

USING THE SPECTRONIC 20D

1: Colors and wavelengths

Turn the machine on and allow to warm up
Half fill a disposable glass test tube with tap water: dry the outside.
Add a pinch of chalk dust, cover with Parafilm and mix
Set the wavelength to 450 nm
Insert the test tube, leave the cover open
Turn the right hand knob as far as it will go
Look straight down into the test tube, note the color.
Increase the wavelength by 50 nm and repeat. Continue to 650 nm

2. Absorption spectra

Take one tube of red dye, one of blue (both at 1600 micrograms/ml)
and one of water
Set the wavelength to 475 nm
Follow the instruction sheet to blank the machine and measure the
absorbance of the red and blue dyes
Increase the wavelength by 25 nm and repeat. Remember: every time
you change wavelength you must reset to 100%T with the blank.
Continue to 650 nm: reset the filter lever at 600 nm
Plot the absorption spectra of both dyes. Look at the high and low areas.
How do these fit with the colors you observed in part 1?

3. Transmission vs Absorbance

Remeasure the absorbance of the red dye (1600 $\mu\text{g/ml}$) at 500 nm
Note the % Transmission
Take 2 mls of the red dye and place it in a fresh tube.
Add 2 mls water, cover with Parafilm, and mix (now at 800 $\mu\text{g/mls}$)
Measure the Absorbance and % Transmission
Keep diluting and measuring down to a 100 $\mu\text{g/ml}$
Plot Concentration on the X axis and % Transmission and Absorbance
on the two Y axes.
Which is closer to a straight line?

USING MICROPIPETTES

1) Choosing and setting the pipette

- Generally always use the size that is the closest equal or larger device.
 - For example, if you wish to measure 60 μL , use a 10-100 μL pipette.
 - If two pipettes both fit, then use the smaller.
- Loosen the locking device: turn the adjustment knob to the correct reading (NEVER force it beyond its limits: it can permanently ruin the pipette)
- Pick up the appropriate tip (usually color coded, but not always)

2) Transferring liquid

- Press down on the plunger until a slight hesitation is felt
- Insert the end of the tip under the liquid
- Slowly release the plunger
- Move the pipette to the destination
- Press down on the plunger to the second stop
- Remove the pipette: release the plunger
- Discard the tip into a waster container by pressing on the release button

3) Checking accuracy and repeatability

- Each member of the group should do this
- Measure 5 mls of water into a test tube
- Add 20 μL of red dye to the tube and mix
- Determine the absorbance at 500 nm
- Compare your results: this is a measure of the variation that comes from errors in handling. Keep this in mind when looking at results in later experiments

You will be using these two skills throughout the semester. I will occasionally quiz you (as a group or individually) at various times. In addition there will be a practical quiz at the end of the semester where you will be required to demonstrate proficiency in both skills.