TECHNICAL APPLICATION NOTE

Contact Angle Measurement in Biotechnology

Wettability, surface free energy and measurement data for protein-, cell- and fluid-contacting materials

Contact angle goniometry is the cheapest, fastest and most sensitive probe of the outermost 1–10 nm of a biomaterial — the layer a protein, a cell or a droplet actually sees. This note covers the underlying physics, the logic for choosing a method, a protocol that produces comparable numbers between laboratories, and four datasets that show how the measurement behaves in real biotechnical work.

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CONTENTS

1 Summary for decision-makers
2 Why wettability governs biotechnical interfaces
3 Physical basis: from Young’s equation to surface free energy
4 Measurement methods and how to choose one
5 Instrumentation, calibration and uncertainty
6 A protocol that gives comparable numbers
7 Measurement data
8 Three application case studies
9 Pitfalls and troubleshooting
10 Specifications, validation and regulatory context
11 Reporting checklist
12 References and appendices

Data provenance: Sections 7.2 to 7.4 reproduce values published in the peer-reviewed literature, cited in place. Section 7.6 is a synthetic dataset built to demonstrate a capability study and is labelled as such. Section 7.5 uses worked-example inputs to demonstrate the calculation chain.

1 Summary for decision-makers

A contact angle is a force balance written as an angle. For a drop of liquid resting on a solid in air it reads $\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta$. Everything useful — and every trap — follows from that equation, because the angle depends on all three phases at once, not on the solid alone.

In biotechnology the measurement answers a small number of commercially important questions: will cells attach, will protein adsorb, will a channel fill by capillary action, will a coating stay bonded, will a lens stay wet, will a membrane foul. Each of those is decided in the first nanometres of the surface, and each correlates with wettability strongly enough to be used as a release test — provided the measurement is specified properly.

Six conclusions that shape a specification

- **Report a method, not a number.** Sessile-drop static, advancing, receding and captive-bubble angles on the same surface can differ by 30° or more. A specification without a method statement is not a specification.
- **The hysteresis window carries the biology.** Advancing and receding angles bracket the states a surface actually visits when it is wetted, dried, sheared or adsorbed onto. A single static angle sits somewhere inside that window arbitrarily.
- **Hydrated materials must be measured wet.** On silicone hydrogels, the dry and immersed readings differ by 30°; on any hydrogel or membrane, the sessile drop in air measures a state the product never sees.
- **Roughness and chemistry are confounded.** In the plasma series in Section 7.2 the angle is non-monotonic with dose because etching competes with oxidation. Contact angle alone cannot separate the two; pair it with roughness or XPS at validation.
- **The measurement uncertainty is roughly ±2–3°.** A process window narrower than about 6° cannot be controlled with a single-drop test. Section 5.5 gives a worked uncertainty budget.
- **Contact angle is a surface characterisation, not a biocompatibility test.** It supports ISO 10993 work; it does not replace any part of it.

> **DESIGN RULE OF THUMB**
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> For protein-contacting surfaces, the literature places a step change in protein adhesion at a water contact angle of about 60–65°: below it, adsorbed protein layers are weaker and more easily displaced; above it, hydrophobic interaction dominates and adhesion rises sharply (Section 7.4). Cell attachment commonly peaks near 70°. A window of 55–70° therefore covers most attachment-critical cell culture ware, while protein-repellent surfaces are usually specified below 40° with low hysteresis.

2 Why wettability governs biotechnical interfaces

2.1 The interfacial cascade

When a material meets a biological fluid, four events occur in sequence and each is conditioned by the surface energy of the solid.

1. **Water restructuring** (picoseconds to nanoseconds). Bound water layers form; a hydrophilic surface organises water strongly and presents a repulsive barrier, a hydrophobic surface disrupts the hydrogen-bond network and makes the interface entropically expensive.
2. **Protein adsorption** (seconds to minutes). Proteins arrive by diffusion and displace water. The Vroman effect describes the subsequent competition: abundant, low-affinity proteins (albumin) adsorb first and are displaced by lower-abundance, higher-affinity ones (fibrinogen, fibronectin, immunoglobulins). Surface chemistry decides which protein wins and in what conformation.

3. **Cell and platelet response** (minutes to hours). Cells do not see the polymer; they see the adsorbed protein layer and its conformation. Fibronectin presented in an extended, integrin-accessible conformation supports attachment; albumin passivates.
4. **Biofilm, clot or fouling** (hours to days). Irreversible attachment consolidates the earlier steps.

Contact angle is the only routine laboratory measurement that reports on step 1 directly and predicts steps 2–4 well enough to be used as a process control. It is fast (seconds), non-destructive in most cases, requires little material, and is sensitive to fractions of a monolayer of contamination — which is why it is also unforgiving of poor technique.

2.2 Where the measurement earns its place

Application	Question answered	Metric normally specified
Cell culture ware (TCPS, microplates, scaffolds)	Is the surface oxidised enough for protein-mediated attachment?	Advancing water angle; lot-to-lot trend
Implantable polymers, catheters, blood-contacting tubing	How strongly will protein and platelets anchor?	Advancing / receding pair, hysteresis, SFE
Contact lenses and ophthalmic devices	Does the surface stay wet in the eye, and how mobile is it?	Captive-bubble angle, hysteresis after soak
Microfluidic chips (PDMS, COC, PMMA)	Will the channel fill by capillarity, and will the bond hold?	Angle after plasma, recovery versus time
Filtration and dialysis membranes	How quickly will the pore structure foul?	Captive bubble, SFE, contact angle of feed
Biosensor and assay surfaces	Did each functionalisation step cover uniformly?	Angle per step, within-slide IQR
Primary packaging (vials, syringes, stoppers)	Is the silicone layer or coating complete?	Angle of the oil film, surface energy
Hydrogels, dressings, porous scaffolds	Will aqueous fluid imbibe and spread?	Captive bubble or Washburn, not sessile

Table 1 — Typical biotechnical uses of contact angle as a decision parameter. The metric column matters more than the application column: choosing the wrong angle type is the most common cause of a specification that cannot be met.

2.3 What the number does not mean

A contact angle is not a biocompatibility result, not a chemistry identification and not a measure of topography. Two surfaces with the same angle can differ completely in chemistry (a fluorinated monolayer and a close-packed methyl monolayer both reach about 110–115°). Conversely a single surface can report different angles depending on the probe liquid, the vapour phase, the humidity and the time since it was cleaned. The correct reading is that wettability is a *system property* — and once that is accepted, it becomes an extremely reliable one, because the same system measured the same way gives the same number.

3 Physical basis: from Young’s equation to surface free energy

3.1 The Young balance

For a drop at equilibrium on an ideal (rigid, chemically homogeneous, atomically smooth) solid, the three interfacial tensions balance along the contact line:

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cdot \cos\theta_Y$$

where γ_{sv} is the solid–vapour, γ_{sl} the solid–liquid and γ_{lv} the liquid–vapour interfacial tension (72.8 mN/m for water at 20 °C). Young’s angle θ_Y is defined only for that ideal case. Real surfaces never satisfy it exactly, which is why a measured angle is always one member of a distribution rather than a single thermodynamic constant.

3.2 Real surfaces: Wenzel, Cassie–Baxter and what roughness does

Roughness changes the apparent angle in one of two ways. If the liquid fills the asperities completely (Wenzel state), the measured angle follows $\cos\theta^* = r \cdot \cos\theta_Y$, where r is the ratio of true to projected area ($r \geq 1$). Roughness therefore *amplifies* the underlying chemistry: a hydrophilic surface becomes more wettable, a hydrophobic surface more repellent, and a surface near 90° barely moves.

If air is trapped beneath the drop (Cassie–Baxter state), the apparent angle is a weighted average of the solid and air contributions, $\cos\theta^* = f_1 \cos\theta_1 + f_2 \cos\theta_2$ with $f_1 + f_2 = 1$. This is the mechanism behind superhydrophobic surfaces above 150°, and it is also a failure mode in porous scaffolds, where the drop may sit on a composite interface that has nothing to do with the material's chemistry.

The practical consequence for biotechnology is direct: any process that changes roughness while it changes chemistry — plasma etching, sandblasting, electrospinning, freeze-drying, laser texturing — produces a contact angle that cannot be interpreted without a roughness measurement.

3.3 Hysteresis is the informative part of the measurement

Advance a drop by adding liquid and the contact line moves forward at the advancing angle θ_a; withdraw liquid and it retreats at the receding angle θ_r. For any real surface $\theta_a \geq \theta_r$, and the difference $\Delta\theta = \theta_a - \theta_r$ is the contact angle hysteresis. A static angle placed on the surface lies somewhere between the two and is therefore not reproducible across operators unless the drop history is controlled.

Hysteresis has three main causes, and they matter differently in biology:

- **Chemical heterogeneity** — patches of different surface energy pin the contact line. In a grafted or adsorbed coating this is a direct measure of coverage uniformity.
- **Topography** — asperities pin the line; hysteresis rises with roughness even when chemistry is constant.
- **Surface reorientation and adsorption** — mobile polymer chains, surfactants and proteins rearrange at the interface during the measurement. This is why a receding angle on a hydrogel or a protein-contacting surface can be irreversibly changed by the first wetting cycle.

A large hysteresis on a chemically uniform surface is diagnostic of a mobile interface: silicone hydrogel lenses, block copolymers, and plasma-treated PDMS all show it, and all of them behave differently in use than their dry static angle suggests.

3.4 Surface free energy

Contact angles of several probe liquids are combined to split the surface energy into components. The Owens–Wendt–Rabel–Kaelble (OWRK) model, the most widely used in industry and the basis of ISO 19403-2, assumes the geometric mean of dispersive and polar contributions:

$$\gamma_l\,(1 + \cos\theta) = 2\left[\,(\gamma_s^{d} \cdot \gamma_l^{d})^{1/2} + (\gamma_s^{p} \cdot \gamma_l^{p})^{1/2}\,\right]$$

Two liquids with known dispersive and polar fractions — typically water (21.8 / 51.0 mN/m) and diiodomethane (50.8 / 0.0 mN/m) — give two equations for the two unknowns γ_s^{d} and γ_s^{p}, and the total surface free energy is their sum. The Wu harmonic-mean model is preferred for low-energy surfaces and for polymers above about 90°; the van Oss–Chaudhury–Good (vOCG) three-liquid acid–base model is preferred when hydrogen bonding is the question, at the cost of needing three well-characterised liquids and a stricter protocol. Section 7.5 works the OWRK calculation through numerically.

Surface free energy is the more transferable number when comparing chemically different materials, because it removes the arbitrary choice of probe liquid. Its weakness is model dependence: the same three angles analysed with OWRK, Wu and vOCG give three different totals. Report the model, the liquids and their source values alongside the result.

3.5 Work of adhesion

Combining Young and Dupré gives the work required to separate the liquid from the solid:

$$W_a = \gamma_{lv}\,(1 + \cos\theta) = \gamma_{sv} + \gamma_{lv} - \gamma_{sl}$$

W_a is the quantity to quote when the engineering question is adhesive: coating retention, ink anchorage, cell spreading, or the energy a cleaning step must supply. It is directly proportional to (1 + cos θ), so it is most sensitive at low angles, which is why hydrophilic process control is easier in relative terms than hydrophobic process control.

4 Measurement methods and how to choose one

Six methods cover essentially all biotechnical needs. They are not interchangeable: they measure different angles of the same system, and they suit different sample geometries.

4.1 Sessile drop

A drop of known volume is deposited on a horizontal surface and its profile is imaged from the side; the angle is extracted from the profile fit at the three-phase contact point. It is the default method for flat, non-absorbent materials: cell culture ware, polymer films, coated slides, device housings.

Two variants must be distinguished in any specification. A **static** angle is captured at a fixed time after deposition and is a single, history-dependent member of the hysteresis window. An **advancing** angle is captured while the contact line is moving outward, either by growing the drop through a needle held inside it or by tilting the stage; it is the reproducible, physically meaningful upper bound and is what ASTM D7334 describes.

4.2 Captive bubble

The sample is immersed in the relevant aqueous phase and an air bubble is brought into contact with its underside. The angle is measured *through the liquid*, which is exactly the configuration a cell, a protein solution or a tear film sees. For the same system the bubble angle and the sessile angle are supplementary (θ_bubble + θ_drop ≈ 180°), so the captive bubble converts a hydrophobic hydrogel into an easily measured high angle and a hydrophilic one into a low angle.

This is the method of choice for hydrogels, contact lenses, swollen membranes, and any surface that dewets or dries during a sessile measurement. It is also the only practical way to measure a surface in its intended buffer, at 37 °C, in the presence of protein — which is what makes it valuable for claim substantiation rather than incoming inspection.

4.3 Wilhelmy plate and single-fibre tensiometry

The sample is suspended from a microbalance and immersed into and withdrawn from the liquid while the force is recorded. The wetting force gives the contact angle through $F = \gamma_{lv} \cdot p \cdot \cos\theta$, where p is the wetted perimeter. Advancing and receding angles come from one immersion cycle, which makes it the fastest route to hysteresis, and it is the only method that works on single fibres, sutures, hollow fibres and thin films of known perimeter. It requires a completely wetting reference liquid for calibration, careful buoyancy correction, and a sample of uniform cross-section.

4.4 Dynamic and forced-wetting methods

Needle-in-drop volume cycling gives θ_a and θ_r under controlled contact-line velocity; the tilt or roll-off method gives the angle at which a drop departs, which is the relevant metric for repellency; oscillation and force-based methods extend the range to superhydrophobic surfaces where optical fitting loses accuracy. In biotechnology the needle-in-drop cycle is the standard way to detect time-dependent adsorption, because the receding angle collapses as the interface is colonised.

4.5 Washburn and capillary rise

For powders, granules and highly porous scaffolds, the liquid is drawn into a packed column and the mass uptake is recorded against time; the Washburn equation converts the slope into a contact angle. It is the only practical method for a powder, but it reports an effective angle for a packed bed, mixes in the bed’s tortuosity, and depends critically on reproducible packing. Treat it as a comparative technique, not an absolute one.

Method	Sample form	Output	Suits biotech when…	Main limitation
Sessile drop (static)	Flat, non-absorbent	θ at fixed time	Fast incoming inspection; large areas	History-dependent; not reproducible without protocol
Sessile drop (advancing)	Flat, non-absorbent	θ_a	Specification and release testing	Needs controlled dosing; edge pinning
Needle-in-drop cycle	Flat, non-absorbent	θ_a, θ_r, $\Delta\theta$	Detecting adsorption and surface mobility	Slow; needle disturbs thin drops
Captive bubble	Any, incl. hydrogel	θ through liquid	Hydrated, physiological, protein-bearing surfaces	Requires immersion cell; bubble stability
Wilhelmy plate / fibre	Fibre, film, tube	θ_a, θ_r from force	Sutures, hollow fibres, curved samples	Perimeter must be known; buoyancy correction
Washburn	Powder, porous	Effective θ	Scaffold granules, powders, packing media	Packing-dependent; comparative only

Table 2 — Method selection. The first decision is whether the surface will be measured dry or immersed; the second is whether a single angle or the hysteresis window is needed.

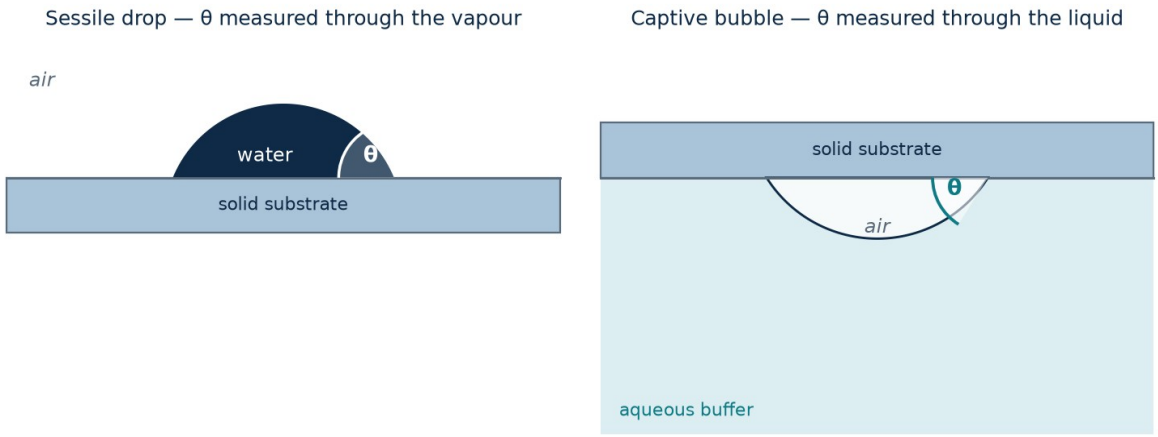


Figure 1 — The two geometries that dominate biotechnical work. In the sessile drop the angle is read through the vapour; in the captive bubble it is read through the liquid, which is the configuration the cells and proteins actually experience.

5 Instrumentation, calibration and uncertainty

5.1 The optical chain and the fit

A modern goniometer is a telecentric lens, a diffuse backlight and a camera, with sub-pixel edge detection on the drop profile. Three elements dominate quality:

- **Baseline placement.** The contact line is the reference for the angle, and on a low-angle drop a one-pixel baseline error moves the result several degrees. Automatic baseline detection is not a substitute for inspection; for angles below about 30° the baseline should be set manually and checked on screen.
- **Profile fit.** Young–Laplace fitting uses the whole drop shape and is the most consistent for axisymmetric drops; polynomial or circle fitting uses only the neighbourhood of the contact point and is more tolerant of a needle in the field of view but more sensitive to local defects. Report which was used.
- **Optical resolution and magnification.** The drop must occupy a large fraction of the field. A 2 µL drop imaged at low magnification carries more angular uncertainty than a 5 µL drop imaged well.

5.2 Calibration and daily verification

Calibrate the instrument against a certified reference or a stable, well-characterised surface, and verify daily with two liquids. Polished PTFE is convenient because its water angle is high and its value is well documented; a clean silicon wafer with native oxide is convenient at the hydrophilic end. A verification record should capture the reference value, the measured value, the probe liquid, the temperature and the operator.

Note the asymmetry: a check at 110° does not prove accuracy at 30°. If the process window sits in the hydrophilic range, verify in that range.

5.3 Environmental control

Water evaporates from a microlitre drop at a rate that changes both the volume and the local vapour concentration; the angle drifts as it does. Control the room to 21–25 °C and 40–60 % relative humidity, capture the angle at a fixed time, and for long dynamic runs enclose the drop in a saturated-vapour cell. For biological relevance use the intended medium — phosphate-buffered saline, cell culture medium, artificial tear fluid — because buffer salts and surfactants change both the surface tension and the interfacial behaviour.

5.4 Drop volume

Gravity distorts the drop when the capillary length is approached; the Bond number compares gravitational to capillary forces. In practice, 2–10 µL of water is the safe range on polymer surfaces, with larger drops preferred at high angles and smaller drops on curved or small parts. The test is empirical: measure the same surface at three volumes; if the angle is volume-independent within uncertainty, the volume is acceptable. Record it.

5.5 Uncertainty budget

The following budget is representative of a well-run laboratory measuring a 60° water angle with a 5 µL drop, ten replicates. It is an example of the arithmetic, not a claim about a specific instrument.

Contribution	Type	Standard uncertainty (°)	How it is controlled
Repeatability (n = 10)	A	1.20	Median of 10 drops, 3 locations
Drop volume 2 µL vs 8 µL	B	0.60	Fixed volume; volume-independence check
Baseline and fit model	B	0.50	Manual baseline below 30°; one fit model
Temperature ±1 K	B	0.30	Air-conditioned room; record T
Operator and placement	B	0.80	Training; annual Gage R&R
Combined standard uncertainty uc		1.67	Root sum of squares
Expanded uncertainty U (k = 2)		3.3	95 % coverage

Table 3 — Example uncertainty budget. Every contribution is expressed as a standard uncertainty in degrees so that they can be combined in quadrature.

The practical consequence: a single-drop reading carries about ±3° at 95 % confidence, and a mean of ten drops about ±1°. Specifications tighter than 5° therefore require replication, and a process window of 6° is the practical minimum for a release test based on contact angle alone.

5.6 Sampling

Contact angle varies across a surface far more than a well-controlled process suggests. Sample at least three locations per part, five drops per location, and report the median with the interquartile range rather than the mean with a standard deviation, because contamination and pinning produce skewed distributions. On a 96-well plate, measure the bottom centre of three sacrificial wells per plate; on a tube, three axial positions and both sides if the tube is flattened.

6 A protocol that gives comparable numbers

The following sequence is written to be transposed into an SOP. It assumes a drop-shape goniometer with a temperature-controlled, humidity-monitored enclosure and a manual or motorised dosing unit. The acceptance criteria are the ones that make the result defensible in a design history file.

Step	Action	Parameter / acceptance criterion
1	Condition the sample	23 ± 2 °C, 50 ± 10 % RH, 2 h minimum; record time since the last process step (cleaning, plasma, coating)
2	Handle without touching the test face	Powder-free nitrile gloves, tweezers at the edge; never wipe a treated surface
3	Verify the probe liquid	Water 72.8 ± 0.5 mN/m at 20 °C (pendant drop or certificate); discard after 7 days open
4	Level the stage	Two-axis bubble level; re-check after mounting each part
5	Set dosing	2–10 µL drop, 1–2 µL/s deposition, needle withdrawn before capture or held inside for advancing
6	Capture	Static: at t = 2.0 s after deposition. Advancing/receding: 0.1–0.5 µL/s, 3 cycles
7	Fit and inspect	Young–Laplace fit; manual baseline below 30°; reject images with a visible needle shadow or bubble
8	Replicate	5 drops × 3 locations minimum; report median and IQR, discard the first drop after loading
9	Record	Instrument ID, calibration date, liquid lot, T, RH, angle type, fit model, operator

Table 4 — Minimum protocol for a defensible contact angle result. Steps 3, 5 and 9 are the ones most often missing from published methods, and they are the ones that make results comparable between laboratories.

Two protocol details deserve emphasis because they explain most inter-laboratory disagreement. The first is the **first-drop effect**: after the dosing line has been idle, the first drop often contains a slightly different volume or a different surfactant history and reads several degrees away from the steady state. Discard it. The second is the **time stamp**: water evaporates, surfactants adsorb and polymer chains reorient on the timescale of tens of seconds, so an angle without a capture time is not a measurement but an anecdote.

7 Measurement data

This section presents four datasets chosen to cover the range of biotechnical practice: reference surfaces for verification, a process-dose series on a polymer, a hydrated soft-material comparison, and a production capability study. The provenance of each is stated. Where a dataset is illustrative rather than published, it is labelled in the section heading and in the caption.

7.1 Reference surfaces for verification

The values below are the ones a laboratory should be able to reproduce within its own uncertainty budget. They are method-dependent by construction: the table therefore states the angle type, the probe liquid and the source for every row.

Surface	Water angle (°)	Type / liquid	Note
PTFE, machined, cleaned	108–117; 117.7 ± 2.1 reported	Dynamic, water	Literature range for water/PTFE; the single quoted value is for a clean machined surface
LDPE, untreated	91 ± 2	Advancing, PBS	Reference state of the plasma series in 7.2
Polystyrene, untreated	≈ 92	Advancing, water	Origin of the plasma series reported for PS

Surface	Water angle (°)	Type / liquid	Note
Polystyrene, IPA plasma	≈ 77	Advancing, water	Angle falls with increasing plasma dose in the same series
Silicone hydrogel lens, from blister	66	Sessile, water	Surfactant-rich packaging solution present
Same lens after 48 h soak and wash	96	Sessile, water	Same polymer; difference is the removed surfactant layer
Borosilicate glass, cleaned	20–30	Sessile, water	Highly process-dependent; contaminated glass drifts to 50–70°

Table 5 — Verification and reference values. Rows 1–4 and 6 are drawn from published measurements; the glass range is a compiled indicative value and should be established locally against a certified reference.

The PTFE row illustrates a general point: the literature range for a common material spans 9°, which is larger than most process windows. A verification surface is only useful when its own value has been established on the instrument in question, with the same liquid and the same angle type.

7.2 Plasma dose series on low-density polyethylene

Published data: low-density polyethylene treated in a glow-discharge plasma at 100 W and about 200 mTorr for 0–150 min, characterised by advancing water contact angle (eight independent measurements, mean ± SD) and by AFM roughness over 2 × 2 µm² [1]. The series is reproduced here because it is the clearest published demonstration that wettability is not a monotonic function of treatment dose.

Plasma exposure (min)	0	15	45	60	90	150
Advancing water angle (°)	91 ± 2	48 ± 2	71 ± 2	71 ± 2	53 ± 3	41 ± 2
Roughness Rq (nm)	4.6 ± 1.0	5.1 ± 0.5	9.8 ± 0.8	12.4 ± 0.8	8.6 ± 0.8	5.5 ± 0.4

Table 6 — Advancing water contact angle and AFM roughness versus plasma exposure, after Xu and Siedlecki [1]. Probe liquid PBS; n ≥ 8 per point.

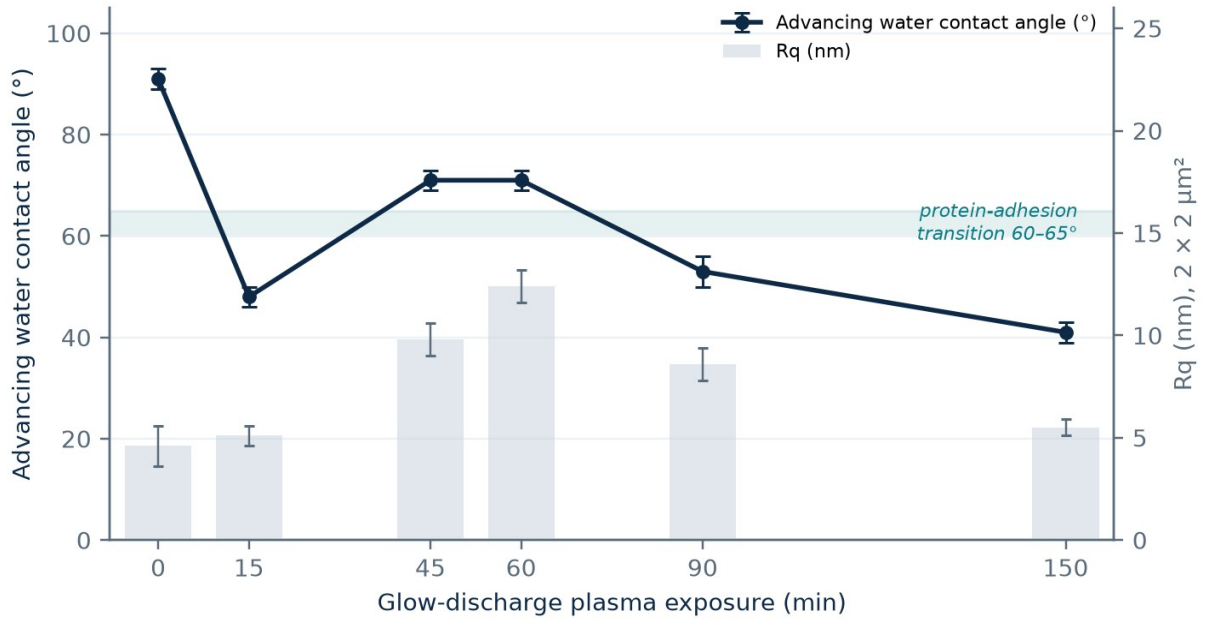


Figure 2 — The same data plotted. Wettability improves sharply at 15 min, partially reverses at 45–60 min, then improves again. The roughness trace follows the reversal.

The series is instructive precisely because it is not monotonic. After 15 minutes the angle has fallen from 91° to 48°: oxygen-containing groups have been incorporated and the surface is substantially more polar. At 45 and 60 minutes it returns to 71°, and only beyond 90 minutes does it fall again to 53° and

then 41°. Roughness rises over the same interval, peaking at 12.4 nm at 60 minutes before falling back to 5.5 nm at 150 minutes.

Two effects are competing. Oxidation lowers the angle; etching, chain scission and redeposition of low-molecular-weight fragments raise it and simultaneously increase the true area available to the drop. The 5° difference between the 53° and 48° points, both measured with 2–3° standard deviations, is at the edge of resolution — which is exactly why the roughness channel is needed to interpret the angle channel.

> **WHAT THIS MEANS FOR A SPECIFICATION**
>
> A specification written as “lower angle = better treatment” would have rejected the 60-minute sample — the one with the highest roughness and, in many cell-culture and bonding applications, the most useful topography. Conversely, a specification written only on angle would accept a 150-minute sample whose surface has been etched back to a smoother state.
>
> Where a plasma or corona step matters functionally, control the angle **and** the roughness, or control the angle at two doses and demonstrate equivalence functionally. A single angle specification is sufficient only when the process is known to sit on the monotonic part of the curve.

7.3 Hydrated soft materials: sessile drop versus captive bubble

Published data: five commercial silicone hydrogel contact lenses measured by both techniques [2]. The results are a compact lesson in method dependence.

Condition	Sessile drop (°)	Captive bubble	Interpretation
As received from the blister	66	Not reported separately	Surface-active agents from the packaging solution lower the dry angle
After 48 h soaking and washing in saline	96	Relatively low value reported for this lens type	Surfactant removed; the true polymer interface is exposed and reads hydrophobic in air

Table 7 — The only lens in the panel with published absolute values before and after washing (p = 0.0002 for the difference), after Maldonado-Codina and Morgan [2].

The 30° shift between the two conditions is not a polymer change. It is the removal of a surface-active layer that had been controlling the interface. Any incoming inspection that measures lenses straight from the blister is therefore measuring the packaging, not the device — a distinction with direct regulatory consequences when wettability is claimed as a performance attribute.

The same study splits the five lenses into two populations. Three lenses showed relatively high sessile angles together with relatively low captive-bubble angles — a large hysteresis, indicating a mobile, reorganising interface. Two showed the opposite pattern: low sessile, high captive, little hysteresis, indicating a more stable interface. The clinically relevant conclusion is that a single dry angle ranks these materials incorrectly; the pair of measurements, and the hysteresis between them, is what separates a surface that restructures on contact with tear fluid from one that does not.

For any hydrogel, membrane or swollen scaffold, the operative rule is therefore: **measure immersed, in the intended medium, after equilibration.** A sessile drop in air on a hydrated material measures a collapsed, dehydrated state that the product never presents to a cell.

7.4 The protein-adhesion threshold

Published data: adhesion forces between three proteins and a series of plasma-treated LDPE surfaces spanning 41–91°, measured by protein-functionalised AFM probes in PBS, with contact times from 0 to 50 s [1]. The finding that matters for specification is a step change rather than a gradient.

Protein	Mass (kDa)	Behaviour across the wettability series	Relevance
Bovine serum albumin	69	Adhesion rises sharply above θ ≈ 60–65°; strongest on the untreated 91° surface	Passivating layer; its displacement governs the later cascade
Human fibrinogen	340	Same threshold and same trend; larger absolute adhesion than FXII	Ligand for platelet integrin αIIbβ3; central to thrombus formation
Human Factor XII	80	Same threshold, smaller adhesion force on all surfaces	Contact activation of the intrinsic coagulation pathway

Table 8 — Protein adhesion on plasma-treated LDPE as a function of surface wettability, after Xu and Siedlecki [1]. Contact time was also varied: increasing it from 0 to 50 s raised adhesion on every surface regardless of wettability.

Two design consequences follow. First, the transition at 60–65° is narrow — about the width of the measurement uncertainty of a single drop — so a surface specified at 61° is not meaningfully different from one at 66° in wettability terms, but may be on opposite sides of a biological threshold. Specify with margin: either clearly below 55° or clearly above 75°, never straddling the transition. Second, adhesion is time-dependent even where wettability is constant, which means a short static contact angle cannot fully predict a long-dwell application such as an implanted catheter or an extracorporeal circuit.

> **DESIGN RULE**
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> For blood- and protein-contacting devices intended to minimise adsorption, target a water angle below about 55° with low hysteresis (Δθ < 15°). For surfaces intended to anchor protein — assay wells, culture substrates, adsorption columns — target above 65° or use a dedicated binding chemistry. Avoid specifications that place the mean within ±5° of 62°, where the biological response changes fastest.

7.5 Surface free energy: a worked OWRK calculation

The following example demonstrates the arithmetic of Section 3.4 end to end. The contact angles are **illustrative inputs**, chosen to represent three common classes of biomaterial surface; the computation is exact for those inputs.

Surface (illustrative)	θ water (°)	θ diiodomethane (°)	γ_s^d (mN/m)	γ_s^p (mN/m)	γ_s total (mN/m)	W_a water (mJ/m²)
A — aromatic polymer, untreated	72.5	45.2	36.9	7.1	44.0	94.7
B — O_2-plasma treated	28.4	38.0	40.6	29.3	69.9	136.8
C — fluoropolymer	115.0	88.0	13.6	0.3	13.9	42.0

Table 9 — OWRK result for three illustrative surfaces. Liquid constants used: water γ = 72.8 (21.8 dispersive / 51.0 polar), diiodomethane γ = 50.8 (50.8 / 0.0). The full calculation chain is given in Appendix B.

Surface B is the informative case. Its dispersive component (40.6) is close to that of surface A (36.9) — the underlying hydrocarbon backbone is unchanged — while its polar component has risen from 7.1 to 29.3 mN/m. That signature is characteristic of oxidation without a change of bulk chemistry, and it is the fingerprint a plasma or corona process should produce. A treatment that raised both components together would suggest a coating rather than a surface modification.

The fluoropolymer case shows the limit of the technique: with a polar component of 0.3 mN/m, the polar term is at the noise floor of the two-liquid method. For surfaces below about 15 mN/m total, use three liquids or the vOCG model, and quote the total with an uncertainty of at least ±1.5 mN/m.

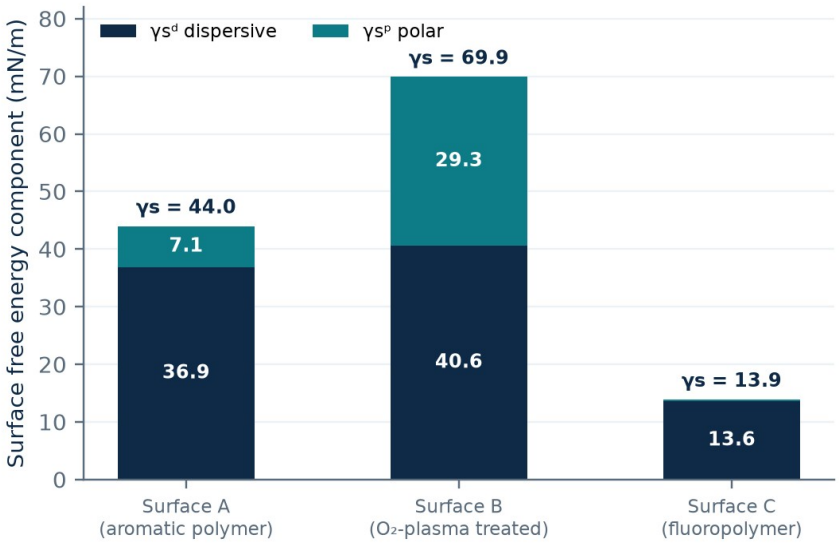


Figure 3 — Dispersive and polar components of the three surfaces. Plasma treatment roughly quadruples the polar term while leaving the dispersive term nearly unchanged: the chemistry added is polar, not a new material.

7.6 Production capability: an illustrative lot study

The dataset in this section is **synthetic**. It was constructed to demonstrate how contact angle data are turned into a release decision, and the numbers should not be read as measurements of any real product. The specification is 62 ° ± 8 ° (LSL 54°, USL 70°), n = 10 drops per lot at three locations.

Lot	Mean (°)	SD (°)	Cpk	Decision	Comment
A	61.4	1.8	1.37	Release	Centred, tight; normal production
B	63.2	2.1	1.08	Release, monitor	Acceptable but capability below 1.33
C	58.9	3.4	0.48	Investigate	Low mean and high spread: suspect partial treatment
D	66.8	2.6	0.41	Reject	Mean close to USL; drift not yet a failure of any single drop
E	64.1	1.5	1.31	Release	Recovered after cell C maintenance

Table 10 — Illustrative capability study. Cpk = min(USL − mean, mean − LSL) / 3σ.

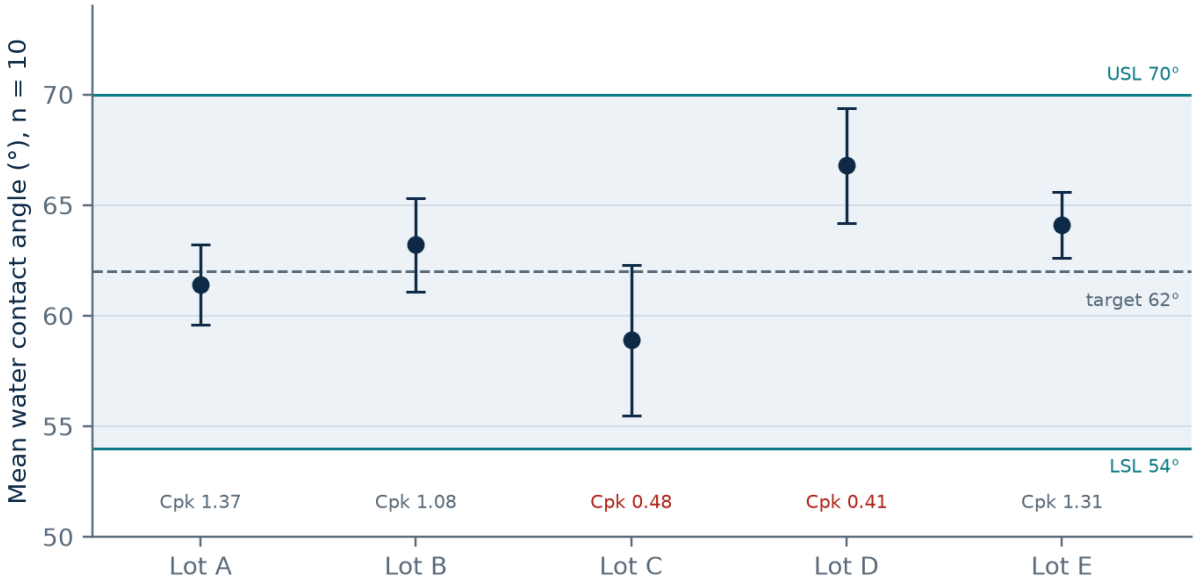


Figure 4 — Lot means with ±1 SD. The specification band is 54–70°; the target is 62°. Lots C and D are inside the specification on their means but fail on capability, which is the distinction a release test must be able to make.

The example makes the central quality argument for contact angle data: lot D would pass a simple “is the mean inside the limits” test in most informal laboratories, yet its position near the upper limit plus a 2.6° spread means a meaningful fraction of individual drops falls outside the window. Cpk below 1.0 should trigger investigation of the upstream process — usually plasma or corona dose drift, lamp ageing, or a change in the mould release agent — not tightening of the specification.

8 Three application case studies

8.1 Cell culture ware: releasing a plasma-treated microplate

Tissue culture polystyrene is the highest-volume contact angle application in biotechnology. Untreated polystyrene sits near 92°; the tissue-culture grade is oxidised by corona, plasma or gamma treatment to a window that supports protein-mediated attachment. Because protein adhesion rises above 60–65° and cell attachment commonly peaks near 70°, the useful window is narrow and lies on a steep part of the response curve.

A workable release strategy has three tiers. **Per lot**, measure the advancing water angle on the bottom of three sacrificial wells per plate, five drops each, and require the median inside 55–70° with IQR below 4°. **Per shift**, verify the instrument against the reference surface and record it. **At validation only**, correlate the angle with a functional assay — attachment efficiency and doubling time against a qualified cell line — and establish the window from that correlation rather than from literature. The functional assay is not repeated per lot; the angle is the surrogate, and its validity rests on that one-time correlation.

The common failure mode is a slow upward drift of two to four degrees over months, caused by lamp or electrode ageing, that nobody notices until customers report uneven growth. A trend chart with a Cpk calculation catches it while the plates are still inside specification.

8.2 Contact lenses: substantiating a wettability claim

A wettability claim on a soft lens is a claim about the interface in the eye, and the measurement must be configured accordingly. Three methodological choices decide whether the data support the claim.

- **Measure immersed.** Captive bubble in the intended packaging or tear-like medium, after equilibration. A dry sessile angle on a silicone hydrogel reports a collapsed surface and can invert the ranking of materials.
- **Control the soak history.** The 66° to 96° shift in Section 7.3 shows that residual packaging surfactants dominate the as-received reading. Specify the rinse and soak protocol, and report both as-received and washed values if the claim concerns the worn device.
- **Report hysteresis, not just the angle.** The population split in the published panel — high sessile with low captive versus the reverse — separates mobile interfaces from stable ones, and mobility is what governs lipid and protein interaction over a wearing period.

For a regulatory submission the method statement matters as much as the result: instrument, liquid, temperature, soak time, number of lenses, number of drops per lens, fit model and the statistical treatment. A claim supported by a single drop per lens on three lenses is not defensible, regardless of how favourable the number is.

8.3 Microfluidics: qualifying a plasma bond on PDMS

Polydimethylsiloxane is hydrophobic as cured — commonly 100–110° — and is rendered hydrophilic by oxygen plasma so that it bonds irreversibly to glass and fills channels by capillarity. The treatment is not permanent: low-molar-mass chains migrate to the interface and the surface recovers toward its hydrophobic state over hours to days, which is why a device that bonded well on the day of manufacture can leak or refuse to fill a week later.

The qualification therefore needs two time points, not one. Measure within 15 minutes of treatment to define the process window (typically requiring an angle below about 40° for reliable capillary fill and bonding), and again after the intended shelf life — 24 hours and 7 days are convenient checkpoints — to

define the storage claim. Plot angle against time since treatment; the recovery curve is the deliverable, not a single number.

Two practical cautions apply. PDMS is permeable and absorbs small molecules, so do not measure on a device that has already been exposed to solvents or surfactants. And because the treated surface is soft, deposit the drop gently and read the angle within two seconds: the surface deforms and the apparent angle creeps.

9 Pitfalls and troubleshooting

Symptom	Probable cause	Corrective action
Values creep upward over weeks with no process change	Hydrophobic recovery or ageing of a treated surface	Fix the time from treatment to measurement; measure at t = 0 and at shelf life
First drop differs from the rest by more than 3°	Dosing line not equilibrated; residual liquid at the tip	Purge two drops; discard the first; keep the tip immersed between drops
Within-sample SD above 5°	Heterogeneous treatment, phase separation, local contamination	Map five locations; report IQR; inspect with XPS or ToF-SIMS
Angle falls continuously during capture	Evaporation, surfactant adsorption, or spreading	Capture at a fixed time; use a saturated-vapour cell; report the time
Implausibly low angle on a porous membrane	Liquid absorbed into the pore structure; no equilibrium	Switch to captive bubble or Washburn; never report a sessile angle on absorbent material
Angle near 0° on every sample	Surfactant or cleaning residue film	Change the cleaning protocol; verify by XPS; check the rinse water
Receding angle erratic and irreproducible	Contact-line pinning on defects or adsorbed layer	Reduce cycling rate to 0.1 µL/s; average five cycles; inspect the surface
Angle depends on drop volume	Gravity at large volume, or roughness at small volume	Test three volumes; use 5 µL unless the surface requires otherwise
Operator-to-operator offset of 2–5°	Baseline placement and image selection	Automatic baseline plus manual check below 30°; annual Gage R&R; shared image set for training
Results differ from a supplier certificate	Different liquid, angle type or fit model	Compare method statements, then re-measure both samples in one laboratory

Table 11 — Diagnostic table. The first three rows account for most of the variability encountered when a contact angle method is transferred between sites.

10 Specifications, validation and regulatory context

10.1 Setting a specification that can be met

A contact angle specification is a statement about a population, not about a drop. Three rules make it workable. Set the window at least six times the expanded measurement uncertainty wide — for U = 3.3°, that means a window of about 20° for single-drop testing, or 6–8° if ten drops are averaged. Place the target away from a biological threshold (Section 7.4) so that ordinary process variation does not cross it. And express the result as a median with an interquartile range, because contamination produces skewed distributions that a mean conceals.

10.2 Method validation and Gage R&R

Treat the method like any other test method in a quality system: demonstrate accuracy against a reference surface, precision by repeated measurement of the same surface, repeatability within operator and reproducibility between operators, and robustness against the parameters that are likely to drift — drop volume, capture time, humidity, fit model. A Gage R&R with ten samples, three operators and three

replicates is the standard design; a gauge contribution below 20 % of total variation is generally acceptable, and between 20 and 30 % is acceptable with justification.

Linearity deserves a note. Contact angle responds non-linearly to surface composition (cos θ is linear in surface energy, not θ itself), so a method that is accurate at 90° is not automatically accurate at 30°. Validate in the range you intend to specify.

10.3 Standards to cite

Standard	Subject	Use it for
ISO 19403-2	Wettability — determination of surface free energy of solid surfaces by contact angle	Surface free energy reporting; liquid set and model
ASTM D7334	Surface wettability of coatings, substrates and pigments by advancing contact angle	Advancing angle on flat industrial surfaces
ASTM D5946 / ISO 15989	Water contact angle of corona-treated polymer film	Incoming and in-process checks on films and webs
ASTM D8597	Contact angle of coatings and substrates using portable goniometers	Field or line-side measurement on large parts
ASTM D7541	Critical surface tension by the Zisman method	Ranking low-energy surfaces with a liquid series

Table 12 — Method standards. Cite the standard that matches the angle type actually measured; a Zisman result is not an OWRK result, and neither is an ISO 19403-2 result unless the specified liquid set and model were used.

10.4 Relationship to ISO 10993

Contact angle data support, but never replace, biological evaluation. ISO 10993-1 requires characterisation of the material and its surface in the context of the intended contact; wettability is part of that characterisation, and it is useful for justifying why a particular biological endpoint is or is not a concern. It is not a biocompatibility test, it does not demonstrate absence of cytotoxicity, and it cannot substitute for extractable or leachable studies. State it in the design file as a physical surface characteristic with a defined measurement method.

10.5 When to add a second technique

Contact angle reports the outermost layer, averaged over the drop footprint, with no chemical specificity. Add XPS or ToF-SIMS when the question is *what* is on the surface rather than *how much* it wets; add AFM or white-light interferometry when roughness changes with the process; add QCM-D or ellipsometry when the question is how much protein adsorbs and how tightly. The combination that answers most biotechnical questions is contact angle for the process control, XPS for the chemistry at validation, and one functional assay to anchor both.

11 Reporting checklist

A contact angle result is only as useful as its method statement. The following items should appear in any report, specification or design document that quotes one.

- **Angle type** — static, advancing, receding, captive bubble, or a named combination, with the value reported for each.
- **Probe liquid and its surface tension** — grade, lot, measured or certified value, temperature.
- **Drop volume and dosing rate**, and evidence from a volume-independence check.
- **Capture time** after deposition, and the total duration of any dynamic cycle.
- **Fit model** — Young–Laplace, polynomial or circle — and whether the baseline was automatic or manual.

- **Environment** — temperature, relative humidity, and whether a saturated-vapour cell was used.
- **Sample history** — cleaning, treatment, time since treatment, storage, soak or rinse protocol.
- **Replication and statistics** — number of parts, locations and drops; median and IQR rather than mean and SD where the distribution is skewed.
- **Instrument and calibration status** — identifier, last verification date, reference value obtained.
- **Surface free energy details**, if reported — model, liquid set, literature values used, and the uncertainty.
- **Limitations** — for example, that a sessile measurement on a hydrogel reports the dehydrated state.

> **ONE-SENTENCE RULE FOR REVIEWERS**
>
> If a document quotes a contact angle without stating the angle type, the probe liquid and the capture time, treat the number as indicative only — it cannot be compared with any other number, including another number from the same laboratory.

12 References

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[9] ASTM D7334-08(2022). *Standard Practice for Surface Wettability of Coatings, Substrates and Pigments by Advancing Contact Angle Measurement.* ASTM International, West Conshohocken, PA.

[10] ASTM D5946 / ISO 15989. *Water contact angle of corona-treated polymer film.* ASTM International and ISO.

Appendix A Symbols and constants

Symbol	Meaning	Value / unit
θ	Contact angle	degrees
θ_Y	Young (ideal) contact angle	degrees
θ_a, θ_r	Advancing and receding angles	degrees
$\Delta\theta$	Contact angle hysteresis, $\theta_a - \theta_r$	degrees
γ_{lv}	Liquid–vapour surface tension	mN/m (= mJ/m²)
γ_s^d, γ_s^p	Dispersive and polar components of solid surface energy	mN/m
W_a	Work of adhesion	mJ/m²
r	Wenzel roughness factor, true / projected area	dimensionless, ≥ 1
f_1, f_2	Area fractions in the Cassie–Baxter state	dimensionless, sum to 1
	Water at 20 °C: γ = 72.8 (21.8 dispersive / 51.0 polar)	mN/m
	Diiodomethane at 20 °C: γ = 50.8 (50.8 dispersive / 0.0 polar)	mN/m

Table A1 — Symbols used in this note, with the liquid constants applied in Section 7.5.

Appendix B OWRK calculation worksheet

Step-by-step evaluation for Surface A of Table 9 (θ water = 72.5°, θ diiodomethane = 45.2°). The same six steps apply to any pair of probe liquids with known dispersive and polar fractions.

Step	Expression	Substitution	Result
1	$\gamma_l(1 + \cos\theta)$ for water	72.8 × (1 + cos 72.5°) = 72.8 × 1.3007	94.7 mN/m
2	Same for diiodomethane	50.8 × (1 + cos 45.2°) = 50.8 × 1.7045	86.6 mN/m
3	$(\gamma_s^d)^{1/2}$ from step 2	86.6 / (2 × √50.8) = 86.6 / 14.256	6.075
4	γ_s^d	(6.075)²	36.9 mN/m
5	$(\gamma_s^p)^{1/2}$ from step 1	(94.7/2 − √(36.9 × 21.8)) / √51.0	2.66
6	γ_s^p, then total	(2.66)²; then 36.9 + 7.1	7.1; 44.0 mN/m

Table B1 — OWRK evaluation. Steps 3 and 5 isolate the two unknowns by using the apolar liquid first; the polar liquid then supplies the remaining equation.

The same worksheet applies to the three-liquid vOCG model, with the difference that the acid (γ^+) and base (γ^-) terms enter as a product and require three liquids to solve. For routine industrial work, two liquids and OWRK remain the most reproducible combination.

— End of document —