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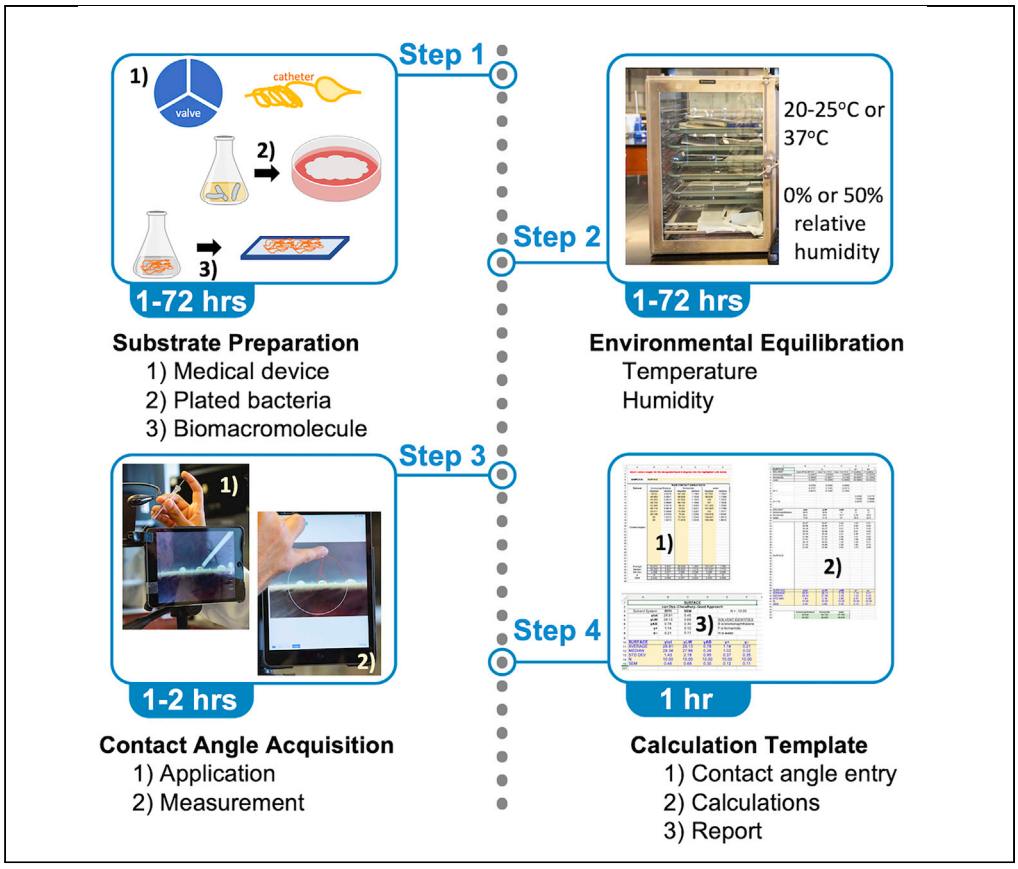
Quantifying interfacial substrate interactions via surface energy analyses



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 (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.

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Highlights

Surface energy (SE) describes interfacial substrate interactions

Every material or biological substrate has a unique, five-component (5K) SE profile

The 5K SE profile may be experimentally determined goniometrically

A calculation template helps determine the 5K SE profile from contact angle data

Cavitt et al., STAR Protocols 2, 100476
June 18, 2021
https://doi.org/10.1016/j.xpro.2021.100476

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Quantifying interfacial substrate interactions via surface energy analyses

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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 macrolens 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 macrolens 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 triacrylate	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).

⚠ CRITICAL: The bacteria should uniformly and thoroughly coat the surface of the plate.

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

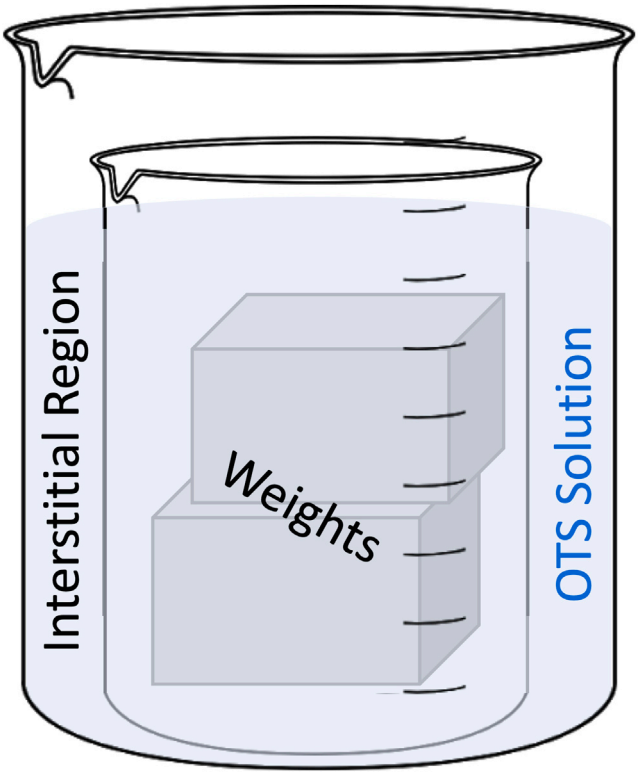


Figure 1. Setup for coating glass slides with OTS solution for adhering a biomacromolecule to a nonpolar substrate

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 #: 155620A
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
Toluene, 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
6× Bubble Spirit Level, 32×7 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
Deposited data		
Calculating the five-component surface energy profile from contact angles (goniometry)*	Cavitt, 2021	https://data.mendeley.com/datasets/y3fxw34g8k/1

*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 macrolens attachment/clip for macrophotography.

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

Note: A relatively inexpensive macrolens 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 macrolens. DO NOT ZOOM.

Figure 2. Contact angle measurement station

Figure 2 labels the components used to obtain the contact angle measurements where (A) is the mounted portable electronic device, (B) is the macrolens clip, (C) is the sample platform, (D) adjustable LED lamp, (E) Bunsen burner to remove humidity when lit, and (F) climate- controlled sample conditioning chamber.

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

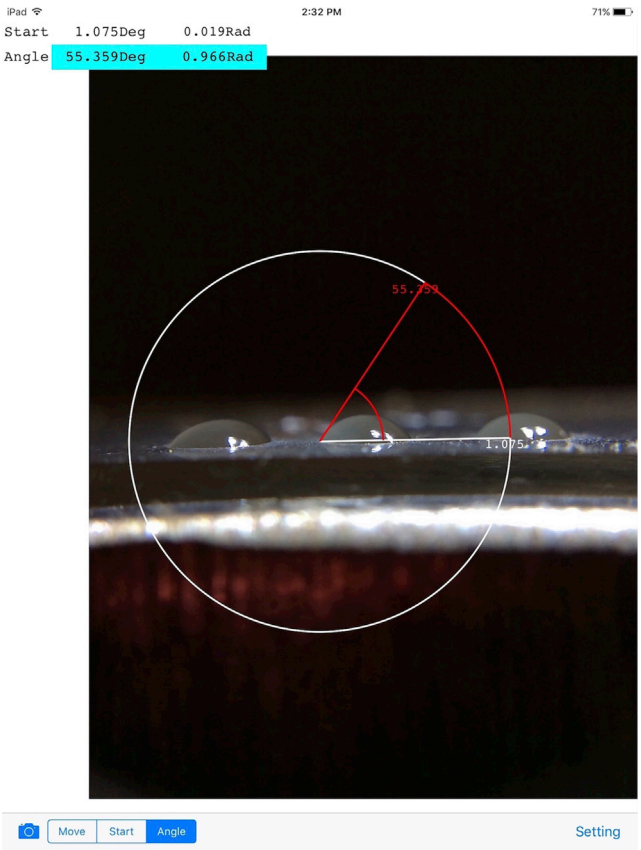


Figure 3. Example contact angles and measurement via a screenshot of the Photo Protractor app

Note: The baseline of the angles often needs to be slightly adjusted to ensure planarity with substrate (i.e., contact angle adjusts to 54.284°).

(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 ($0.65° \leq SEM \leq 1.39°$). 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):

Table 1. Surface energy (mJ/m^2) profile of liquids used (Lide, 2009)

Liquid	γ_l^{LW}	γ_l^+	γ_l^-	γ_l^{AB}	γ_l^{tot}
Bromonaphthalene	44.4	—	—	—	44.4
Formamide	39.5	2.28	39.6	18.7	58.2
Water	21.8	25.5	25.5	52	72.8

$$(1 + \cos\theta_{sl})\gamma_l^{tot} = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+}\right) \quad \text{(Equation 1)}$$

$$\gamma_s^{AB} = 2\sqrt{\gamma_s^+\gamma_s^-} \quad \text{(Equation 2)}$$

$$\gamma_s = \gamma_s^{LW} + \gamma_s^{AB} \quad \text{(Equation 3)}$$

The total goniometric surface energy is designated as γ_s which is the sum of the nonpolar component (γ_s^{LW}) and the polar component (γ_s^{AB}). The polar component is the geometric mean of the acid (γ_s^+) and the base (γ_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., γ_s, γ_s^{LW}, γ_s^{AB}, γ_s^+, and γ_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.

Table 2. Example of raw contact angle data (in degrees) of a silicone substrate measured via a second generation iPad Mini equipped with a macrolens

Sample	Bromonaphthalene	Formamide	Water
1	48.530	65.105	97.763
2	48.992	66.659	99.628
3	49.353	67.853	100.000
4	49.733	68.134	101.000
5	53.395	69.23	101.352
6	55.114	70.02	101.922
7	55.511	70.284	103.000
8	56.108	70.44	104.816
9	60.000	70.733	106.647
10	60.000	71.819	108.345

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

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 γ_s and γ_s^{LW} 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_s^{AB}, \gamma_s^+$, and γ_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 (γ_s), which is often reported as the singular surface energy in the published literature, as calculated herein is very reliable.

Note: The interfacial Gibbs energy of a substrate (ΔG_{sl}) interacting with a liquid is given in Equation 4 where θ_{sl} is the contact angle in radians of the liquid with the substrate and γ_l^{tot} is the total surface energy of the liquid:

$$\Delta G_{sl} = (1 + cos\,\theta_{sl})\gamma_l^{tot} \quad \text{(Equation 4)}$$

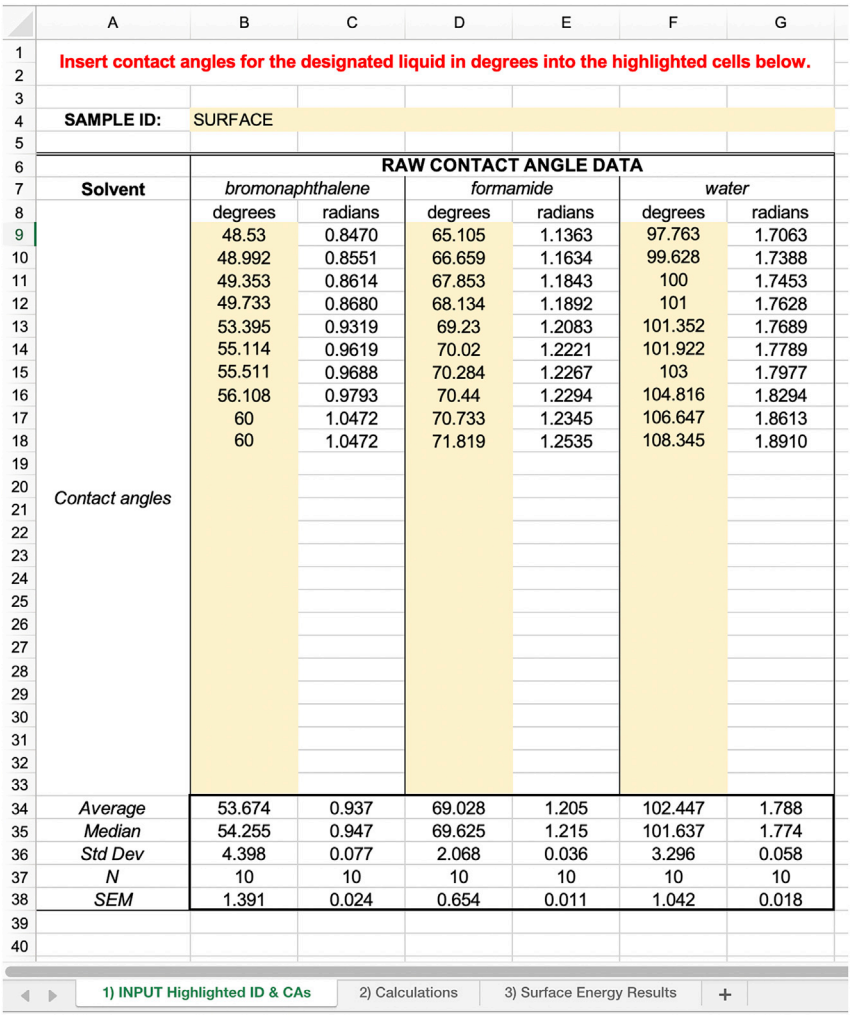
LIMITATIONS

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

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

Statistic	γ_s	γ_s^{LW}	γ_s^{AB}	γ_s^+	γ_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



Insert contact angles for the designated liquid in degrees into the highlighted cells below.

SAMPLE ID: SURFACE

Solvent	RAW CONTACT ANGLE DATA					
	bromonaphthalene		*formamide*		*water*	
	degrees	radians	degrees	radians	degrees	radians
Contact angles	48.53	0.8470	65.105	1.1363	97.763	1.7063
	48.992	0.8551	66.659	1.1634	99.628	1.7388
	49.353	0.8614	67.853	1.1843	100	1.7453
	49.733	0.8680	68.134	1.1892	101	1.7628
	53.395	0.9319	69.23	1.2083	101.352	1.7689
	55.114	0.9619	70.02	1.2221	101.922	1.7789
	55.511	0.9688	70.284	1.2267	103	1.7977
	56.108	0.9793	70.44	1.2294	104.816	1.8294
	60	1.0472	70.733	1.2345	106.647	1.8613
	60	1.0472	71.819	1.2535	108.345	1.8910
Average	53.674	0.937	69.028	1.205	102.447	1.788
Median	54.255	0.947	69.625	1.215	101.637	1.774
Std Dev	4.398	0.077	2.068	0.036	3.296	0.058
N	10	10	10	10	10	10
SEM	1.391	0.024	0.654	0.011	1.042	0.018

1) INPUT Highlighted ID & CAs | 2) Calculations | 3) Surface Energy Results

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)

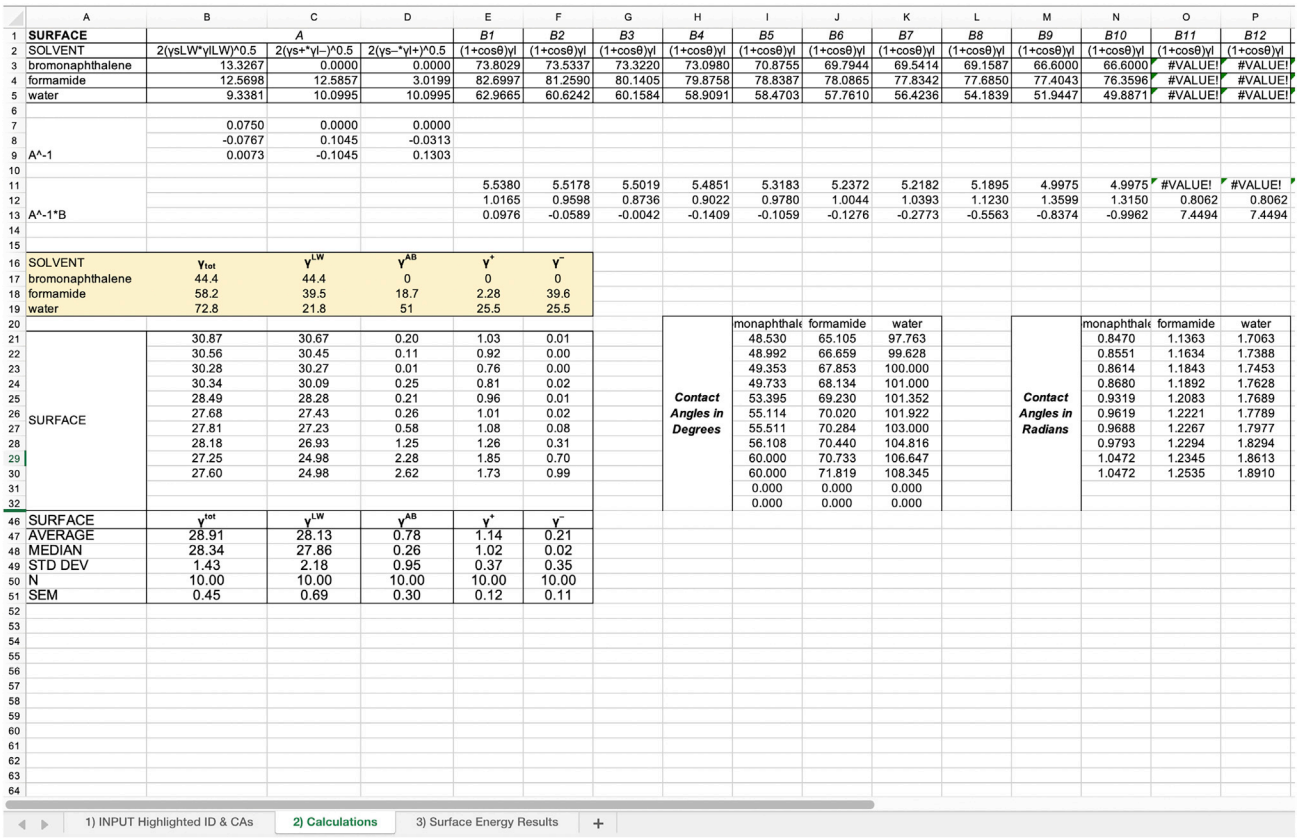
controlled. Dust may also inhibit accurate goniometric measurements; therefore, a dust-free environment must be sustained during measurement. A strength of goniometric measurements resides in the diversity of samples that can be examined including highly crystalline and highly amorphous substrates.

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")



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	SURFACE	A			B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
2	SOLVENT	2(ysLW*ylLW)^0.5	2(ys+*yl–)^0.5	2(ys–*yl+)^0.5	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl	(1+cosθ)yl
3	bromonaphthalene	13.3267	0.0000	0.0000	73.8029	73.5337	73.3220	73.0980	70.8755	69.7944	69.5414	69.1587	66.6000	66.6000	#VALUE!	#VALUE!
4	formamide	12.5698	12.5857	3.0199	82.6997	81.2590	80.1405	79.8758	78.8387	78.0865	77.8342	77.6850	77.4043	76.3596	#VALUE!	#VALUE!
5	water	9.3381	10.0995	10.0995	62.9665	60.6242	60.1584	58.9091	58.4703	57.7610	56.4236	54.1839	51.9447	49.8871	#VALUE!	#VALUE!
6																
7		0.0750	0.0000	0.0000												
8		-0.0767	0.1045	-0.0313												
9	A^-1	0.0073	-0.1045	0.1303												
10																
11					5.5380	5.5178	5.5019	5.4851	5.3183	5.2372	5.2182	5.1895	4.9975	4.9975	#VALUE!	#VALUE!
12					1.0165	0.9598	0.8736	0.9022	0.9780	1.0044	1.0393	1.1230	1.3599	1.3150	0.8062	0.8062
13	A^-1*B				0.0976	-0.0589	-0.0042	-0.1409	-0.1059	-0.1276	-0.2773	-0.5563	-0.8374	-0.9962	7.4494	7.4494
14																
15																
16	SOLVENT	γ_{tot}	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}										
17	bromonaphthalene	44.4	44.4	0	0	0										
18	formamide	58.2	39.5	18.7	2.28	39.6										
19	water	72.8	21.8	51	25.5	25.5										
20									monaphthale	formamide	water			monaphthale	formamide	water
21		30.87	30.67	0.20	1.03	0.01			48.530	65.105	97.763			0.8470	1.1363	1.7063
22		30.56	30.45	0.11	0.92	0.00			48.992	66.659	99.628			0.8551	1.1634	1.7388
23		30.28	30.27	0.01	0.76	0.00			49.353	67.853	100.000			0.8614	1.1843	1.7453
24		30.34	30.09	0.25	0.81	0.02			49.733	68.134	101.000			0.8680	1.1892	1.7628
25		28.49	28.28	0.21	0.96	0.01		Contact	53.395	69.230	101.352		Contact	0.9319	1.2083	1.7689
26	SURFACE	27.68	27.43	0.26	1.01	0.02		Angles in	55.114	70.020	101.922		Angles in	0.9619	1.2221	1.7789
27		27.81	27.23	0.58	1.08	0.08		Degrees	55.511	70.284	103.000		Radians	0.9688	1.2267	1.7977
28		28.18	26.93	1.25	1.26	0.31			56.108	70.440	104.816			0.9793	1.2294	1.8294
29		27.25	24.98	2.28	1.85	0.70			60.000	70.733	106.647			1.0472	1.2345	1.8613
30		27.60	24.98	2.62	1.73	0.99			60.000	71.819	108.345			1.0472	1.2535	1.8910
31									0.000	0.000	0.000					
32									0.000	0.000	0.000					
46	SURFACE	γ^{tot}	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}										
47	AVERAGE	28.91	28.13	0.78	1.14	0.21										
48	MEDIAN	28.34	27.86	0.26	1.02	0.02										
49	STD DEV	1.43	2.18	0.95	0.37	0.35										
50	N	10.00	10.00	10.00	10.00	10.00										
51	SEM	0.45	0.69	0.30	0.12	0.11										
52																
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64																

Figure 5. Depiction of Sheet 2 "Calculations" illustrating the breadth of calculations performed to obtain the surface energy profile boxed at bottom left (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

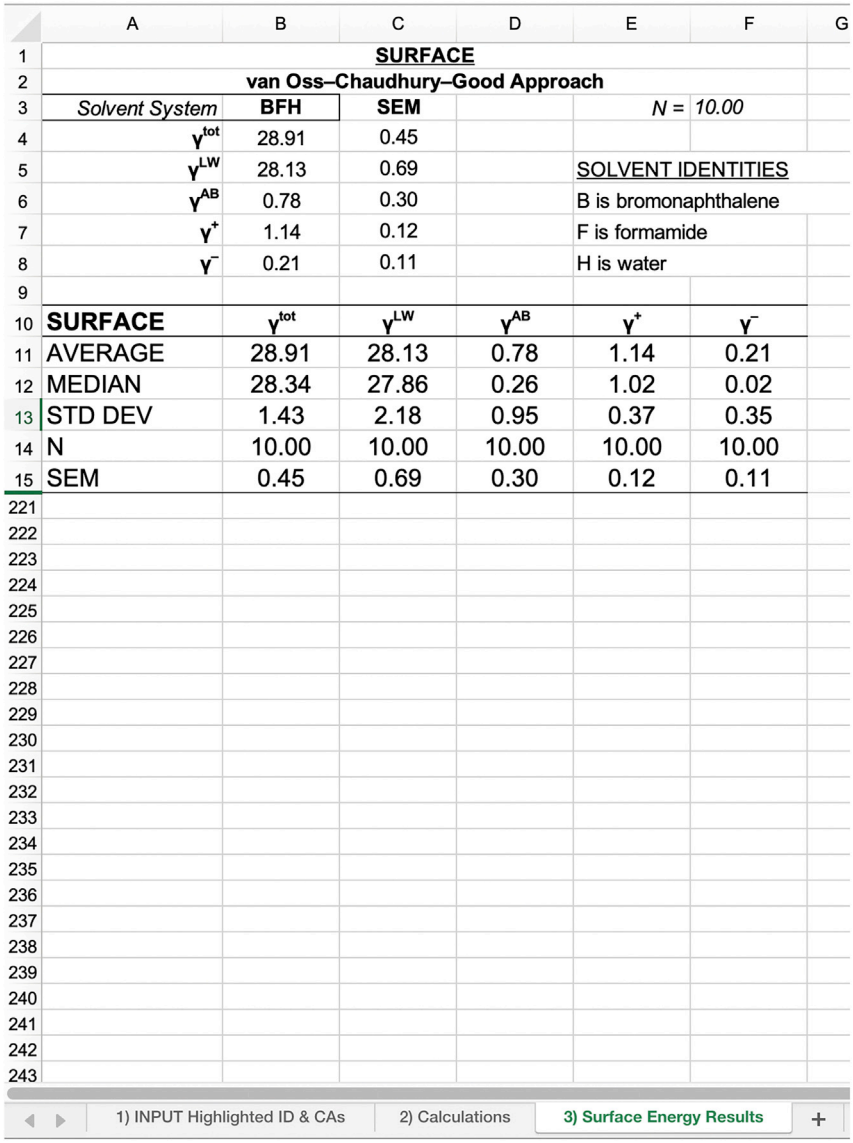
Substrate damage from application of fully characterized liquid (step 1 in "step-by-step method details")

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")



	A	B	C	D	E	F	G
1			SURFACE				
2			van Oss–Chaudhury–Good Approach				
3	*Solvent System*	**BFH**	**SEM**		*N =*	*10.00*	
4	γ^{tot}	28.91	0.45				
5	γ^{LW}	28.13	0.69		SOLVENT IDENTITIES		
6	γ^{AB}	0.78	0.30		B is bromonaphthalene		
7	γ^{+}	1.14	0.12		F is formamide		
8	γ^{-}	0.21	0.11		H is water		
9							
10	**SURFACE**	γ^{tot}	γ^{LW}	γ^{AB}	γ^{+}	γ^{-}	
11	AVERAGE	28.91	28.13	0.78	1.14	0.21	
12	MEDIAN	28.34	27.86	0.26	1.02	0.02	
13	STD DEV	1.43	2.18	0.95	0.37	0.35	
14	N	10.00	10.00	10.00	10.00	10.00	
15	SEM	0.45	0.69	0.30	0.12	0.11	
221–243							

1) INPUT Highlighted ID & CAs | 2) Calculations | 3) Surface Energy Results | +

Figure 6. Depiction of Sheet 3 "Surface Energy Results" providing surface energy data in tabular form for facile reporting (See also Data S1)

Potential solution

The solution to Problem 3 assumes that Problem 1 and 2 have either been solved or are nonextant. 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 $\pm$2–3°, 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.

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