Agilent 8453 UV/Vis Spectrometer with DAD Detector

Updated November 14, 2017

Instrument instructions can be found at:
http://academic.bowdoin.edu/chemistry/resources/instructions.shtml

If you have any problems with the instrument or would like to get trained, please contact
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1. Protocol

a. **Read instructions carefully before using instrument**. Reading the bold sentences in each category will tell you what you need to know to run the instrument.
   i. Bullets are under the bold sentences when more detail is required.
   ii. At the end of the instructions is a frequently asked questions/troubleshooting section.

2. Startup Procedure

a. **Turn on computer and login** (use your Bowdoin account).
   i. First time users only.
      1. Create folders to store your data.
         a. Right click on START, then click Explore, c:\Chem32\1\Data.
         b. Right click in the right side of the screen where the folders are, click on New>Folder, then type in the new name when "New Folder" is highlighted. If it isn't highlighted, right click on "New Folder" and select "Rename" You can type in your name or initials.

b. **Turn on instrument. (button on front, lower left of second box)**
   i. Wait until the light on front of the instrument is green before proceeding to next step. Be sure that the CAG Bootp Server is open. If you click on it, you should see lines of communication between the instrument and the computer. If you don't see this, you'll need to turn off the instrument and turn it back on again to make the connection. You may also need to re-open the CAG Bootp program. The lamps will not turn on if the spectrophotometer is not on.

c. **Open "Instrument 1 Online" (icon on the desktop).**
   i. (Start > All Programs > UV-Visible ChemStations > Instrument 1 Online).
   ii. In the Password window, click OK. No password necessary.

d. **Enter sample information you are going to run onto UV log sheet next to the instrument.**

3. Create/Edit Method

a. **Load method** (File > Load Method) **or create new method** (File > New Method).

b. **Setup spectrophotometer** (Instrument > Setup Spectrophotometer).
   i. Scan range (Limits 190 – 1100 nm).
   ii. Integration (usually 0.5 seconds is good)
   iii. Interval (usually 1 nm).

c. **Turn on lamps** (Instrument > Lamp(s)).
    i. Lamps need to warm up for at least 15 minutes before running a sample.
d. **Select Spectrum/Peaks** (Method > Spectrum/Peaks) – if already selected, you will not get the popup window. You can open the popup window in Method > Setup Analysis.
    i. Enter parameters in popup window.
        1. Select find and annotate peaks/valleys – enter number of peaks/valleys to find (maximum 19).
        2. Data type should be absorbance.
        3. Display spectrum – use the same as your scan range.
e. **Save method** (File > Save Method As).

4. Measure Blank

a. **Load blank into sample chamber.**
    i. The load/unload lever is located on the left side of the sample chamber.
        1. When the sample chamber is closed, the lever is facing down. When the chamber is open, the lever will be pointing toward you.
    ii. Open chamber (if necessary) – rotate lever counter clockwise one-quarter turn.
    iii. Load sample.
    iv. Close chamber - rotate lever clockwise one-quarter turn. If cell is empty, it will not snap or lock into place, so be careful not to over-rotate it.
b. **Measure blank** (Measure > Blank), or click on the **BLANK** icon in the "Sampling" window.
    i. When finished, the "Last Blank Spectrum" window will popup.
c. **Remove blank from sample chamber.**

5. Measure Sample

a. **Load sample into sample chamber.**
b. **Measure sample** (Measure > Sample), or click on the **SAMPLE** icon in the "Sampling" window.
    i. When finished, spectrum and peak pick results will appear on screen.
c. **Remove sample from sample chamber.**
d. **Multiple samples can be analyzed under the same blank unless the solvent of the sample is different from the blank that was measured.**

6. Save Spectrum

a. **Save spectrum** (File > Save > Sample As). Will save as a .SD file.
b. **To save the data points for transfer to Excel, go to File>Export Selected Spectrum as>CSV Format, then type in a name for the file. **Note**--You must click on the actual spectra before exporting, or it will give you a message that reads nothing was selected.**

7. Generate Report

a. **Print report** (File > Print > Current View).

8. Shutdown Procedure

a. **Turn off lamps** (Instrument > Lamps).
b. **Load default method** (File > Load Method > stdefmet.m).
c. **Close ChemStation** (do not save changes/configuration if asked) and CAG Bootp Server.
d. **Shutdown computer and turn off instrument.**
e. **Make sure you clean up your samples.**

1. How do I add the local printer (HP Deskjet 5550)?

a. **Make sure the HP Deskjet 5550 is on and connected to computer.**
b. **Go to Start > Printers and Faxes.**
c. **Go to File > Add Printer**.
d. **The "Add Printer Wizard" will open up.**
    i. Click Next.
    ii. Select "Local Printer Attached to this Computer".
    iii. Select "Automatically detect and install my Plug and Play printer".
    iv. Complete wizard.
e. **Set printer as default.**
    i. Start > Printers and Faxes.
    ii. Right click on printer you just added.
    iii. In the menu, select "Set as Default Printer".

2. How do I select a printer?

a. **Complete the following steps in the UV-Visible ChemStation program.**
b. **Go to File > Printer Setup…**
c. **In the group "Printer" there are two choices.** Select "Specific Printer" and use the pull down menu to select the printer you would like to use.
d. **Click OK.**

3. How do I save just one spectrum when many are in the graph window?

a. **Select the spectrum you would like to save in the graph window.**
b. **Points will appear along the spectrum when it is selected.**
c. **Go to File > Save > Selected Spectra As.**

4. How do I load spectra?

a. **Go to Load > Samples.**

5. How do I clear all the spectra on the screen?

Note: You will not be able to call up spectra that were cleared unless you have saved them.

a. **Go to Edit > Clear > Samples.**
b. **All spectra in the graph window will be cleared.**

Quick Guide for Agilent 8453 UV/Vis

1. Write name and sample information on **Sample Log Sheet**
2. Turn on computer and log-in with your own user name and password
    a. Make a folder on the local disk c:\Chem32\Data
3. Turn on the instrument (bottom left corner of second box)
4. When light is green, open "**Instrument 1 Online**" from the desktop
5. Load or create method under **File>New** or load a method already created
6. Set up spectrophotometer parameters under **Instrument** tab
    a. Range 190-1100nm
    b. Integration-0.5s
    c. Interval-1nm
7. Turn on lamps (under **Instrument** tab) and let warm up for 15 minutes
8. Select **Spectrum/Peaks** under **Method**, Save Method
9. Load and measure blank
10. Load and measure sample
11. Save spectrum and print current view

Shutdown

1. Turn off lamps
2. Load default method (File>Load Method>stdefmet.m: c:\Chem32\1\Methods\stdefmet.m)
3. Close ChemStation (do not save changes/config. is asked)
4. Shutdown computer and instrument