Quantifying interfacial substrate interactions via surface energy analyses

Determination of a substrate’s surface energy profile is a facile and inexpensive method to indicate the substrate’s interfacial thermodynamics with another substance (e.g., microorganisms, biomacromolecules, medical devices, etc). The following protocol details a goniometric method to calculate a substrate’s surface energy profile which (1) directly correlates to a substrate’s interfacial Gibbs energy ($\Delta G$) and (2) predicts the interfacial interactions with other substances. We also provide a calculation template using advanced mathematics to expedite surface energy profile determination.
Protocol

Quantifying interfacial substrate interactions via surface energy analyses

T. Brian Cavitt, 1,2,3,* Jasmine G. Carlisle, 1 Rachel A. Brooks, 1 Lauren G. Scott, 1 and Pooja R. Patel 1

1Department of Chemistry and Biochemistry, Lipscomb University, One University Park Drive, Nashville, TN 37217 USA
2Technical contact
3Lead contact
*Correspondence: tbcavitt@lipscomb.edu
https://doi.org/10.1016/j.xpro.2021.100476

SUMMARY

Determination of a substrate’s surface energy profile is a facile and inexpensive method to indicate the substrate’s interfacial thermodynamics with another substance (e.g., microorganisms, biomacromolecules, medical devices, etc). The following protocol details a goniometric method to calculate a substrate’s surface energy profile which (1) directly correlates to a substrate’s interfacial Gibbs energy (ΔG) and (2) predicts the interfacial interactions with other substances. We also provide a calculation template using advanced mathematics to expedite surface energy profile determination. For complete details on the use and execution of this protocol, please refer to Cavitt et al. (2020).

BEFORE YOU BEGIN

Refer to “materials and equipment” for a list of equipment needed for this protocol.

Goniometric station setup

© Timing: 1 h

While there are many goniometric instruments of varying costs that measure both static and dynamic contact angles, most of these instruments are benchtop apparatuses that require a computer interface. To distinguish this protocol, we advocate the use of ubiquitous, inexpensive mounted portable electronic devices equipped with a high-resolution camera and one of many available protractor apps to determine the contact angle of a solvent on a substrate. The availability of the goniometric station described herein without compromising the reliability of the contact angle measurements makes this protocol useful for a much wider and diverse research and professional audience.

1. Mount a portable electronic device to a tripod or similar. (Alternatively, a digital camera with macrophotographic capability may be used.)
2. Equip the portable electronic device with a macro lens clip to ensure that a high-resolution image is captured without enhancement via the zoom function.
3. Ensure that the platform on which the sample will be macrophotographed is:
   a. Secure, stable, and not easily jostled;
   b. Level by using a two-dimensional bubble level or similar;
   c. Placed within 1–2 cm of the macro lens for best photography;
   d. Equipped with a neutral, solid-colored background; and
   e. Backlit with an appropriate light source to illuminate and differentiate the droplets.
Note: The goniometric station should be setup in a room or enclosed space with active filtration to reduce the potential for contact angle variations due to dust or other airborne particulates.

Note: The sample platform’s backlighting works best with a white or red LED light source; such will avoid prematurely evaporating the applied droplet. The red light in reduced lighting has traditionally been used with goniometry; however, modern advances in digital photography and editing allow more flexibility.

The following three substrate preparation steps are essential for ensuring that the substrate is properly conditioned for accurate and precise contact angle measurements.

**Medical device substrate preparation**

© Timing: 1 h–3 days

Due to the diversity of medical devices, the specific preparation of the device is omitted. Each device must be prepared according to manufacturer and/or laboratory specifications. A model silicone substrate was used in this study.

Note: The model silicone substrate was comprised of the following formulation, applied to clean glass slides at a thickness of 100 μm, and cured upon exposure to full-arc ultraviolet (UV) radiation until completely solid.

| Reagent                        | Final concentration | Amount |
|--------------------------------|---------------------|--------|
| 2,2-Dimethoxyphenyl acetophenone | 1 weight percent    | 0.1 g  |
| Trimethylolpropane triacylate   | 10 weight percent   | 1 g    |
| Silmer ACR D4                   | 29.7 weight percent | 2.97 g |
| Silmer ACR Di-400              | 59.3 weight percent | 5.93 g |
| Total                          | 100 weight percent  | 10 g   |

4. Select a planar and uniform portion of the device that will be in contact with biological material (minimum dimensions: 2 cm × 0.5 cm).
5. Clean and sterilize the material according to the manufacturer specifications.
6. If the device is water permeable, allow the device to equilibrate in the dust-free environmentally controlled chamber.
   a. Suggested humidity: 0% or 50%
   b. Suggested temperature: 20°C (approximating room temperature) or 37°C (body temperature) depending on device application
   c. Suggested equilibration time: 2–3 days if water permeable or immediate measurement if water impermeable
7. Remove device from the environmentally controlled chamber, and perform contact angle measurements immediately.

**Plated bacterial substrate preparation**

© Timing: 1 – 3 days

Bacteria must be plated according to specific methodology, much of which is published in journals or instructional manuals; therefore, the procedure for plating bacteria are omitted herein (Zimbro and Power, 2009).
8. Prepare plated biologic sample according to accepted/published methodology (Zimbro and Power, 2009).

Note: Differing bacteria require differing agar plates based on the nutrition required. Luria Broth, Prepoured Agar Plates or Anaerobe Blood Agar, Prepared Media Plates are generally used for many bacteria.

9. Select a planar and uniform portion of the plated sample (minimum dimensions: 2 cm × 0.5 cm).

10. Using a cutting tool, cut out and remove an adequately sized portion for analysis (minimum dimensions: 2 cm × 0.5 cm).

11. Perform contact angle measurements immediately.

Biomacromolecular substrate preparation

Timing: 1 – 3 days

Generally, any isolated and purified biomacromolecule (e.g., polymeric carbohydrate, high molecular weight lipid, protein, enzyme, or nucleic acid) may be used as the substrate of which the surface energy could be determined. The adsorption of every biomacromolecule varies to some degree; appropriate literature procedures for the specific biomacromolecule’s adsorption should be followed accordingly.

△ CRITICAL: The biomacromolecule should uniformly and thoroughly coat the surface of the glass slide.

12. Thoroughly scrub a brand-new glass slide with acetone-soaked non-abrasive wipe to remove any protective chemicals.

13. If adsorbing the biomacromolecular sample to glass, follow the procedure described in the literature or by the supplier, and then skip to step 16.

14. If adsorbing the biomacromolecular sample to a substrate tailored to bind with the aforementioned, follow the procedure described in the literature or by the supplier, and then skip to step 16.

15. If adsorbing the biomacromolecular sample to a nonpolar substrate, follow the procedure described immediately below adapted from Wasserman et al., 1989.
   a. Prepare a solution of 0.387 g (0.394 mL) octadecyltrichlorosilane (OTS) in anhydrous toluene to produce 1.00 L of total solution.
   b. A weighted 100 mL beaker was placed in a 400 mL beaker containing the OTS solution (Figure 1).
   c. Glass slides were placed vertically into the interstitial region containing enough of the OTS solution to completely submerge the slides and allowed to stand covered for 45 min.
   d. The OTS-coated glass slides were then removed from solution and rinsed well with anhydrous toluene.
   e. After sonication in toluene for 6 min, the slides were again rinsed with anhydrous toluene.
   f. Allow to dry in a well-ventilated area.
   g. To adsorb the biomacromolecular sample to the OTS-coated substrate, follow the procedure described in the literature (e.g., Wasserman et al., 1989) or by the supplier.

Note: The following biomacromolecular samples (i.e., insoluble collagen from bovine Achilles tendon and bovine collagen solution) were adsorbed to the OTS-coated glass slide to illustrate that both soluble and insoluble proteins may be used effectively. Both samples were used at concentrations of 100 μg/mL with the insoluble collagen suspended in a 5% glucose
solution at pH=2.7 (adjusted with concentrated hydrochloric acid and dilute sodium hydroxide solution) and the collagen solution dissolved in a 1× phosphate-buffered saline (PBS) solution.

Note: In steps 13, 14, and 15g, the actual procedure for preparing the biomacromolecular samples is not detailed because most biomacromolecular samples require a unique procedure to coat a sample. Therefore, the procedure was purposefully written generically.

16. Allow the glass slide coated with the biomacromolecular sample to equilibrate in a humidity and temperature controlled environment.
   a. Suggested humidity: 50%
   b. Suggested temperature: 37°C (body temperature)
   c. Suggested equilibration time: 1–2 days
17. Remove the biomacromolecular sample from the environmentally controlled chamber, and perform contact angle measurements immediately.

Note: In step 16, thermal and humidity conditions are suggested for equilibration; however, actual equilibration conditions may vary depending on the biomacromolecular sample.

△ CRITICAL: All samples should be equilibrated identically to ensure that subsequent contact angle measurements are consistent across all samples. Temperature and humidity can significantly affect contact angle measurements and cause surface energy discrepancies, especially when comparing differing substrates’ surface energies.
## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| *Escherichia coli* K12, Living, Bacteriophage Host | Carolina Biological Supply Company | Catalog #: 124500 |
| *Pseudomonas aeruginosa*, MicroKwik Culture®, Vial | Carolina Biological Supply Company | Catalog #: 155250A |
| *Staphylococcus aureus* (coagulase positive), MicroKwik Culture®, Pathogen, Vial | Carolina Biological Supply Company | Catalog #: 155554A |
| *Streptococcus pneumoniae*, MicroKwik Culture®, Pathogen, Vial | Carolina Biological Supply Company | Catalog #: 155420A |
| *Salmonella typhimurium*, MicroKwik Culture®, Pathogen, Vial | Carolina Biological Supply Company | Catalog #: 155351A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Collagen from bovine Achilles tendon | Sigma-Aldrich | Catalog #: C9879 |
| Bovine collagen solution | Sigma-Aldrich | Catalog #: 804614 |
| 1-Bromonaphthalene* | Sigma-Aldrich | Catalog #: B73104 |
| Formamide* | Sigma-Aldrich | Catalog #: F7503 |
| Luria Broth, Prepoured Agar Plates | Carolina Biological Supply Company | Catalog #: 216600 |
| Anaerobe Blood Agar, Prepared Media Plates, 100 × 15 mm, Pack of 10 | Carolina Biological Supply Company | Catalog #: 821192 |
| Sodium hydroxide solution, 0.1 M | Supelco | Catalog #: 1091411000 |
| D- (+)-Glucose solution, 100 g/L in water | Sigma-Aldrich | Catalog #: G8644 |
| PBS (Phosphate-buffered saline), 1×, sterile | Alfa Aesar | Catalog #: J61196AP |
| Hydrochloric acid (step 15 g) | Sigma-Aldrich | Catalog #: H1758 |
| Octadecyltrichlorosilane | Sigma-Aldrich | Catalog #: 104817 |
| Toluen, anhydrous | Sigma-Aldrich | Catalog #: 244511 |
| Silmer ACR Di-400 | Siltech Corporation | N/A |
| Silmer ACR D4 | Siltech Corporation | N/A |
| Trimethylolpropane triacrylate | Sigma Aldrich | Catalog #: 246808 |
| 2,2-Dimethoxy-2-phenylacetophenone | Sigma-Aldrich | Catalog #: 196118-50G |
| **Other** | | |
| Carolina® Microscope Slide, Glass, Standard, 25 × 75 mm, 0.8–1.00 mm, Box of 36 | Carolina Biological Supply Company | Catalog #: 631920 |
| Apple iPad Mini, Generation 2* | Apple | Model #: A1489 |
| Longay 3 in 1 Universal Clip+Fish Eye+Wide Angle+Macro Lens for iPhone for Samsung & Smart Phone Tablet (Black) * | Amazon | N/A |
| ThermoPro TP50 Digital Hygrometer Indoor Thermometer Room Thermometer and Humidity Gauge with Temperature Humidity Monitor* | Amazon | N/A |
| Hamilton™ 701 N Microliter Syringes* | Fisher Scientific | Catalog #: 14-824 |
| 6x Bubble Spirit Level, 32x7 mm Circular Level Bubble for RV, Travel Trailer, Tripod, Phonograph, Turntable* | Amazon | N/A |
| (2021 VERSION) LENCENT Book Light, (70 h) Rechargeable 7 LED Reading Light with 3 Brightness × 3 Color, Eye Protection Clip Light, Bed Lamp For Kids&Bookworms (Warm/White/Mixed) * | Amazon | N/A |
| Thermo Scientific™ Forma™ Environmental Chamber Model 3911, 311.5 L, Stainless Steel | Fisher Scientific | Catalog #: 13-987-065, GS07F161BA |

*Starred Reagent or Resources in the Key Resources Table are Critical Reagents/Resources.

---

## MATERIALS AND EQUIPMENT

Several pieces of equipment must be available including:

- A digital photographic device, device mount, and tripod;
  - Device could be a portable electronic device or digital camera.
Device needs to be equipped for macrophotography. If a portable electronic device is used, the device will require a macro lens attachment/clip for macrophotography.

**Note:** A second generation Apple iPad Mini was the digital photographic device used in this study.

**Note:** A relatively inexpensive macro lens attachment/clip is available from a number of online retailers and tends to be uniquely designed for each device.

- A dust-free environmentally controlled chamber capable of maintaining constant temperature and humidity.

  **Note:** If a custom chamber is built, an inexpensive digital hygrometer/thermometer combination can be purchased from a number of online retailers.

- Microliter syringes (10 µL capacity).
- A two-dimensional bubble level available from a number of online retailers.
- An LED light source (red or white) to illuminate the applied droplets.

  **Note:** The LED light source should not heat the droplets as an incandescent bulb would. The LED light source should avoid prematurely evaporating the applied droplet. The red light in reduced lighting has traditionally been used with goniometry; however, modern advances in digital photography and editing allow more flexibility.

A suggested contact angle (e.g., goniometric) station is described in before you begin and shown in Figure 2.

**STEP-BY-STEP METHOD DETAILS**

**Contact angle measurements**

© Timing: 1 – 2 h

The following sessile drop method is a classic goniometric experiment designed to determine the contact angle of a liquid when applied to a substrate. The contact angle is the interior angle formed at the air-liquid-substrate interface. Static measurements of the contact angles produced via two or three fully characterized liquids yields the surface energy of the substrate which can then be related to Gibbs energy (i.e., the spontaneity of interfacial interaction).

**Note:** Ideally, the fully characterized liquids (i.e., solvents) should be nonspreading solvents.

**Note:** The spreading nature of the solvent should be investigated prior to application. Spreading solvents, where wetting occurs quickly and/or the contact angle is very small, should be avoided if possible; however, if a spreading solvent is selected, the suggested equilibration time should allow for the contact angle measurement though the reliability may be reduced.

1. Place a 2 µL droplet on the substrate.
2. Allow the droplet to equilibrate for 10–15 seconds to avoid evaporation.
3. Following equilibration, photograph the drop using a mounted second generation iPad Mini equipped with a macro lens. DO NOT ZOOM.
4. Obtain left and right contact angle measurements via a protractor app, e.g., Photo Protractor (Figure 3).
5. A minimum sample size of \((N \geq 6)\) is necessary for each liquid used.
6. Obtain statistical averages for each contact angle measurement \((N \geq 6)\) by averaging all measurements.
7. Repeat steps 1–6 for the other two liquids.
8. Input contact angle data into the calculation template (Data S1).

Note: The three recommended liquids for contact angles on biological materials include bromonaphthalene, formamide, and deionized water based on their 1) relatively high surface energies (Table 1), 2) low volatility, and 3) potential for diverse intermolecular interactions with the substrates.

EXPECTED OUTCOMES

The above experimentation upon a model silicone substrate yields contact angle measurements that immediately illustrate the degree of interaction with liquids of differing polarities as shown in Table 2.

Small contact angles indicate favorable, adhesive interactions with the liquid while large contact angles indicate repulsive interactions with the liquid. A static contact angle of 90° illustrates equivalent adhesive and repulsive interactions between the liquid and substrate. The contact angles shown in Table 2 for a silicone substrate show favorable interactions with a nonpolar liquid.
(e.g., bromonaphthalene) and increasingly repulsive interactions as the liquid’s polarity is increased. Such behavior in this case is consistent with the nonpolar nature of silicone substrates. However, to finalize the experimentation, quantification of the contact angles and subsequent statistical analysis must occur as described.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Table 3 shows the statistical analysis for the measured contact angles of three preferential solvents upon a model silicone substrate. The standard deviation is within 8.2% for bromonaphthalene and approximates 3% for both formamide and water indicating that the individual contact angle measurements are reasonably accurate. Furthermore, the standard error of mean (SEM) – a more reliable statistical validation – is very favorable (0.65° ≤ SEM ≤ 1.39°). The SEM demonstrates that there is a 95% confidence that mean of the contact angle measurements will be within 1.4° of the standard deviation of the sample set having relatively large contact angles. Comparing contact angle measurements using differing substrates and two different static contact angle goniometers with average SEMs of 2.42° and 1.08°, the reliability of contact angle measurements acquired via this protocol indicates accurate contact angle determination (Boo et al., 2018; Van Der Merwe et al., 2018).

For brevity, the underlying mathematics for calculating the surface energy profiles of the substrates are explained elsewhere (Cavitt et al., 2020). Herein, we exemplify the data acquired and interpretation thereof to obtain the surface energy profiles from Equations 1–3 (van Oss et al., 1987; van Oss et al., 1988):
The goniometric surface energy is designated as $\gamma_s$ which is the sum of the nonpolar component ($\gamma_{LW}$) and the polar component ($\gamma_{AB}$). The polar component is the geometric mean of the acid ($\gamma_s^+$) and the base ($\gamma_s^-$) components. Equation 1 illustrates the individual interactions of the solvent with the substrate as the geometric mean thereof. In general, as the total surface energy of a substrate increases, 1) the polarity of the substrate increases, and 2) the cohesive internal interactions become overwhelmed by the adhesive interfacial interactions.

In order to simplify the quantification and statistical analysis, a calculation template (Data S1) has been developed to process the experimentally determined contact angles from the three preferential solvents for biologically relevant substrates provided in Table 1. By inserting up to 25 contact angle measurements for each solvent (e.g., Table 2), the surface energy profile (e.g., $\gamma_s^+$, $\gamma_{LW}$, $\gamma_{AB}$, $\gamma_s^+$, and $\gamma_s^-$) may be determined for the sample, a silicone substrate in this case (Table 4).

The template (Data S1), based on analysis via bromonaphthalene, formamide, and water, has three sheets titled: 1) INPUT Highlighted ID & CAs, 2) Calculations, and 3) Surface Energy Results. Sheet 1 is the only sheet requiring data input [i.e., the sample identity and measured contact angles (CAs), both highlighted] (Figure 4).

Upon inputting the data, Sheet 2 performs the calculations required to determine the surface energy of the desired substrate and requires little to no modification (Figure 5). Of important note, Sheet 2 performs calculations only when CA data are input into Sheet 1. If a fully characterized solvent other than bromonaphthalene, formamide, and water is used, the highlighted table in Sheet 2 should be modified accordingly with the relevant data.

$$ (1 + \cos \theta_0)\gamma_s^{tot} = 2 \left( \sqrt{\gamma_{LW}^2 - \gamma_1^LW} + \sqrt{\gamma_1^s \gamma_1^-} + \sqrt{\gamma_s^- \gamma_s^+} \right) $$  \hspace{1cm} (Equation 1)

$$ \gamma_{AB}^+ = 2 \sqrt{\gamma_s^+ \gamma_s^-} $$  \hspace{1cm} (Equation 2)

$$ \gamma_s = \sqrt{\gamma_s^LW + \gamma_{AB}^+} $$  \hspace{1cm} (Equation 3)
To simplify data processing and entry into reports and/or papers, Sheet 3 aggregates the surface energy results and meaningful statistics into two different tables by which the researcher may report the findings for that substrate (Figure 6).

The aforementioned template (Data S1) provides the information shown in Table 4, including statistical analysis, on a single sheet (i.e., Sheet 3) for easy insert into a report or paper. The SEM for both $\gamma_s$ and $\gamma_{1W}$ of the silicone substrate show a 95% confidence within $\pm 0.45$ mN/m ($\pm 1.6\%$) and $\pm 0.69$ mN/m ($\pm 2.4\%$), respectively. Because of the very small numerical values of the polar components ($\gamma_{AB}^s$, $\gamma_+^s$, and $\gamma_{/C0}^s$) for silicones, the SEM is a bit more disperse (i.e., less reliable). For substrates with larger values for the polar components, the SEM is expected to be much more reliable. Regardless, the overall surface energy of a substrate ($\gamma_s$), which is often reported as the singular surface energy in the published literature, as calculated herein is very reliable.

Table 4. Surface energy (mJ/m$^2$) profile and statistics of sample silicone substrate

| Statistic          | Mean $\gamma_s$ | $\gamma_{1W}$ | $\gamma_{AB}^s$ | $\gamma_+^s$ | $\gamma_{/C0}^s$ |
|--------------------|-----------------|----------------|-----------------|--------------|-----------------|
| Mean               | 28.91           | 28.13          | 0.78            | 1.14         | 0.21            |
| Median             | 28.34           | 27.86          | 0.26            | 1.02         | 0.02            |
| Standard deviation | 1.43            | 2.18           | 0.95            | 0.37         | 0.35            |
| Number (N)         | 10              | 10             | 10              | 10           | 10              |
| Standard error of the mean (SEM) | 0.45 | 0.69 | 0.30 | 0.12 | 0.11 |

Table 3. Contact angle (degrees) statistics of sample silicone substrate

| Statistic       | Bromonaphthalene | Formamide | Water     |
|-----------------|------------------|-----------|-----------|
| Mean            | 53.67            | 69.03     | 102.45    |
| Median          | 54.25            | 69.62     | 101.64    |
| Standard deviation | 4.398        | 2.068     | 3.2962    |
| Number (N)      | 10               | 10        | 10        |
| Standard error of the mean (SEM) | 1.391          | 0.6538    | 1.042     |

Note: The interfacial Gibbs energy of a substrate ($\Delta G_{sl}$) interacting with a liquid is given in Equation 4 where $\theta_{sl}$ is the contact angle in radians of the liquid with the substrate and $\gamma_{1mt}$ is the total surface energy of the liquid:

$$\Delta G_{sl} = (1 + \cos \theta_{sl})\gamma_{1mt}$$  \hspace{1cm} (Equation 4)

LIMITATIONS

Static goniometric contact angle measurements are one of several methodologies by which surface energies may be obtained. The platform upon which the contact angles are measured must be level to ensure consistent contact angle measurements. Alternative methodologies sometimes yield discrepant surface energy values due to embedded assumptions. For example, the use of density functional theory (DFT) to determine surface energy profiles requires the use of a carefully determined basis set to underpin the calculations. Furthermore, the literature seems to indicate the necessity of a highly crystalline sample/substrate for successful DFT determination of surface energies (Tran et al., 2016). The older cleaving method, such as those reported by Gilman and also Jaccodine, may also be used to determine surface energies for crystalline and ordered substrates; however, amorphous substrates may yield divergent and inconsistent surface energies (Gilman, 1960; Jaccodine, 1963).

Goniometric measurements are sensitive to temperature and humidity and can vary widely with differing temperatures and humidity; therefore, the environmental conditions must be carefully controlled.
controlled. Dust may also inhibit accurate goniometric measurements; therefore, a dust-free environment must be sustained during measurement. A strength of goniometric measurements resides in the diversity of samples that can be examined including highly crystalline and highly amorphous substrates.

Substrates often interact with an applied liquid disrupting the continuity of the surface (e.g., dissolution, orange peeling, swelling, softening, etc.). If a solvent disrupts the substrate’s surface, there are a plethora of fully characterized liquids from which to choose (Lide, 2009). It is highly recommended that relatively non-volatile, high surface energy solvents are used to maximize the apparent contact angle and simplifying the determination thereof.

TROUBLESHOOTING

Problem 1
Uneven microdroplet and/or inconsistent contact angle measurements (step 1 in “step-by-step method details”).

---

Figure 4. Depiction of Sheet 1 “INPUT Highlighted ID & CAs” illustrating the highlighted regions requiring the input of the sample name and contact angles, CAs (See also Data S1)
Potential solution

An uneven microdroplet is present when the left and right contact angles are not equivalent within reasonable error (±2°).

First, the level of the platform supporting the substrate should be verified in both the x (left to right) and z (front to back) directions. Once level, the tip of the microsyringe should be examined. Regardless of the tip style (i.e., tapered or flat), application of the microdroplet should be such that the tip is parallel to the substrate surface. Upon equilibration, the left and right contact angles should be equivalent within the aforementioned error.

Problem 2

Substrate damage from application of fully characterized liquid (step 1 in “step-by-step method details”)

Potential solution

In some cases, the fully characterized liquid will damage the substrate surface upon application. Contact angles obtained from liquids that damage the substrate should not be reported. Damage could include, but is not limited to, dissolution, etching, blistering, swelling, delamination (Weldon, 2009). The liquid should be exchanged for a differing fully characterized liquid (Lide, 2009). The damaged portion of the surface should not be used again for any contact angle measurements.

Problem 3

Uneven bacterial coverage (step 9 in “before you begin”)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|----|----|----|
| A | B | C | D | E | F | G | H | I | J | K | L |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
| Surface | Angle | Degree | Contact | Angle | Contact | Angle | Monophenol | Formamide | Water | Monophenol | Formamide | Water |
Potential solution
The solution to Problem 3 assumes that Problem 1 and 2 have either been solved or are nonexistent. If the contact angle data are inconsistent for the chosen plated bacterial sample, another sample should be obtained, either via the same plate or a new plate. All contact angle measurements should be recorded for comparison to subsequent data. If the contact angle data are inconsistent for multiple samples, the data for all samples should be aggregated, and the statistics should be obtained (i.e., average, median, standard deviation, number of measurements, and standard error of the mean (SEM)). The larger data set will often mitigate the inconsistency in the contact angle measurements. If the SEM is no larger than $G^2/C1^4$, the contact angle data should yield reasonable surface energies, especially since reported high quality goniometric data have similar reported error (Boo et al., 2018; Van Der Merwe et al., 2018).

Problem 4
Uneven biomacromolecular coating on glass slides (step 15 g in “before you begin”)
Potential solution
Assuming Problems 1 and 2 have been addressed, an uneven biomacromolecular coating of the slide can present as deviant and inconsistent contact angle measurements. In many cases, the potential solution is very similar to that in Problem 3 where measuring the contact angles of multiple samples may provide statistically reliable data.

Another potential solution may be to repeat the adsorption procedure several times to build up the biomacromolecular coating. By doing so, many of the surface defects (i.e., unevenness) may be addressed.

However, some biomacromolecular samples do not easily form uniform coatings and yield statistically unreliable data. In these cases, the use of non-destructive instrumentation such as scanning electron microscopy, x-ray diffractometry, and atomic force microscopy should be employed to determine the surface uniformity. Many of the aforementioned instruments allow for some degree of mapping such that a uniform surface may be found and used. In extreme circumstances where published methodologies to adsorb the biomacromolecule to a glass slide are difficult to reproduce, other experimental methodologies should be explored or developed.

Problem 5
Error in the calculation template (Data S1) (step 8 in “step-by-step method details”)

Potential solution
If the surface energies are very deviant from what might be expected, the solvents on Sheet 2 should be confirmed as those used. If one or more solvents are incorrect, appropriate changes should be made.

Occasionally the calculation template (Data S1) will produce errors reported as #VALUE!, #DIV/0!, or #REF!. In most circumstances, the contact angle data have not been input properly. First, the data should be input anew. If the error persists, the program should be exited and a fresh, unaltered template (Data S1) should be opened. Then the data should be input into the fresh template (Data S1) whereupon the error should be resolved.

RESOURCE AVAILABILITY
Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. T. Brian Cavitt (tbcavitt@lipscomb.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The published article includes all silicone substrate datasets generated or analyzed during this study. Using the aforementioned silicone substrate datasets, the calculation template for surface energy determination is provided as a supplemental Excel file (.xlsx) to this protocol (Data S1). Additionally, the dataset is available from Mendeley Data (Cavitt 2021).

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100476.

ACKNOWLEDGMENTS
Funding for the aforementioned project was gratefully provided by Lipscomb University’s Office of the Provost and the Department of Chemistry and Biochemistry.
AUTHOR CONTRIBUTIONS
Conceptualization, T.B.C.; investigation, J.G.C., R.A.B., L.G.S., and P.R.P.; writing – original draft, T.B.C.; writing – review and editing, T.B.C.; funding acquisition, T.B.C.; and supervision, T.B.C.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
Boo, C., Hong, S., and Elimelech, M. (2018). Relating organic fouling in membrane distillation to intermolecular adhesion forces and interfacial surface. Environ. Sci. Technol. 52, 14198–14207.

Cavitt, B. (2021). Calculating the Five-Component Surface Energy Profile from Contact Angles (Goniometry) (Mendeley Data, V1).

Cavitt, T.B., Carlisle, J.G., Dodds, A.R., Faulkner, R.A., Garfield, T.C., Ghebranious, V.N., Hendley, P.R., Henry, E.B., Holt, C.J., Lowe, J.R., et al. (2020). Thermodynamic surface analyses to inform biofilm resistance. iScience 23, 101702.

Gilman, J.J. (1960). Direct measurements of the surface energies of crystals. J. Appl. Phys. 31, 2208–2218.

Jaccodine, R.J. (1963). Surface energy of germanium and silicon. J. Electrochem. Soc. 110, 524–527.

Lide, D.R. (2009). CRC Handbook of Chemistry and Physics, Ninetieth Edition (Boca Raton, FL: CRC Press), pp. 6–162–6–164.

Tran, R., Xu, Z., Radhakrishnan, B., Winston, D., Sun, W., Persson, K.A., and Ong, S.P. (2016). Surface energies of elemental crystals. Sci. Data 3, 160080.

Van Der Merwe, S.M., Bouropoulos, N., Katsamenis, D.A., Lampou, O.L., and Fatouros, D.G. (2018). Preparation and characterization of large unilamellar vesicles mixed with trimethylchitosan (TMC): the effect of polyelectrolyte concentration. Open Biotechnol. J. 12, 134–139.

van Oss, C.J., Chaudhury, M.K., and Good, R.J. (1987). Monopolar surfaces. Adv. Colloid Interface Sci. 28, 35–64.

van Oss, C.J., Good, R.J., and Chaudhury, M.K. (1988). Additive and nonadditive surface tension components and the interpretation of contact angles. Langmuir 4, 884–891.

Wasserman, S., Tao, Y., and Whitesides, G. (1989). Structure and reactivity of alkylsiloxane monolayers formed by reaction of alkyltrichlorosilanes on silicon substrates. Langmuir 5, 1074–1087.

Weldon, D.G. (2009). Failure Analysis of Paints and Coatings (John Wiley & Sons, Ltd.), pp. 275–346.

M.J. Zimbro, and D.A. Power, eds. (2009). Difco & BBL Manual: Manual of Microbiological Culture Media, Second Edition (Dickinson, and Company).