



A Microfuge Tube Diode Array Spectrophotometer for Undergraduate Laboratories

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Problems encountered with traditional spectrophotometric methods

- **Precipitates**
- **Gases in solution**
- **Solution transfer into measurement cell**

Fuge tube spectrometer

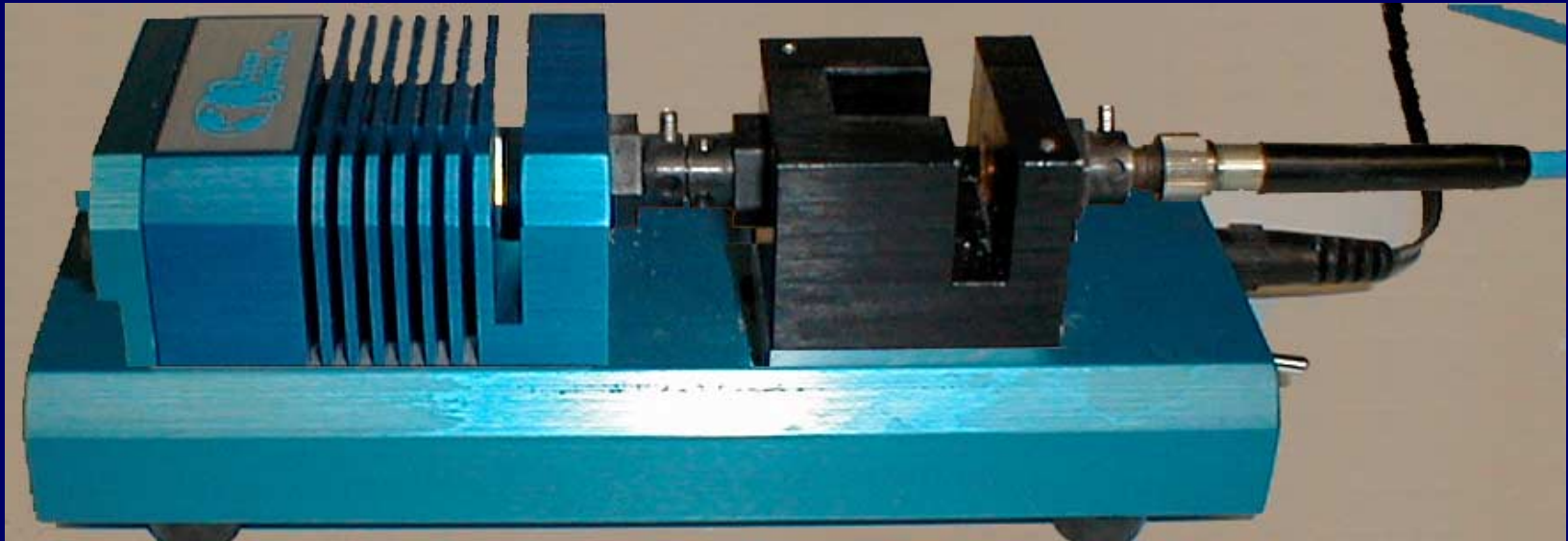
- One vessel experiments
- Can centrifuge to remove precipitates
- Will be able to use for spectrophotometric measurements

What is a fuge tube?

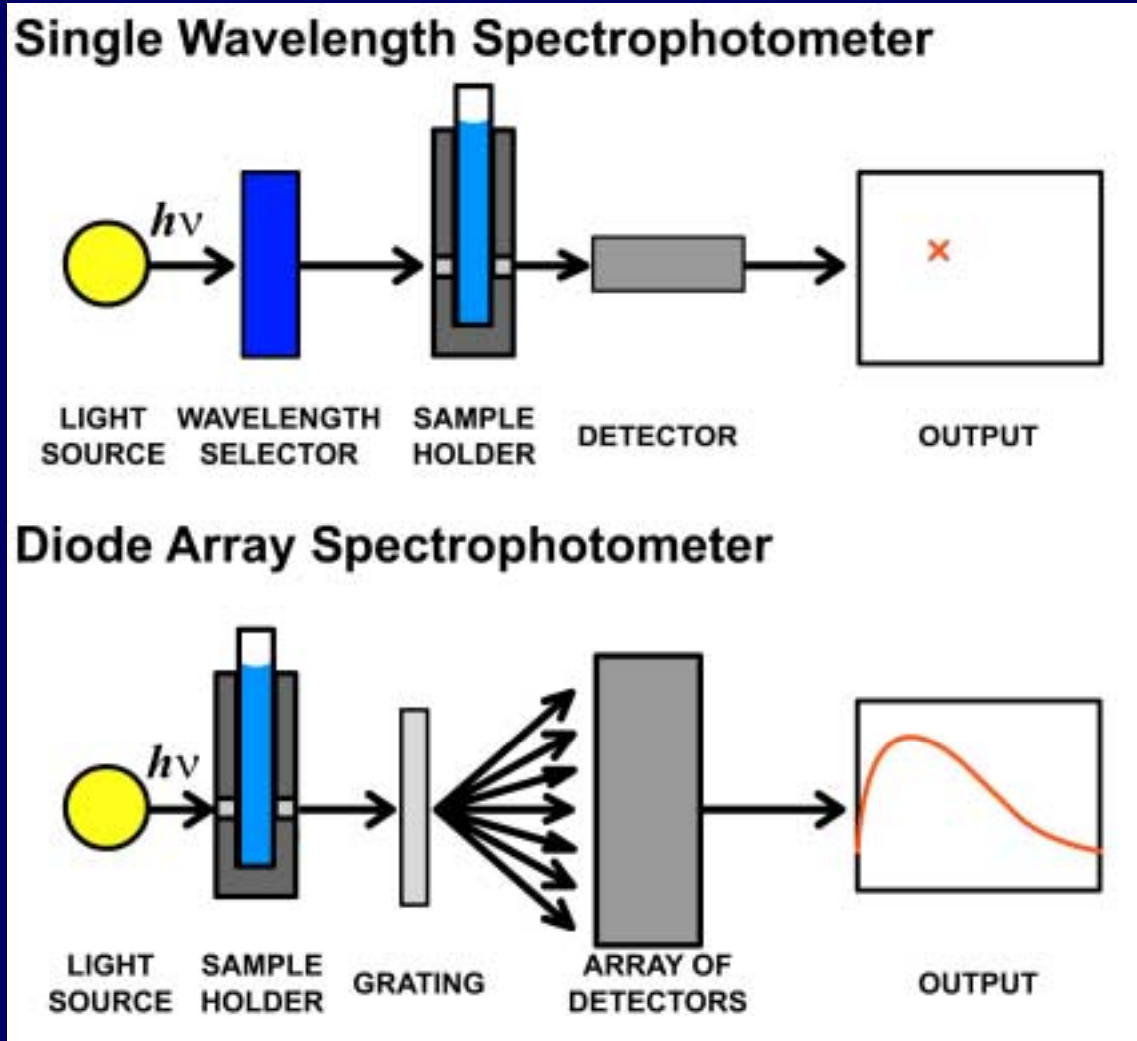
- Standard biotech sample vessel
- 1.5 mL Volume
- Plastic
- Cheap - pennies per tube



Diode array spectrometer

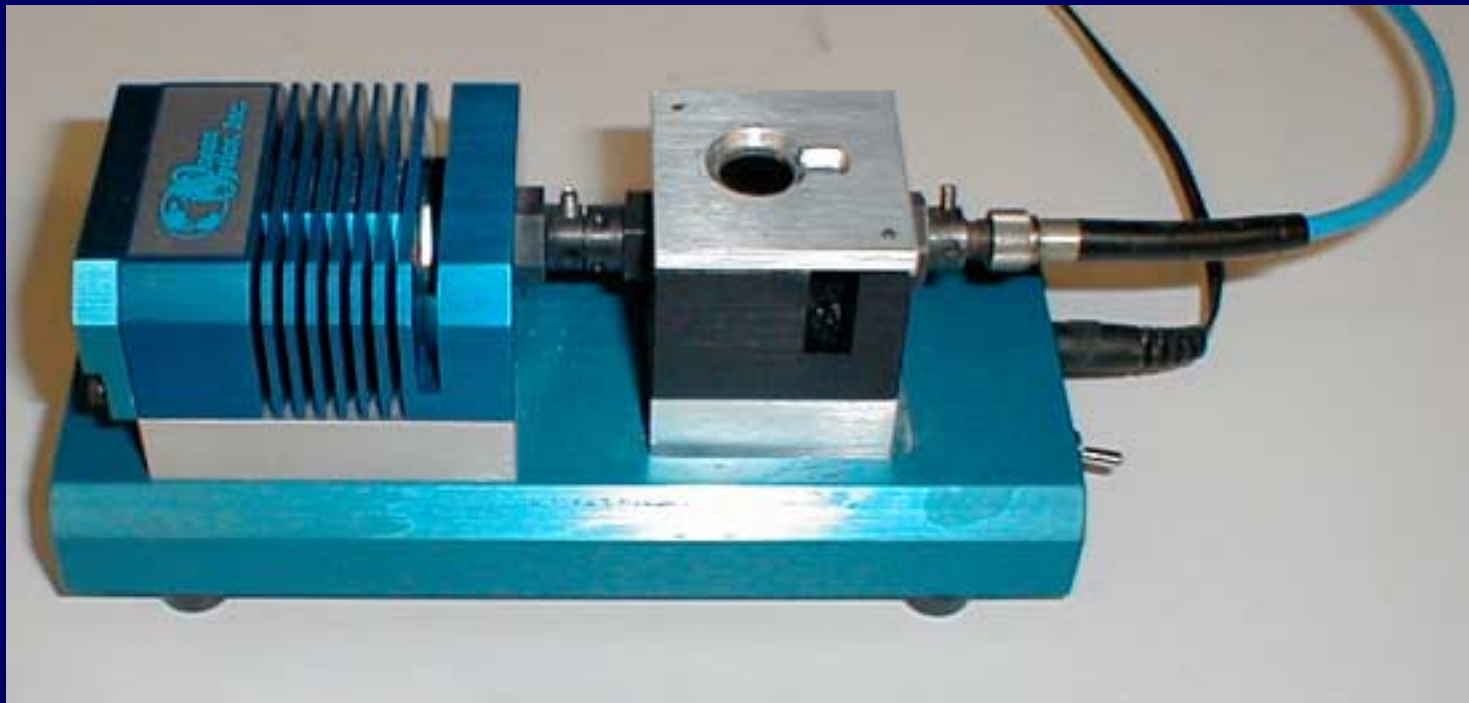


Diode array spectrometer



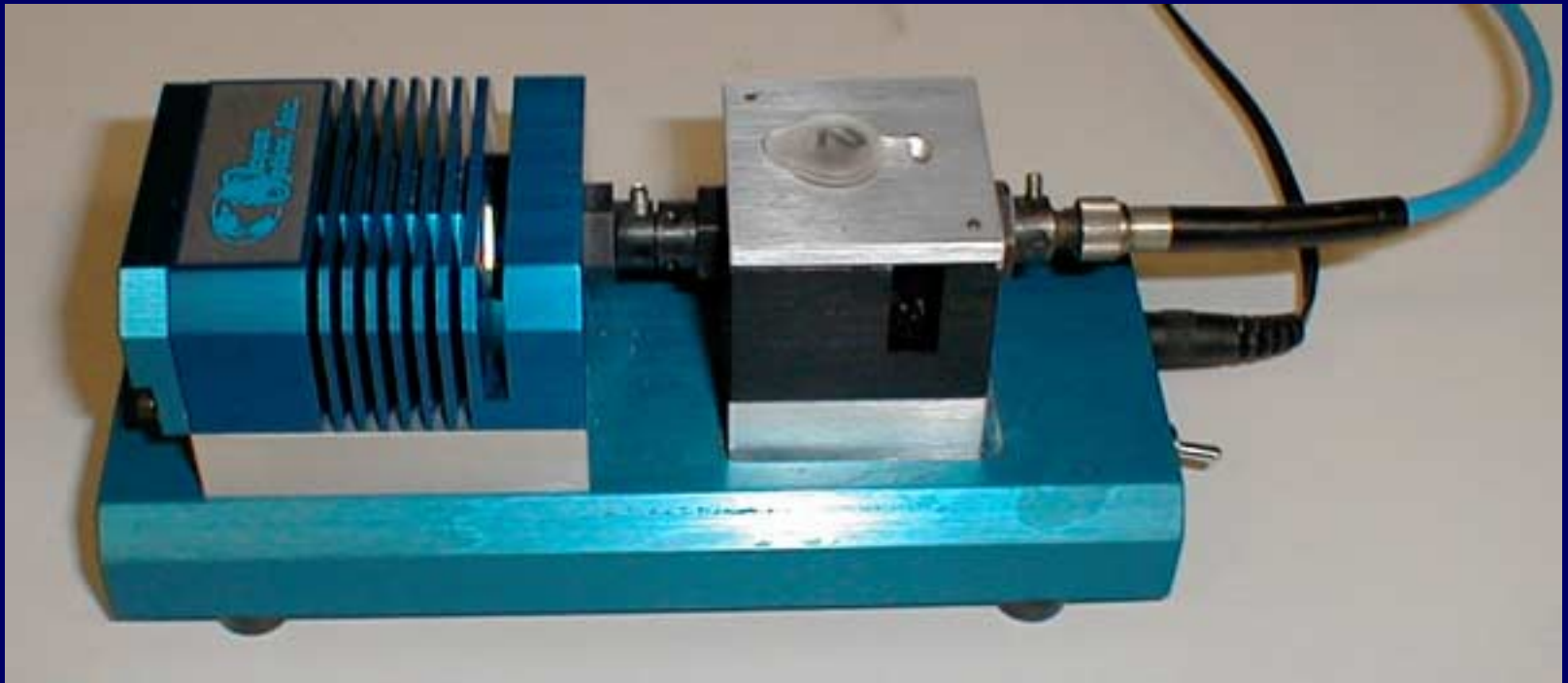
Combine the two

- Modify sample chamber to hold a fuge tube



Combine the two

- Fuge tube aligned in sample chamber



Precipitation Reaction



Blue

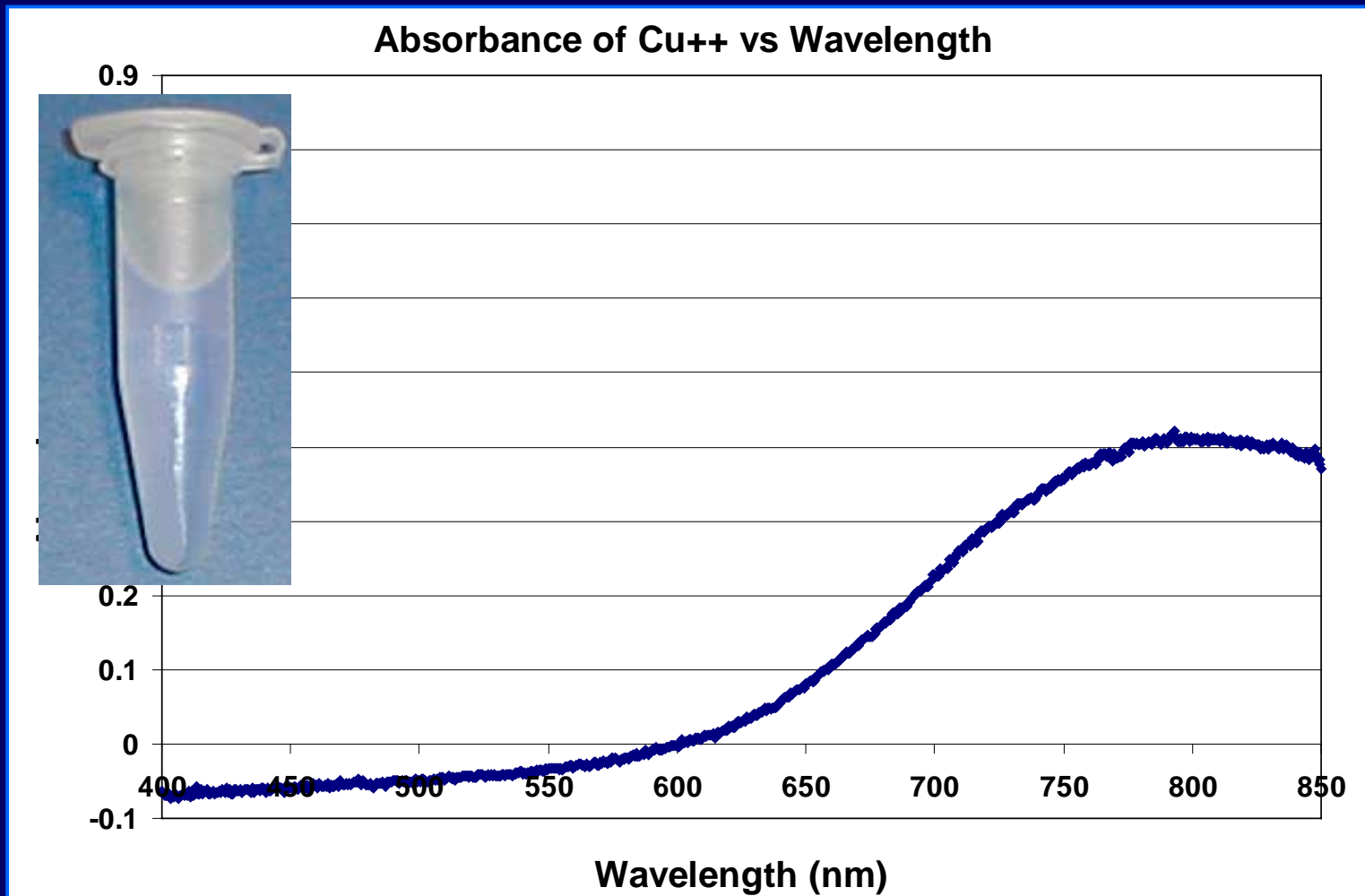
Bluish White

Start with Cu Solution

- Picture of Cu solution in tube



Spectra of Cu Solution

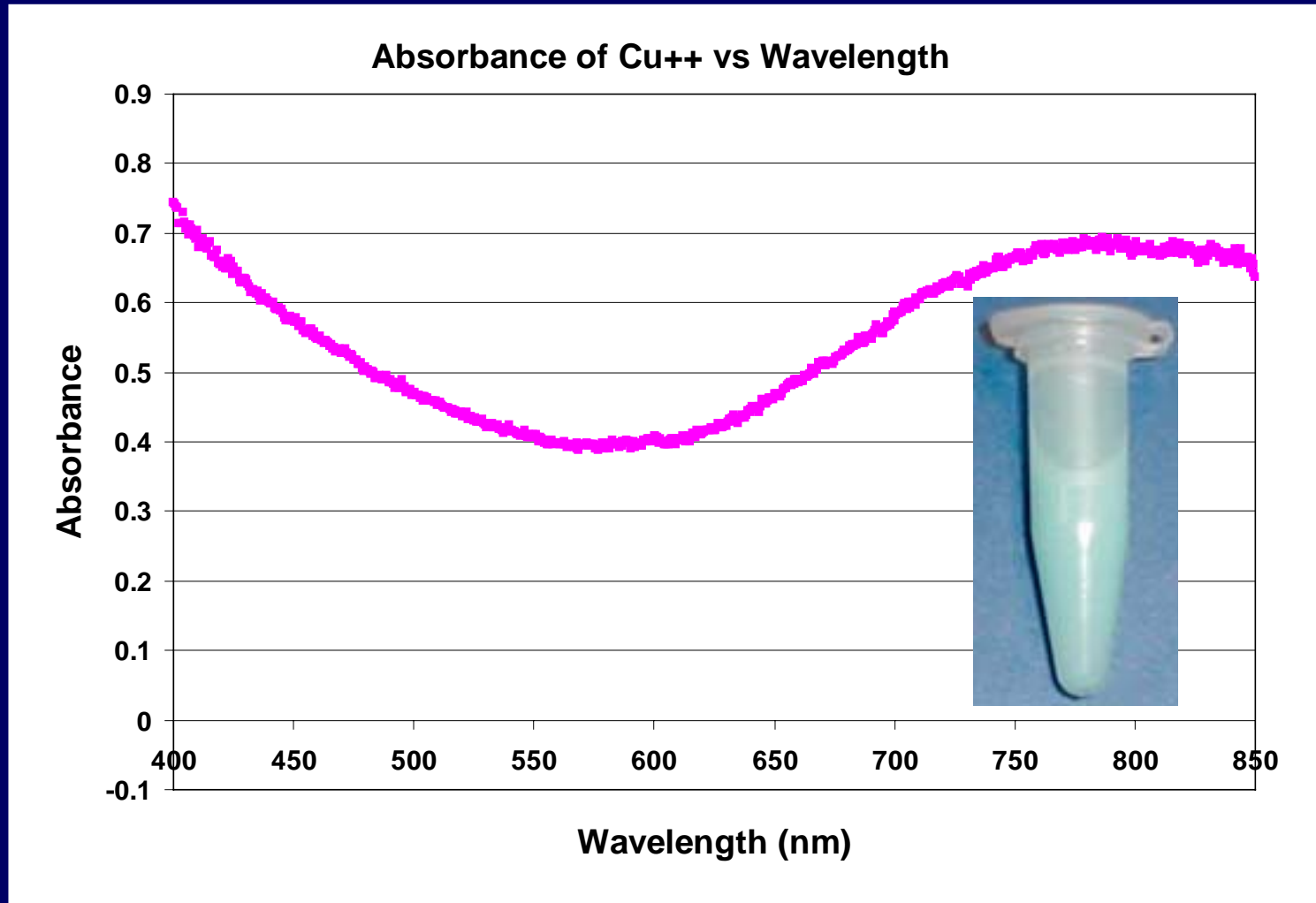


Add Hydroxide

- Picture of reaction mixture



Spectra of Reaction mixture



Centrifuge the Reaction Mixture

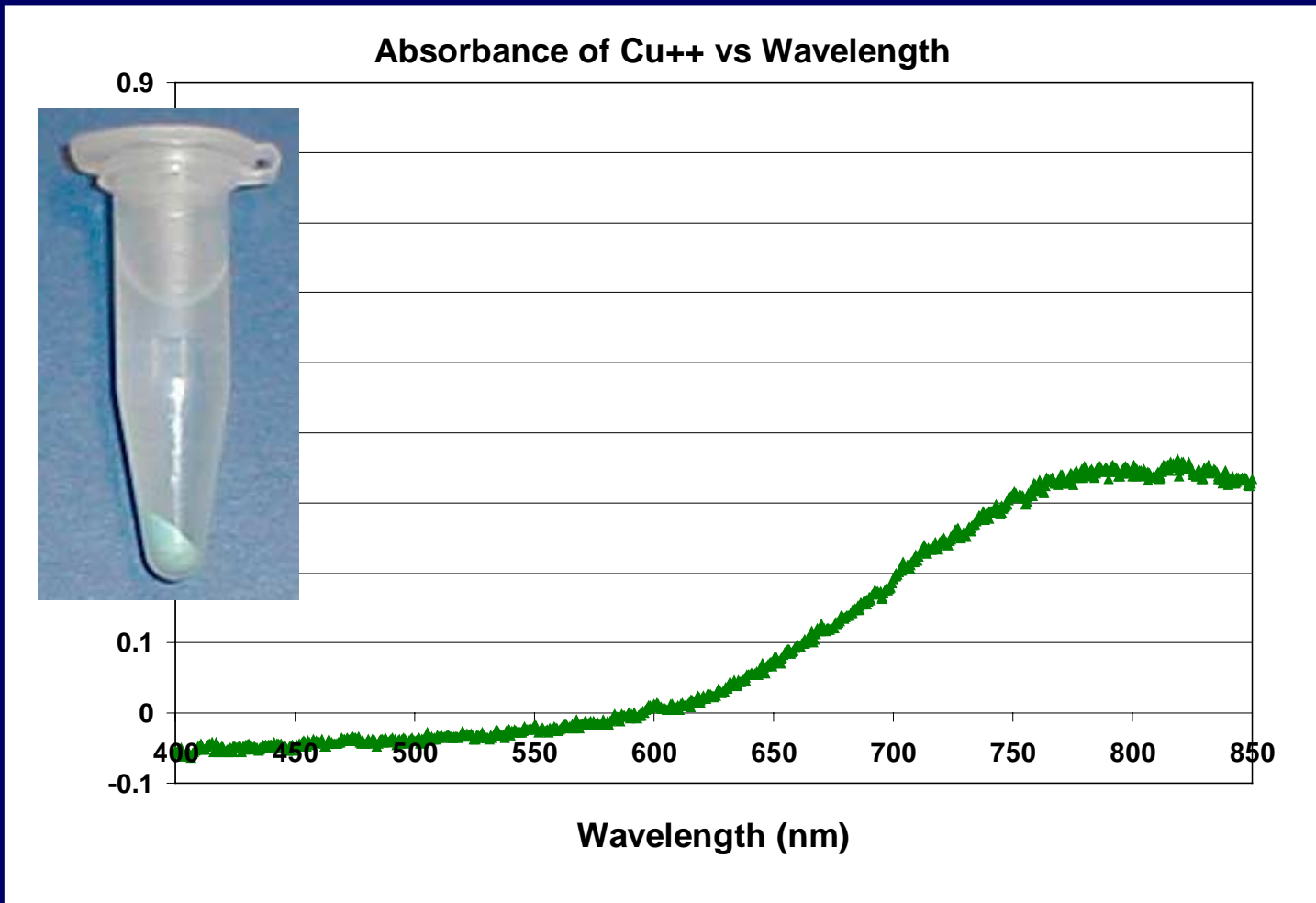
Before



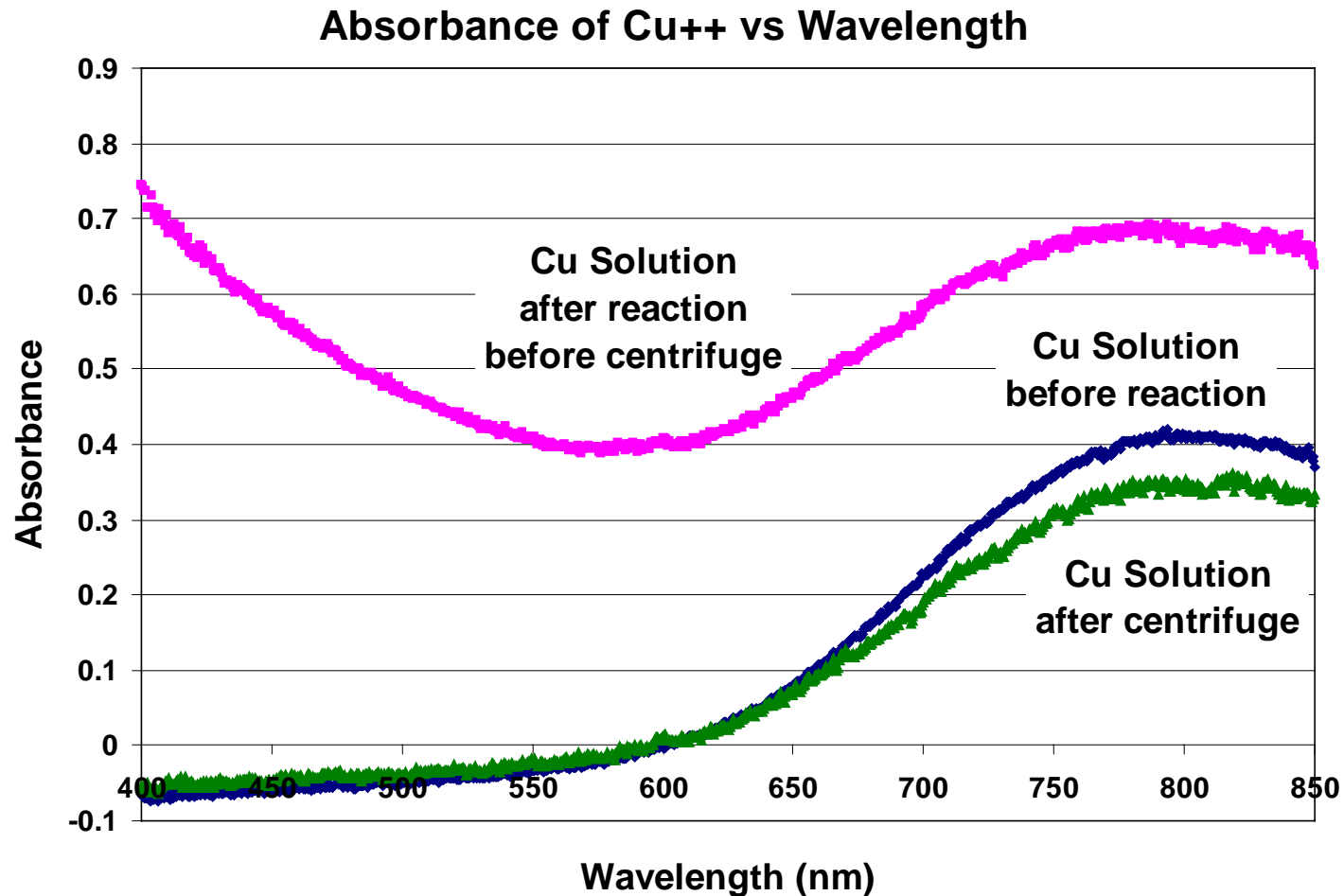
After



Spectra of Supernatant



Combination of Spectra



Prepare Several Solutions of NaOH

- Vary the concentration of NaOH added

Tube 1 0.200 mL of 0.000 M NaOH added

Tube 2 0.200 mL of 0.063 M NaOH added

Tube 3 0.200 mL of 0.125 M NaOH added

Tube 4 0.200 mL of 0.250 M NaOH added

Tube 5 0.200 mL of 0.375 M NaOH added

Tube 6 0.200 mL of 0.500 M NaOH added

Follow the stoichiometry with spectra

$$[\text{Cu}^{2+}]_{\text{original}} = 0.050 \text{ M}$$

In tube 2 after reaction:

$$[\text{Cu}^{2+}]_{\text{final}} = \frac{[\text{Cu}^{2+}]_{\text{original}} * V_{\text{Cu}^{2+}} - 0.5 * ([\text{OH}^-] * V_{\text{OH}^-})}{V_{\text{final}}}$$

$$[\text{Cu}^{2+}]_{\text{final}} = \frac{0.050 \text{ M} * 1.000 \text{ mL} - 0.5 * (0.063 \text{ M} * 0.200 \text{ mL})}{1.2 \text{ mL}}$$

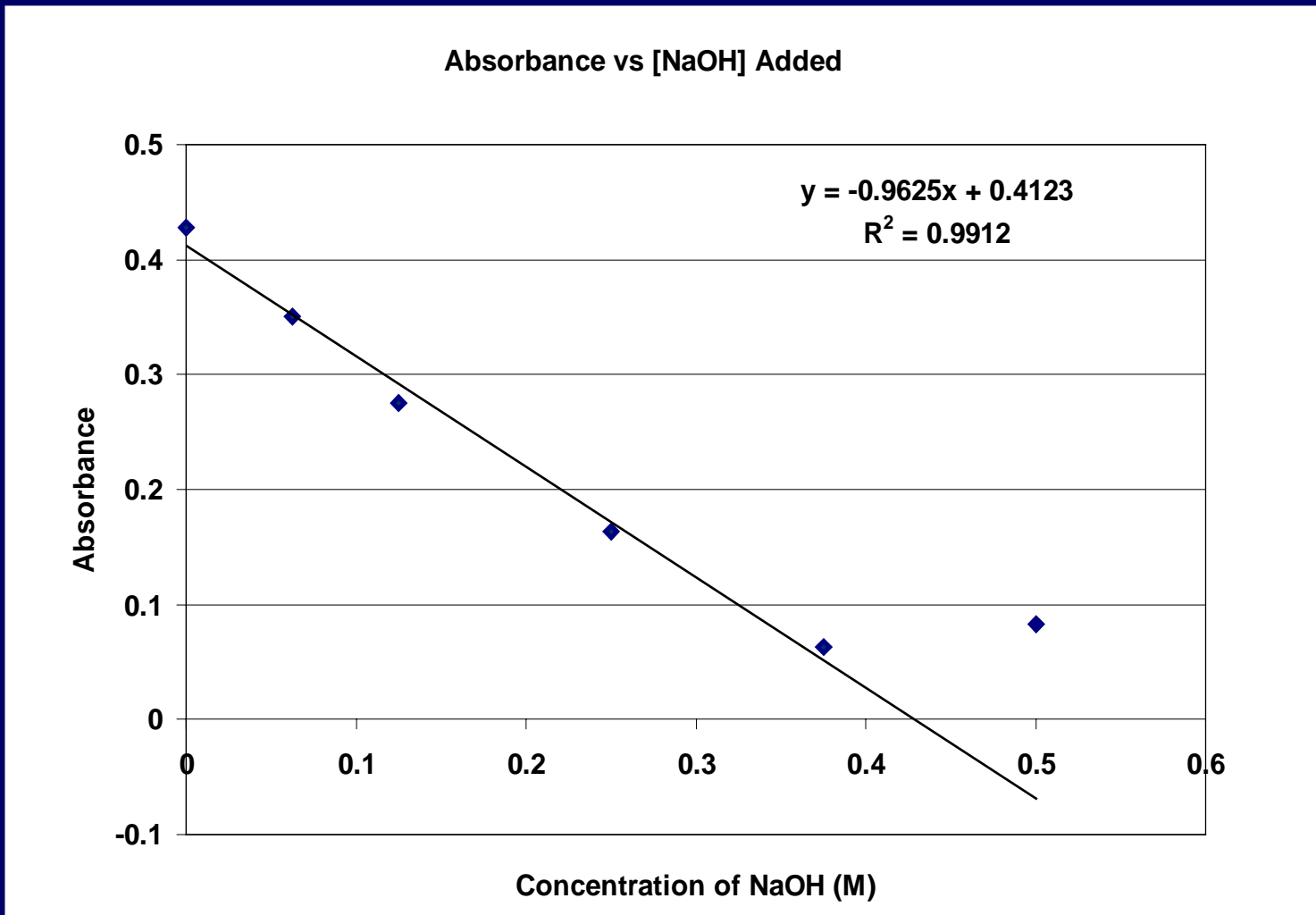
$$[\text{Cu}^{2+}]_{\text{final}} = 0.044 \text{ M}$$

Follow the stoichiometry with spectra

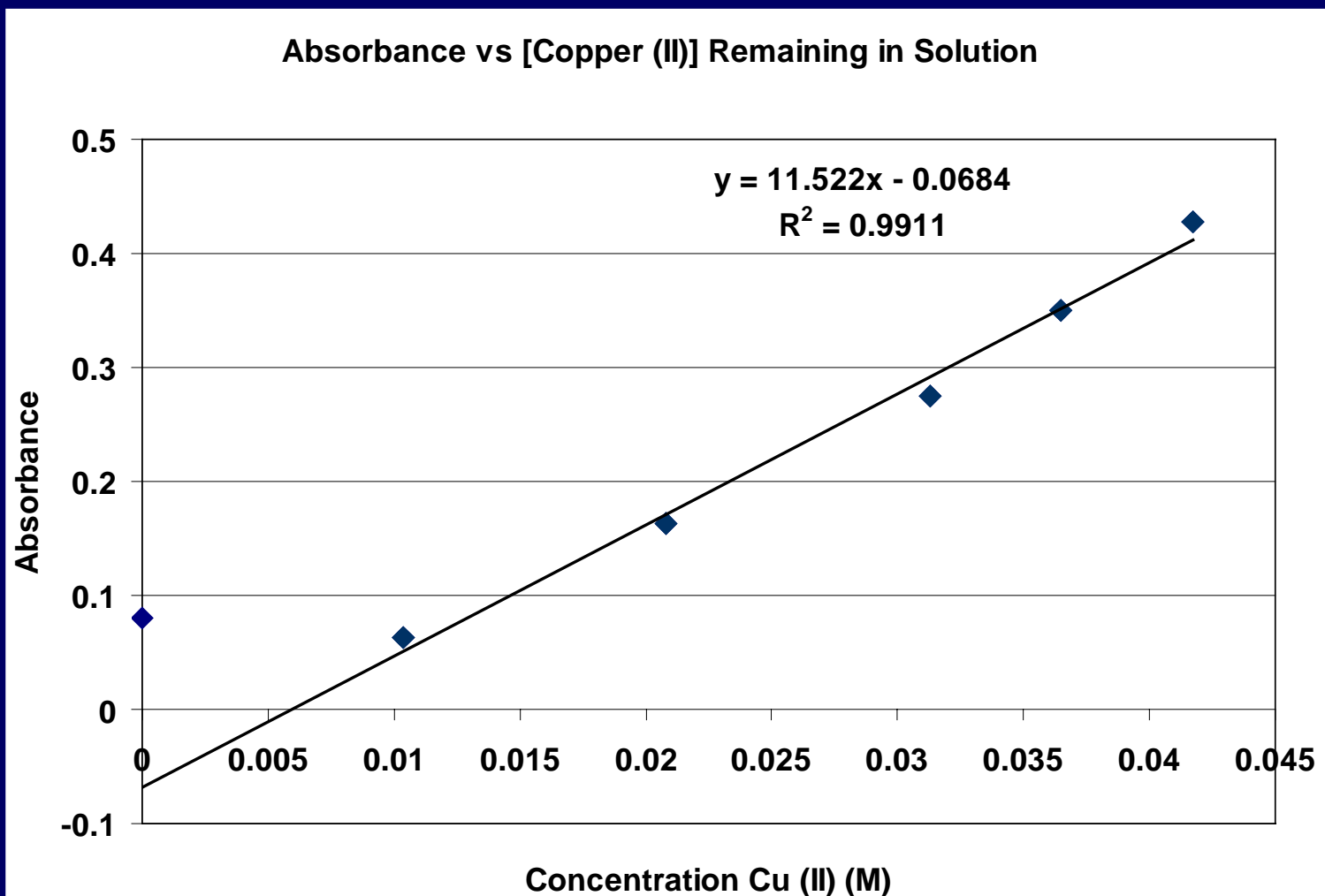
- Calculations of concentration with measured absorbancies


	<u>Final Concentration</u>	<u>Absorbance</u>
Tube 1	0.042 M Cu ²⁺	0.428
Tube 2	0.036 M Cu ²⁺	0.350
Tube 3	0.031 M Cu ²⁺	0.275
Tube 4	0.021 M Cu ²⁺	0.163
Tube 5	0.010 M Cu ²⁺	0.063
Tube 6	0.000 M Cu ²⁺	0.080

Plot of Concentration vs Absorbance



Plot of Concentration vs Absorbance





**Fuge tube allows for
spectrometric analysis of
reactions that produce
precipitates**

Also used for Beers Law Measurements

- Standard curves developed for species at 420 nm, 500 nm, and 620 nm
- All produce a linear response from 0.05 - 2 AU, one species linear to 3 AU
- Correlation coefficients are 0.99 or better

Blanking

- **Concern:**
Different fuge tubes may have different absorbances

Blanking

- **Original experiments showed acceptable results**
- **Further experiments showed some fuge tubes vary in absorbance**

Blanking

- However, the variance seems to occur in sets
- Possible to select a group of fuge tubes with similar blank absorbance

Blanking

- Possible solutions

- Use a baseline correction

- Optically blank the different tubes before use

Conclusion

- A fuge tube spectrophotometer has been developed
- Precipitates can be spun out of solution and the supernatant absorbance can be measured
- The system is linear throughout the visible light region

Future experiments

- **Solutions to blanking problems**
- **Gas evolution reactions**
- **Biochemical Assays**
- **Spectrophotometric Titrations**



THANK YOU!