Cary 60 UV-Vis Spectrophotometer Standard Operating Procedure

NOTE: Before reading this you MUST read the 'SOP - Energy and environmental impacts under normal, abnormal and emergency conditions' which is Mills group web site, https://www.profandrewmills.com/leaf-documents/. This addresses general energy and environmental impacts under normal, abnormal and emergency conditions considerations which you NEED to be cognisant of before conducting any experiment. If you identify anything in an SOP which can be improved, please contact the LO and PI to discuss the proposed change(s) before putting them into effect.

1. Switching on the UV-Vis spectrophotometer

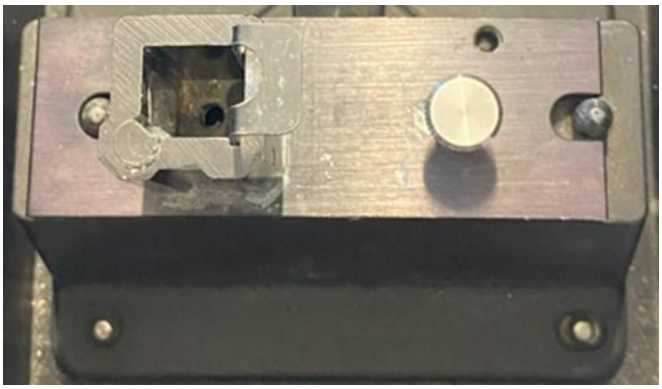
Below is a photograph of the CARY 60 UV-vis spectrophotometer (note that when the on/off button for the spectrophotometer is green-this means that it is ON):

To switch the spectrophotometer on, simply press the on/off button at the bottom right hand side. Once pressed, the on/off button will become red and will flash continuously while the spectrometer calibrates itself. Ensure that the cell holder in the spectrophotometer is empty (i.e., does not contain a cell) while this process occurs.

When the spectrometer is fully calibrated and ready to use, the on/off button will become a solid green colour as shown below.

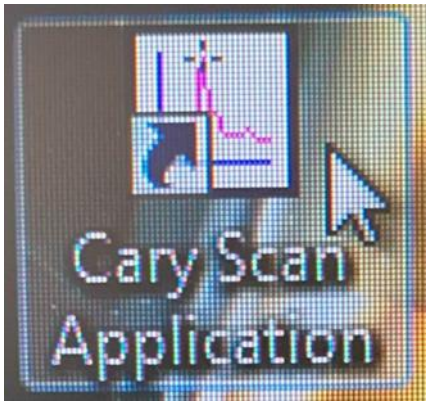
Slide the housing at the top of the spectrophotometer back to reveal the cell holder inside the spectrophotometer.

This is what the cell holder looks like when empty.

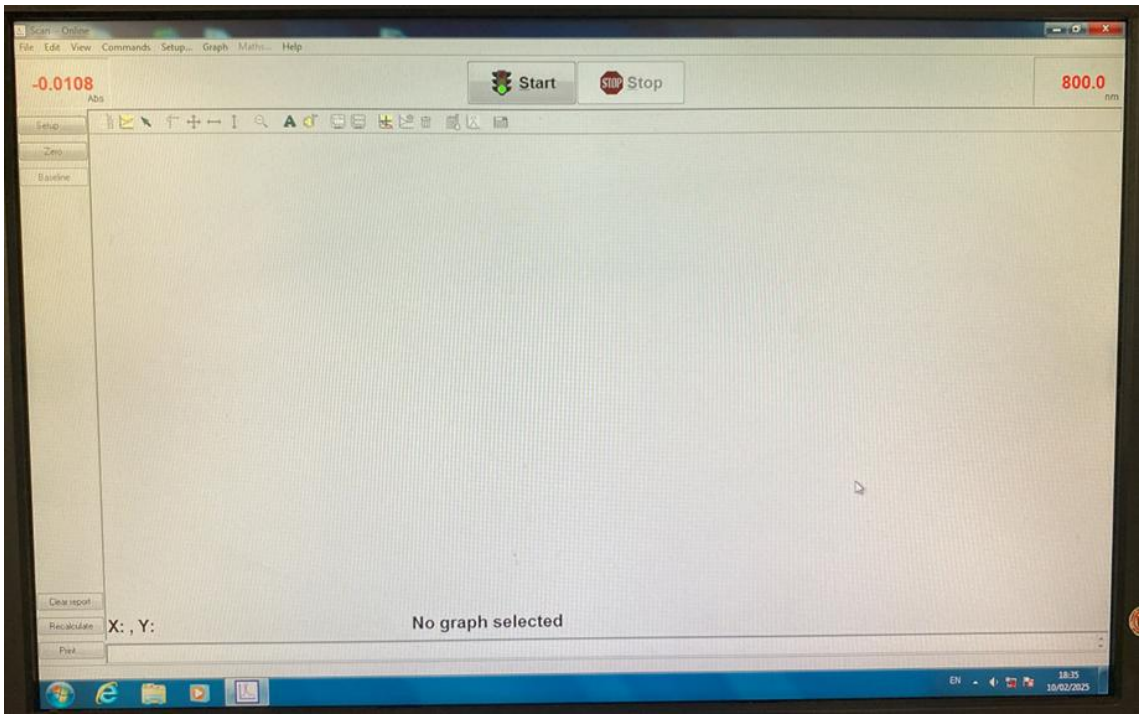


2. Loading and scanning samples

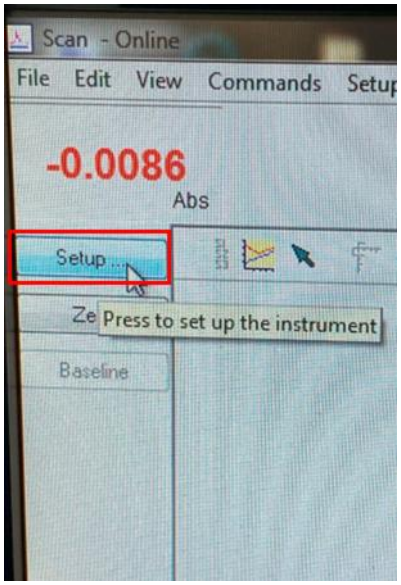
Double-click the ‘Cary Scan Application’ pinned to the desktop to open it.



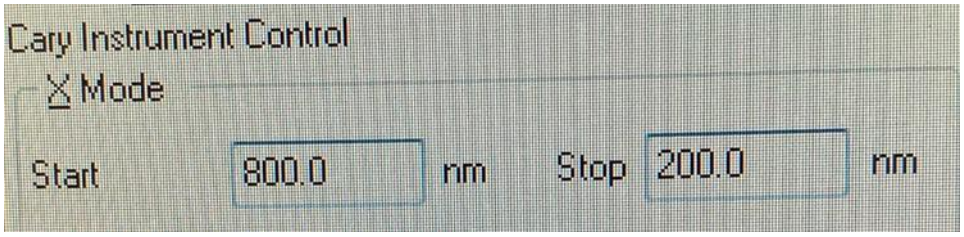
Once opened, the Scan application will display a blank chart on a white background, with ‘Start’ appearing at the top middle of the screen to indicate that the machine is ready to run.



Before starting the scanning of a sample, click on ‘Setup’ located at the top left hand corner of the screen:

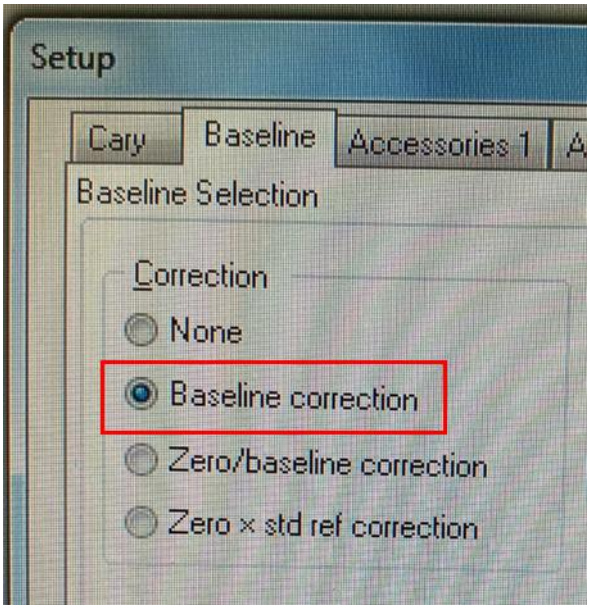


The Setup window will appear. Here, on this first tab entitled 'Cary', under the heading 'X Mode', ensure that the 'Start' value is set to 800 nm and the 'stop' value is set to 200 nm, as spectra are always run at 200-800 nm.

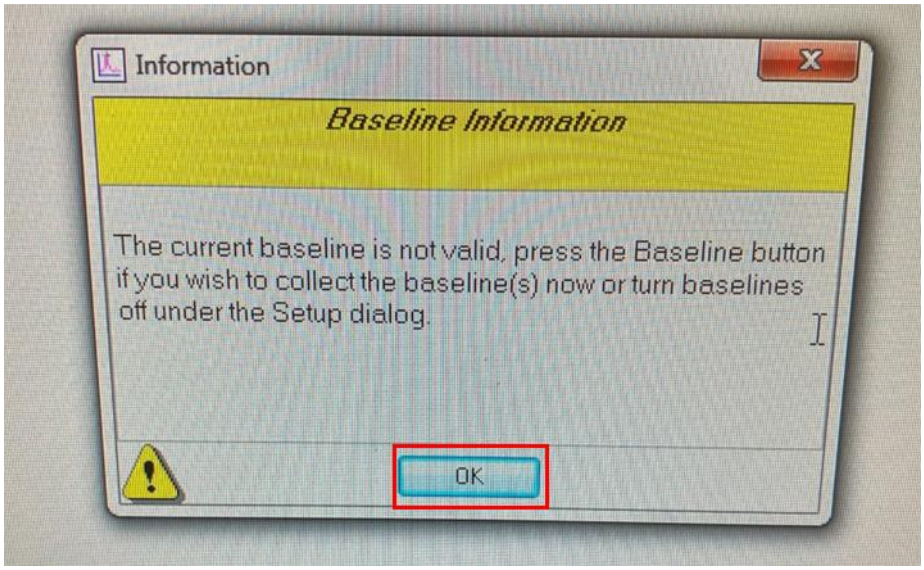


Then, ensure that under the heading 'Scan Controls' the 'Simple' option is selected and 'Fastest' scanning mode is selected (indicated by the square being lit up blue).

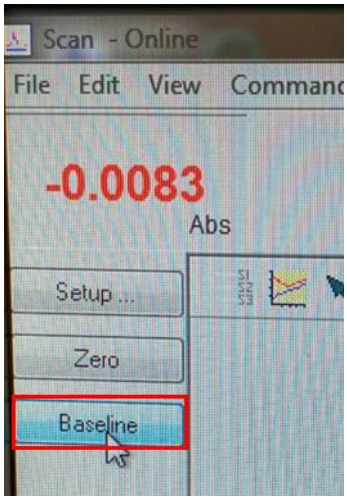
Next, it is essential to set a baseline. To do so, click on the second tab in the 'Setup' window, which is entitled 'Baseline'. Under the heading 'Correction', select the option 'Baseline correction'.



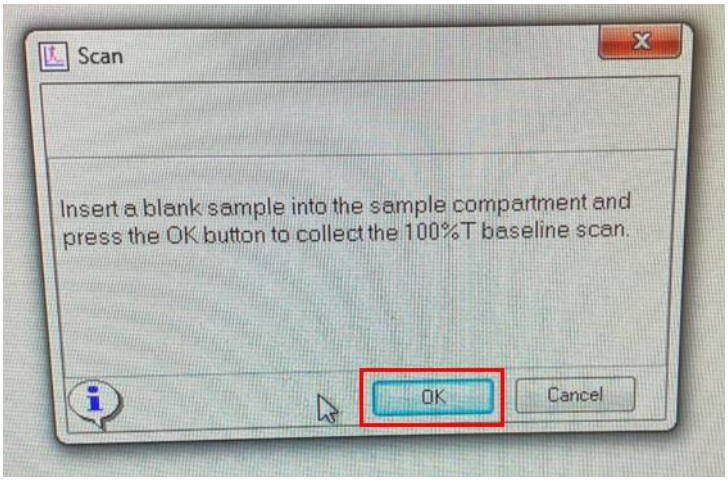
Next, click the button labelled 'OK' at the bottom of the tab. The following window will appear, reminding the user to collect a baseline.



To collect a baseline, click the 'Baseline' button in the top left corner of the screen.

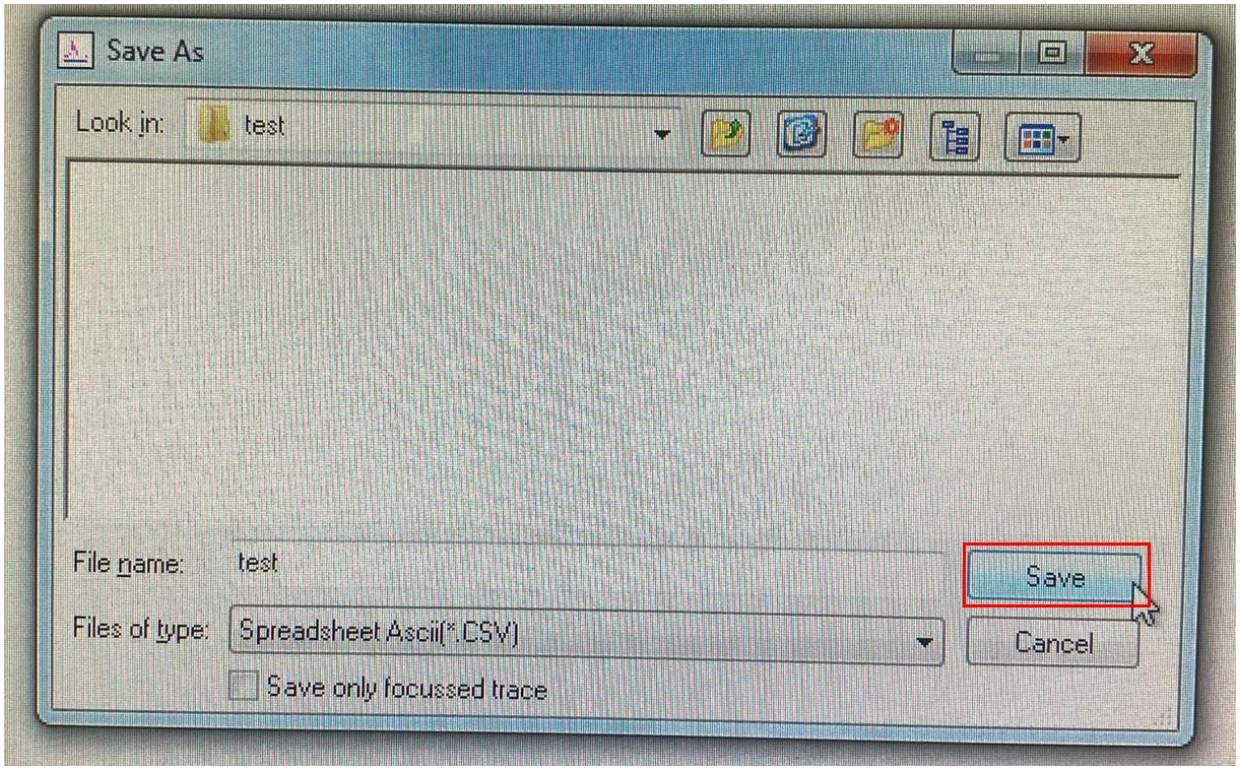


The following window will appear. Check there is no sample inside the machine and then press 'OK'.

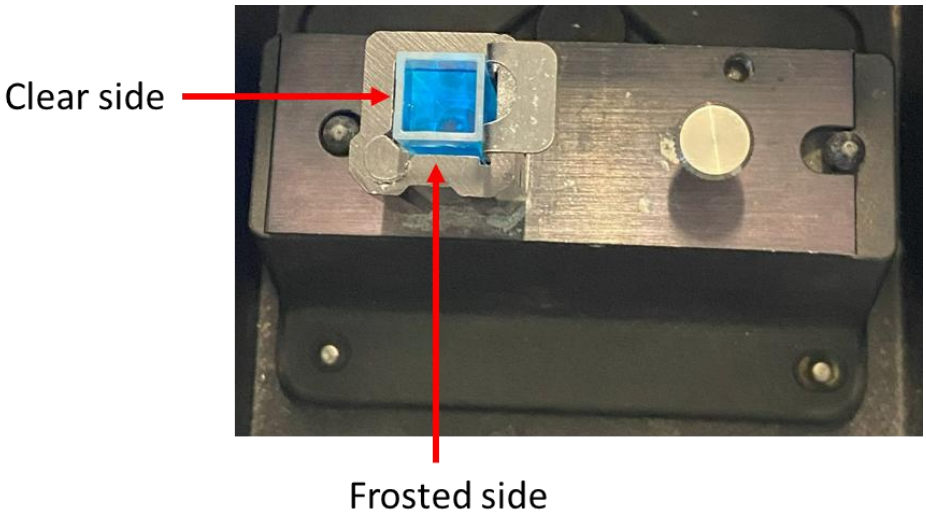


The machine is now ready to run.. To begin running a sample, click on 'Start' at the very top of the screen.

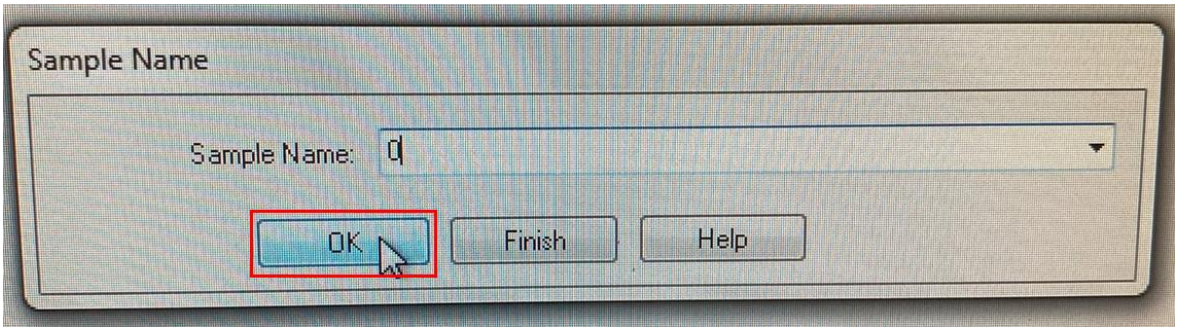
A window will appear, asking you to type a name for your file. Type the name in, then press 'Save'.



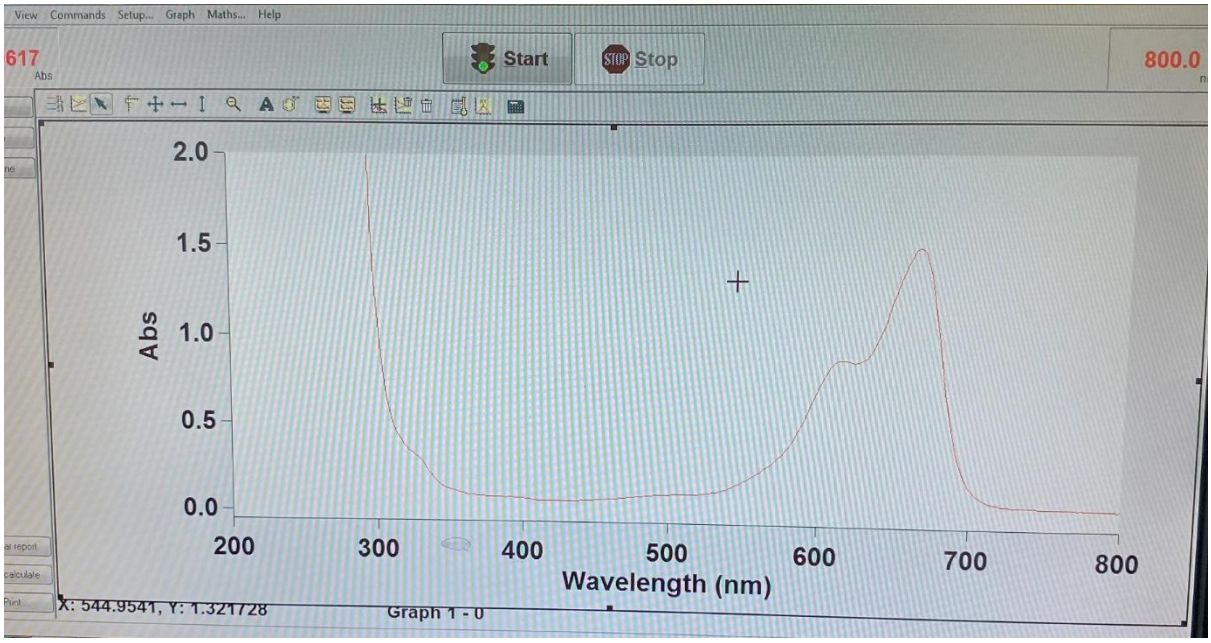
Insert the cuvette into the sample holder with the frosted sides pointed out towards you as below. Cuvettes should be roughly ¾ full.



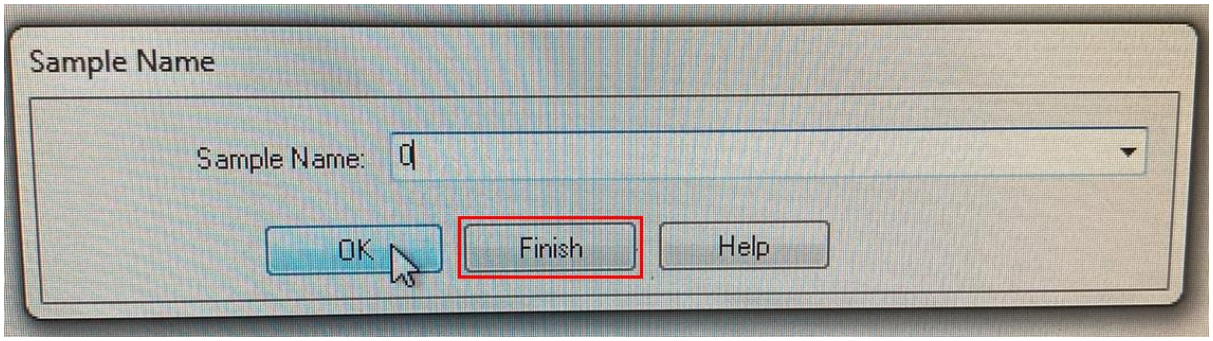
Type your sample name into the pop up box onscreen, followed by 'OK'- then your sample will run.



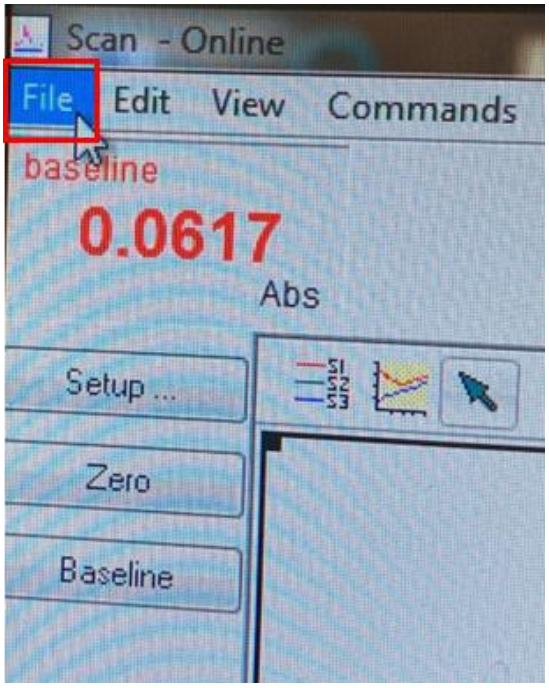
The UV-Vis spectrum (a plot of absorbance vs wavelength) for the sample will appear on screen.



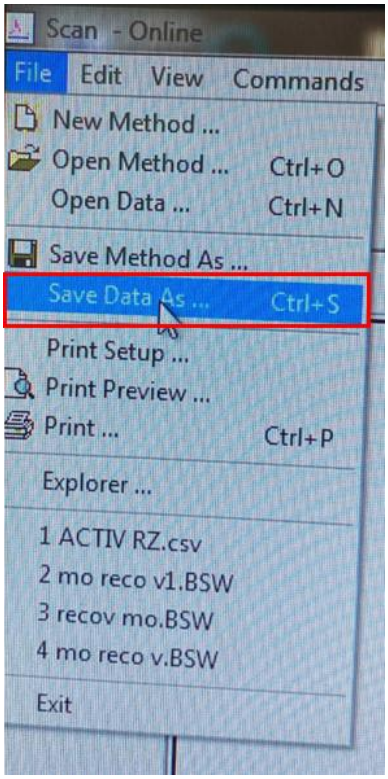
Press ‘Finish’ to finish collecting.



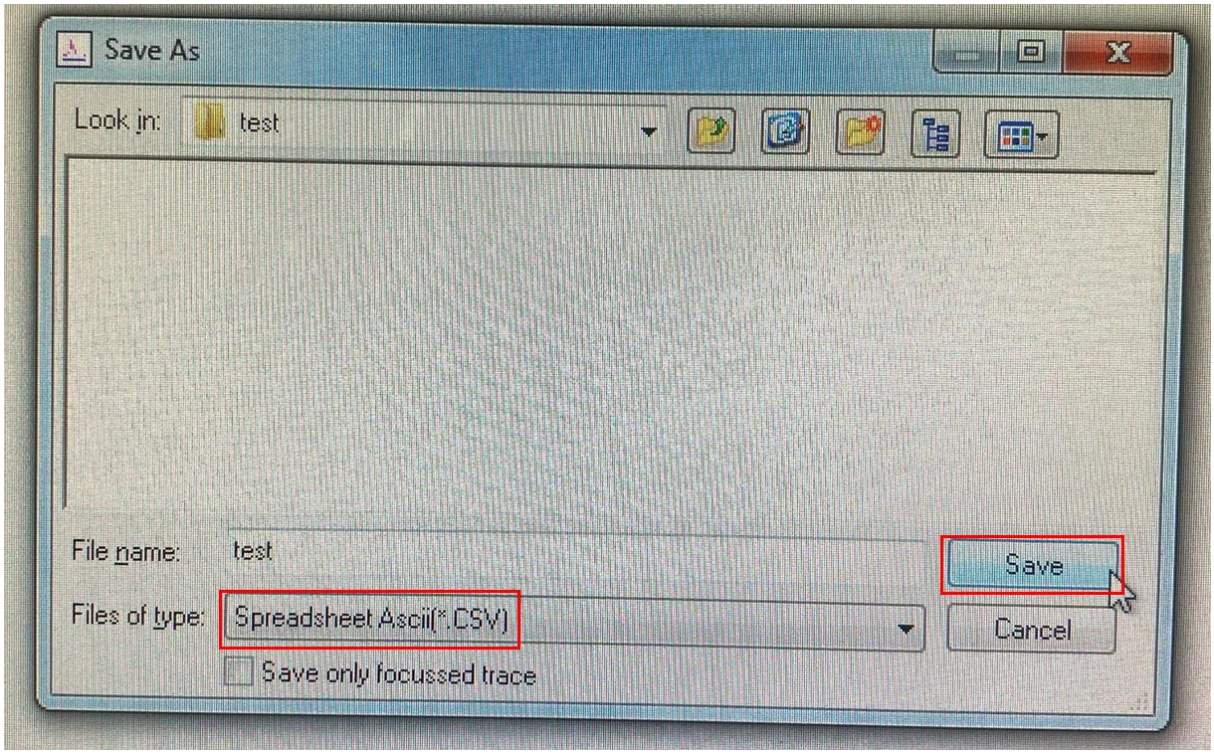
To save your data in a format compatible with Excel, click ‘File’ in the upper left corner of the screen.



Then navigate to, and click, 'Save Data As'.



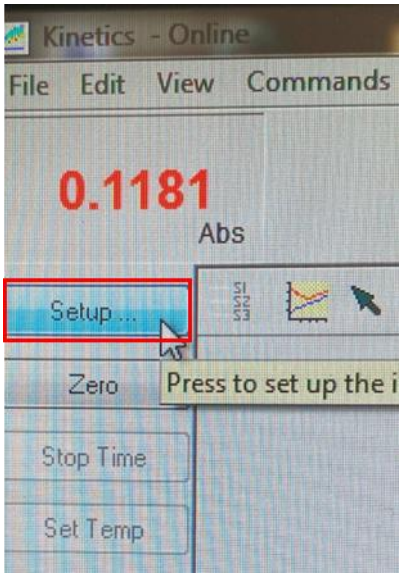
When the following window appears, type in your file name and then set the 'Files of type:' option to 'Spreadsheet Ascii(*.CSV)' from the dropdown list, then hit 'Save'. In this format, the file can be opened with Excel.



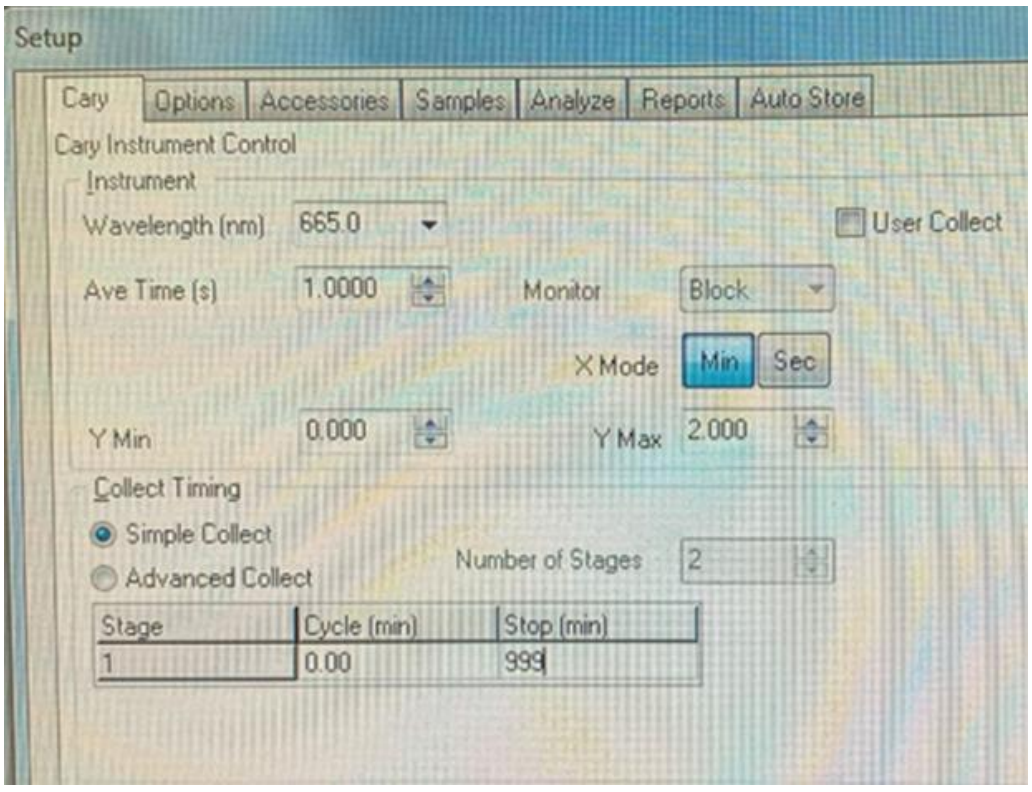
3. Using the Cary Kinetics Application

Firstly, double click to open the Cary Kinetics Application, pinned to the desktop of the PC.

The application opens on a blank white chart; press 'Setup' in the upper left corner of the screen.

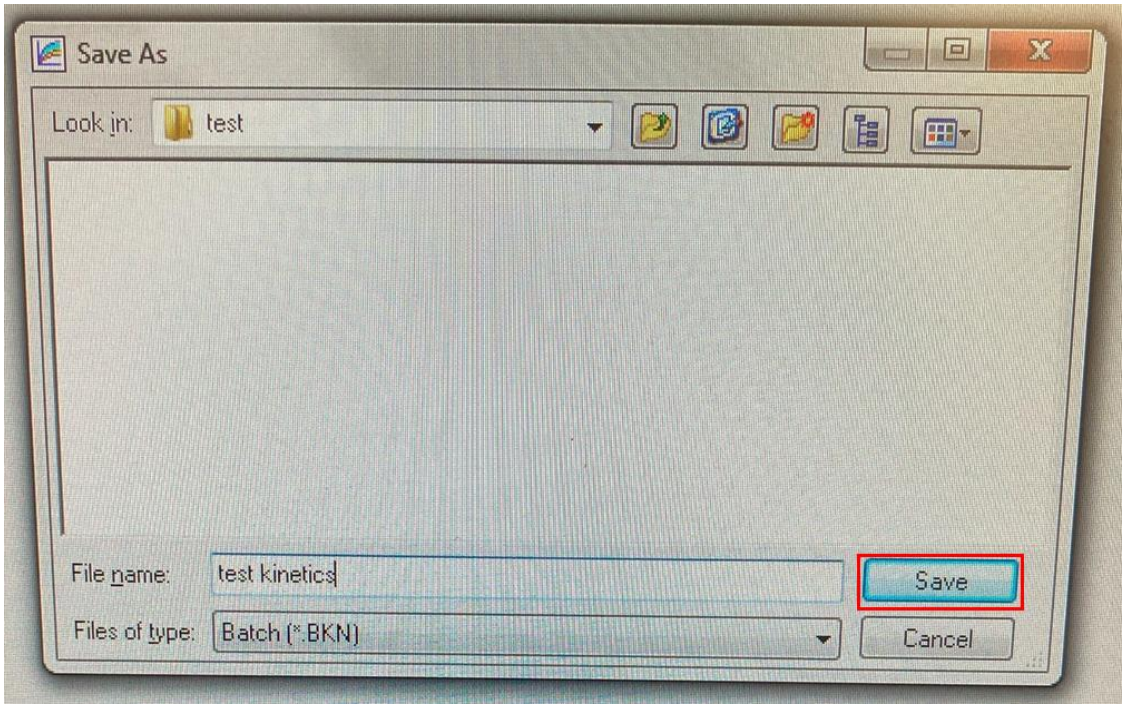


The Setup window appears; here, you can alter the wavelength, sampling time, Y axis value, and length of the experiment. For example, the below scan is set to run at a wavelength (ʎ)= 665 nm, sampling every 1 s, up to a Y axis (absorbance) value of 2, for 999 minutes. Always keep the 'Simple Collect' option checked. Press 'OK' at the bottom of the window.

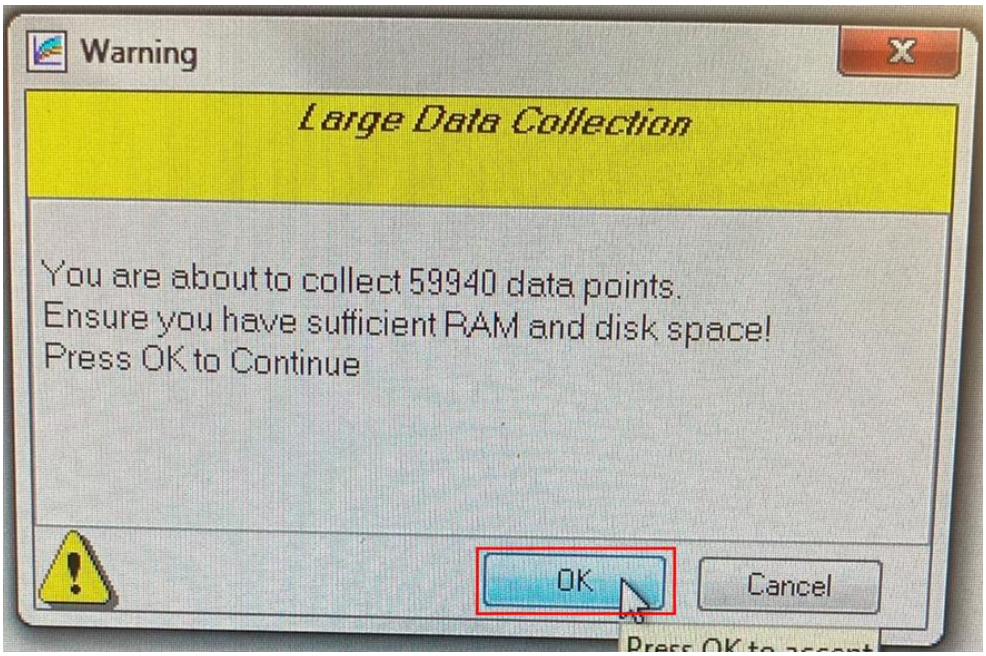


Press 'Start' at the top of the page.

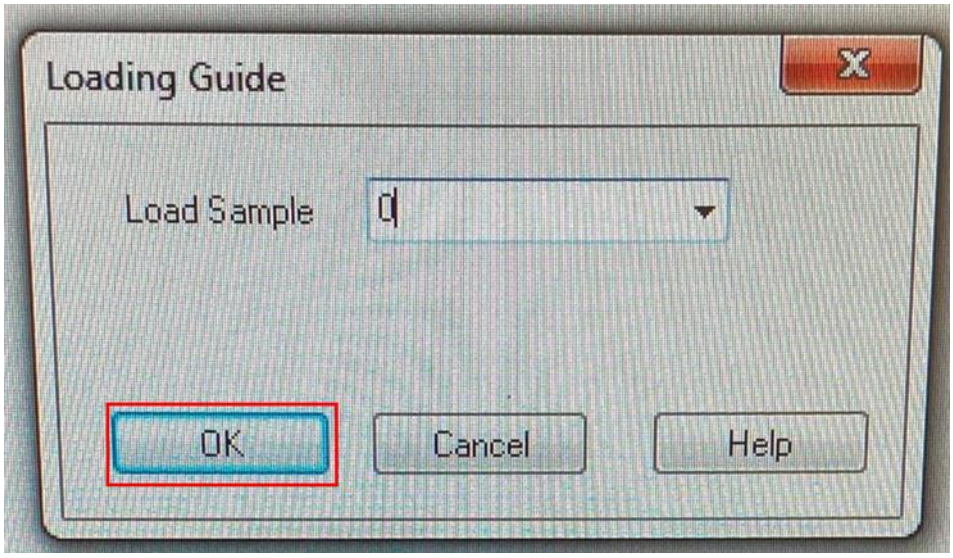
The 'Save As' window will pop up. Enter your file name, then press 'Save'.



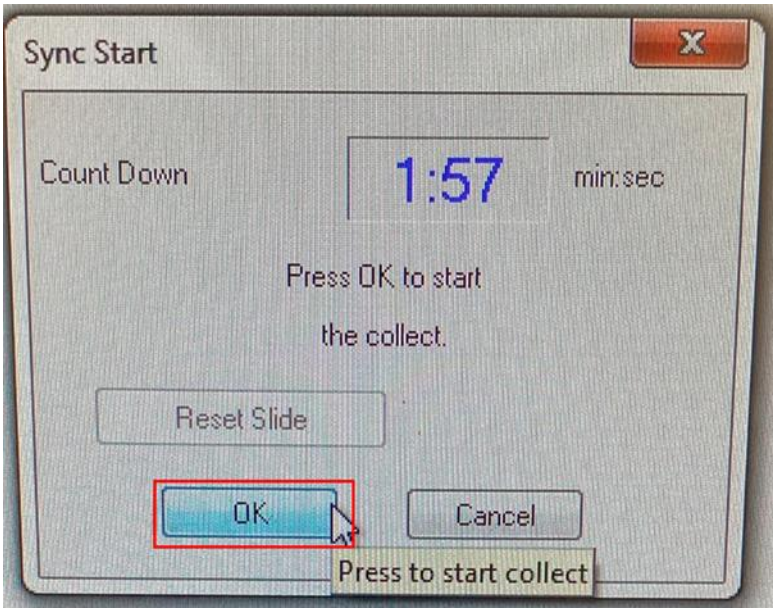
A warning window may pop up, to tell you that you're collecting a large number of data points. Press 'OK'.



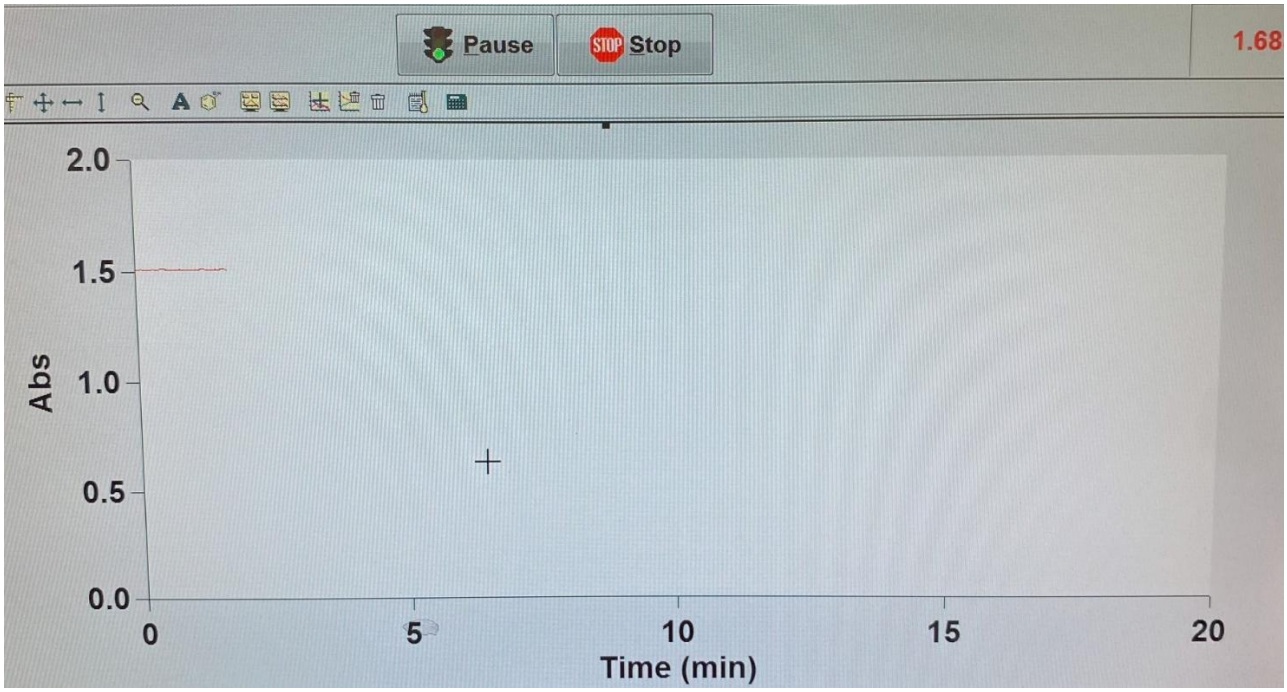
Load your sample into the machine as before with the Scan application. A 'Loading Guide' window appears onscreen- alter your sample name and, when you're ready to run, press 'OK'.



A 2 minute countdown to the start of data collection appears on screen. Press 'OK' when you are ready to start data collection.

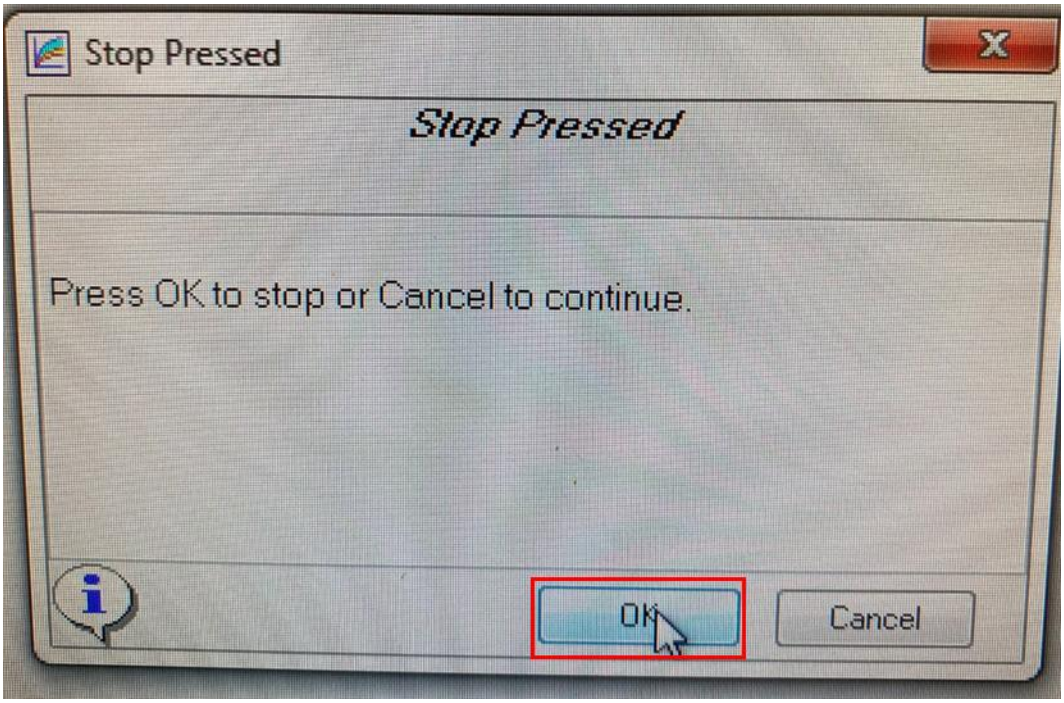


Data appears onscreen as a plot of absorbance vs time.



To stop the collection at any time, press the stop button at the top of the screen.

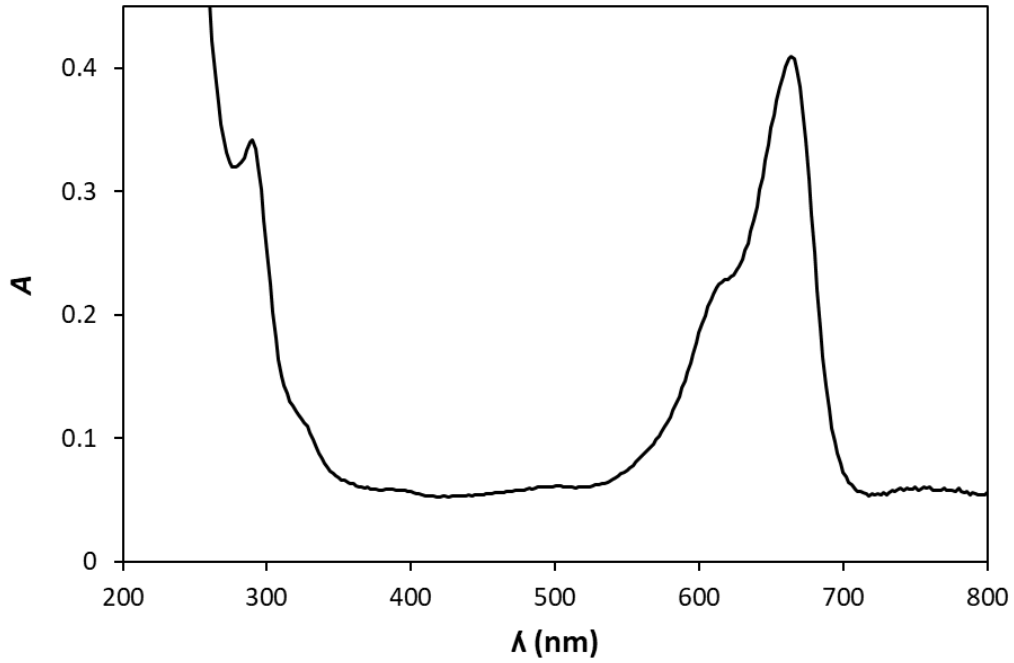
The following window appears- press 'OK'. The data is saved exactly as described before, for the Scan application.



4. Example results

Cary Scan application

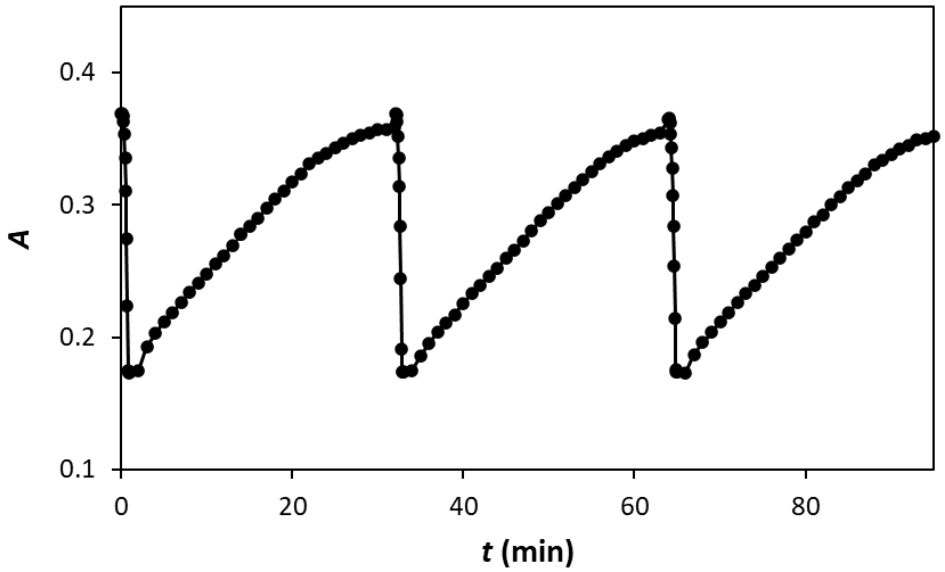
Below is an example UV-Vis spectrum generated using the Cary Scan software for an aqueous solution of methylene blue dye (conc= 6.38 x 10^{-6} M). The program was set to scan between wavelengths of 200-800 nm, with a Y-axis (absorbance) value of 0-0.45, on the 'Fastest' option under scan controls. A baseline correction was applied to the data by the spectrophotometer. From this plot, the wavelength at which the dye absorbs most strongly, λ_{max}(D), could be deduced (= 665 nm).



UV-Vis spectrum (absorbance vs wavelength) of an aqueous solution containing 6.38 x 10^{-6} M methylene blue dye.

Cary Kinetics Application

Below is an absorbance vs time plot, generated using the Cary Kinetics Application, for a redox indicator containing methylene blue dye (λ_{max}(D)= 665 nm), when exposed to alternating streams of H_2 and O_2.



Absorbance (A) vs time (*t*) profile generated by the Cary Kinetic application for a methylene blue H_2 indicator exposed to alternating streams of H_2 and air (*i.e.* 21%O_2).

The program used to generate these results was as follows:

Instrument control

- Wavelength (nm)= 665 nm
- Ave time (s)= 5
- Y Min= 0.1
- Y Max= 0.45
- X Mode= Min

Collect timing

- Simple collect, Stop(Min)= 90