



Agilent 8453E UV-visible Spectroscopy System



Operator's Manual



Notices

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In This Guide...

This handbook describes what you have to do to install and operate your new Agilent 8453E UV-visible spectroscopy system. The first part of the handbook deals with installing a complete system, that is, spectrophotometer, handheld controller and printer. In the second part of this handbook you will find additional information about installing accessories and operating and maintaining your Agilent 8453 spectrophotometer. In the last main part of the manual, you will find information about the structure and contents of the software. It also describes in brief how to use the software.

1 Installation and Start Up

Use this chapter if you need more detailed information to begin with.

2 Good Measurement Practices

Use this chapter if you require information about general operating conditions to perform accurate and reliable measurements (for example, handling samples, solvents and cells).

Other chapters contain the following information:

3 Operating your Agilent 8453E UV-visible Spectroscopy System

This chapter gives you detailed informations about the features of the software and how to operate the handheld controller.

4 Troubleshooting

This chapter gives you hints and tips how you can resolve technical problems of the Agilent 8453E UV-visible spectroscopy system.

5 Service and Maintenance

In this chapter you will find information on service and maintenance features supported by the Agilent 8453E UV-visible spectroscopy system.

6 Installing Accessories

A brief description of accessories compatible with the Agilent 8453E UV-visible spectroscopy system and how to install them is given in this chapter.

A Software and Firmware Upgrade

In this chapter you will find information on how to upgrade the software of the handheld controller and firmware of the spectrophotometer.

B Data Export

This chapter gives you hints and tips how you can export data of the Agilent 8453E UV-visible spectroscopy system to other software applications.

C Safety Information

This chapter contains relevant safety information.

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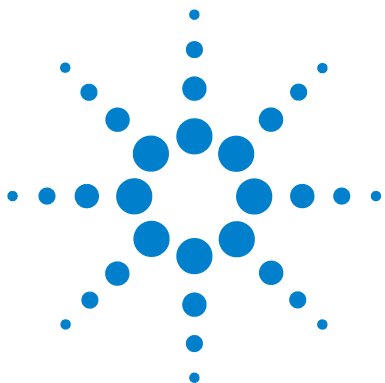
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The following topics are covered in this section:

- “[Site Requirements for Your Agilent 8453 Spectrophotometer](#)” on page 13 contains information about electrical requirements, i.e. power lines, power cords, etc. It also gives recommendation for minimum available bench space and environmental conditions in the laboratory.
- “[Unpacking Your Agilent 8453E UV-visible Spectroscopy System](#)” on page 16 contains information about what you do when the packing material is damaged or the shipment is incomplete. It also gives a list of items which come with the instrument and handheld controller.
- “[Installing Your Agilent 8453 Spectrophotometer](#)” on page 17 gives an overview of the instrument and explains pushbuttons, indicators and line power switch. It gives instructions where and how to do line power connection, communication connections and where to install the waste drain tubing.
- “[Starting the Agilent 8453E UV-visible Spectroscopy System](#)” on page 22 describes the turn-on procedure of the spectrophotometer and the warm up time required to fulfil all specifications.



1 Installation and Start Up

- “[Installation Checkout](#)” on page 25—use this section to check if your spectrophotometer in combination with the handheld controller software is in proper operating condition. Optionally there are instrument tests which can be used for a quick check out of the spectrophotometer. These instrument tests can only be executed after 45 minutes warm-up time of the instrument.

Site Requirements for Your Agilent 8453 Spectrophotometer

Power considerations

Your spectrophotometer can operate on a line voltage of 100–120 or 220–240 V AC $\pm 10\%$ with a line frequency of 50 or 60 Hz $\pm 5\%$. Maximum power consumption is 220 VA.

The instrument power supply has wide ranging capability. It accepts any line voltage supplied at the above mentioned range. Consequently there is no voltage selector at the rear of the spectrophotometer. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

WARNING

To disconnect the instrument from line, pull out the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.

No external power supply is needed for your handheld controller, it is powered by the CAN interface.

The DeskJet printer accepts a nominal line voltage of 110 or 220 V AC with a line frequency of 60 or 50 Hz. For more information, see the documentation that comes with your DeskJet or LaserJet printer.

WARNING

If you connect your instrument to a line voltage that is higher than specified, shock hazard or damage to your instrument can occur.

1 Installation and Start Up

Site Requirements for Your Agilent 8453 Spectrophotometer

Power Cords

Different power cords are offered as options with the spectrophotometer. The female end of each of the power cords is identical. It plugs into the power-input socket at the rear of the spectrophotometer. The male end of each of the power cords is different and designed to match the wall socket of a particular country or region.

WARNING

Always operate your instrument from a power outlet which has a ground connection. Always use the Agilent Technologies power cord designed for your region.

Space

The spectrophotometer is 34.4 cm (13.5 inches) wide, 56.0 cm (22.0 inches) deep and 18.5 cm (7.3 inches) high (see [Figure 5](#) on page 22). It weighs only 14 kg (30.86 lbs) so it will fit on almost any desk or laboratory bench. It needs an additional 2.5 cm (1.0 inches) of space on either side to allow for the circulation of air for cooling and approximately 8 cm (3.1 inches) in the rear for electrical connections.

Additional space is required for your printer and accessories like pumps.

Environment

Your spectrophotometer will operate within specifications at ambient temperatures of 0 to 55 °C (32 to 131 °F).

WARNING

If you use the instrument at environmental temperatures higher than 50 °C (122 °F), the rear of the instrument may get hot.

The spectrophotometer can be shipped or stored where the ambient temperature is between -40 and 70 °C (-4 and 158 °F).

WARNING

Do not store, ship or use your instrument under conditions where temperature fluctuations could cause condensation within the instrument. Condensation will damage the electronics. If your instrument was shipped in cold weather, leave it in its box and allow it to warm up slowly to room temperature to avoid condensation.

You can operate your spectrophotometer in environments with relative humidity up to 95 % (non-condensing) provided the temperature is between 25 and 40 °C (77 to 104 °F).

Avoid air circulation of varying temperatures (like from refrigerators or air conditioners) and exposure to direct sun light to ensure the instrument operates within performance specifications.

Your spectrophotometer can be stored at altitudes up to 4,600 m (14,950 ft) and operated at altitudes up to 2,000 m (6,500 ft).

Unpacking Your Agilent 8453E UV-visible Spectroscopy System

Upon receipt of your spectrophotometer, handheld controller and printer, inspect the shipping containers for any signs of damage. If the containers or cushioning material are damaged, save them until the contents have been checked for completeness and the spectrophotometer, handheld controller or printer have been mechanically and electrically checked. If the shipping container or cushioning material is damaged, notify the carrier as well as Agilent Technologies. Save the shipping material for the carrier's inspection.

Check the contents of the shipping container against the list below. If any items are missing or obviously damaged, call Agilent Technologies.

- ✓ Unpack the spectrophotometer. You should have the following components:
 - Power cord to connect the spectrophotometer to line power.
 - Waste tubing to connect the sample pan of your spectrophotometer to a waste container.
 - Standard cell holder
 - Standard Quartz Cell.
 - Checkout sample (caffeine solution).
- ✓ Unpack the handheld controller. You should have the following components:
 - handheld controller with flexible connector cable (fixed).
 - Serial/parallel printer cable for connecting parallel port of printer with serial port (RS 232) of the spectrophotometer.
 - 4 MByte PC-card.
- ✓ Unpack the printer. You should have the following components:
 - Power module.
 - Printer accessories, for example, ink cartridge in case of a DeskJet printer.

Installing Your Agilent 8453 Spectrophotometer

Your spectrophotometer is very easy to install. This section provides an overview of the instrument and a checklist of the main installation procedure. If you have accessories for the spectrophotometer which are not covered in this section, see [Chapter 6](#), “Installing Accessories” or refer to the documentation that was delivered with your accessories for detailed information.

Installing the Waste Tubing

There is a waste outlet for the sample area. It is used to drain any liquids which may come from the sample area in case of a leak or a spill to a waste container. To connect your waste tubing to the spectrophotometer:

- 1 Lift the front part of the spectrophotometer up so that you can locate the waste outlet underneath the sample pan as shown in [Figure 1](#).

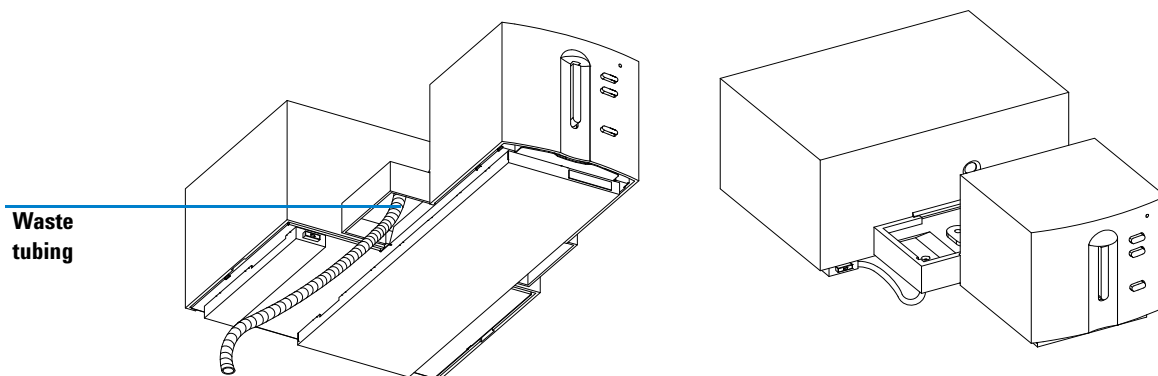


Figure 1 Connecting the Waste Tubing

- 2 Connect the waste tubing which comes with your spectrophotometer to the waste outlet underneath the sample pan.

1 Installation and Start Up

Installing Your Agilent 8453 Spectrophotometer

- 3 Put the other end of the waste tubing into the waste container.
- 4 Make sure that the waste tubing has a constant downslope from the spectrophotometer to the waste container.

Putting in the Cell Holder

- 1 Put the cell holder into the sample compartment and tighten the two fixing screws. See [Figure 2](#).

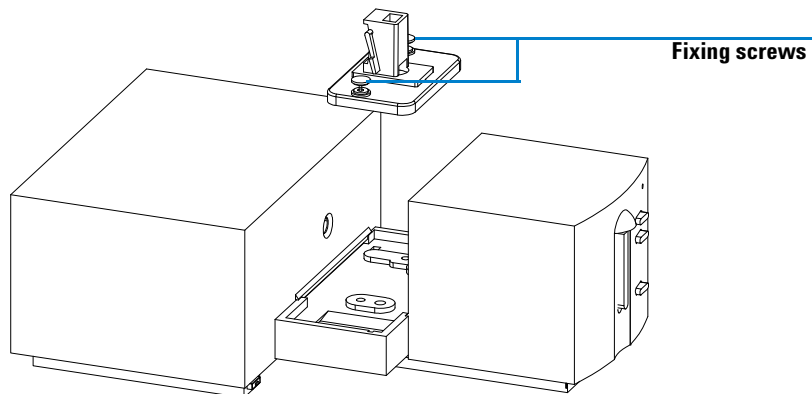


Figure 2 Mounting the Cell Holder

Connecting the Spectrophotometer to the Line Power

- 1 Your line voltage must be between 100–120 or 220–240 V AC $\pm 10\%$ with a line frequency of 50 or 60 Hz $\pm 5\%$. There is no voltage selector on your spectrophotometer because the power supply has automatic wide ranging capability.
- 2 Make sure the power switch of the spectrophotometer is in the off position, that is, the pushbutton stands out.
- 3 Plug the female end of the power cord into the power input socket at the rear of the spectrophotometer. Plug the male end of the power cord into your electrical outlet.

Connecting the Handheld Controller

Connect the flexible CAN cable of the handheld controller to the right CAN interface at the rear of the spectrophotometer. See [Figure 3](#).

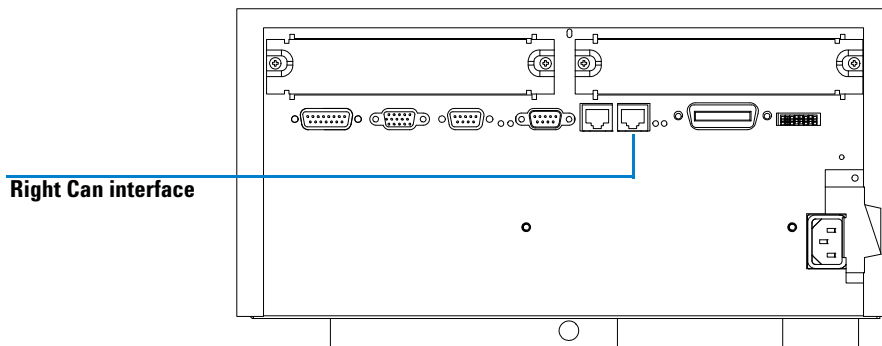


Figure 3 Connecting the CAN Cable of the Handheld Controller

The handheld controller is powered by the CAN interface of the spectrophotometer. It does not need any additional power supply.

The software of the handheld controller is started automatically as soon as the Agilent 8453 is powered on and the controller connected to the spectrophotometer.

Installing the Printer

Detailed instructions for installing your printer are specific to the type and configuration of the printer and are provided with it. This section gives a checklist of the main installation steps; refer to the documentation that was delivered with your printer for detailed information.

Setting up the Printer

- ✓ For more detailed instructions how to setup the printer, refer to the documentation that comes with the printer.
 - Remove all safety shipping material from the printer
 - Put in the ink cartridge(s) that come with the DeskJet printers
- ✓ Connect the printer cable with the serial end (9 Pins) to the RS 232 interface of the Agilent 8453 spectrophotometer and with the parallel end (centronics interface) to the printer. See [Figure 4](#).

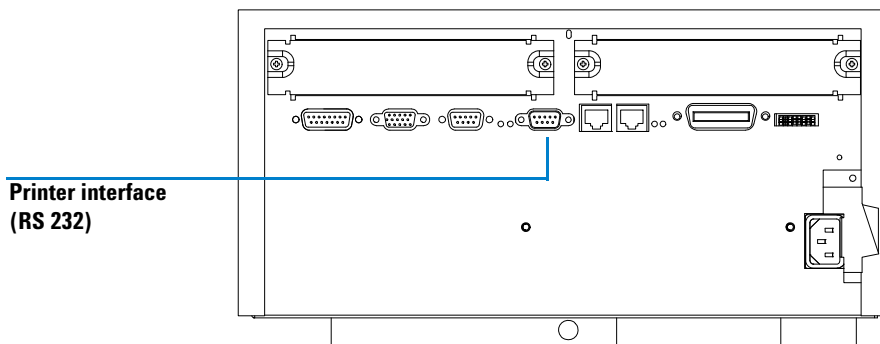


Figure 4 Connecting the Printer Cable to the Agilent 8453 Spectrophotometer

- ✓ Connect the printer to line power using the power module, and switch it on.
- ✓ For details how to setup the printer options in the software of the handheld controller, refer to [“Printing your Results”](#) on page 75 and [“Printer / Printer Configuration”](#) on page 100.

Starting the Agilent 8453E UV-visible Spectroscopy System

Before you turn on your spectrophotometer, make sure the light path through the sample compartment is clear. All filters should be removed from the light path. Anything blocking or absorbing part of the light beam could cause an error indication when you turn on the spectrophotometer.

Turning On

- 1 Turn on your spectrophotometer with the pushbutton at the lower left part of the instrument. The pushbutton will stay pressed in and light green if power is present.

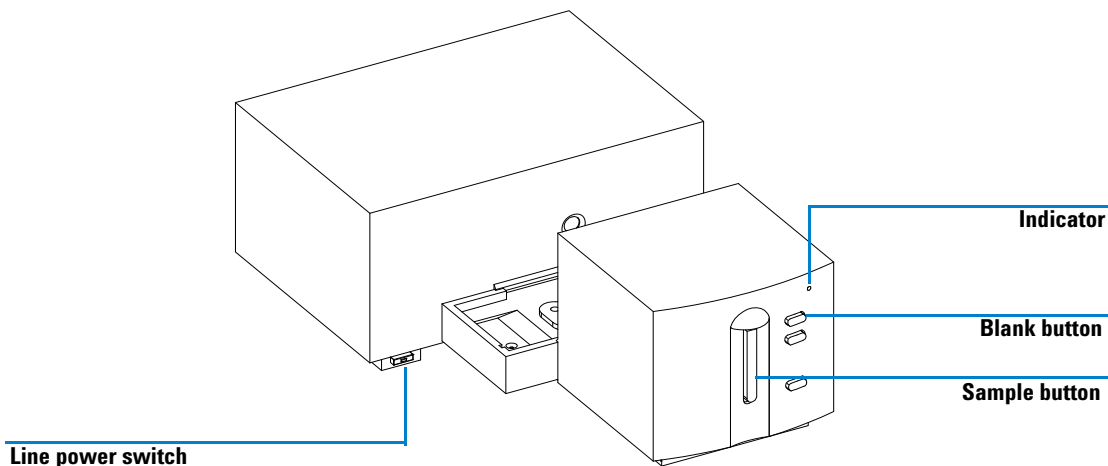


Figure 5 Turning on the Agilent 8453E UV-visible Spectroscopy System

- 2 The fan will start turning.
- 3 The indicator at the upper right corner of the front panel will light in three different colors. The yellow color which means busy will stay on during the

start up and self test routines, until at least one of the lamps is ignited and the spectrophotometer is ready to operate.

- 4 The turn on procedure including electronic self tests lasts about 20 seconds before the lamps start turning on. Under cold ambient conditions the lamp may not ignite and the instrument will repeat the ignition cycle automatically. The entire turn-on and self-test process will take about 1–2 minutes. Optical self tests are performed which last a few seconds.
- 5 When the green indicator at the upper right corner of the front panel comes on, the instrument is ready to measure.

NOTE

As soon as the handheld controller is connected to the spectrophotometer and the spectrophotometer is turned on, the software of the handheld controller is started automatically.

If the front panel indicator remains yellow the system has been configured for both lamps to be off at start up.

If the indicator shows a red color, there is an error condition. Try turning on the spectrophotometer again. If the red indicator persists check [Chapter 4](#), “Troubleshooting” of this manual, or the Service Manual of the Agilent 8453 spectrophotometer.

Selecting the Appropriate Language of the Software

If you have 2-MB version of the handheld controller (see label on rear of controller to identify whether it’s a 2-MB or 1-MB version), you can choose between english and french language versions of the software.

- 1 In the Startup dialog box that appears, use the up and down arrow keys to select the appropriate language and then press Enter.
- 2 To exit this dialog and to confirm your settings, press the F6-key (Done).

Changing the Language of the Software

You select the language of the software of your handheld controller at the first startup of your system. Having selected the language of the software in the start-up dialog, this dialog will not reappear when the system is restarted.

To change the language of the software you have to do the following:

- 1** Go into one of the task dialogs (e.g. Single WL).
- 2** In the task dialog, press the F5-key (System).
- 3** In the System dialog, press the m-key and select Language in the list box using the left and right arrow keys.
- 4** Select the appropriate language, then press Enter and the F6-key (Done) to confirm your settings.
- 5** In the dialog that appears, highlight the Yes button and press Enter to restart the system.

After restart of the system, the new language setting will be active.

Instrument Warm-up

Technically, once your spectrophotometer has passed the self-tests it is ready to run samples. For good results you may want to let the spectrophotometer warm up for at least 15 minutes. This time allows the system to thermally equilibrate. If the instrument was not stored at room temperature before it was turned on, allow more time for the instrument to stabilize.

In general, the life of your spectrophotometer will be increased by not switching it off at frequent intervals. Leave the instrument powered up but switch off the deuterium lamp if it is not expected a measurement will be made for the next 2 hours. Lamp intensity of the deuterium lamp and lifetime of lamps decrease with use.

Installation Checkout

Installation checkout is used to determine if the Agilent 8453E UV-visible spectroscopy system is functioning properly.

- 1 Be sure to verify the successful completion of the turn-on selftest of the spectrophotometer, that is, the indicator LED on the front panel of the Agilent 8453 spectrophotometer is lit and is green.
- 2 Use the vertical arrow keys of your handheld controller to select the *Single-WL* task, then press *Enter*. To exit the dialogue coming up press the F6-key (*Done*).
- 3 Fill the Quartz cell, which is shipped with the system, with distilled water and take a blank measurement by pressing the *Blank* button at the front of the instrument (see [Figure 5](#) on page 22). Empty the cell and fill it with the caffeine sample which is also shipped with your system. Take a sample measurement by pressing the *Sample* button at the front of the instrument (see [Figure 5](#) on page 22). Press the F6-key (*Graphic*) to display the spectrum on the screen of your handheld controller. The spectrum should resemble the one shown in [Figure 6](#).

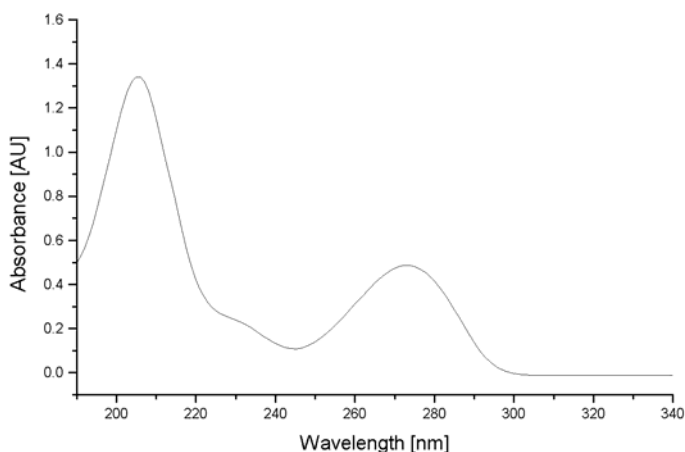
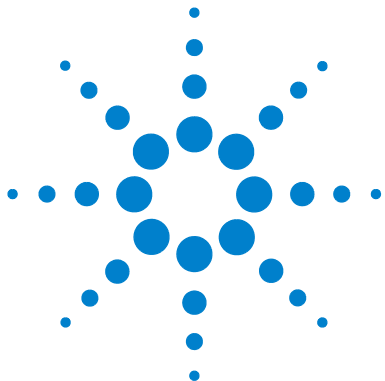


Figure 6 Spectrum of Caffeine (measured with a 10 mm Standard Quartz Cell)

1 Installation and Start Up
Installation Checkout



2 Good Measurement Practices

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Inserting a Cell	39

This chapter describes:

- making measurements
- selecting material, optical specification and type of cell
- handling and maintaining cells
- checklist for good results
- solvents selection,
- sample preparation,
- use of filters,
- stirring and temperature control of sample,
- how to insert cuvettes into the cell holder.



General Considerations

There are many factors that can affect the results of your measurements. This section provides brief discussions of some of the more important ones.

Spectrophotometer Design

The sample compartment of the Agilent 8453 spectrophotometer is open. Unlike conventional instruments the Agilent 8453 does not suffer from ambient stray light. The open sample area makes it easier to access it generally and to connect tubing to a flow cell or thermostatable cell holder.

Making Measurements

Blank (Reference) and Sample Measurement

Your spectrophotometer is a single beam instrument so you must measure a blank before you measure a sample. For the high accuracy measurements, the blank and the sample measurement should closely follow each other.

In general, a blank measurement should be repeated as often as is practical. Even in a thermally stable environment, a blank measurement should be taken every half hour to ensure accurate results.

Chemically, the only difference between the blank and the sample should be the presence of the analyte(s). For measurements with liquid samples, the blank should be a sample cell filled with the solvent you plan to use.

Sample Cell or Cuvette Material

Quartz sample cells (cuvettes) or sample cells with quartz face plates are required if you want to use the full 190 to 1100 nm wavelength range of your spectrophotometer.

If you plan on working only in the visible and/or short-wave near-infrared range of 350 to 1100 nm, you can use good quality glass cells.

Disposable plastic sample cells, for measurements in the range 400 - 1100 nm, are also available. The quality of these cells varies and they are generally not recommended.

Optical Specifications of Cells

The accuracy of the readings of a diode array instrument is very sensitive to spatial shifts of the analysis light beam. Cells having non parallel opposite faces, or so called wedge shaped cells, lead to a spatial shift of the light beam (see Figure 7). Therefore, the opposite cell walls illuminated by the analysis light beam have to very parallel. The parallelism is measured in terms of the angle between the two opposite cell walls. We recommend to use 10 mm path length cells with an angle which is below 0.1 degrees of an arc.

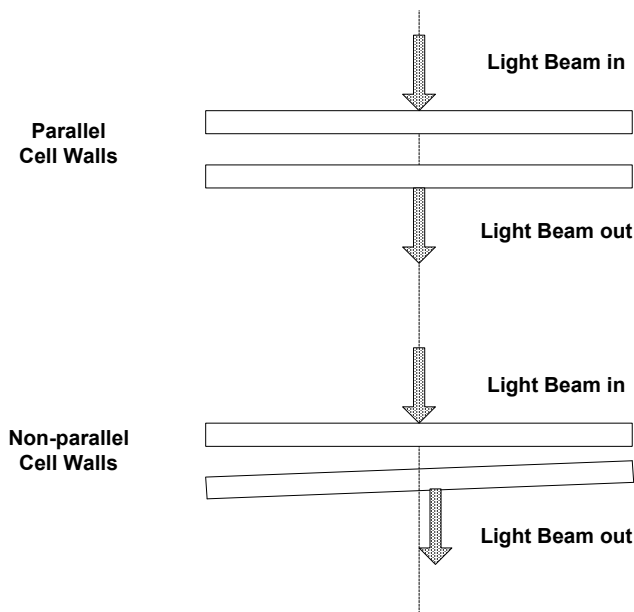


Figure 7 Shift of the Instrument Light Beam due to non Parallel Cell Walls

Apertured Cells or Cuvettes

In applications where sample volume is limited, *apertured* or microcells are used. The width of these cells is reduced to reduce the volume and the *blank* part of the cell must be blackened to avoid unwanted transmission and reflection through the side walls. If the side walls are not blackened the result will be poor photometric accuracy and, if different concentrations are measured, poor linearity.

The disadvantage of apertured and microcells is that part of the light beam is blocked. Not all the light passes through the sample and there can be some loss in sensitivity. See [Figure 8](#) for recommended and [Figure 9](#) for cells you should not use with the instrument.

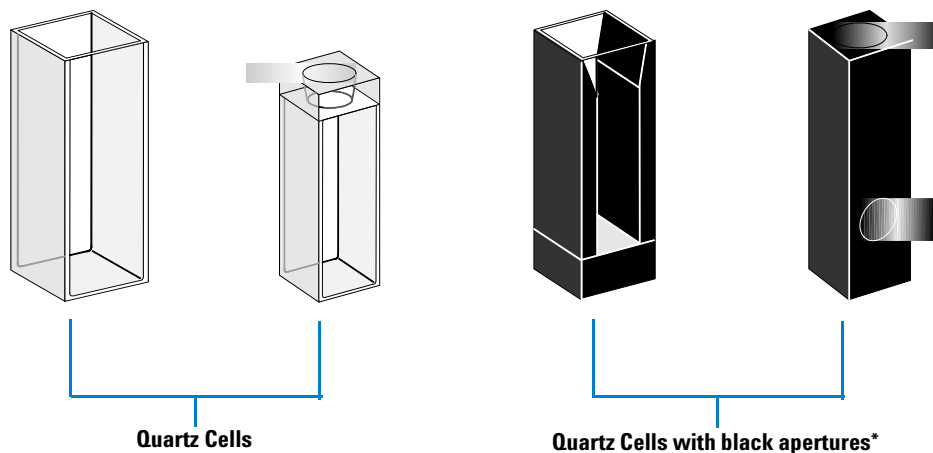


Figure 8 Recommended Cells

CAUTION

* Quartz Cells with apertures having a width of less than 2mm, when used with a multicell transport, can lead to measurements of poor reproducibility.

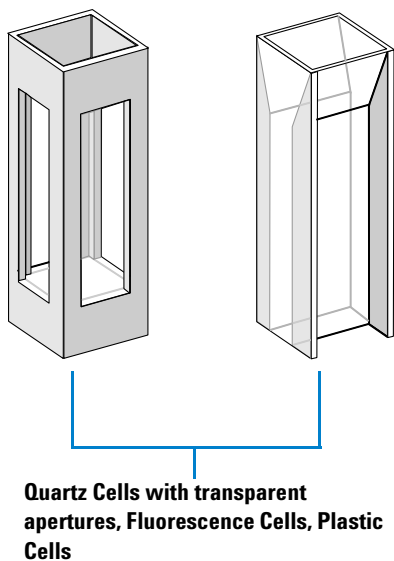


Figure 9 Cells You Should Not Use With the Instrument

Flow Cells

We recommend a sipper system with a flow cell for obtaining the high precision measurements. Using a flow cell eliminates the necessity of moving the cell between blank measurement and sample measurement. Also, the cell can be rinsed thoroughly with the solution to be measured.

The design of the flow cell should minimize entrapment of bubbles and flow *channeling* to provide the most reliable results.

Handling and Maintaining Cells or Cuvettes

Passivating New Cells

When filling a non passivated new cell with your sample, you will observe that air bubbles stick on the windows of your cell. To prevent the formation of sticky bubbles, rinse the cell with cleaning and passivating fluid (part number 5062-8529). The cleaning procedure you should apply is described on the label of the cleaning fluid container.

Cleaning Cells

The fats in fingerprints are significant absorbers in the UV region and, if left on optical surfaces, can cause erroneous results. Wipe off all fingerprints and contaminants before using a sample cell.

Use only high quality lens tissues (part number 9300-0761) and never dry the inside of a cell with lens tissues. Dry the inside of the cell with pressurized, oil free air, that prevents the cell from getting contaminated with tissue particles, or rinse the cell with blank or sample solution. Floating particles in the cell will deflect the light beam and so lead to a very poor quality of the measured spectrum.

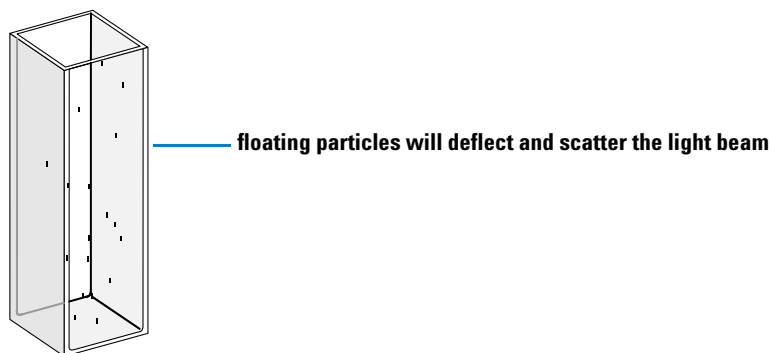


Figure 10 Floating Particles in a Cell

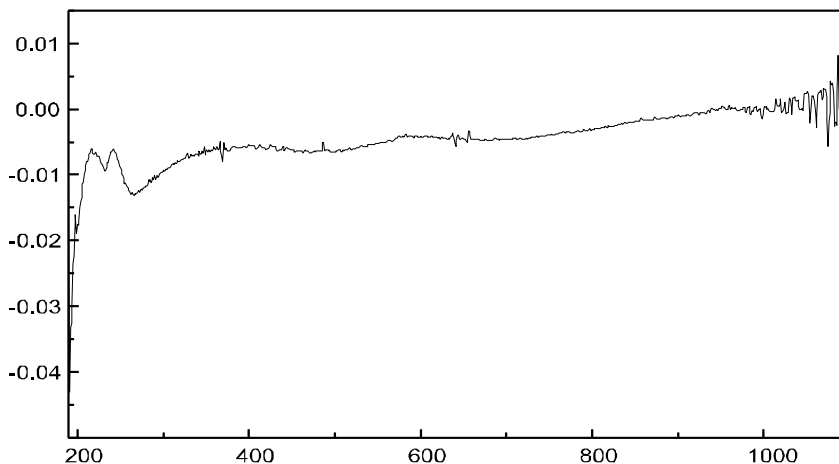


Figure 11 Spectrum Taken With Floating Particles in the Light Path

Lens tissues for glasses or other uses often contain detergents or lubricants which can affect your measurements. If possible avoid cleaning the faces of your cell between blank and sample measurements.

Handling Cells

Always install a cell so that it faces the same direction to minimize problems with cell non-uniformity. For best results with microcuvettes, leave your sample cell clamped in position throughout the measurement sequence. Solutions should be removed and replaced by pipette or use flow cells.

Solvents

Your choice of solvents should be based primarily on the solvent's absorbance characteristics over the wavelengths of interest, its suitability as a solvent for the analyte, and on experimental conditions. [Table 1](#) lists common solvents and the lower limit of their useful wavelength range.

Table 1 Lower Limit of UV Transmission for Some Common Solvents

Lower Limit	Solvent
180–195 nm	Sulfuric acid (96%) Water Acetonitrile
200–210 nm	Cyclopentane n-Hexane Glycerol 2,2,4-Trimethylpentane Methanol
210–220 nm	n-Butyl alcohol Isopropyl alcohol Cyclohexane Ethyl ether
245–260 nm	Chloroform Ethyl acetate Methyl formate
265–275 nm	Carbon tetrachloride Dimethyl sulfoxide Dimethyl formamide Acetic acid
280–290 nm	Benzene Toluene m-Xylene
Above 300 nm	Pyridine Acetone Carbon disulfide

WARNING

Many of the solvents in [Table 1](#) are hazardous. Be sure you fully understand their properties before using them.

When using volatile solvents such as acetone or methylene chloride, make sure that the sample cell is stoppered. Evaporation of a solvent can change the solute concentration or cause *solution noise* due to solute convection currents. Both of these will affect the accuracy of your measurements. We also recommend stirring and temperature control when you use volatile solvents.

When using water as solvent we recommend using UV grade or HPLC grade water to reduce unwanted absorbance from the water. If you are using the sipper/sampler system the water should be degassed to avoid bubble formation in the flow cell, especially if the water comes from a pressurized water supply.

Sample Preparation

The sample cell should be rinsed three to five times with your intended solvent before you fill it with the pure solvent that will be used in the measurement. Turning the cell upside down on a small stack of absorbent tissues will help remove any residual solvent. This treatment will minimize contamination from previous experiments.

Samples which contain colloidal dispersions, dust or other particulate matter should be filtered, centrifuged or allowed to settle. If not, the overall attenuation-of-transmittance spectrum due to light scattering and/or reflection will hide the spectral information from the analyte.

Photosensitive Samples

A few substances are very photosensitive. They degrade or undergo photochemical reactions if exposed to light. This can be easily seen by a decrease of sample absorbance over time.

Use of Filters

At shorter wavelengths, higher-energy UV light is most likely to degrade photosensitive samples. If you have a problem, you can selectively block portions of the UV spectrum with a UV cut-off filter. An optical filter wheel assembly with three cut-off filters is available for the spectrophotometer. The cut-off wavelength of the filter you choose should be low enough that it does not eliminate important spectral information but high enough that it blocks the light that could degrade your sample. If you use a filter with your samples, you must use the same filter when you make your blank measurement.

For further information about the filter wheel, see [“Optical Filter Wheel”](#) on page 115.

Turning the D₂-Lamp off

The short wavelength radiation leading to photodegradation comes from the light of the D₂-Lamp. For application where readings are taken at wavelengths above 400 nm, the D₂-Lamp can be turned off. The light intensity supplied by the Tungsten lamp is sufficient for a good signal to noise ratio over the wavelength range 400 nm - 1100 nm. When using cells with small apertures, you should check the signal to noise ratio by making sample measurements under conditions of your application.

Stirring and Temperature Control

Solution homogeneity can be a problem, especially for viscous solutions. There are cases where, due to convection induced gradients, rapid absorbance changes may give irreproducible data. These changes can be observed spectroscopically by taking measurements with short integration times. To minimize convection effects keep the temperature of your sample the same as the cell holder or environmental temperature. Problems like these can also be minimized by using a thermostatable cell holder and/or a stirring module.

A similar effect can occur in cases of incomplete mixing. This is especially true where the specific gravities or miscibilities of the solvent and analyte are quite different. Again, stirring is a way to prevent this kind of problem.

In an unstirred cell, it is sometimes possible to observe local photodegradation of sensitive analytes. Because the actual volume of the sample in the light path is very small, stirring the sample will reduce the time any given analyte molecule is in the light path. This minimizes the photodegradation and increases homogeneity. Using a flow cell with continuous flow can yield similar results.

Checklist for Best Results

Cell:

- ✓ Cell is made of quartz or glass
- ✓ Apertured cell has black sides
- ✓ Apertured cell has an aperture greater than or equal to 3 mm
- ✓ Cell windows are free of fingerprints and other contamination
- ✓ Flow cell used instead of an apertured standard cell

Measurements:

- ✓ Solution in cell is free of floating particles
- ✓ Solution in cell and cell walls are free of bubbles
- ✓ Solution in cell is mixed homogeneously
- ✓ Blank measured on same solvent as sample
- ✓ Blank measurement shows a flat baseline (Figure 12 and Figure 13 on page 38 show a good and a poor baseline)
- ✓ Cell orientation of blank and sample measurements is the same
- ✓ Ideally the cell is not removed between the measurements, which means the cell is filled/rinsed using a pipette or a flow cell is used
- ✓ Time between blank and sample measurement should be short

2 Good Measurement Practices

General Considerations

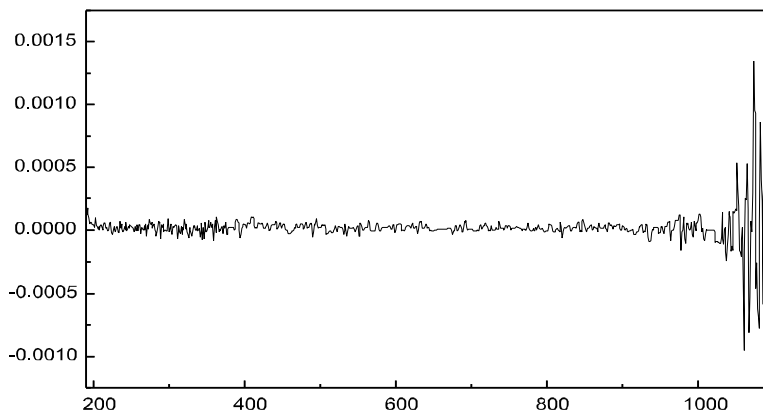


Figure 12 Example of a Blank on Water Showing a Good Baseline

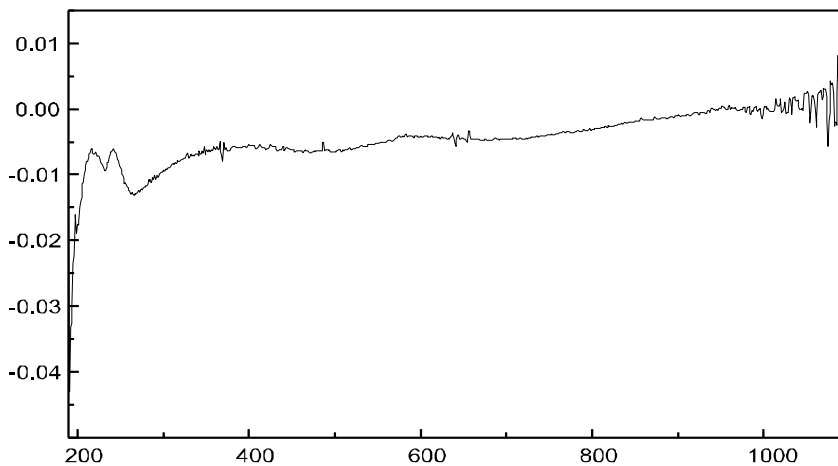


Figure 13 Example of a Blank on Water with Bubbles Causing a Poor Baseline

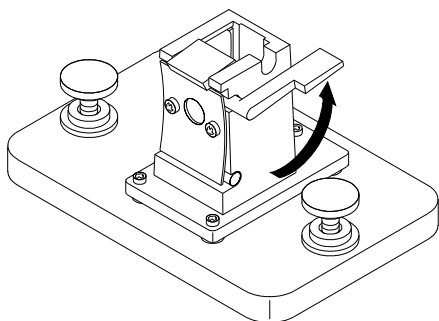
NOTE

If your blank or spectra shows artifacts similar to the one in [Figure 13](#), see [“Solvents”](#) on page 34 to optimize the measurement procedure.

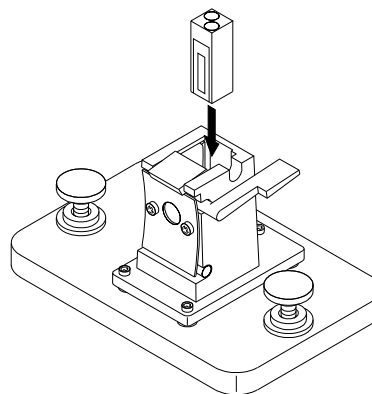
Inserting a Cell

Your spectrophotometer is shipped with the standard single-cell cell holder you first have to install in the sample compartment. This cell holder accommodates standard cells or flow cells. To insert a sample cell (cuvette) in the cell holder:

1 Move the locking lever to its up position.



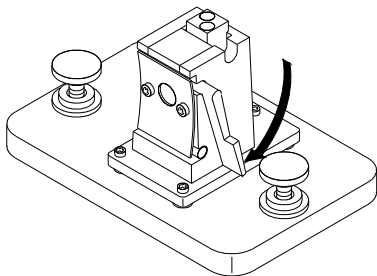
2 Insert the sample cell, making sure you orient it correctly. The frosted (non-clear) sides of the sample cell *should not* be in the path of the light beam.



2 Good Measurement Practices

Inserting a Cell

3 Lock the sample cell in place by pushing the locking lever back down.



Small volume flow cells and particularly any cells with less than a 2 mm aperture may require use of the optional adjustable cell holder. The adjustable cell holder helps you ensure the cells are properly centered in the light path.



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3 Operating your Agilent 8453E UV-visible Spectroscopy System

This chapter gives you an overview of:

- definitions of keys on the control module,
- definitions of the keys on the spectrophotometer,
- software features,
- navigating through the tasks,
- setting the parameters in a task,
- saving, loading and deleting of data,
- saving, loading and deleting of a method,
- printing your results,
- setting up parameters for your accessories,
- switching the lamps on an off,
- changing your PC-card, and
- triggering the Agilent 8453E UV-visible spectroscopy system from an external device.

Handheld Controller Overview

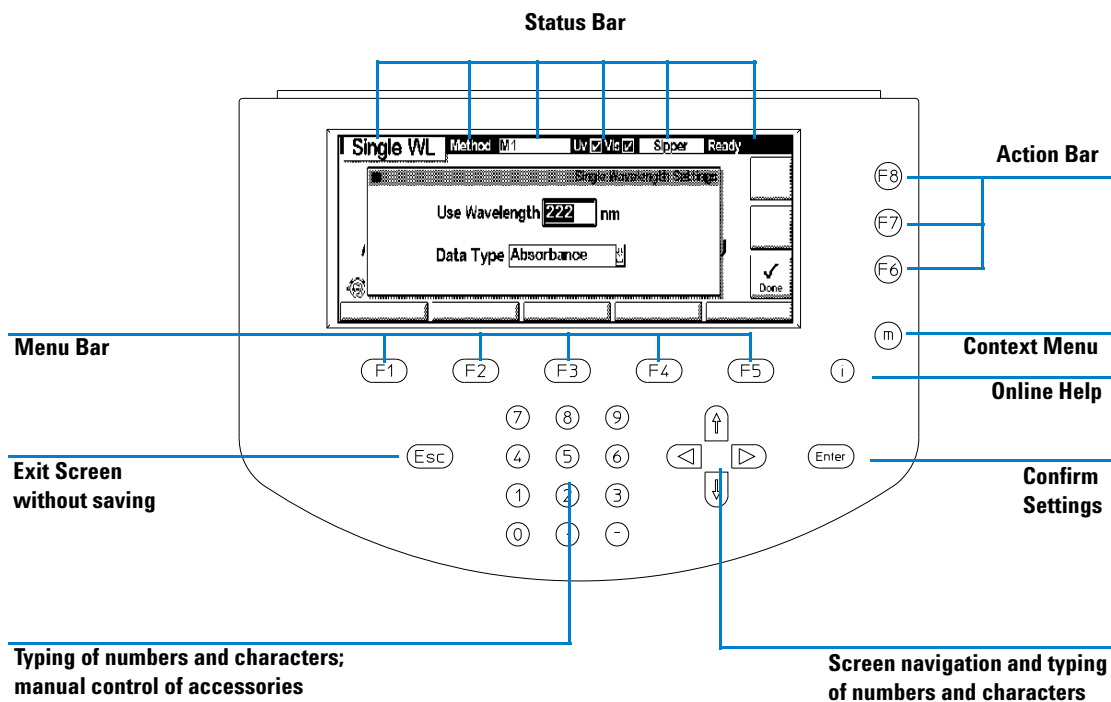


Figure 14 Description of Control Module Keys

NOTE

Press the i-key of the handheld controller for online help.

NOTE

The actual definitions of the keys change with task and context.

Spectrophotometer Overview

The front view of the spectrophotometer is shown in [Figure 15](#).

The spectrophotometer is shipped with the standard single-cell holder. Standard and accessory cell holders can be removed and replaced in seconds with few or no tools. See “[Installing Accessories](#)” on page 105 for more details.

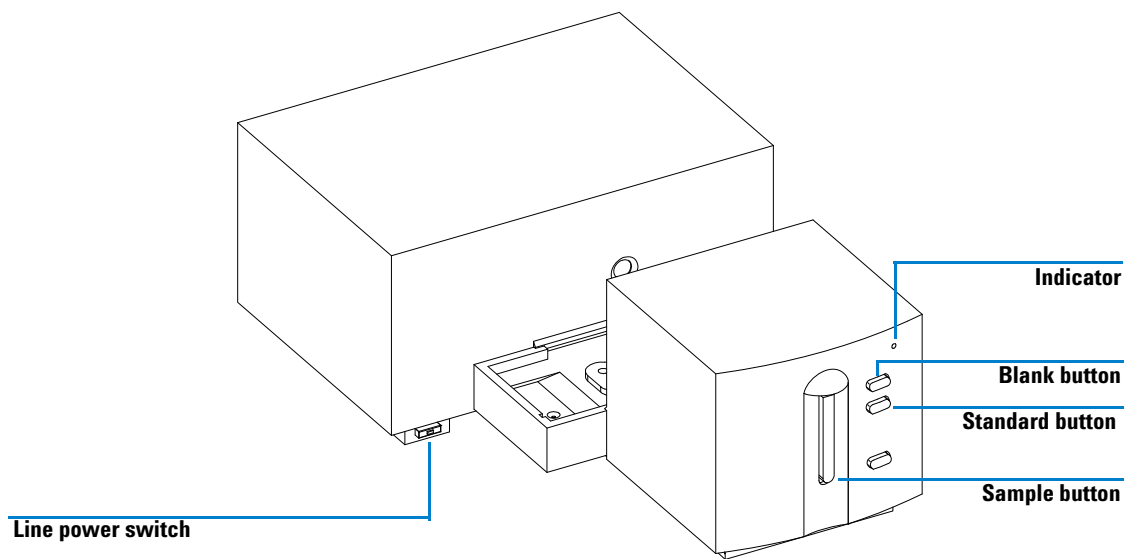


Figure 15 Front View and Keys of the Spectrophotometer

The four push-buttons on the front panel cause the following actions to be performed and the resulting data to be sent to the handheld controller.

- blank: the instrument takes a blank measurement, that is, a reference measurement of solvent without the analyte. This is used in all subsequent sample measurements until a new blank measurement is taken.
- sample: the instrument takes a sample measurement or starts a series of measurements. This depends on the parameters set in your software.

- standard: the instrument takes a measurement of a standard. Additional information, such as concentration and so on, have to be entered in the operating software.
- stop: this button is not active with the handheld controller.

The line power switch is located at the lower left part of the instrument. Pressing it will turn on the instrument. It stays pressed in and shows a green light when the instrument is turned on. When the line power switch stands out and the green light is off, the instrument is turned off.

Software Overview

This section provides you with a general overview over the features of the software of the handheld controller and how you can get access to them.

All software features are grouped by the definition of the keys.

Tasks (F1-key)

When booting up the system, the software comes up with a screen giving the user the possibility to choose between six different software tasks:

- 1 Single Wavelength
- 2 Multi Wavelength
- 3 Equation
- 4 Spectrum
- 5 Quantification
- 6 Kinetics

The general feature set of the individual tasks is shown in [Table 2](#).

Table 2 Feature Set of Software Tasks

Task	Number of Wavelengths	Set of Readings	Actions	Task Specials	Accessories
Single WL	1	Absorbance Transmittance 1. Derivative ... 4. Derivative	Blank Sample	• Display of Sample Spectrum	Multicell Transport or Sipper
Multi WL	4	Absorbance Transmittance 1. Derivative ... 4. Derivative	Blank Sample (Start)	• Display of Sample Spectra • Background Correction • Measurement Sequence (Multicell Transp.)	Multicell Transport or Sipper

Table 2 Feature Set of Software Tasks (continued)

Task	Number of Wavelengths	Set of Readings	Actions	Task Specials	Accessories
Equation	4	Absorbance Transmittance 1. Derivative ... 4. Derivative	Blank Sample (Start)	<ul style="list-style-type: none"> • Display of Sample Spectra • Measurement Sequence (Multicell Transp.) • Data Evaluation with user defined Equation 	Multicell Transport or Sipper
Spectrum	Complete range	Absorbance Transmittance 1. Derivative ... 4. Derivative	Blank Sample (Start)	<ul style="list-style-type: none"> • Display of Sample Spectra • Measurement Sequence (Multicell Transp.) • Mathematical Operations on Spectra 	Multicell Transport or Sipper
Quantification	1	Absorbance Transmittance 1. Derivative ... 4. Derivative	Blank Sample Standard (Start)	<ul style="list-style-type: none"> • Display of Sample/Standard Spectra • Background Correction • Measurement Sequence (Multicell Transp.) • Calibration • Display of Calibration Curve • Single Component Analysis 	Multicell Transport or Sipper
Kinetic	1	Absorbance	Blank Run	<ul style="list-style-type: none"> • Time resolved Measurement • Display of Timetrace • Rate Calculation 	Multicell Transport or Sipper

Settings (F2-key)

Under *Settings* you have access to, depending on the task you are in, the

- setpoints of your task (e.g. wavelength, data type),
- parameters of your accessories (e.g. pump time of sipper pump, measurement sequence of multicell transport,
- display range of graphic,
- settings of your calibration (analyte name, unit, curve type)
- recalculation of your results (only *Kinetics* task).

File / Print (F3-key)

When you press the *File/Print* key you can

- load results (samples, standards, timetraces) from the PC-card,
- save results (samples, standards, timetraces) to the PC-card,
- delete results (samples, standards, timetraces) on the PC-card,
- delete methods on the PC-card,
- save methods to the instrument
- copy methods from the instrument to the PC-card,
- delete methods from instrument and PC-card,
- load methods into the buffer of the instrument,
- print your results.

System (F5-key)

The *System* key takes you into a new screen, where all the F-keys have new meanings.

Control (F1-key)

Control provides access to

- turning the lamps on and off,
- moving the multicell transport,
- starting the pump (only pump and no multicell transport connected).

Configure (F2-key)

Configure provides access to

- spectrophotometer settings (e.g. wavelength range, integration time),
- system options (e.g prompt for task settings),
- display contrast,
- printer settings.

Tests (F3-key)

Tests provides access to

- performance tests for the spectrophotometer (e.g. photometric accuracy)
- maintenance and diagnostic tests for the whole system (e.g. dark current, wavelength recalibration).

Records (F4-key)

The *Records* key brings you into another screen where you can check

- EMF limits (e.g. lamp usage in hours)
- firmware revision of the spectrophotometer and software revision of the handheld controller,
- system logbook (e.g. shutter open, UV lamp on),
- error logbook (e.g. visible lamp current),
- maintenance logbook (firmware upgrade done, lamp exchanged),
- info logbook
- firmware/software update interface.

Math (F4-key, only in Equation task)

The *Math* key allows you to choose one of the following mathematical operations

- Absorbance,
- Transmittance,
- Derivative,
- Add,
- Add Scalar,
- Subtract,
- Multiply Scalar.

Samples / Standards (F4-key, only in Quantification task)

In *Quantification* you can change from the *Calibration* screen to the *Evaluation* screen.

In *Calibration* you setup your calibration curve. In *Evaluation* you analyze your samples by means of your calibration curve. The concentrations of your samples are displayed on the screen.

Context Menu (m-key)

Depending on the *Task* you are in, you have direct and easy access to the following actions when you press the m-key:

- Lamps on/off,
- Clear sample measurement(s),
- Clear standard measurement(s) (only Quantification task),
- Clear timetrace measurement(s) (only Kinetic task),
- Export sample/standard (as *.wav file),
- Export timetrace (as *.csv file),

When you go to *System* the definition of the *Context Menu* actions changes to:

- *Date & Time*: set date and time,
- *About*: show system revision,
- *Restart*: restart the control module.

Under *Records*, which is an item under *System*, you will have the actions

- *Info Log*: show latest instrument messages,
- *About*: show system revision.

In the *Graphic* screen you can, except for *Single WL* and *Kinetics*,

- overlay up to three spectra of same data type.

Action Keys (F6, F7, F8-key)

Task Menu

In all software tasks of the handheld controller the action keys start default or previously defined measurements (F7- and F8-key). The definition of key F6 is such that you can display a selected spectrum (*Single WL*), or timetrace (*Kinetics*), or up to three selected spectra (all other tasks). The spectra you want to overlay in the graphics screen are marked by using horizontal arrow keys.

Blank (F8-key)

When pressing the *Blank-key* a reference measurement is carried out. You cannot view the blank spectrum, but it is internally stored in the RAM of the Agilent 8453 spectrophotometer. All sample or standard measurements always refer to the latest blank measured and there is always only one blank spectrum stored in the RAM.

Sample / Standard / Start/ Run (F7-key)

Depending on the *Task* you are in, pressing the F7-key starts either a

- Sample measurement,
- Standard measurement (only in *Quantification*),
- Measurement sequence defined for the multicell transport (not in *Single WL* and *Kinetics*)
- Kinetic run (only in *Kinetics*).

Graphic (F6-key)

Displaying up to three spectra of the samples or standards taken over the complete or a defined wavelength range of the spectrophotometer, can be achieved by pressing the F6-key (not in *Single WL* and *Kinetics*).

The wavelength range displayed can be changed when you choose *Graphic* in the *Settings* menu.

Miscellaneous Menus and Key Definitions

The action keys are not only used in the *Task* menus, but also in various different contexts like the *Settings* menu, or other sub-menus.

The definition of the keys changes with context and sub-menu. Their meaning can be easily deduced from the icon displayed and the action to be carried out. Only the most important definitions are given here.

Done (F6-key)

In the *Settings* menu, screens are displayed, allowing you to enter parameters for your task. To save your entries and exit the screen, press the *Done-key*. Whenever you want to exit a screen without saving your entries, press the *Escape-key*.

Print (F6-key)

In the *Records* sub-menu you can list the log book entries of several logbooks. To get a hardcopy of these logbooks, select the appropriate logbook and press F6.

In the *Test* submenu you can printout the test results by pressing the F6-key.

Navigation Keys (Arrow keys)

In all dialogs where you can

- edit entryboxes
- select options,
- select items from a list box,
- select and unselect check boxes,
- turn fields on and off

the arrow keys and alphanumeric keys are activated and help in setting up your parameters.

For all edit boxes where you have to

- type in names (of samples, standards, files, methods),

the arrow keys and alphanumeric keys are defined such, that you can enter names. The horizontal arrow keys allow you to move the cursor, whereas the vertical arrow keys are used to select a letter.

Numerical Keys

The keys of the numerical keyboard can have to different meanings, depending on the menu or sub-menu you are in.

- In the task menus you can control your accessories, like moving the multicell transport to different positions or starting the pump to turn clockwise, counterclockwise or stop.
- In screens with edit boxes (e.g. file save, analyte name) where you enter names, you can use the alphanumeric or arrow keys to type numbers and letters of the alphabet.

You can type names using the numeric keys (0–9). The “0” key contains zero and special characters. Each other key contains its corresponding number and three letters. Pressing “1” once gives you the number “1”, pressing “1” twice gives the letter “A”, pressing “1” three times gives “B” and pressing “1” four times gives “C”. The “2” key continues with “2”, then “D”, “E” and “F”, and so on.

Use the vertical arrow keys to move to the next position and the horizontal arrow keys to scan the alphabet and to find special characters. Press *Done* (function key F6) once you have finished to return to the main window.

Table 3 Text

Key	Characters Behind Key	Key	Characters Behind Key
0	!, ?, ~	5	M, N, O
1	A, B, C	6	P, Q, R
2	D, E, F	7	S, T, U
3	G, H, I	8	V, W, X
4	J, K, L	9	Y, Z

Escape and Enter Keys

Escape-Key

The *Escape-key* is always the appropriate key, when you want to exit a screen without saving your settings.

Enter-Key

In dialogs, you

- confirm the settings you typed in,
- select an item in a list box,

by pressing the *Enter-Key*.

In all task screens, except for *Single WL* task, you can select individual samples, standards or timetraces in a list box. Select one of the samples, standards or timetraces and press *Enter*. This will bring you in a dialog where you can edit the name of this sample, standard or timetrace.

Online Help (i-key)

Context Sensitive Help

When pressing the *i-key* the software brings you into the help system. The help topic which appears is always context sensitive, that is, it is related to the screen you are in, or the item you highlighted. You can select different items in the help menu by using the arrow keys and pressing enter to get more details on these topics.

Previous (F6-key)

When you browse through the help menu, a history file is logged internally. This log file holds the different items/screens you have been chosen since you entered the help menu.

With the *Previous* key you can scroll backwards in this history log file, successively going you back into the screens you have been before.

Related (F7-key)

Because the context sensitive help brings you always to topics directly linked to the screen you are in (or the topic you highlighted), your access to the help system is limited. With the *Related* key the help system leads you through, starting from your context sensitive help screen, new help topics which have similar or related contents.

Search (F8-key)

The help system not only includes topics on how to operate the handheld controller, but also information on

- installing your Agilent 8453E UV-visible spectroscopy system,
- maintaining your Agilent 8453E UV-visible spectroscopy system,
- configuring your Agilent 8453E UV-visible spectroscopy system,
- spectroscopic idioms (e.g. background correction),
- good measurement practice (e.g. cell selection, sample handling),
- task related information (e.g. Single Wavelength, Quantification),
- hardware related information (e.g. installing accessories),

3 Operating your Agilent 8453E UV-visible Spectroscopy System

Software Overview

- error messages from the handheld controller (message boxes like: ‘lamp door open’, ‘to high current through Tungsten lamp’ appear),
- error messages from the spectrophotometer (e.g. Red LED, instrument does not turn on)
- troubleshooting (e.g. how to do a blank measurement, or a sample measurement)
- tests (e.g. multicell transport test calibration, dark current, intensity).

Pressing the *Search* key gives you access to the choice to enter the help either via the

✓ Contents item,

or the

✓ Index item.

Choosing *Contents* brings you to the *Table of Contents* of the help system. How the table of contents is structured is shown in [Figure 16](#).

When you select *Index* you have the possibility to browse for words which are in the help system. The words are grouped in alphabetic order and have links to help screens. As soon as you choose one of the words from the list, the appropriate help screen is displayed.

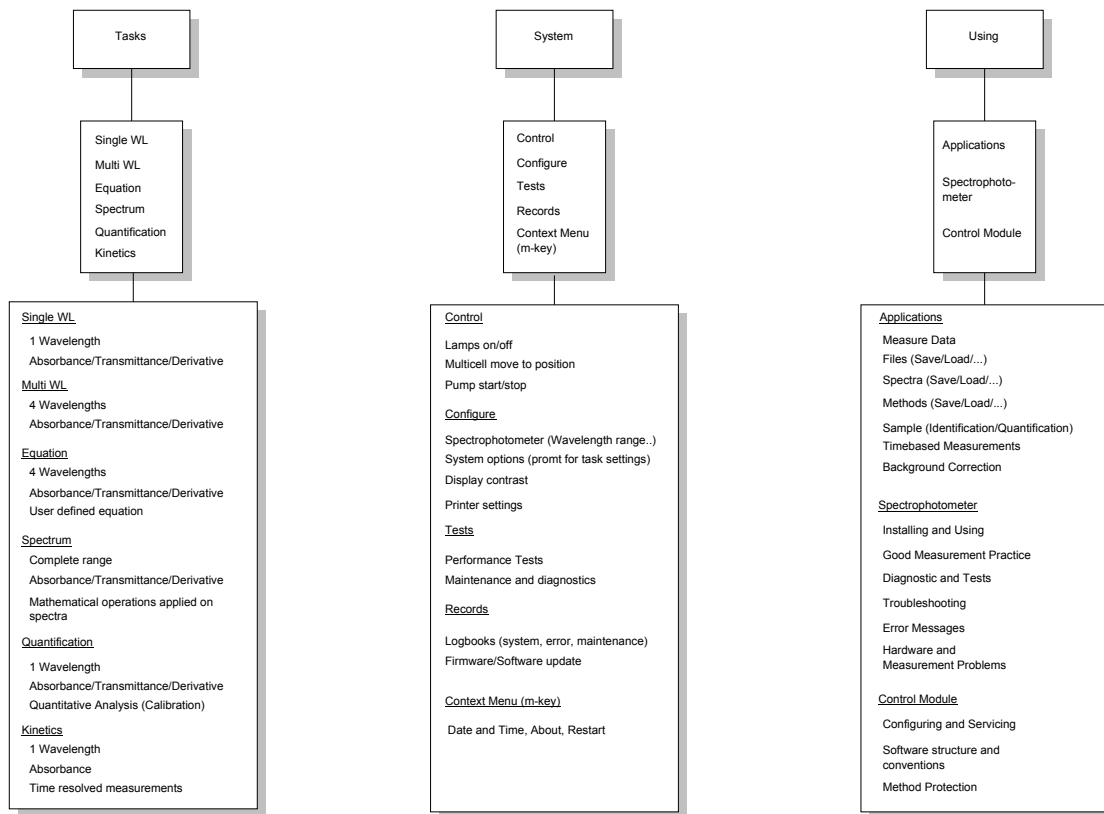


Figure 16 Table of Contents of the Help System

General Navigation Through the Tasks

A roadmap which helps you to access different tasks, settings, actions and informations in the software of the handheld controller is given in [Figure 17](#).

First try to locate the item you are interested in, in the roadmap. For example *Wavelength Recalibration*.

Then follow the lines, connecting this item with boxes, back, until you finally hit the upper left corner (task list box).

Now follow your path from the upper left corner down to the selected item. When leaving a box you have to press the appropriate key connected to the line you follow.

For the *Wavelength Recalibration* example you will get the following path:

- 1 Select one of the tasks, e.g. *Single WL*.
- 2 In the task screen press the *System* key.
- 3 In the system screen press *Tests*.
- 4 Then select *Diagnostics & Maintenance* and press *Enter*.
- 5 In the *Diagnostics & Maintenance* screen press the *Maintenance* key and select *Wavelength Recalibration*.
- 6 Follow the instructions on the screen.

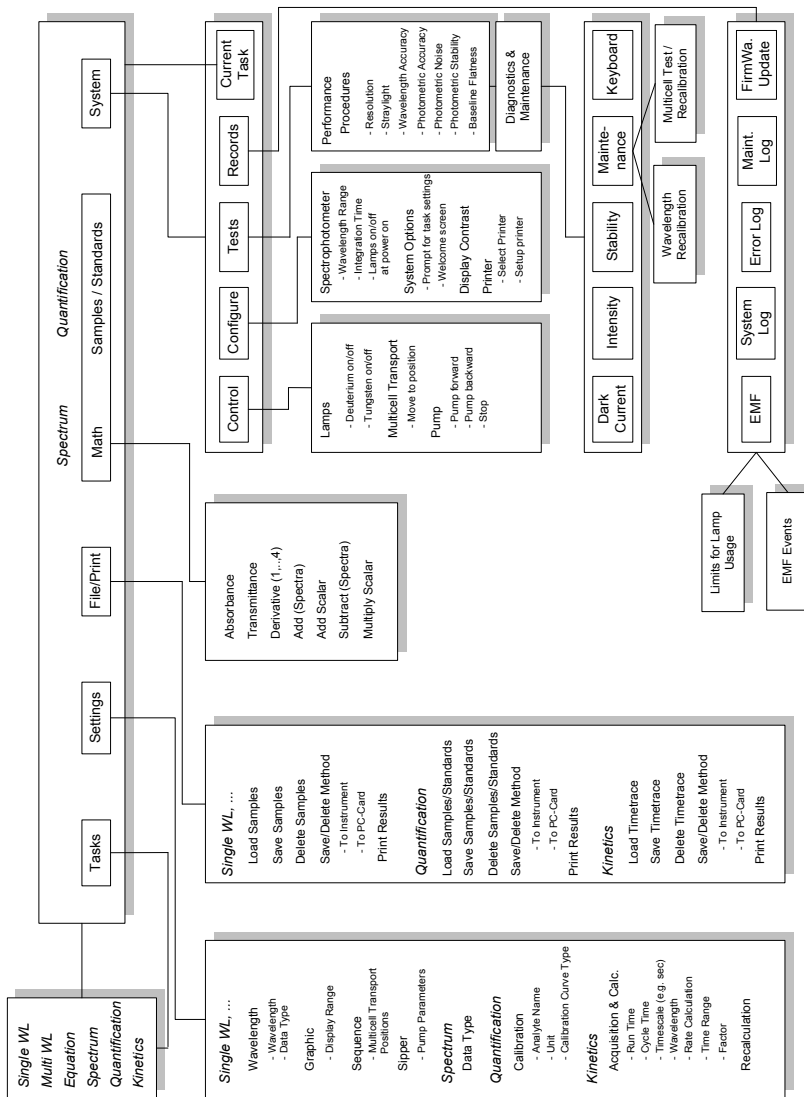


Figure 17 Roadmap for Software Navigation

Single Wavelength Task

- 1 Select *Single WL* from the task list box.
- 2 Type in the appropriate analytical *Wavelength* using the numerical keys.
- 3 Select your *Data Type* using the horizontal arrow keys.
- 4 Press the F6-key (*Done*) to save your settings and exit the screen.
- 5 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 6 Press the F7-key (*Sample*) to make a measurement on your sample.
- 7 The result of your measurement is automatically displayed on the screen.
- 8 Press the F6-key to display the sample spectrum on the screen.

NOTE

How you save your results and your measurement parameters is described in [“Saving, Loading and Deleting Your Samples, Standards and Timetraces”](#) on page 69 and [“Saving, Loading and Deleting Your Methods”](#) on page 72.

Multi-wavelength Task

- 1 Select *Multi WL* from the task list box.
- 2 Type in the appropriate analytical *Wavelength(s)* using the numerical keys.
- 3 Select your *Data Type* using the horizontal arrow keys.
- 4 Select a *Background Correction* if necessary and type in the *Reference Wavelength(s)*.
- 5 Press the F6-key (*Done*) to save your settings and exit the screen.
- 6 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 7 Press the F7-key (*Sample*) to make a measurement on your sample.
- 8 The result of your measurement is automatically displayed on the screen.
- 9 Select a sample by using the horizontal arrow keys and press the F6- key to display a sample spectrum on the screen.

NOTE

How you save your results and your measurement parameters is described in “[Saving, Loading and Deleting Your Samples, Standards and Timetraces](#)” on page 69 and “[Saving, Loading and Deleting Your Methods](#)” on page 72.

Equation Task

- 1 Select *Equation* from the task list box.
- 2 Type in the appropriate analytical *Wavelengths* using the numerical keys.
- 3 Select your *Data Type* using the horizontal arrow keys.
- 4 Select a *Unit* using the horizontal arrow keys.
- 5 Type in your equation for evaluation using the arrow-, F1-, F2-, F3-, F4 and F5- keys. Use the F7-key (*Delete*) to delete a single entry and the F8-key (*Clear*) to delete the complete equation.
- 6 Press the F6-key (*Done*) to save your settings and exit the screen.
- 7 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 8 Press the F7-key (*Sample*) to make a measurement on your sample.
- 9 The result of your measurement is automatically displayed on the screen.
- 10 Select a sample by using the horizontal arrow keys and press the F6-key to display a sample spectrum on the screen.

NOTE

How you save your results and your measurement parameters is described in “[Saving, Loading and Deleting Your Samples, Standards and Timetraces](#)” on page 69 and “[Saving, Loading and Deleting Your Methods](#)” on page 72.

Spectrum Task

- 1 Select *Spectrum* from the task list box.
- 2 Select your *Data Type* using the horizontal arrow keys.
- 3 Press the F6-key (*Done*) to save your settings and exit the screen.
- 4 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 5 Press the F7-key (*Sample*) to make a measurement on your sample.
- 6 If you want to apply a mathematical operation on the spectra, press the F4-key (*Math*) and select an operation. Follow the instructions on the screen.
- 7 When the mathematical operation has been applied, the screen is automatically updated.

NOTE

How you save your results and your measurement parameters is described in “[Saving, Loading and Deleting Your Samples, Standards and Timetraces](#)” on page 69 and “[Saving, Loading and Deleting Your Methods](#)” on page 72.

Quantification Task

Calibration Curve

- 1 Select *Quantification* from the task list box.
- 2 Type in the appropriate analytical *Wavelength* using the numerical keys.
- 3 Select your *Data Type* using the horizontal arrow keys.
- 4 Select a *Background Correction* if necessary and type in the *Reference Wavelength(s)*.
- 5 Press the F6-key (*Done*) to save your settings and exit the screen.
- 6 Press the F2-key (*Settings*) and select *Calibration*.
- 7 Type in the name of your *Analyte* using the horizontal arrow keys.
- 8 Select a *Unit* and *Calibration Curve Type*.
- 9 Press the F6-key (*Done*) to save your settings and exit the screen.
- 10 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 11 Press the F7-key (*Standard*) to make a measurement on your standard.
- 12 Press Enter-key and type in the concentration of your standard using the numerical keys. Press F6-key to exit the screen.
- 13 The calibration curve is automatically calculated and displayed.
- 14 Select a standard by using the horizontal arrow keys and press the F6-key to display a standard spectrum on the screen.

Analysis

- 1 Press the F4-key (*Samples*) to get access to the *Evaluation* screen.
- 2 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 3 Press the F7-key (*Sample*) to make a measurement on your sample.
- 4 Select a sample using the vertical arrow keys and press *Enter*.
- 5 Type in the *Sample Name* using the horizontal arrow keys.
- 6 Type in a *Dilution Factor* using the numerical keys.

- 7 Press the F6-key (*Done*) to save your settings and exit the screen.
- 8 The result of your analysis is displayed on the screen.
- 9 Select a sample by using the horizontal arrow keys and press the F6- key to display a sample spectrum on the screen.

NOTE

How you save your results and your measurement parameters is described in “[Saving, Loading and Deleting Your Samples, Standards and Timetraces](#)” on page 69 and “[Saving, Loading and Deleting Your Methods](#)” on page 72.

Kinetics Task

- 1 Select *Kinetic* from the task list box.
- 2 Type in a *Run Time* (total time of analysis), a *Cycle Time* (time between successive measurements) using the numerical keys and select a *Timescale*.

NOTE

For *Cycle Times* below 3 seconds plus twice the integration time, the shutter will be kept open during the kinetic run. Thus the samples will be irradiated with the full lamp light during the complete *Run Time*.

NOTE

For *Cycle Times* below 2 seconds plus twice the integration time, the stray light correction will not be applied. This can lead to a decrease in instrument linearity in the UV region.

- 3 Type in a *Wavelength* using the numerical keys and select a *Rate Calculation*.
- 4 When a *Rate Calculation* has been selected, specify a *Calculation Time Range*, a *Factor* and if necessary a new unit (*to convert to*).
- 5 Press the F6-key (*Done*) to save your settings and exit the screen.
- 6 Press the F8-key (*Blank*) to make a measurement on your solvent.
- 7 Press the F7-key (*Start*) to start your kinetic run.
- 8 The result of your measurement is automatically displayed on the screen.
- 9 After the kinetic run has finished, press the F6-key to view the timetrace.
- 10 If you want to recalculate your rate coefficient based on the actual timetrace, go to F2 (*Settings*) and select *Recalculation*.
- 11 Select a *Rate Calculation*, specify a *Calculation Time Range*, a *Factor* and if necessary a new unit (*to convert to*).
- 12 Press the F6-key (*Done*) to save your settings and exit the screen.
- 13 The result of your recalculation is automatically displayed on the screen.

NOTE

How you save your results and your measurement parameters is described in “[Saving, Loading and Deleting Your Samples, Standards and Timetraces](#)” on page 69 and “[Saving, Loading and Deleting Your Methods](#)” on page 72.

Displaying of Data on the Screen

You can display spectra or timetraces on the screen of your handheld controller. The maximum number of spectra you can overlay on the display is three.

You cannot overlay several spectra in *Single WL* task and you cannot overlay several timetraces in *Kinetics*.

Displaying a Spectrum or Timetrace on the Screen

- 1 Select a task from the task list box (e.g. *Single WL*).
- 2 After you did the appropriate settings in the task dialog, measure a blank. Then measure a sample, standard or timetrace, depending on the task you are in.
- 3 Select the data you want to display by highlighting the appropriate item in the list box of the screen using the vertical arrow keys.
- 4 Press the F6-key (*Graphic*) to display the data.

Displaying Multiple Spectra on the Screen

- 1 Select a task from the task list box, except for *Single WL* and *Kinetics*.
- 2 After you did the appropriate settings in the task dialog, measure a blank. Then measure a sample or standard, depending on the task you are in.
- 3 Select the data you want to display by first highlighting the appropriate item in the list box of the screen and then marking it using the vertical arrow keys. Do the same for the up to two additional samples or standards you want to overlay in the graphic window.
- 4 Press the F6-key (*Graphic*) to display the samples or standards you select.

Saving, Loading and Deleting Your Samples, Standards and Timetraces

Saving Your Samples, Standards and Timetraces

NOTE

When you save your data by selecting the save item in the *File/Print* list box, all data is always saved to the PC-card of your handheld controller. Saving data while no PC-card is installed, will lead to an error message.

Saving Samples / Standards (only in *Quantification*)

- 1 Press the F3-key (*File/Print*), select *Save Samples / Standards* with the vertical arrow keys and press *Enter*.
- 2 Use horizontal, vertical arrow keys and alpha-numerical keys to type in a file name for your samples / standards. The default file extension is *.sp.
- 3 Confirm your file name by pressing *Enter*.
- 4 To save your samples / standards press F6 (*Done*).

NOTE

If you want to exit a dialog without saving your samples / standards press *Esc*.

Exporting a selected Sample / Standard as ASCII File

- 1 Press the m-key.
- 2 Select *Export Selected Sample* from the task list box
- 3 Use horizontal, vertical arrow keys and alpha-numerical keys to type in a file name for your samples / standards. The default file extension is *.wav.
- 4 Confirm your file name by pressing *Enter*.
- 5 To save your samples / standards press F6 (*Done*).

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Saving, Loading and Deleting Your Samples, Standards and Timetraces

Saving a Timetrace (only in *Kinetics*)

- 1 Press the F3-key (*File/Print*), select *Save Timetrace* with the vertical arrow keys and press *Enter*.
- 2 Use horizontal, vertical arrow keys and alpha-numerical keys to type in a file name for your timetrace. The default file extension is *.tr.
- 3 Confirm your file name by pressing *Enter*.
- 4 To save your timetrace press F6 (*Done*).

NOTE

If you want to exit a dialog without saving your timetrace press *Esc*.

Exporting a Timetrace as ASCII File

- 1 Press the m-key.
- 2 Select *Export Selected Timetrace* from the task list box.
- 3 Use horizontal, vertical arrow keys and alpha-numerical keys to type in a file name for your timetrace. The default file extension is *.csv.
- 4 Confirm your file name by pressing *Enter*.
- 5 To save your timetrace press F6 (*Done*).

Loading Your Samples, Standards and Timetraces

Loading Samples / Standards (only in *Quantification*)

- 1 Press the F3-key (*File/Print*), select *Load Samples / Standards* with the vertical arrow keys and press *Enter*.
- 2 Use the vertical arrow keys to select your samples / standards and press *Enter*.
- 3 To load your samples / standards press F6 (*Done*).

NOTE

If you want to exit a dialog without loading your samples / standards press *Esc*.

Loading a Timetrace (only in *Kinetics*)

- 1 Press the F3-key (*File/Print*), select *Load Timetrace* with the vertical arrow keys and press *Enter*.
- 2 Use the vertical arrow keys to select your timetrace and press *Enter*.
- 3 To load your timetrace press F6 (*Done*).

Deleting Your Samples, Standards and Timetraces

Deleting Samples / Standards (only in *Quantification*) from the PC-card

- 1 Press the F3-key (*File/Print*), select *Delete Samples / Standards* with the vertical arrow keys and press *Enter*.
- 2 Use the vertical arrow keys to select the samples / standards you want to delete and press *Enter*.
- 3 To delete your samples / standards press the F8-key (*Delete*).

Deleting a Timetrace (only in *Kinetics*) from the PC-card

- 1 Press the F3-key (*File/Print*), select *Delete Timetrace* with the vertical arrow keys and press *Enter*.
- 2 Use the vertical arrow keys to select the timetrace you want to delete and press *Enter*.
- 3 To delete your timetrace press the F8-key (*Delete*).

Deleting Your Samples, Standards and Timetraces from the volatile memory of the controller

- 1 In the appropriate task (e.g. kinetics for Timetrace) press the m-key.
- 2 Use the vertical arrow keys to select either "*Clear all samples/ standards/ timetraces*" or "*Clear selected samples/ standards/ timetraces*"
- 3 To delete your *samples/ standards/ timetraces* press *Enter*.

Saving, Loading and Deleting Your Methods

The method file holds information on the settings of your system during measurement like:

- Wavelength range of the instrument,
- Integration time of the instrument,
- Used wavelength(s),
- Data type,
- Graphic settings.

In *Quantification* you will have additionally:

- Unit,
- Calibration curve,
- Analyte name.

In *Kinetics* you will have additionally:

- Kinetic timing,
- Kinetic Calculation,
- Rate unit.

When a multicell transport or a peristaltic pump is connected to the instrument the method furthermore holds the

- Measurement sequence for the multicell transport,

and the

- Pump parameters (e.g. pump time) for the peristaltic pump.

NOTE

When you save a method, it is always saved to the instrument. To save it to the PC-card, you have to download it from the instrument to the card.

Saving a Method

- 1 Press the F3-key (*File/Print*), select *Method...* with the vertical arrow keys and press *Enter*.
- 2 Press the F8-key (*Save As*).
- 3 Use horizontal and vertical arrow keys or alphanumeric keys to type in a *file name* for your method.
- 4 Confirm your method name by pressing *Enter*.
- 5 As additional information you can mark the *Protected* check box, type in a *User Id* and a *Comment*.

NOTE

You can prevent your method from being changed by marking the *Protected* check box.

- 6 To save your method to the instrument press F6 (*Done*).
- 7 If you want to copy a method to the PC- card press the F2-key (*PC- card*).
- 8 Press the F7-key (*<<Copy*) and the method will be stored on the PC- card.

Loading a Method

- 1 Press the F3-key (*File/Print*), select *Method...* with the vertical arrow keys and press *Enter*.
- 2 Press the F2-key (*PC- card*).
- 3 Select a method, using the vertical arrow keys, in the left list box and press the F8-key (*>>Copy*). The method will be automatically loaded to the instrument.

Deleting a Method on the Instrument

- 1 Press the F3-key (*File/Print*), select *Method...* with the vertical arrow keys and press *Enter*.
- 2 Press the F1-key (*Instrument*).
- 3 Press the F6-key (*Delete*).
- 4 Select whether you want to delete a *Selected Method* or *All Methods* and press *Enter*.

3 Operating your Agilent 8453E UV-visible Spectroscopy System

Saving, Loading and Deleting Your Methods

Deleting a Method on the PC-card

- 1 Press the F3-key (*File/Print*), select *Method...* with the vertical arrow keys and press *Enter*.
- 2 Press the F2-key (*PC- card*).
- 3 Select a method, using the vertical arrow keys, in the left list box and press the F6-key (*Delete*).
- 4 Select whether you want to delete a *Selected Method* or *All Methods* and press *Enter*.

Printing your Results

In each of the tasks you can print your results. Before applying the following procedure you should ensure the printer parameters are setup correctly. Refer to “[Printer / Printer Configuration](#)” on page 100 to get hints how you have setup the parameters for your printer.

- 1 Press the F3-key (*File/Print*), select *Print Results* with the vertical arrow keys and press *Enter*.
- 2 In the Dialog Box that comes up enter
 - the number of copies you want to print,
 - if you want to print to a PCL-file or a printer.
- 3 Press the F6-key, or select *Print* and press *Enter*, to start printing.

NOTE

You can stop and resume as well as delete print jobs from the spooler by pressing the F7-key.

Defining a Sequence for the Multicell Transport

Your multicell transport can be used with the Agilent 8453E UV-visible Spectroscopy System as an autosampler with 8 cell positions (G1120A) or 7 cell positions (89075D).

The software allows you to setup a measurement sequence, which defines the cell position used for your

- reference measurement (Blank),
- sample measurements (Sample or Standard).

When defining a sequence which includes a reference measurement, the Agilent 8453 spectrophotometer acts as a pseudo dual beam instrument.

Defining a Measurement Sequence for Blank and Samples

- 1 Choose a task.
- 2 If you are in *Quantification* task, make sure you are in the *Evaluation* not in the *Calibration* screen. In *Calibration* press the F2-key (*Samples*) to change to the *Evaluation* screen.
- 3 Press the F2-key (*Settings*), select *Sequence* with the vertical arrow keys and press *Enter*.
- 4 In the *Setup Sequence* user interface press enter to mark the box for defining the blank measurement.
- 5 Use the horizontal arrow key to activate the edit box for the cell number and use the numerical keys to type in a cell position number.
- 6 Press the horizontal arrow key again to highlight the check box for the sample measurements and press *Enter* to mark the check box.
- 7 Use the horizontal arrow key(s) to activate the edit box for the cell number(s) and use the numerical keys to type in cell position number(s).
- 8 To save your settings press F6 (*Done*).

Defining a Measurement Sequence for Blank and Standards (only in Quantification)

NOTE

The measurement sequence you will define under *Calibration* in the *Quantification* task (*Standards*) is completely independent of the measurement sequence you have defined under *Evaluation* in the *Quantification* task (*Samples*).

- 1 Choose the *Quantification* task.
- 2 Press the F4-key (*Standards*), if you are not already in the *Calibration* screen of the *Quantification* task.
- 3 Press the F2-key (*Settings*), select *Sequence* with the vertical arrow keys and press *Enter*.
- 4 In the *Setup Sequence* user interface press enter to mark the box for defining the blank measurement.
- 5 Use the horizontal arrow key to activate the edit box for the cell number and use the numerical keys to type in a cell position number.
- 6 Press the horizontal arrow key again to highlight the check box for the standard measurements and press *Enter* to mark the check box.
- 7 Use the horizontal arrow key(s) to activate the edit box for the cell number(s) and use the numerical keys to type in cell position number(s).
- 8 To save your settings press F6 (*Done*).

Setting the Parameters for the Sipper System

For high precision measurements, a sipper system (part number 89068D) has to be used with the Agilent 8453E UV-visible Spectroscopy System. It comprises a flow cell, connectors and tubings and a peristaltic pump.

In the software of the handheld controller you can define parameters for the peristaltic pump like

- Pump time (in s),
- Wait time (in s),
- Sample return (in %).

Setting the Pump Parameters

- 1 Choose a task.
- 2 Press the F2-key (*Settings*), select *Sipper* with the vertical arrow keys and press *Enter*.
- 3 In the *Sipper Settings* user interface use the horizontal arrow key to activate the edit box for the *Pump Time* and use the numerical keys to type in your pumping time.
- 4 Do the same for the *Wait Time* and *Sample Return*.
- 5 To save your settings press F6 (*Done*).

Switching the Lamps On and Off

Turning the Lamps On and Off in the System Menu

- 1 Choose a task.
- 2 Press the F5-key (*System*) and then the F1-key (*Control*).
- 3 In the *Control* list box select *Lamps* and press *Enter*.
- 4 Use the horizontal arrow keys to select a lamp and vertical arrow keys to turn it on or off.
- 5 To save your settings press F6 (*Done*).

Turning the Lamps On and Off - Shortcut

- 1 Choose a task.
- 2 Press the m-key, select *Lamps on/off* using the vertical arrow keys and press *Enter*.
- 3 Use the horizontal arrow keys to select a lamp and vertical arrow keys to turn it on or off.
- 4 To save your settings press F6 (*Done*).

Changing the PC-card of the Handheld Controller

Data like for example sample spectra can be saved to and retrieved from the PC- card of the handheld controller. How the PC- card is changed is described in the following procedure.

- 1 Disconnect the control module from the spectrophotometer before removing or inserting a PC-card.
- 2 Insert the connecting side of the PC- card into PC- card slot of the handheld controller until you feel it will not go in any further.
- 3 Reconnect the control module to the spectrophotometer to restart the software.
- 4 To remove the PC-card, press the card-eject button.

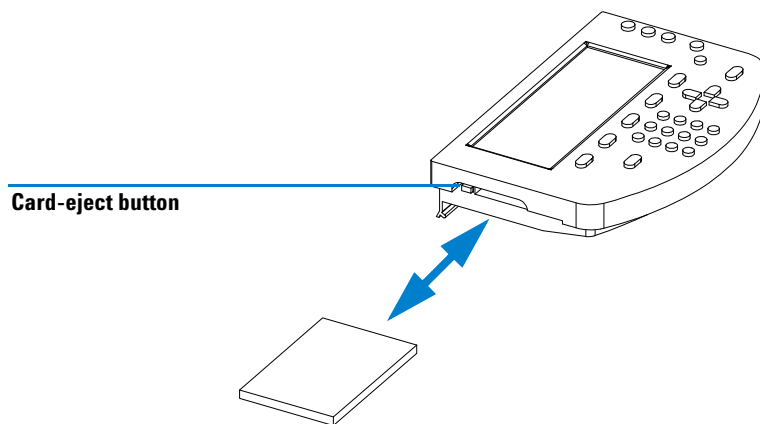


Figure 18 Changing the PC-card

Triggering the Spectrophotometer from an External Device

External Triggering

Unless the peristaltic pump is connected to the spectrophotometer through the GPIO interface, the GPIO port can be used to trigger a measurement from an external device. Three types of measurements can be triggered:

- Blank measurement,
- Sample measurement,
- Standard measurement (only in *Quantification*).

The trigger lines use the GPIO connector pins as shown in [Table 4](#).

Table 4 Trigger Inputs

Pin	Function
9	Trigger blank measurement
10	Trigger sample measurement
11	Trigger standard measurement (only in <i>Quantification</i> task)
15	Ground

Measurements are activated on contact closure to ground (pin 15).

NOTE

By default the trigger lines 9, 10, 11 are always active in all tasks, except for *Kinetics* task.

3 Operating your Agilent 8453E UV-visible Spectroscopy System

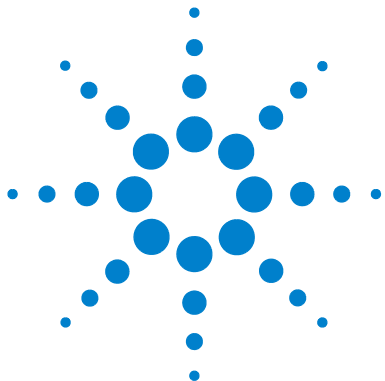
Triggering the Spectrophotometer from an External Device

External Triggering and Multicell Transport

You can setup two separate sequences for the multicell transport:

- Sample sequence,
- Standard sequence.

When triggering a sample/standard measurement, the software of the handheld controller will start performing the associated sample/standard sequence you have defined for your multicell transport.



4 Troubleshooting

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Error Messages 88

When your Agilent 8453E UV-visible Spectroscopy System does not come up or does not work properly, you have to apply troubleshooting procedures.

This chapter provides with troubleshooting procedures for the

- Agilent 8453 spectrophotometer,
- handheld controller,
- Agilent 8453E UV-visible Spectroscopy System.



Troubleshooting Your Spectrophotometer

This chapter gives you hints and tips how you can troubleshoot your Agilent 8453 spectrophotometer by checking the starting sequence of the instrument.

Starting Sequence of the Spectrophotometer

When the spectrophotometer is turned on, it runs through a selftest sequence. The different stages of the selftest sequence are indicated by the

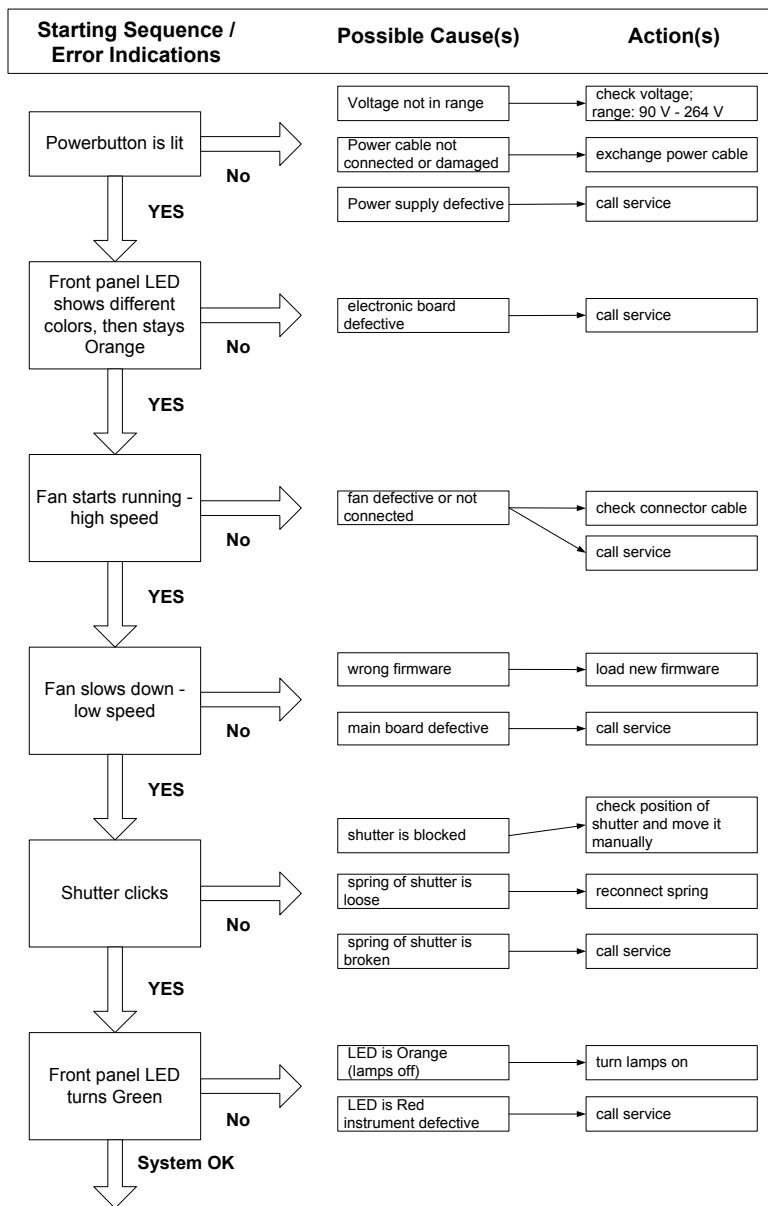
- colour of front panel LED,
- speed of fan,
- movement of shutter.

To troubleshoot your instrument, switch the instrument off. Then push the power button of the spectrophotometer to switch it on again and check the starting sequence of the instrument as shown in [Figure 19](#).

NOTE

The handheld controller should be connected to the spectrophotometer while you are troubleshooting the system.

When a check of the hardware of the spectrophotometer is necessary, e.g. check cable connection of the fan, refer to the *Reference Manual* of the Agilent 8453 spectrophotometer or call your service representative.



* refer to the Reference Manual of the Agilent 8453

Figure 19 Starting Sequence for Troubleshooting the Agilent 8453 Spectrophotometer

Troubleshooting Your Handheld Controller

Resetting the Software

If your handheld controller does not work correctly, disconnect the handheld controller CAN connector from the rear of the spectrophotometer and reconnect it. This will restart the software of the handheld controller.

If the problem still remains, then

- 1 power off the spectrophotometer and wait 1 minute, then power the spectrophotometer on again.
- 2 check if the LED at the front of the spectrophotometer turned Green after the boot-up sequence. In case the LED stays Orange or Red, refer to the “[Troubleshooting Your Spectrophotometer](#)” on page 84 for more help.

If the problem still remains, call your service representative.

PC-card not Recognized by the Controller

When your PC-card is not recognized by the handheld controller, either the card is defective or the card has not been inserted when the Agilent 8453E UV-vis Spectroscopy System was turned on.

To resolve the problem apply the following procedure:

- 1 Disconnect the handheld controller from the spectrophotometer by unplugging the Can connector.
- 2 Insert the PC-card.
- 3 Reconnect the handheld controller to the spectrophotometer by plugging in the Can connector in the right Can interface.

If the problem still remains, then

- 4 insert another PC-card and redo step 1 through 3 to check if your PC-card was defective.

If the problem still remains, call your service representative.

Checking the Keyboard

When the handheld controller is used very frequently, the keys might show aging effects and do not work properly any more. To check the performance of the keys of your keyboard individually, apply the following procedure:

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F5-key (*Keyboard*).
- 5 Press all keys of the keyboard successively. When a key is working properly, the symbol on the screen representing the key will turn dark.
- 6 To exit the keyboard test, press *Escape* twice in a row.

Troubleshooting Your Agilent 8453E UV-visible Spectroscopy System - Error Messages

Table 5 gives you a complete list of error codes and messages which may be created by the firmware of the Agilent 8453 spectrophotometer, whenever a internal error is detected. The software will prompt you for the error by displaying an error message box with the appropriate description.

To troubleshoot your Agilent 8453E Spectroscopy System refer to columns “Possible Cause(s)” and “Action(s)” in Table 5.

Table 5 System Error Messages and Troubleshooting Procedures

Error Message	Possible Cause(s)	Action(s)
Multicell Transport Home Position Not Found	Multicell transport mechanism is jammed	Check if fixing screws of Multicell Transport are screwed down Check manually if the multicell transport can move freely along it's range
	Electronics failed	Check for defective HOME switch, defective MCT cable, defective motor, defective MCT interface.
No Current Through VIS Lamp	Ignition failed Lamp is probably disconnected Lamp is defective Lamp power supply defective Main board defective	Retry to start the lamp Reconnect tungsten lamp* Replace tungsten lamp* Replace SLS (lamp power supply) board* Replace SPM (main) board*
No Voltage at VIS Lamp	see error ' <i>No Current Through VIS Lamp</i> '	See error ' <i>No Current Through VIS Lamp</i> '
UV Lamp Heater Overcurrent	Ignition failed Deuterium lamp defective Lamp power supply defective Main board defective	Retry to start the lamp Replace Deuterium lamp* Replace SLS (lamp power supply) board* Replace SPM (main) board*
VIS Lamp Overcurrent	see error ' <i>UV Lamp Heater Overcurrent</i> '	see error ' <i>UV Lamp Heater Overcurrent</i> ', but replace Tungsten lamp instead

Table 5 System Error Messages and Troubleshooting Procedures (continued)

Error Message	Possible Cause(s)	Action(s)
Cooling Fan Defective	Fan is disconnected Cycles per minute sensed at fan is too low - Fan sticks or is defective Main board defective	Reconnect cooling fan Move cooling fan manually and replace if necessary Replace SPM (main) board*
Lamp Door Open	Lamp door is open Light switch is not covered by metal door Light switch is defective	Close lamp door Reposition or replace metal door* Replace SLS (lamp power supply) board*
Digital Signal Processor Error	Communication error between the main processor and the digital signal processor on the SPM (main) board Communication error between SPM (main) board and SDA (data acquisition) board Flat ribbon cable defective	Replace SPM (main) board* Replace SDA (data acquisition) board* Replace flat ribbon cable*
Wavelength Calibration Failed	Deuterium lamp disconnected Deuterium lamp is defective Filter assembly defective Optical components misalignment Processing of calibration data invalid	Reconnect deuterium lamp* Screw down or replace deuterium lamp* Replace shutter/filter assembly* Screw down lamps or replace optical unit* Replace SPM (main) board*
Wavelength Recalibration Data Lost	Dip switch settings of 8 bit configuration switch are wrong Firmware has been updated SPM (main) board has been replaced	Check dip switch settings and perform a wavelength recalibration* Perform a wavelength recalibration Perform a wavelength recalibration
Raw Data Buffer Overflow	Spectra acquired cannot be transferred fast enough to handheld controller	Reduce number of spectra acquired, or raise cycle time, or reduce wavelength range, or increase memory on SPM (main) board*
Excessive Diode Dark Current	SDA (data acquisition) board unable to set dark current Photo Diode(s) defective	Replace SDA (data acquisition) board of spectrophotometer* Replace optical unit*

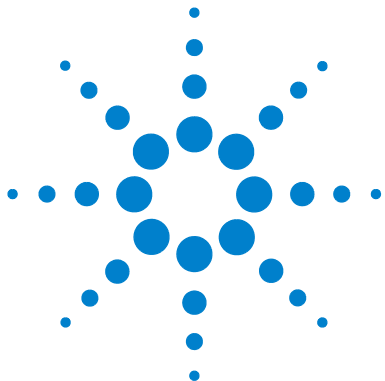
4 Troubleshooting

Troubleshooting Your Agilent 8453E UV-visible Spectroscopy System - Error Messages

Table 5 System Error Messages and Troubleshooting Procedures (continued)

Error Message	Possible Cause(s)	Action(s)
Invalid Data Points in Spectrum	Blank has higher absorbance than sample spectrum (Sticky) Bubbles or floating particles in cell deflect and obscure light beam Blank or / and sample medium is / are not mixed homogeneously enough Impurity in blank medium Variation of Absorbance during blank/sample measurement due to photodegradation or evaporation or thermal expansion of medium Deuterium lamp is defective Electronics and/or optics defective	Ensure sample and blank use same solvent or chemical matrix Clean and passivate cells (see “ Good Measurement Practices ” on page 27) Wait till blank/sample medium equilibrates or stir blank/sample medium Clean cell /blank medium Use topped cells for blank/ sample measurement and/or use UV cut-off filter for blank/sample measurement Screw down or replace deuterium lamp * Replace SPM (main) board, and/or SDA (data acquisition) board, and/or optical unit*
No Current Through UV Lamp	Deuterium lamp is disconnected Deuterium lamp is defective Electronics failed	Reconnect Deuterium lamp* Replace Deuterium lamp* Replace SLS (lamp power supply) board, and/or SPM (main) board*
No Voltage at UV Lamp	see error ‘ <i>No Current Through UV Lamp</i> ’	see error ‘ <i>No Current Through UV Lamp</i> ’
UV Lamp Ignition Failed	see error ‘ <i>No Current Through UV Lamp</i> ’	see error ‘ <i>No Current Through UV Lamp</i> ’
No UV Lamp Heater Current	see error ‘ <i>No Current Through UV Lamp</i> ’	see error ‘ <i>No Current Through UV Lamp</i> ’

* for detailed instructions, refer to the *Service Manual* of the Agilent 8453 UV-visible spectrophotometer which comes with your system.



5 Service and Maintenance

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This chapter describes the service capabilities of your Agilent 8453E UV-visible Spectroscopy System such as

- logbook entries,
- lamp counters,
- system tests,
- printer configuration,

and basic maintenance tasks which are supported by the software of the handheld controller such as

- recalibrating the wavelength scale of the spectrophotometer,
- recalibrating the multicell transport,
- replacement parts.

It also provides you with compatibility information on software, firmware and hardware.



Handheld Controller and Spectrophotometer / Logbooks

If the Agilent 8453E UV-visible spectroscopy system does not work properly, the logbooks for the spectrophotometer and the handheld controller will help you to find out what specific reasons cause the malfunction of the system.

Table 6 shows the logbooks available for the Agilent 8453 spectrophotometer and the handheld controller.

Table 6 Logbooks Available for the Handheld Controller and Spectrophotometer

Module	System Logbook	Error Logbook	Maintenance Logbook	Info Logbook	EMF Logbook
Agilent 8453 spectrophotometer	YES	YES	YES	YES	YES
Handheld controller	YES	NO	NO	NO	NO

Spectrophotometer Logbooks

Messages coming from the firmware of the spectrophotometer are logged in the following logbooks:

- ✓ System Logbook (*System Log*), which logs all actions carried out or seen by the spectrophotometer such as
 - UV lamp ignition,
 - UV lamp off,
 - Multicell transport disconnected,
 - Blank,
 - Shutter open, closed,
 - Stray light filter open, closed,
 - Analysis (making a Sample or Standard measurement),
 - Method saved, deleted,
 - System parameters changed.

For a complete list refer to the help system (i-key).

All entries of the system logbook will be lost, after the spectrophotometer has been powered down.

- ✓ Error Logbook (*Error Log*), which logs all errors coming from the spectrophotometer or the software of the handheld controller such as
 - Vis lamp overcurrent
 - Lamp door open,
 - raw data buffer overflow,
 - no current through Tungsten lamp.

For a complete list refer to the help system (i-key) or the service handbook of the Agilent 8453 spectrophotometer.

The entries in the error logbook are stored on the mainboard of the spectrophotometer. They are reloaded when the system is booted up again.

- ✓ Maintenance Logbook (*Maint Log*), which logs maintenance tasks like
 - Firmware upgrade,
 - UV or Vis lamp replaced,
 - Filter assembly replaced,
 - Optical unit replaced.

The entries in the maintenance logbook are stored on the mainboard of the spectrophotometer. They are reloaded when the system is booted up again.

- ✓ Info Logbook (*m-key* in *Records* screen), which logs maintenance tasks like
 - UV lamp ignition,
 - UV lamp off,
 - Multicell transport disconnected,
 - Blank,
 - Shutter open, closed,
 - Stray light filter open, closed,
 - Analysis (making a Sample or Standard measurement),
 - Method saved, deleted,
 - System parameters changed.

- ✓ EMF Logbook (*m*-key in *Records* screen), which logs maintenance tasks like
 - Number of ignitions for UV-Lamp exceeded,
 - Number of ignitions for Vis-Lamp exceeded,
 - Lamp burn time for UV-Lamp exceeded,
 - Lamp burn time for Vis-Lamp exceeded.

Handheld Controller Logbook

Messages coming from the software of the handheld controller are logged in the following logbooks:

- ✓ System Logbook (*System Log*), which logs all actions carried out or seen by the handheld controller such as
 - Error during calculation,
 - Memory consumed.

Viewing and Printing the System Logbooks

- 1 Choose a task (e.g. *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F4-key (*Records*), and select one of the logbooks by either pressing
 - F2 - System Logbook,
 - F3 - Error Logbook,
 - F4 - Maintenance Logbook,
 - m - Info Logbook.
- 4 Press F6 (*Print*) to print out the chosen logbook.

NOTE

Troubleshooting tools are implemented in the software. Refer to the sections [“Troubleshooting”](#) on page 83, [“Checking the Keyboard”](#) on page 87, [“Spectrophotometer / Tests”](#) on page 96, [“Spectrophotometer / Recalibrating the Wavelength Scale”](#) on page 99 and [“Accessories / Recalibrating the Multicell Transport”](#) on page 101.

Spectrophotometer / Lamp History and Limits

The software of the handheld controller has the capability to prompt you when the hours of usage of the lamps or the number of ignitions exceeds a certain limit. Limits can be set individually for both lamps by the user.

Checking the Lamp History and Setting the Lamp Limits

The number of ignitions and the hours the lamps have been used is displayed on the *Spectrophotometer EMF Limits* screen.

To check or set the lamp hours and ignitions do the following:

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*) and then press the F4-key (*Records*)
- 3 In the *Records* screen, press the F1-key (*EMF*), select *Setup Limits* and press *Enter*.
- 4 In the *Spectrophotometer EMF Limits* screen use horizontal keys to select the appropriate edit box and use the numerical keys to type in your limits.
- 5 Press the F6-key (*Done*) to leave the screen.

Resetting the Lamp Counters

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*) and then press the F4-key (*Records*)
- 3 In the *Records* screen, press the F1-key (*EMF*), select *Setup Limits* and press *Enter*.
- 4 Press the F7-key (*Reset*). You will be prompted for resetting the lamp usage of the Deuterium and Tungsten lamp separately.

Spectrophotometer / Tests

Tests, checking the optical and electronic performance of the Agilent 8453 UV-visible spectrophotometer can be carried out from the software. The list of tests that can be done are:

- Dark Current test,
- Intensity test,
- Stability test.

Dark Current Test

To check the electronic performance of the diode array, do the dark current test.

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F1-key (*Dark Current*) and the test will start.
- 5 By pressing the F8-key (*Take*) you can redo the test.
- 6 Press the F6-key (*Print*) to print out your results.
- 7 Press *Escape* to exit the dark current test.

The dark current test recommendations are listed in [Table 7](#).

Table 7 Recommended values for the Dark Current Test

Lower Limit of Counts	Upper Limit of Counts
0	12.000

Intensity Test

To check the performance of the lamp, the optical system, the A/D converter and the diode array do the intensity test.

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F2-key (*Intensity*) and the test will start.

NOTE

For the intensity test the light path of the spectrophotometer must be free from any obstructions (e.g. cells, filters).

- 5 By pressing the F8-key (*Take*) you can redo the test.
- 6 Press the F6-key (*Print*) to print out your results.
- 7 Press *Escape* to exit the intensity test.

The intensity test recommendations are listed in [Table 8](#).

Table 8 Recommended Values for the Intensity Test

Wavelength Range	Number of Counts
190 – 220 nm	> 2000
190 – 350 nm	> 5000
350 – 500 nm	> 2000
500 – 950 nm	> 4000
950 – 1100 nm	> 200

Stability Test at 340 nm

To check the stability of your baseline do the stability test.

NOTE

During this test, the Agilent 8453 spectrophotometer must not be exposed to any airflow, ambient temperature changes and spikes coming from the line power.

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F3-key (*Stability*) and the test will start.
- 5 Press the F6-key (*Print*) to print out your results.
- 6 Press *Escape* to exit the stability test.

The stability test recommendations are listed in [Table 9](#).

Table 9 Recommended Values for the Stability Test

Allowed Range in Counts

100

Spectrophotometer / Recalibrating the Wavelength Scale

Changes of the wavelength scale of the Agilent 8453 are normally negligible. This is mainly due to the fact that there are no moving parts in the spectrophotometer doing the wavelength selection.

After changing lamps, or the shutter assembly, or severe mechanical shock, there may be a slight chance of a wavelength shift.

To check, and if necessary, recalibrate the wavelength scale of your spectrophotometer, apply the following procedure:

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F4-key (*Maintenance*), select *Wavelength Recalibration* and press *Enter*.
- 5 Press the F8-key (*Execute*) to execute the test.
- 6 Select *Adjust* and press *Enter* to recalibrate the wavelength scale, or select *Cancel* to leave the screen without recalibrating the wavelength scale.
- 7 Press the F6-key (*Done*) to leave the screen.

Printer / Printer Configuration

This section provides you with a procedure how you select your printer and set it up in the software.

- 1** Select a task (e.g. Single WL).
- 2** Press the F5-key (*System*).
- 3** Press the F2-key (*Configure*), select *Printer* with the vertical arrow keys and press *Enter*.
- 4** In the Dialogue Box that comes up select the printer model (Deskjet or Laserjet) using the vertical arrow keys and press *Enter*.
- 5** In the *Connected to* list box select *Agilent 8453* using the vertical arrow keys and press *Enter*.
- 6** To print a test page, by highlight the *Print Test Page* button and press *Enter*.
- 7** Press the F6-key (*Done*), to exit the dialogue and save your settings.

Accessories / Recalibrating the Multicell Transport

Changes of the optimum positions of the individual cells of the multicell transport (G1120A as well as 89075D) can be checked by using the *MCT Test/Calibration* tool.

To check, and in case, recalibrate the wavelength scale of your spectrophotometer, apply the following procedure:

- 1 Choose a task (e.g *Single WL*).
- 2 Press the F5-key (*System*).
- 3 Press the F3-key (*Tests*), select *Diagnostics & Maintenance* and press *Enter*.
- 4 In the *Tests* screen press the F4-key (*Maintenance*), select *MCT Test/Calibration* and press *Enter*.
- 5 Select the cell positions you want to test by marking the appropriate check boxes.
- 6 Press the F8-key (*Execute*) to execute the test.
- 7 Select *Adjust* and press *Enter* to recalibrate the wavelength scale, or select *Cancel* to leave the screen without recalibrating the wavelength scale.
- 8 Press the F6-key (*Done*) to leave the screen.

Replacement Parts

This section gives an overview over the replacement parts and part numbers available for the Agilent 8453E UV-visible spectroscopy system.

For replacement parts of the multicell transport (G1120A), the sipper system (89068D) and the Agilent 8453 spectrophotometer (G1103A), refer to the individual service and user handbooks of these devices.

Handheld Controller

Table 10 helps you to identify the replacement parts and related part numbers for your handheld controller.

Table 10 Replacement Parts for Handheld Controller

Part	Part Number
Handheld Controller	G1819A

Printer Interface

Table 11 helps you to identify the replacement parts and related part numbers for your printer interface.

Table 11 Replacement Parts for Printer Interface

Part	Part Number
Serial/Parallel Printer Cable	5181-1529

Compatibility

Spectrophotometer Hardware

You connect the handheld controller through the CAN connector to the spectrophotometer. The handheld controller is powered by and communicates through the CAN interface with the spectrophotometer.

Only spectrophotometers with serial number

DE60400900, US53400500

or higher are compatible with the handheld controller.

Spectrophotometer Firmware

Only spectrophotometers having firmware revisions

Rev.A.03.20

or later are compatible with the handheld controller.

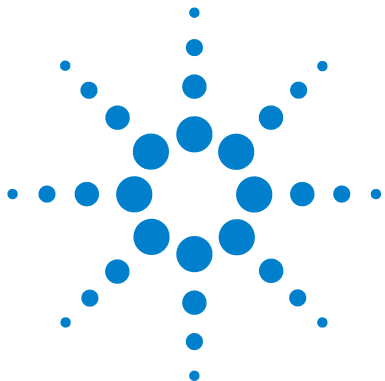
NOTE

For detailed information how to upgrade the firmware of the Agilent 8453 spectrophotometer, refer to [“Upgrading the Firmware of the Spectrophotometer”](#) on page 137.

Agilent ChemStation for UV-Visible Spectroscopy

The Agilent 8453 spectrophotometer can, instead of connecting a handheld controller, be controlled by a PC with Agilent ChemStation software loaded.

5 Service and Maintenance
Compatibility



6 Installing Accessories

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Sipper System	126

Many simple, mechanical/optical accessories are available to expand the capabilities of your Agilent 8453 spectrophotometer. The first part of this section briefly describes these accessories and outlines how to install them. These accessories include:

- Thermostatable cell holder
- Cell stirring module
- Long path cell holder
- Optical filters

The following more complex accessories with electronic components are available to enhance the functionality and capabilities of your spectrophotometer. These accessories are described in the second part of this chapter.

- Multicell transport
- Sipper system.

Detailed information on the multicell transport and the sipper system are included with the accessories in separate handbooks.



Thermostattable Cell Holder

The Agilent 89054A thermostattable cell holder (see [Figure 20](#)) is a single-cell cell holder with a manifold around the cell. Water from a thermostatted water bath can be circulated through the manifold to hold the sample cell at a uniform temperature. Up to 1 cm open-topped cells and flow cells can be used in a thermostattable cell holder.

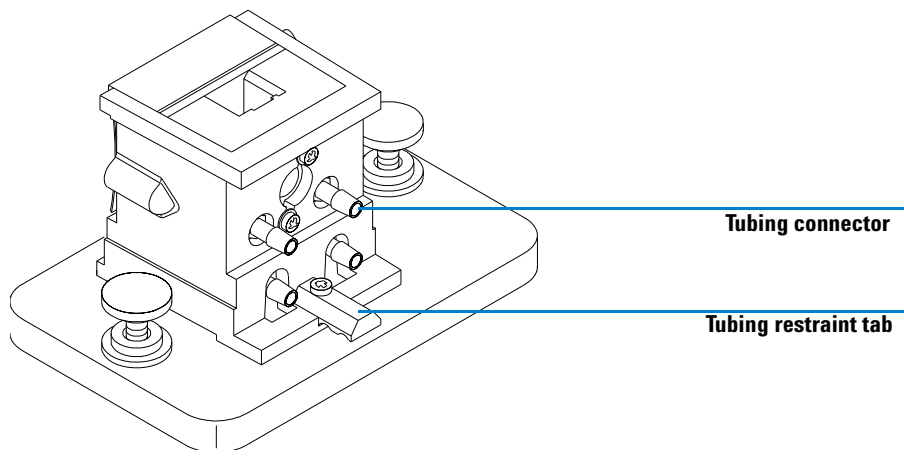


Figure 20 Thermostattable Cell Holder

Installing a Thermostatable Cell Holder

- 1 Loosen the hold-down screws and remove the current sample holder from the sample compartment.
- 2 Orient the thermostatable cell holder the same way the standard cell holder was oriented and lower it onto the sample compartment mounting guides.
- 3 Tighten the hold-down screws.
- 4 Connect tubing (silicon, 3/16 inch i.d.) to the inlet and outlet of the manifold. It does not matter which side is used as the inlet and which side is used as the outlet. If necessary, route the tubing under the tubing restraint tab to keep it from interfering with the light beam. Make sure the tubing is securely attached; it expands when heated and if it is not secure a leak will result.
- 5 Insert the sample cell and lower the lever down to lock the cell in place.
- 6 Begin pumping thermostatted water through the manifold. Wait for the sample cell and sample to reach thermal equilibrium before you make any measurements. The time required to reach thermal equilibrium will vary depending on the water temperature and the sample but generally should take about five minutes.

When the thermostatable cell holder is not in use, it is best to disconnect the tubing and drain the manifold.

Cell Stirring Module

The Agilent 89055A cell stirring module shown in [Figure 21](#), mounts inside the base of the thermostatable cell holder. It provides the capability of magnetic induction stirring inside a standard 1 cm sample cell. The cell stirring module contains a plastic-encased magnetic impeller that can be driven by water or pressurized air. When you put a magnetic stirring bar (9301-1161) into the filled sample cell, the stirring bar rotates in tandem with the impeller magnet, thus stirring the sample.

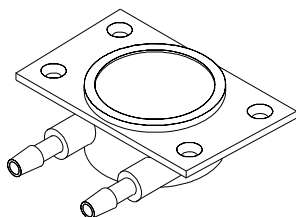


Figure 21 Cell Stirring Module

Installing the Cell Stirring Module

- 1 Remove the thermostatable cell holder from the sample compartment.
- 2 Remove the three screws that hold the thermostatable cell holder to its base. Remove the cell holder and turn it upside-down.
- 3 Insert the cell stirring module into the cavity in the base of the thermostatable cell holder, see [Figure 22](#).

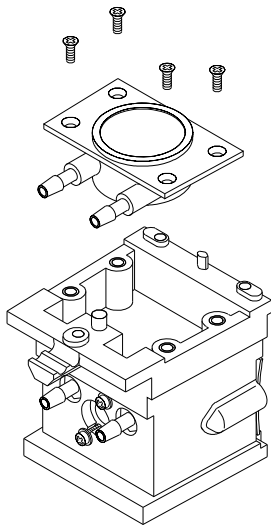


Figure 22 Installing the Cell Stirring Module

- 4 Tighten the four screws (flathead, M3×6 mm) that will hold the cell stirring module in place.
- 5 Attach the thermostatable cell holder to its base.
- 6 Mount the thermostatable cell holder back in the sample compartment.

Tubing Connections and Operation

The cell stirring module can be driven by either water or air. Water is probably the fluid of choice because it is more easily controlled. Water temperature can be up to 100 °C (212 °F) with a maximum allowable back pressure of 4 bar (approximately 60 psi). If air is used to drive the cell stirring module, the pressure should be less than 0.3 bar (approximately 5 psi) when the stirrer is started. Again, the maximum pressure is 4 bar (approximately 60 psi).

There are several ways you can connect a water or air supply to the cell stirring module, see [Figure 23](#). In all cases we recommend using 3/16 inch i.d. silicon tubing between the water supply and the stirring module.

- The stirring module can be used alone, without the thermostatable functions of the cell holder.
- The same water supply can be used to regulate sample cell temperature and to power the cell stirring module.

If you need a faster stirring speed than can be obtained by connecting the manifold and cell stirring module in series, you can connect separate supplies to the manifold and stirring module.

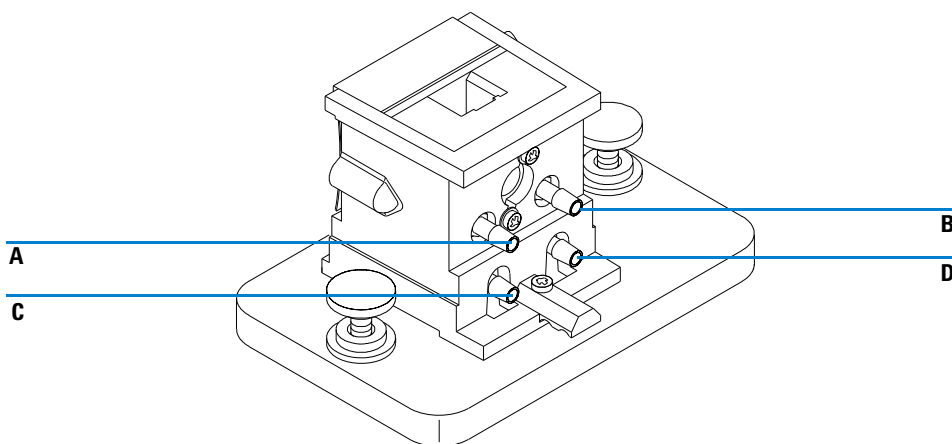


Figure 23 Tubing Connections for a Cell Stirring Module

Table 12 Tubing Connections for a Cell Stirring Module

Connection	Comment
A (in), B (out)	Without stirring
C (in), D (out)	Without cell thermostating
A (in), B to D, C (out)	In series with cell thermostating
A and C (in), B and D (out)	In parallel with cell thermostating

Long Path Cell Holder

The Agilent 89076A long path cell holder is a sample cell holder which can be adjusted to hold most cylindrical and rectangular sample cells with path lengths of up to ten centimeters. It uses the same mounting system as the other sample cell holders.

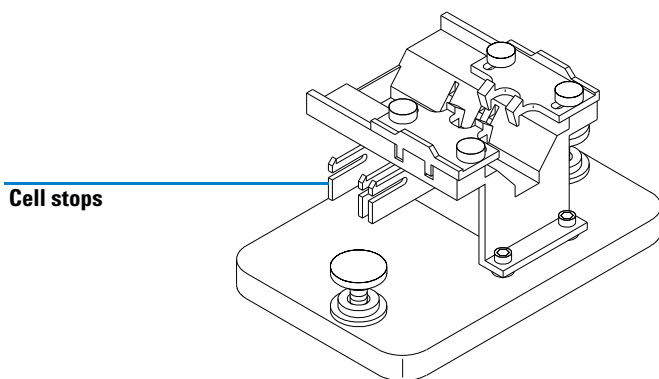


Figure 24 Long Path Cell Holder

The long path cell holder includes built-in cell stops for accurate positioning of 1, 2, 5, and 10-cm sample cells.

The optical filter wheel (08451-60302) can be mounted on the long path cell holder in the same way it is mounted on other cell holders, see “[Optical Filter Wheel](#)” on page 115 for details. However, instead of a screwdriver, a 2.5-mm hex wrench is required to install the optical filter wheel on the long path cell holder.

Installing the Long Path Cell Holder

- 1 Loosen the hold-down screws and remove the current sample holder from the sample compartment.
- 2 Orient the long path cell holder so that the clamps that hold the sample cell in place are on the right. Lower the cell holder onto the sample compartment mounting guides.
- 3 Tighten the hold-down screws.

Installing a Sample Cell

- 1 Position the cell stops according to the path length of the sample cell you will be using. For example if your sample cell is a 1-cm cell.

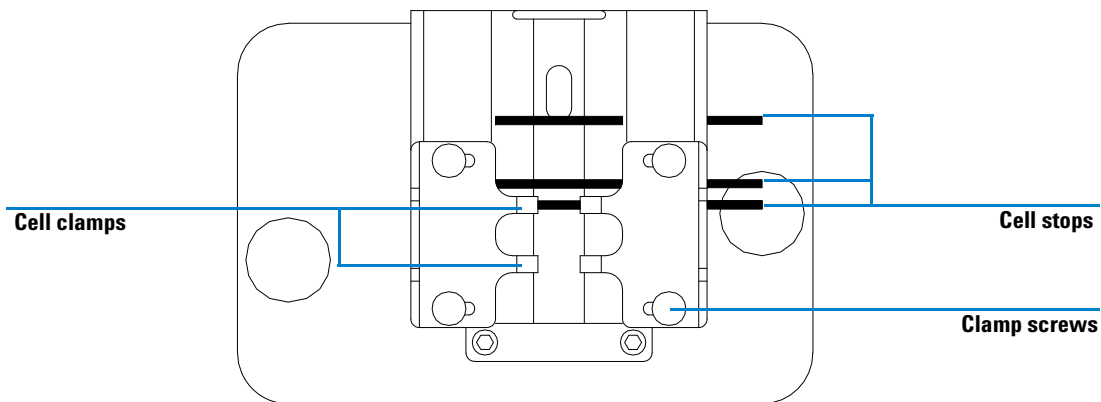


Figure 25 Positioning the Cell Stops for a 1-cm Sample Cell

- 2 If your sample cell is not one of the standard lengths, position the cell stops as for a long path sample cell, i.e. move all cell stops in [Figure 25](#) to the left.
- 3 Because the light beam is collimated, absolute positioning of the sample cell is not important; however, consistent cell positioning gives more consistent results. The cell stops make it easier to consistently position your sample cells.

6 Installing Accessories

Long Path Cell Holder

- 4 Install the sample cell between the clamps. Slide it against the cell stop. If your sample cell is not one of the standard lengths, position it so the clamps are gripping the mid-point of the cell.
- 5 Allow the clamps to close against the sample cell, see [Figure 26](#). Tighten the four screws that hold the sample cell in place.

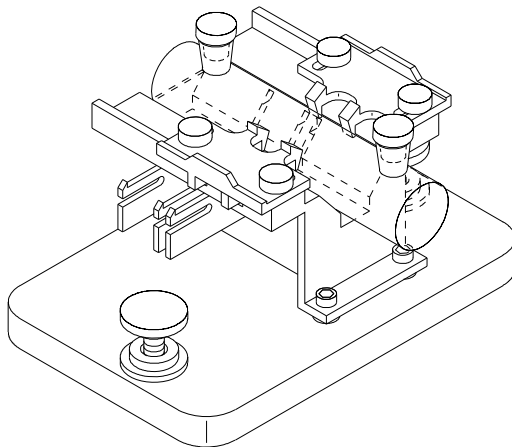


Figure 26 Installed 10-cm Cylindrical Cell in the Long Path Cell Holder

Optical Filter Wheel

To achieve optimum measurement conditions, optical filters may be necessary for spectral measurements of photosensitive samples. Agilent Technologies offers an optical filter wheel assembly (08451-60302), see [Figure 27](#), which can be mounted on the standard cell holder, the adjustable cell holder or the thermostatable cell holder. An optical filter wheel with the same filters but with a different mounting can be used with the multicell transport, see *User's Guide* of the multicell transport.

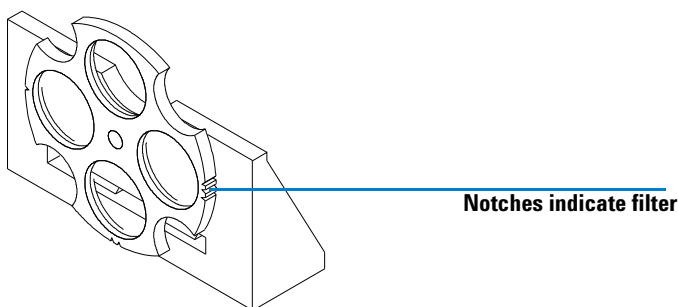


Figure 27 Optical Filter Wheel Assembly

The filter wheel rotates to four different positions. Position zero is empty and permits 100% transmittance at all light wavelengths. Positions one, two and three have filters which absorb varying amounts of the UV spectrum. [Figure 28](#) shows the transmission characteristics of each filter.

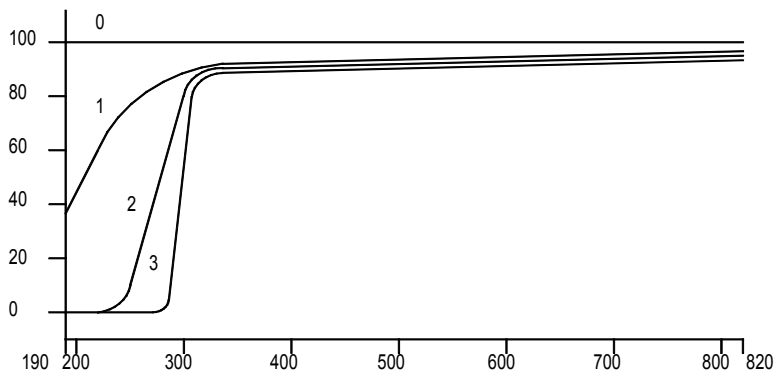


Figure 28 Transmission Characteristics of Filter Wheel Filters

Table 13 Filter Wheel Positions

Position	Filter
0	None
1	UV roll-off
2	265 nm UV cut-off (at 50 %T)
3	295 nm UV cut-off (at 50 %T)

The choice of when to use a filter and which filter to use should be made only after considering all the factors that affect your measurements. If you decide that a filter is needed, you should choose a filter that transmits the wavelengths needed for analytical information and that blocks the wavelengths that contribute to sample degradation. Often, your final choice will be a compromise since sometimes even the light at the wavelengths of analytical interest can affect your sample.

If you do not need a filter for a group of measurements, you can move the filter wheel to position zero. This permits full light transmission. When you will not be using the filter wheel for an extended period of time you should remove the filter wheel assembly from the cell holder and store it in a clean plastic bag.

The optical filters of the filter wheel must be kept clean. Dirty filters interfere with light transmission and lower the spectrophotometer performance. These filters may be cleaned with isopropanol and photographic lens tissues.

Installing the Filter Wheel Assembly

Installing the filter wheel assembly is fairly simple. The procedure is the same for all three cell holders. You will need a Phillips screwdriver to install the filter wheel assembly. However, if you install the filter wheel on the long path cell holder, instead of a screwdriver, a 2.5-mm hex wrench is required.

- 1 Remove the cell holder from the sample compartment.
- 2 Remove the two screws shown in [Figure 29](#).
- 3 Position the filter wheel assembly clamp as shown in [Figure 29](#). Replace the two screws but do not tighten them.

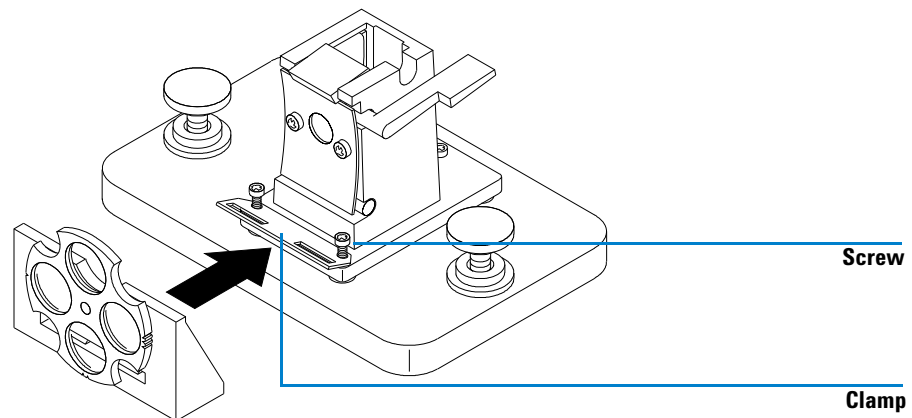


Figure 29 Installing the Optical Filter Wheel Assembly

- 4 Tilt the outer edge of the clamp up slightly.
- 5 Bring the filter wheel assembly against the cell holder so that the clamp fits in the slot of the filter wheel assembly. The mounting ridges on the filter wheel assembly should fit into the holes in the clamp. The base of the filter wheel assembly should be against the base of the cell holder.
- 6 Tighten the screws that hold the clamp.

Removing the Filter Wheel Assembly

- 1** Loosen but do not remove the two screws that hold the clamp.
- 2** Separate the filter wheel assembly from the cell holder.
- 3** The filter wheel clamp may be left in place or removed. In either case, retighten the two screws.
- 4** Store the filter wheel in a clean plastic bag.

Eight-position Multicell Transport

The eight-position Multicell Transport (part number G1120A) is an accessory for the Agilent 8453 spectrophotometer. It is a multi-sample holder with multiple-position capability, see [Figure 30](#). The multicell transport cell holder holds eight cells in a rack which can be held at a constant temperature with water circulated from a thermostatic bath (provided by the user and not by Agilent Technologies).

A brief description of how to install the multicell system is given here. For more detailed information, e.g. technical specifications, refer to the documentation that is shipped with the eight-position Multicell Transport.

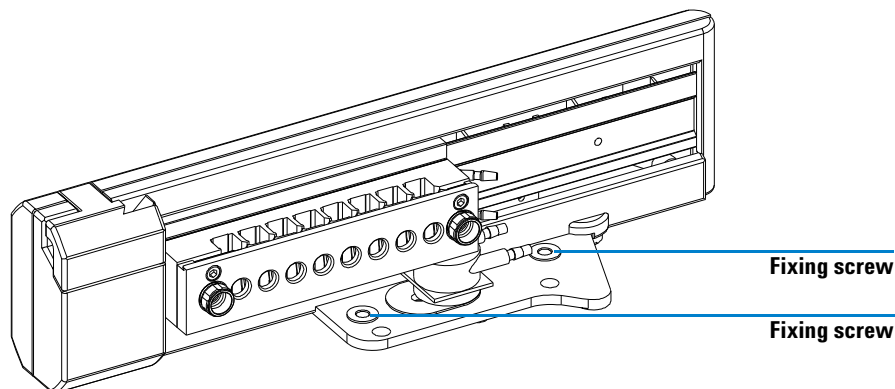


Figure 30 The Eight-position Multicell Transport

Installing the Eight-position Multicell Transport

The power for the multicell transport is supplied by the multicell transport interface of the spectrophotometer. As soon as the multicell transport connector is plugged in, and the spectrophotometer turned on, the multicell transport will be powered on and does a self calibration. No additional power connection is needed.

6 Installing Accessories

Eight-position Multicell Transport

- 1 Place the multicell transport in the sample compartment of the spectrophotometer. See [Figure 31](#).

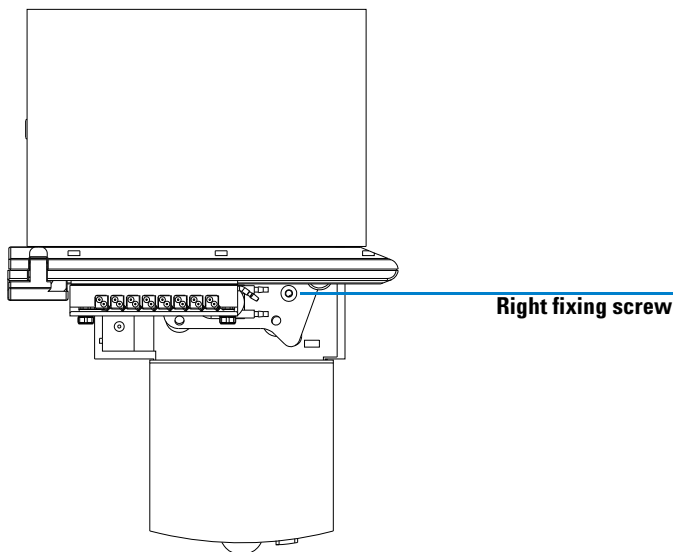


Figure 31 Top View of the Multicell Transport in Spectrophotometer

- 2 Move the cell holder of the multicell transport manually to the left-most position and tighten the screw at the right side. Move it now to the right-most position and tighten the screw at the left side.
- 3 Plug in the connector of the multicell transport at the rear of the spectrophotometer. See [Figure 32](#) on page 121.

NOTE

Turn the Agilent 8453 spectrophotometer off before plugging in the connector of the multicell transport. Plugging the multicell connector in while the instrument is running could damage the electronics of the multicell transport.

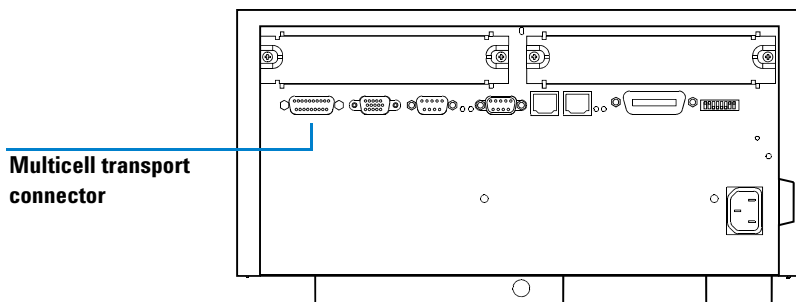


Figure 32 Connector at Rear of Spectrophotometer

- 4 Turn on the spectrophotometer at the lower-left part of the instrument. The transport initializes, that is, the turnabouts moves to the HOME-position.
- 5 Wait until the boot process of the software has finished.
- 6 To verify proper installation, use the numerical keys of the handheld controller to move the multicell to different positions. See [Table 14](#) for key numbers and related multicell transport positions.

Table 14 Key Numbers and related Multicell Transport Positions

Key Number	Multicell Transport Position
0	HOME-position
1	Cell 1
2	Cell 2
3	Cell 3
4	Cell 4
5	Cell 5
6	Cell 6
7	Cell 7
8	Cell 8

For controlling the eight-position multicell transport from the software of the handheld controller, refer to “[Defining a Sequence for the Multicell Transport](#)” on page 76.

Accessories for the Eight-position Multicell Transport

Three accessories are available for the multicell transport to enhance the capabilities of your multicell transport

- Stirring Module
- Filter Kit
- Cell Holder.

A brief description of the features of the different accessories will be given here. For more details refer to the manual of the eight-position multicell transport.

Stirring Module

The stirring module (part number G1120-60006) is an optional accessory for the eight-position multicell transport. The stirring module comprises the stirring assembly, four tubing clamps and 2 meters of 3/8 inch i.d. flexible tubing.

Two positions for mounting the stirring assembly are available. One under the cell in the light path, as shown in [Figure 33](#) on page 123, and one under the cell at the left side of the cell in the light path. You can turn the stirring assembly by 90 degrees about the axis perpendicular to the base plate of the multicell transport, thus bringing the fittings to three different positions.

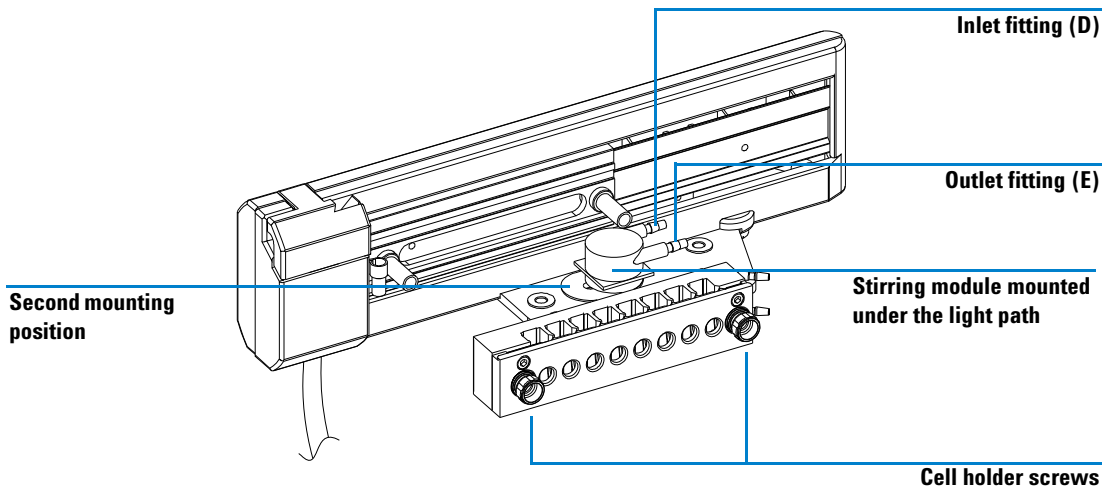


Figure 33 Mounting the Stirring Assembly

Filter Kit

Some compounds are photosensitive and may be decomposed during measurement. This effect can be reduced or eliminated by using a filter to cut off the highly energetic low-wavelength UV part of the spectrum.

The filter kit (part number G1120-68707) comprises three threaded ring assemblies, see [Figure 34](#), having different filters. The cut-off wavelengths of the filters are different and absorb therefore varying amounts of the UV spectrum. All threaded rings are labelled with the corresponding cut-off wavelength of the filters mounted.

Remove the multicell transport from the sample area. Screw the threaded ring, which holds the filter appropriate for your application, in the circular opening located at the rear part of the multicell transport. If no threaded ring is screwed in, the complete light coming from the lamps is let through.

6 Installing Accessories

Eight-position Multicell Transport

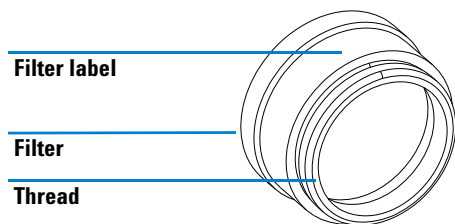


Figure 34 Threaded Filter Ring for Multicell Transport

Figure 35 shows the transmission characteristics of each filter.

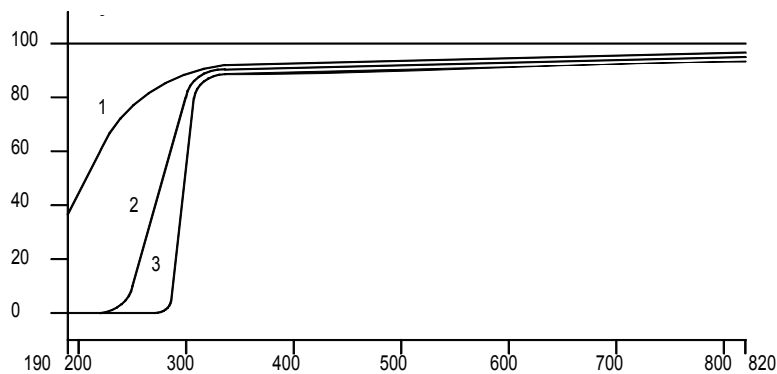


Figure 35 Transmission Characteristics of Filters

Table 15 shows the type of filters available.

Table 15 Filter Types and Labels

Label	Filter
1	UV roll-off
2	265 nm UV cut-off (at 50 %T)
3	295 nm UV cut-off (at 50 %T)

Cell Holder

The cell holder has a capacity for eight cells. The outside dimensions of individual cells must be 12.5×12.5 mm and the height from base of the cell to the sample area 15 mm.

During an analysis, the position of individual cells is maintained by a flat spring inside each of the cell compartments. The spring alone fixes the cell. No additional fixing mechanism is needed, see [Figure 36](#).

The cell holder can be thermostated by an external water bath. The tubing connectors for the water bath are shown in [Figure 36](#).

You can remove the cell holder very easily by loosening the two fixing screws, as shown in [Figure 36](#). The Multicell cell holders are manufactured to such a precision that you can exchange cell holders, leaving the multicell transport base in place, without readjusting the multicell transport at all. Thus you can exchange very easily batches of up to eight cells.

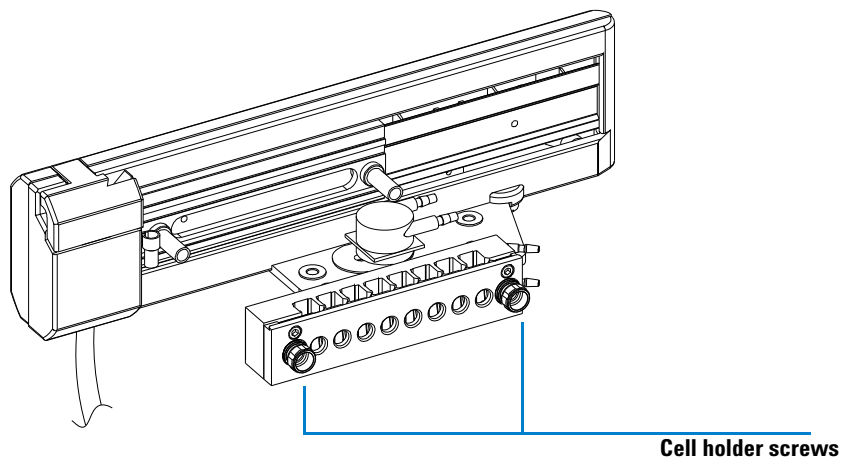


Figure 36 Removing the Cell Holder Assembly

Sipper System

The sipper system (part number 89068D) is an accessory for the Agilent 8453 spectrophotometer. It comprises a flow cell, connectors and tubings and a peristaltic pump.

By eliminating the handling of cuvettes and the errors that can result from cuvette repositioning, the sipper system provides a simple method of improving the productivity and reproducibility of spectrophotometric analyses. The sipper system comprises a single-channel peristaltic pump, quartz flow cell, PTFE tubing and remote cables.

In operation, the sipper system aspirates a liquid sample into a flow cell where it is measured and then returned or pumped to waste.

A brief description of how to install the sipper system is given here. For more detailed information, e.g. technical specifications, refer to the documentation that is shipped with the system.

Connecting the Peristaltic Pump to the Line Power

Your peristaltic pump draws power from an external source. It operates at a line voltage of 100 - 120 V or 220 - 240 V AC +/- 10% with a line frequency of 50 or 60 Hz +/- 5% respectively.

The appropriate power cord is supplied with the sipper system. It plugs into the power-input socket at the rear of the pump, see [Figure 37](#) on page 127. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

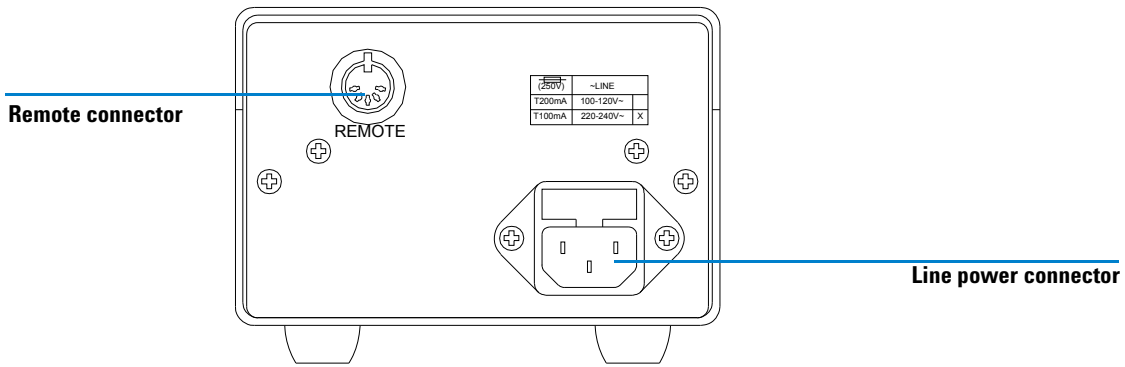


Figure 37 Rear View of Peristaltic Pump

Installing the Pump Cables

- 1 Read the label on the rear panel of your pump to check the pump has the correct voltage option (100–120 V, 60 Hz or 220–240 V, 50 Hz) for operation from your line power supply.

CAUTION

It is not possible to select a line voltage different from that on the label of your pump.

- 2 Push the connector of the power cord into the line power connector on the rear panel of the pump, see [Figure 37](#).
- 3 Push the other plug of the power cord into the line power source.
- 4 Push the connector of the sipper-GPIO cable (G1103-61609) or sipper/sampler-GPIO cable (part number G1103-61608) into the remote control connector on the rear of the pump, see [Figure 37](#).

When this cable is connected, the control switch on the front panel does not function. This prevents you using the pump manually during automatic operation.

6 Installing Accessories Sipper System

- 5 Connect the other end of the cable to the GPIO connector on the rear of the spectrophotometer.

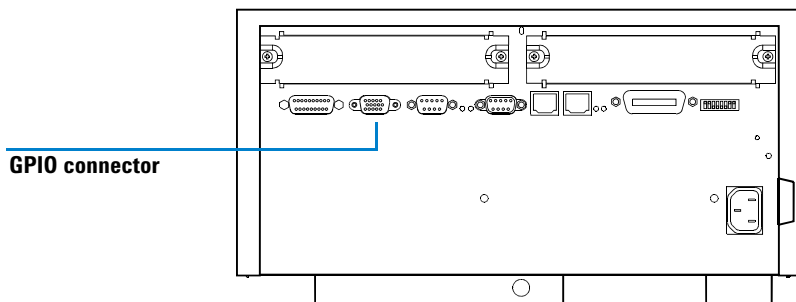


Figure 38 Connector at Rear of Spectrophotometer

Installing the Pump Tubing

- 1 Remove the tubing cassette from the pressure wheel on the front of the pump by pressing the snap-lock lever to the left and upwards.

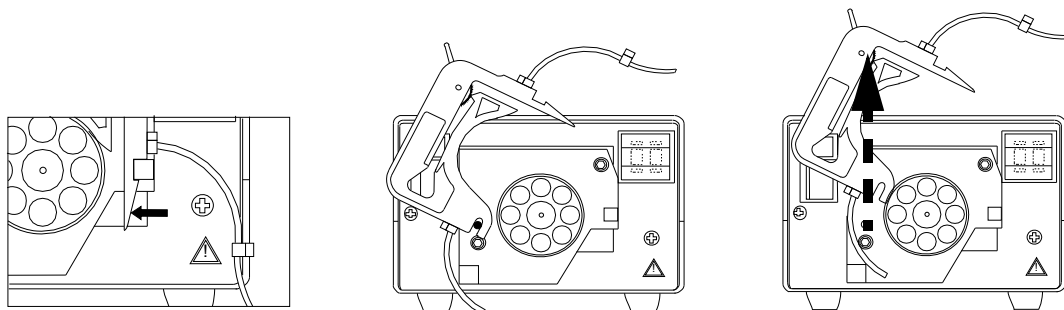


Figure 39 Removing the Tubing Cassette

- 2 Move the pressure-control lever of the tubing cassette (see [Figure 39](#)) clockwise to the right-most position, that is, the lever is horizontal.

- Put the pump tubing in the tubing cassette, locating the fixing collars on each side of the cassette in the locating holes. Make sure the fixing-collars are pointing upwards and the tubing cannot slide out of the cassette.

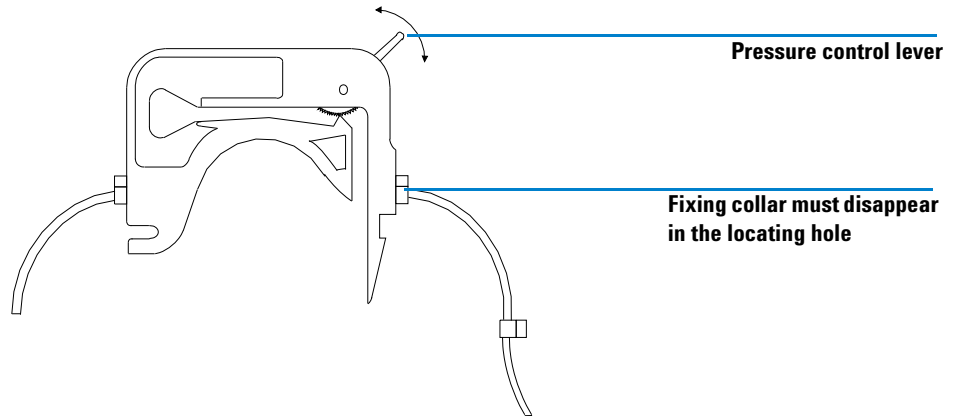


Figure 40 Tubing Cassette with Pump Tubing

- Make sure the pressure control lever of the tubing cassette is in the right-most position, that is, the lever is horizontal.
- Put the tubing cassette on the pressure wheel by placing the cassette on the mounting pivot.

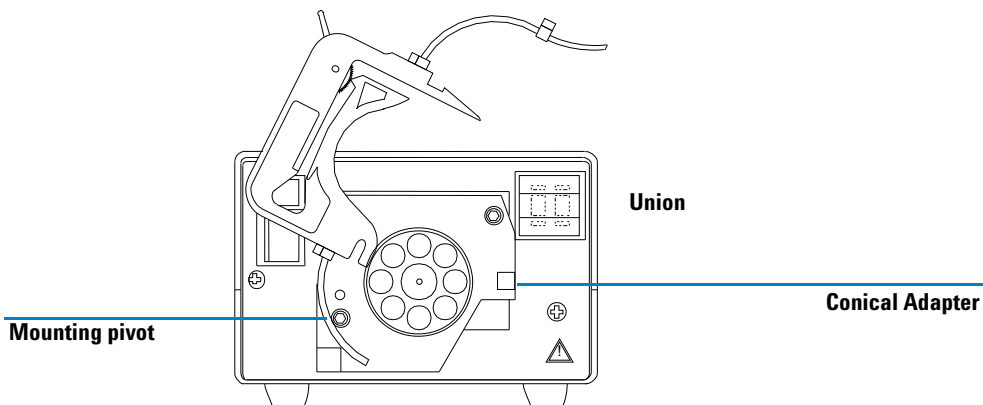


Figure 41 Replacing the Tubing Cassette

6 Installing Accessories Sipper System

- 6 Press the cassette down to lock the right-side snap lever, on the locking bar.
- 7 Connect the pump tubing to the other accessories, see relevant handbook.

Connecting the Sipper Tubing

- 1 Connect the tubing with the short cell fitting to the outlet of the flow cell. The outlet of the flow cell is the connector that is *not* marked with an arrow.

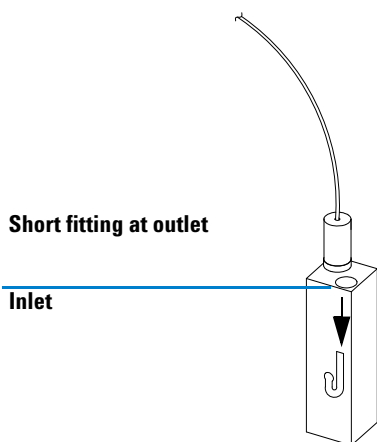


Figure 42 Connecting Tubing to Flow Cell

- 2 Insert the flow cell into the cell holder of the spectrophotometer.

- 3 Connect the fitting and ferrule to a union.

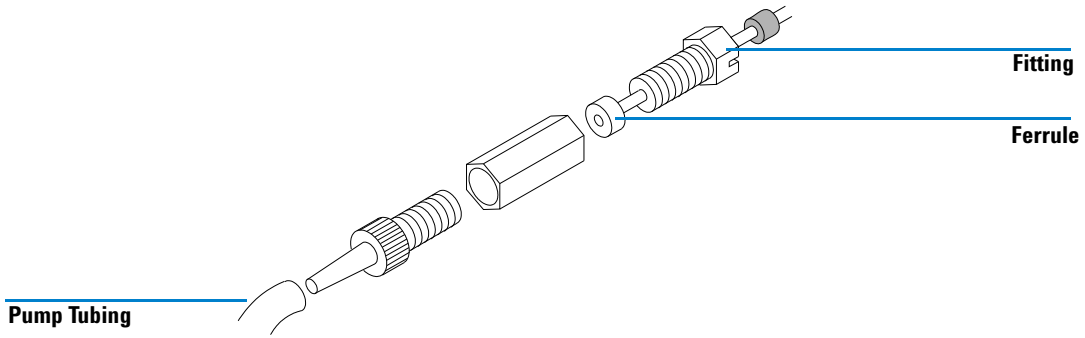


Figure 43 Connecting Tubing to Pump

- 4 Connect the conical adapter to the union and insert the conical adapter in the pump tubing at the inlet side (left side) of the pump.
- 5 Choose the 0.35 m long tubing with the long cell fitting on one end and the other end open. Connect the long cell fitting to the inlet. The inlet of the flow cell is at connector that is marked with an arrow.

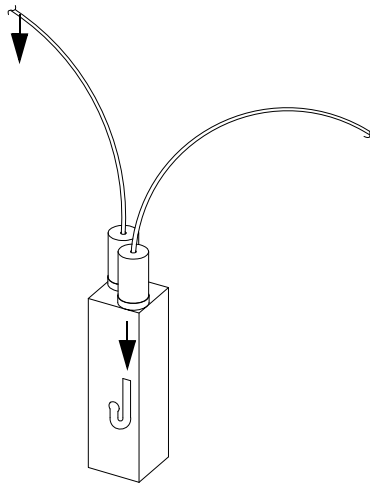


Figure 44 Connecting Tubing to Flow Cell

6 Installing Accessories Sipper System

- 6 Insert the other end of the tubing in the hole in the front cover of the spectrophotometer and push in the tubing until it comes out at the top part of the front panel of the spectrophotometer.

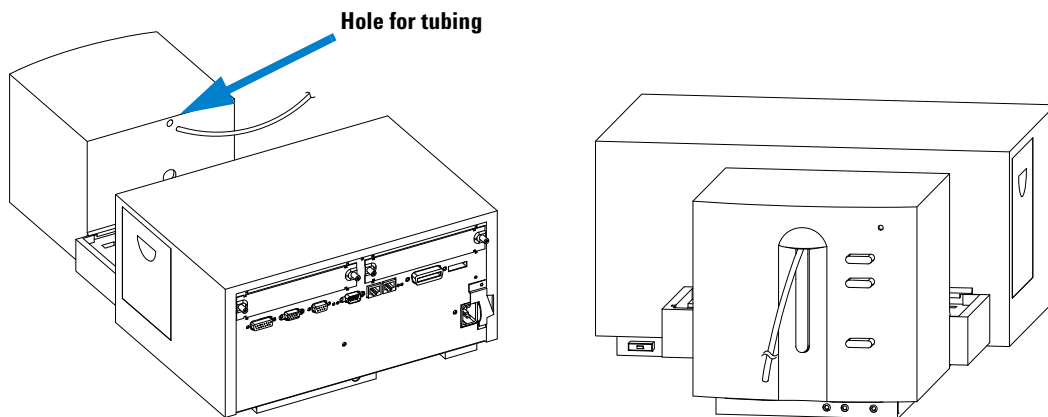


Figure 45 Installing Tubing in the Spectrophotometer

- 7 Adjust the length of the sipper tubing that comes out of your front panel until it meets with the requirements of your sample containers.

Waste Tubing Connection

- 1 To install waste tubing, choose the 1.5 m long tubing with the two hexagonal fitting on either end. On one end of the tubing, connect the fitting and ferrule to a union.

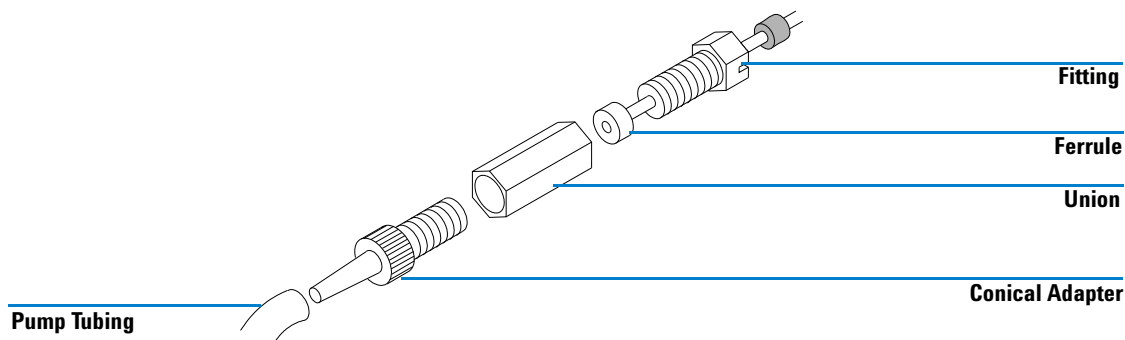


Figure 46 Connecting Waste Tubing to Pump

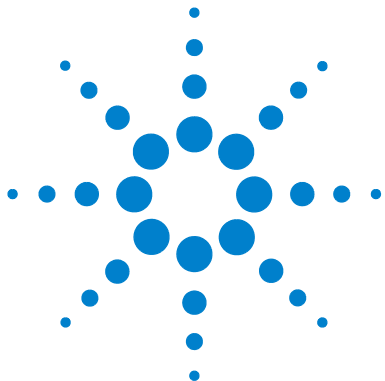
- 2 Connect the conical adapter to the union and insert the conical adapter in the pump tubing at the outlet side (right side) of the pump.
- 3 Put the other end of the waste tubing in your waste container.
- 4 To verify proper installation, use the numerical keys of the handheld controller to start and stop the pump. See [Table 16](#) for key numbers and related pump actions.

Table 16 Key Numbers and Related Pump Actions

Key Number	Pump Action
1	pump clockwise
2	pump counterclockwise
0	stop pumping

For controlling the peristaltic pump from the software of the handheld controller, refer to “[Setting the Parameters for the Sipper System](#)” on page 78.

6 **Installing Accessories** Sipper System



A Software and Firmware Upgrade

Upgrading the Software of the Handheld Controller [136](#)

Upgrading the Firmware of the Spectrophotometer [137](#)

How you upgrade the

- software of the handheld controller,

or the

- firmware of the spectrophotometer

is described in this section. The upgrade procedures can only be applied when a PC-card with the appropriate system files loaded is available.



Upgrading the Software of the Handheld Controller

As outlined in the last section of this chapter “[Compatibility](#)” on page 103 the software of the handheld controller and the firmware of the spectrophotometer have to match. This section describes the procedure, how the software of the handheld controller is upgraded.

NOTE

Prerequisite for this task is a PC-card with the new software loaded.

- 1 Disconnect the handheld controller from the Agilent 8453 spectrophotometer.
- 2 Insert the PC-card into the PC-card slot of the handheld controller.
- 3 Reconnect your handheld controller to the Agilent 8453 spectrophotometer.
- 4 Choose a task (e.g. *Single WL*).
- 5 Press the F5-key (*System*).
- 6 Press the F4-key (*Records*).
- 7 Select *Controller (G1819A)* in the *Records* list box using the vertical arrow keys and press *Enter*.
- 8 Press the F5-key (*FW Update*) which brings you into the *Control Module Firmware Update* screen.
- 9 In the list box select the appropriate file and press the F8-key (*Execute*) to start the update process.

NOTE

At the end of the software update process, the module will automatically restart with the new software loaded.

Upgrading the Firmware of the Spectrophotometer

As outlined in the last section of this chapter “[Compatibility](#)” on page 103 the software of the handheld controller and the firmware of the spectrophotometer have to match. This section describes the procedure, how the firmware of the spectrophotometer is upgraded.

NOTE

Prerequisite for this task is a PC-card with the new spectrophotometer firmware loaded.

- 1 Disconnect the handheld controller from the Agilent 8453 spectrophotometer.
- 2 Insert the PC-card into the PC-card slot of the handheld controller.
- 3 Reconnect your handheld controller to the Agilent 8453 spectrophotometer.
- 4 Choose a task (e.g. *Single WL*).
- 5 Press the F5-key (*System*).
- 6 Press the F4-key (*Records*).
- 7 Select *Agilent 8453 (Module)* in the *Records* list box using the vertical arrow keys and press *Enter*.
- 8 Press the F5-key (*FW Update*) which brings you into the *Module Firmware Update* screen.
- 9 Press the F7-key (*Transfer*) and wait until the system swaps to the resident system. This will be indicated by a message on the screen.

NOTE

During the switch to the resident system, the fan of the spectrophotometer will speed up and run at a higher speed.

A Software and Firmware Upgrade

Upgrading the Firmware of the Spectrophotometer

- 10 Press the F4-key (*Records*), F5-key (*FW Update*) and choose the appropriate *.dlb file in the list box using the vertical arrow keys and press *Enter*.
- 11 Now press the F8-key (*Execute*) to start the firmware upgrade. The system will display the current status of the upgrade process.
- 12 When the upgrade process is finished, press the F7-key (*Transfer*) to restart the handheld controller and the spectrophotometer.

NOTE

After the firmware upgrade has been completed, the fan will slow down and the spectrophotometer will do a self test.



B **Data Export**

Exporting Data 140

This chapter describes how you can export data of the Agilent 8453E UV-visible spectroscopy system, in ASCII format, to other software applications.

The ASCII data format can be read for example by the Agilent ChemStation UV-visible software and so allows you to load data (e.g. sample spectra) measured with the Agilent 8453E UV-visible spectroscopy system into the Agilent ChemStation software for further evaluation.



Exporting Data

Files created by the Agilent 8453E UV-visible spectroscopy system can be exported as ASCII files to the PC-card of the handheld controller. From the PC-card the ASCII files can then be retrieved into the Agilent ChemStation UV-visible software or other software packages having an ASCII filter for data import.

There are different file types used by the Agilent 8453E UV-visible Spectroscopy system in different tasks. Accordingly, different file types and extensions are used for data export, to reflect the contents and data structure of the file.

File Extensions

The file extension used by the Agilent 8453E UV-visible spectroscopy system when saving timetraces in *Kinetics* is *.tr.

Data saved in all other modes will be stored under filenames having the extension *.sp.

When data is exported in ASCII format in *Kinetics*, the files will have file extension *.csv, whereas files exported in all other modes will have file extension *.wav. This is summarized in [Table 17](#).

Table 17 File Extensions used by the Agilent 8453E UV-visible Spectroscopy System

Task	File extension for saving data (internal format)	File extension for exporting data (ASCII format)
Single WL	*.sp	*.wav
Multi WL	*.sp	*.wav
Equation	*.sp	*.wav
Spectrum	*.sp	*.wav

Table 17 File Extensions used by the Agilent 8453E UV-visible Spectroscopy System (continued)

Task	File extension for saving data (internal format)	File extension for exporting data (ASCII format)
Quantification	*.sp	*.wav
Kinetics	*.tr	*.csv

File Structure of *.wav and *.csv Files

File Structure of *.wav files

All files stored under the extension *.wav contain

- a header to identify the type of data and method parameters,
- wavelength information (e.g. Absorbance values).

The structure of *.wav file is shown in [Figure 47](#).

Line 1	Filetype, (not used), Time, Date
Line 2	Resolution, Integration Time, (not used)
Line 3	Derivative Order
Line 4	(not used)
Line 5	Name of Spectrum, (not used)
Line 6	Concentration, (not used)
Line 7	(not used), Data Type, No. of Points, (not used)
Line 8	Wavelength1, Wavelength2
...	...
Line ..	Data Point
...	...

Figure 47 Structure and Contents of *.wav Files

A detailed description of the meanings of the different items in [Figure 47](#) is given in [Table 18](#).

Table 18 Description of *.wav File Items of [Figure 47](#)

Item	Description
Filetype	Number which represents the type of file, default 11
Time	Time at which the data were measured
Date	Date at which the data were measured
Resolution	Resolution of instrument, default 1(nm)
Integration Time	Integration time, default 0.5 (sec)
Derivative Order	Order of derivative of data (e.g. 1 for first derivative)
Name of Spectrum	Name assigned to spectrum in software
Concentration	Concentration of a standard (only Quantification)
Data Type	Number represents data type: 1- Absorbance, 2 - Derivative, 3 - Transmittance
No. of Points	Number of data points in file
Wavelength1	Lowest wavelength of spectrum
Wavelength2	Highest wavelength of spectrum
Data Point	Values (listed by ascending wavelengths)

File Structure of *.csv Files

All files stored under the extension *.csv contain

- a header to identify the method parameters,
- data points (time versus absorbance).

The structure of *.csv file is shown in [Figure 48](#).

Line 1	Name of Timetrace
Line 2	Time, Date
Line 3	Integration Time
Line 4	Wavelength
Line 5	Run Time, Cycle Time, Time Scale
Line 6	No. of Pairs of Data
...	...
Line ..	Time/Absorbance
...	...

Figure 48 Structure and Contents of *.csv Files

A detailed description of the meanings of the different items in [Figure 48](#) is given in [Table 19](#).

Table 19 Description of *.csv File Items of [Figure 48](#)

Item	Description
Name of Timetrace	Name assigned to timetrace in software
Time	Time at which the data were measured
Date	Date at which the data were measured
Integration Time	Integration time, default 0.5 (sec)
Wavelength	Wavelength at which data has been measured (nm)
Run Time	Total measurement time
Cycle Time	Time interval for data acquisition
Time Scale	0 - seconds, 1 - minutes

Table 19 Description of *.csv File Items of Figure 48 (continued)

Item	Description
No. of Pairs of Data	Number of data pairs of timetrace
Time/Absorbance	Elapsed time/absorbance value

Loading *.wav Files into the Agilent ChemStation UV-visible Software

The data export functionality of the Agilent 8453E UV-visible spectroscopy system can be used to transfer data from the handheld controller to the Agilent ChemStation UV-visible software.

This feature is not applicable to timetraces exported as *.csv files, only to spectra exported as *.wav files.

NOTE

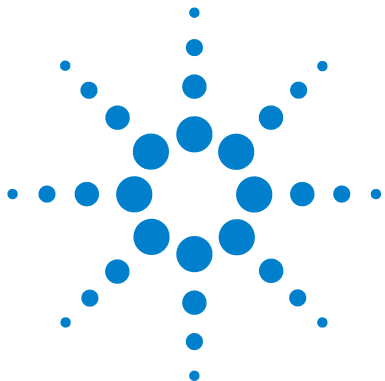
The PC used for running the Agilent ChemStation software and loading the data of the handheld controller must have a PC-card drive.

Apply the following procedure to export data to the Agilent ChemStation software:

- 1 Select a task (e.g. Single WL)
- 2 Press the m-key, select *Export ...* and press *Enter*.
- 3 In the Dialog Box that comes up type in a file name by using horizontal and vertical arrow keys. Press *Enter* to confirm your file name.
- 4 Press the F6-key to export a spectrum to the PC-card (*.wav file extension).
- 5 Remove the PC-card from the handheld controller and put it into the PC-card drive of your PC.
- 6 Load the data into the Agilent ChemStation software by selecting the *Import Samples/Standards/Auxiliary* item from the *File* menu item.

NOTE

The *.wav files can be loaded in all modes of the Agilent ChemStation except for *Kinetics* mode and *Thermal Denaturation* mode. *Auxiliary* spectra can only be loaded in *Advanced* or *Dissolution* (single-bath and multi-bath) mode.



C **Safety Information**

Safety Information [146](#)

Solvent Information [149](#)

Lithium Batteries Information [151](#)

Agilent Technologies on Internet [152](#)



Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

General

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

Operation

Before applying power, comply with the installation section. Additionally the following must be observed.

Do not remove instrument covers when operating. Before the instrument is switched on, all protective earth terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective earth via a ground socket. Any interruption of the protective earth grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, and so on) are used for replacement. The use of repaired fuses and the short-circuiting of fuseholders must be avoided.

Some adjustments described in the manual, are made with power supplied to the instrument, and protective covers removed. Energy available at many points may, if contacted, result in personal injury.

Any adjustment, maintenance, and repair of the opened instrument under voltage should be avoided as much as possible. When inevitable, this should be carried out by a skilled person who is aware of the hazard involved. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not replace components with power cable connected.





Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Capacitors inside the instrument may still be charged, even though the instrument has been disconnected from its source of supply. Dangerous voltages, capable of causing serious personal injury, are present in this instrument. Use extreme caution when handling, testing and adjusting.

Safety Symbols

Table 20 Safety Symbols used on Instruments and in Manuals

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	Eye damage may result from directly viewing light produced by deuterium lamps used in detectors and spectrophotometers. Always turn off the deuterium lamp before opening the lamp door on the instrument.

C Safety Information

Safety Information

WARNING

A warning alerts you to situations that could cause physical injury or damage to the equipment. Do not proceed beyond a warning until you have fully understood and met the indicated conditions.

CAUTION

A caution alerts you to situations that could cause a possible loss of data. Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Solvent Information

Observe the following recommendations on the use of solvents.

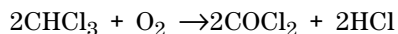
Flow Cells

Avoid the use of alkaline solutions (pH > 9.5) which can attack quartz and thus impair the optical properties of flow cells.

Solvents

Always filter solvents, small particles can permanently block capillaries. Avoid the use of the following steel-corrosive solvents:

- Solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on).
- High concentrations of inorganic acids like nitric acid, sulfuric acid especially at higher temperatures (replace, if your analysis method allows, by phosphoric acid or phosphate buffer which are less corrosive against stainless steel).
- Halogenated solvents or mixtures which form radicals and/or acids, for example:



This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.

- Analysis-grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether) such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1-% solution of acetic acid in methanol will attack steel.

C Safety Information
Solvent Information

- Solutions containing strong complexing agents (for example, EDTA, ethylene diamine tetra-acetic acid).
- Mixtures of carbon tetrachloride with 2-propanol or THF.

Lithium Batteries Information

WARNING

Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer.



Do not dispose of lithium batteries in domestic waste.

Transportation of discharged lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed. Discharged lithium batteries must be disposed of locally according to national waste disposal regulations for batteries.

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In This Book

This handbook describes what you have to do to install your Agilent 8453E UV-visible spectroscopy system.

Most of the handbook deals with installing a complete system, that is, spectrophotometer, handheld controller and printer. However, in the second half of this handbook you will find additional information about installing mechanical and optical accessories, accessory boards, GPIB connections and configuration, and software installation.

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