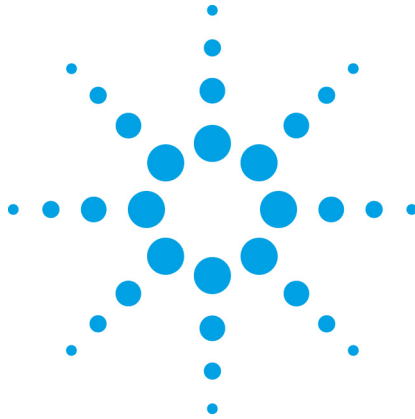


# **Agilent Cary 8454 UV-Visible Spectroscopy System**



## **Operator's Manual**



**Agilent Technologies**

# Notices

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This handbook is for B.05.xx revisions of the Agilent ChemStation software, where xx is a number from 00 through 99 and refers to minor revisions of the software that do not affect the technical accuracy of this handbook.

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## Safety Notices

### CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

### WARNING

A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

## In This Guide...

This book is an introduction to your Agilent Cary 8454 UV-Visible spectroscopy system. It includes step-wise procedures and examples for basic operations and tasks.

For more detailed information see the following manuals:

- *Agilent Cary 8454 UV-Visible Spectroscopy System Installation Guide*
- *Understanding Your UV-Visible Spectroscopy System*
- *Agilent Cary 8454 Service Manual*
- *UV-Visible ChemStation Help*

### **1 Safety Information**

Introduces your UV-Visible spectrophotometer and the Agilent ChemStation software.

### **2 Introduction to Your System**

Introduces your UV-Visible spectrophotometer and the Agilent ChemStation software.

### **3 Installation and Start Up**

Summarizes the system installation process, and explains how to start a measurement session.

### **4 Good Measurement Practices**

Provides guidance for good measurement practices.

### **5 Using your UV-Visible Spectroscopy System**

Provides examples of basic measurements and related tasks.



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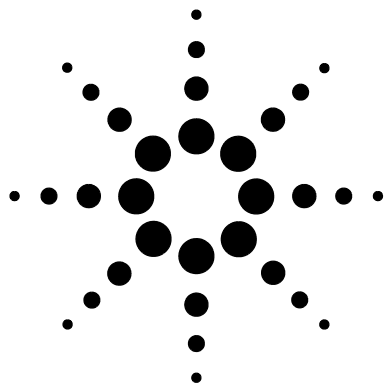
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# 1

## Safety Information

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## Safety Information

Agilent products must only be used in the manner described in the Agilent product user guides. Any other use may result in damage to the product or personal injury. Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

### Verifying Safe State

The following general safety precautions must be observed during all phases of operation, maintenance and service of this instrument. To ensure continued safety of the instrument after maintenance or service procedures, verify the instrument is returned to a safe state for the customer and service personnel user. This includes running performance checks to verify the instruments safety systems are functioning correctly. Check the general condition of the instrument during operation, and for wear or signs of corrosion that are likely to inhibit function or safety.

### Warning Symbols

To avoid personal injury, death, or damage to the instrument, the operator must refer to an explanation in the manual. This symbol is placed on the product where it is necessary for you to refer to the manual in order to understand a hazard.



The following symbols appear on the instrument for your information. The hazard they describe is also shown.



Single phase alternating current.



When attached to the rear of the instrument, indicates that the product complies with the requirements of one or more EU directives.



Indicates a protective 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.

## **Precautions**

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

If the instrument is used in any manner not specified by Agilent, the protection provided by the equipment may be impaired.

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.

## Solvent Information

### CAUTION

Organic and aqueous solvents can be hazardous if handled improperly. Always follow the precautions listed below to protect both operator and instrument.

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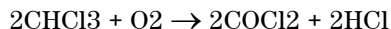
## Flow Cells

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.

## **1 Safety Information**

### **Precautions**

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

## Other Precautions

Use of the spectrometer system and accessories may involve materials, solvents and solutions which are flammable, corrosive, toxic or otherwise hazardous. Careless, improper or unskilled use of such materials, solvents and solutions can create explosion hazards, chemical burn hazards, fire hazards, toxicity and other hazards that can result in death, serious personal injury or damage to equipment. Apply all necessary precautions including use of lab coats, safety goggles and other appropriate forms of personal protection. All wastes should be disposed of in accordance with local regulatory requirements.

### Protective Earth

#### WARNING

##### Shock Hazard

Connecting the Agilent Cary 8454 UV-Vis to a power source that is not equipped with a protective earth contact creates a shock hazard for the operator and can damage the instrument. Always operate your instrument from a power outlet which has a ground connection.

---

### Disconnecting Device

#### WARNING

##### Shock Hazard

To disconnect the instrument from line power, disconnect the power cord from the supply. The power supply still uses some power and a shock hazard remains inside the instrument, even if the power switch on the front panel is turned off.

For safety reason do not position the equipment so that it is difficult to operate the disconnecting device.

---

## Allowing Hot Instrument to Cool

### **WARNING**

#### **Hot Surface**

The lamps and its surroundings remain hot for up to five minutes after the lamp is turned off. To avoid burns, allow the lamp compartment to cool before carrying out any work in this area.

---

## UV Light Source

### **WARNING**

#### **Eye Hazard**

Eye damage may result from directly viewing light produced by deuterium lamps. Always turn off the deuterium lamps before opening the lamp door on the instrument. Direct viewing of ultraviolet rays without protection causes permanent blindness or vision impairment.

---

## Power Cord

### **NOTE**

Do not replace the mains power cord with one that is inadequately rated. When replacing the mains power cord, use only a mains power cord with a rating equivalent to the one supplied with the instrument.

---

## Lithium Batteries Information

### **WARNING**

#### **Explosion Hazard**

The Lithium battery can be found on the Spectrometer Processor Main Board (SPM). 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.

---



# Environmental Conditions and Power Requirements

**Table 1** Suitable environmental conditions for the 8454 UV-Vis

| Condition                              | Altitude                      | Temperature                     | Humidity (%RH)<br>non-condensing |
|--|-------------------------------|---------------------------------|----------------------------------|
| Non-operating<br>(Storage or shipping) | Up to 4,600 m<br>(14,950 ft.) | - 40 to 70 °C<br>(-4 to 158 °F) | 90%                              |
| Operating within<br>specifications     | Up to 2,000 m<br>(6,500 ft.)  | 5-40 °C<br>(41-104 °F)          | <95%                             |

## NOTE

For optimum analytical performance, it is recommended that the ambient temperature of the laboratory be between 20-25 °C and be held +/- 2 °C throughout the entire working day.

## WARNING

### Hot Surface

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

## CAUTION

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.

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.

## Pollution level

The pollution level is 2, based on IEC61010-1. Pollution level describes the degree to which a solid, liquid or gas that deteriorates dielectric strength is adhering. '2' applies to a normal indoor atmosphere, where only non-conductive pollution occurs.

## Regulatory Information

### CE Compliance

Your Agilent instrument has been designed to comply with the requirements of the Electromagnetic Compatibility (EMC) Directive and the Low Voltage (electrical safety) Directive (commonly referred to as the LVD) of the European Union. Agilent has confirmed that each product complies with the relevant Directives by testing a prototype against the prescribed EN (European Norm) standards.

Proof that a product complies with these directives is indicated by:

- the CE Marking appearing on the rear of the product, and

the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the directives listed above, and shows the EN standards to which the product was tested to demonstrate compliance.

## Electromagnetic Compatibility

### EN55011/CISPR11

**Group 1 ISM equipment:** group 1 contains all Industrial, Scientific and Medical (ISM) equipment in which there is intentionally generated and/or used conductively coupled radio- frequency energy which is necessary for the internal functioning of the equipment itself.

**Class A equipment** is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments due to conducted as well as radiated disturbances.

Operation is subject to the following two conditions:

- 1** This device may not cause harmful interference.
- 2** This device must accept any interference received, including interference that may cause undesired operation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

- 1** Relocate the radio or antenna.
- 2** Move the device away from the radio or television.
- 3** Plug the device into a different electrical outlet, so that the device and the radio or television are on different electrical circuits.
- 4** Make sure that all peripheral devices are also certified.
- 5** Make sure that appropriate cables are used to connect the device to peripheral equipment.
- 6** Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.

Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

### **ICES/NMB-001**

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est conforme à la norme NMB-001 du Canada.

## **1 Safety Information**

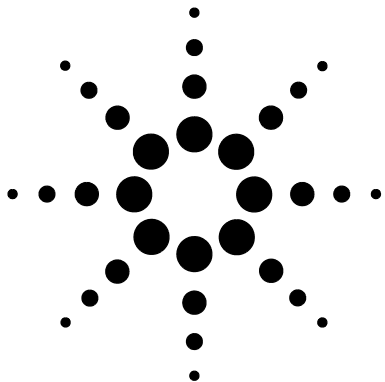
### **Environmental Conditions and Power Requirements**

#### **South Korean Class A EMC declaration**

A 급 기기 ( 업무용 방송통신기자재 )

This equipment is Class A suitable for professional use and is for use in electromagnetic environments outside of the home.

이 기기는 업무용 (A 급 ) 전자파적합기기로서 판 매자 또는 사용자는 이 점을 주의하시기 바라 며 , 가정외의 지역에서 사용하는 것을 목적으로 합니다 .



## 2 Introduction to Your System

Agilent Cary 8454 UV-Visible Spectrophotometer — Overview 22

General Purpose Agilent ChemStation Software for UV-Visible  
Spectroscopy — Overview 30

This chapter introduces the principles of data acquisition, data evaluation and data handling to help you to run your system successfully.

Your spectroscopy system is based on a UV-Visible spectrophotometer with general purpose Agilent ChemStation software for UV-Visible spectroscopy, running on a PC with a supported Microsoft Windows operating system. The spectrophotometer and PC are linked by a network connection. This type of link is very flexible. It can be used for a direct connection between the spectrophotometer and the PC, as well for integration in a enterprise network with network access from the PC to the spectrophotometer.

The spectrophotometer acquires and provides absorbance data which is handled by the PC's application software. All of the data display, evaluation and long term storage is done under software control on the PC.



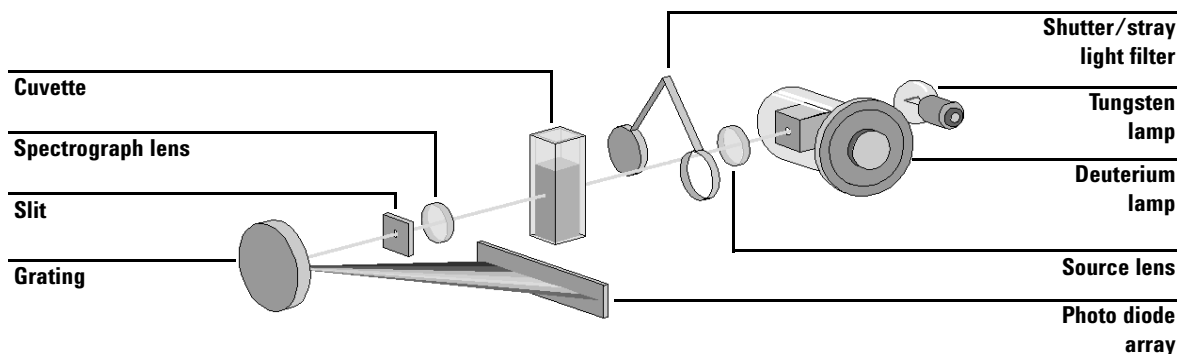
## **Agilent Cary 8454 UV-Visible Spectrophotometer — Overview**

This section gives an overview of the optical system and explains the spectrophotometer front and back panels. It also explains the layout and construction of the spectrophotometer including the electronic and mechanical assemblies inside the spectrophotometer.

### **Optical System Overview**

#### **Optical System**

The optical system of the spectrophotometer is shown in Figure 1. Its radiation source is a combination of a deuterium-discharge lamp for the ultraviolet (UV) wavelength range, and a tungsten lamp for the visible and short wave near-infrared (SWNIR) wavelength range. The image of the filament of the tungsten lamp is focused on the discharge aperture of the deuterium lamp by means of a special rear-access lamp design which allows both light sources to be optically combined and to share a common axis to the source lens. The source lens forms a single, collimated beam of light. The beam passes through the shutter/stray-light correction filter area then through the sample to the spectrograph lens and slit. In the spectrograph, light is dispersed onto the diode array by a holographic grating. This allows simultaneous access to all wavelength information. The result is a fundamental increase in the rate at which spectra can be acquired.



**Figure 1** Optical system of spectrophotometer

- Lamps

The light source for the UV wavelength range is a deuterium lamp with a shine-through aperture. As a result of plasma discharge in a low pressure deuterium gas, the lamp emits light over the 190 nm to approximately 800 nm wavelength range. The light source for the visible and SWNIR wavelength range is a low-noise tungsten lamp. This lamp emits light over the 370 nm to 1100 nm wavelength range.

- Source Lens

The source lens receives the light from both lamps and collimates it. The collimated beam passes through the sample (if one is present) in the sample compartment.

- Shutter

The shutter is electromechanically actuated. It opens and allows light to pass through the sample for measurements. Between sample measurements it closes, to limit exposure of sample to light. If the measurement rate is very fast, you can force the shutter to remain open (using Agilent ChemStation software) or it stays open automatically (handheld controller software).

- **Stray-Light Correction Filter**

In a standard measurement sequence, reference or sample intensity spectra are measured without and then with the stray-light filter in the light beam. Without the filter, the intensity spectrum over the whole wavelength range from 190–1100 nm is measured.

The stray-light filter is a blocking filter with 50 % blocking at 420 nm. With this filter in place, any light measured below 400 nm is stray light only. This stray-light intensity is then subtracted from the first spectrum to give a stray-light corrected spectrum. Depending on the software, you can switch off the stray light correction (using Agilent ChemStation software) if you want to do very fast repetitive scans, or it is switched off automatically.

- **Sample Compartment**

The spectrophotometer has an open sample compartment for easier access to sample cells. Because of the optical design a cover for the sample area is not required. The spectrophotometer is supplied with a single-cell holder already installed in the sample compartment. This can be replaced with the Peltier temperature control accessory, the thermostatable cell holder, the long path cell holder or the multicell transport. All of these optional cell holders mount in the sample compartment using the same simple mounting system. An optical filter wheel is also available for use with the spectrophotometer and most of the accessories.

- **Spectrograph**

The spectrograph housing material is ceramic, to reduce thermal effects to a minimum. Its main components of the spectrograph are the lens, the slit, the grating and the photo diode array with front-end electronics. The mean sampling interval of the diode array is about 0.9 nm over the wavelength range 190 nm to 1100 nm. The nominal spectral slit width is 1 nm.

- **Spectrograph Lens**

The spectrograph lens is the first of the parts which are collectively known as the spectrograph. It is mounted on the housing of the spectrograph. The spectrograph lens refocuses the collimated light beam after it has passed through the sample.



- Slit

The slit is a narrow aperture in a plate located at the focus of the spectrograph lens. It is exactly the size of one of the photo diodes in the photo diode array. By limiting the size of the incoming light beam, the slit makes sure that each band of wavelengths is projected onto only the appropriate photodiode.

- Grating

The combination of dispersion and spectral imaging is accomplished by using a concave holographic grating. The grating disperses the light onto the diode array at an angle linear proportional to the wavelength.

- Diode Array

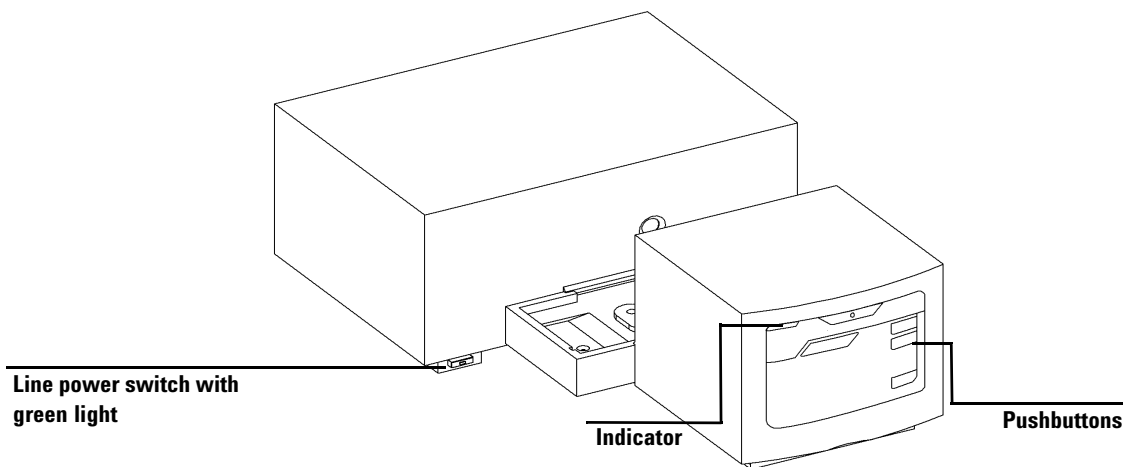
The photodiode array is the heart of the spectrograph. It is a series of 1024 individual photodiodes and control circuits etched onto a semiconductor chip. With a wavelength range from 190 nm to 1100 nm the sampling interval is about 0.9 nm.

## Spectrophotometer Description

Your spectrophotometer is very easy to use. It has a line power indicator, a status indicator and some push buttons. All electrical connections are made at the rear of the spectrophotometer.

### Front View

The front view of the spectrophotometer is shown in Figure 2. Notice that the sample compartment is open. Unlike conventional spectrophotometers the Agilent Cary 8454 UV-Visible spectrophotometer does not suffer from ambient false light. The open sample area makes it easier to access for cuvette handling and to connect tubing to a flow cell or thermostatable cell holder. 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.



**Figure 2** Front view of spectrophotometer

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

On the front panel of the spectrophotometer is a status indicator which will display different colors depending of the actual condition of the spectrophotometer.

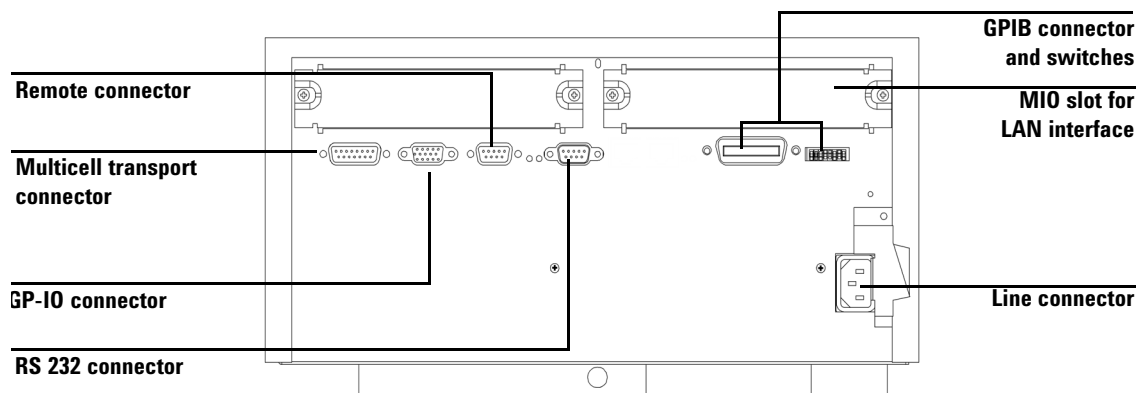
- Green—the spectrophotometer is ready to measure.
- Green, blinking—the spectrophotometer is measuring.
- Yellow—the spectrophotometer is in not-ready state, for example, turning one of the lamps on or if both lamps are switched off.
- Red—error condition, that is, the spectrophotometer does not pass one of the self-tests which are run when the spectrophotometer is turned on or an error occurred during operation. In this case the UV-Visible operating software gives a detailed error message and possible explanations are in the online help system and in your *Service Manual* Chapter 3 “Diagnostics and Troubleshooting”.
- Red, blinking—error condition of the spectrophotometer processor system. Because in this case there is no communication with the computer there will be no error message. The online help system and your *Service Manual* Chapter 3 “Diagnostics and Troubleshooting” give more information about troubleshooting.

The four measure push buttons on the front panel cause the following actions to be performed and the resulting data being sent to the computer. The push button functionality is controller by the UV-Visible ChemStation software and adjusted to the current measurement task.

- Blank—the spectrophotometer takes a blank measurement. This comprises a reference measurement that is used in all subsequent sample measurements until a new blank measurement is taken. Following the reference measurement an additional baseline spectrum is measured and displayed on the PC.
- Sample—the spectrophotometer takes a sample measurement or starts a series of measurements. This depends on the parameters set in your software.
- Standard—the spectrophotometer takes a measurement of a standard. Additional information such as concentration and so on, have to be entered in the operating software.
- Stop—the spectrophotometer and/or software aborts any ongoing activity and returns to a to ready state.

### **Rear View**

All connections are made at the rear of the spectrophotometer, see Figure 3.



**Figure 3** Rear view of spectrophotometer

- The multicell connector allows you to connect a multicell transport.
- The GPIO (general-purpose input/output) connector allow you to control a sipper and autosampler or other accessories depending on the software you are using.
- The remote connector is currently not used by Agilent instrument control software. It can be used e.g. to synchronize instruments.
- The RS232C connector may be used to control the spectrophotometer from a computer through RS232 connection, using appropriate software. The Multiple Instrument Firmware Tool provided can be used with RS232 communication. The UV-Visible ChemStation software currently supports LAN communication only.
- The MIO board slot is reserved for a LAN interface board.
- The accessory board slot is reserved for future use.

- The power input socket does not have a voltage selector because the power supply has wide-ranging capability, for more information see your *Service Manual* Chapter 1 “Specifications”. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply. The security lever at the power input socket prevents that the spectrophotometer cover is taken off when line power is still connected.

### **Side of the Spectrophotometer**

On the right side of the spectrophotometer is a double door for access to the lamps. For exchanging lamps both doors, the plastic door and the sheet-metal door must be opened. Safety light switches are automatically turning off the lamps when the inner sheet metal door is opened.

## **General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview**

This section gives an overview of the elements of the user interface implemented with your Agilent ChemStation software and the data analysis concept behind. It explains how data are processed and what the advantages of this processing are from a practical point of view.

## User Interface Elements

Your general purpose Agilent ChemStation software for UV-Visible spectroscopy facilitates operation of your diode-array-based UV-Visible spectrophotometer in daily routine operation. The focus of this software is on ease-of-use and ease-of-learning. A graphical user interface visualizes the spectrophotometer operation and usage. This user interface consists of a number of elements as described in the following sections.

**Method name**

**Tool bar**

**Menu bar**

**Analysis panel**

**Data files**

**Navigation bar**

**Modes**

**Instrument panel**

**Tabular results**

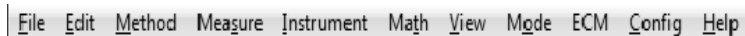
**Spectra**

| # | Name | Peaks(nm) | Abs[AU]   |
|---|------|-----------|-----------|
| 1 |      | 623.0     | 0.54155   |
| 1 |      | 257.0     | 0.44652   |
| 1 |      | 412.0     | 0.36484   |
| 1 |      | 713.0     | 3.3587E-2 |
| 1 |      | 720.0     | 3.2845E-2 |

## 2 Introduction to Your System

### General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview

#### Menu



The more traditional menu interface at the top of the Agilent ChemStation window provides access to all operations. When you choose an item from the menu bar, a list of commands and submenus is displayed. An operation is performed by choosing a command (mouse click or Enter key).

#### Toolbar



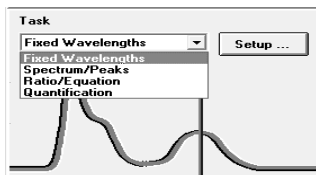
The tool bar below the menu bar provides direct access to basic operations such as printing result reports, loading methods, and saving methods and data.

#### Navigation Bar

The navigation bar consists of mode switching bars and a file browsing tree view for saved files. The navigation bar is dockable, and can be hidden or resized. The navigation bar window provides fast, direct access to file system and mode switch functionality. The size and position of the analysis and instrument panels are fixed, but are a function of your display's resolution. Files can be loaded from the navigation bar tree view by double-clicking. Clicking on the "Browse" section allows access to any available data directory.

#### Analysis Panel

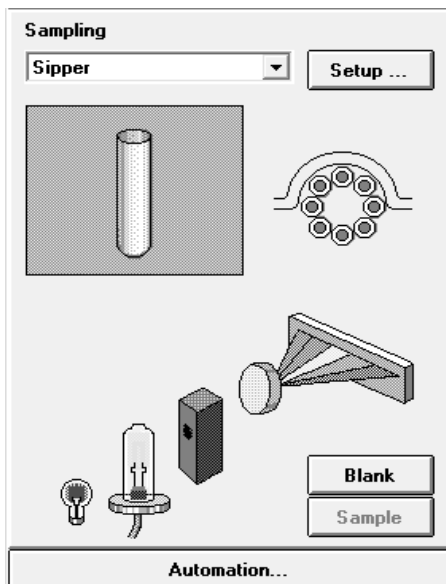
The analysis panel gives you a graphical visualization of the current working context. In addition, it provides access to the setup dialog of your actual task by means of the Setup button.





## Instrument Panel

The instrument panel is below the analysis panel. It displays and controls your sampling devices and spectrophotometer. Some of the graphical elements on this panel are active items which are used, for example, to switch lamps on or off, or run a peristaltic pump.



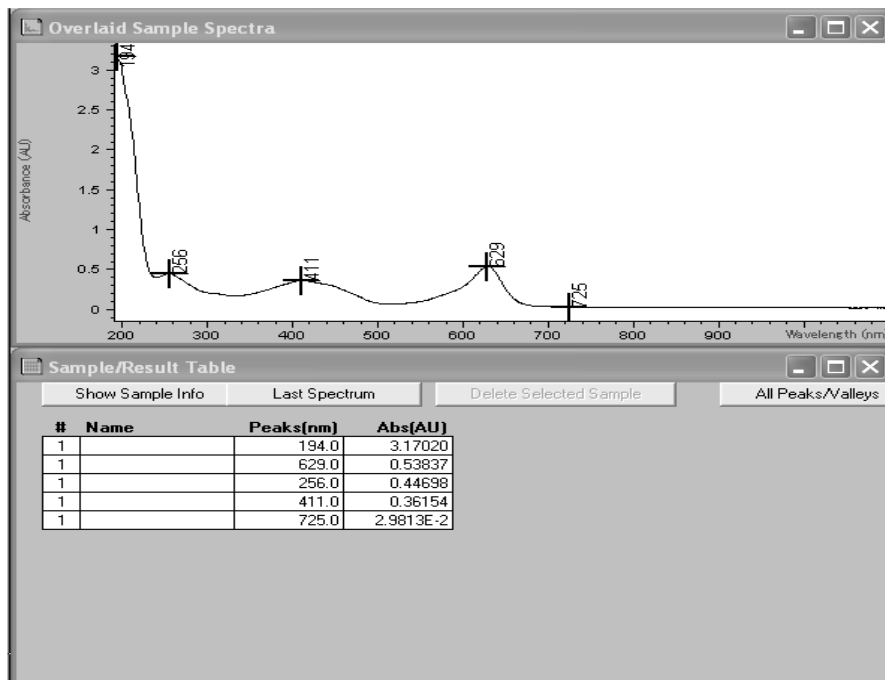
When you move the mouse across an active area, the cursor changes. Click in an active area to bring up a small menu with selections, or to perform an operation.

In addition you can select a sampling system from the list of available systems. Its parameters can be adjusted by means of the Setup button.

## 2 Introduction to Your System

### General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview

#### View



The area on the right side of the side panels provides a view on a certain aspect of your current task. A view consists of one or more separate windows. These windows provide information such as a graph showing your measured sample spectra, or a table with the calculated results.

Views are usually handled automatically by the operation you performed, but you may also use the view menu's commands to select the view you prefer.

## Software Structure

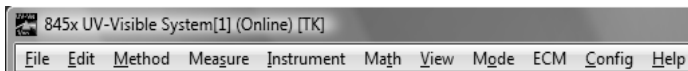
The software is divided into specific applications, called modes. In addition levels of operation are available, and there is support for data evaluation sessions without spectrophotometer control.

### Agilent ChemStation Sessions

Your Agilent ChemStation belongs to the Agilent's ChemStation family of instrument control software. An installation of Agilent ChemStation software can control up to four different UV-Visible instruments on a single PC. Each of these instruments has its own session



If you start a session, its name is indicated in the main application's window title bar, for example, 845x UV-Visible System[1].



Each instrument session is available for data analysis only, or for instrument control. An instrument control session has the suffix (Online) and can be started as the first instance only on your PC. In addition a data analysis session, which has the suffix (Offline), can be launched. The offline session allows recalculations based on stored data, and is useful in the development of analytical methods.

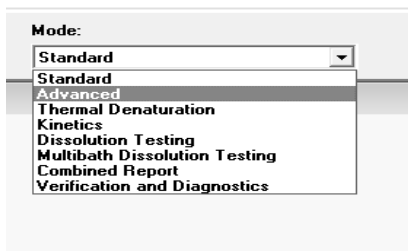
### Operation Levels

The operation levels *manager level* and *operator level* apply to all modes and allow managing an application and running an application only. In the managing level of an application, methods can be developed and stored permanently to disk. The manager level of operation is password protected. This assures the integrity of predefined methods and operation sequences.

In the operator level, a reduced set of functions is available. Functions which may affect the integrity of an analytical procedure are not available. However, an operator may use their own settings. This is flagged on the tool bar and is indicated on printed reports.

### Agilent ChemStation Modes

The Agilent ChemStation modes are application oriented. Each mode has its own mode-specific menu, panels, operations and set of views. Your general purpose software for UV-Visible spectroscopy is the platform for all modes. It is split into a *Standard* mode, an *Execute Advanced Method* mode and a *Verification and Diagnostics* mode.



Depending on your needs, modes are available for *Advanced* operation, *Dissolution Testing* runs, *Multibath Dissolution Testing* runs, *Combined Reports* evaluation, *Kinetics* measurements, *Thermal Denaturation* studies and *Color Calculations*.

These modes of operation can be switched within a running Agilent ChemStation session. All current raw data will be preserved during such a switch. This allows you to view your data from different perspectives.

Most of the modes offer you the ability to define your analytical task by a set of parameters and, if necessary, data. A set of parameters and data can be saved as a method. This allows you to repeat your analysis task under defined conditions simply by loading a method and running your samples.

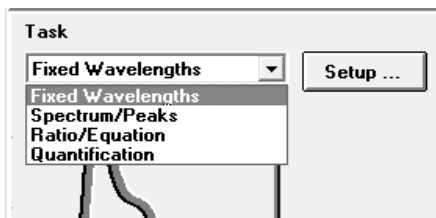
## Standard Mode Tasks

The Agilent ChemStation modes also offer the ability to focus on specific tasks.

The standard mode of your general purpose software for UV-Visible spectroscopy is oriented towards the most common tasks performed in a analytical laboratory that employs UV-Visible spectroscopy. Four tasks are available:

- Fixed Wavelength
- Spectrum/Peaks
- Ratio/Equation
- Quantification

A task is selected and activated from the analysis panel's selection box.

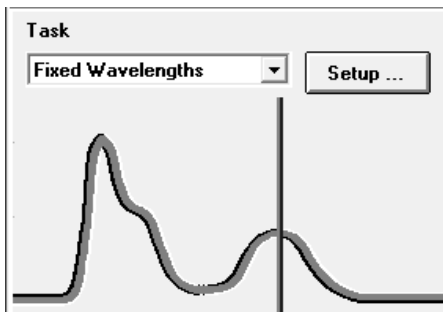


The task orientation lets you quickly adjust the software to give you the right view and answers based on your data. These tasks have been derived from a survey of the most common UV-Visible spectroscopic tasks done on a routine basis in analytical laboratories. Historically, these tasks have been developed on filter photometers or scanning spectrophotometers.

With your spectrophotometer you have the advantage that, by default, the entire UV-Visible spectrum of your samples is available. So these four tasks offer different views of your acquired data.

A task switch within the Standard mode is much faster than an entire mode switch. An additional benefit of these tasks is that the definition of a method is done within a single dialog.

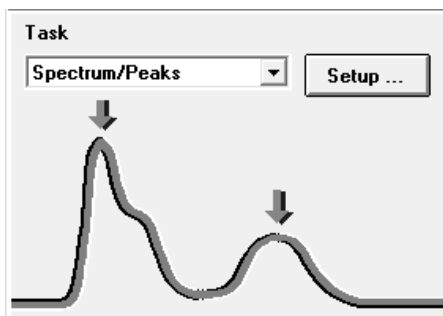
#### Fixed Wavelength



The Fixed Wavelength task is used to look at measured sample data at up to six different wavelengths. This data is available as absorbance, transmittance and first to fourth derivative.

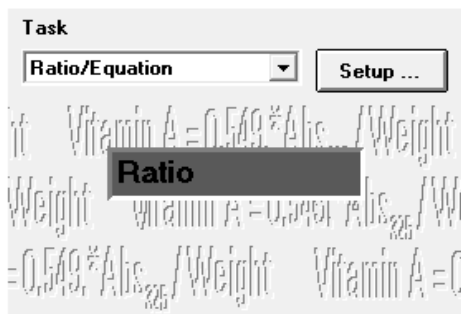
Due to the spectral acquisition, additional techniques such as internal reference or three-point, drop-line background corrections can be applied.

#### Spectrum/Peaks



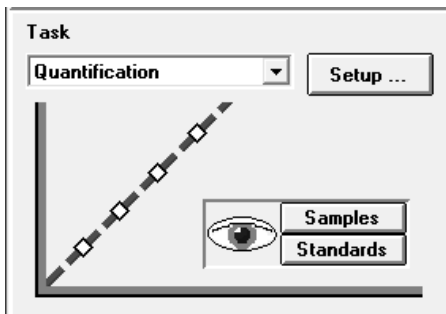
In the Spectrum/Peaks task, you can view absorbance minima and maxima. The focus here is more on the wavelength scale, but the relevant absorbance readings are also provided.

## Ratio/Equation



The Ratio/Equation task is used to evaluate a user-definable equation based on measured data and sample information. An equation can be set up using sample data at up to six wavelengths. Weight and volume data is entered with the samples measured. By means of an equation, for example, analysis results based on chemical test kits can be automatically calculated and reported. Another application for this task is to use a ratio of data values to check for the identity or the purity of a sample.

## Quantification



The Quantification task allows you to do single component analysis based on four different types of calibration curves and a set of standards. Due to the spectral acquisition, background corrections can also be applied to your data.

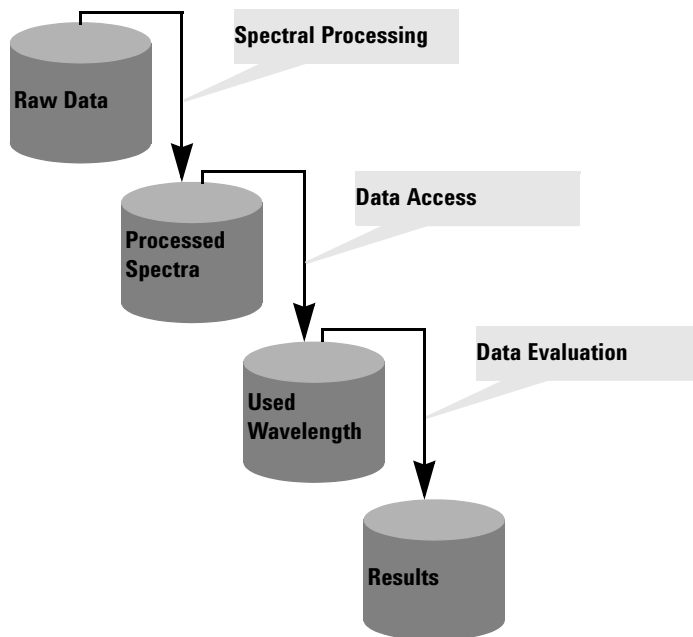
The calibration can be optimized for your concentration range of interest by changing the wavelength used for calibration. A new calibration and a new analysis is automatically performed, based upon your current standard data.

## Standard Mode Data Processing

### General Data Processing

Although knowledge of the internal design of the data flow and processing is not required to use Agilent ChemStation software, it can be useful to understand how Agilent ChemStation processes your data and how this processing is controlled by your method parameters.

The data processing can be described using a model of data containers and operations visualized in a flow diagram.

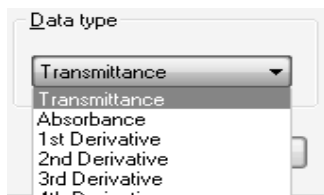


All basic data go into a *raw data* container. This container is empty when you start your Agilent ChemStation session and it is filled by measuring data or loading data from a file.

The *raw data* container held the originally acquired data as specified with your acquisition parameters and stamped with, for example, acquisition date and time and the acquisition operator's name.

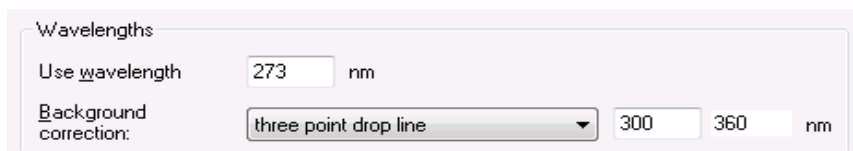


## Spectral Processing



Your method defines how this data is analyzed. The first processing step is spectral processing. The processed raw data spectra are transferred automatically into a second container for *processed spectra*. This allows you to examine the results of this processing step by viewing the *processed spectra* container's content. If, for example, you specified the first derivative data type, the first derivative spectra of all your raw data are available in the *processed spectra* container after analysis. The type of spectral operation is defined by your method settings.

## Used Wavelength

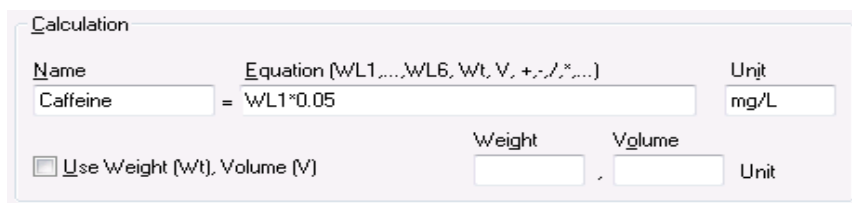


The next step in the data analysis process is the provision of data for further evaluation specified in terms of wavelength and background correction operations such as an internal reference calculation or three-point, drop-line calculation.

This data is stored in the *used wavelength* container. In the Fixed Wavelength task, for example, a tabular view of this data is available with the Sample/Results Table window.

## Results

An additional step is the evaluation of the accessed data.

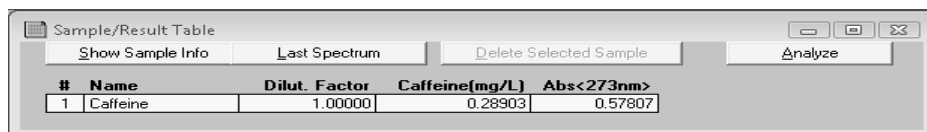


## 2 Introduction to Your System

### General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview

In the Ratio/Equation task, for example, this *used wavelength* data is processed by an evaluation of the equation specified which generates the calculation results. These calculated result values are stored in the *results* container.

The results are available with the Sample/Results Table.



| # | Name     | Dilut. Factor | Caffeine(mg/L) |
|---|----------|---------------|----------------|
| 1 | Caffeine | 1.00000       | 0.28903        |

Abs<273nm> 0.57807

#### Summary

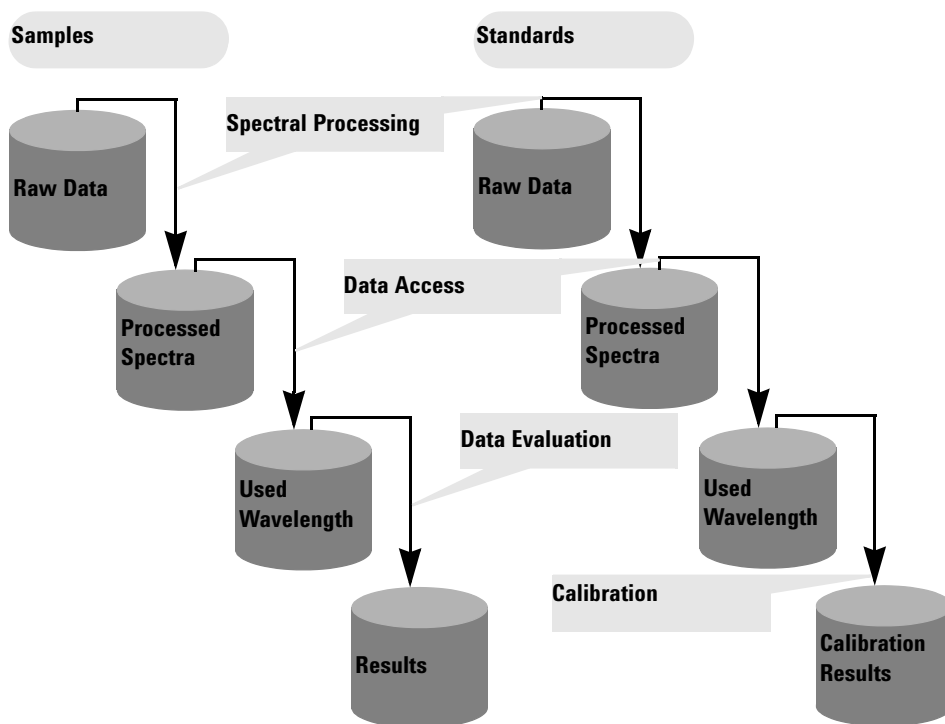
The generalized basic data processing is divided into three steps.

- 1 spectral processing
- 2 data access
- 3 data evaluation

These steps are always performed in the above order identically for all spectra in the *raw data* container. The results are placed in the *results* container. The previous content of the *processed spectra*, *used wavelength* and *results* container is replaced.

## Processing with Standards

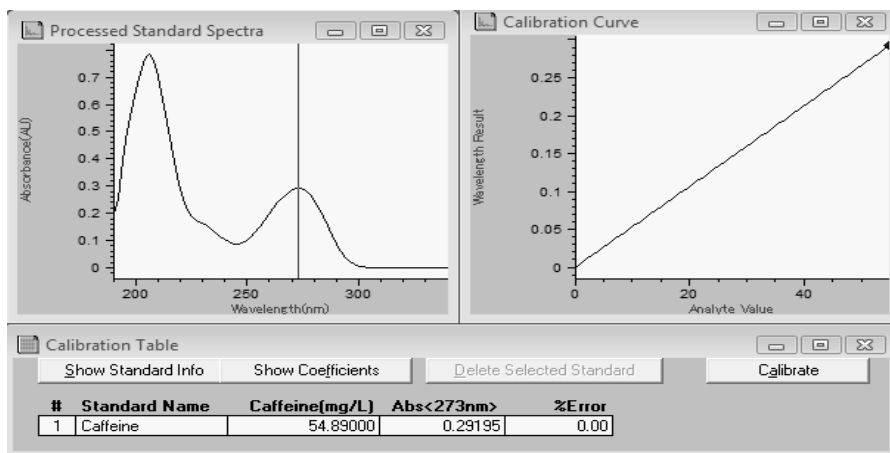
In the fourth task, standards are used in addition to your sample data. This requires an extension to the above concept to handle standards. Two independent sets of containers are implemented, one for standards, and another for samples. All processing containers are also duplicated. As with the sample-only processing, all evaluation steps are done in parallel on both the samples and the standards.



The evaluation in quantification is now a calibration using standards. The coefficients are calculated based on the method's settings and the current standards in the Agilent ChemStation memory. This means that results now become a function of a measured set of standards and of the standard concentrations specified.

## 2 Introduction to Your System

### General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview



These coefficients are then used to calculate concentration results for the samples currently in memory. The same processing steps, applied to sample and standard data, lead to the most precise results.

### Advantages

A diode-array-based spectrophotometer, in combination with powerful data evaluation based on Agilent ChemStation, offers many advantages over traditional spectrophotometer systems. Some of these advantages are:

- Virtually unlimited number of standards

Measure your standards before or after your samples and, as well as the minimum required number of standards, use as many standards as you wish with your calibration.

- Easy optimization

The availability of all raw data—samples and standards—means you can easily optimize your method's settings by choosing a different calibration wavelength and re-calibrating your system. Also, by simply removing this standard from your standard data set, you can eliminate outliers in your calibration.

- Calibrated method

When you save your method, the standards currently in memory are always saved with the method. After loading a method, you can directly analyze your samples.

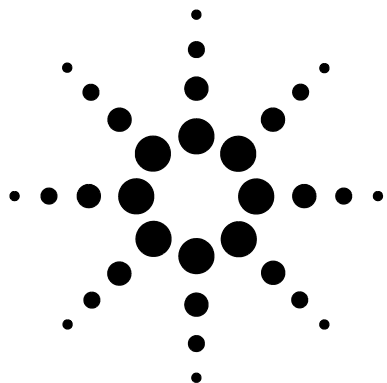
- Optimization for a particular sample

You can optimize your wavelength settings for a sample outside the linear range of your actual calibration. Due to the excellent wavelength reproducibility of your spectrophotometer, you can switch to a wavelength with a lower extinction coefficient for precise analysis of such a sample.

In summary, the availability of spectral raw data offers you many additional opportunities to optimize your calibration and analysis for best results. This optimization can be done quickly, just by setting new method parameters. You get new answers almost instantaneously with consistent and reliable results.

## **2 Introduction to Your System**

### **General Purpose Agilent ChemStation Software for UV-Visible Spectroscopy — Overview**



### 3 Installation and Start Up

|   |    |
|---|----|
| Installation Summary for Your General Purpose UV-Visible System | 48 |
| Starting a Measurement Session                                  | 50 |

This chapter does not replace the information available with the *Agilent Cary 8454 UV-Visible Spectroscopy System* Installation Guide. It is intended as a reminder of the key steps of the installation and system startup.



# Installation Summary for Your General Purpose UV-Visible System

## General

A detailed description of your UV-Visible system is given with the manual *Agilent Cary 8454 UV-Visible Spectroscopy System* Installation Guide. This summary reminds you of the key points of installation.

## Spectrophotometer

- ✓ Make sure that your spectrophotometer has the LAN interface card installed.
- ✓ Check that your spectrophotometer is either connected to your PC directly using a crossover LAN cable, or to your LAN using a direct connection.

### CAUTION

**The Agilent Cary 8454 UV-Visible spectrophotometer does not have a CAN interface.**

**Do not connect the LAN adapter of your PC to the CAN interface of the Agilent 8454 spectrophotometer, otherwise the LAN adapter of the PC will be seriously damaged, because the operating voltage of the CAN interface (12 V) is higher than the operating voltage of the LAN adapter (5 V).**

- 
- ✓ Check that your spectrophotometer is connected to a power outlet.

### WARNING

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

- 
- ✓ Before you switch on your spectrophotometer, make sure that the Agilent Bootp Service is installed on your PC or your network administrator has assigned an IP address to your spectrophotometer. For details see the chapter “LAN Communication, Installation, Connection and Configuration” in your *Agilent Cary 8454 UV-Visible Spectroscopy System* Installation Guide.



## PC

- ✓ Make sure that all of your PC components are connected to line power.
- ✓ Make sure that your general purpose software for UV-Visible spectroscopy is installed.
- ✓ A printer must be configured on your PC.
  - Adjust paper size (for example, Letter, A4)
  - Set Orientation to Portrait
- ✓ The TCP/IP protocol must be installed and configured on your PC.
- ✓ Your spectrophotometer must be configured with its IP address.
- ✓ If you connected your spectrophotometer directly to your PC be sure that the Agilent Bootp Service is installed and configured. If you connect to your spectrophotometer using a LAN make sure that your network administrator assigns the IP address to your spectrophotometer.

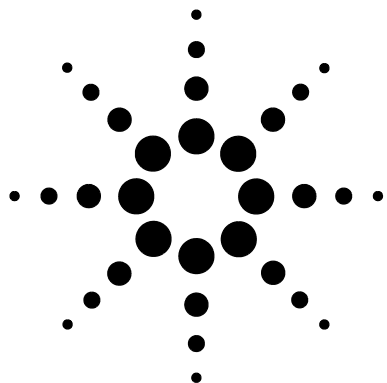
## Starting a Measurement Session

With a network connection to your spectrophotometer, it is important that your spectrophotometer is recognized by the software. This requires the assignment of a unique IP address to your spectrophotometer when it is powered on. The assignment is done either by the Agilent BootP Service application running on your PC with a direct connection to the spectrophotometer, or by a server application on your LAN. Therefore it is important that either one of these applications is up and running before you switch your spectrophotometer on.

- ✓ Switch on your PC and boot your PC operating system. If a printer is connected to your system, switch the printer on.
- ✓ Make sure that the Agilent BootP Service is running or you are logged onto your LAN.
- ✓ Switch your spectrophotometer on and wait until the spectrophotometer's indicator light turns to green. This process includes the spectrophotometer's self test and takes about one minute. For details on the startup sequence see chapter "Installation and Start Up" in your *Agilent Cary 8454 UV-Visible Spectroscopy System* Installation Guide.
- ✓ Launch your measurement session by pressing Start > All Programs or All Apps > UV-Visible ChemStations > Instrument 1 online.
- ✓ You are ready to use your system, if the blue *busy* status display on the system's bottom message line turns off.
- ✓ The first measurement you have to perform is a reference measurement. After this alignment you are ready to measure absorbance data and spectra.

### NOTE

It takes about 15 minutes for the lamps to reach stable state conditions. For best results, do not perform measurements before this period of time has elapsed.



## 4 Good Measurement Practices

General Considerations 52  
Inserting a Cell 65

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 cells 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 spectrophotometer is open. Unlike conventional instruments the UV-Visible spectrophotometer does not suffer from ambient false 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 at least 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 Material**

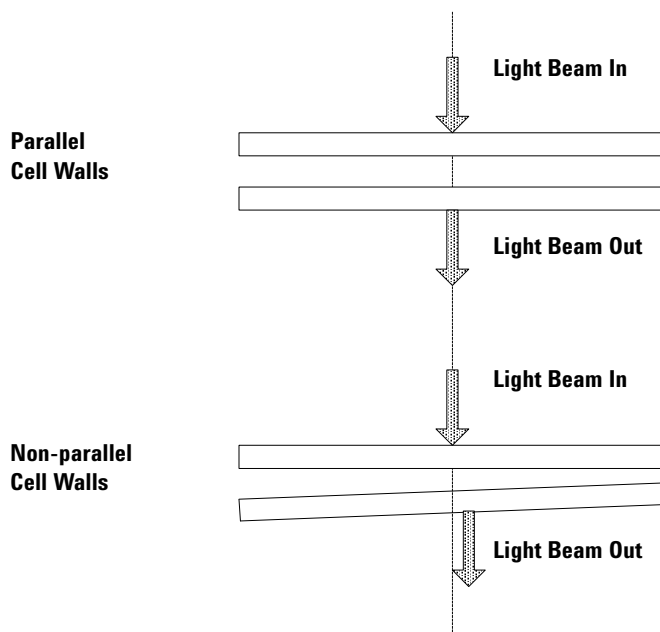
**Quartz sample cells** or sample cells with quartz face plates are required if you want to use the full 190 to 1100 nm wavelength range of your spectrophotometer.

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 spectrophotometer is very sensitive to spatial shifts of the measurement 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 4). Therefore, the opposite cell walls illuminated by the analysis light beam have to be very parallel. The parallelism is measured in terms of the *angle between the two opposite cell walls*. We recommend that you use 10 mm path length cells with *an angle that is below 0.1 degrees of an arc*.

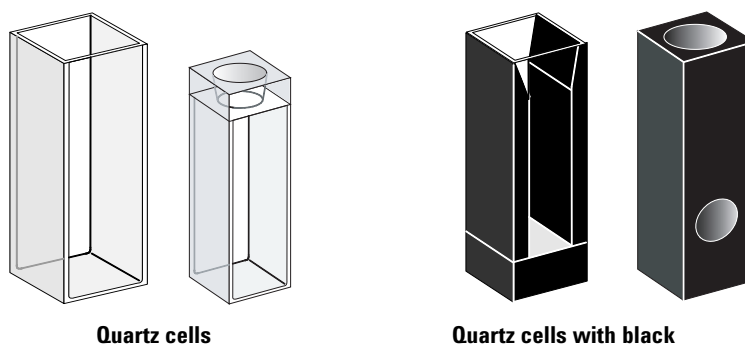


**Figure 4** Shift of the spectrophotometer light beam due to non-parallel cell walls

## Apertured Cells

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.

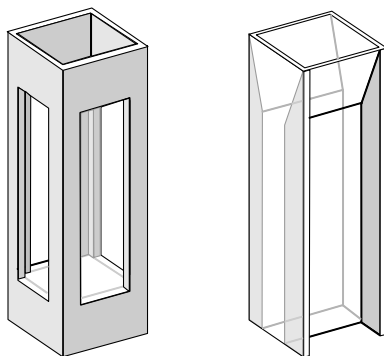
The disadvantage of apertured and microcells is that part of the light beam is blocked. Not all the light passes through the sample and there can be some loss of sensitivity. See Figure 5 for recommended and Figure 6 for cells you should not use with the instrument.



**Figure 5** Recommended cells

### CAUTION

\* Quartz cells with black apertures smaller than 2 mm, when used with a multicell transport, can lead to measurements with poor reproducibility.



**Quartz cells with transparent apertures,  
fluorescence cells, plastic cells**

**Figure 6** Cells you should not use with the instrument

## Flow Cells

We recommend a sipper system with a flow cell for obtaining 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

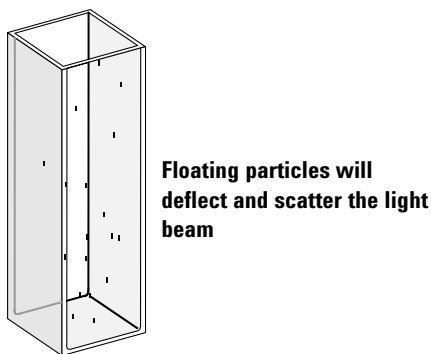
### Passivating New Cells

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 is described on the label of the cleaning fluid container.

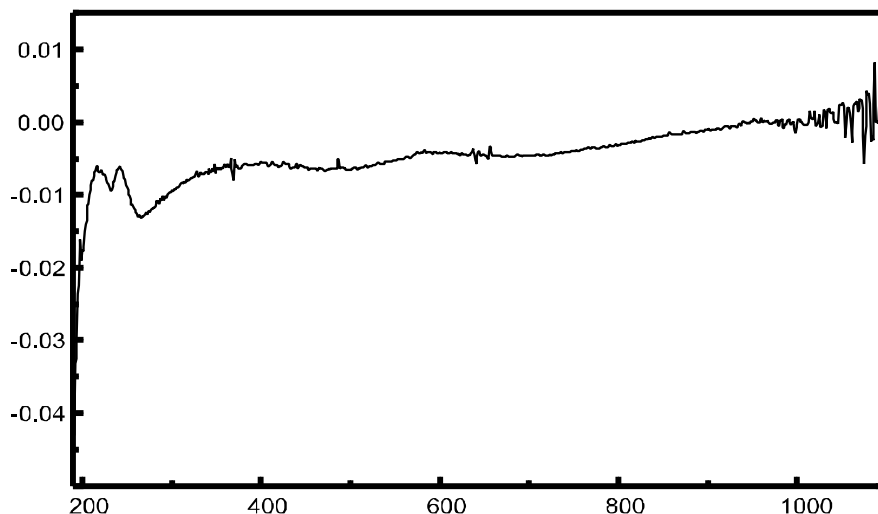
### Cleaning Cells

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 contamination with tissue particles, or rinse the cell with blank or sample solution. Floating particles in the cell will deflect the light beam and result in very poor quality of the measured spectrum.



**Figure 7** Floating particles in a cell



**Figure 8** 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 microcells, leave your sample cell clamped in position throughout the measurement sequence. Solutions should be removed and replaced by pipette or use flow cells.

#### CAUTION

If glass pasteur pipettes are used, make sure that the optical windows of the cell are not touched or scratched by the pipette.

## 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 2 lists common solvents and the lower limit of their useful wavelength range.

**Table 2** Lower limit of UV transmission for some common solvents

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

**WARNING**

**Many of the solvents in Table 2 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 does change the solute concentration and can 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 impurities in 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

The shorter wavelength, higher-energy UV light is most likely to degrade photosensitive samples. If this is 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.

### 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 400–1100 nm. When using cells with small apertures, you should check the signal to noise ratio by making sample measurements under the 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**

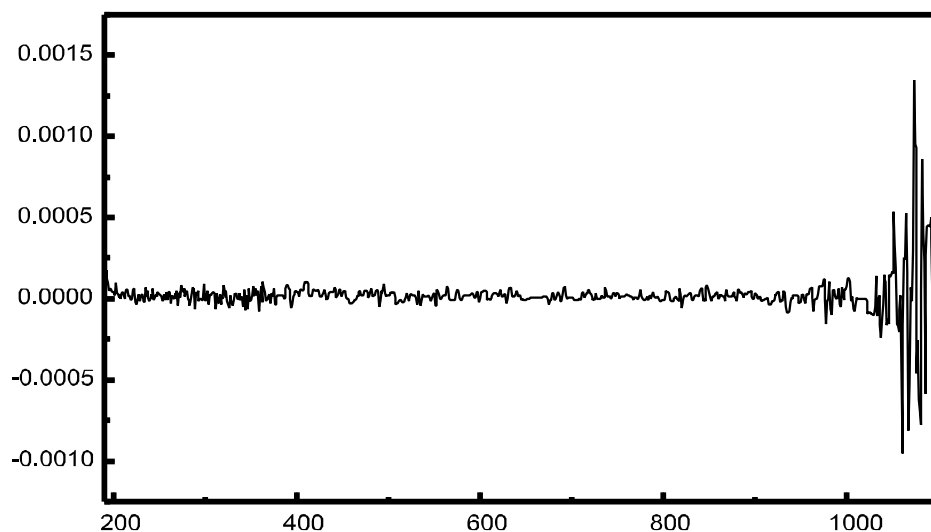
Cell:

- ✓ 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

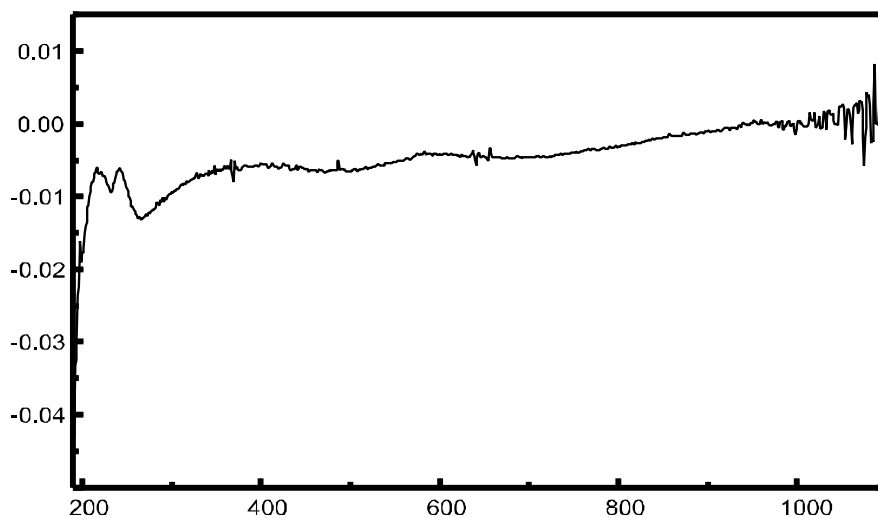
- ✓ Blank measurement shows a flat baseline (Figure 9 and Figure 10 on page 64 show a good and a poor baseline)
- ✓ Cell orientation of blank and sample measurements is the same
- ✓ Ideally the cell is not removed between the measurements, which means the cell is filled/rinsed using a pipette or a flow cell is used
- ✓ Time between blank and sample measurement should be short



**Figure 9** Example of a blank on water showing a good baseline

## 4 Good Measurement Practices

### General Considerations



**Figure 10** Example of a blank on water with bubbles causing a poor baseline

#### NOTE

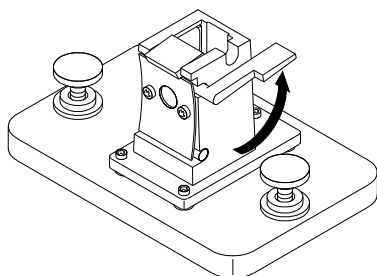
If your blank or spectra shows artifacts similar to those in Figure 10, see "Solvents" on page 59 to optimize the measurement procedure.



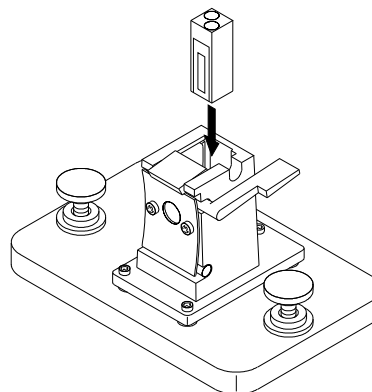
## Inserting a Cell

Your spectrophotometer is shipped with the standard single-cell holder which you must install in the sample compartment. This cell holder accommodates standard cells or flow cells. To insert a sample cell in the cell holder:

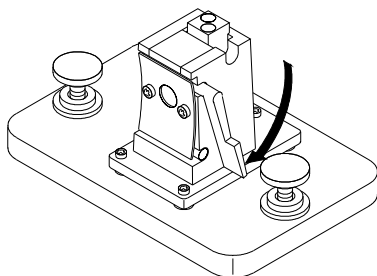
1 Move the locking lever to its up position.



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.



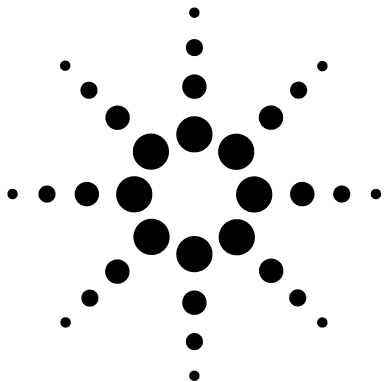
3 Lock the sample cell in place by pushing the locking lever back down.



Utmost care should be taken when using small volume flow cells and particularly any cells with less than a 2 mm aperture. Here it is important that the cells are properly centered in the light path. The cell holder is designed for cells with 15 mm center height. If applicable, these cells should not be removed between the respective reference (blank) measurement and the sample measurement.

## **4 Good Measurement Practices**

### **Inserting a Cell**



## **5**

# **Using your UV-Visible Spectroscopy System**

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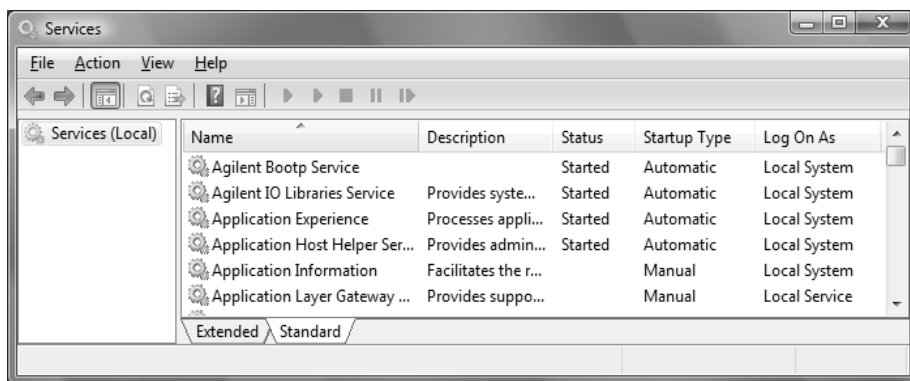
## Starting Your First Measurement Session

- 1 Make sure that your spectrophotometer has been installed correctly.

For details of installation see your *Agilent Cary 8454 UV-Visible Spectroscopy System* Installation Guide.

- 2 Switch on your PC, monitor and printer.
- 3 Log on to your PC operating system.

Confirm that your Agilent BootP Service is running using the Control Panel's Administrative Tools Services dialog, or make sure that your network administrator has integrated your spectrophotometer into your local network.



- 4 Switch on your spectrophotometer.

A running BootP service will now assign the configured IP address to your spectrophotometer. In the standard installation, the Agilent BootP Service performs this task.

- 5 Start your measurement session by selecting Instrument 1 online from the menu.

Your Instrument Panel shows you the current state of the spectrophotometer and the Blank button is enabled.

- 6 The first task is to measure a reference. Typically the cell containing the solvent used with your samples is put in the measurement position and a

blank measurement performed. To start this measurement, click the Blank button on your Instrument Panel or press the spectrophotometer's Blank button.

A blank measurement is a reference measurement combined with the measurement of a baseline spectrum. A baseline spectrum gives you additional hints on the absorbance of the cell windows and the solvent. Areas with high noise indirectly indicate high absorbance.

**NOTE**

For high precision measurements, wait until the spectrophotometer and the lamps have reached thermal equilibrium. The time required is a function of the environmental conditions. Your spectrophotometer should be ready after 45 minutes.

---

- 7 The next measurement is your sample measurement. To get the most precise results, use the same cell in the same orientation to the measurement beam. Flush your cell about three times with your sample solution and start the measurement by clicking the Instrument Panel's Sample button, or by pressing the spectrophotometer's Sample button.

**NOTE**

For details on how to mount your cell, see "Inserting a Cell" on page 65.

---

## **Starting Your UV-Visible Software**

This section describes how to start an Agilent ChemStation session on your PC. If you want to perform measurements, you can start an online session, or you can start an offline session for optimizing the analytical parameters of a method, recalculating results or printing reports.

An online session can be started only as the first instance on your PC. An offline session can be started in parallel. This allows you to optimize your method settings by direct comparison based on identical sets of data.

- 1** Switch on your PC, monitor and printer.
- 2** Log on to your PC's operating system
- 3** Start your Agilent ChemStation session by selecting Instrument 1 online, for a measurement session, or Instrument 1 offline, for method optimization and data evaluation.
- 4** Enter your name to log on to your Agilent ChemStation session. If you protected your manager level by password, you must enter the correct password. The system will then come up with the last-used mode and method.

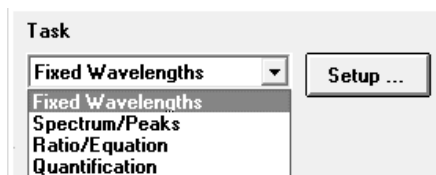
## Measuring Caffeine Absorbance at 273 nm

This section describes how to measure the caffeine sample that was shipped with your spectrophotometer. Measurement of this caffeine sample is also used for the IQ (installation qualification) of your spectrophotometer.

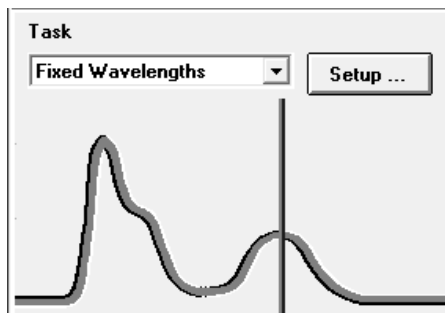
- 1 Make sure that you are in Standard mode. The mode is indicated on the toolbar of your Agilent ChemStation session.



- 2 Select the Fixed Wavelength task in the analysis panel's selection box.



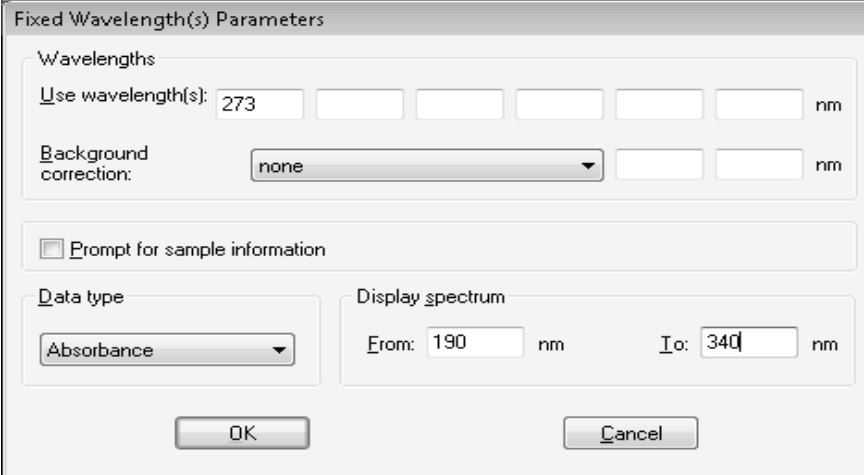
- 3 Click Setup in the analysis panel to open the parameter dialog.



## 5 Using your UV-Visible Spectroscopy System

### Measuring Caffeine Absorbance at 273 nm

- 4 Type the wavelength of interest in the Wavelengths section of the Fixed Wavelength(s) Parameters dialog. Adjust your spectral display to a wavelength range from 190 nm to 400 nm in the Display spectrum section. Click OK to set your parameters.

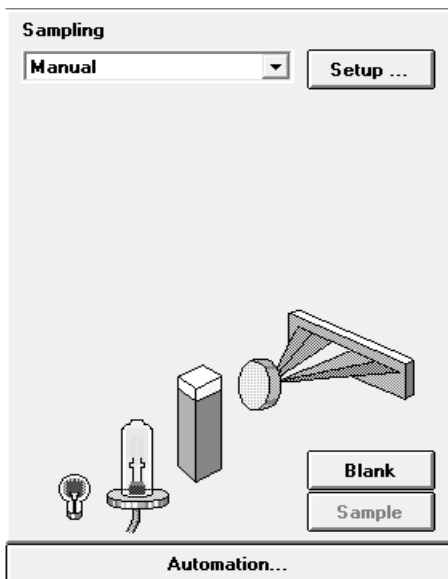


The image shows a software dialog box titled "Fixed Wavelength(s) Parameters". It contains several input fields and buttons. In the "Wavelengths" section, the "Use wavelength(s):" field has "273" entered, followed by five empty boxes and the unit "nm". Below this, the "Background correction:" dropdown menu is set to "none", followed by two empty boxes and the unit "nm". A checkbox labeled "Prompt for sample information" is unchecked. In the "Data type" section, the dropdown menu is set to "Absorbance". In the "Display spectrum" section, the "From:" field has "190" and the "To:" field has "340", both followed by the unit "nm". At the bottom, there are "OK" and "Cancel" buttons.

- 5 Fill your 1 cm path length quartz cell with distilled water. Lift the lever at the left side of your cell holder. Put the cell in the cell holder and make sure the transparent windows face towards the front and the back of the spectrophotometer. Push the lever down to secure your cell in the cell holder.



- 6 Press the Blank button on the front of the spectrophotometer or click Blank on the Instrument Panel to start the measurement.



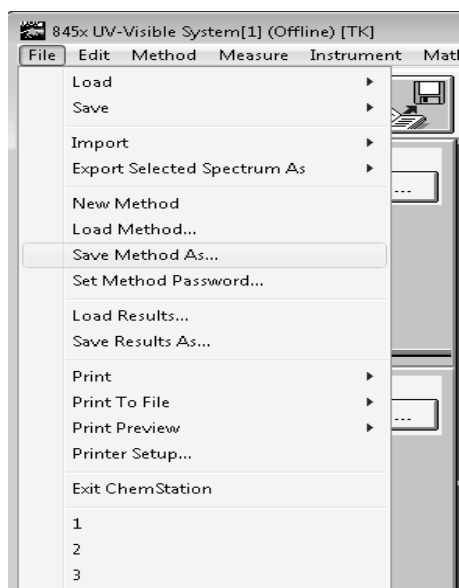
- 7 Remember the orientation of the cell in the cell holder. Lift the level to release the cell, remove it and flush it three times with about 1 ml of your caffeine sample. Then fill the cell with about 3 ml of the caffeine sample. Make sure that the cell windows are clean and reposition the cell in the same orientation as for the reference measurement. Close the cell holder's level.
- 8 Press the Sample button on the front of your spectrophotometer, or click Sample on the Instrument Panel to start the measurement.
- 9 The view shows you the spectrum of your caffeine sample with a vertical line indicating your wavelength of interest. Below the spectrum is the Sample/Result Table which shows the absorbance reading at 273 nm.

## Saving Your Parameters as a Method

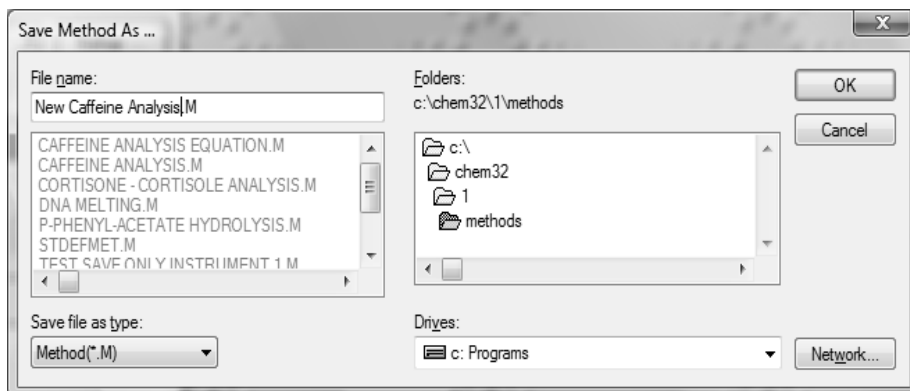
This section describes how to save your settings for a future Agilent ChemStation session. Simply by loading your method you adjust your Agilent ChemStation to repeat your measurement. A library of methods facilitates routine laboratory work.

Let's assume that your current settings are defining your method to analyze a caffeine sample. To be able to repeat such an analysis all set parameters can be stored permanently to disk. This allows you to load such a method on your system or even transfer such a method to your colleague with a Agilent ChemStation system.

- 1 Choose Save Method As... from the File menu or click the icon.

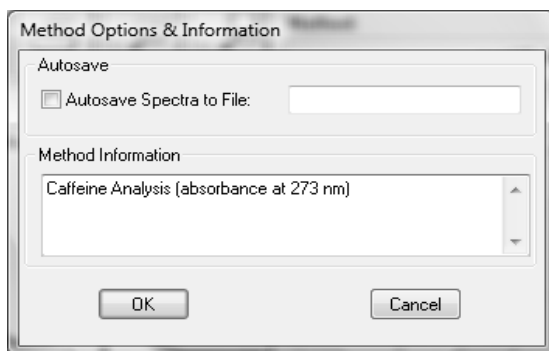


- 2 This displays the Save Method As... dialog box. Type the method name in the File name field, for example, Caffeine.m. Click OK to save your method.



## NOTE

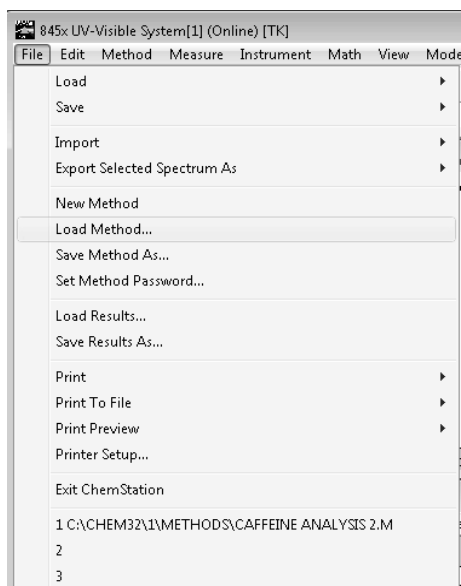
If you generate many methods, you can use Options & Infos... from the Method menu to add text for documentation purposes and to simplify method access. This displays the Method Options & Information dialog box. In the Method Information section you can enter a descriptive text which becomes part of your method.



## Retrieving and Printing a Method

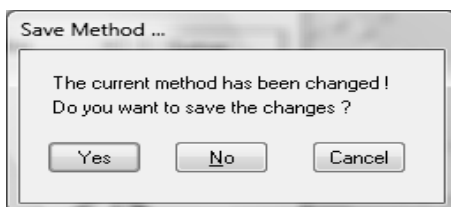
This section describes how to access methods and print a method report.

- 1 Choose Load Method... from the File menu or click the icon in the toolbar.

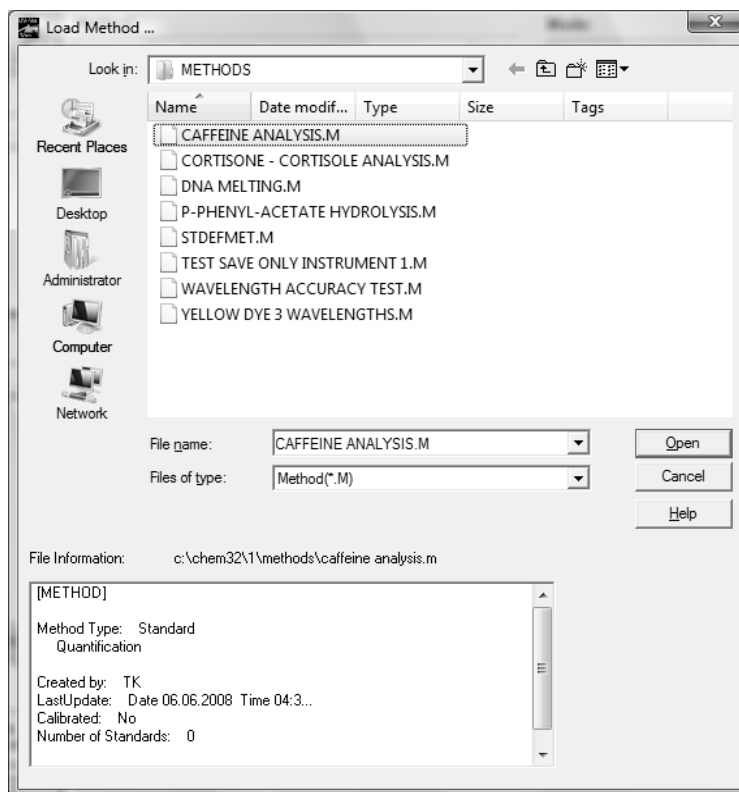


### NOTE

If your current method has been modified, a dialog box will ask you whether you want to save or ignore these changes.



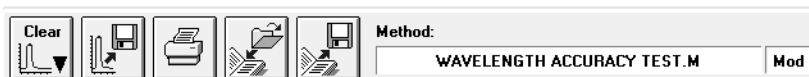
- 2 The Load Method... dialog is displayed. The selected method information is shown in the File Information section of the dialog box.



- 3 If you want to load this method, click OK.

#### NOTE

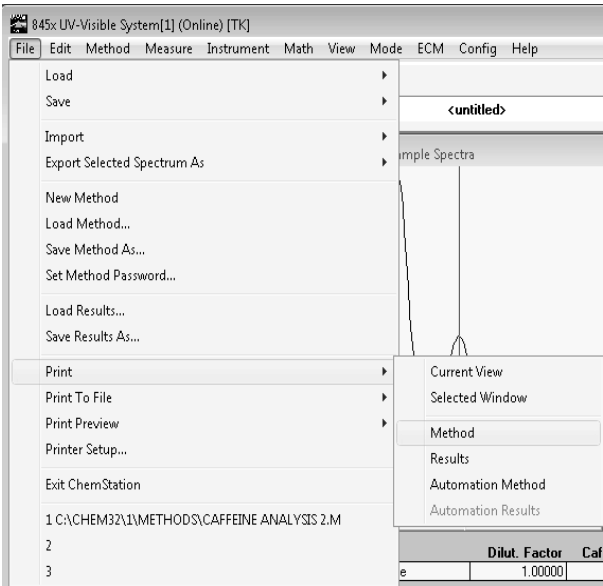
Whenever you change a parameter of the current method, an indication is displayed in the toolbar's modification field.



This triggers the reminder dialog mentioned above.

**5 Using your UV-Visible Spectroscopy System**  
**Retrieving and Printing a Method**

**4** To print a method, choose Print, Method from the File menu.



**NOTE**

To print your method report, your printer must be properly configured and online. If your printer is currently not online, you can view the print preview on the screen.

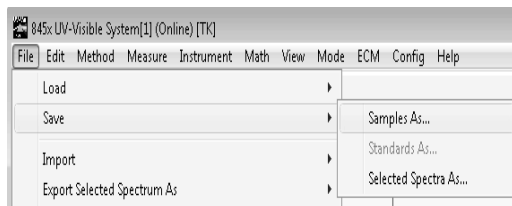
## Saving and Retrieving Data

This section explains how to save and retrieve measured data. This data can be used for archiving, for method development at a later stage, or for exchange with other Agilent ChemStations.

Your Agilent ChemStation can store and retrieve your data using a binary, checksum-protected data format (extension \*.sd, \*.std). All current spectra -samples or standards- can be saved to disk for permanent storage. Saving and loading of data is possible using local and network drives. In addition a single spectrum can be selected for storage.

### Saving your Samples

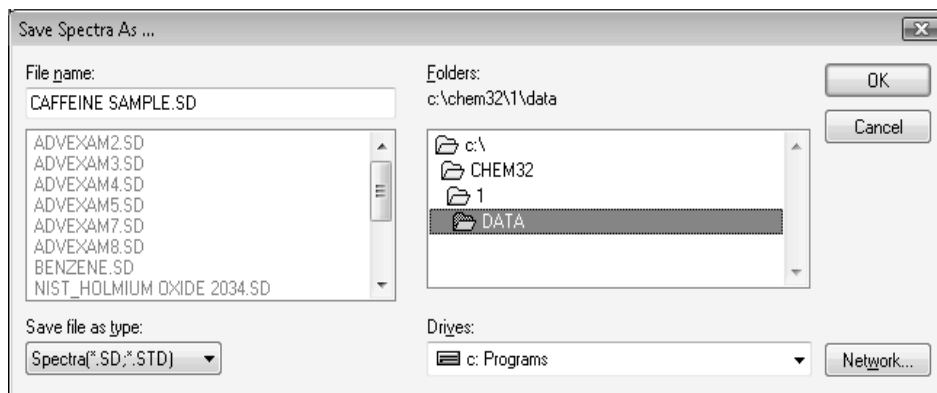
- 1 Choose Save, Samples As... from the File menu or click the toolbar icon.



## 5 Using your UV-Visible Spectroscopy System

### Saving and Retrieving Data

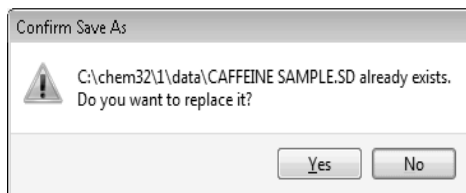
- 2 Select a data files in the File name selection box of the Save Spectra As... dialog or type a valid file name into the File name edit box.



#### NOTE

A valid file name consist of eight alphanumeric characters and the file extension .sd or .std. Usually the extension .std is used for standards only.

If the file name exists, a message box is displayed allowing you to abort or continue with the operation.

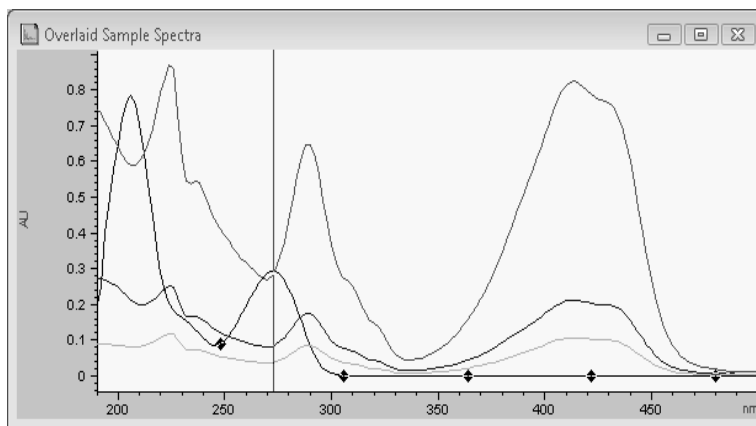


- 3 Click OK to start the operation.



## Saving a Selected Spectrum

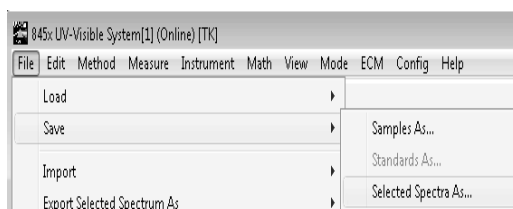
- 1 Select the spectrum of interest in the graphic window.



Or, in the tabular Sample/Results Table window.

| Sample/Result Table |          |               |                |                        |
|---------------------|----------|---------------|----------------|------------------------|
| Show Sample Info    |          | Last Spectrum |                | Delete Selected Sample |
| #                   | Name     | Dilut. Factor | Caffeine(mg/L) | Abs<273nm>             |
| 1                   | Caffeine | 1.00000       | 0.28903        | 0.57807                |

- 2 Choose Save, Selected Spectra As... from the File menu.



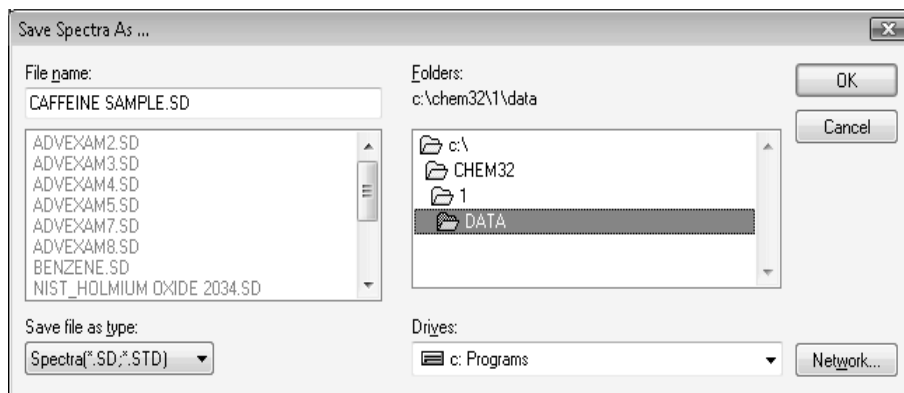
### NOTE

If you did not select either the Overlaid Sample Spectra window or the Sample/Results Table window, you may get the warning, Select/activate a window!, on the message line.

- 3 Select a data files in the File name selector box of the Save Spectra As... dialog or enter a valid file name into the File name edit box.

## 5 Using your UV-Visible Spectroscopy System

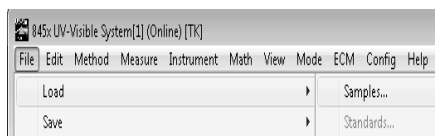
### Saving and Retrieving Data



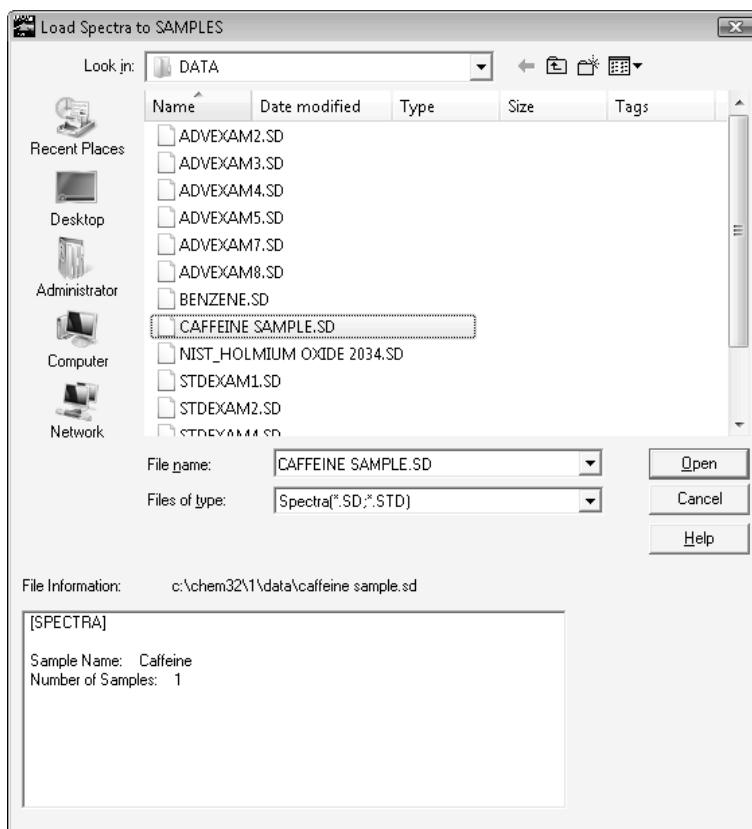
4 Press OK to start the operation.

## Retrieving Spectra

- 1 Choose Load, Samples... from the File menu.



- 2 Select the data file in the File name selector box of the Load Spectra to SAMPLES dialog box.



## 5 Using your UV-Visible Spectroscopy System

### Saving and Retrieving Data

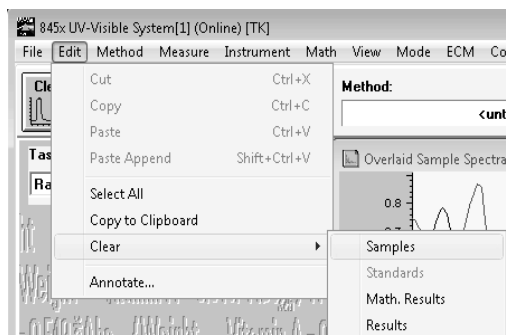
#### NOTE

Select a file in the file selector box to view file information for that file.

- 3 Click OK to start the operation. The spectra available with your data file are added to the files currently in the Agilent ChemStation sample container.

## Deleting Current Spectra

- 1 Choose Clear, Samples from the Edit menu or click the toolbar icon.



#### NOTE

Clear, Standards and Clear, Math. Results can be used to delete the current standards and the current mathematical results spectra from the Agilent ChemStation memory.

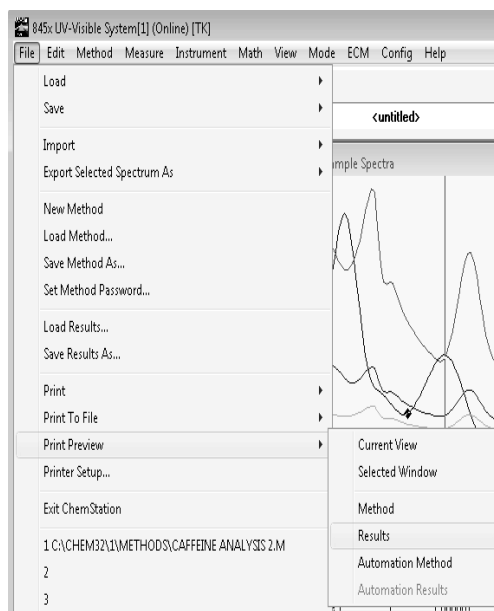
## Print Preview of Reports

Print preview allows you to view the report in a separate Agilent ChemStation window based on the currently configured printer. All types of available reports can be checked page by page in the preview window. The number of pages generated and the layout also can be checked. In addition the currently displayed report can be printed.

### Print Preview of a Results Report

The print previews work similarly for all available types of reports. The example below shows you how to preview your results report.

- 1 Choose Print Preview, Results from the File menu.



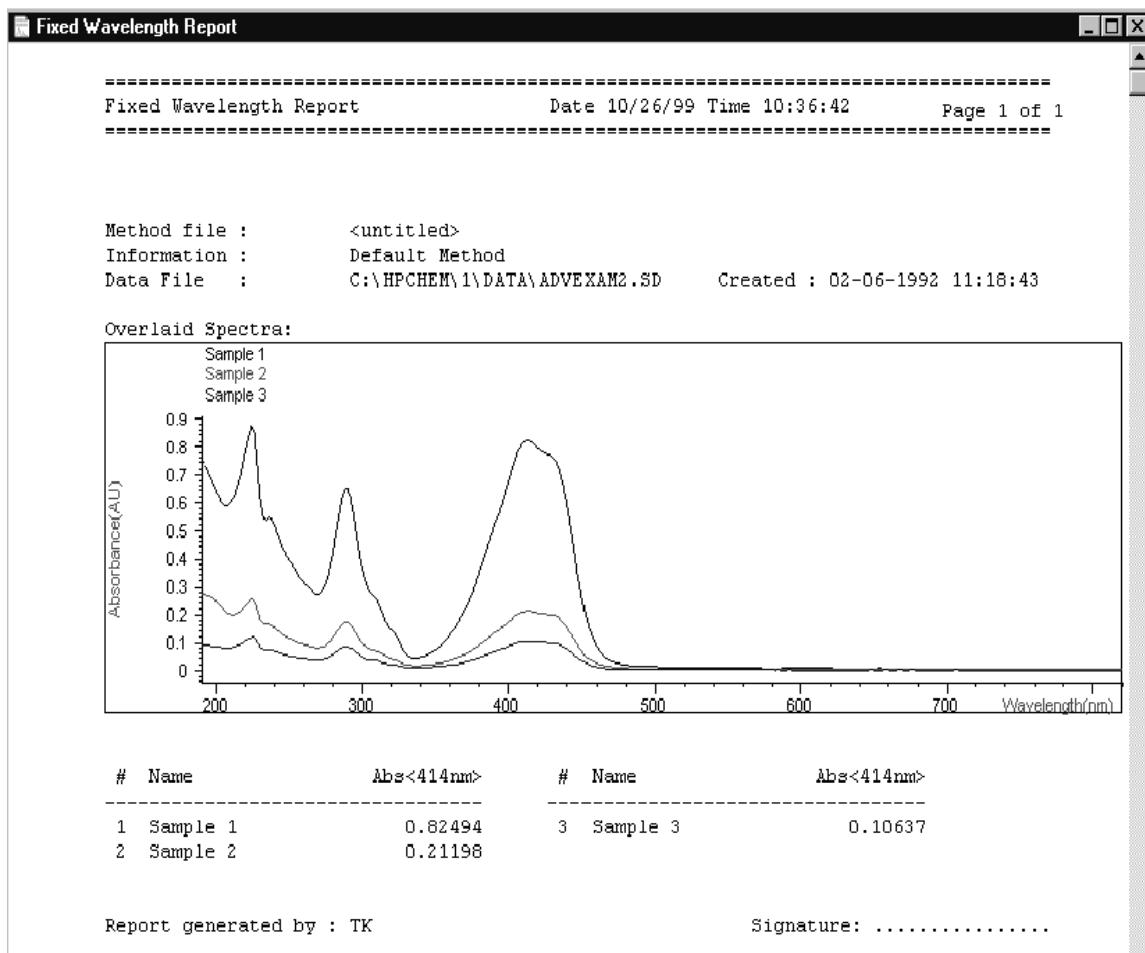
#### NOTE

Printing a results report requires you to have set all parameters properly and have data available for evaluation. If you do not have data, you may get the message *No results present!* on the message line.

## 5 Using your UV-Visible Spectroscopy System

### Print Preview of Reports

- 2 The report generated will be displayed in the preview window.



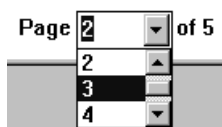
This window allows you to look at your report page by page. Scroll bars are available if a page does not fit into the actual preview window.

In addition, you may use a different size for your preview display. Three sizes are available with the size selection box. Depending on your display resolution select the one which best fits your needs.



The following functions are available with the print preview window:

- Browse through the report pages with the Prev and Next buttons.
- Jump to a page directly using the selection box.



- The Print button sends the report to the printer, which is displayed in the lower left corner of the print preview window.

HP LaserJet 5L (PCL) on LPT1:  
A4 210 x 297 mm/Portrait

- The Close button closes your print preview window and discards the report.

## Finding the Caffeine Absorbance Maximum

This section describes how you find the absorbance maximum for your caffeine IQ sample.

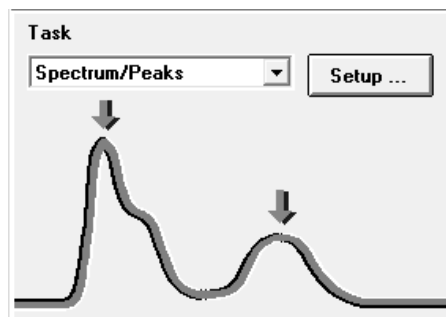
- 1 Make sure that you are in the Standard mode. The mode is indicated on the tool bar of your Agilent ChemStation session.



- 2 Select the Spectrum/Peaks task in the analysis panel's selection box.



- 3 Use the Setup button of the analysis panel to open the parameter dialog.





- 4 Type 2 for the number of peaks to find and uncheck the Valley find option. Set your data type to absorbance, and adjust your spectral display to a wavelength range from 190 nm to 400 nm in the dialog's Display spectrum section. Click OK to set your parameters.

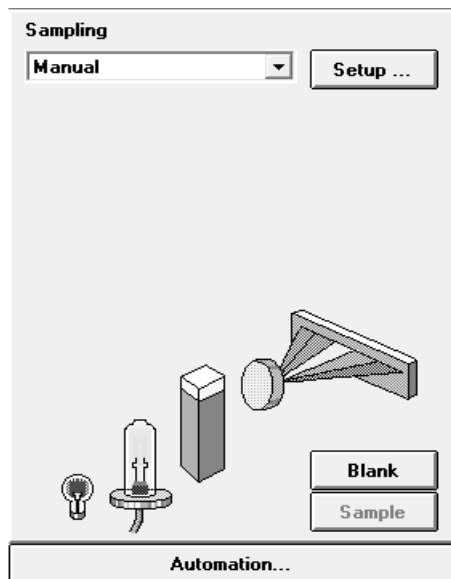
The image shows a software dialog box titled "Spectrum/Peaks Parameters". It contains several settings for spectral analysis. Under the "Peak/Valley find" section, the option "Find and annotate up to" is checked and set to 2 peaks, while "Find and annotate up to" is unchecked and set to 3 valleys. There is also an unchecked option "Prompt for sample information". The "Data type" is set to "Absorbance" in a dropdown menu. The "Display spectrum" section shows a wavelength range from 190 nm to 400 nm. At the bottom, there are "OK" and "Cancel" buttons.

- 5 Fill your 1-cm path-length quartz cell with distilled water. Lift the lever at the left side of your cell holder. Put the cell in the cell holder and make sure the transparent windows face towards the front and the back of the spectrophotometer. Push the lever down to secure your cell in the cell holder.

## 5 Using your UV-Visible Spectroscopy System

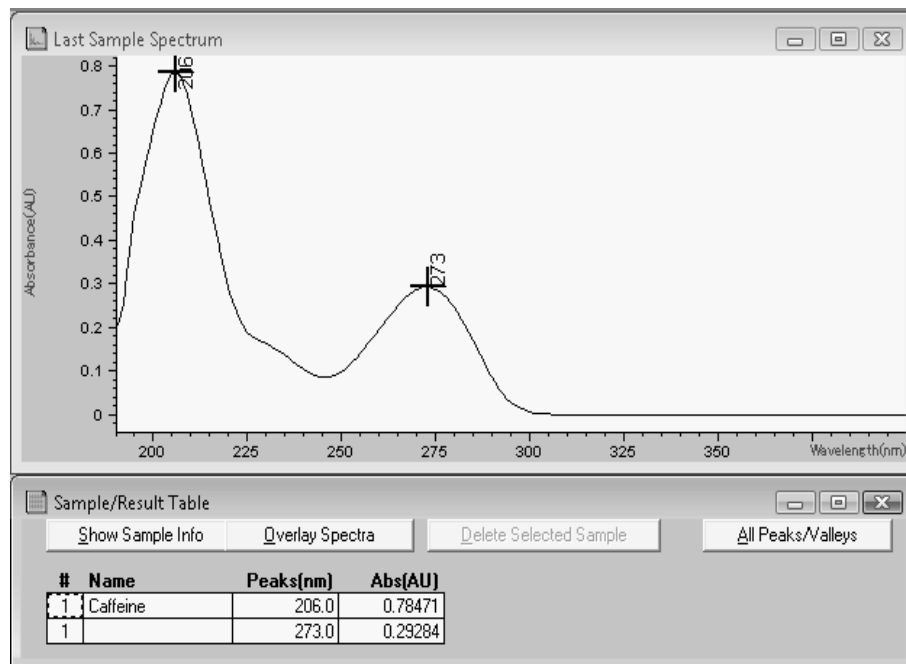
### Finding the Caffeine Absorbance Maximum

- 6 Press the Blank button on the front of the spectrophotometer or click Blank on the Instrument Panel to start the measurement.



- 7 Remember the orientation of the cell in the cell holder. Lift the level to release the cell, remove it and flush it three times with about 1 ml of your caffeine sample. Then fill the cell with about 3 ml of the caffeine sample. Make sure that the cell windows are clean, and reposition the cell in the same orientation as for the reference measurement. Close the cell holder's level.
- 8 Press the Sample button on the front of your spectrophotometer or click Sample on the Instrument Panel to start the measurement.

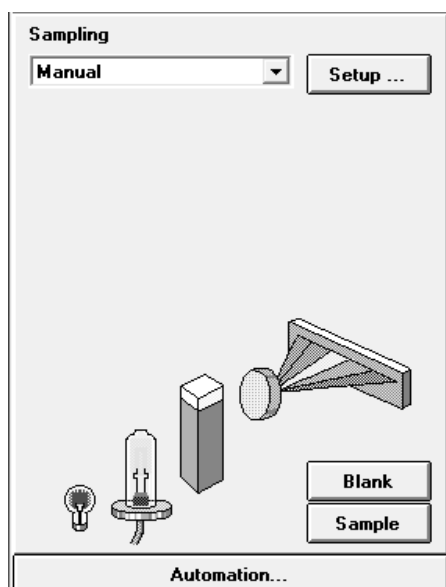
- 9 The view shows you the spectrum of your caffeine sample. Two peaks were found and are annotated with the wavelength. Below the spectrum graph the Sample/Result Table shows the wavelength of the peaks found and the measured absorbance values.



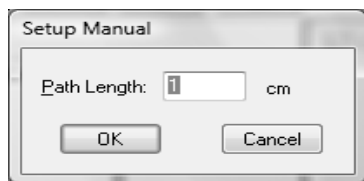
## Entering your Cell's Path Length

The cells used for your measurements are specified with the sampling system parameters. In quantitative calculations, these parameters are used in results calculations. You must provide the correct value for the path length setting. Usually you get this information from the supplier of your cells. You set the path length in manual mode cell handling as follows.

- 1 Click Setup on the Instrument Panel.



- 2 Type the path length in cm in the Setup Manual dialog box.



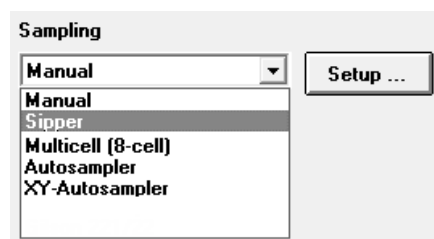
- 3 Click OK to set the specified path length.

## Controlling your Sipper System

A sipper system transfers your sample by means of a peristaltic pump into a flow cell for the measurement. To control your sipper system through the Agilent ChemStation software, you must adjust your current sampling system for sipper introduction.

In addition, due to the length of tubing, the dead volume of your flow cell and the flow rate of your pump, you must adjust your sipper system parameters. For details see the *Installing and Operating Your Sipper System* manual.

- 1 Select the Sipper in the Instrument Panel selection box.



- 2 Click Setup on the Instrument Panel. Type the path length of your flow cell in cm and click OK.



- 3 Click Setup again, and access the Sipper Parameter dialog by clicking Parameter. The parameters required can be determined using the Flow Test task of your Verification and Diagnostics mode.

## 5 Using your UV-Visible Spectroscopy System

### Controlling your Sipper System

Sipper Parameter

|            |    |   |                 |    |   |
|------------|----|---|-----------------|----|---|
| Pump Time: | 20 | s | Pump Direction: | CW |   |
| Wait Time: | 3  | s | Sample Return:  | 0  | % |
| Wash Time: | 0  | s | Air Segment:    | 0  | s |

OK Cancel

- 4 Click OK in the Sipper Parameter dialog and click OK in the Sipper dialog to set the parameters.

#### NOTE

Every measurement you start by clicking one of the Instrument Panel measurement buttons or by pressing the spectrophotometer buttons uses the sipper for sample introduction. Sipper introduction is also used by an automated sequence.

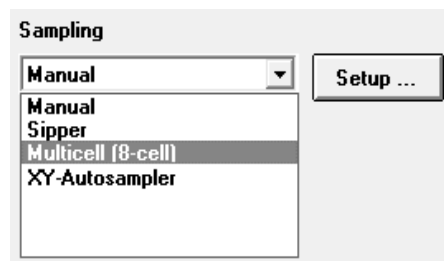
## Using your Multicell Transport

The multicell transport is a cell changer which allows you to position up to 8 cells in the measurement position automatically. You may use different cells in each measurement position. The path length can be specified individually for each of the cell positions.

### NOTE

For details of your multicell transport see the *Installing and Operating Your Multicell Transport* manual.

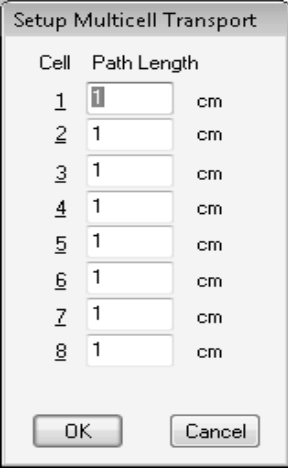
- 1 Select Multicell (8-cell) in the Instrument Panel selection box.



- 2 Click Setup on the Instrument Panel. Type the path lengths of all cells used in cm and click OK.

## 5 Using your UV-Visible Spectroscopy System

### Using your Multicell Transport



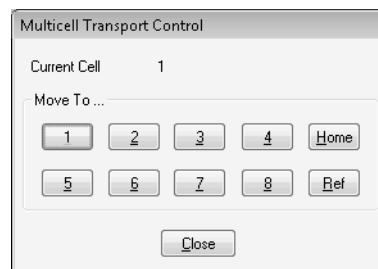
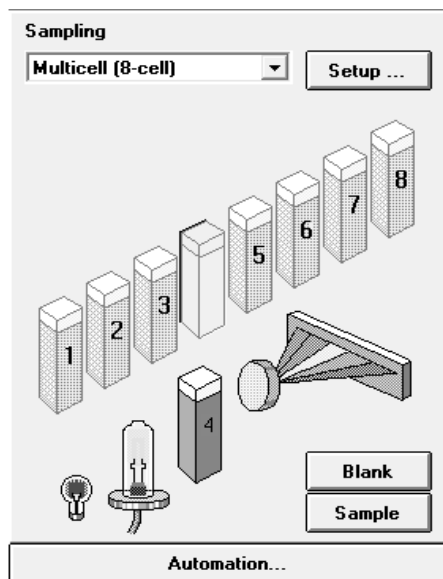
The dialog box is titled "Setup Multicell Transport". It contains a table with two columns: "Cell" and "Path Length". The "Cell" column lists cells 1 through 8. The "Path Length" column shows a dropdown menu for cell 1 (currently set to 1) and text boxes for cells 2 through 8, all followed by "cm". At the bottom are "OK" and "Cancel" buttons.

| Cell | Path Length |
|------|-------------|
| 1    | 1 cm        |
| 2    | 1 cm        |
| 3    | 1 cm        |
| 4    | 1 cm        |
| 5    | 1 cm        |
| 6    | 1 cm        |
| 7    | 1 cm        |
| 8    | 1 cm        |

OK Cancel

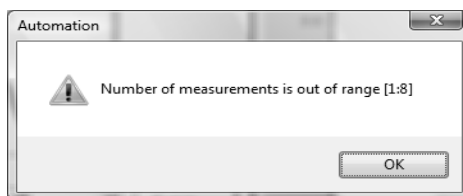
- 3 To move your cell for the next measurement into the measurement position, click the cell on the Instrument Panel or choose Multicell Transport Position from the Spectrophotometer menu to access the Multicell transport Control dialog. In the Multicell Transport Control, press one of the position buttons to move the multicell transport.





## NOTE

In an automated sequence, the multicell transport can be used for automatic sample introduction. A maximum of 8 samples can be introduced. If you specify more than 8 measurements this warning is displayed:



## **Quantitative Analysis using a Calibration with Standards**

Your quantitative analysis task is based on a calibration with standards. After a successful calibration the measured standards can become part of your method. Such a method can be used directly for quantitative analysis of samples.

After having set up your method, calibrated samples can be analyzed. Several views of both your standards and the calibration, as well as of your samples and results are available.

As a quick introduction, a calibration using Beer's law with a single standard and the analysis of a sample is described. The only limitation on the number of samples and standards is the memory capacity of your Agilent ChemStation.

For the practical experiment we use the IQ caffeine sample as standard and a 1:1 dilution with distilled water as sample. For the calibration we use absorbance data at 273 nm.

## Setup

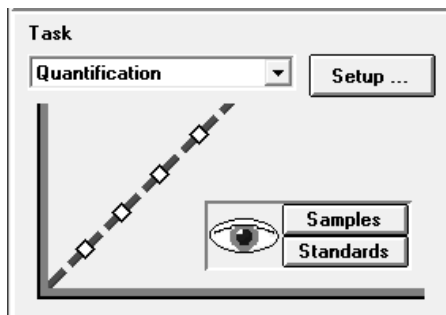
- 1 Make sure that you are in Standard mode. The mode is indicated on the tool bar of your Agilent ChemStation session.



- 2 Select the 'Quantification' task in the analysis panel's selection box.



- 3 A new task panel is displayed and the Quantification Parameters dialog is opened automatically.



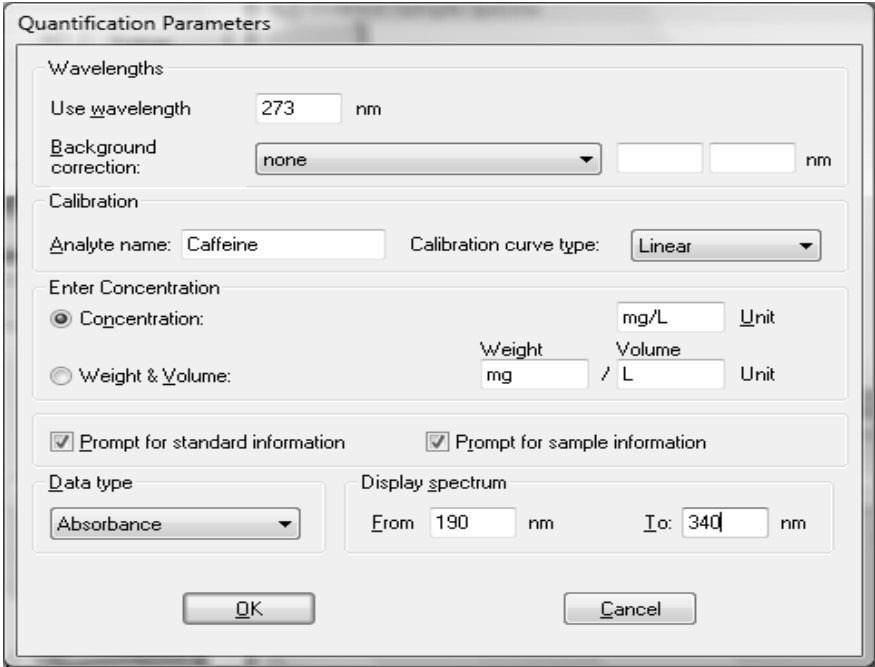
### NOTE

If you are already in the Quantification task, use the Setup button on the analysis panel to open the parameter dialog.

## 5 Using your UV-Visible Spectroscopy System

### Quantitative Analysis using a Calibration with Standards

- 4 Setup your analysis wavelength at 273 nm (Use wavelength), type Caffeine for the Analyte name, set the Calibration curve type to Linear, select Concentration entry and use mg/l as Unit. Check the Prompt for standard information and the Prompt for sample information. Select Absorbance as Data type and set Display spectrum From 190 nm To 340 nm.



The image shows a 'Quantification Parameters' dialog box with the following settings:

- Wavelengths:** Use wavelength: 273 nm
- Background correction:** none
- Calibration:** Analyte name: Caffeine; Calibration curve type: Linear
- Enter Concentration:** Concentration: mg/L Unit
- Prompt for standard information:** ☒
- Prompt for sample information:** ☒
- Data type:** Absorbance
- Display spectrum:** From 190 nm To 340 nm

Buttons: OK, Cancel

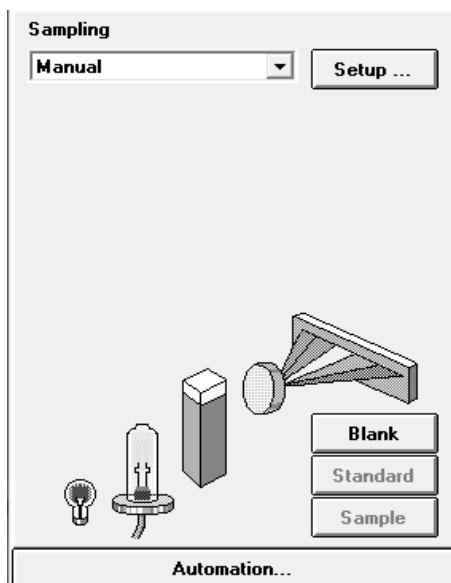
- 5 Click OK to set your parameters.

#### NOTE

Now you are ready to run your measurements.

## Calibration

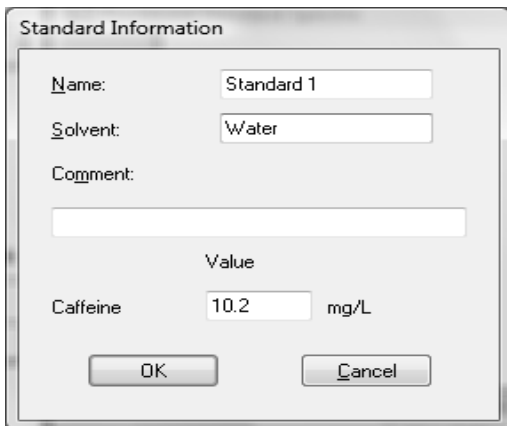
- 1 Fill your 1-cm path-length quartz cell with distilled water. Lift the lever at the left side of your cell holder. Put the cell in the cell holder and make sure the transparent windows face towards the front and the back of the spectrophotometer. Push the lever down to secure your cell in the cell holder.
- 2 Press the Blank button on the front of the spectrophotometer or click Blank on the Instrument Panel to start the measurement.



- 3 Remember the orientation of the cell in the cell holder. Lift the level to release the cell, remove it and flush it three times with about 1 ml of your caffeine sample. Then fill the cell with about 3 ml of the caffeine sample. Make sure that the cell windows are clean and reposition the cell in the same orientation as for the reference measurement. Close the cell holder's level.
- 4 Press the Standard button on the front of your spectrophotometer, or click Standard on the Instrument Panel to start the measurement.

## 5 Using your UV-Visible Spectroscopy System

### Quantitative Analysis using a Calibration with Standards

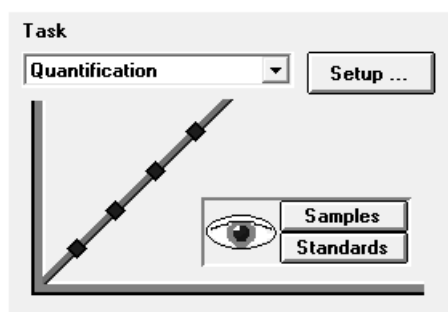


A dialog box titled "Standard Information" with the following fields and controls:

- Name:** Standard 1
- Solvent:** Water
- Comment:** (empty text box)
- Value:** (empty text box)
- Caffeine:** 10.2 mg/L
- Buttons:** OK, Cancel

- 5 Enter your standard's information in the Standard Information dialog box and click OK.

Your Agilent ChemStation software automatically calibrates and displays the calibration results. After a successful calibration the task panel's calibration curve shows green. This indicates that your method is ready for analysis.



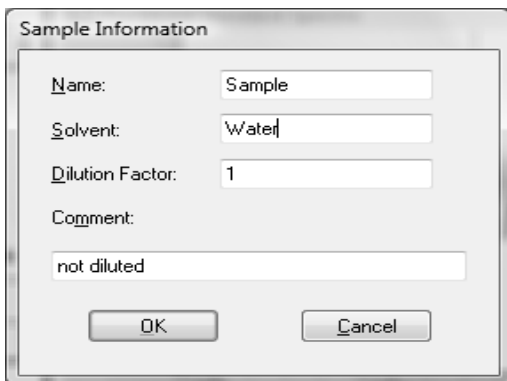
#### NOTE

The Task panel's Samples and Standards buttons can be used to switch your current view to the samples or standards view.

At this point you can also save your method for future use. See "Saving Your Parameters as a Method" on page 74 for more information.

## Analysis

- 1 Remember the orientation of the cell in the cell holder. Lift the level to release the cell, remove it and flush it three times with about 1 ml of your caffeine sample (1:1 dilution with distilled water). Then fill the cell with about 3 ml of the caffeine sample. Make sure that the cell windows are clean and reposition the cell in the same orientation as for the standard measurement. Close the cell holder's level.
- 2 Press the Sample button on the front of your spectrophotometer or click Sample on the Instrument Panel to start the measurement.

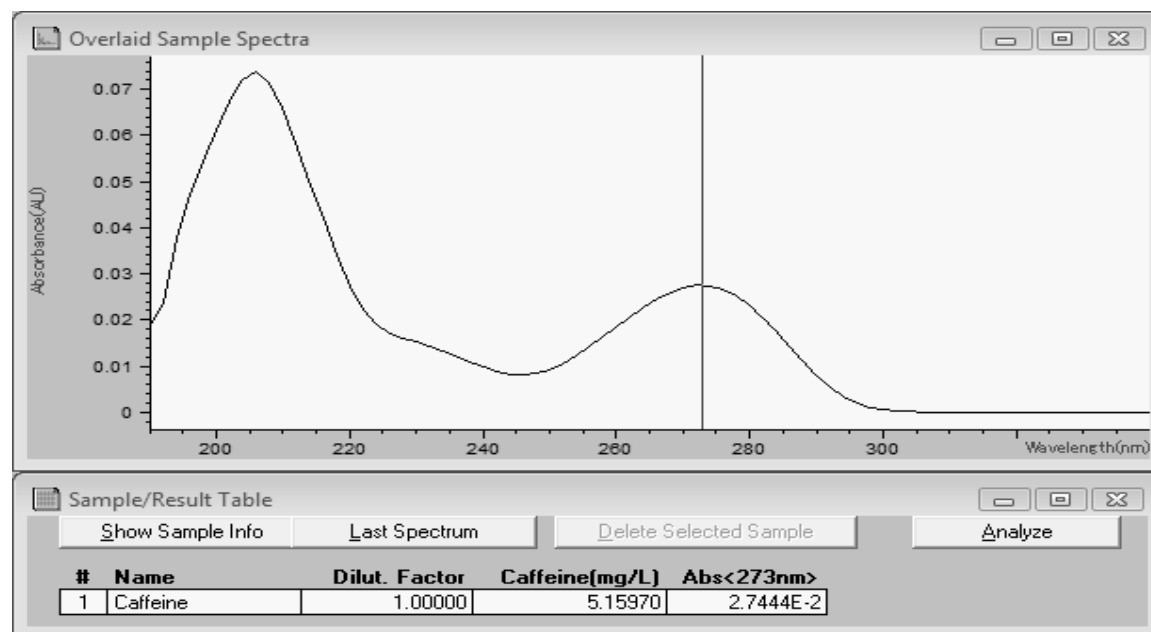


A screenshot of a 'Sample Information' dialog box. It contains four labeled text input fields: 'Name' with the value 'Sample', 'Solvent' with the value 'Water', 'Dilution Factor' with the value '1', and 'Comment' with the value 'not diluted'. At the bottom of the dialog are two buttons: 'OK' and 'Cancel'.

- 3 Enter your sample's information in the Sample Information dialog box and click OK. The view switches to the samples and your quantitative results will be displayed with the Sample/Result table.

## 5 Using your UV-Visible Spectroscopy System

### Quantitative Analysis using a Calibration with Standards



#### NOTE

To save your data for future use or for documentation purposes, see "Saving and Retrieving Data" on page 79 for more information.



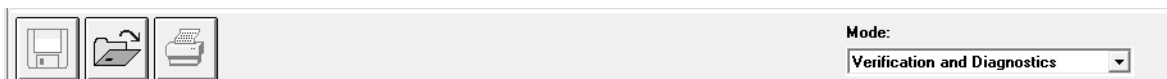
## How Can I Be Sure That My Spectrophotometer Works Properly?

The quality of your measurement data is dependent on the performance of your spectrophotometer. For a full performance verification external standards are required. Standard kits are available for operational qualification and performance verification (OQ/PV). OQ/PV kits for the Agilent Cary 8454 UV-Visible spectrophotometer are available as spare parts.

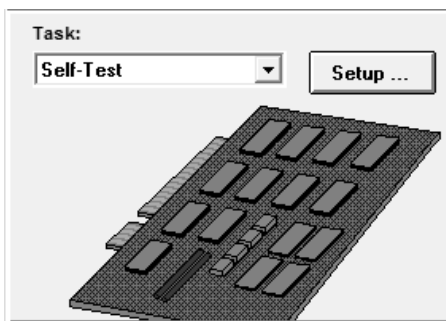
A quick check without the need of standards can be performed by the Verification and Diagnostics mode's self test. This test can be performed always after starting the spectrophotometer.

### Spectrophotometer Self test

- 1 Make sure that you are in the Verification and Diagnostics mode. The mode is indicated on the tool bar of your Agilent ChemStation session.



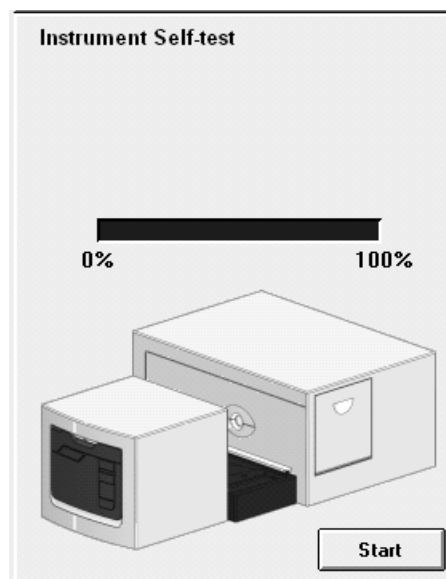
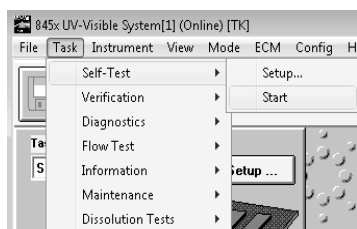
- 2 Select the Self-Test task in the analysis panel's selection box.



## 5 Using your UV-Visible Spectroscopy System

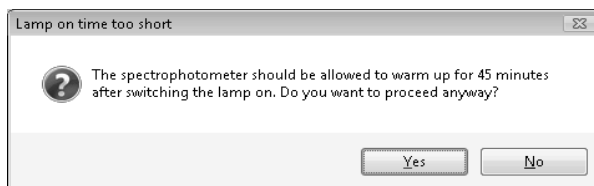
### How Can I Be Sure That My Spectrophotometer Works Properly?

- 3 Choose Self-Test > Start from the Task menu or click Start to start the self-test.



#### NOTE

The spectrophotometer should be in stable working conditions before you initiate the test. If these conditions are not met, you may get a warning message.



- 4 The self-test results are displayed with pass/fail criteria.

#### NOTE

Self-test results can be stored with the spectrophotometer. Storing the self-test results with the spectrophotometer allows you to monitor the performance over time. Graphical representations of the self-test histories can be generated.

## What Maintenance Does My Spectrophotometer Require?

### Cleaning

Any spills should be wiped up immediately.

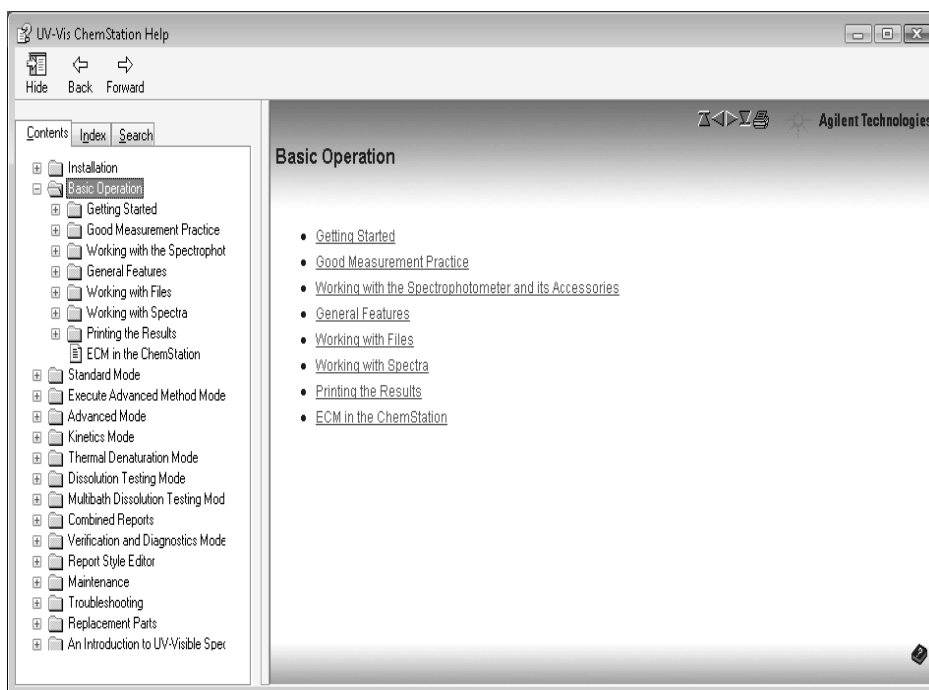
The exterior surfaces of the spectrophotometer should be kept clean. All cleaning should be done with a soft cloth. If necessary, this cloth can be dampened with water or with a mild detergent. Do not use organic solvents or abrasive cleaning products.

### Spare Parts

For spare parts and consumables ordering information, refer to the Agilent Technologies website at [www.agilent.com](http://www.agilent.com).

## How Can I Get a Deeper Understanding of UV-Visible Spectroscopy?

The basic principles of UV-Visible spectroscopy are covered with your help system. This covers Basic Principles through to details of Derivative Spectroscopy.



In addition, solutions to selected topics in UV-Visible analysis are described. There you can find help on topics such as Enhancing Sensitivity or Determining Purity.

More specific examples, including data, are available in the Examples section of the Standard Mode help. These can be used to run your Agilent ChemStation software for the special purpose described.



## **When Do I Have to Measure a Blank?**

The measurement data acquired by your spectrophotometer is instrument independent. To achieve this independence, a reference measurement must be performed. All consecutive measurements refer to the last measured reference.

In the Agilent ChemStation software, the reference measurement is combined with a baseline measurement. The baseline displayed is information about the quality of the current reference. In absorbance mode, the data should be close to 0 AU and in transmission mode the data should be close to 100%.

Typically, reference measurements for dissolved samples are made on the cell filled with the solvent used. The absorbance properties of the cell and the solvent also influence the reference data. In wavelength ranges where your solvent or cell is absorbing, the noise of the baseline spectrum is high. Reliable sample data cannot be expected in these areas.

Therefore new reference or blank measurements are required when:

- you change your measurement cell or its orientation relative to the measurement position.
- you use a different solvent or even a different lot of the same solvent.
- the time between your reference measurement and the sample measurement get too long.
- the time conditions are depending on the aging rate of your lamps and possible changes of your environmental conditions—typically the last blank measurement should not be older than half an hour.

To ensure the highest accuracy of sample measurement, the blank measurement should be taken immediately prior to the actual sample measurement.

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## **In This Book**

To be able to use your new Agilent Cary 8454 UV-Visible spectroscopy system quickly, this book gives you step-wise procedures and examples for basic operations and tasks.

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