

HOW TO USE THE CARY 50 SPECTROPHOTOMETER

JWG
VERSION 7
26/2/01

TURNING THE INSTRUMENT ON

The instrument gets all its power from the computer, so all you have to do is to press the big power button on the front of the computer and wait for the computer to boot up. If you do need to turn it off, it is best to shut down “properly” using the software on the screen, but in an emergency you can turn it off by pressing the on/off button IN for more than 4 seconds (child-proof protection). There is NO reset button. The lamp in the instrument is only turned on (flashes) at the instant that a measurement is being made, so there is no point in turning the instrument off between uses. I recommend leaving it turned on all the time. The lid can be open during measurements, but close it when you finish to keep out dust.

SIMPLE MEASUREMENTS

Double click on the “Simple Read” icon on the desktop.
Press CONNECT.
Click on SETUP.
Enter desired wavelength from the keyboard.
Press ENTER.
Press OK.

THE FOLLOWING BIT IS REALLY IMPORTANT

Push the cuvette with blank buffer in the holder, as far down as it will go.
Then, pull the little tab in the cuvette holder up as far as it will go, to ensure that the bottom of the cuvette is near the bottom of the light path. **THIS IS PARTICULARLY IMPORTANT FOR SMALL VOLUMES.** (When you put a cuvette containing a liquid sample in the machine, make sure that you have a look at where the light beam passes through the cuvette. It should be obvious that the light path passes ABOVE the little knurled screw on the cuvette holder. If the liquid sample is not above this level, your readings will make no sense at all. **I strongly recommend that all first time users check the position of the light beam as shown on the next page.**

Click on ZERO.
It will zero the instrument and remember the wavelength.
Remove cuvette, and fill it with your sample.
Click on READ.
The OD of your sample will appear on screen.
You can then take out cuvette and do another measurement.

PRINTING OUT

Click on the PRINT icon.
You will then get a printout.

MEASUREMENT AT SEVERAL WAVELENGTHS

In the SIMPLE MEASUREMENT mode, choose the desired wavelengths separated by a semicolon
260;280;350;400
Then zero the instrument with blank sample in cuvette.
The instrument will then remember the zero settings for all wavelengths.
Insert the cuvette with your sample.
Press READ.

TO WRITE COMMENTS IN YOUR DATA:

Click on EDIT REPORT. You can then enter text from the keyboard.

TO SEE THE BEAM

One of the most common causes of error in measurements is due to the light beam striking the bottom of the cuvette or the meniscus of the sample, or possibly the sides of the cuvette. This can be checked by inserting a piece of white paper/card in the light path and looking at where the light spot falls in relation to the cuvette.

Exit the current application by clicking on the X in the top right hand corner of the current window.

This should take you back to the desktop.

Double click on the CaryWinUV icon and let it start up.

Double click on the ALIGN icon.

Choose a wavelength that is visible (eg 500 nm).

Click on ZERO ORDER.

Click APPLY.

Put paper in light path.

Look at where the spot is in relation to the walls/bottom of the cuvette, and also in relation to the top of the sample. Obviously, the light must go through the middle of the sample without touching the top, bottom or sides.

The height of the cuvette can be altered by a screw under the cuvette holder, but this should not normally need to be changed.

Note that the light beam goes very high above the bottom of the cuvette, and for small volumes it is essential to push the cuvette to the bottom and then pull it back up with the small tab in the cuvette holder, to ensure that the bottom of the cuvette is above the silver knurled screw on the cuvette holder.

TO CLEAR THE DATA REPORT FROM THE SCREEN:

Press CLEAR REPORT.

TO CONVERT AN OD TO A CONCENTRATION:

There is a function called a CONCENTRATION MACRO, which is a simple programming exercise.

TO SAVE DATA TO AN ASCII OR EXCEL FILE:

This can't be done with the SIMPLE READ mode. You have to use the ADVANCED READS mode.

SCANS, KINETICS ETC:

You will have to read the online manual and figure this out yourself!

HELP:

The manual is online and available at any time, and any part of it can be printed out.

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MEASUREMENT OF PROTEIN CONCENTRATION BY PIERCE DYE BINDING ASSAY

Version 4

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The computer is capable of accepting the standard values, plotting the curve and calculating the unknowns. Setting up the computer is rather slow and tedious, but once it is set up, making readings is extremely fast and the computer will graph out your results and calculate concentrations, so it is worth it.

Make sure that the printer has paper in it.

Double click on CaryWinUV

Double click on Concentration icon

Click on Setup and choose SetupChoose wavelength (**562 nm**)

Averaging time: 0.1 second (default)

Y minimum = 0

Y maximum = 3 (or 2.5, if you wish)

Click in the box marked "show status display"

Click on OK

Click on Setup and choose Standards (standards MUST be in ascending order of conc.)

Click in box marked "calibrate during run"

Choose the desired units for concentration (usually mg/ml)

Choose number of standards that you will use (max = 30) and enter concentration of standards in ascending order. **It is important that the first standard should be a blank (ie 0 mg/ml).**

I generally use 6 standards, as follows:

Standard 1: 0

Standard 2: 0.125 mg/ml

Standard 3: 0.25 mg/ml

Standard 4: 0.5 mg/ml

Standard 5: 1.0 mg/ml

Standard 6: 2.0 mg/ml

Choose Fit Type (quadratic is best for dye binding data because it is a curve and not a straight line)

Minimum r²: default is 0.95, which is OK.

Click in box saying "show status display"

Click on OK.

Click on Setup and choose Samples

Nominate how many samples you have (up to 500)

Corrections: leave blank

Click on OK

Click on Setup and choose Reports

Enter name, date and any comments

Choose Autoprint and graph ON. (or you can print manually)

Click on OK.

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Click on Setup and choose Auto Store

Choose Storage ON, prompt at start. (asks you to nominate a storage file name and location before you start).

Status display: OFF

Click OK.

Press Zero

You will be asked to insert cuvette with blank sample. Do so and then press OK.

Press START

You will get a screen saying "Selected for Analysis".

Click on Std 1, and press OK.

You will get a screen with folders and space for file names on it.

Enter your desired filename (NB Windows won't allow full stops, slashes or spaces!)

I suggest a simple name like JWG191200. Press SAVE.

You will then get a prompt saying "Present Standard 1"

Put standard 1 in the machine and press OK to read it.

You will then be asked to Present Standard 2.

Put standard 2 in the machine and press OK to read it.

Follow this through to the end of the unknowns.

When all the standards and unknowns have been measured, the computer will automatically print out the results and the graph.

Re-analysis: You can go back and re-analyse the data in a different way after you have finished, by pressing the "Recalculate" button. Printout will be automatic.