Ring distraction technique for measuring surface tension of sputum: relationship to sputum clearability

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Albers, G. M., R. P. Tomkiewicz, M. K. May, O. E. Ramirez, and B. K. Rubin. Ring distraction technique for measuring surface tension of sputum: relationship to sputum clearability. J. Appl. Physiol. 81(6): 2690–2695, 1996.—Poor sputum clearance has been related to sputum adhesion tension. In this study, we describe a modified du Nouy ring method for measuring the surface tension (γ) of small samples of sputum and for comparing the calculated work of adhesion (Wad) for sputum specimens with the measured mucociliary transportability (MCTR) and cough transportability (CTR). The γ, as measured by this method, correlates with γ measured by sputum contact angle on a low-surface-energy solid (R² = 0.368, P = 0.03). There is a small but significant difference in measurements made by these two methods (P = 0.03). Wad calculated from the surface tension ring method is inversely correlated with CTR (R² = 0.181, P = 0.004) but has no correlation with MCTR in this study. The miniaturized ring method gives accurate and reproducible measurements of the surface tension of small amounts of respiratory secretions. Because sputum behaves enough like a liquid that the assumptions made in using the Young equation to calculate Wad appear valid, we also showed that the Neumann equation can be used to determine the surface tension of sputum by its contact angle on tetrafluoroethylene (Teflon).

physical properties of sputum; adhesion; surface properties; cystic fibrosis; chronic bronchitis

RESPIRATORY MUCUS is a viscoelastic gel that is secreted into the airways and spreads over the surface of the airway epithelium in contact with the tips of the cilia and the periciliary fluid layer (12, 18, 20). Normally, mucus is cleared to the proximal airway by the direct interaction of beating cilia with the surface of the mucus. When there is hypersecretion or ciliary damage, secretions are cleared by cough, which is dependent on the flow of air to remove the mucus accumulated in the airway (10). In both of these forms of clearance, the physical properties of mucus play a role. These properties include viscoelasticity as well as surface interactions as mucus forms an interface with the underlying epithelium. Work must be done to overcome the forces that are active at the mucus–epithelium interface. The work required to separate 1 cm² of an interface between two substances is called the work of adhesion (Wad)

\[ W_{ad} = \gamma_1 + \gamma_2 - \gamma_{12} \]  

where \( \gamma_1 \) is the surface tension of one substance at an interface, \( \gamma_2 \) is that of the other substance, and \( \gamma_{12} \) is the interfacial tension between the two (1). When a liquid rests on the surface of a solid, in most instances the liquid will not completely wet the solid and will form a drop on the surface with a discrete contact angle (θ). When this system is at equilibrium, Young's equation (Eq. 2) applies, where \( \gamma_{lv} \) is the interfacial tension between the liquid phase and its vapor, \( \gamma_{sv} \) is the interfacial tension between the solid and vapor, \( \gamma_{sl} \) is the interfacial tension between the solid and the liquid, and \( \theta \) is the discrete contact angle that the liquid forms on the solid (Fig. 1).

\[ \gamma_{lv} \cos \theta = \gamma_{sv} - \gamma_{sl} \]  

Equations 1 and 2 can be combined to give

\[ W_{ad} = \gamma_{lv}(1 + \cos \theta) \]  

To calculate Wad for a mucus-surface interface from Eq. 1, we must be able to measure the surface tension of the mucus, the surface tension of the surface on which the mucus is resting, and the interfacial tension between the two. However, if we assume that mucus behaves enough like a liquid to accept the Young equation, we can calculate the Wad for a particular mucus interface by using Eq. 3, in which we only need to measure the surface tension of the mucus and its contact angle on the surface in question.

The gel-like properties of mucus make determinations of surface tension difficult. At least two methods for measuring the surface tension of sputum have been reported. Puchelle and colleagues (15) described use of the du Nouy ring distraction method to directly measure surface tension \( \gamma_{ring} \) using a standard ring, 10-mm in diameter and 0.7-mm thickness. This technique is limited by the relatively large amount of sputum (250 µl) needed for the measurement. Smaller samples can be used in a second method described by Pillai et al. (14), which treats mucus as a solid and calculates γ from the contact angle formed on the mucus by a liquid with known surface tension (glycerol). This ap-
METHODS

also assessed the relationship between the Wad for an iridium ring for determining the surface tension of gel. We wanted to validate the use of a small platinum-ring for surface properties of airway secretions so that the role that surface properties have on the transportability (MCTR) of the sputum samples. This was done to evaluate the role that surface properties have on the transportability of airway secretions by using a variety of specimens from patients with chest disease.

METHODS

Sputum samples were obtained by expectoration from patients with cystic fibrosis (CF) lung disease and chronic bronchitis (CB) who were not currently experiencing an exacerbation of their disease. The patients were asked to swallow all saliva before expectoration, and then the sputum was separated from any remaining saliva before conducting our analyses. Specimens were stored at -70°C until analysis. None of the CF patients had been treated with recombinant human deoxyribonuclease or were using any mucoactive medication. Approval to collect and use sputum from patients for in vitro analysis was obtained from the St. Louis University School of Medicine Institutional Review Board.

Surface tension measurements. Surface tension measurements were made with the use of the du Noyer ring technique. A custom ring was fashioned of 90% platinum/10% iridium with a diameter of 5.4 mm, circumference of 1.57 cm, and width of 0.7 mm for use in a commercial surface tensiometer (Surface Tensimat model 21; Fisher Scientific, Indiana, PA). The tensiometer was calibrated at each use with a standard 6-cm-circumference ring with a known mass, and then calibration was verified against water with a known surface tension in air of γ = 72.8 dyn/cm. The small-ring calibration was then checked against water. Values were read from the tensiometer and were corrected for the difference in size of the ring (the circumference of the small ring is 3.8 times less than the standard ring).

With the use of a positive displacement pipette, a sample of sputum (30 µl volume) was placed on a glass slide at room temperature (24°C). To minimize the effect of drying, measurements were made in room air within 1 min of the sample's being placed on the slide. The ring was allowed to come into contact with this sample with a dwell time of <2 s. The contact pressure in all cases was equal to the weight of the ring, as the ring is suspended from a lever arm and cannot be "pushed" into the surface. The force required to completely separate the ring from the sample was directly measured as the apparent surface tension in dynes per centimeter. The Fisher Tensimat model 21 vernier dial is calibrated to read dynes per centimeter for a 6-cm ring, so that the actual values from the dial were multiplied by 3.8 to account for the difference in the circumference of the ring. Several determinations were made on each sample within a 2-min period, and these were averaged. A determination was considered valid only if there was a clean separation of the sputum from the entire circumference of the ring.

Contact angles. The θ of all sputum samples were also measured on acid-washed, ethanol-dried glass slides (θglass). A random subset of samples was chosen for measurement on tetrafluoroethylene (TFE, or Teflon) as well. These same sputum samples had surface tension calculated with the use of the Neumann equation from their advancing θ on TFE, a low-energy solid with a surface tension of 18 dyn/cm (13, 14). In both instances, analysis was done with a sample volume of 30 µl in 100% relative humidity by a modification of a previously described image analysis technique (5, 19).

The θ of a series of liquids with known surface tension were measured on glass by the same method to generate a best-regression fit of θ on glass against known surface tension. The liquids were chosen because their surface tensions spanned a range of 21.97 to 63.4 dyn/cm (ethanol to glycerol, respectively). These measurements were made in the same chamber as the sputum samples but without additional humidification. The temperature of the chamber was 25 ± 1°C.

MCTR. MCTR was measured by using the frog palate technique (16, 17). Palates isolated from frogs (Rana pipiens), spontaneously depleted of native mucus but still cilioactive, were placed in a Plexiglas chamber controlled for temperature (25°C) and 100% relative humidity. Samples ~2 µl in volume were placed on the palate, and the trailing edge velocity was computed from the transit time over a 5-mm path at the midline of the palate. On the average, three to eight measurements were taken for each sample to calculate a mean velocity. To take into account the variability of transport rates on the palates, the values were standardized by expressing them as the ratio of the mean sample velocity over the mean velocity of native frog mucus before and after the human sample on the same palate. This normalized ciliary transportability rate is presented as MCTR.

CTR. CTR of the samples was measured in a simulated cough machine with constriction (2). The cough machine is a Plexiglas model tractua connected to an 8-liter tank containing air pressurized to 6 pounds/inch² (psi). A solenoid valve controls air release through a flow-constrictive element used to mimic the airflow pattern of a natural cough. A sinusoidal constriction (length 7.7 cm, height 8 mm) is used to decrease the airway diameter, while minimizing the turbulence of the system with a small airway model. The peak lobar air velocity is ~120 m/s, with the 4-mm gap at a flow rate of 11 liters/s. A sample of ~30 µl is spread in a thin line of ~0.5-mm depth across the base of the tractua. The distance traveled by the
sputum under the effect of the airflow, measured in millimeters, is presented as CTR.

Statistical analysis and graphing calculations. The graphs for Eq. 4 were produced with the use of either Maple V5.3, Waterloo Maple Software, or Differential Systems 3.0, Drexel University (1991) on an Apple Power PC 7100. The Neumann equation was simplified, and computations were done using xFunctions 2.2, written by David Eck with support from National Science Foundation Grant USE-905158313 (13, 14). Statistical analysis was performed using the StatView 4.1 statistics package (Abacus Concepts, Berkeley, CA). Results of the analyses are reported as means ± SD. Equality of means was tested by the analysis of variance (ANOVA), and association between characteristics was tested by regression analyses. In all cases, \( P \leq 0.05 \) was considered significant.

RESULTS

Surface tension values measured by the ring method are reported for all CF and CB samples, and surface tensions measured by the contact angle method are reported for a subset of these in Table 1. There is a significant correlation between values determined by the two methods (Fig. 2, \( R^2 = 0.368, P = 0.03 \)), although there is a difference between surface tension measurements by the two methods (\( P = 0.03 \)), with the values by contact-angle method usually being higher when \( \gamma < 90 \) dyn/cm. We also report in Table 1 the measured contact angles on glass that are used to calculate \( W_{ad-glass} \) by Eq. 3, as well as the contact angles measured on TFE that were used to calculate \( \gamma \)-contact angle by Eq. 4. \( W_{ad} \) was calculated from Eq. 3 using \( \gamma \) calculated by either the ring method or the contact angle method. So that both calculations of \( W_{ad} \) described the same interface (sputum-glass), the contact angle on glass was chosen to represent \( \theta \).

In Fig. 3, the mean values ± SD of \( \theta_{glass} \) and \( \gamma_{ring} \) of CF and CB sputum were superimposed onto a regression curve made from the contact angles measured on glass of a series of liquids plotted against their known surface tension (8). This was done to demonstrate the close relationship to the tail of the regression curve that \( \gamma_{ring} \) for these sputum samples have.

The MCTR and CTR of samples of sputum were measured and compared with \( W_{ad} \) on glass to describe the relationship between the surface properties and the ability to clear these secretions from the airway. A significant inverse correlation between \( W_{ad} \) and CTR (\( R^2 = 0.181, P = 0.004 \)) is shown in Fig. 4. However, there was no correlation between \( W_{ad} \) and MCTR, as seen in Fig. 5.

Table 1. Surface tension and contact angle measurements made by ring and contact-angle methods with calculated \( W_{ad-glass} \) for CF and CB sputum

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ring method ( \gamma ), dyn/cm</th>
<th>Contact angle ( \theta )</th>
<th>( \theta_{glass} )</th>
<th>( \gamma_{ring} )</th>
<th>( W_{ad-glass} ) ( \gamma )-Ring</th>
<th>( W_{ad-glass} ) ( \gamma )-Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>81.1 ± 14.4</td>
<td>92.4 ± 8.1</td>
<td>45.5 ± 10.8</td>
<td>128.3 ± 9.2</td>
<td>137.1 ± 28.2</td>
<td>162.3 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>n 18</td>
<td>7</td>
<td>18</td>
<td>7</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>CB</td>
<td>72.1 ± 11.1</td>
<td>84.8 ± 5.8</td>
<td>31.9 ± 13.6</td>
<td>119.8 ± 5.8</td>
<td>128.3 ± 22.87</td>
<td>152.5 ± 17.6</td>
</tr>
<tr>
<td></td>
<td>n 30</td>
<td>6</td>
<td>30</td>
<td>6</td>
<td>30</td>
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</tr>
</tbody>
</table>

Values are means ± SD. \( \gamma \), Surface tension; \( \theta \), contact angle; \( W_{ad} \), work of adhesion; CF, cystic fibrosis; CB, chronic bronchitis; TFE, tetrafluoroethylene (Teflon); n, no. of experiments. Although the 2 methods have a correlation, there is a statistical difference between \( \gamma \) by ring and \( \gamma \) by \( \theta \); \( P = 0.03 \).

Fig. 2. Surface tension (\( \gamma \)) of sputum measured by its \( \theta \) on TFE (or Teflon) plotted against \( \gamma \) measured by ring method. CF, cystic fibrosis; CB, chronic bronchitis. There is a significant correlation between \( \gamma \) measured by 2 methods; \( R^2 = 0.368, P = 0.03 \). Shaded line represents line of identity.

Fig. 3. Comparison of \( \theta \) on glass (\( \theta_{glass} \)) compared with \( \gamma \) (dyn/cm) for series of liquids with known surface tension. Mean ± SD of \( \gamma \) by ring method and \( \theta_{glass} \) of CF and CB sputum are superimposed on this curve displaying their relative positions. Dotted portion of line represents extrapolation of curve past measured known liquids.
DISCUSSION

This study was undertaken to develop a simple, reproducible method for measuring the surface tension of small samples of mucus or sputum. The method reported here is a modification of the ring technique described by Puchelle et al. (15). Although Puchelle’s du Noüy ring method requires a sample size of 250 µl, we have been able to accurately measure \( \gamma \) in samples of sputum as small as 30 µl by using this smaller ring. The values for \( W_{ad} \) for CF and CB sputum, shown in Table 1, are very similar to those reported by Girodet al. (6, 7).

In the report of Puchelle et al., \( \gamma \) was measured for CF sputum and known liquids with the ring, and a decrease in measured surface tension was demonstrated after adding sodium dodecyl sulfate, a surface tension-lowering agent, to the sputum. We have shown that the small ring accurately and reproducibly can measure \( \gamma \) and that these values are consistent with surface tension measurements calculated from the contact angle measured on TFE. The ring method appears to give accurate surface tension measurements of sputum in that the two methods arrive at values in the same range. However, the miniaturized ring technique gives sputum surface tension values that are usually lower than those calculated from contact angles, except, it appears, when there are much higher surface tensions.

This may in part be due to the effect of cohesion which could contribute to the apparent surface tension by the sample stretching before release from the ring. Precision of measurements will remain an issue because of the heterogeneity of the samples.

Mucus or sputum is a non-Newtonian liquid, so that with spreading on a surface, some energy may be stored as the elastic modulus. Therefore the contact angle may not truly represent the change in energy that occurred to increase the surface as the mucus spread to form the interface. When the Young relationship is used to describe mucus surface properties, the liquidlike behavior of mucus is assumed. This can introduce some error; specifically, if a sample of mucus does not spread completely, surface tension will tend to be underestimated, but relative changes in surface tension will be evaluable. Further, the significant but rather small difference between \( \gamma \) measured by the ring method, which does not depend directly on the liquid behavior of mucus, and \( \gamma \) measured by contact angles, also suggests that any error in assuming liquid behavior is small as well.

An alternative method for evaluating the surface tension of mucus or sputum is to assume that the sputum behaves much like a solid and then to evaluate the spreading of an ideal liquid placed on the mucus layer by using the Neumann equation of state of approach, as used by Pillai and colleagues (14). To understand the limitations that the Neumann equation-of-state approach (Eq. 4) imposes on measuring surface tension, we graphically solved Eq. 4 as shown in Fig. 6 (13)

\[
\cos \theta = \frac{(0.015 \gamma_{sv} - 2.00)(\gamma_{sv} \gamma_{lv})^{0.5} + \gamma_{lv}}{\gamma_{lv}[0.015(\gamma_{sv} \gamma_{lv})^{0.5} - 1]}
\]

In looking at Fig. 6, the values of \( \gamma_{lv} \) (surface tension of the liquid drop) and \( \gamma_{sv} \) (surface tension of the solid surface) that give a constant value for \( \cos \theta \) were

![Graphical solution of Neumann equation (Eq. 4) for \( \gamma_{lv} \) vs. \( \gamma_{sv} \) with \( \cos \theta \) plotted in regions where values are constant. Shaded triangular region between \( \cos \theta = -1 \) and \( \cos \theta = 1 \) is where \( \cos \theta \) remains constant and equation is solvable. Line \( \cos \theta = 1 \) is line of identity, where \( \gamma_{lv} = \gamma_{sv} \). At this point, \( \theta \) has approached 0, and surface is completely wetted. It is impossible to solve for \( \gamma_{sv} > \gamma_{lv} \).]

Fig. 6. Graphical solution of Neumann equation (Eq. 4) for \( \gamma_{lv} \) vs. \( \gamma_{sv} \) with \( \cos \theta \) plotted in regions where values are constant. Shaded triangular region between \( \cos \theta = -1 \) and \( \cos \theta = 1 \) is where \( \cos \theta \) remains constant and equation is solvable. Line \( \cos \theta = 1 \) is line of identity, where \( \gamma_{lv} = \gamma_{sv} \). At this point, \( \theta \) has approached 0, and surface is completely wetted. It is impossible to solve for \( \gamma_{sv} > \gamma_{lv} \).
plotted. It is apparent that a given $\gamma_{lv}$ cannot solve for a $\gamma_{sv}$ that is equal to or greater than itself. This is demonstrated in the figure as the line where $\cos(\theta) = 1$. This makes physical sense in that when $\cos(\theta)$ approaches 1, the contact angle approaches 0, and the liquid has completely wetted the solid. The range of valuable solid-surface tensions can be broadened by use of the complementary angle when evaluating surfaces other than very-low-surface energy solids, but to use this method to measure the surface tension of a “solid”, the liquid chosen must have a surface tension above the possible range suspected for the solid. If we use the equation differently, using a very low-energy surface such as TFE ($\gamma_{sv} = 18$ dyn/cm) as the known, and solve for an unknown $\gamma_{lv}$ in the graphic solution there are valid solutions in an approximate range of $\gamma_{lv}$ from 18 to 110 dyn/cm (Fig. 6). A $\gamma_{sv}$ of 80 dyn/cm or greater, can only be solved outside the triangular region on the graph described by the plots of $\cos(\theta) = -1$ and $\cos(\theta) = 1$, where the equation makes sense. This demonstrates that treating mucus as the liquid phase and TFE as the solid phase allows us the widest range in which to evaluate the surface tension of mucus, and this is the only valid use of the state-of-approach equation in this situation.

After mathematical verification that the Neumann state-of-approach equation could solve for higher surface tensions when a true low-energy solid was used to calculate $\gamma$, we showed that this method could be used to calculate the surface tension of mucus as well. Using this method is most consistent with the basic assumption in the Neumann equation that the solid is a low energy, homogeneous, nondeformable surface.

We demonstrated that the relationship of contact angle to surface tension holds on glass surfaces, as used by Puchelle and colleagues (15) as well. However, because acid-washed, ethanol-dried glass slides are wettable and are not low energy solids, they cannot be used in the same way as TFE to derive surface tension. We used a series of liquids with known surface tensions to demonstrate the association between contact angle on glass of those liquids with their known surface tensions. The close relationship that our values for sputum had to the regression curve for this series of pure liquids further demonstrates that, for purposes of surface measurements, it is safe to assume that sputum behaves much like a Newtonian liquid. If the contact angle is measured on this glass surface, it is possible to use Fig. 3 to extrapolate surface tension.

An attempt was made to compare the ring method directly to the technique described by Pillai et al. (14). When there was a large difference in the values noted between the two methods in a pilot study (not reported here), the mathematical assumptions of the Neumann state-of-approach equation (13) were reviewed. It was noted that, when performing measurements as described by Pillai et al., if the surface energy of the “solid” (mucus) is equal to or greater than the test liquid (glycerol), the equations solve to an invalid answer. The surface tension for CF sputum was determined by the ring method to be $81.1 \pm 3.36$ dyn/cm. Therefore, when using glycerol with a $\gamma$ of 63.4 dyn/cm, it is not possible to measure sputum surface tension. Furthermore, this technique, although using advancing contact angles to minimize the effect of heterogeneity and deformability of the mucus surface, makes the assumption that glycerol is chemically inert in regards to the mucus. Glycerol, however, is miscible with water and actually absorbs water from the air (3). We attempted to reproduce this technique, using mercury as a nonmiscible liquid with surface energy significantly higher than the possible values for mucus ($\gamma = 485.48$ dyn/cm at $25^\circ$C), but the surface of the mucus readily deformed, invalidating the assumption that the mucus would behave like a solid.

We also demonstrated that the surface properties of mucus affect its transportability. The $W_{ad}$ of sputum inversely correlates with the ability to clear these secretions as assayed by a simulated cough. This correlation has been previously reported by King and colleagues (11) and is logical, as cough must separate the mucus-epithelial interface to propel the mucus forward. The lack of a correlation between $W_{ad}$ and MCTR may be due to a more complex dependency of mucociliary clearance on other rheologic characteristics, especially viscoelasticity (4, 9).

$W_{ad}$ is a characteristic of two substances, each with unique surface tensions, that share an interface. It is the work that is done to overcome the interfacial tension and effect a separation of these two substances. When describing the $W_{ad}$ for respiratory secretions, we must specify the interface that is being evaluated, i.e., mucus-glass, mucus-TFE. The ideal would be to describe the mucus-epithelium interface. Differences in the interface may arise in either component of the system. Just as mucus specimens have different surface characteristics, so do the surfaces we measure them on. Differences in the $W_{ad}$ of a particular interface may better reflect the differences in the surface than the mucus. For example, acid washing renders the surface of slides negatively charged; differences in $W_{ad}$ between specimens calculated from these surfaces may be influenced by covalent forces that do not apply at an epithelial surface. This points out the need to develop further ways of studying mucus as part of a mucus-epithelium system in which the unique characteristics of each component lead to a unique interaction.

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REFERENCES

2. Agarwal, M., M. King, B. K. Rubin, and J. B. Shukla. Mucus transport in a miniaturized simulated cough machine: effect of