

## APPENDIX VIII: OPERATION OF THE SHIMADZU UV-1601PC UV/VISIBLE SPECTROPHOTOMETER

### A. Instrument Setup

1. Push the **power** switch on the lower left side of the Shimadzu UV-1601PC UV/visible spectrophotometer to turn on the instrument.
2. Then push the **power** buttons on the Micron computer, the monitor and the Epson printer.
3. Double-click the **UVProbe** icon, followed by the **OK** button. The UV Probe software will load and the Spectrum window will appear.

### B. Baseline Correction

1. Open the spectrophotometer's sample-chamber cover. Make sure that neither the sample beam nor the reference beam is obstructed, and that there is **no sample** in the sample compartment.
2. Add **2 mL of the solvent** to a quartz cuvette and place the cuvette in the sample compartment. (**Caution:** Quartz cuvettes are **very** expensive! Only touch the frosted, not the clear surfaces. When preparing a solution for analysis, be careful not to contaminate the sample nor the equipment with acetone, which has a large absorption maximum at 268 nm.) Close the sample-chamber cover.
3. Click the **Connect** button, followed by the **Baseline** button. The Baseline Parameters window will appear. Enter the appropriate **wavelength limits**. Then click the **OK** button to acquire a background spectrum.

### C. Method Preparation

1. Select the **File > Open** menu item. Then choose the desired **method** (e.g., **caffeine.smd**) and click the **Open** button. The Spectrum Method window will appear.
2. Set the following parameters in the Measurements subwindow.
  - a. Wavelength Range: Choose the appropriate **wavelength limits**.
  - b. Scan Speed: **Medium**.
  - c. Sampling Interval: **1.0**
  - d. Scan Mode: **Single**.

3. Click on the **Instrument Parameters** tab. Then choose the **Absorbance** Measuring Mode. Click the **OK** button to send the method parameters to the instrument.

#### D. Sample Preparation

1. Open the sample-chamber cover and remove the cuvette. Empty the cuvette. Then use a solution of the sample dissolved in the solvent to **rinse the cuvette** twice. Use about 1/2 mL for each rinse.
2. Once the cuvette is rinsed, add approximately **2 mL of the sample solution** and place the cuvette in the sample compartment. Close the sample-chamber cover.

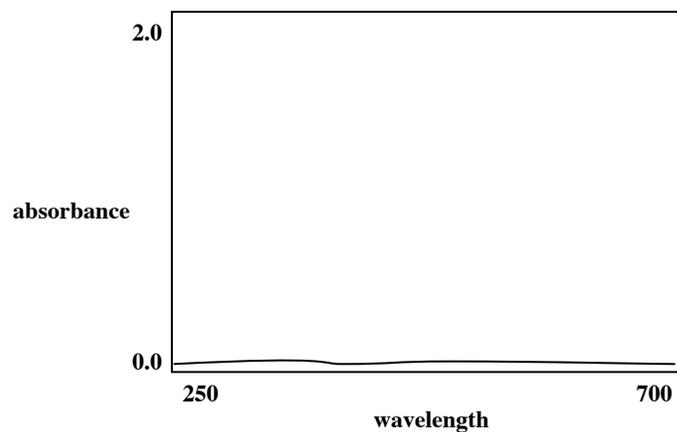
#### E. Data Acquisition

1. Click the **Overlay** tab to place the spectrum in Overlay mode. Click the **minimum absorbance** value on the Y-axis and set it to 0. Then click the **maximum absorbance** value on the Y-axis and set it to 2. (Note: If the acquired spectrum does not display properly on the graph, right-click the mouse and choose the Auto Scale function to adjust the graph's coordinates.)
2. Click the **Start** button to begin the scan. Watch the spectrum trace in the graph pane to see that the absorbance values do not exceed the upper limit during the acquisition. If the upper limit is exceeded, the scan will need to be repeated with a larger upper absorbance limit and/or a more dilute solution.
3. The New Data Set window will appear after the scan is complete. Enter an appropriate **File name**.
4. Click the **Print** button to print the spectrum.
5. After an acceptable spectrum has been printed, open the sample-chamber cover, **remove the sample** from the instrument and close the sample-chamber cover. (Three spectra are shown on the next page to illustrate the preferred appearance of a uv/visible spectrum.)
6. **Clean the cuvette** thoroughly (without acetone!) and return it to the instructor.

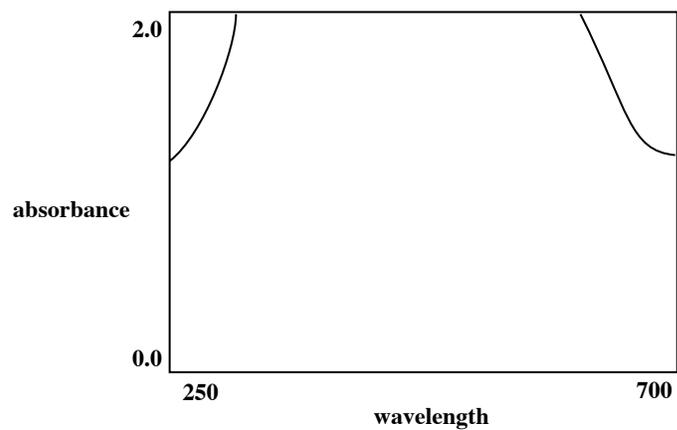
#### F. Instrument Shutdown

1. Select the **File> Exit** menu item to close the UV Probe software.
2. Push the **power** switch in the lower left corner to turn off the spectrophotometer.

a. Too dilute a sample. The spectrum is barely visible from the baseline. Unacceptable.



b. Too concentrated a sample. The spectrum goes off-scale. Also unacceptable.



c. Proper sample concentration. Just right!

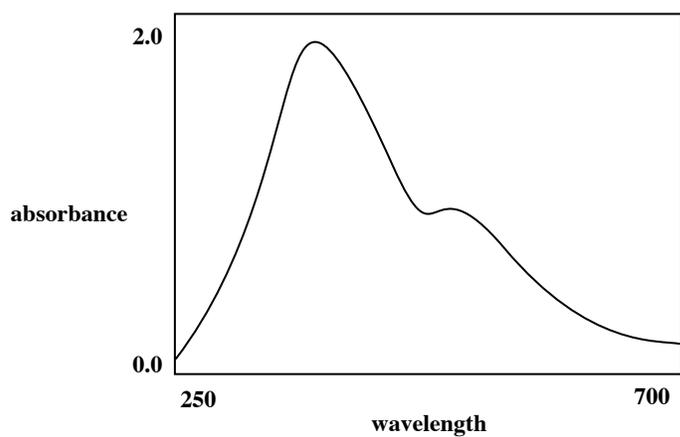


Figure 49. Appearance of an ultraviolet/visible spectrum.