Cuvette Spectrophotometers = Clear Samples Only.
CLARiTY Integrating Cavity UV/Vis Spectrophotometer = Clear and Not Clear!

Cuvettes offer convenience. The cuvette is commonly one centimeter on a side, i.e. 1 cm². Short pathlength models are available for high absorbance studies; masked cuvettes are available for low volume samples. Quartz cuvettes are used for UV spectroscopy; lower cost glass and plastic cuvettes are suitable for visible spectroscopy.

Results from a cuvette spectrophotometer:
Here, we see one protein concentration in four solvents of increasing turbidity.

The answer changes from accurate to inaccurate to ultimately nonsense in the face of increasing turbidity. The only correct answer is the blue on the far left.

The terms “turbidity,” “haze,” and “scatter” are used interchangeably in this document.

Two limitations of the cuvette:
The measurement light from the spectrophotometer passes through a small section of the cuvette, so that:

1. the sample volume must be to at least this Z-axis level, and
2. only the portion of the sample through which the light passes is involved in the measurement and thus in the experimental results.

A third limitation occurs when the sample must be perfectly clear. The more hazy the sample, the more flawed results are. The dramatic and disruptive effect of scatter is shown in the figure above.
In 1955¹, a group of oceanographers used a filled integrating sphere to achieve high sensitivity measurements and dubbed their design an “integrating cavity.”

Turbidity does not change the absorbance measurement. This example, published in Analytical Chem, May 2018, confirms that a given protein concentration is measured exactly the same whether in a clear or opaque medium. These are the identical sample preparations shown in figure 1, page 1 (lower left).

An Integrating Cavity solves the problem of light escaping detection. Now, all light entering the sample holder is available to the detector. Only absorbance by the sample changes the light level. No light is outside of the range of the detector.

Plus, all of the sample is involved in the measurement.

Our favorite shape is round, but an integrating cavity can take other forms.

A round-bottom quartz flask was our first design. Round is the perfect shape. And, it leaves the sample nowhere to hide. The flask is blown of high quality quartz, inert to effectively every sample and cleaning agent.

Highest sensitivity is achieved when the cavity is filled with the sample.

The most popular volume is 8 mL. Example sample preparations are (a) 50 uL of protein in 8 mL of solvent, (b) $10^7$ cells/mL of living cells or bacteria, and (c) a test tube containing 2 mL of nanoparticles.

The sample can also be:
A, B: A very small or partial volume in the flask
C, D: A lesser volume introduced within a 2 mL test tube, a solid lowered or coiled into the flask, or an immobilized solid within a solvent bath.

As of June 2019, the standard volume cavity is 8 mL. With a low absorbance sample, the resulting pathlength is 30 cm. Sensitivity is on the nanomolar range.

Absorbances from roughly 0.001 AU/cm to 3 AU/cm are successful in the filled cavity.

Higher absorbing samples which cannot be diluted can be measured in this large flask by using a lesser volume.
Sensitivity, Volume, and Pathlength

<table>
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<th>physical path</th>
<th>optical path</th>
<th>upper limit (abs/cm)</th>
<th>volume (mL)</th>
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Flow-through, short pathlength:
For ease of handling very high absorbance samples which must be measured in very short pathlength integrating cavities, we opted for flow-through cells. Such samples include undiluted whole milk, red wine, and highly colored flowable ingredients.

Choose from the five flow-through cavities shown on the chart.

The integrating cavity is within the DSPC.

The integrating cavity is permanently packed inside a housing, which we call a DSPC, for DeSa Suspension Presentation Chamber.

Between the quartz integrating cavity and the high density black housing is a highly reflective material, encasing the integrating cavity and its contents.

The light inside the cavity reflects endlessly off this reflective surface until it is either absorbed by the sample or exits the DSPC to the detector.

Quartz rods -- straight or curved, short or long – can be used to direct light in and out of the DSPC.

A lab might have two or three DSPCs to accommodate different sample types. These sample holders are extremely easy to exchange one for the other. Stirring and Peltier control are available.

The measurement light is fully diffused as a “gas of photons.”

All photons are trapped within DSPC until they pass through a port to the awaiting detector.

Roughly 5% of the light in escapes as detected light.

Roughly 0.1% of the light is absorbed by the reflective surface.