Agilent 8453 UV-Vis Operating Instructions
02/07/2012 S.V.

Location: **1240 Hach Hall**
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Safety

All researchers working in 1240 Hach Hall must complete the EH&S course: *"Fire Safety and Extinguisher Training"*. When preparing samples in this room, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available in 1238A Hach Hall. If solvents are involved, consider preparing your sample in room 1238A.

Properly dispose of glass pipettes in the container provided. Waste solvents can be disposed of in the waste containers provided in 1238A. All of the computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Introduction

The Agilent 8453 UV-Vis instrument is a simple but powerful diode-array spectrophotometer capable of quickly acquiring data in the spectral range from 190 to 1100 nanometers. The instrument was purchased in 2006.


Agilent 8453 UV-Vis Spectrophotometer

The instrument is normally available for walk-on use. As part of your training, a login account will be established for you on the acquisition PC.

Instrument Startup

1. Turn on the instrument. The switch is at the lower left corner.

2. Log on to the computer using the username and password established during your training. The username should be same as your ISU netID.
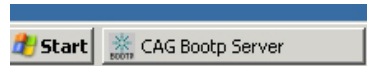

NOTE: A special program called "CAG BootpServer" will start when you login. This program must be running in order for communication to be established between the PC and the instrument.


3. Double-click on the UV-Vis program icon to start the program. A program login box will appear. We do not use this feature of the software. **Simply press "cancel".**
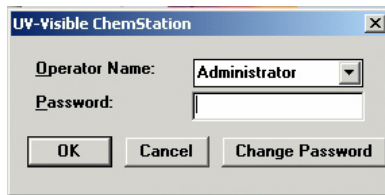
**4**. The software should now open in the standard view.
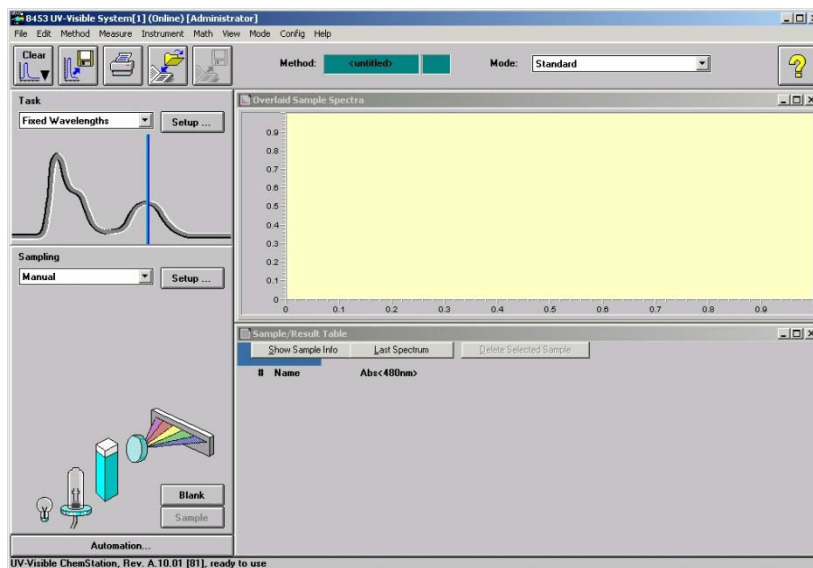
5. Click on each lamp icon in succession to turn them on. The lamps take a few seconds to light. For critical work, you should allow the lamps to stabilize for about twenty minutes. Routine measurements can be performed immediately.
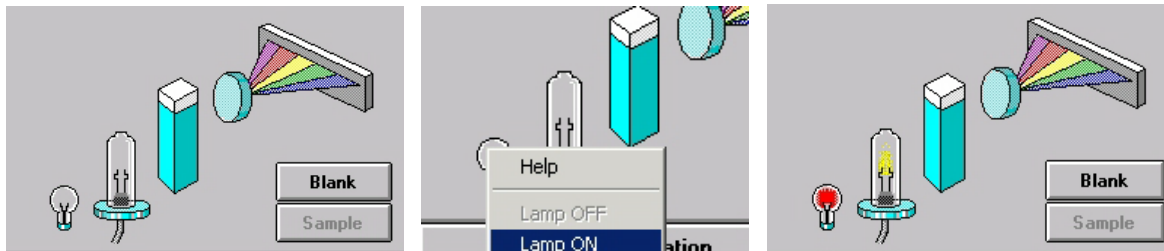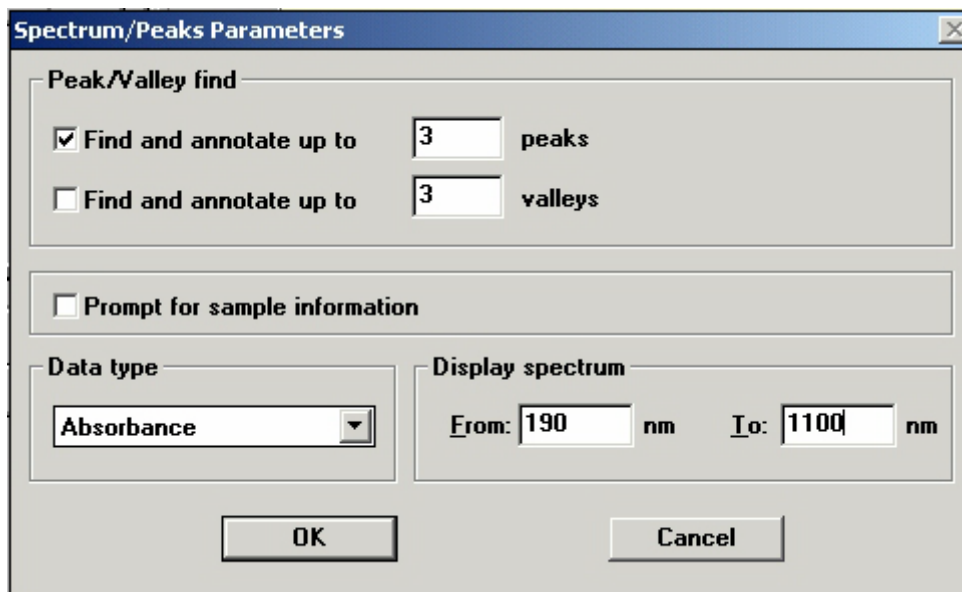

Lamp ignition sequence.

6. The software opens in "Fixed Wavelength" task mode. Most users will want to change this to "Spectrum / Peaks" mode.
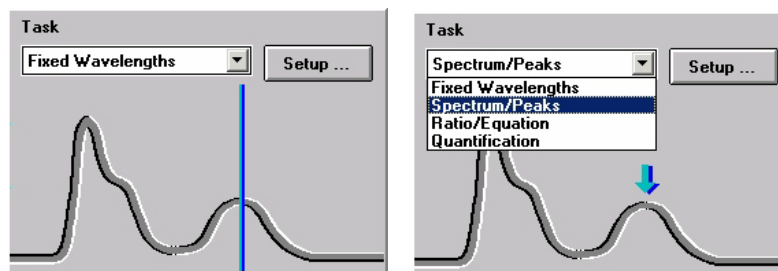
Change the Task mode to "Spectrum / Peaks"

Changing to a different Task mode will automatically open the "Setup…" dialog box. Adjust these settings to meet your needs. The allowable limits are from 190 nm to 1100 nm.

Acquiring Data

1. With nothing in the sample holder, select "Blank" from the source pane.
If the lamps have been allowed to stabilize, the resulting "instrument blank" spectrum should be straight, with the noise level below +/- 0.002 AU in the range from 190 nm to 1000 nm. The noise will be higher in the region above 1000 nm.
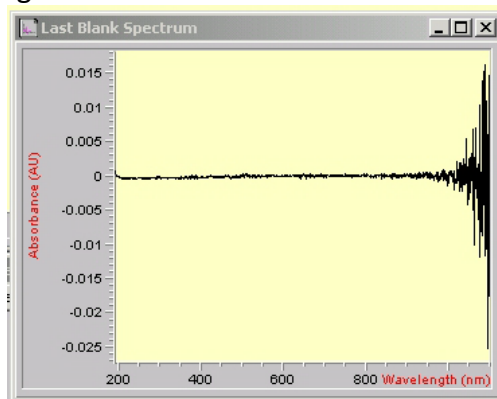
Typical "Blank" spectrum

2. After running an instrument blank, the previously grayed out "Sample" icon will now be accessible. Place your sample in the holder, select "Sample". The measurement will only take a few seconds.
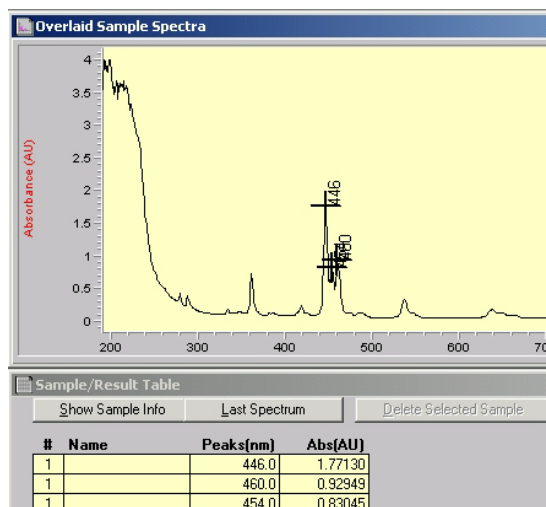
Cuvette mounted in the sample holder   *Typical Sample Spectra and Results Table*

NOTE: The proper measurement procedure will depend on the nature of your sample. Typically, the cuvette with solvent would be run as "Sample" after running the instrument blank. This allows you to profile the absorption characteristics of your cuvette and the solvent.

If the cuvette/solvent results are acceptable, the <u>cuvette with solvent</u> would then be run as a "Blank". Then the <u>cuvette with solvent and sample</u> would be measured as "Sample".

Processing Data

Data is not stored automatically. Each successive "Sample" measurement is overlaid in the Overlaid Sample Spectra view and added to the Results Table. You may manipulate the current data; including zooming in, annotating more or fewer peaks, and printing results, but when you exit the program your data will not be automatically saved.

1. Use the **Printer** icon located in the tool bar to print the spectrum currently in the view and a tabular report of the peaks.   Alternatively, use *File -> Print -> Selected window* to print a highlighted view (the spectrum or results table).
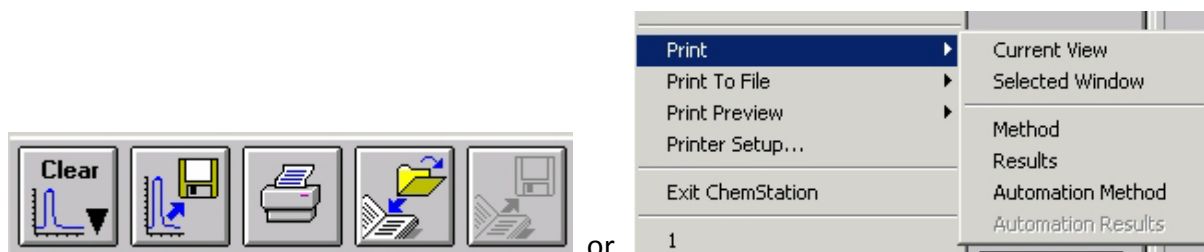

2. A spectral region may be selected by dragging a box around the area of interest.
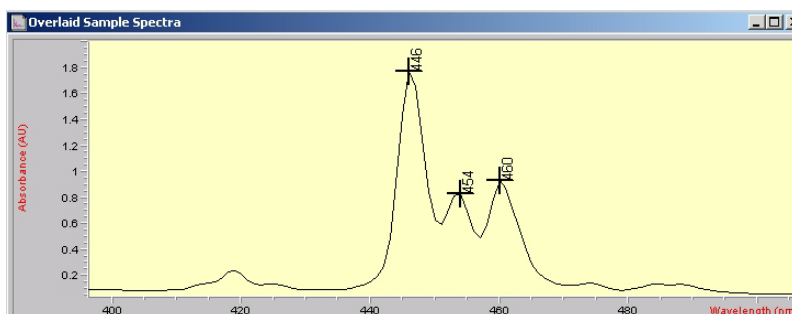
Use *View -> Reset Current View* to return to the full spectral display.

3. Additional spectra may be acquired and overlaid. To delete a spectrum from the view, select it by clicking on the appropriate trace in the Sample Spectra view. Diamond-shaped points will appear on the selected trace, and a "Delete Selected Sample" button option will appear below the Sample Spectra view. The selected trace may now be deleted.
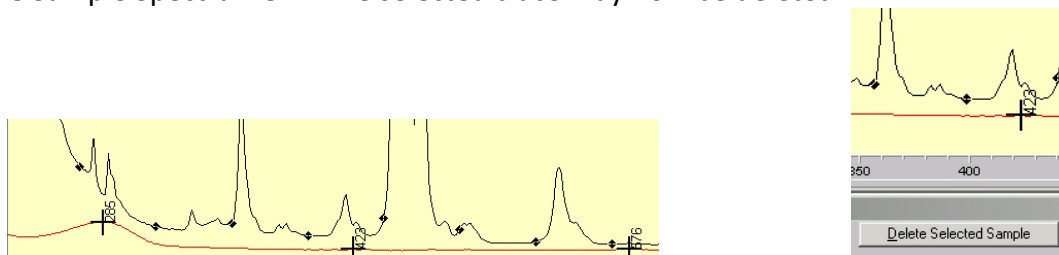
4. To save data files, click on the **Spectrum to Disk** icon located in the tool bar. Alternatively, select *File -> Save -> Samples As* from the File pull down menu. Only store your files in the data

directory that has been established for you. You may then e-mail the files to yourself. To avoid possible virus transmission, please do not attach USB thumb drives to any of our computers unless the thumb drive has been scanned for viruses and approved for use.


Finishing Up

**1.** Remove your cell form the instrument sample holder.

2. Choose the **Clear** icon and select *Samples* to clear the display.

3. Choose the **Lamp** icons and select *Lamp Off* from the menu. Both lamps must be turned off.

4. From the File pulldown menu, select *Exit ChemStation*.   The "Close 8453 UV-Visible System" dialog box will open. Do NOT select Save Configuration unless you are developing specific methods for your use only. Select *OK* .
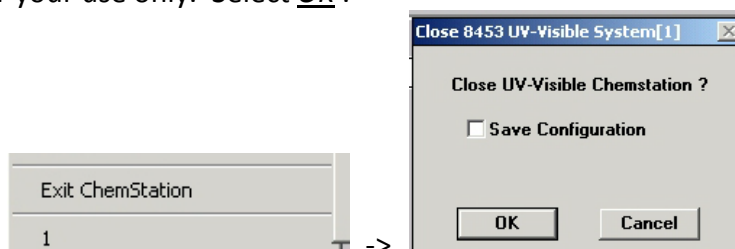
5. When you returned to the Windows Desktop, you must Close the **CAG Bootp Server** program, or the Windows XP operating system may not allow you to log off.
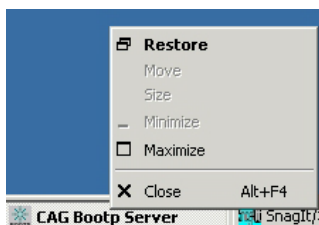
6. Be sure to log off the computer. You are charged based upon the logon / logoff records.

7. Please turn off the instrument power unless you plan to return the same day.