

## Ultraviolet-Visible (UV-Vis) Spectrophotometer Standard Operating Procedure

### Starting Procedure:

1. Turn on the machine with the switch on the front, bottom left corner.
2. Make sure that the proper sampling accessory is loaded.
  - Open the top door and remove the white box on front.
  - Typically, the cuvette holder should be in the machine. With this you can do single- or dual-beam experiments with one sample at room temperature.
  - To take out the accessory, use a screwdriver to unscrew it from its brace and remove it.
3. If you want to perform temperature-controlled experiments or run it for multiple samples, you can use the sample block, which allows for 12 samples and is temperature controlled. Ask the CBIC staff for help setting up the sample block, but in general:
  1. Turn on the water circulator underneath the UV-Vis for temperature control.
  2. Hook up the cables underneath the sample block, the large and small plugs.
  3. There is a removable plate on the white box you removed before. There is a similar one attached to the sample block that allows water to circulate while still blocking out light.
  4. Screw in the sample block to the brace inside the machine
  5. Replace the white box, sliding the plate attached to the sample block into the white box.
4. Another available accessory is the integrating sphere, which is in what looks like a gray tackle-box.
  - To install, first plug in the cable underneath the accessory, but this time the correct plug is actually located inside, toward the front of the machine. Then screw in the accessory to the brace.
  - The sample is typically loaded onto a slide which is placed into the front of the accessory. The wedge in the accessory allows you to do diffuse reflectance with the sample held at an angle. You can also take out the wedge to do the sample straight-on for specular reflectance.
  - To zero the accessory, use the included pure-white puck (of course without letting in stray light)

### Performing Experiments:

Note: There are a great number of experiments this machine can do, but for this procedure I will explain basic ones using the standard cuvette holder. Please ask CBIC staff for help if there's a different experiment you want to perform, or if you have any questions.

1. All of the different modes are separate programs, which are located on the desktop in the "Cary WinUV" folder. I will walk you through the most commonly used programs, which are "Simple Reads" and "Scan".
2. "Simple Reads" measures the absorbance for a single wavelength at a particular point in time. To use "Simple Reads":
  1. Start the program.

2. Place a blank cuvette (empty or only solvent) into the holder and close the top door. Click “Zero” on the left side of the screen. The absorbance should go to 0.
3. Place your sample inside. There should now be an absorbance at that particular wavelength.
4. To change the parameters, click “Setup”. Here you can change the wavelength, as well as whether the output is absorbance, transmission, etc.
3. “Scan” collects a spectrum at a variety of wavelengths. To use “Scan”:
  1. Start the program.
  2. Click “Setup” to change the parameters.
    - In the “Cary” tab, you can customize the range of wavelengths as well what you want the output to be (absorbance, transmission, etc.). You can also adjust the averaging time and/or the data interval, but the defaults should be fine unless you have a reason to adjust these parameters.
      - Note: if you want to scan in the ultraviolet range, make sure you use quartz cuvettes as glass and plastic will absorb UV.
      - If you have a different accessory in the machine, there may be additional options in the “Accessory” tabs.
  3. If you want to collect a baseline, click the “Baseline” tab and select an option (in this case, “Zero/baseline correction”).
    - To do this correction, place the blank sample inside and click “baseline”. It will collect the 100% transmission. Then, to collect the 0% transmission, block the beam on the left side with the plate.
  4. Place your sample inside and click “Start” at the top.
  5. It will ask you about where to save the data it collects.
    - If you don’t have a folder, make one with your username.
    - Write a filename for the data to be collected, and choose your folder as the save location. Regarding filetype, “.CSV” is the easiest to deal with so you may want to save it as such.
    - In the next “Sample name” window, you can name your sample, which allows for multiple samples in a single file.
  6. You can also save your data after it was collected by clicking “File” —> “Save Data As” and filling out the filename, file type, location, etc.
4. Other programs that are available include: “Thermal”, which monitors a single wavelength as a function of temperature; “Kinetics”, which monitors a single wavelength as a function of time; and several others for more complicated experiments.

#### Shutting Off:

1. Close the window.
2. Make sure the temp-control water supply is off if it was used.
3. Take out your sample.
4. Turn off the spectrophotometer.