

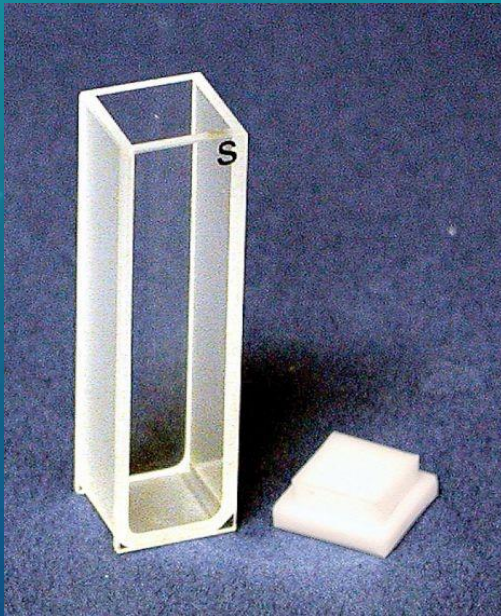


# CONSIDERING VOLUMES

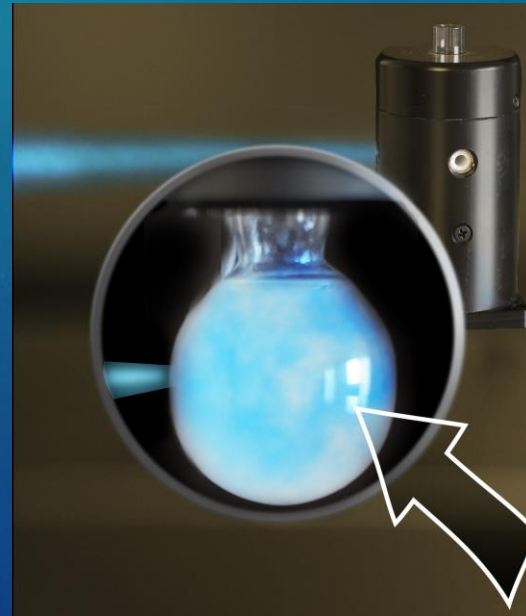
EVERYONE TENDS TO GET A BIT UNSETTLED WHEN WE  
MENTION “8 ML” FOR A CLARITY.

INTERESTINGLY, THIS IS ROUGHLY 2X AS MUCH AS A 1 CM<sup>2</sup> CUVETTE

4.375 mL to fill; 3.5 mL to reach  
measurement light path



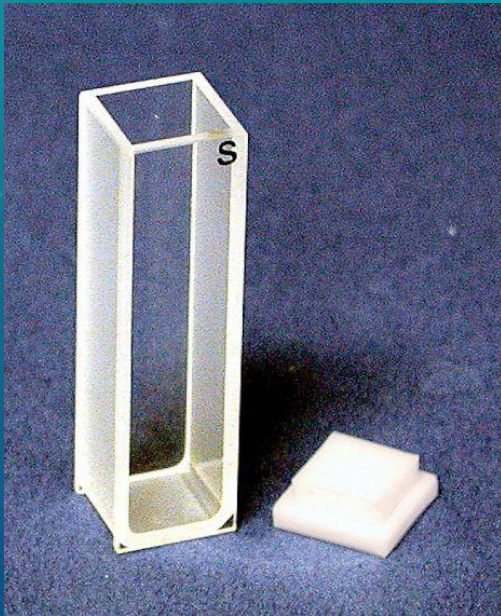
8 mL



## WHAT IS THE PATHLENGTH?

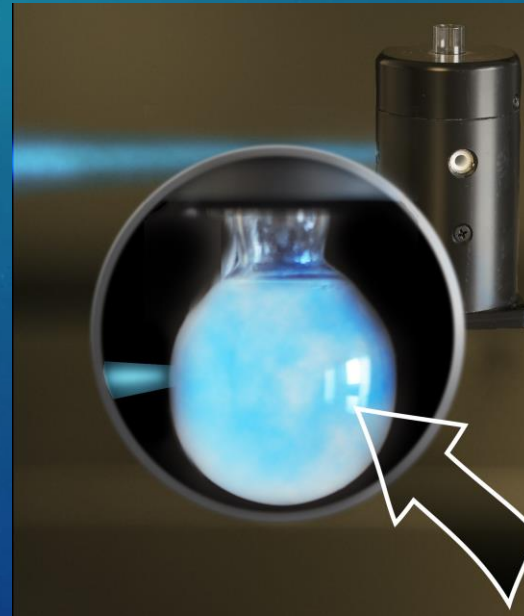
1 cm

Shorter and longer cuvettes are available



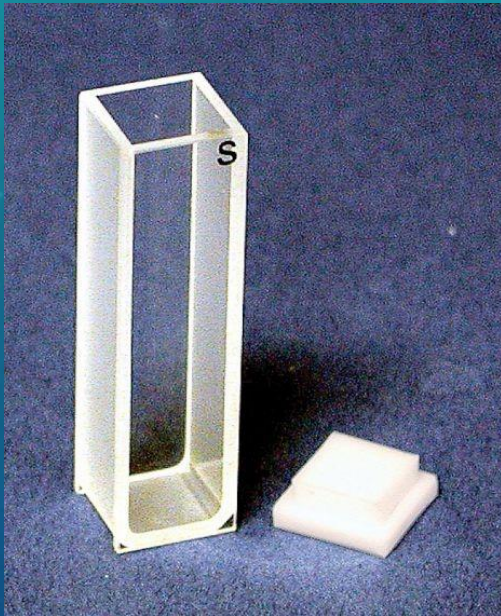
Approaching 30 cm

The photons traverse the interior of the cavity 'endlessly,' until either absorbed or exiting to the detector.

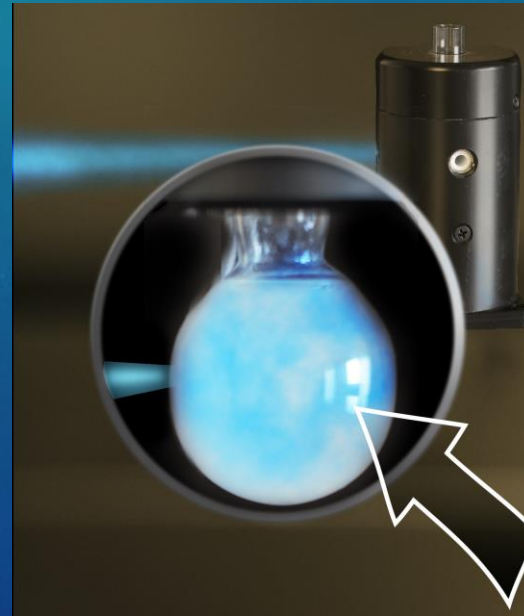


# AND THUS THE SAMPLE CONCENTRATION?

What you usually use



Upwards of 30x less concentrated



# WHAT VOLUME IS USED IN THE MEASUREMENT?

Exactly the volume through which the focused measurement beam passes. (Not much!)

100%

The majority of the sample is not included in the measurement, but is required to fill the cuvette to the necessary Z-height.

100% of the solution, solid, or suspension in the cavity is within the measurement light.

## WHAT HAPPENS WHEN THE SAMPLE IS NOT CLEAR?

Some of the photons are deflected from their path, so that not all of the photons reach the detector.

Therefore, the absorbance calculated is higher than it actually is. One calls this “apparent high absorbance”.

Zero photons are deflected from the path of the detector; all of the photons which should reach the detector do.

Therefore, the absorbance calculated is accurate.

WHAT HAPPENS WHEN THE SAMPLE IS NOT CLEAR?

The answer is incorrect.

The answer is correct.

# SUMMARY

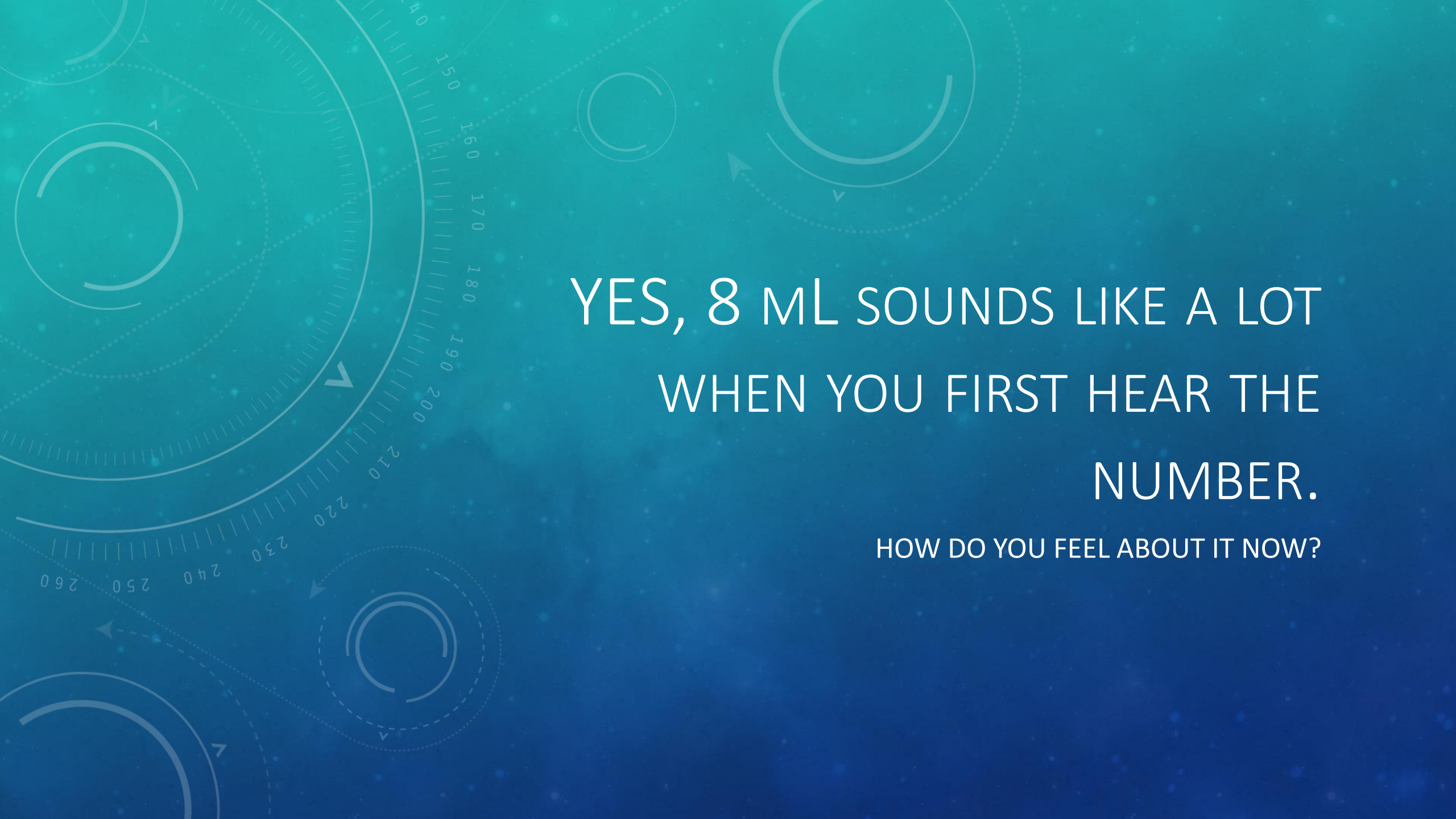
## A spectrophotometer with a rectangular (1 cm<sup>2</sup>) cuvette

- At least 3.5 mL is used
- A small percentage of the sample is used in the measurement
- The pathlength is 1 cm
- If the sample is not perfectly clear, the returned answer is wrong.

## A spectrophotometer with an integrating cavity (i.e., a CLARiTY UV/Vis)

- A few particles up to 8 mL of sample is used. The lower limit is that which produces a signal of 0.0001 AU/cm or larger.
- 100% of this sample is used in the measurement; there is nowhere for molecules to hide.
- When the filled cavity volume is used, the sample is up to 30x more dilute than in a 1 cm cuvette.
- The only obtainable answer is the correct absorbance. The answer is pathlength-corrected to Abs/cm when the volume is known (not always possible with solids).





YES, 8 mL SOUNDS LIKE A LOT  
WHEN YOU FIRST HEAR THE  
NUMBER.

HOW DO YOU FEEL ABOUT IT NOW?