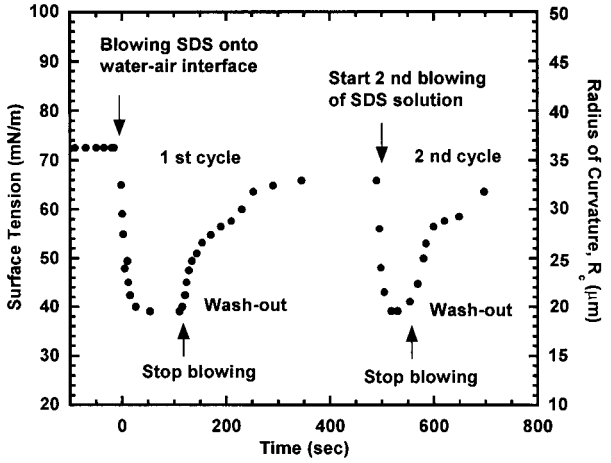

Figure 7. Dynamic surface tension measurement of SDS (10 mM) with the micropipet technique: adsorption and desorption cycles for SDS solutions at the water−air interface are tracked by changes in the surface tension.

surfactant exchange. Subsequent SDS blowing at the SDS-free water−air interface shows again a rapid approach to the minimum equilibrium surface tension value, followed by desorption upon removal of the flow pipet. Thus, this simple surfactant delivery experiment provides a direct measure of the rate and extent of adsorption of a soluble surfactant and also measures the rate and extent of desorption for the same interface. The micropipet method gives the whole time history of surfactant exchange for microscopic liquid−air or liquid−liquid interfaces by providing a convenient monitoring of the changes in interfacial tension and its approach to equilibrium.

5.3. The Adsorption of PEG-40-Stearate at the Water−Air Interface. After verifying that interfacial tension, cmc, and area per molecule can be accurately measured with this technique, we then characterized PEG-40-Stearate, a molecule that has been used as one of the stabilizers in formulations of microbubbles in our laboratory.[6,7] Figure 8A shows a plot of surface tension as a function of log concentration of PEG-40-Stearate. Note that the cmc region for PEG-40-Stearate was relatively broad and found to be centered at 40 ± 2 μM. The

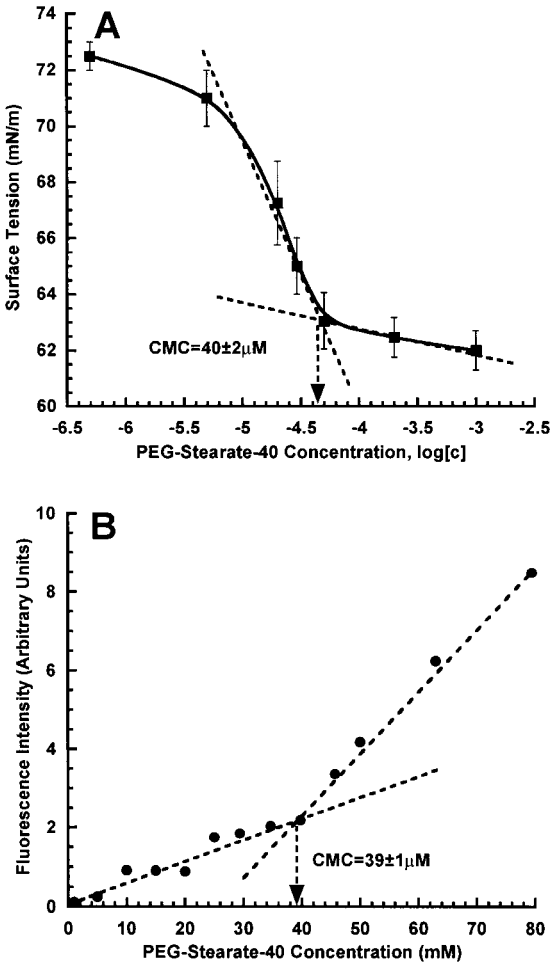

Figure 8. (A) Surface tension vs log of the concentration of PEG-40-Stearate in aqueous solution at 23 °C at the water−air interface measured by the micropipet technique. The cmc range is found to be 40 ± 2 μM. (B) Fluorescence intensities of DPH/PEG-40-Stearate aqueous samples as a function of the PEG-40-Stearate concentration. The cmc, determined from the intersection of the linear fits to the low and high concentration regimes of PEG-40-Stearate, is approximately 39 ± 1 μM.

polydispersity of the PEG[31] may account for this breadth. This cmc measurement was also verified independently with a fluorescence technique that uses solubilization of a fluorescent dye, diphenylhexatriene (DPH), which fluoresces in solution only when solubilized in the hydrophobic center of micelles.[22] A plot of fluorescence intensity of the DPH/PEG-40-Stearate samples as a function of PEG-40-Stearate concentration, shown in Figure 8B, displays a steep rise in fluorescence intensity at PEG-40-Stearate concentrations higher than ~40 μM. The cmc is obtained from the intersection of the linear fits to the low and high concentration regimes of PEG-40-Stearate. From the results of Figure 8B, the cmc of PEG-40-Stearate was 39 ± 1 μM, which is consistent with the value determined from surface tension measurement using the micropipet technique. From the Gibbs adsorption equation, the surface excess concentration was found to be 1.4×10^{-6} mol/m^2, with an area per molecule of 1.19 nm^2. This relatively large area per molecule is consistent with the large PEG molecule exerting a lateral pressure at the interface. The ratio of cross-sectional areas between the hydrophilic (1.19 nm^2) and hydrophobic (0.4 nm^2) regions of the molecule differs by a factor of 3, implying that a pure PEG-40-Stearate monolayer is not as closely packed at the surface as pure SDS or phospholipids (see part 2[8]), a feature reflected in its relatively high equilibrium surface tension of 62 mN/m.

6. Concluding Remarks

We have demonstrated that this new micropipet technique is a robust and convenient method to measure interfacial tensions at both liquid−air and liquid−liquid interfaces. Area per molecule and cmc can also be measured by this method, and results are in excellent agreement with literature values typically measured on macroscopic interfaces, confirming the accuracy and reliability of the technique. The micropipet technique also provides a convenient means of rapidly exchanging the solution in the vicinity of the interface inside the measuring pipet to monitor the adsorption/desorption of the surfactant at the clean interface. Application of this technique to study the adsorption of PEG-40-Stearate as a monolayer at a liquid−air interface showed that PEG-40-Stearate monolayers have a relatively high surface tension of 62 mN/m with a large area per molecule of 1.19 nm^2, suggesting that this amphipathic molecule does not have a strong surface tension lowering effect and that PEG inhibits close packing of the lipid molecule. As these results attest, the micropipet technique provides a unique and convenient means of studying a host of interfacial phenomena, including equilibrium and dynamic interfacial tension of microscopic liquid−gas and liquid−liquid interfaces. In addition, low maintenance and the ability to reproducibly produce clean interfaces in rapid succession for multiple measurements provide a complementary technique to other conventional approaches. In a companion paper, we report additional new equilibrium and dynamic data on phospholipid monolayers.[8]

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