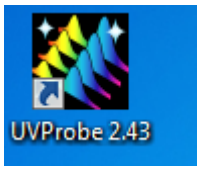
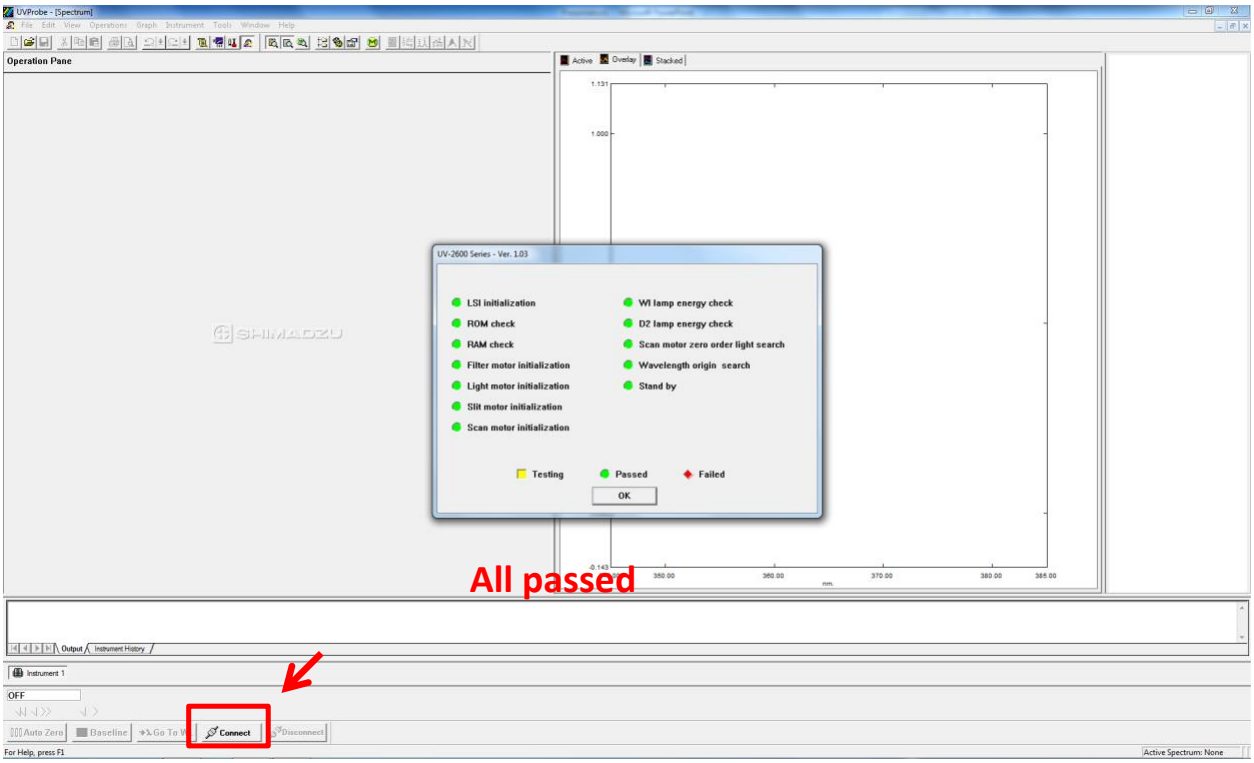
Standard Operating Procedure (SOP) for Shimadzu UV-2600 UV-VIS Spectrophotometer

1. Turn of the UV-2600 UV-VIS spectrophotometer.

2. Launche the UVProbe 2.43 software

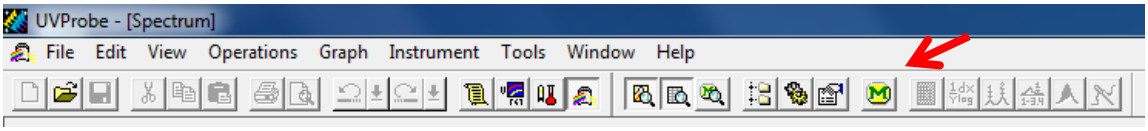


3. Click the Connect button on the bottom of the window and wait until it has performed all of its tests (they should all pass and have green dots next to them.

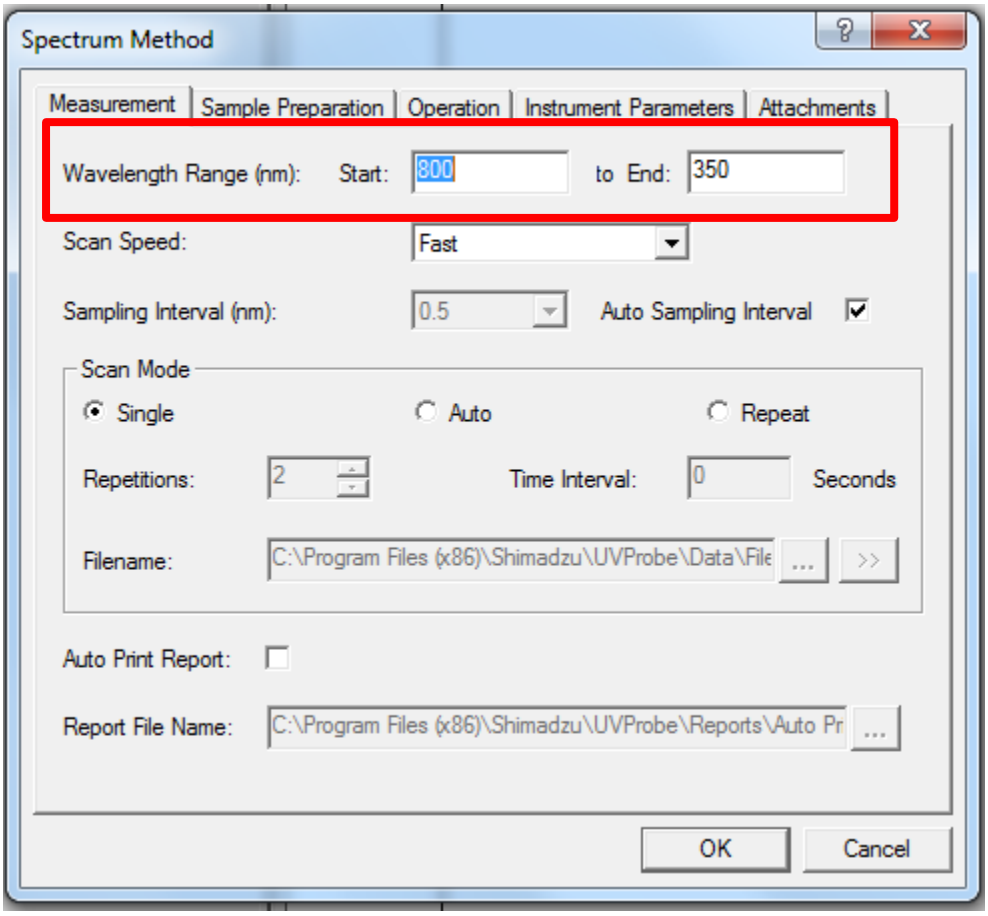


click OK

4. Click on the Edit method tab in the tool bar (the green M in a yellow circle)



5. Variables to change will typically only be wavelength range



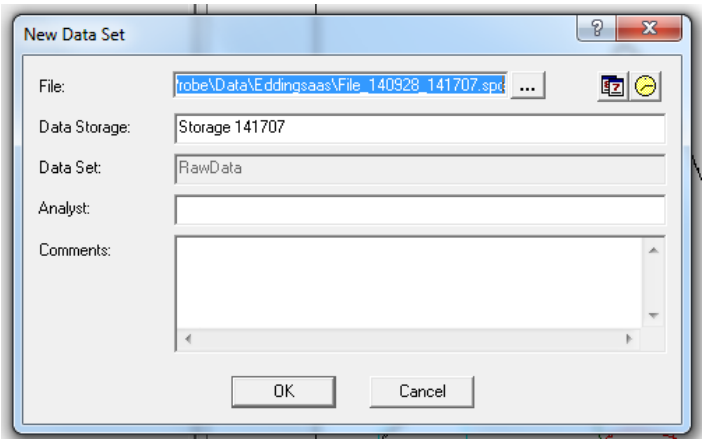
If other parameters need to be changed we will discuss it.

6. Zero the instrument by clicking the Auto Zero button at the bottom of the window.

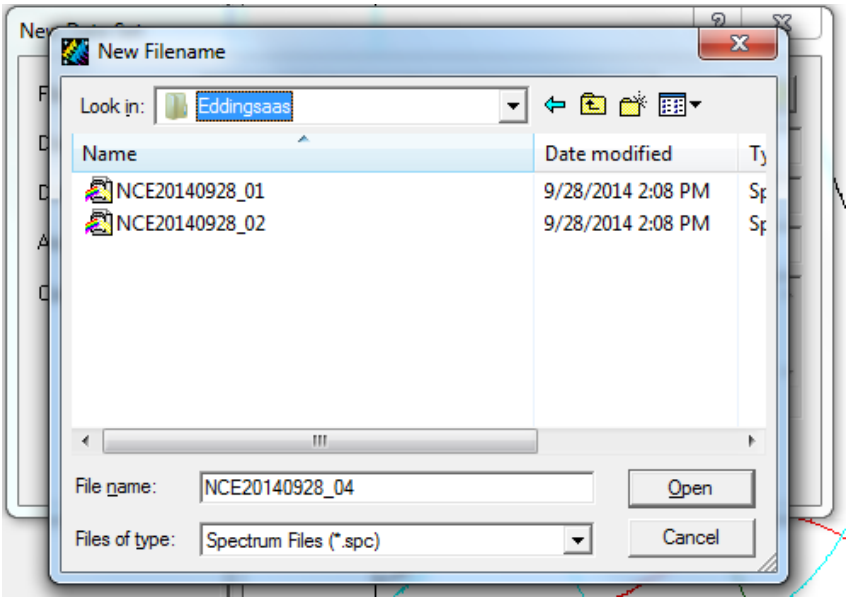
7. Create a baseline using a solution that contains everything in your sample besides the analyte (or as best that you can). This is your reference.
   Put your reference solution into a cuvette and place it into the front sample holder in the instrument. Make sure that clear sides of the cuvette is placed into the instrument so that the light will pass through (notice that the cuvettes have two clear and two semi-opaque sides, be careful to use the clear sides). With the reference in the sample holder, close the door and click the Baseline button next to the Auto Zero.

8. When the baseline scan is done you will hear a prompt. Open the door and remove your reference and place your first sample into the same sample holder and click the Start button two over from the Baseline button. Once again make sure the top door is closed.

9. When the spectrum has been taken and converted into absorbance units you will see a new window pop up:



This is where you will give the sample a file name. To the right of the File blank is three dots, click this to make sure you are storing your data where you want. It should be stored in the data folder, if you want you can back a folder within the data folder with your name on it.

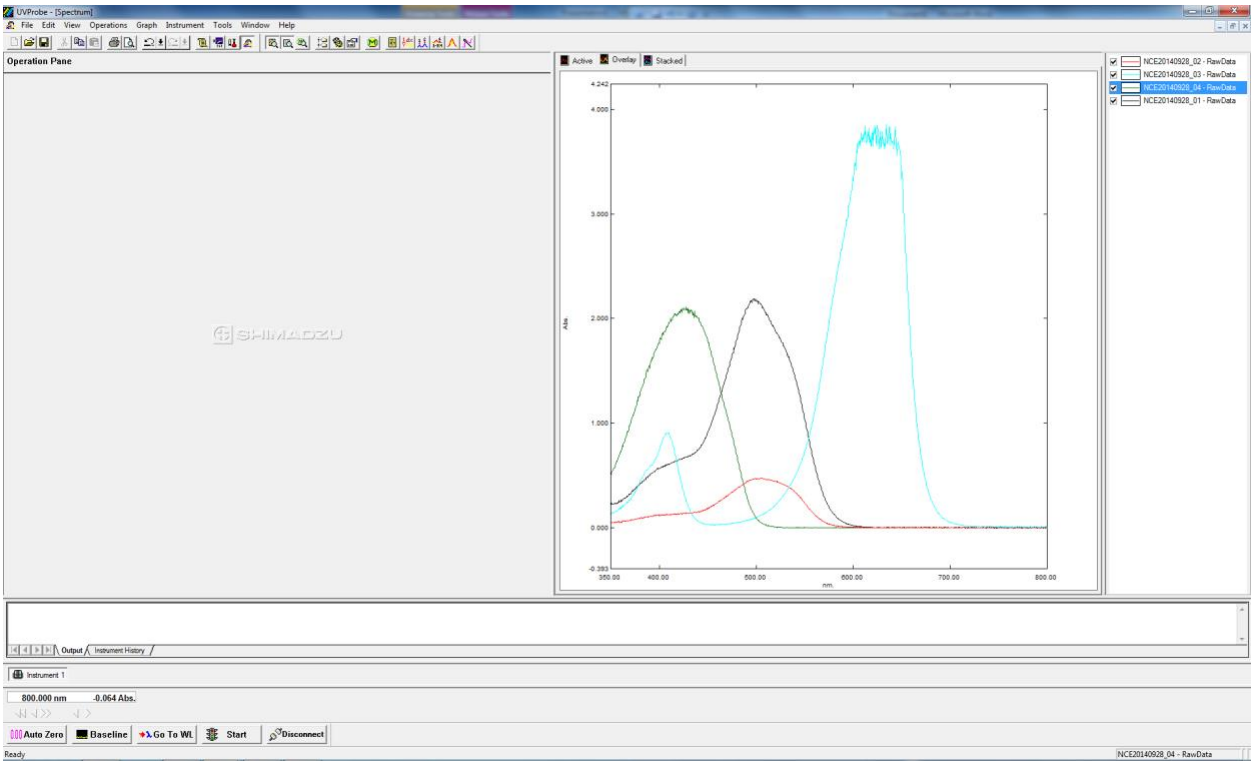


And fill out the comment section with information about the sample.

Note: this does not save your data just sets up the raw data to be manipulated or saved as the file time you so choose.

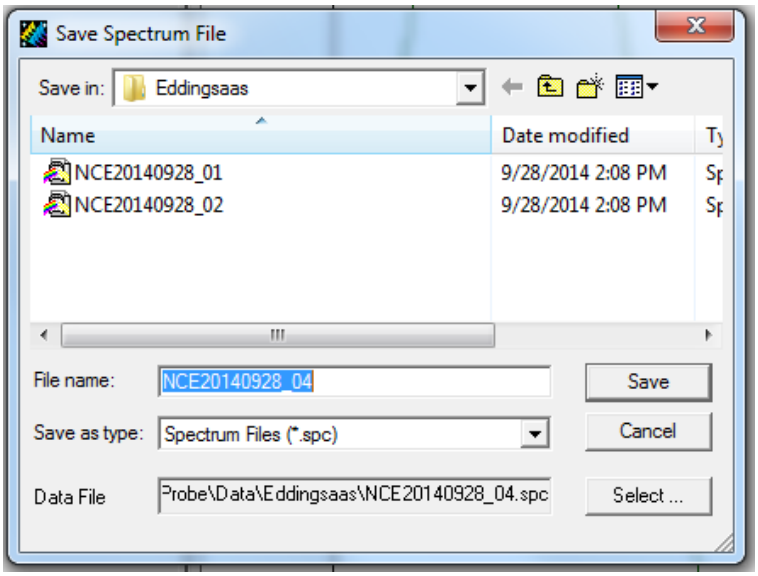
10. Now your raw data will be displayed on the graph on the right hand side of the window. As long as you do not change any parameters or use a different solvent for your sample you can use the background you have already generated and run more samples by putting in your new sample and hitting the Start button. This will place all of your raw data onto one graph. Remember to save each spectrum as with its own file name and comment.

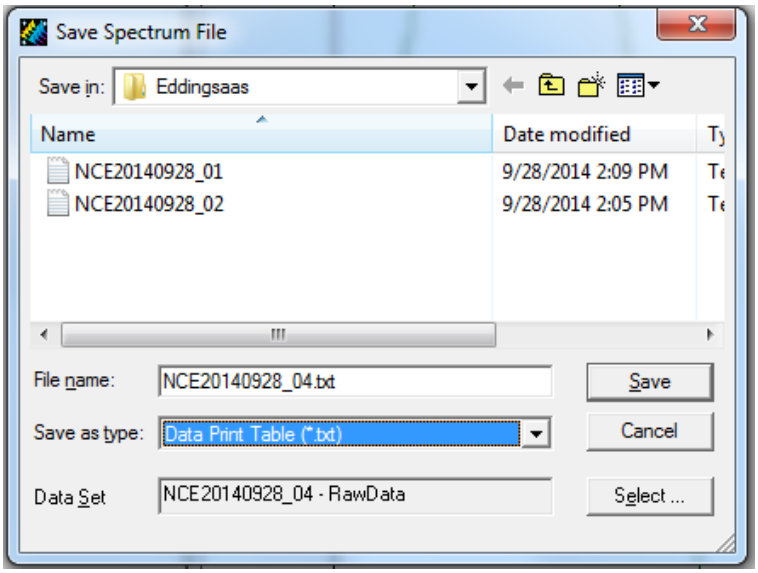
11. Once you have taken some spectra your window will look something like this:



Notice that many of the solutions were too concentrated, if you have an absorbance that is greater than 2 you are much too concentrated. An absorbance of 2 is telling you that 99% of the light was attinuated, or another way of putting it, only 1% of the light made it through. Notice that all spectra are displayed. If you want to look at only one you can either uncheck all of the spectra but the one you want or better way is to click on the graph menu at the top and click the active button which will only display the spectrum that is active. While in the active graph setup, to observe another spectrum simply double click on its name in the list to the right of the active spectrum.

12. We want to save the data in two formates, one that can be displayed using this software and one that you can import into Excell. While in the active spectrum mode and having only one spectrum displayed, click on file and save as, make sure you are in the folder that you want your data saved in then save the file (already named) as “Save as type: Spectrum Files (*.spc)”. Then do it again and save as “Save as type: Data Print Table (*.txt). This last one will be able to be opened using Excell.





Do both of theses saves to all of your data.

13. There are a number of things you can do to manpulate graph displayed. Right click on the graph and there are two features to take note of; Auto scale and cross hair. Auto Scale will make sure all of the data is displayed and cross hair lets you move around within the window and see what wavelength and abosrbance a peak has.

14. Your data will be stored somewhere near here:
    C:\Program Files (x86)\Shimadzu\UVProbe\Data\Eddingsaas
    You should transfer all of your saved data onto a USB flash drive.

15. When done taking data click on the Disconnect button at the bottom of the window.

16. After disconnecting you can exit the program then turn off the instrument.