Design and implementation of an experimental device by the pendant drop method for measuring surface tension of pulmonary fluid

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Design and implementation of an experimental device by the pendant drop method for measuring surface tension of pulmonary fluid

Zhenglong Chen, Dan LV, Chunyuan Zhang, Yuanyang Liu, Jingxin Sha, Linhong Deng, Ming Zhong, Yuanlin Song

Abstract
The quantitative measurement of pulmonary fluid surface tension in many lung diseases including respiratory distress syndrome (RDS), pneumonia, COVID-19 and acute respiratory distress syndrome (ARDS) has great physiological and clinical significance. The conventional techniques like film balances and bubble methods, however, are hard to use in the clinical practice due primarily to a limitation of small sample volume. In this work, we developed an experimental device based on the pendant drop method to clinically measure surface tension of pulmonary fluids in ARDS patients. We provided a detailed discussion of the theoretical background to the design of hardware and software as well as validation of measurement precision. The measured surface tensions of the two test liquids, pure water and dimethyl silicone oil, are 72.35±1.00 mN/m and 21.38±0.61 mN/m, respectively, in good agreement with the corresponding literature values of 72.02 mN/m and 21.10 mN/m at room temperature of 25℃. Using this experimental setup, we tentatively measured surface tensions of four pulmonary fluid samples from several mechanically ventilated ARDS patients. The results showed that the measured surface tension at 25±1℃ is within the range of 23.8~53.9 mN/m, comparable with reported values in the literature. Further, the calculation of the Worthington number showed that the data obtained are sufficiently accurate. We came to the conclusion that the pendant drop tensiometry is a powerful tool in the studies of pulmonary fluid surface properties due to a small sample size, high degree automation and freedom from contamination.

Keywords: Lung mechanics; Pulmonary fluid; Surface tension; Pendant drop method; Acute respiratory distress syndrome; COVID-19
Introduction

Human airways and alveolar surface are lined with a thin fluid continuum, referred to as airway surface liquid or pulmonary fluid more popularly [12, 27]. In large airways, this film of fluid is a bilayer consisting of a mucus gel layer and a periciliary layer, whereas in the distal bronchioles and alveoli, it transitions to a single layer of an aqueous subphase covered by a film of pulmonary surfactant. The low surface tension of pulmonary fluid plays an important role in maintaining the normal respiratory mechanics [7, 10]. The presence of pulmonary surfactant renders alveolar surface tension to decrease as alveolar size varies with inspiration and expiration. Therefore, according to the Laplace equation, the inward elastic recoil of the alveolus is reduced, so the likelihood of alveolar collapse during expiration. Similarly, low surface tension minimizes the magnitude of negative pressure in airway liquid layer thus deceeding tendency for airway compliant collapse. In addition, pulmonary transudation of fluid is also affected by surface forces[14]. For example, in patients with pulmonary edema, an increase in surface tension of the alveolar lining fluid may cause a greater pressure gradient between interstitial fluid and intra-alveolar fluid, leading to excessive fluid transudation from the interstitial space into the alveolar air spaces.

In acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS), alterations in the alveolar surface tension may cause volutrauma or atelectrauma during mechanical ventilation[2]. The most frequent causes of ARDS include sepsis, severe pneumonia, aspiration of gastric contents, and trauma[26]. Particularly, Wu et al. reported recently that patients with severe COVID-19 may quickly progress to ARDS[28]. Protective ventilation strategies have used higher positive end-expiratory pressures (PEEPs) and recruitment maneuvers to prevent lung atelectrauma[1]. Computational models of ventilator induced lung injury showed that surface tension of pulmonary fluid has a crucial effect on the lung recruitment pressures or airway reopening pressures[1, 31]. Premature infants may suffer from neonatal respiratory distress syndrome (NRDS) due to surfactant deficiency. The postnatal delivery of exogenous surfactant has been established as a standard therapeutic intervention in the management of preterm infants with NRDS[15]. A recent study by Filoche and colleagues showed that surface tension of the instilled surfactant mixture determines its delivery efficiency and distribution homogeneity in the pulmonary airways during surfactant replacement therapy (SRT)[8].

Therefore, measuring the surface tension of pulmonary liquid has an important clinical significance in both designing lung protective
ventilatory strategy and optimizing surfactant replacement therapy. A number of measuring methods, for example film balances, bubble methods and drop shape techniques, have been developed for assessing surface tension[7]. Among these methods, drop shape techniques are found to be particularly appropriate for the study of surface tension of pulmonary fluid as they require only a small amount of lung liquid sample, are free of film leakage and allow for measurements of dynamic and ultralow surface tensions[4, 19]. In this study, we developed an experimental pendant drop tensiometry to measure surface tension of pulmonary fluid. We provided a detailed discussion on the theory of the pendant drop method, design of hardware and software, experimental results, measurement precision, as well as potential sources of error.

Methods

The principle of pendant drop method

Axisymmetric drop shape analysis techniques have been developed for the measurement of interfacial tension. Among these, the pendant drop is the one most extensively used. In the pendant drop setup, a drop is suspended at the end of a needle or a small flat circular holder. The shape of the pendant drop depends on the combined action of surface tension and external forces, such as gravity, as reflected mathematically in the Laplace equation of capillarity. It describes the relationship among the pressure difference across a liquid-fluid interface, the curvature of the interface and surface tension:

\[ \Delta P = \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \gamma \]  

where \( \Delta P \) represents the pressure difference across the interface, \( R_1 \) and \( R_2 \) are the two principal radii of curvature at point P (see Fig.1), and \( \gamma \) is the interfacial tension.

Referring to a pendant drop below a needle shown in Fig.1, we set a coordinate system with the apex of the drop as the origin. The x axis is tangent to the curved interface and normal to the axis of symmetry, and z is the vertical height measured from a reference horizontal plane through the apex. Provided that the drop is not subjected to external forces other than gravity, the pressure difference can be expressed as the sum of a reference pressure \( \Delta P_0 \) at \( z = 0 \) and a hydrostatic pressure \( (\Delta \rho)gz \),

\[ \Delta P = \Delta P_0 + (\Delta \rho)gz \]  

Where \( \Delta P_0 \) is the pressure difference at the reference plane, \( \Delta \rho \) is the density difference between two bulk phases, \( g \) is the local gravitational
acceleration, and $z$ is the vertical height of the given point $P$ on the surface.

\[ \frac{d\varphi}{ds} = \frac{2}{R_0} + \frac{(\Delta \rho)g}{\gamma} z - \frac{\sin \varphi}{x} \quad (3a) \]
\[ \frac{dx}{ds} = \cos \varphi \quad (3b) \]
\[ \frac{dz}{ds} = \sin \varphi \quad (3c) \]

where $R_0$ is the radius of curvature at the origin, and $\varphi$ is the turning angle between the tangent to the interface at the point $P$ and the horizontal plane. The associated boundary conditions are

\[ x(0) = z(0) = \varphi(0) = 0. \quad (4) \]

Therefore, for given $R_0$ and the capillary constant $c \equiv (\Delta \rho)g/\gamma$, the theoretical shape of curve can be obtained by a numerical integrating simultaneously the above set of equations. Then, experimental drop profiles are matched with the theoretical drop profiles with the surface tension as one of the adjustable parameters. The best fit identifies the operative surface tension. Details of these mathematical procedures can be found in Refs [13, 16, 17].
Briefly, suppose that $U_i, i = 1, 2, \cdots, N$ are a set of experimental profile points and $u = u(s)$ is a calculated Laplacian curve. The objective function is defined as the sum of the individual errors

$$E = \sum_{i=1}^{N} e_i = \frac{1}{2} \sum_{i=1}^{N} d_i^2 = \frac{1}{2} \sum_{i=1}^{N} [(x_i - X_i)^2 + (z_i - Z_i)^2], \quad (5)$$

where the error for the i-th point is computed as the square of the normal distance $d_i$ between an experimental point $(X_i, Z_i)$ and the point on the Laplacian curve closest to it, as illustrated in Fig.1(b). Obviously, the value of objective function $E$ is a function of a set of parameters, i.e. $b = 1/R_0, c, x_0$ and $z_0$ ($x_0$ and $z_0$ are the coordinates of the apex). When the objective function assumes a minimum value at one point in the parameter space of $E$, the optimum parameters that give the best fit between the experimental points and a Laplacian curve are obtained. So surface tension can be calculated based on the optimum value of the capillary constant $c$.

Experimental setup

The experimental setup for axisymmetric drop shape analysis is shown in Fig.2. A medical cannula with a blunt needle tip is used to suspend a pendant droplet. The volume of the droplet is manually controlled a syringe. The drop is illuminated from behind with a circular LED light source (Cogstek, YRL-74-60-W, China). A ground-glass diffuser is used to reduce the effect of chromatic aberration for a clear image of the drop at the edge. The pendant droplet is contained inside a glass cuvette to prevent contamination and to avoid oscillation induced by air currents.

The drop images are acquired using an industrial digital camera (DAHENG, MER-500-14GC, China) equipped with a microscopic lens (Computar, M3Z1228C-MP, Japan). The camera has a color CMOS digital image sensor featuring frame rate up to 14 fps. The acquired images of the pendant drop are transmitted to a personal computer for further analysis and computation using the drop shape analysis technique. The resolution is fixed at 640×480 for all the images.
During the experiment, much attention must be paid to the following key points. First, focusing has a major influence on the quality of the image, hence the position of the CCD camera with respect to the drop must remain relatively constant. Second, in order to facilitate determination of the drop profile coordinates in the next image analysis, both the needle and the camera must be vertically aligned with a plumb line. Third, the measurement precision for pendant drop tensiometry depends on the shape of the drop. It is operatively required to control syringe for generating a large drop with a well-deformed shape, i.e., a drop with inflection points in the neck area. And finally, the entire experimental setup, except the computer, needs to be mounted on an anti-vibration table to minimize the effects of random vibrations.

Once a drop image acquisition is completed by the CCD digital camera, a computer can establish communication with the camera through a software kit GigE IP Configurator and then load the image by DAHENG MER-Series Viewer. Following this procedure, a computational routine developed in Microsoft Visual Studio 2017 environment is used to perform the image analysis and numerical optimization to detect the experimental profile and calculate surface tension of the drop, respectively. The routine can be divided into the steps shown schematically in Fig.3.

1. The image preprocess, including grayscale transform, smoothing filtering, image zooming and edge detection, are performed by calling OpenCV software library. The edge detection process has a considerable impact on the accuracy of surface tension. This will be discussed in detail below.

2. Extract the drop profile coordinates by Mat.at function in OpenCV software library traversing all pixel points of the drop profile image obtained at the step 1, and perform an image coordinate transformation by calculating the image magnification factor according to cannula size.
3. Select arbitrarily several accurate coordinate points from the experimental drop profile on the screen of the computer using a mouse.

4. Solve simultaneous Eqs. (3a), (3b) and (3c) and the objective function using a fourth-order Runge-Kutta integration procedure and Newton–Raphson method, respectively, obtaining the value of the optimal parameter $c$.

5. Input known values of physical properties, i.e. density difference $\Delta \rho$ and gravitational acceleration $g$ and determine surface tension by the equation $c = (\Delta \rho)g/\gamma$.

![Fig.3 Flow chart of software for axisymmetric drop shape analysis method.](image)

Extracting the drop profile from an experimental image is a most basic problem in edge detection. Hoofar and Neuman [11] compared the effects of various edge detection techniques (i.e., Sobel, Roberts, Prewitt, Laplacian of Gaussian(LoG), SUSAN, and Canny) on the surface tension values of cyclohexane. Their studies reveal that SUSAN and Canny are the two most satisfactory edge detectors for experimental drop profiles[6, 25]. However, the traditional Canny operator is susceptible to noise in images, due to its differential nature. In this case, setting an empirical Gauss filtering parameter is not a piece of cake. In addition, it only calculates the gradient in the horizontal and vertical directions, and hence might lose some important edge information, especially one in the oblique edge. In
this study, an improved Canny operator is used to detect the drop profile [5].
The algorithm is described briefly as below.

1. Add the salt and pepper noise to the original drop image.
2. Apply morphological open and close operations to the image for noise reduction.
3. Similarly to the traditional Canny method, calculate the gradient magnitude of the smoothed image in the horizontal and vertical directions, defined as $G(X)$ and $G(Y)$ respectively. The gradient direction is determined as $\theta = \arctan[\frac{G(X)}{G(Y)}]$. Next make non-maximum suppression to get the gradient image $G_1$.
4. Calculate the gradient magnitude of the smoothed image in two diagonal directions using two diagonal templates as shown below
   \[
   \begin{bmatrix}
   0 & 1 & 2 \\
   -1 & 0 & 1 \\
   -2 & -1 & 0
   \end{bmatrix}
   \text{ and }
   \begin{bmatrix}
   -2 & -1 & 0 \\
   -1 & 0 & 1 \\
   0 & 1 & 2
   \end{bmatrix}
   \]
   and get the gradient image in the oblique direction, $G_2$.
5. Compare the gradient magnitudes of the corresponding elements in $G_1$ and $G_2$, and take the maximum of the two magnitudes as the final gradient value, thereby obtaining the improved gradient image $G$.
6. Process the gradient image $G$ with double thresholds method and get the final edge image of the drop.

Figure 4 shows a water droplet suspended from a needle (a) and its profile extracted by the improved Canny operator.

**Fig.4** Water droplet image (a) and its edges extracted by the improved Canny operator (b).

**Results**

**Validation of the experimental setup**

To demonstrate the capabilities of the current experimental setup, two common liquids, i.e., pure water and dimethyl silicone fluid, were tested
for surface tension measurements. Dimethyl silicone fluid was purchased from The Dow Chemical Company (USA), 99% purity or greater. The literature values for the surface tension of pure water and dimethyl silicone fluid are 72.02 \(\text{mN/m}\) and 21.10 \(\text{mN/m}\) at a temperature of 25\(^\circ\text{C}\), respectively. The cannula used in this study has an outer diameter of 0.8 mm. Each test liquid was tested repeatedly at least 30 times. All measurements were carried out at room temperature (25±1\(^\circ\text{C}\)). The results of the measurements are shown in Figure 5. The surface tensions of pure water and dimethyl silicone measured by the current experimental setup are 72.35 ± 1.00\(\text{mN/m}\) and 21.38 ± 0.61\(\text{mN/m}\), respectively. Compared to the literature values, the relative error of measured surface tension is 0.48\% for pure water, and 1.33\% for dimethyl silicone fluid. This measurement precision indicates that the current experimental setup is applicable to determine surface tension of pulmonary fluids.

![Figure 5](image-url)

**Fig.5** Surface tension of pure water and dimethyl silicone fluid measured by the experimental setup. The error bars represent SD, n=10.

**Surface tension measurements of pulmonary fluid**

The pulmonary fluid samples from ARDS patients, who were admitted to the surgical intensive care unit of Zhongshan Hospital Fudan University, were collected using a bronchoalveolar lavage catheter (cat. No. CF12; XiangSheng Medical Products, Shanghai, China). The study was granted by the institutional review board of the hospital, and written informed consent was obtained from the patients or their surrogates. Using the current
experimental setup, we tentatively measured surface tension of 4 pulmonary fluid samples in total. Due to a relatively small and different sample volumes (sometimes only 2 milliliters or less), the number of independent measurements for each pulmonary fluid sample was varied. The results are reported in Figure 6. All data are expressed as mean ± standard deviation (SD). The measured surface tensions for samples 1, 2, 3 and 4 are 23.83±1.93, 45.91±1.63, 38.53±4.13 and 52.94±1.09 mN/m, respectively. There are statistically significant differences among four samples for the measured surface tension ($P<0.0001$, one-way analysis of variance [ANOVA]). It is also noted that the standard deviation from twelve measurements for samples 3 is markedly higher than those for samples 1, 2, and 4, indicating there may exist a significant error in measured surface tensions for sample 3. These points will be discussed more fully in the following Discussion.

![Fig.6](image-url) Surface tensions of four pulmonary fluid samples measured by the experimental setup. The error bars represent SD, $n_1=15$, $n_2=10$, $n_3=12$ and $n_4=10$.

**Discussion**

In this study, we designed and implemented an experimental apparatus based on pendant drop tensiometry, and further validated its measurement accuracy using test liquids with known surface tension values. Using this experimental setup, we tentatively measured surface tensions of four pulmonary fluid samples from several mechanically ventilated ARDS
patients. The results showed that the measured surface tension covered a range of 23.83~52.94 mN/m at room temperature (25±1°C). By use of microdroplet method, Schürch et al. [21, 22] measured alveolar surface tension directly in an excised normal lung to be approximately 7~29.7 mN/m at 37°C. Using lung pressure-volume curves, Smith and Stamenovic [24] reported surface tension in the normal lung ranging from near 0 mN/m at low lung volumes to high values near 40 mN/m during inflation, and the value of equilibrium surface tension to be approximately 28 mN/m at 22°C. Therefore, surface tensions of pulmonary fluid samples determined with the current experimental setup are commensurate with the above literature values.

A significant difference was found in the mean surface tension among four pulmonary fluid samples studied. Except for sample 1, the mean surface tensions of the rest three samples are all larger than the normal value of equilibrium surface tension about 28 mN/m. These findings reveal that the surface tension of pulmonary fluids in ARDS may vary from patient to patient and also day to day. By an approximate relationship between the capillary pressure ($P_{\text{cap}}$) and surface tension, $P_{\text{cap}} \approx 8\gamma / R$, where R is the radius of airway, the increase in the surface tension of the air-liquid interface would subsequently lead to increase in the airway opening pressure[9]. Therefore, quantitative measurements of pulmonary fluid surface tension may help better set the value of positive end-expiratory pressure in mechanically ventilated ARDS patients. Additionally, we also noted that there existed a relatively large deviation among multiple repeated measurements on the same sample, particularly on samples 3, as described earlier in Results. The possible reasons for this measurement error are discussed in detail below.

Drop volume effect. It is well documented that a small drop volume is the primary source of errors in most practical pendant drop experiments [3, 20, 29]. Several different criteria or parameters, such as the capillary constant, Bond number, shape parameter, Worthington number and Neumann number, have been introduced to quantify the effect of drop volume on measurement precision[29]. Due to its simplicity and reliability, Worthington number, $W_o$, is considered here as a post-measurement check to give an indication of the likely measurement precision. $W_o$ is denoted by Berry et al. as [4],

$$W_o = \frac{\Delta \rho g V}{\gamma \pi D_t},$$  \hspace{1cm} (6)

where $V$ is the actual drop volume, and $D_t$ is the needle diameter. $W_o$, by definition, scales from 0 to 1. In general, a $W_o$ larger than 0.58 is
required for accurate surface tension measurements with pendant drop constellation.

It can be seen from Figure 7 that for the majority of measurements (41 out of 47 in total) the Worthington number is larger than the critical value 0.58, suggesting a negligible effect of drop volume on measurement accuracy. It is also noted that the proportion of measurements with \( Wo < 0.58 \) in sample 2 (30\%, 3 out of 10) is slightly larger than that in sample 3 (25\%, 3 out of 12), but the standard deviation of the sample 2 (SD=1.63 mN/m) is appreciably less than that of the sample 3 (SD=4.13 mN/m). This fact further rules out the possibility that the relatively large deviation on the sample 3 originates from insufficient deformation of the drops.

**Fig.7** The surface tension and Worthington number obtained from fitting droplets of pulmonary fluid samples.

*Measurement uncertainty.* Shardt and Elliott [23] reported that the effect of temperature on the surface tension is less than 0.25 mN/m/K for a broad range of compounds. The variation of temperature in the experiment is tightly controlled within one degree. Therefore, the uncertainty associated with the variation of temperature is less than 0.25 mN/m/K. Considering the experimentally measured maximum surface tension of pulmonary fluids to be less than 53 mN/m, this measured uncertainty approximately corresponds to a relative error of 0.5\%. Moreover, applying the principles of error propagation [30] to the equation \( c = (\Delta \rho)g/\gamma \), the relative error of the surface tension can be calculated as

\[
\frac{|\Delta \gamma|}{\gamma} = \frac{|\Delta \rho|}{\rho} + \frac{|\Delta g|}{g} + \frac{|\Delta c|}{c}.
\]
The first term on the right-hand side of equation (7) represents the uncertainty of fluid density measurement that is generally less than 0.2%, and the gravitational acceleration is broadly constant, hence the second term is usually negligible. The relative error on the optimization parameter originates from random perturbations in edge detection with real images. Saad and Neumann [18] showed that pendant drop techniques are capable of producing accurate surface tension values with error less than 1%. Taken together, the general uncertainty that has been propagated to $\gamma$ from each error source is less than 2%.

As discussed above, given the large Worthington number and the small relative error in the experiment, the relatively large standard deviation on samples 3 could not be explained by the drop volume effect and the measurement uncertainty of the experiment setup. A close inspection of sample 3 showed that the pulmonary fluid looked reddish due to mixing with blood, and a small number of micro-bubbles were trapped in it. These micro-bubbles were not easy to remove because of highly viscous confinement by pulmonary fluid. On the other hand, the Laplace equation (1) describes the mechanical equilibrium for two homogenous fluids separated by an interface. Therefore, in the case of the inhomogeneous or impure fluid, it is no longer appropriate to calculate surface tension by the Laplace equation. In other words, the large standard deviation observed on sample 3 is most likely caused by inhomogeneity of pulmonary fluid component. This preliminary experiment shows that before determining surface tension of pulmonary fluids by using the pendant drop technique, it is necessary to filter and degas fluid samples for the most accurate and precise measurements.

**Conclusions**

In this work we developed an experimental device that utilized axisymmetric drop shape analysis technique to perform surface tension measurements, validating also its accuracy with standard test liquids. Using this experimental setup, we attempted to measure surface tension of pulmonary fluid samples from ARDS patients. The measured surface tension is within the range of $23.8 \sim 53.9$ mN/m, comparable with surface tensions as obtained by means of conventional techniques like microdroplet method and pressure-volume method. Further, the calculation of the Worthington number shows that the data obtained are sufficiently accurate. Most importantly, we observed a significant difference between samples in the surface tension of pulmonary fluid, indicating that
properties of pulmonary fluid in ARDS patients may vary from individual to individual and day to day. Further measurements in more ARDS patients are needed to confirm this observation. In this regard, the pendant drop tensiometry is a powerful tool for investigators due to a small sample size, high degree of automation and freedom from contamination.

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Declarations

Ethical Approval The study was granted by the institutional review board of Zhongshan Hospital Fudan University (B2017-021R), and written informed consent was obtained from the patients or their surrogates.

Conflicts of Interests The authors have no relevant financial or non-financial interests to disclose.

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