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Flow
VPE

$m = \frac{A}{l} = \alpha c$

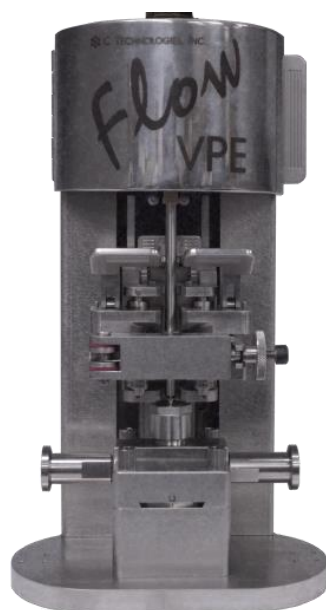
$A = \alpha l c$
 $y = m x + b$

Slope
spectroscopy

$m = \frac{A}{l} = \alpha c$

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C TECHNOLOGIES, INC.
BRIDGEWATER, NEW JERSEY



FlowVPE User Manual

*Flow*
VPE

User Manual

Revision 01

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1 Introduction

1.1 Congratulations on the purchase of your new FlowVPE

The FlowVPE (pr. flō vē-pē-ē) is a unique and powerful instrument that will help you and your lab save time and money by providing real-time concentration measurements for in-line product processing and manufacturing. This manual will help introduce you to the technology and serve as a reference for use. It is recommended that it be kept close to the system to serve as a training document and reference resource.

1.2 Manual Conventions

The following conventions have been used throughout this manual:

- Menus, on-screen controls and user interface elements are typed in bold face, for example “click **Done**” or “from the Edit menu choose **Copy**”.
- All CAPITAL LETTERS indicates keyboard commands, for example, “Press ENTER” or “Press CTRL+F6”



- This graphical symbol indicates that more detailed information is available on the specific topic in the documentation that is provided with the Cary Spectrophotometer, the Fiber Optic Coupler or the system computer.



- This graphic symbol indicates a Key Concept or Capability



- This graphic symbol indicates a useful tool or tip.



- This graphic symbol indicates a useful step by step procedure or work instructions.



- This graphic symbol indicates the need for verification or close inspection.



- This graphic symbol indicates a secured feature.

1.3 Definitions

There are a number of terms, definitions and naming conventions that are helpful to know and understand. This section of the manual will provide an overview of these concepts and serve as a handy quick reference.

- **Absorbance** – A percentage of total transmitted light that is lost or consumed by the solution being measured. Total Absorbance contains the percent of light lost due to Scatter as well, and must be corrected depending on the composition of the solution through which Absorbance is being measured.
- **Absorbance Plateau** – A 2-dimensional data set created from Pathlength versus Wavelength data at a specific (constant) Absorbance level. Generally obtained by taking a slice from a Variable Pathlength Surface.
- **ADL** – Application Development Language is a powerful programming language provided with the Cary WinUV software suite. It can be used to customize existing programs that work with Agilent spectrophotometers and your FlowVPE as well as to develop custom applications.
- **ADL Shell** – The ADL Shell is program template provided with the Cary WinUV software suite. It provides the basic environment and common functionality associated with all of the Cary WinUV applications. It is the platform on which customer applications can be developed for use with Agilent’s line of spectrophotometers and is the foundation of the FlowVPE control Software.
- **Averaging Time** – The Cary spectrophotometer setting that configures how long the system will collect data at each wavelength. Configured in seconds, this number corresponds to a number of pulses of the Xenon flash lamp. One pulse corresponds to 1/80th of a second or 0.0125 seconds, therefore each second is 80 pulses of the lamp.
- **Baseline Measurement** – The beginning point, based on an evaluation of output over a period of time, used to determine the process parameters prior to any improvement effort; the basis against which change is measured.
- **Beer-Lambert Law** – The law of physics which describes the proportional relationship between absorbance, Pathlength and concentration.
- **Continuum** – the name used to describe a collection of X-Y data points stored in the software or computer memory. An example of a Continuum is a spectral curve or Spectrum which is a series of points defined by a wavelength value and an Absorbance value.
- **CSV File** – A comma delimited text file that can be used to transfer Continuum data between different applications. Most frequently accessed with commonly available spreadsheet software.
- **Database** – This is a compilation of all data stored from FlowVPE testing, when data is configured to be exported.
- **Delivery Fiber** – The Fiber Optic cable that brings the light from the Cary spectrophotometer to the Flow Head.

- **Detector Module** – A removable unit that contains the Optical Detector for the FlowVPE. A dovetail top surface mates with the Flow Cell to lock the two together before use.
- **Discrete Mode** – A configuration mode that can be applied to either the Cary spectrophotometer or the FlowVPE unit. In this configuration, Absorbance data will be collected at up to ten individual wavelengths specified by the user.
- **Extinction Coefficient** – An intrinsic property of a substance, described by a numerical value which quantifies the propensity of a substance to absorb electromagnetic radiation at a specific wavelength. It is one of the terms in the Beer-Lambert Law.
- **Fiber Optic Coupler** – Directs light from the Cary 60 into the Delivery Fiber, for transmission to the FlowVPE Instrument.
- **Fibrette Loading Position** – The position the FlowVPE system drives to in order to load the Flow Cell and Fibrette. Clicking **Load Controls** in the software will take the FlowVPE to this position.
- **Fibrette Flange** – The part of the Flow Fibrette that is physically clamped in the Flow Fibrette Clamp.
- **Fixed Slope** – A user-defined slope measurement parameter consisting of max Pathlength, step size and wavelength.
- **Flow Cell** – The interface between the FlowVPE and the process flow to be measured. Creates a seal around the Flow Fibrette to create an enclosed flow path. Comes in 3mm and 10mm flow diameter varieties.
- **Flow Cell Window** – The bottom surface of the Flow Cell which allows light to pass through to the Detector Module.
- **Flow Fibrette** – The component of the FlowVPE system that delivers light to the sample. Its position in the solution determines the measurement Pathlength as the Flow Head moves it up and down in the Flow Cell. There is a different Flow Fibrette for the 10 mm Flow Cell and the 3 mm Flow Cell.
- **Flow Head** – Naming convention that indicates the hardware of the FlowVPE system, not including the Flow Cell and Detector Module.
- **Flow Validation Cuvette Adapter (FVCA)** – An optional accessory that works exclusively with FlowVPE Variable Pathlength spectrophotometer system. The FVCA was developed to provide an additional technique for validating the FlowVPE device; specifically allowing the use of filters and standards based on a cuvette form factor.
- **Home Position** – This is the fully raised position of the FlowVPE motion control system.
- **KF** – The abbreviation used for the “Fixed Slope Kinetics” FlowVPE program.
- **KQ** – The abbreviation used for the “Quick Slope Kinetics” FlowVPE program.
- **Load** – The entire process of mounting Flow Cell and Flow Fibrette hardware into the Flow Head, and the corresponding user interaction with the software.
- **Mount** – The physical interaction of the user with the Flow Head during the Flow Cell and Fibrette loading process.
- **Optical Detector** – The component of the Flow Head that captures the light output from the Flow Cell. Located in the Detector Module.

- **Pathlength** – The distance the measured light travels through the sample when making absorbance spectroscopy measurements based upon the Beer-Lambert Law. In the FlowVPE, this distance, generally expressed in millimeters, is defined by the physical gap between the bottom of the Flow Cell containing the solution and the tip of the Flow Fibrette that is submerged in the sample.
- **R² Value** – Coefficient of determination. A statistical value, between 0.0 and 1.0, that indicates the quality of the linear relationship between two variables in a data set. An R² value near 1.0 indicates a near perfect linear relationship between the data. In Slope Spectroscopy®, Section data is regressed and this value is used to identify the level of compliance with the Beer-Lambert Law within Pathlength regions.
- **Scanning Mode** – A configuration mode that can be applied to either the Cary spectrophotometer or the FlowVPE unit. For the Cary spectrophotometer, Scanning Mode allows the collection of spectral data across a range of wavelengths, based upon a user specified starting wavelength, stopping wavelength and wavelength Step Size or Data Interval. For the FlowVPE, Scanning Mode allows the collection of Absorbance information across a range of Pathlengths based upon a user specified starting Pathlength, stopping Pathlength and Pathlength Step Size.
- **Scatter Correction** – An Absorbance value correction that subtracts out Absorbance contributions associated with the molecular interaction with the incident light or particle dispersion.
- **Section Plot** – An Absorbance versus Pathlength graph that is generated in real time as data acquisition is occurring, and displayed in the FlowVPE software window.
- **Slope Inquiry** – A Variable Pathlength software utility that rapidly creates Section data using the FlowVPE system. By making measurements at various Pathlengths, the utility searches for the linear range of the sample, in compliance with the Beer-Lambert Law. Once found, Section data (Absorbance vs. Pathlength) is collected, and a linear regression is performed on the data set to determine the optimized slope value which can then be used for quantitation.
- **Slope Spectroscopy®** – An analytical technique based upon the Beer-Lambert Law which utilizes the slope term of a statistically analyzed Absorbance vs. Pathlength plot to make calculations and predictions of sample properties.
- **Special Characters** – There are a number of special characters that the FlowVPE software uses and these should be avoided in sample names. The special characters are explained as follows:
 - “{” – Open Bracket designates the beginning of the sample name.
 - “}” – Closed Bracket designates the end of the sample name.
 - “@” – The “at” symbol indicates the specific or Pathlength or wavelength at which the Continuum was acquired or constructed.
 - “,” – Used to separate fields in a comma delimited file.
- **Spectrum / Spectra** – In absorbance spectroscopy, an X-Y dataset(s) that is made up of absorbance data as a function of wavelength at a fixed Pathlength.
- **Step Size (Data Interval)** – The distance between adjacent Pathlength measurements.

- **Threshold** – a user specified limit specified in Absorbance units that the FlowVPE system will scan the Pathlength range to find, if possible.
- **Trace** – a Continuum that is currently displayed or plotted on a graph as a curve.
- **Variable Pathlength Surface** – a 3-dimensional surface composed of Absorbance, Pathlength and Wavelength Data. Orthogonal slices of the surface will allow the display of *Section* (Abs vs. Pathlength), *Spectra* (Abs vs. Wavelength) and *Absorbance Plateau* (Wavelength vs. Pathlength) datasets.
- **Vessel Holder** – an accessory to the FlowVPE that allows a vessel to be mounted into the path of light for absorbance readings through a standard or sample dispensed into a vessel.

1.4 Data Naming Conventions

Each of the different Continua (trace/curve) that the FlowVPE software generates has specific naming conventions. Table 1-1 describes each data naming convention and type and provides examples in order to make it easier to interpret the curve types.

Table 1-1: Data Naming Conventions

Program	Continuum Type	Example	Notes
KQ	Section	PLXQ{Sample[#]}@500.00nm	# = Current Cycle
	Threshold Search	THXQ{Sample[#]}@500.00nm	# = Current Cycle
	Baseline	BLXQ{Sample}@500.00nm	
	Concentration	KQC{Sample}@500.00nm	
	Slope	KQS{Sample}@500.00nm	
	R-Squared	KQR2{Sample}@500.00nm	
	User Result	KQUR{Sample}@500.00nm	
	Scan Section	KQSx{Sample}@500.00nm	
KF	Section	Plxf#{Sample}[#]@500.00nm	# = Configuration Index (1-5) # = Current Cycle
	Scan	ssf#{Sample}[#]@500.00nm	# = Configuration Index (1-5) # = Current Cycle
	Baseline	Blxf{Sample}@500.00nm	
	Concentration	KC#{Sample}@500.00nm	# = Configuration Index (1-5)
	Slope	KM#{Sample}@500.00nm	# = Configuration Index (1-5)
	R-Squared	KR2#{Sample}@500.00nm	# = Configuration Index (1-5)
	Absorbance	KA#<500.00>{Sample}@0.750mm	# = Configuration Index (1-5)
	User Result	KUR#{Sample}	# = Configuration Index (1-5)

1.5 File Types

There are a number of different files types that the Cary WinUV Software and the FlowVPE software create and use. Table 1-2 below summarizes the most commonly used file types:

Table 1-2: Commonly Used File Types

<i>File Type</i>	<i>Extension</i>	<i>Description</i>
<i>Method</i>	*.MKF *.MKQ	A specific type of Cary WinUV computer file that contains a complete snapshot of the measurement parameters and hardware and software configurations of the FlowVPE System which can be secured, transported, backed up, recalled and applied as a quick and easy way to prepare the FlowVPE system for use.
<i>Data</i>	*.DKF *.DKQ	Data files save only the data.
<i>Report</i>	*.RKF *.RKQ	Report files save only the report.
<i>Batch</i>	*.BKF *.BKQ	A specific type of Cary WinUV computer file that contains a comprehensive record of FlowVPE System activity including method information, data, graphic information, report content, security information and audit trails.
<i>ASCII – Comma Delimited</i>	*.CSV	Text file format useful for transferring data between applications.
<i>RTF</i>	*.RTF	Text file format that includes some simple formatting of fonts.
<i>Regression</i>	*.CSV	A data file that summarizes the FlowVPE regression results on a dataset.
<i>ADL</i>	*.ADL	A program file created with the Application Development Language.

2 System Overview



This chapter provides a brief introduction to the FlowVPE hardware system and the software environment from which the FlowVPE hardware is controlled. It explains the interoperability of the FlowVPE hardware and the Agilent Technologies' Cary 60 hardware as well as the relationship between the FlowVPE software and the Agilent Cary WinUV software. It highlights the power, capabilities and benefits of the unique system structure and provides tips on how to maximize the utility of the FlowVPE system.

2.1 C Technologies, Inc. or Agilent Technologies?

Agilent Technologies is a world leader in the design and manufacture of laboratory instruments, as well as many other scientific and medical devices. C Technologies, Inc. designs and manufactures various optical instruments, devices and accessories including the SoloVPE and FlowVPE. C Technologies is the sole designer and provider of the FlowVPE hardware, and leverages the capabilities of Agilent's Cary 60 in order to deliver the power of the Variable Pathlength technology for real-time concentration measurements.

2.2 FlowVPE System Hardware

The FlowVPE system hardware is composed of a number of individual components that are integrated together to enable the Variable Pathlength technology. The primary components of the system are the spectrophotometer engine, which serves as the light source and detection platform, and the FlowVPE

device, or Flow Head, along with the Flow Cell, Flow Fibrette and Detector Module. The current FlowVPE system ships exclusively with the Agilent Cary 60 UV-Vis Spectrophotometer.

The FlowVPE device is the key component that enables the Variable Pathlength technology. Much like its predecessor, it is an extension of the spectrophotometer and serves as the in-line material measurement device which allows dynamic adjusts of the optical Pathlength between 0.005 mm (5 microns) and 8.000 mm. Acting as an optical bridge, the fiber optic coupler redirects the beam of the spectrophotometer into the FlowVPE. The final component is the computer that runs the software that integrates the hardware control, data acquisition, analysis and reporting. These hardware components work seamlessly together to create the Variable Pathlength platform that enables accurate measurements of various processes, including UDF, capturing elution peaks, and more.



Figure 2-1: FlowVPE and Cary 60 Engine

2.2.1 The Engine: Agilent Technologies' Cary 60



The FlowVPE has been designed to function as an extension of the Agilent Technologies Cary 60 UV-Vis spectrophotometer. The FlowVPE seamlessly integrates the Agilent Cary 60 detection and control technology creating a powerful and robust implementation of C Technologies Inc.'s Variable Pathlength technology. All power and motion control to the Flow Head and transmission data from the Flow Head is transferred in the single connector at the back of the Flow Head. The only remaining interconnect is the light delivering optical fiber which connects to the optical coupler inside of the sample compartment of the Cary 60. See Section 3 for more details on the interconnections between the Flow Head and the Cary 60.

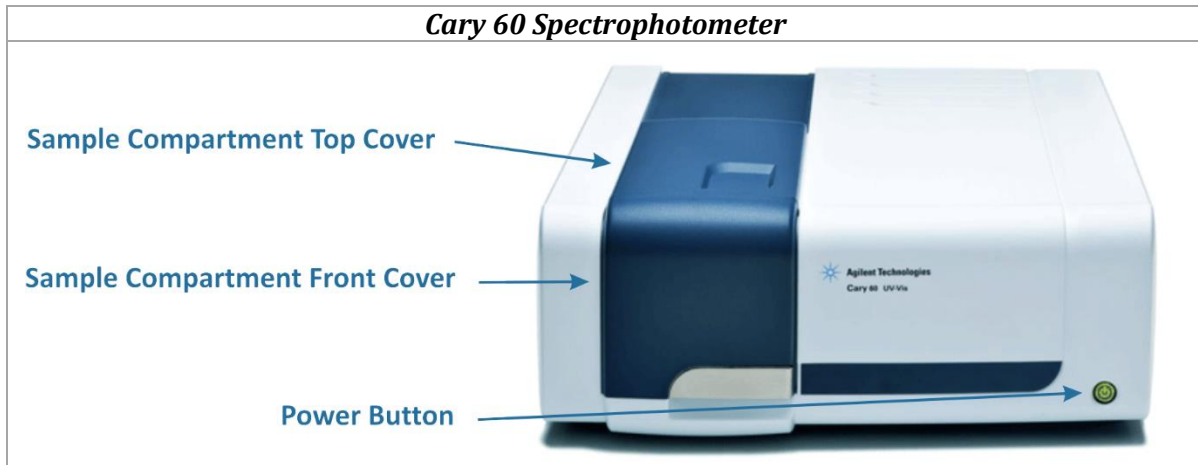


Figure 2-2: Cary 60 Spectrophotometer with callouts

2.2.2 The Extension: The FlowVPE Hardware

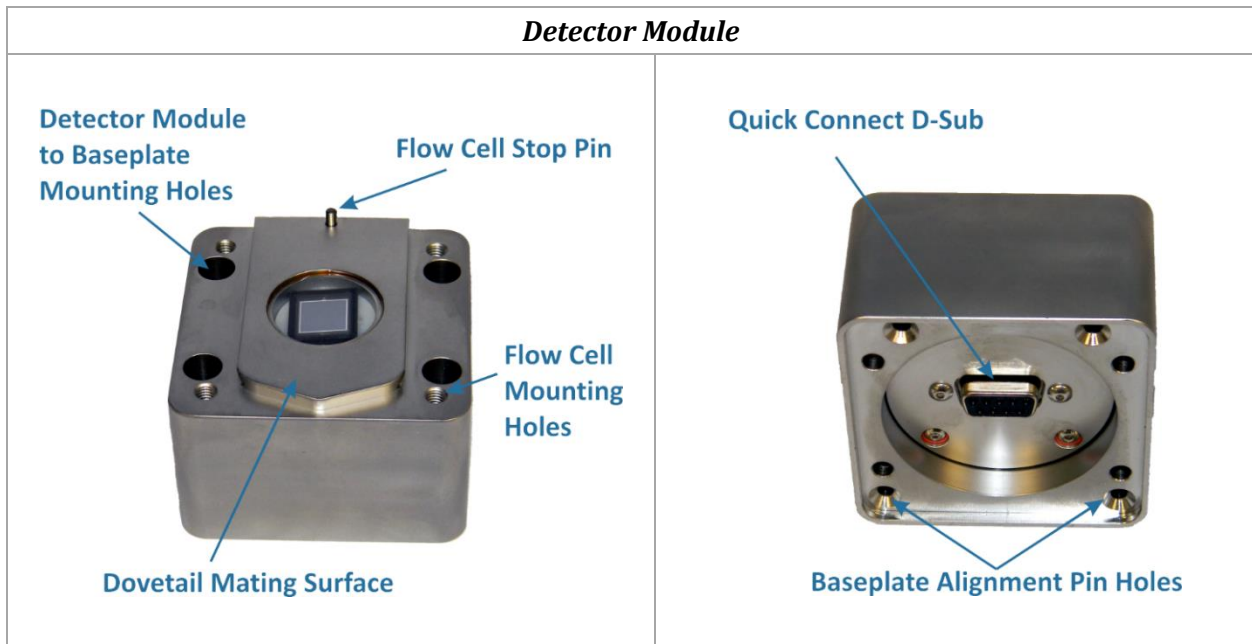
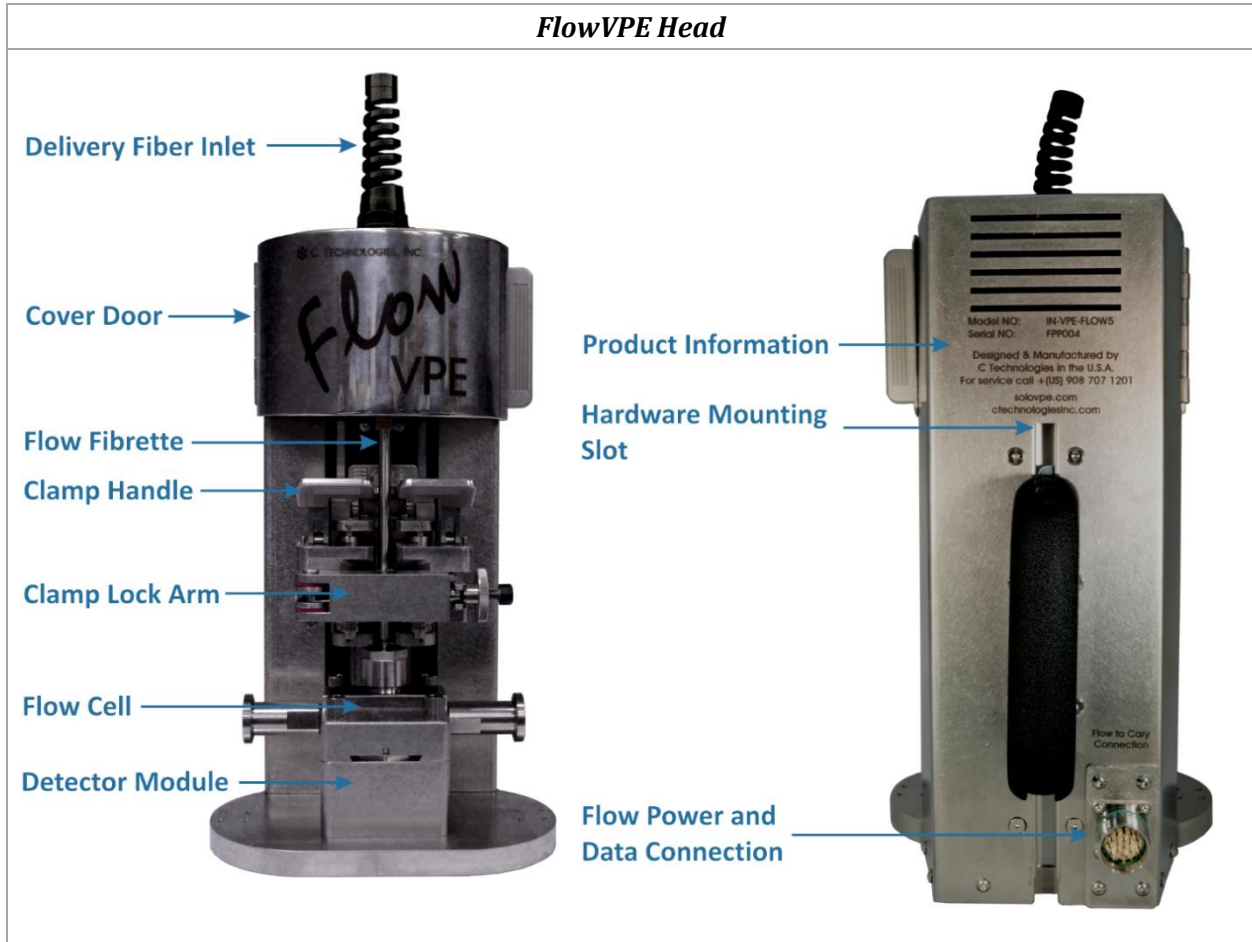


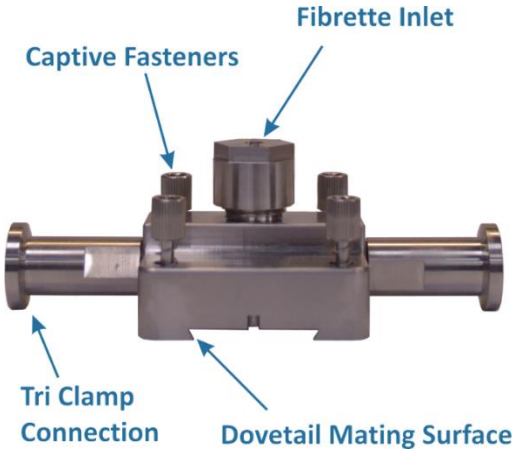
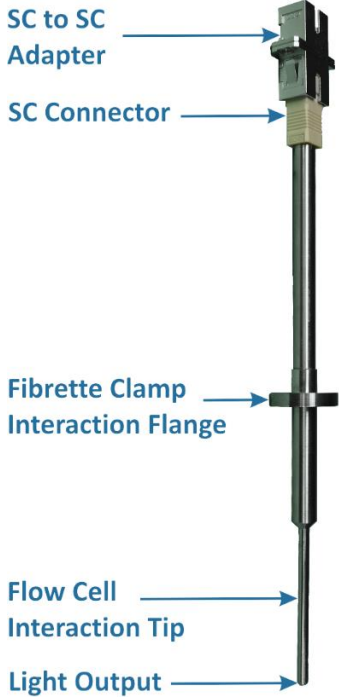
Like the SoloVPE, the FlowVPE device is one-of-a-kind in the field of in-line concentration measurements, and the only product available that allows Variable Pathlength measurements for process flow. The FlowVPE uses the Slope Spectroscopy® measurement technique for real-time sample measurements, saving time and money in process development.

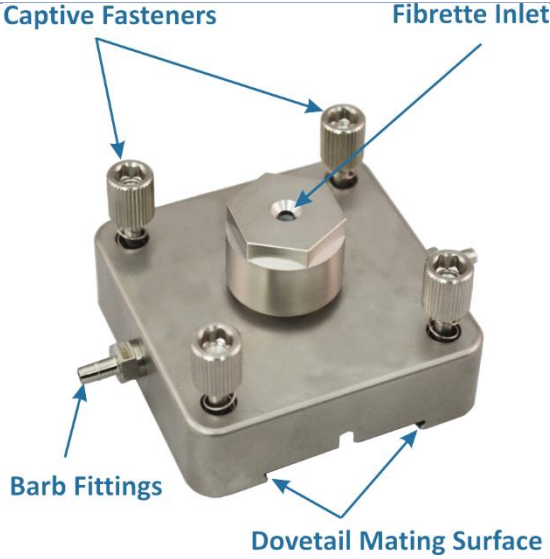
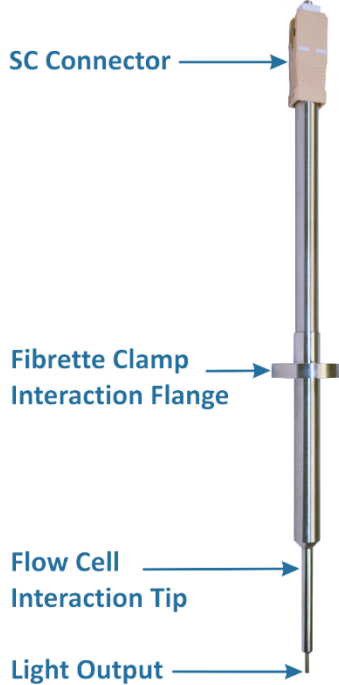
As a user it is important to familiarize yourself with the key components and features of the FlowVPE device. The following pictorial dictionary can be used as a resource during training and when working with the system.



Table 2-1: FlowVPE Device Pictorial Dictionary



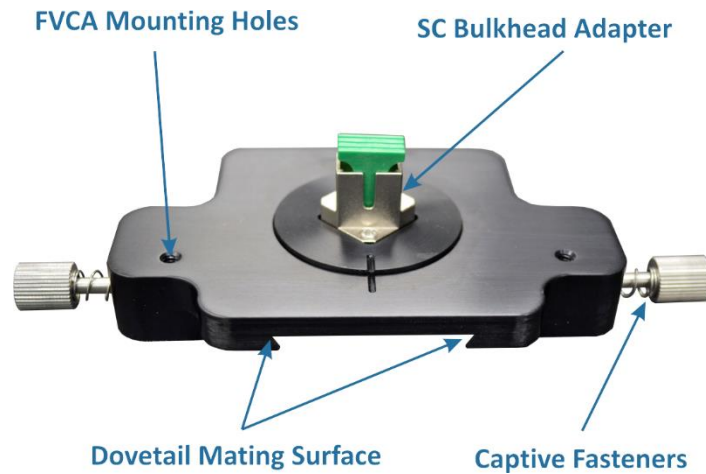
10 mm Flow Cell	10 mm Flow Fibrette
 <p>Captive Fasteners Fibrette Inlet Tri Clamp Connection Dovetail Mating Surface</p>	 <p>SC to SC Adapter SC Connector Fibrette Clamp Interaction Flange Flow Cell Interaction Tip Light Output</p>

3 mm Flow Cell	3 mm Flow Fibrette
 <p>Captive Fasteners Fibrette Inlet Barb Fittings Dovetail Mating Surface</p>	 <p>SC Connector Fibrette Clamp Interaction Flange Flow Cell Interaction Tip Light Output</p>

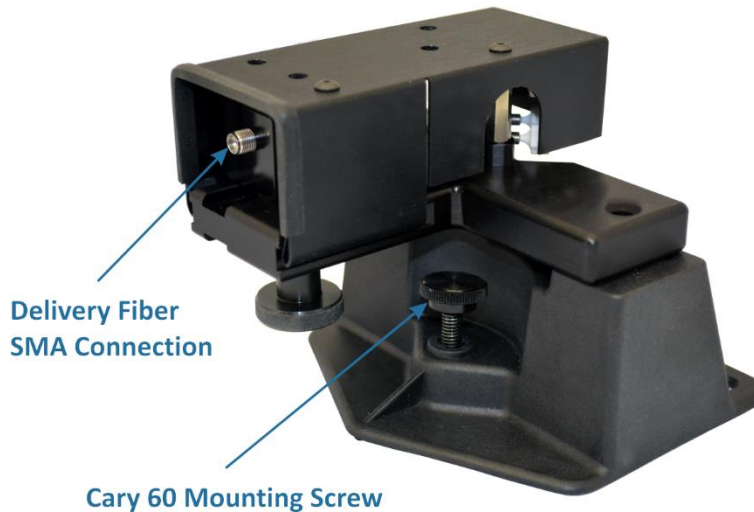
FlowVPE Vessel Holder



Transmission Tool



FlowVPE Fiber Optic Coupler



2.2.3 Detector Module

The FlowVPE detector has been designed to be modular, meaning it can be easily removed and installed into the Flow Head for detector validation using the FVCA and for cleaning. The Detector Module is fitted with a 9 pin D-Sub connector that plugs directly into a D-Sub in the Flow Head baseplate.



The Detector Module comes preinstalled into the Flow Head with blue tape covering the window. This blue tape must be removed before operation of the Flow System. For details on removal and reinstallation of the Detector Module, see Chapter 7.

2.2.4 Flow Cell and Flow Fibrette

The FlowVPE Fibrette has been specifically designed to fit into the Flow Cell that allows vertical motion to take continuous slope measurements. The Flow Cell has been designed not only to accept the Flow Fibrette and create a seal around it, but also to allow continuous flow through the Cell without leaking or contamination of the process fluid, as well as to allow light to pass through to the detector for absorbance readings.

2.2.5 Flow Fibrette Clamp

This subassembly of the FlowVPE is intricately designed to automatically set the Flow Fibrette to its zero position each time the clamp is operated. This works by the top plate of the clamp pushing the Flow Fibrette down into the Flow Cell until the tip is touching the window of the Flow Cell. At this point, continuing motion of the clamp brings the bottom plate up to meet the Flow Fibrette Flange and

sandwich it between the top and bottom plates of the Fibrette Clamp. The handle will toggle into place when the Flange is clamped, and an auto-locking mechanism will fall into place behind the handle. After the Flange is clamped in place, a secondary lock is implemented to ensure that the zero position does not change during operation of the Flow Head. This is done by rotating the Lock Arm into place, and tightening the thumb nut on the Lock Arm to a hard stop. This puts a strong locking force onto the shafts of the Flow Fibrette clamp, maintaining the set Zero Position. See Section 4.2 for detailed loading instructions and pictures.



WARNING: The Fibrette Clamp controls the Loading Position, and must be operated strictly as this manual details. Deviation may result in inaccurate measurements or damage to the FlowVPE hardware.



2.2.6 Cover and Cover Door

The cover of the Flow Head serves as a protection for the internal components, as well as support for the Delivery Fiber. The top of the cover holds a strain relief boot where the Delivery Fiber is inserted, and the underside of this boot throughput is accessed by opening the door of the cover. Once the Delivery Fiber is connected, the door can remain closed to protect the fiber as the Flow Head is being operated.

2.2.7 FlowVPE Vessel Holder

The FlowVPE Vessel Holder mounts directly to the Detector Module, and can be used to acquire absorbance readings for standards that can be dispensed into a vessel, thus verifying the proper functionality of the FlowVPE hardware, specifically the Fibrette Clamp, Lock and linear motion of the Flow Head. It loads into the Flow Head in the same fashion as the Flow Cell and Fibrette (See Section 4.2 for details).

2.2.8 FlowVPE Transmission Tool

The Flow Transmission Tool mounts directly to the Detector Module and is used to take transmission readings directly from the Delivery Fiber. This is used to get a reading of the overall system transmission, verifying the optical path is clear before introducing the Flow Fibrette and Flow Cell. See Section 7.2.4 for instructions on using the Transmission Tool.

2.2.9 FlowVPE Validation Cuvette Adapter (FVCA) *[Optional Accessory]*

The FVCA is an optional accessory (sold separately) that works exclusively with the FlowVPE Variable Pathlength spectrophotometer system, and is used to validate the function of the spectrophotometer and FlowVPE using USP and EP filter and liquid standards. See DOC0063 for usage instructions, or contact C Technologies for more information.

2.2.10 The Bridge: The FlowVPE Fiber Optic Coupler

The fiber optic coupler functions as an optical bridge by intercepting the beam of light from inside the Cary instrument and coupling it into an optical fiber that brings it to the FlowVPE and the sample itself. Leveraging the remote detection capability of the Cary 60, the absorbance response is acquired by a remote detector position within the Detector Module, directly below the Flow Cell.

2.2.11 The Brain: The FlowVPE System Computer

The brain of the FlowVPE is the computer that runs the software that controls all aspects of configuration, data acquisition, analysis and reporting. The software environment is the Agilent Cary WinUV software that ships with the Cary instruments. Integrated into the software package is a development language that was used to create the FlowVPE Software, which is a single user interface for controlling all aspects of the FlowVPE system.

2.2.12 The FlowVPE Accessory Kit

The FlowVPE Accessory Kit is included in every FlowVPE system purchase. It includes the required tools to attach and remove the Detector Module from the Flow Head baseplate and to tighten the captive fasteners in the Flow Cell onto the Detector Module. Additionally, this is where the FlowVPE Vessel Holder with a spare Flow Fibrette and sample vessels to be used with the Vessel Holders is kept. The Accessory Kit also contains the SC to SC adapter needed to connect the Delivery Fiber to the Flow Fibrette for use, and the FlowVPE control cable that connects the FlowVPE to the Cary 60.

2.2.13 FlowVPE Data Acquisition Hardware *[Optional Accessory]*

The FlowVPE system has been designed to allow for integration of data acquisition hardware that is available for purchase from C Technologies. There is great flexibility with the DAQ hardware to be set up and configured as needed by the user. The FlowVPE software has been written to include an Analog Output signal configuration that works in conjunction with this DAQ hardware. See DOC0064 for installation instructions, or contact C Technologies for more information.

2.3 FlowVPE System Software

2.3.1 Agilent Technologies Cary WinUV Software



The Agilent Cary 60 spectrophotometers are purchased with the Cary WinUV software suite, which is a collection of programs designed to work with the spectrophotometer. The Cary WinUV suite controls the whole family of Agilent Technologies spectrophotometers. It includes a variety of applications designed to address specific types of UV/Vis measurements. The Agilent Cary WinUV software manual explains the types of measurements and studies that can be performed using the programs provided.

2.3.2 ADL and the ADL Shell Application



Included with the Cary WinUV software suite is powerful programming language called the “Applications Development Language” or “A-D-L” for short. The programming language is similar to Visual Basic, but has been specifically adapted for spectroscopic applications and the Agilent line of UV/Vis/NIR instruments. It provides control of the instrument and the software environment allowing customization of Cary WinUV applications or creation of new applications designed to meet a user’s specific requirements.

The Cary WinUV Suite comes with an application called the ADL Shell. The ADL Shell program is a predesigned software environment template upon which custom applications can be written using ADL. The ADL Shell provides the core functionality and interface for the new ADL program to run. The FlowVPE control software takes advantage of the power of ADL and has been designed and tested in the ADL Shell environment. One of the most powerful features of this design is that new programs and applications can be written to your specification that can take advantage of all of the power of the FlowVPE, the Cary 60 and the Cary WinUV software suite. Please contact C Technologies, Inc. for more information.

2.3.3 FlowVPE Control Software Programs

There are two FlowVPE control programs included with the FlowVPE system. The first is dubbed “Quick Slope Kinetics,” or KQ, and the second “Fixed Slope Kinetics,” or KF. Both programs will control the FlowVPE device and the Agilent Cary 60. They were written using the Applications Development Language provided with the Agilent Cary WinUV software suite as described in Section 2.3.2. The KQ control software is specially formulated for tracking all concentration changes during data collection. Inherent in the software is a searching algorithm that takes time to find the linear range of the Slope Spectroscopy[®] equation before measurements. The KF control software is formulated for the user who knows the appropriate measurement Pathlength range to be captured, and therefore can input this directly into the software and achieve more measurements in less time. More details about the differences between KQ and KF are given in Section 6.



3 System Installation

Follow these instructions to quickly and easily setup your FlowVPE system. **Please read and understand these instructions prior to making any connections.** These step by step directions will guide you through the process of making the primary connections required between the computer, the Agilent Cary 60 and the FlowVPE. The basic computer connections (e.g. monitor, mouse, power etc.) are not explained. For those instructions please see the documents that came with that equipment. Depending on whether you purchased a complete FlowVPE System or the FlowVPE Extension for use with your own Agilent Cary 60, some of these steps may have already been completed.

3.1 Basic Installation Instructions

The following steps should be carried out after speaking with a certified C Technologies Representative in order to get confirmation on setting up the system. These instructions do not specify a footprint configuration of the system, as the system is intended for integration into a process line. The end-user can and should customize hardware placement based on the needs of the process line to be utilized.

Table 3-1: FlowVPE Install Instructions

Step 1	Unpack & Verify
	<p>Carefully unpack the equipment and all accessories. Confirm the presence of all items against the checklist included with the shipment to become familiarized with the components and make following this procedure easier.</p> <p><i>Any blue tape found on the components has been placed there for surface protection and should be removed during installation and before use of the Flow VPE.</i> </p> <p>If you have any questions or concerns, be sure to call C Technologies, Inc. or your authorized representative to obtain clarification prior to proceeding.</p>

Step 2



Remove the Cary 60 Access Panel

Using a Phillips head screw driver, remove the Cary 60 *Sample Compartment Access Panel* which is located at the back of the Cary 60 and secured by two Phillips head screws. With the Access Panel removed, reinstall the two screws to prevent them from getting lost.

Step 3



Remove Top and Front Sample Compartment Covers





Step 4

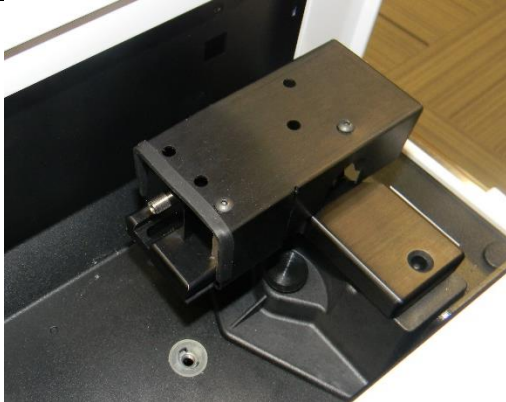



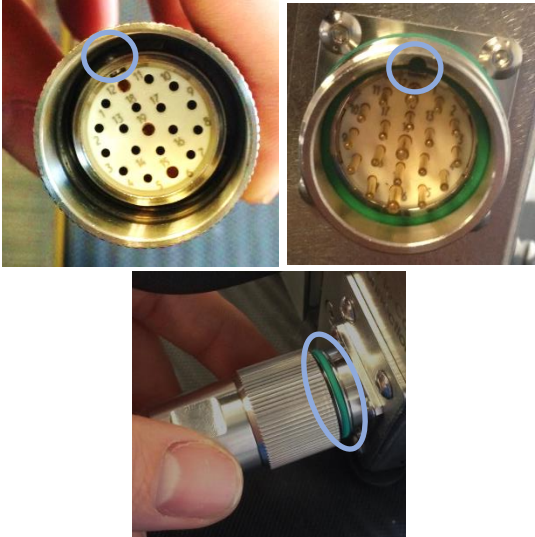
Feed D-Sub and Right Angle Connector into Cary 60

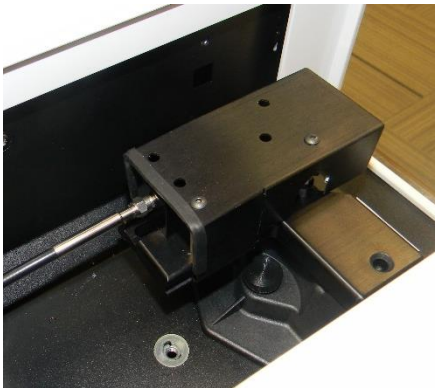

Unwind the Flow to Cary cable and feed the bifurcated end into the back of the Cary 60. This is the end with the D-Sub and right angle connection that will connect to the inside of the Cary 60.


Step 5	Connect the D-Sub Connector
	<p>With the cabling of the D-Sub facing down, push the D-Sub into place on the right side of the Cary sample compartment, just behind the Fiber Optic Coupler. Hand-tighten the D-Sub screws to ensure the connection.</p>


Step 6	Connect Right Angle Connector to the Cary 60
	<p>The connection port is on the left wall of the sample compartment. Orient the connector so the cable is pointed away from you, and push the connector into place.</p>

Step 7	Install the Fiber Optic Coupler into the Cary 60
	<p>The C Technologies, Inc. Fiber Optic Coupler (FOC) is the device that captures the light from the Cary 60 and routes it through the Delivery Fiber. The FOC installs quickly and easily into the Cary 60 Sample Compartment. Two raised pins in the base of the Sample Compartment assure proper alignment. Position the FOC above the pins and lower into place making sure it mates securely. Screw down the coupler with the large thumb nut until it is securely fastened.</p>

Step 8	Connect the FlowVPE
	<p>Slide the large circular connector onto the threaded connection port on the back of the FlowVPE. The connector will slide into place when oriented properly. Look for a ball in the cable connector end, and a round slot in the Flow connector end. Screw in the connector over the green o-ring to secure it in place.</p> 

Step 9	Connect Delivery Fiber to Fiber Optic Coupler
	<p>Feed the SMA end (with hex nut) of the Delivery Fiber into the access port in the back of the Cary.</p> <p>Insert the SMA ferrule into the Fiber Optic Coupler and secure by tightening the hex nut.</p> <p>Note: You will need to follow the alignment procedure in the Fiber Optic Optimization document (DOC0111) in order to maximize the transmission through the Fiber Optic Coupler. </p>

Step 10	Attach Strain Relief
	<p>The FlowVPE is shipped with the Delivery Fiber strain relief packed separately. Attach the strain relief by screwing it onto the mating thread at the top of the FlowVPE.</p>

Step 11	Insert Delivery Fiber Through Strain Relief
	<p>The Delivery Fiber needs to be inserted through the strain relief in order to be properly connected to the Flow Fibrette when the Flow Cell is installed. Feed the SC end (plastic rectangular connector) into the strain relief and let it rest in place.</p>

Step 12	Reinstall Cary 60 Sample Compartment Covers
	

Step 13

Connect the Power Cable to the Cary 60



Connect the power cord that was supplied with the Cary 60 to the back of the Cary 60. The transformer has a separate cord that runs to the wall outlet to allow for the various power connectors around the globe.

Step 14

Connect the USB Cable to the Cary 60




Connect the USB Cable that came with the Cary 60 to the back panel of the Cary 60. Do not connect the opposite end to the computer until directed to do so.


Step 15


Power On the Computer




Press the Power button on the computer to turn it on.

Step 16	Follow the Manual Directions To Install All
	<p>Your system should come with all pertinent software already installed. If it has not been installed, log onto the computer with Administrator privileges and install the Cary WinUV software and FlowVPE software. Each installer is available on their respective disc or USB key.</p>

Step 17	Power On the Cary 60
	<p>With the USB Cable disconnected from the computer, press the Power button at the front of the Cary 60. Power up the Cary 60 and allow it to fully initialize.</p>

Step 18	Allow Cary 60 to Complete Startup Initialization
	<p>When you power on the Cary 60, the hardware will initialize and calibrate. As it is doing this the Power button will light up and flash an Orange color. If the system fails to initialize and calibrate, the Power button will turn Red. When it is successful, the power button will glow steadily green. After pressing the power button to turn on the Cary 60, wait for it to turn green. <u>Note: If it fails to startup successfully, turn it off and turn it on again.</u></p>

Step 19	Connect the USB Cable to the Computer
	<p>With the Computer and Cary 60 powered up, make the final connection of the USB Cable to the Computer. The computer will audibly acknowledge that a USB Device was connected and automatically detect and install the drivers for the Cary 60.</p>

Congratulations!

Your FlowVPE System Should Be Ready For Use!

4 FlowVPE Hardware Operation

The FlowVPE has been designed for a powerful application of the Slope Spectroscopy® technology in an in-line processing environment. To ensure accurate measurements, it is important to become familiar and comfortable with handling the various components of the system as well as correctly following the loading and unloading procedures outlined in this chapter. The following chapter details these procedures, as well as the setup required for the FlowVPE hardware prior to the loading procedure.

4.1 FlowVPE Pre-Load Setup

All of the items in this section must be completed before the Loading procedure in Section 4.2 can begin, and assume that all steps in Section 3.1 have been completed. If your Flow system has been installed by a C Technologies Service Representative, then you are ready to proceed with this section. These items will be discussed in detail, with a summary in Table 4-1 at the end of this section.

4.1.1 Flow Cell and Fibrette

First, remove the Flow Cell from its packaging, and remove all coverings – most importantly the blue tape over the Flow Cell Window. Pull the Flow Fibrette out of its packaging and remove the caps on both optical surface ends of the Fibrette. Before inserting the Flow Fibrette into the Flow Cell, clean off the tip with some IPA (IsoPropyl Alcohol) and a cotton swab. Wipe once per side of the cotton swap, and wipe in one direction across the entire face of the optical tip. Blow off any remaining fibers or debris with an air can.



Make sure to remove and discard the blue tape from the underside of the Flow Cell. Failure to do so will hinder accurate measurements. Do not reuse the blue tape once it has been removed!

4.1.2 Flow Head Preparations

First, remove the blue tape from the Detector Module. This will obstruct the light transmission, and is only in place to protect the glass on the Detector Module for shipment. Discard the blue tape after removal, and do not reuse it!

Make sure the Fibrette Clamp is open. The Lock Arm will be pointed out, and the Clamp Handle will be pushed back. Open the door on the cover of the Flow Head. This is the curved metal sheet that displays the FlowVPE logo. Figure 4-1 is a depiction of a Flow Head that is ready for Flow Cell and Fibrette mounting.

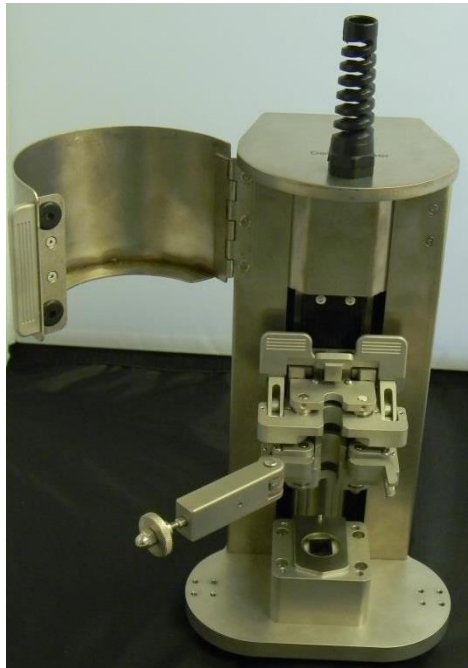


Figure 4-1: FlowVPE Pre-Mount Configuration

4.1.3 Computer, Cary and Software

Boot up the computer and log in. Turn on the Cary 60 by pushing the button on the front of the Cary. The Cary will boot up and run initial calibration, during which the power button will flash orange. When calibration is complete, it will glow a steady green. If calibration failed, the light will glow a steady red. See Section 7.3 for what to do for a failed calibration.

Select the appropriate Flow control software: KQ or KF. Double click on the icon to open the program. The Cary will again run through a quick calibration, and send the Flow Head to the Home Position.

The Flow hardware is now ready for the mounting and loading process.

4.1.4 Preload setup Checklist

Table 4-1: FlowVPE Preload Setup Checklist

- | | |
|--------------------------|---|
| <input type="checkbox"/> | Remove all caps and plugs from Flow Cell |
| <input type="checkbox"/> | Remove and discard blue tape from Flow Cell and Detector Module. |
| <input type="checkbox"/> | Remove caps from optical surfaces, or end faces, of Flow Fibrette. |
| <input type="checkbox"/> | Ensure cleanliness of Detector Module window, Flow Cell window, and end faces of Flow Fibrette. |
| <input type="checkbox"/> | Open the cover door of the Flow Head. |
| <input type="checkbox"/> | Open the Fibrette Clamp Lock Arm. |
| <input type="checkbox"/> | Open the Fibrette Clamp by pushing the handle back. |
| <input type="checkbox"/> | Turn on computer and Cary 60. |
| <input type="checkbox"/> | Open appropriate Flow Software. |

4.2 Loading the Flow Cell and Fibrette

This section details specifics about how to properly load the Flow Cell and Fibrette into the Flow Head for accurate measurements. In order to describe this procedure, some software components have been included. All introductory and explanatory information regarding the FlowVPE software is found in Chapters 5 and 6.



WARNING: *These instructions must be followed very closely, as they ensure proper loading and operation of the FlowVPE. Failure to do so may result in damage to the Flow hardware.*



You are a Critical Link between Software and Hardware

You must tell the software when the Flow Cell is mounted and demounted. The software is set up to prompt you accordingly, but as the user you must be aware of the current state of the hardware for safe and effective use of the FlowVPE system.



How To: Loading the Flow Cell and Fibrette

1. Click on **Load Controls** in the FlowVPE Sidebar:

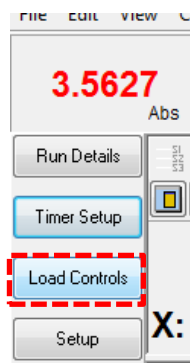


Figure 4-2: Load Controls Button

The Flow Head will move to the Loading Position and open the Load Controls Window.

2. Make sure the proper Flow Cell is selected in the drop down menu.

*Select "FC-03-SS" for a 3mm Flow Cell
Select "FC-10-SS" for a 10mm Flow Cell*

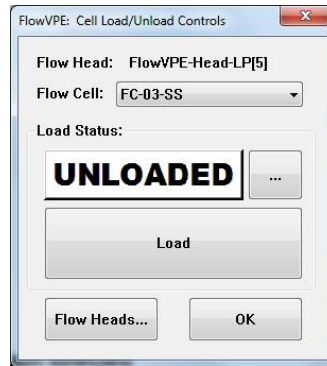


Figure 4-3: Cell Load/Unload Controls Box



DO NOT CLICK **LOAD UNTIL YOU HAVE MOUNTED THE FLOW CELL AND FIBRETTE**



3. **Ensure once more that the Flow Head is ready to accept the Flow Cell and Fibrette. Review Section 4.1 and Figure 4-1 to ensure all pertinent items have been completed.**
4. Insert the Flow Fibrette into the top of the Flow Cell.



Figure 4-4: Inserting Flow Fibrette into Flow Cell



The Flow Cell has two seals that the Flow Fibrette fits into, which will be felt during the insertion of the Flow Fibrette. The first seal will be encountered immediately upon insertion of the Fibrette, and the second seal is roughly 10-20 mm below the first. After pushing through the second seal, the window is another 10-15 mm lower. Be sure to insert the Fibrette with care.

Ensure the Flow Fibrette is pushed all the way into the Flow Cell, and is contacting the Flow Cell Window. The Fibrette location can be seen by flipping the Flow Cell over and looking through the Window.

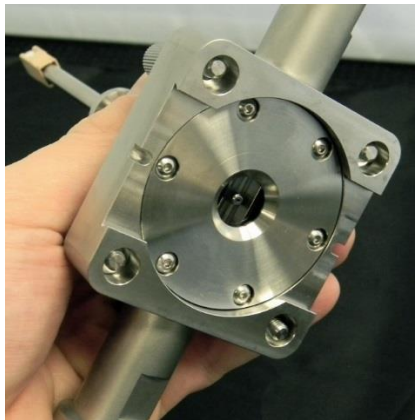


Figure 4-5: Checking Flow Fibrette Location through Flow Cell Window

5. Attach the process flow tubing to the Flow Cell.

For the tri-clamp adapters on the 10 mm Flow Cell it is helpful to orient the clamps so the wing nuts are facing out or slightly downward. This will aid in the attachment of the Flow Cell to the Detector Module.



It is recommended to begin a fluid flow through the Flow Cell at this point, so that the Flow Cell can be inverted to visually ensure there are no bubbles in the process flow.

6. Line up the dovetail on the Flow Cell with the dovetail on the Detector Module, and push the Flow Cell on halfway.

7. Ensure both sides of the dovetail are engaged, otherwise the Flow Cell and Fibrette will appear crooked and not clamp properly. Ensure the Fibrette Flange will fit into place in the Fibrette Clamp. If it appears it will not fit, refer to Chapter 7 for troubleshooting help.

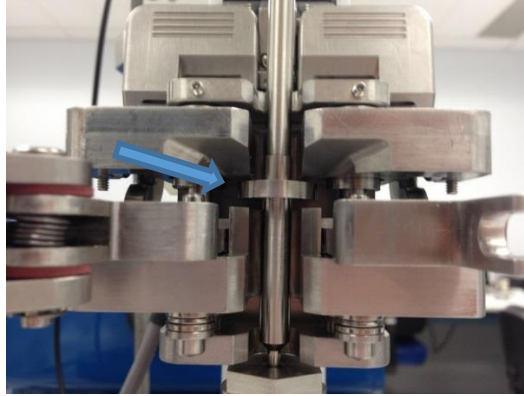


Figure 4-6: Flow Fibrette Flange Fit into Fibrette Clamp

8. Push the Flow Cell and Fibrette into place, where the Flow Cell will make a hard stop on the pin on the back of the Detector Module, and the Fibrette will slide into the Fibrette Clamp.

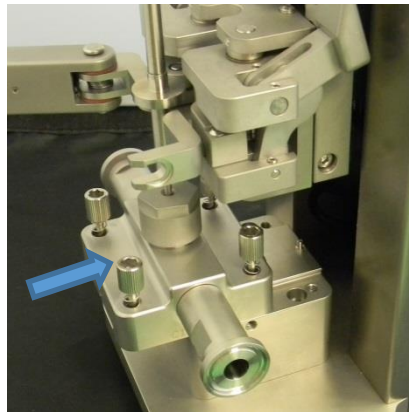


Figure 4-7: Flow Cell Mounting to Flow Head

9. Screw in the Flow Cell to the Detector Module using the 4 captive fasteners.



Figure 4-8: Securing Flow Cell to Detector Module

10. Push up on the Handle Lock on top of the Fibrette Clamp to move it out of the way of the Clamp Handle, and then pull the Clamp Handle towards yourself to close the Fibrette Clamp over the Fibrette Flange. Apply pressure until the Handle Lock falls into place behind the Handle.

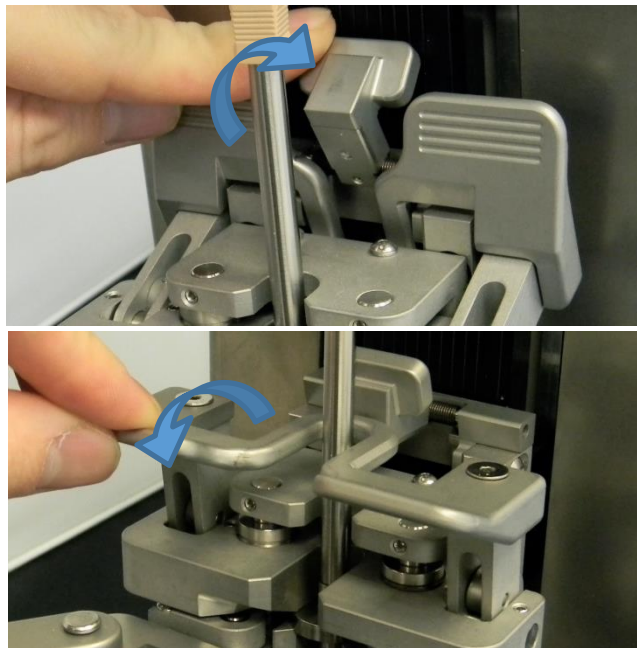


Figure 4-9: Closing Fibrette Clamp over Flow Fibrette

11. Swing the Clamp Lock Arm all the way to the right, and seat the thumb nut into the mating counter bore. Tighten the thumb nut into the mating counter bore until it makes a hard stop. **Do not overtighten the thumb nut!**

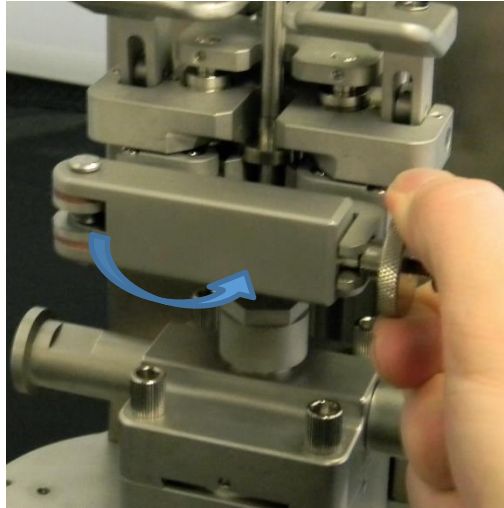


Figure 4-10: Locking Fibrette Clamp Position

12. Using the SC Adapter, connect the Fibrette to the Delivery Fiber. Push the tan pieces on the Delivery Fiber and Fibrette into the SC Adapter. They will click into place, and are properly connected when the small spline on the connector is pulled into the SC adapter.

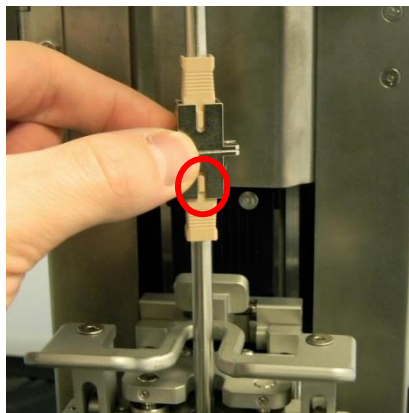


Figure 4-11: Connecting Flow Fibrette to Delivery Fiber

13. Close the Cover Door.

- Click **Load** to tell the program that the Flow Cell and Fibrette are mounted into the Flow Head properly.

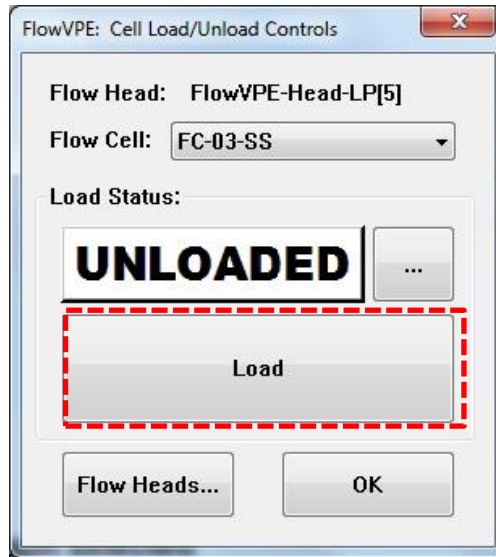


Figure 4-12: Cell Load/Unload Controls Box

The Load Status in the center of the “Load Controls” box will turn blue and say “LOADED” to confirm that the Flow Cell is loaded.

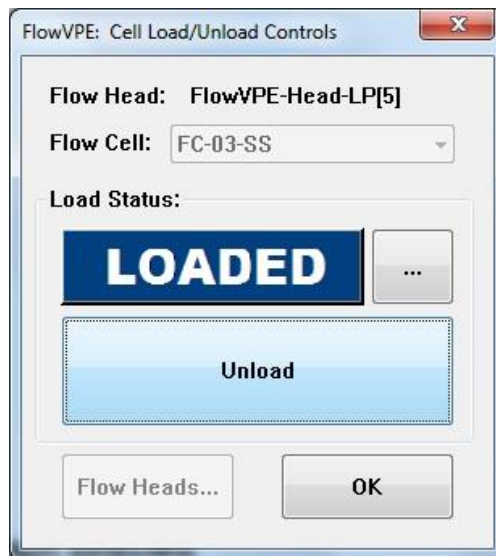


Figure 4-13: Flow Cell Loaded Load/Unload Controls Box

When the Flow Cell and Fibrette have been mounted onto the Flow Head properly, the FlowVPE will appear as shown in Figure 4-14.



Figure 4-14: FlowVPE with Mounted Flow Cell and Flow Fibrette

4.3 Unloading the Flow Cell and Fibrette

The following details the Flow Cell unloading procedure. Any time it is necessary to unclamp the Fibrette Clamp, it is imperative to follow the complete unload instructions to avoid damaging the Flow hardware. Do not make “small” changes during a run. If something needs to be changed or fixed, stop data collection, and unload the Flow Cell properly.



WARNING: Anytime the Fibrette Clamp must be unlocked or unclamped, the entire unload process instructions must be followed to avoid damaging the Flow Hardware.





- It is recommended to flush the Flow Cell with a cleaner before unloading and removal of the Fibrette. This helps keep all optical surfaces clean and ready for the next measurement setup.
- When measurement collection is completed, and the Flow Cell is to be removed for storage, do not disconnect process tubing until the unloading procedure is completed. After Flow Cell dismounting, tip the Flow Cell up on end to ensure all fluid has drained out before disconnecting process tubing.



How To: Unloading the Flow Cell and Fibrette

1. Click on **Load Controls** in the FlowVPE Sidebar. If not already there, the Flow Head will move to the Loading Position.

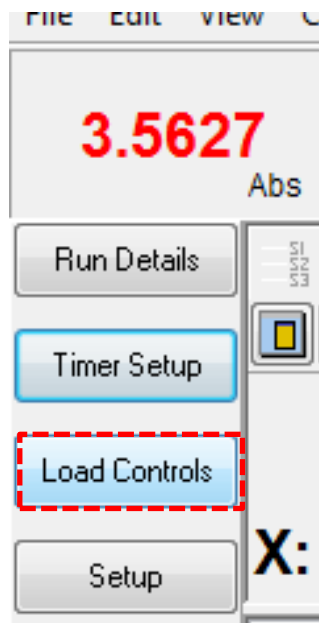


Figure 4-15: Load Controls Button

2. Open the cover and disconnect the Delivery Fiber from the Fibrette by pulling up on the adapter and down on the plastic cover of the Fibrette SC Connector.

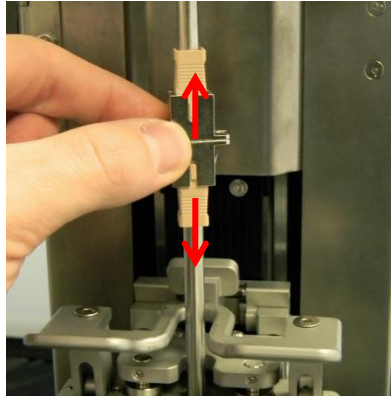


Figure 4-16: Disconnecting Flow Fibrette from Delivery Fiber

3. Unscrew the thumb screw and allow the rectangular Lock Arm to swing open

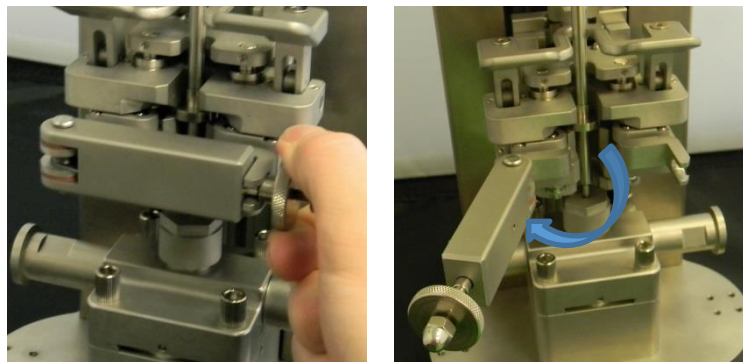


Figure 4-17: Unlocking Flow Fibrette Clamp

4. Lift the Handle Lock, and then lift the Handle by pushing up and back.

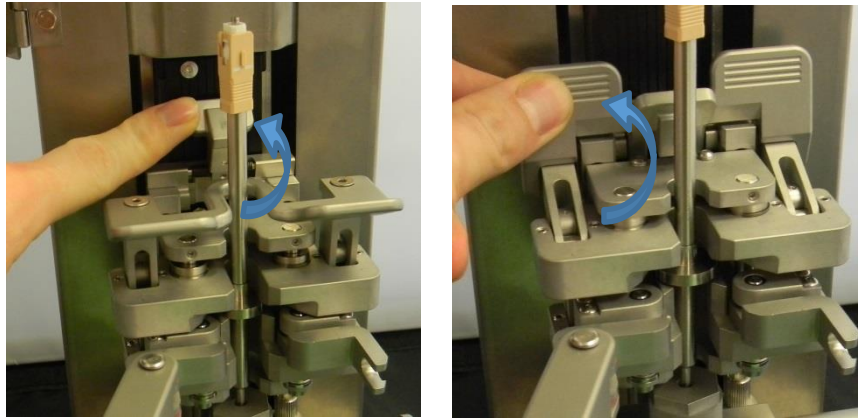


Figure 4-18: Unclamping Flow Fibrette Clamp

5. Unscrew the captive fasteners in the Flow Cell from the Detector Module. They will spring up when they are free from the Detector Module.

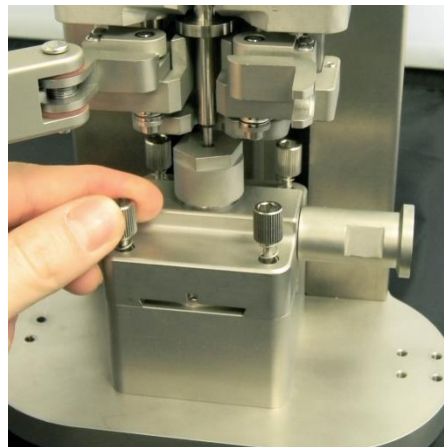


Figure 4-19: Unscrewing Flow Cell from Detector Module

- Slide the Flow Cell and Fibrette out until the Flow Cell is free of the dovetail on the Detector Module.

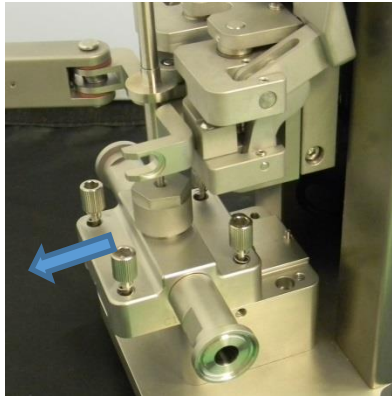


Figure 4-20: Removing Flow Cell and Fibrette from Flow Head

- With the Flow Cell demounted from the Flow Head, click on **Unload** on the Load Controls screen.

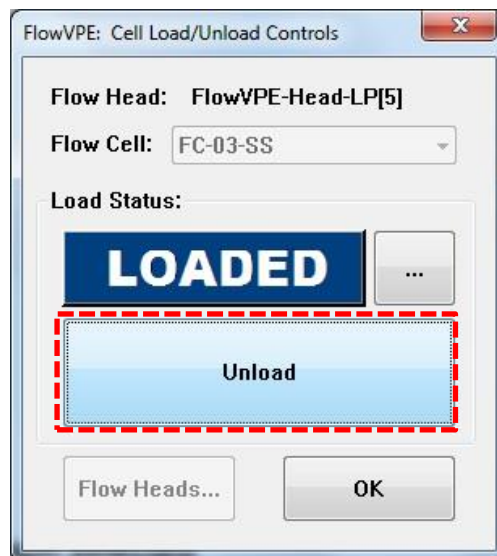


Figure 4-21: Unloading in Load/Unload Controls Box

5 FlowVPE Software Operation

5.1 Understanding the FlowVPE Software Environment

The FlowVPE software was developed to integrate with the Cary WinUV software suite. For individuals with experience using the Cary WinUV software, the FlowVPE control software will be very familiar. For those without familiarity, it will be necessary to learn the various tools and functions of the Cary WinUV suite as only the Flow specific functions of the program will be covered in this manual. The computer that came with the Flow system will already have desktop shortcuts to two Flow specific programs installed on it. They are Quick Slope Kinetics (KQ) and Fixed Slope Kinetics (KF) programs.



Figure 5-1: KF and KQ Program Icons

These programs can also be launched by clicking on **Start > Computer > C: > Program Files > CTECH** and selecting the appropriate program from that folder. Both programs are similar in form and function, differing mainly in the Setup screen accessible through the Flow Sidebar, and the physical measurement capabilities. As explained in Sections 2.3.3 and 5.1.1.4, KQ runs a search algorithm to find an appropriate Pathlength range for the given solution to be measured, and KF is configured for a specific Pathlength range prior to data collection. When either program starts, the ADL Shell application opens and the FlowVPE Splash Screen is displayed as the FlowVPE environment is prepared. The startup sequence initializes the Cary Spectrophotometer and the FlowVPE hardware and creates the software interface by configuring the functionality unique to the FlowVPE system. When the software has fully initialized, the splash screen will disappear signifying program is ready for use. The FlowVPE environment is shown in Figure 5-2 followed by a brief explanation of the various sections of the screen.

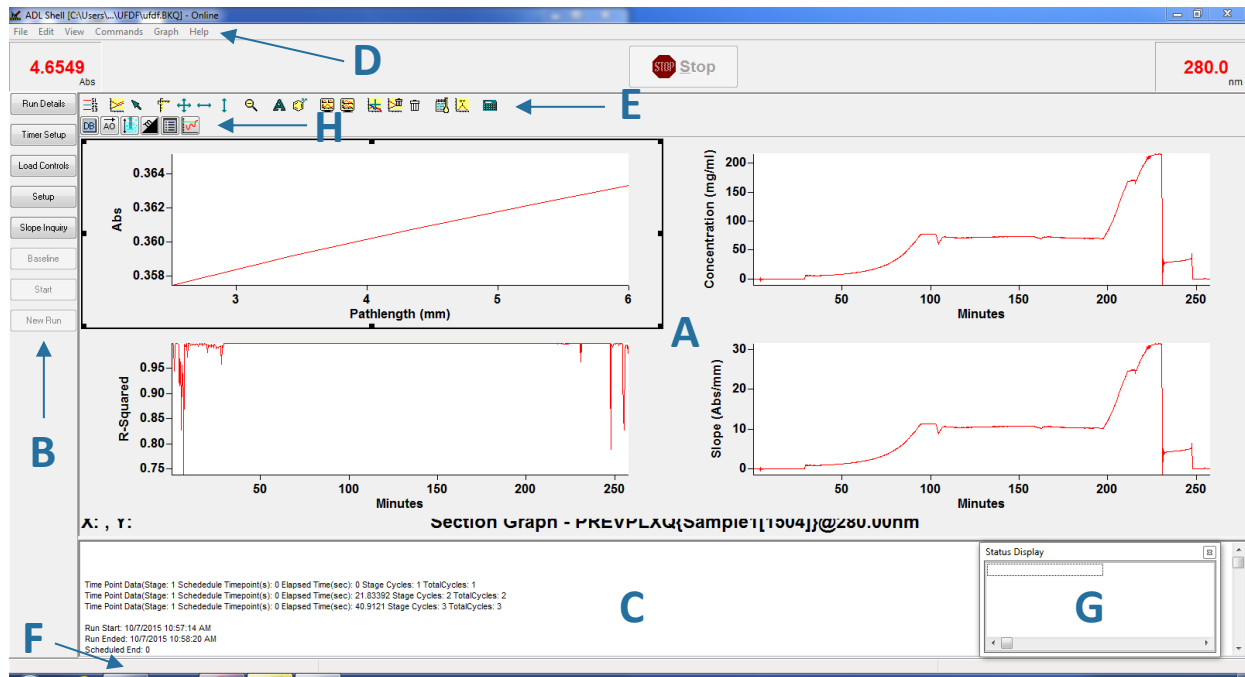








Figure 5-2: The FlowVPE Environment in the Cary WinUV ADL Shell

The major sections of the FlowVPE Control Software application window are described in detail in Table 5-1, with greater detail included in the following sections.

Table 5-1: FlowVPE Environment Description





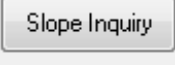
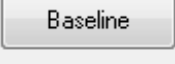
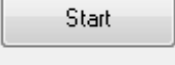
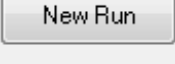
Element	Name	Description
A	Graphics Window	The Graphics Window is where data (curves) are displayed on graphs as traces. Users can display or hide the Graphics Window by toggling it on and off under the View menu. 
B	FlowVPE Sidebar	The FlowVPE Sidebar is a collection of buttons that provide the user primary access to the hardware (both the Cary 60 and the FlowVPE) as well as the unique features and functionality associated with the FlowVPE's Variable Pathlength technology.
C	Report Window	The Report Window is the destination for standard and user created report formats. 
D	Menu Bar	The Menu Bar provides the user access to the core set of Cary WinUV software functionality typically integrated with the computer's operating system. 

E	Graphics Toolbar	<p>The Graphics Toolbar provides the user access to the functions required to manipulate the graphics window including the ability to configure which Continua are visible and their appearance. Available functions also include the ability to add and remove graphs and control their scaling as well as the ability to add and remove traces. The Cary WinUV Calculator is also accessible at the click of a button to allow powerful data analysis.</p> 
F	Status Bar	<p>The Status Bar provides basic information regarding the status of the hardware and software.</p> 
G	Status Window	<p>The Status Window can be toggled on and off in the FlowVPE software modules and provides more detailed information about the status of the hardware and software.</p> 
H	FlowVPE Toolbar	<p>The FlowVPE Toolbar is a collection of buttons that provide the user access to some additional capabilities of the FlowVPE system.</p>

5.1.1 The FlowVPE Sidebar

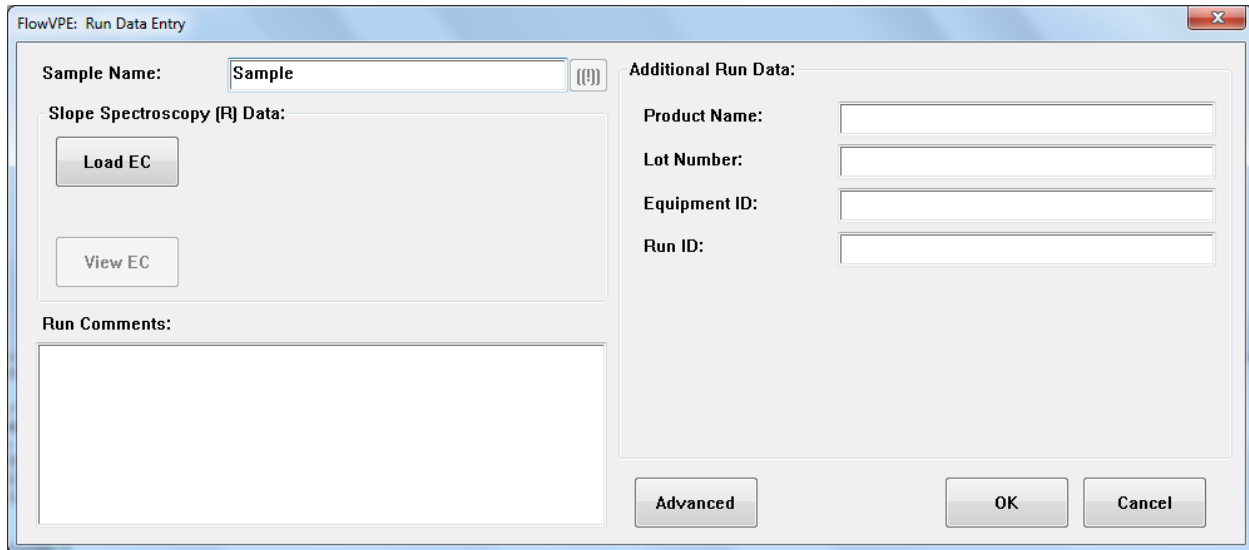
The FlowVPE Sidebar is the primary way users interact with the FlowVPE System. The displayed buttons allow you to configure the software and hardware for data acquisition, initiate the data collection sequences (background collection and data collection) and to analyze data. Table 5-2 summarizes the core functions available on the FlowVPE Sidebar, which will be described in further detail in the following sections.

Table 5-2: FlowVPE Sidebar Functions

	Run Details	Input specifics about the test to be saved with compiled results.
	Timer Setup	Sets a predetermined run time for the flow testing.
	Load Controls	Moves the FlowVPE to the Load or Home Position. Allows user to tell the software when the Flow Cell and Fibrette are mounted in the Flow Head.
	Setup	Opens details configuration screen for the FlowVPE and the Cary.
	Slope Inquiry	Takes a single wavelength slope concentration measurement
	Baseline	Initiates baseline data collection based upon parameters entered into setup configuration.
	Start	Initiates the collection of data based upon parameters entered into setup configuration.
	New Run	Clears the graphics and report window panes and removes data.

5.1.1.1 Run Details

This feature gives the user a location to store more data about the sample to be measured with the FlowVPE. This is an excellent way to capture miscellaneous data that can be used for tracking measurements and product records after data collection. The details that can be input here include a sample name, a product name, lot number, equipment ID number, run ID number, extinction coefficient data, and any comments about the measurement run to be completed on the FlowVPE.



The dialog box is titled "FlowVPE: Run Data Entry". It contains the following fields and controls:

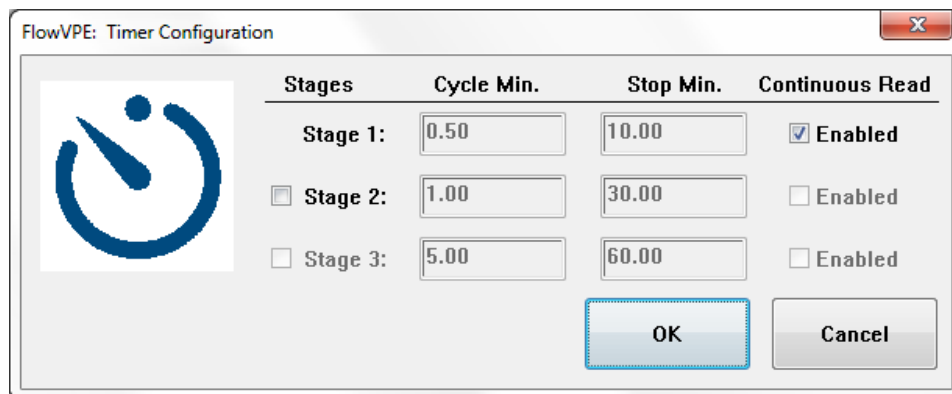
- Sample Name:** A text input field containing the word "Sample" and a help icon.
- Slope Spectroscopy (R) Data:** A section with two buttons: "Load EC" and "View EC".
- Run Comments:** A large empty text area.
- Additional Run Data:** A section with four text input fields: "Product Name:", "Lot Number:", "Equipment ID:", and "Run ID:".
- Buttons:** "Advanced", "OK", and "Cancel" are located at the bottom of the dialog.

Figure 5-3: Run Data Entry Box

All of the data input in the Run Details box is saved in WinUV specific file extensions, and visible when opening or loading these files into the FlowVPE software. The data will remain in the Run Details box in each scenario.

5.1.1.2 Timer Setup

The Timer Setup button is where the run time is set for the measurements. The default setting is for a continuous read, meaning measurements must be stopped manually. Stages can be configured to allow for three different time periods of measurement, with a reading taken at intervals specified in "Cycle Min." and stage duration specified in "Stop Min."



The dialog box is titled "FlowVPE: Timer Configuration" and features a clock icon on the left. It contains a table for configuring measurement stages:

Stages	Cycle Min.	Stop Min.	Continuous Read
<input checked="" type="checkbox"/> Stage 1:	<input type="text" value="0.50"/>	<input type="text" value="10.00"/>	<input checked="" type="checkbox"/> Enabled
<input type="checkbox"/> Stage 2:	<input type="text" value="1.00"/>	<input type="text" value="30.00"/>	<input type="checkbox"/> Enabled
<input type="checkbox"/> Stage 3:	<input type="text" value="5.00"/>	<input type="text" value="60.00"/>	<input type="checkbox"/> Enabled

At the bottom of the dialog are "OK" and "Cancel" buttons.

Figure 5-4: Timer Configuration Box

5.1.1.3 Load Controls

This feature in the FlowVPE Sidebar enables the user to communicate with the Flow software about the current configuration of the Flow Head. As described in Section 4, the user must tell the software that the Flow Cell and Fibrette are properly mounted on the Flow Head, which completes the Load process. The Cell Load/Unload Controls box gives the user the ability to specify what size Flow Cell is mounted on the Flow Head (“FC-03-SS” for 3mm Cell, “FC-10-SS” for a 10mm Cell), as well as to switch between available Flow Heads in the software.



The Flow Head selection feature is password protected.

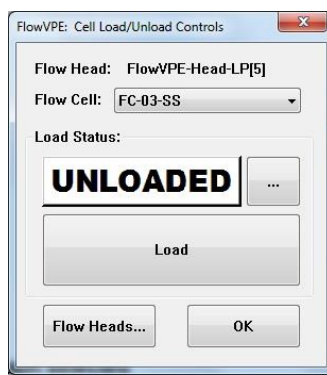


Figure 5-5: Cell Load/Unload Controls

5.1.1.4 Setup

This is where the specific parameters of the measurement run are input. The parameters to be selected will be different between the KQ and KF FlowVPE programs. See Chapter 6 for details on configuring KQ and KF setups, as well as step-by-step instructions for running each program.

5.1.1.5 Slope Inquiry

Slope Inquiry is a helpful tool available in both KF and KQ programs that enables the user to take a quick scan of the solution to be measured to determine an appropriate Pathlength range for data collection. Input the appropriate wavelength (WL) extinction coefficient (EC), datapoints to collect, Cary averaging time, and a target threshold absorbance. The Slope Inquiry uses the same target absorbance algorithm as the KQ program uses for its measurement parameter determination.

Figure 5-6: Quick Slope Inquiry Box

The resulting data will be plotted on the home screen of the FlowVPE software, with a box giving a readout of the resulting data from the Slope Inquiry.

5.1.1.6 Baseline

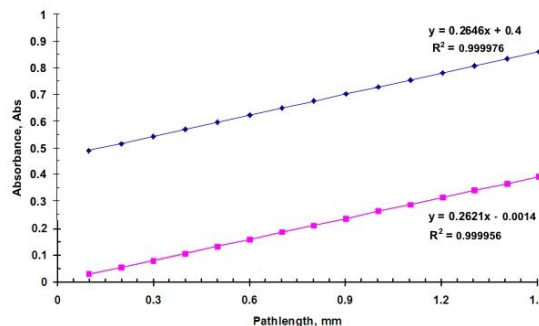
To run a baseline of a solution, baseline must first be enabled in the Setup box (see Section 5.1.1.4).



In most cases, baseline is not required.

Slope Spectroscopy® is a very useful technique that enables users to create very powerful analysis methods. ***One of the most unique and popular features associated with Slope Spectroscopy® is that Baseline Correction is often times unnecessary.*** Why and When that is the case are the two most frequently asked questions.

Why No Baseline? Because Slope Spectroscopy® relies on the change in absorbance associated with changes in Pathlength, the absolute absorbance level does not matter, only the rate of change matters (the slope). The chart to the right shows 2 data sets, the pink set has been baseline corrected, and the blue set has not. The two lines are essentially parallel, which means the two data sets have the same slope. If the slopes are equal, the concentrations calculated from those slopes will be equal. Therefore, you can frequently save the time, effort and costs associated with running the baseline.



When are baselines required? Baselines are required when the background media absorbance scans show Pathlength dependence, meaning the absorbance values change with Pathlength. In terms of Section Data (Absorbance vs. Pathlength), the slope of a section plot will not be zero. This can quickly be determined during method development by scanning the background media and checking its slope. If the slope of the section plot does not equal zero, baseline correction is required.

If baseline correction is required, clicking on “Baseline” in the FlowVPE Sidebar will run a baseline measurement of the contents of the Flow Cell. The resulting plot will be displayed on the home screen of the program, and be stored for data collection integration.

For more information on Baseline, see Section 8.2.1 in the Appendix.

5.1.1.7 Start

The Start button in the FlowVPE sidebar initiates data collection. The button itself will be grayed out until the Flow Cell is loaded and the Setup parameters have been input and approved. Clicking “Start” will bring up a dialog box to select a location for the data file export, and then data collection will begin.

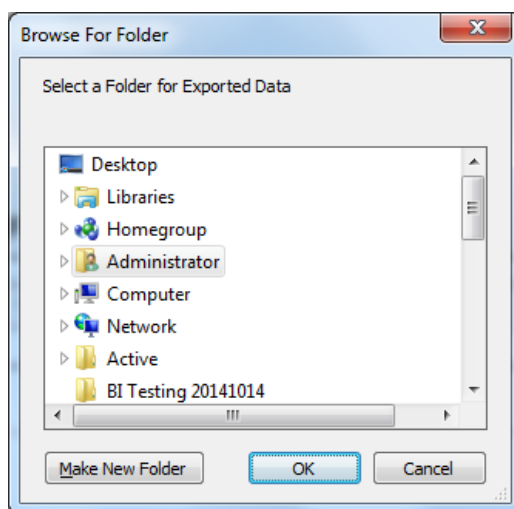


Figure 5-7: Data File Export Save Location Box

5.1.1.8 New Run

After a measurement run has been completed or stopped manually, a new run can be configured using this button. This will clear all traces on the home screen, so ensure all pertinent data has been saved before continuing. After clearing all traces, run through all necessary setup for the new run. All parameters previously used will remain unchanged, so it is only necessary to open dialog boxes where parameters are changing from the previous run.

5.1.2 The FlowVPE Toolbar

The FlowVPE Toolbar contains access to features that complement the main features found in the FlowVPE sidebar functions. It is important to note that the Toolbar is an element within the Graphics pane of the ADL Shell environment. If the Graphics pane mode is not visible using the View menu, the Toolbar will disappear. It will return when the Graphics pane is made visible again. Figure 5-8 shows the FlowVPE Toolbar and Table 5-3 presents the various button icons available in the FlowVPE Toolbar and provides a brief description of the function they provide. Each will be discussed in further detail in later sections.









Figure 5-8: FlowVPE Toolbar Functions




FlowVPE Toolbar Exists Within the Cary WinUV Graphics Pane

It is important to remember that the FlowVPE Toolbar is an object within the Cary WinUV Graphics pane. If the Graphics Pane visibility is toggled on and off using the View Menu options, the FlowVPE toolbar will appear and disappear accordingly.

Table 5-3: FlowVPE Toolbar Functions

	Database	Database node editor
	Analog Output	Features and settings for DAQ output
	Flow Cell Flush Cycle	Cycles Flow Fibrette to various Pathlengths for a flush cleaning cycle
	Slope Tool	Opens a feature that provides quick regression tools.
	Status Window Toggle	Open and close status window
	Optimize Tool	Opens a tool that helps user optimize coupler transmission

5.1.2.1 Database Node Editor 



This tool of the FlowVPE Toolbar allows for some editing of the database from within the FlowVPE software environment. This is included in the Flow software for use by authorized C Technologies Service personnel, and will not be needed by the user.

5.1.2.2 Analog Output 

This feature of the FlowVPE toolbar is used to configure the analog output signal for the Flow data collection runs. In order to use this feature, it must be enabled in the Setup box located in the FlowVPE sidebar first (see Section 5.1.1.4). Additionally, the data acquisition hardware must be setup and recognized by the computer. C Technologies has a data acquisition hardware set available that has been vetted for use with the FlowVPE system.

Clicking on the AO icon will bring up the following screen:

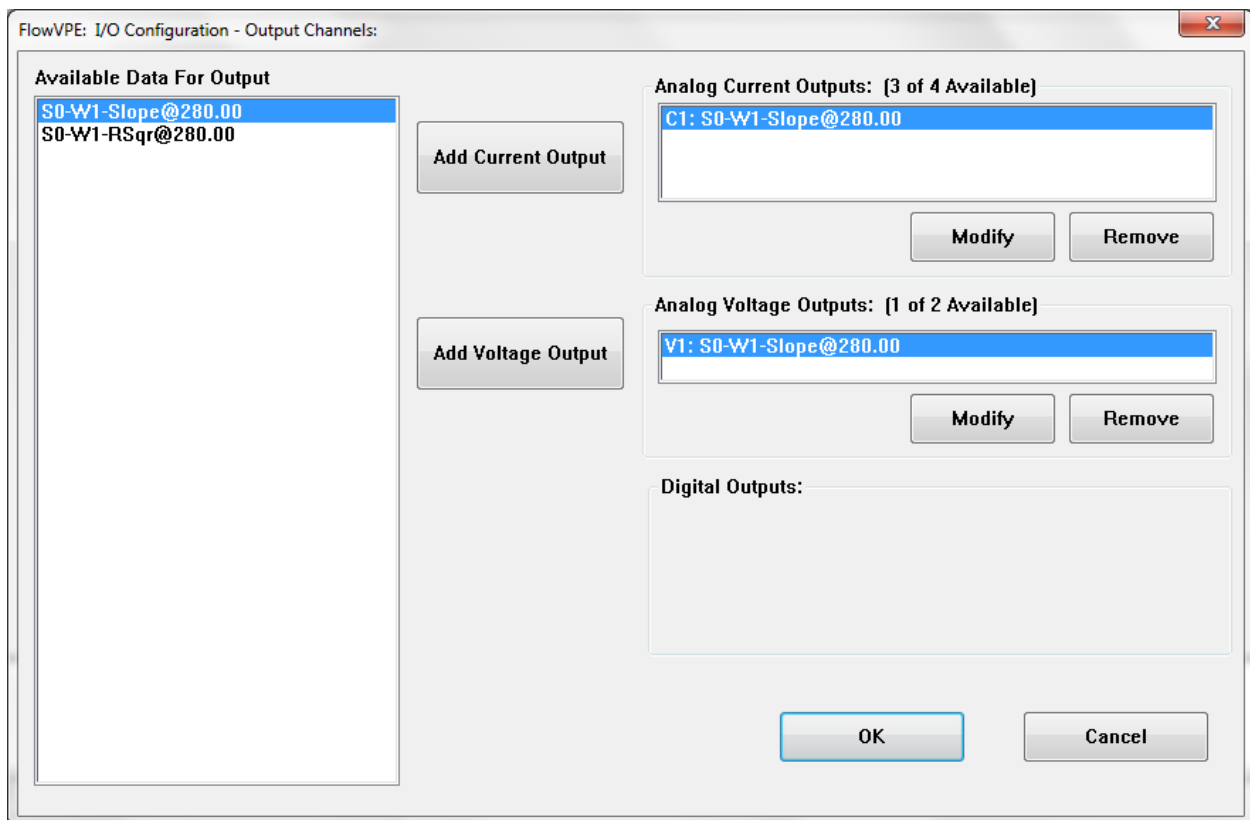


Figure 5-9: I/O Configuration Box

Based on the parameters entered in Setup, the “Available Data for Output” list will be populated. It is important to complete all other steps of the test run method prior to configuring the analog output signal.

To configure a signal, select the data to be output from the available data list, and click “Add Current Output” or “Add Voltage Output” to configure the signal. Clicking on one of the Add Output buttons will bring up a second box with more configuration options:

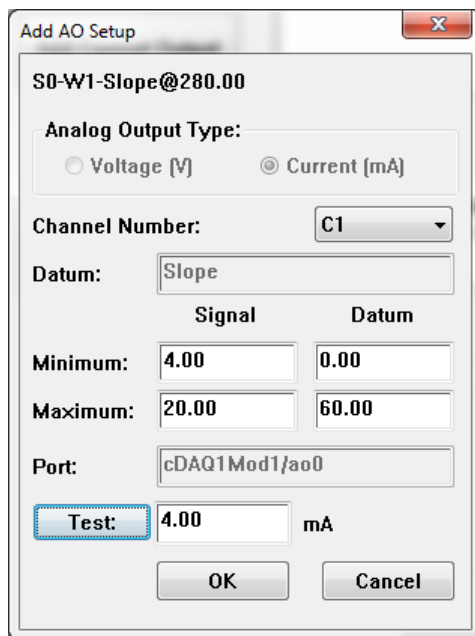


Figure 5-10: Add AO Setup Box

Input the parameters for the signal output, including channel number, and min and max signal and Datum values. The Datum value signifies the actual experimental value of the data being output, and the signal value signifies the corresponding value that is being output. For the values shown in Figure 5-10, an example slope value of 0 at a wavelength of 280 nm would have a corresponding output of 4 mA.

To change the output signals after configuration, use the “Modify” or “Remove” buttons in the I/O Configuration box (See Figure 5-9).

5.1.2.3 Flow Cell Flush Cycle

The Flow Cell Flush Cycle feature has been included to actuate the Flow Fibrette up and down continuously while a cleaning agent is flushed through the Flow Cell. This up and down actuation will repeat until stopped by the user.

5.1.2.4 The Slope Tool



Figure 5-11: The Slope Tool Window

The Slope Tool has many regression analysis capabilities and can switch between available Section curves. This means that all section plots to be analyzed must be included in the FlowVPE program window. For details on data export and configuring data to be kept in the software for use of the Slope Tool, see Section 6.3 for KF and Section 6.1 for KQ.

This tool gives the user the ability to add and remove data points from the regression set, the ability to use the R^2 optimization function and the ability to calculate either the concentration based upon the regression results and a user specified Extinction Coefficient, or the Extinction Coefficient based upon the regression results and a user specified concentration. Clicking **Print** will output a short report to the report window based on the results found with this tool.

The small form factor of the Slope Tool makes it easy to see the graphics window while working with the data. The **Section Selector** allows users to switch between Section data sets without closing and opening the window. The Slope Tool window provides visual tips as to the validity of the regression results with indicator bars and icons that show up when the linearity results appear less than optimal, the number of data points are too few, and when the absorbance levels themselves appear to be either too high or too low. Such indicators help users maintain the highest levels of accuracy when using the FlowVPE system.

5.1.2.5 Status Window Toggle



This feature turns on and off the Status Window of the FlowVPE software. The status window appears during the run and displays the current concentration, how many cycles have been completed (cycle count), and the R-squared and slope values for the last data point set. Even though the window will not display until beginning the run, clicking the icon will display a momentary message confirming if the Status Window is "On" or "Off".

5.1.2.6 Optimize Tool



The Optimize Tool is a helpful feature that allows the user to view a transmission plot in real time. Using this tool, the optical path can be manipulated to maximize the transmission from the Cary 60 to the FlowVPE Detector. This is used primarily for Fiber Optic Coupler Optimization. As shown in Figure 5-12

below, the green line displays the max transmission achieved, and the red line plots the transmission in real time. See DOC0111 for more information about how to use this tool.

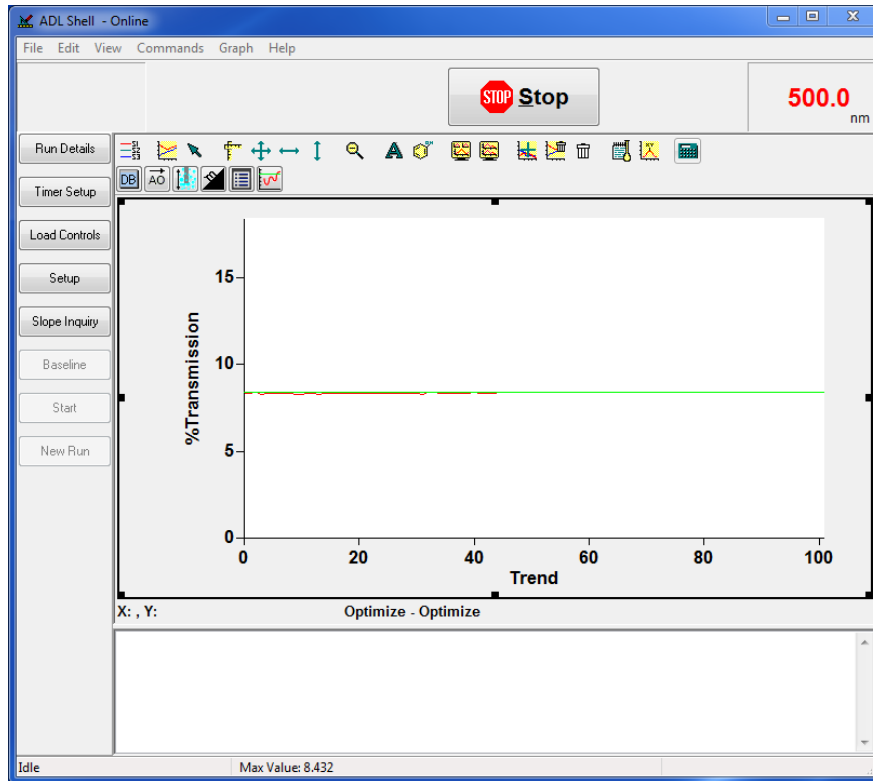


Figure 5-12: Optimize Tool

5.1.3 Software Help

The FlowVPE computer requirements are identical to those of the Agilent Cary 60. The Agilent software includes extensive on screen Help functionality. In addition to this manual, the online help for the FlowVPE system is being enhanced.

6 FlowVPE Program Operation KQ/KF

This chapter details the use of the Setup function of the two FlowVPE programs, Quick Slope Kinetics (KQ) and Fixed Slope Kinetics (KF). Also, this section will walk the user through a software setup of the FlowVPE for each specific program.

6.1 Quick Slope Kinetics (KQ) Setup

The setup box for KQ, or Quick Slope Kinetics, only requires a wavelength and extinction coefficient, with an option to enable a baseline and/or scatter correction, as well as an Analog Output signal.

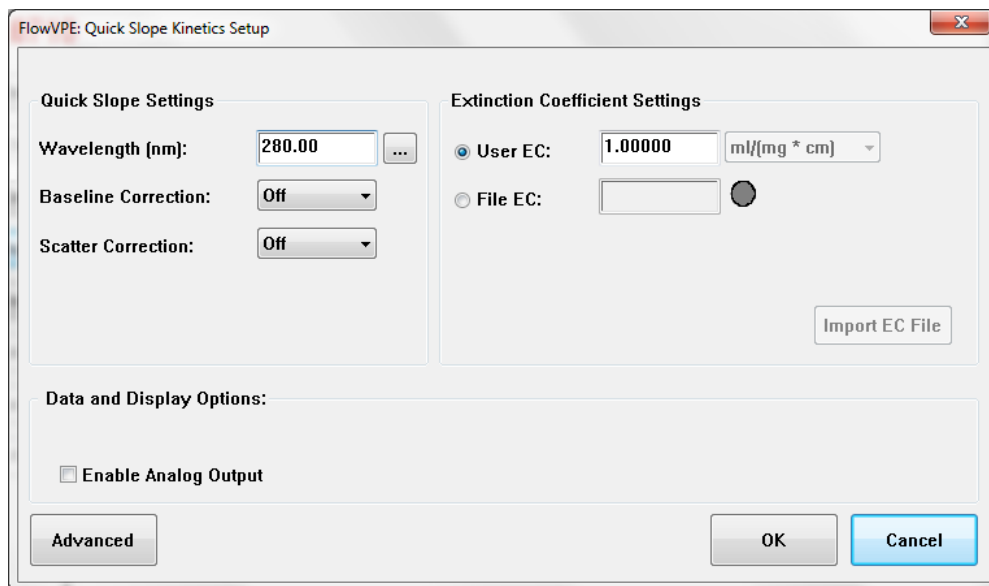
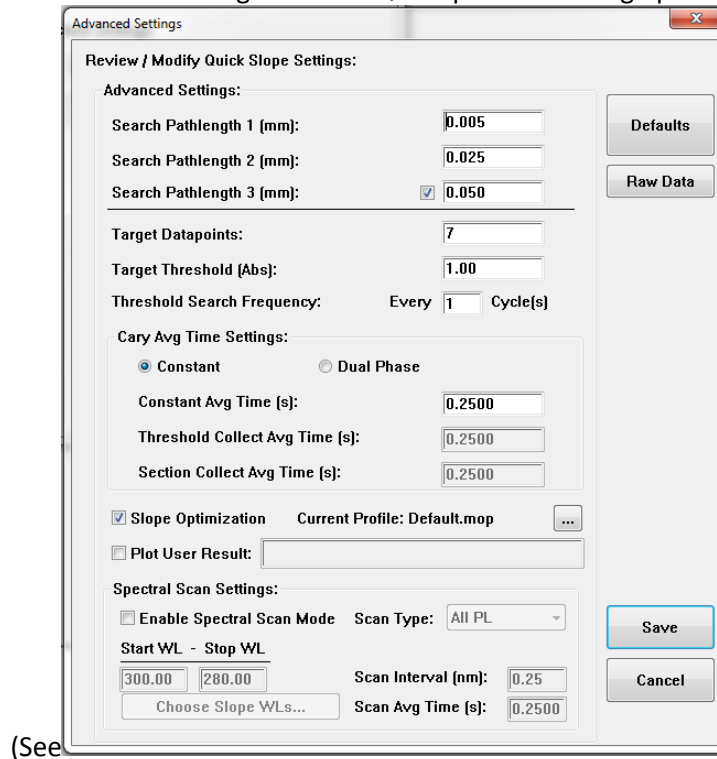


Figure 6-1: Quick Slope Kinetics Setup Box

Clicking on the “...” icon next to wavelength in the KQ Setup box will bring up an Advanced Settings box



(See Figure 6-2). Given the expected rate of concentration change, the frequency of threshold search can be configured here, meaning how often the software will scan for an appropriate range of measurement Pathlengths for data collection in the linear range of the Slope Spectroscopy® equation. Additionally, the Cary averaging time can be changed to optimize measurement rapidity and accuracy, and a spectral scan can be enabled as well.

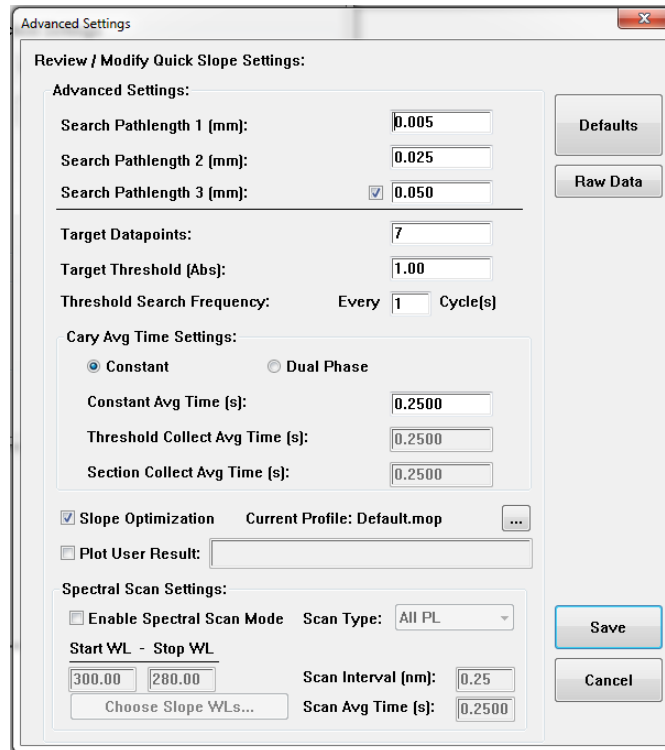


Figure 6-2: KQ Advanced Settings Box

The spectral scan will make an absorbance measurement of all wavelengths at specified intervals in a specified range. The scan will be taken for each of the selected Pathlengths from the quick slope algorithm.

Clicking “Advanced” in the KQ Setup box (

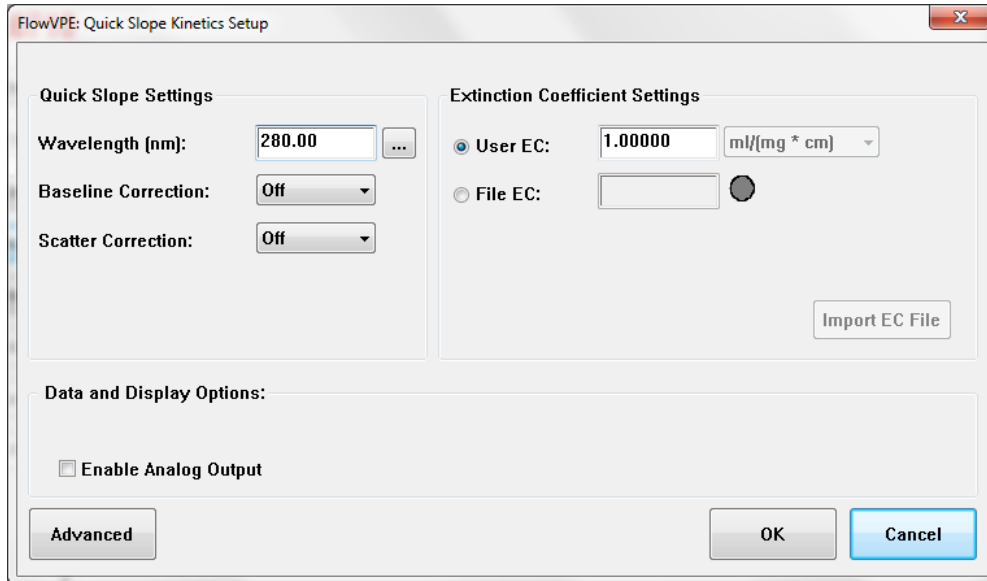


Figure 6-1) allows the user to select the type of data to be exported during the measurement run.

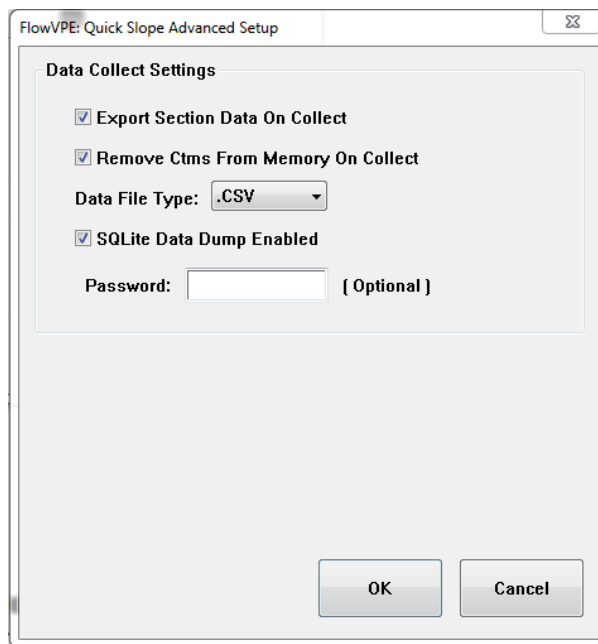


Figure 6-3: Quick Slope Data Collect Settings

The program will, by default, export all section data to an external file to free up system RAM. Unchecking the “Export Section Data” box will make all data collected plot on top of each other in the report window. For longer runs or collection of larger data files (e.g. spectra), it is recommended to have the section data export option enabled, as well as the “Remove Ctms” option. The drop down menu also allows selection of the desired data file type for export, either .CSV or .DKQ.

There is an option included as well to encrypt the SQL data file if desired. This is done by entering a password in the box provided. ***This password must be retained by the user, and will not be available to find through the software after it has been created.***



If system RAM is exceeded during data collection, measurements will cease. Though having all data plotted in the FlowVPE software is helpful for analysis, it is recommended to configure the data to be exported during collection for longer runs or runs with multiple data sets (e.g. spectra).

6.2 Quick Slope Kinetics Program Operation

The Quick Slope Kinetics FlowVPE program allows the user to take measurements based solely on a user-defined wavelength and extinction coefficient. The software finds what max Pathlength gives a predetermined absorbance reading at a user-defined cycle interval, then collects slope data at that max Pathlength. This simplified tool proves powerful for runs where the concentration may shift out of the measurement range of a fixed Pathlength run.



Figure 6-4: Quick Slope Kinetics Splash Screen



KQ is best suited for low flow rates and low rates of concentration differential. User experimentation will be needed to determine applications and algorithm run intervals suitable for accurate use of the Quick Slope Kinetics program.



How To: Configuring and Executing a KQ Measurement Run

1. If a Method file (.MKQ) has been saved previously that will be used for this run, open the Method file and skip to number 10. To set all run parameters, continue with the following:
2. Click on **Timer Setup** in the FlowVPE Sidebar to set the run time parameters.

The default setting is for a continuous read. Stages can be configured to allow for three different time periods of measurement, with a reading taken at intervals specified in "Cycle Min." and stage duration specified in "Stop Min."

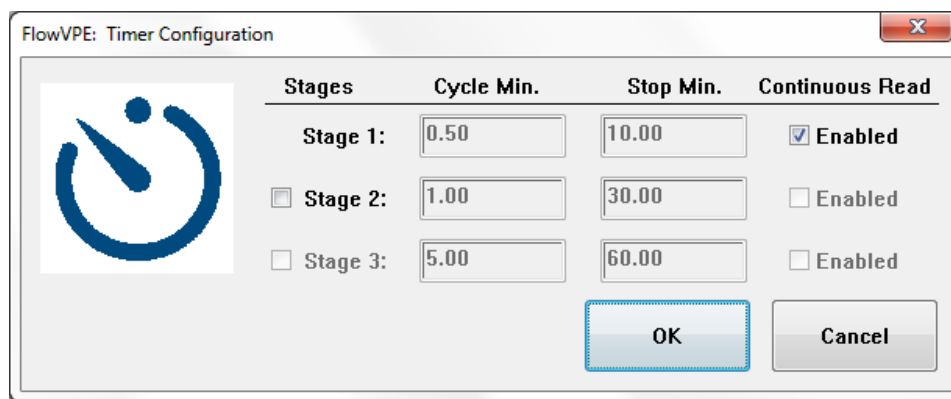


Figure 6-5: KQ Timer Configuration Box

1. Click **OK** to return the main screen.
2. Click on **Setup** in the Sidebar.
3. Input the desired wavelength and appropriate extinction coefficient. Select other features as needed (Baseline Correction, Scatter Correction, Analog Output).

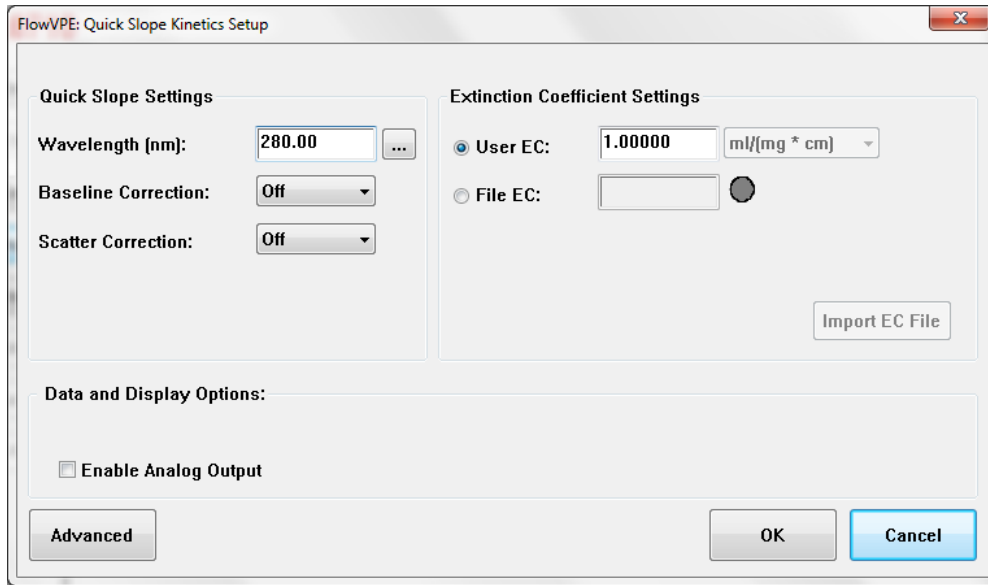


Figure 6-6: KQ Setup Box

4. Click on the “...” to change the threshold absorbance algorithm parameters, as well as the desired number of slope data points.

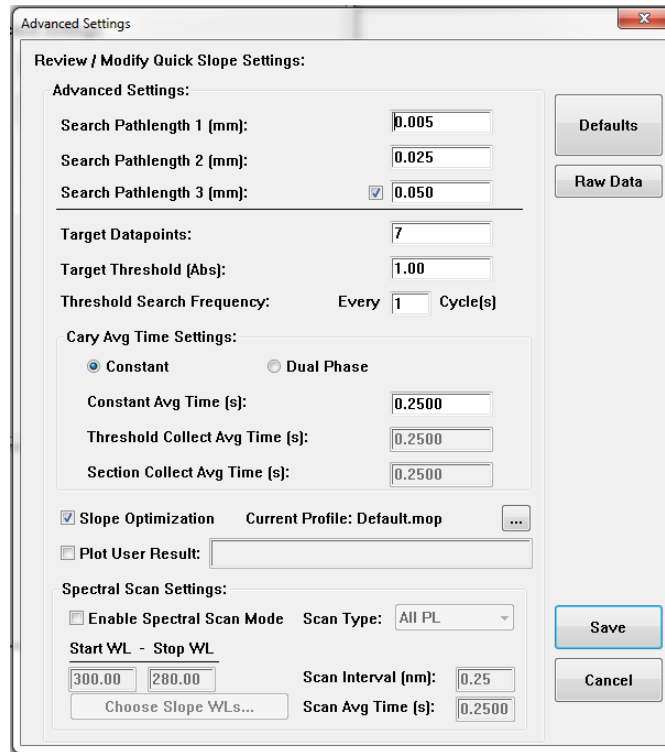


Figure 6-7: KQ Advanced Settings Box

A Spectral Scan Mode can be turned on and configured as well. This feature will require user experimentation to determine the best operating parameters for the application.

5. Click on **Advanced** and make sure all check boxes under “Data Collect Settings” are selected. Select the desired data file type from the drop down menu. All section plot data collected during the measurement run will be saved in this format (.DKQ for an ADL specific data file, .CSV for a comma separated value list).

*Deselecting the check boxes under “Data Collect Settings” will cause the program to plot each consecutive slope overlaid on the previous one(s). Depending on program configuration, the software may crash after too much data has been collected. Therefore it is **HIGHLY RECOMMENDED** to have all data dumped to an external file to avoid interrupting measurements.*

6. Click **OK** on all setup boxes to return to the home screen.

7. If the previously set parameters will be useful again in the future, save out a Method file (.MKQ). A Method file captures all set parameters and can be reloaded to save the user time in run configuration.
8. To execute the Fixed Slope run, click **Start** in the FlowVPE Sidebar.
9. Select or create a folder to have data exported and saved into. This is where the .CSV or .DKQ files specified earlier will be saved.
10. Input any additional run information by clicking **Run Details** in the FlowVPE Sidebar. This information will be saved with all data in a Batch File.
11. Click **OK** to finalize the folder location selection. The Flow Head will begin to operate, and charts will populate on the screen.
12. To pause or end the run, click the **Stop** button in the top middle of the screen. The run will pause until either **End Run** or **Resume Run** is selected.



WARNING: *Do not use the pause run feature to change anything with the Flow Cell, Fibrette or Flow Clamp hardware. If the Flow Cell and Fibrette need to be unloaded and/or reloaded, click End Run and follow the proper procedures in Sections 4.2 and 4.3.*



13. To stop collecting data, click **End Run**. To continue collecting data, click **Resume Run**.
14. To save the data on the screen, click “File – Save Data As”. Select .BKQ under file type, and select the folder to save the .BKQ file into and click “Save”.

It is recommended to save the .BKQ in the same folder as selected previously for the .CSV or .DKQ file export.

To start a new run, click on **New Run** in the FlowVPE Sidebar. Click **Setup** to confirm or change parameters, then click **Start**. Repeat steps 13-18 as needed.



All previous data will be cleared from the screen and deleted. Be sure to have saved all data needed before clicking New Run.

6.3 Fixed Slope Kinetics (KF) Setup

The setup box for KF requires a desired start Pathlength, step size, number of data points to be collected, and wavelengths, input in series separated by a comma. One slope will plot for each wavelength entered, consisting of the Absorbance at the starting Pathlength as well as the Absorbance for each consecutive Pathlength up to the input number of data points in increasing intervals of the input step size.

Multiple slopes can be configured using the dropdown menu next to “Slope Configurations:”. Additionally, baseline correction and an Analog Output signal can be enabled with the check boxes in the lower left of this setup box.

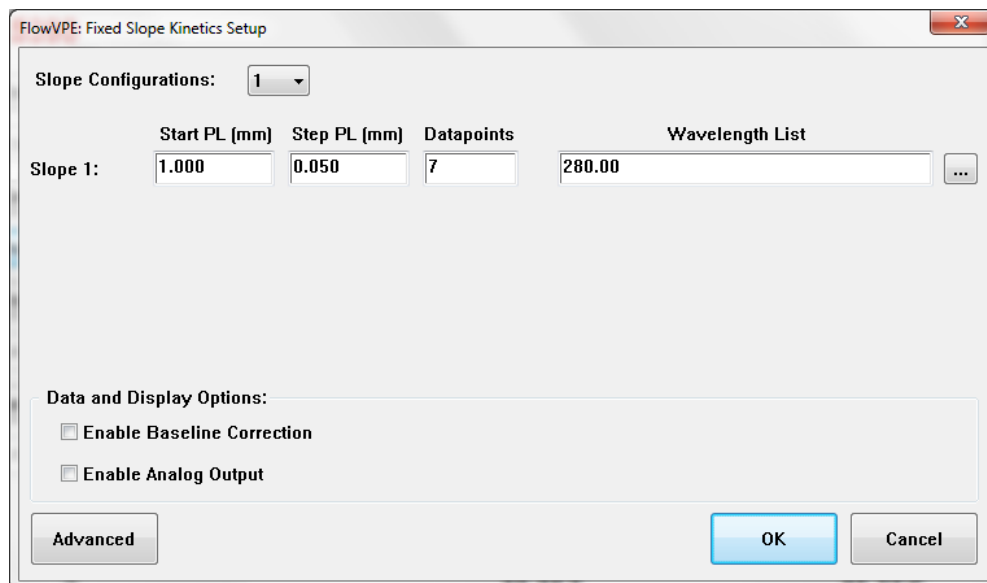
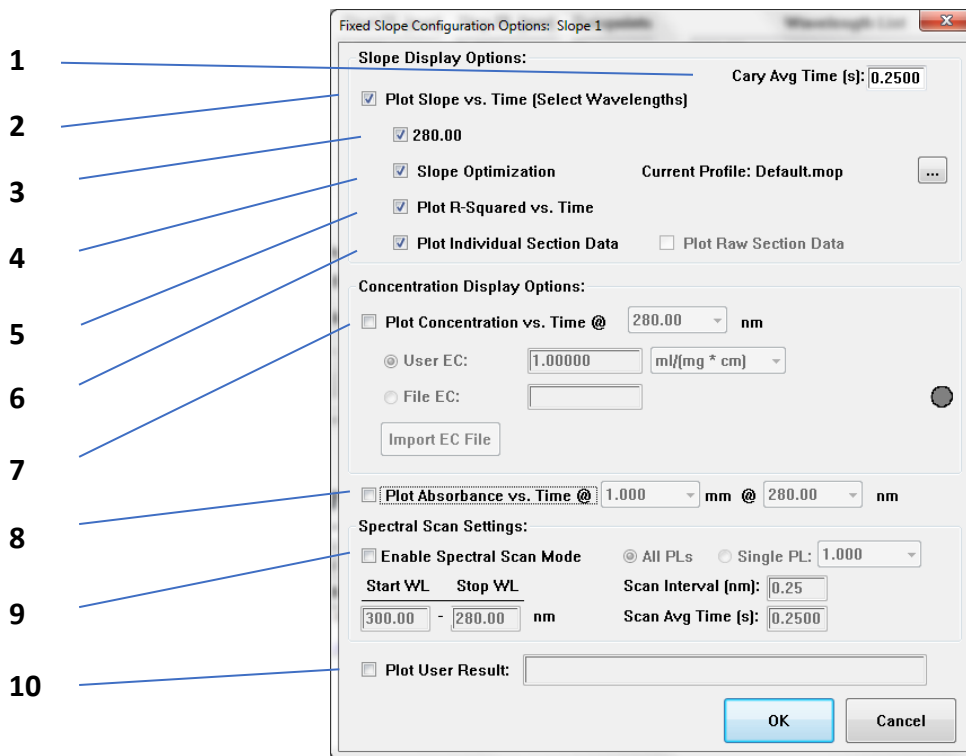


Figure 6-8: Fixed Slope Kinetics Setup Box

Clicking on the “...” icon next to wavelength in the KF Setup box will bring up an Advanced Settings box (See Figure 6-9). The user can turn on or off different data plots in this box, as well as enable a spectral scan. The spectral scan will take absorbance readings at specified wavelength intervals within a specified range for each Pathlength data point specified. Additionally, the Cary averaging time can be configured here as well, increasing the rapidity of measurement. For each wavelength configuration selected, these Advanced Settings will be available. Each item in Figure 6-9 below is described in Table 6-1 below.



The **Current Profile** feature is locked, for use by authorized C Technologies personnel only.

Figure 6-9: KF Slope Configuration Options

Table 6-1: KF Settings Details

Item Number	Description
1	Change the duration of each measurement.
2	Selection will plot the Slope vs Time, and enable options 3-6.
3	Selection will plot Slope vs Time for the specified wavelengths.
4	Selection will take best slope, and drop upper and lower points if plateaued.
5	Selection will plot the R ² value with respect to Time.
6	Selection will plot each section plot. Selection is necessary to export data to database.
7	Selection will plot the concentration with respect to time.
8	Selection will plot the Absorbance at the specified Pathlength and wavelength with respect to time.
9	Selection will enable the spectral scan, which will run during measurement at the specified parameters
10	Selection will plot user defined parameters.

To plot a user-defined result, select the check box. When clicking in the blank to input the user-defined result, a calculator window will open with the available results that can be plotted. The user can select the results like variables, and run mathematic operations on them for the plot.

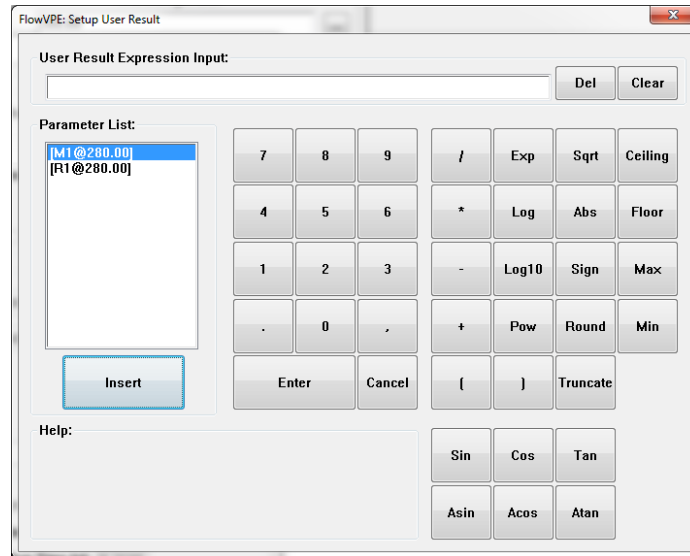


Figure 6-10: Setup User Result Box

Clicking “Advanced” in the KF Setup box (See Figure 6-11) allows the user to select the type of data to be exported during the measurement run.

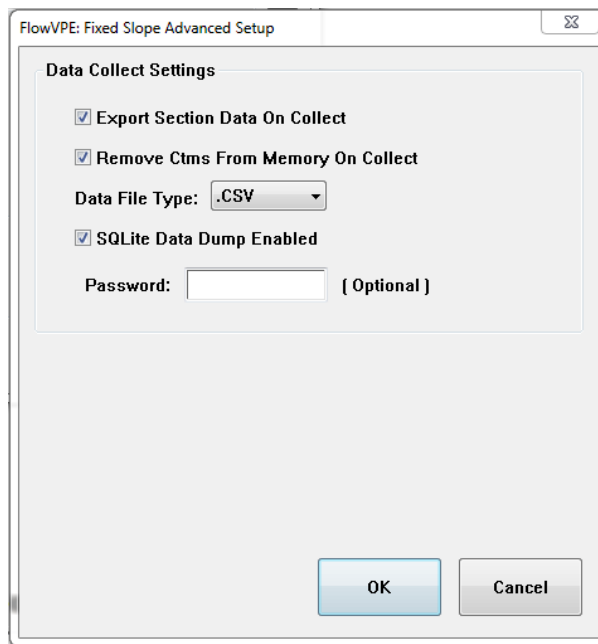


Figure 6-11: Fixed Slope Data Collect Settings

The program will, by default, export all section data to an external file to free up system RAM. Unchecking the “Export Section Data” box will make all data collected plot on top of each other in the

report window. For longer runs or collection of larger data files (e.g. spectra), it is recommended to have the section data export option enabled, as well as the “Remove Ctms” option. The drop down menu also allows selection of the desired data file type for export, either .CSV or .DKF. For data export, ensure the option to plot individual section data is selected (see Table 6-1).

There is an option included as well to encrypt the SQL data file if desired. This is done by entering a password in the box provided. ***This password must be retained by the user, and will not be available to find through the software after it has been created.***



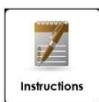
If system RAM is exceeded during data collection, measurements will cease. Though having all data plotted in the FlowVPE software is helpful for analysis, it is recommended to configure the data to be exported during collection for longer runs or runs with multiple data sets (e.g. spectra).

6.4 Fixed Slope Kinetics Operation

The Fixed Slope Kinetics FlowVPE program allows the user to set specific wavelengths, Pathlengths, step sizes and time durations for the pertinent sample measurement. Multiple sets of Pathlength vs. wavelengths to be measured can be configured. The KF Flow program is a powerful tool for the user who already knows in what specific range their data will fall. Before continuing, ensure the Flow Cell and Fibrette are loaded properly - see Section 4.2 for details.



Figure 6-12: Fixed Slope Kinetics Splash Screen



How To: Configuring and Executing a KF Measurement Run

1. If a Method file (.MKF) has been saved previously that will be used for this run, open the Method file and skip to number 8. To set all run parameters, continue with the following:
2. Click on **Timer Setup** in the FlowVPE Sidebar to set the run time parameters.

The default setting is for a continuous read. Stages can be configured to allow for three different time periods of measurement, with a reading taken at intervals specified in "Cycle Min." and stage duration specified in "Stop Min."

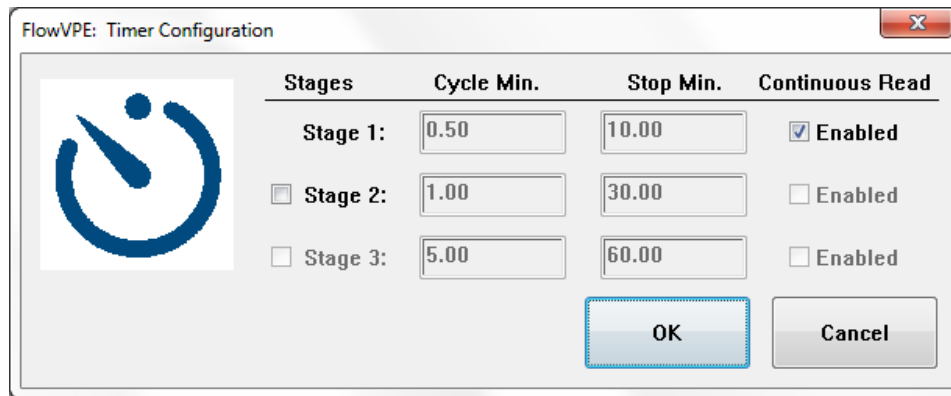


Figure 6-13: KF Timer Configuration Box

3. Click **OK** to return the main screen.
4. Click on **Setup** in the Sidebar.
5. Input the desired start Pathlength, step size, number of data points to be collected, and wavelengths separated by a comma.

One slope will plot for each wavelength entered, consisting of the Absorbance at the starting Pathlength as well as the Absorbance for each consecutive Pathlength up to the input number of data points in increasing intervals of the input step size.

6. For multiple slope configurations, use the drop down menu next to **Slope Configurations**.

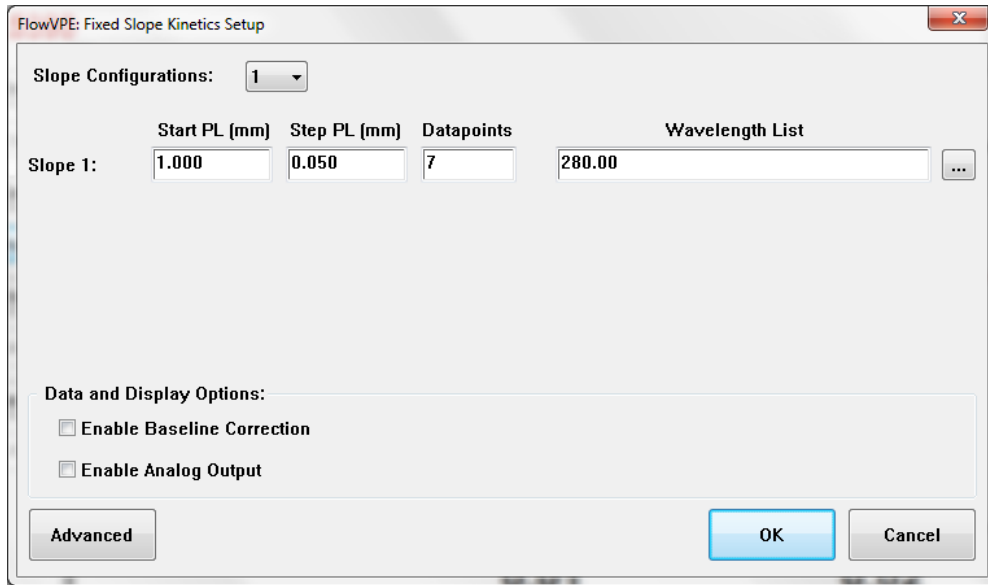


Figure 6-14: KF Setup Box

7. Click on the ... to select which plots will display in the graphics window.

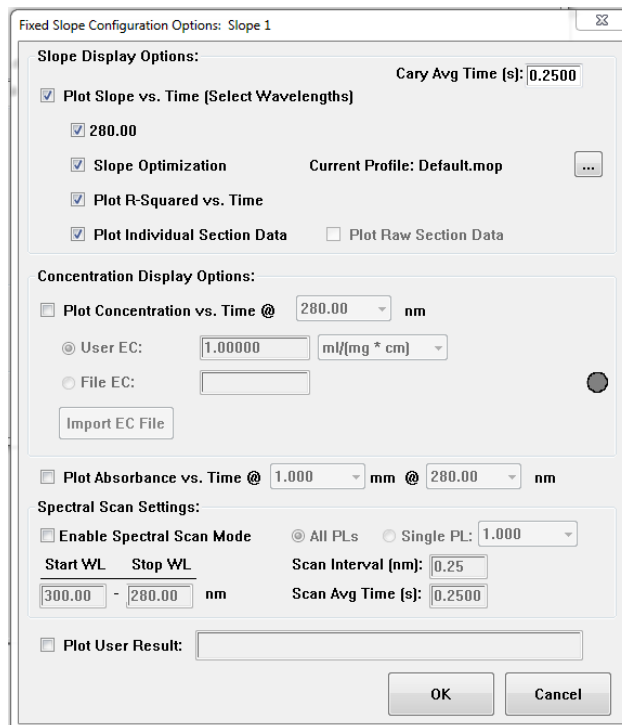


Figure 6-15: KF Setup Slope Configuration Options Box

To plot the concentration, an extinction coefficient must be entered for the solution to be measured.

Additionally, the Cary averaging time can be changed to speed up absorbance readings, and a Spectral Scan Mode can be turned on and configured as well. Both features will require user experimentation to determine the best operating parameters for the application.

8. Be sure to check off the pertinent **Data and Display Options** as needed for baseline correction and Analog Output. See Section 5.1.1.6 for more information on Baseline, and Section 5.1.2.2 for more information on Analog Output Signal configuration.
9. Click on **Advanced** and make sure all check boxes under “Data Collect Settings” are selected. Select the desired data file type from the drop down menu. All section plot data collected during the measurement run will be saved in this format (.DKF for an ADL specific data file, .CSV for a comma separated value list).

*Deselecting the check boxes under “Data Collect Settings” will cause the program to plot each consecutive slope overlaid on the previous one(s). Depending on program configuration, the software may crash after too much data has been collected. Therefore it is **HIGHLY RECOMMENDED** to have all data dumped to an external file to avoid interrupting measurements.*

10. Click **OK** on all setup boxes to return to the home screen.
11. If the previously set parameters will be useful again in the future, save out a Method file (.MKF). A Method file captures all set parameters and can be reloaded to save the user time in run configuration.
12. Input any additional run information by clicking **Run Details** in the FlowVPE Sidebar. This information will be saved with all data in a Batch File.
13. To execute the Fixed Slope run, click **Start** in the FlowVPE Sidebar.
14. Select or create a folder to have data exported and saved into. This is where the .CSV or .DKF files specified earlier will be saved.

15. Click **OK** to finalize the folder location selection. The Flow Head will begin to operate, and charts will populate on the screen.
16. To pause or end the run, click the **Stop** button in the top middle of the screen. The run will pause until either **End Run** or **Resume Run** is selected.



WARNING: Do not use the pause run feature to change anything with the Flow Cell, Fibrette or Flow Clamp hardware. If the Flow Cell and Fibrette need to be unloaded and/or reloaded, click End Run and follow the proper procedures in Sections 4.2 and 4.3.



17. To stop collecting data, click **End Run**. To continue collecting data, click **Resume Run**.
18. To save the data on the screen, click “File – Save Data As”. Select .BKF under file type, and select the folder to save the .BKF file into and click “Save”.

It is recommended to save the .BKF in the same folder as selected previously for the .CSV or .DKF file export.

To start a new run, click on **New Run** in the FlowVPE Sidebar. Click **Setup** to confirm or change parameters, then click **Start**. Repeat steps 13 - 18 as needed.

*All previous data will be cleared from the screen and deleted. Be sure to have saved all data needed before clicking **New Run**.*

7 Maintenance and Troubleshooting

7.1 Routine Maintenance



In order to keep your FlowVPE system operating normally, it is necessary to follow the recommendations in this section. Ignoring these maintenance items may result in inaccurate data and/or decreased product life. For more detailed instructions on proper care refer to *DOC0110 FlowVPE Best Practices* and *DOC00108 Flow Service and Support Options* for more details.

Table 7-1: FlowVPE System Routine Maintenance Schedule

<i>Instrument Maintenance</i>	<i>FlowVPE</i>	<i>Cary Instrument</i>
<i>Daily (or per run)</i>	<ul style="list-style-type: none"> • Transmission Check (See 7.2) • Clean Flow Cell and Fibrette • Unload and Reload Flow Cell and Fibrette 	N/A
<i>Weekly (or per run)</i>	<ul style="list-style-type: none"> • Check Flow Cell window. Clean with IPA if necessary • All Daily items 	<ul style="list-style-type: none"> • Restart the Cary 60 as recommended by Agilent for consistent performance.
<i>Monthly</i>	<ul style="list-style-type: none"> • Run provided standard CHEM013 or your current UV standard in FlowVPE Vessel Holder (See 7.2) • All Daily and Weekly Items 	N/A
<i>Annually</i>	<p><i>For optimal performance have an Authorized C Technologies Service Provider Service your system.</i></p> <ul style="list-style-type: none"> • <i>Services Options:</i> <ul style="list-style-type: none"> ○ <i>Standard Service Contract - Includes Annual PM</i> ○ <i>Standard Multi-Year Contract - Includes Annual PM's</i> ○ <i>GxP Contract - Includes Bi-Annual PM's</i> ○ <i>Preventative Maintenance – Single Day Visit</i> ○ <i>Service Repairs</i> 	

7.2 FlowVPE System Checking and Cleaning

The FlowVPE system has been designed to be compatible with bioprocessing standards, meaning all materials that come in direct contact with process flow and internal cleaning processes are 316 stainless steel, silicone, EPDM or glass. This means that the flow path of the FlowVPE is compatible with all in-

line, internal cleaning flushes, detergents and operations. With respect to cleaning the external surfaces, the vast majority of the rest of the Flow Head is also constructed of 316 stainless steel, including the cover, major Flow Fibrette clamp components, Detector Module and baseplate. However, a main component of the FlowVPE which is exposed under the cover is constructed mainly from anodized aluminum and hardened steel, and is packed with grease. These different materials should be considered when choosing an appropriate cleaning process for the exterior surfaces of the FlowVPE.

7.2.1 Detector Module



For a thorough cleaning, the Detector Module must be removed from the Flow Head. To remove the Detector Module, loosen the 4 screws with the long 9/64" hex driver included in the accessory kit, and lift the Detector Module off of the Flow Head baseplate. This will expose the D-Sub connection in the baseplate of the Flow Head. If there was a large spill previously, the Detector Module may be adhered to the baseplate. Use an appropriate solvent to dissolve and remove any adhering solution. Once the Detector Module is removed, clean or validate it as necessary, and then reinstall on the FlowVPE baseplate.

To clean the Detector Module, take care to not scratch the window by always using a clean, lint-less implement with appropriate solvent, wiping one time per surface of the implement, and only in a single direction. Do not rub back and forth or reuse surfaces of the cleaning implement as this may collect and drag debris across the window causing scratches.

For more information on Validation of the Detector Module, please reference DOC0063.

To reinstall the Detector Module to the baseplate, first ensure proper orientation: the pointed side of the Detector Module dovetail should point out towards the user. Slide the Detector Module down onto the baseplate pins, and press it firmly onto the D-Sub connector in the Flow Head baseplate. Use the long 9/64" driver to tighten the 4 screws of the Detector Module into the base plate.

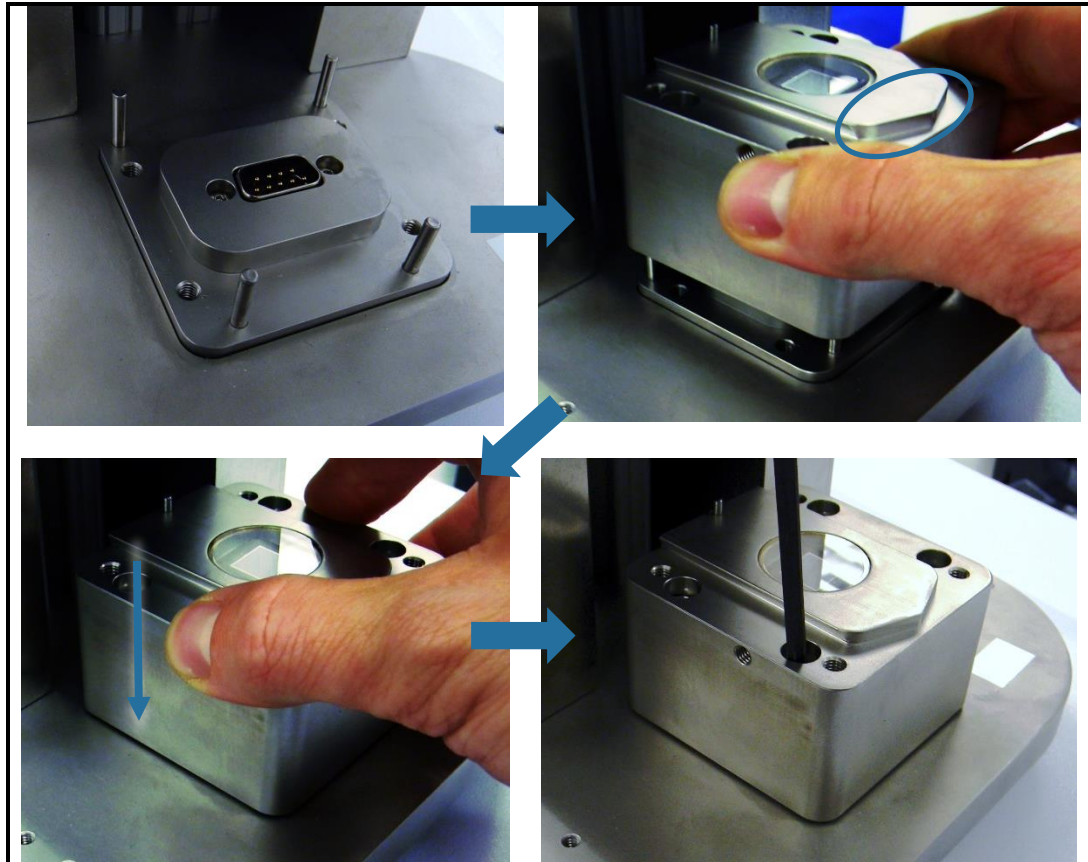


Figure 7-1: Reinstallation of Detector Module

7.2.2 Flow Cell and Flow Fibrette

An interior and exterior cleaning may be necessary for these components. Each Flow Cell ships with blue tape on the exterior of the Flow Cell covering the window. This tape must be removed before operation. If the external of the window needs to be cleaned, take care not to scratch the window. Use a clean, lintless implement with appropriate solvent, wiping one time per surface of the implement, and only in a single direction. Do not rub back and forth or reuse surfaces of the cleaning implement as this may collect and drag debris across the window causing scratches. The Flow Fibrette ships with both ends capped to protect the optical polishes. Remove those caps and, with similar care as the Flow Cell window, wipe each optical surface to ensure cleanliness before insertion into the Flow Cell or connection with the Delivery Fiber.

An internal cleaning process is completed with the Flow Cell and Flow Fibrette properly loaded into the Flow Head; a flush cycle can be run to clean out the interior of the Flow Cell, which includes the optical surfaces of each component. To run a flush cycle, use the flush feature located in the FlowVPE Toolbar (see Section 5.1.2.3). Flushing the system with an appropriate detergent regularly in this fashion will prevent and remove buildup on the Flow Cell window and Flow Fibrette.

7.2.3 Optical Path

Though all the optical surfaces are covered when in use, dust can still collect on them and cause low transmission or other problems. Understanding the optical path and how to clean and check each component will be valuable as it may be a routine maintenance need. Table 7-2 below details all of the optical components in the FlowVPE in sequential order from the Cary 60 sample compartment to the Detector Module on the Flow Head, and how to check them.

Table 7-2: Flow VPE System Optical Surfaces Cleaning Guide

Component	Cleaning and Checking
Coupler Mirror	<ul style="list-style-type: none"> • Remove black cover of the Fiber Optic Coupler. • Wipe with a cotton swab or lintless cleaning implement and IPA. Wipe in one direction and do not reuse cleaning surfaces. • Replace black cover.
Delivery Fiber	<ul style="list-style-type: none"> • Remove from coupler in Cary and FlowVPE. • Clean both end faces of the connectors with a cotton swab or other lintless implement and IPA. Wipe in one direction at a time and do not reuse. • Check the continuity of the Delivery Fiber. While looking at one end of the Delivery Fiber, move the other end in and out of a light source. A broken fiber will not transmit the light or will appear dim, whereas a good Delivery Fiber will have a strong light output.
Flow Fibrette	<ul style="list-style-type: none"> • Remove the Flow Cell and Flow Fibrette. • Clean both end faces of the Flow Fibrette in similar fashion as the Delivery Fiber. • Check the continuity of the Flow Fibrette in similar fashion as the Delivery Fiber.
Flow Cell	<ul style="list-style-type: none"> • Both the interior and exterior surface of the window must be clean and unobstructed. • Ensure the blue tape is removed from the bottom of the Flow Cell • Use a system flush to clean the interior surface of the window. • Wipe the exterior surface of the window in similar fashion as described above.
Detector Module	<ul style="list-style-type: none"> • Ensure the blue tape is removed from the top of the Detector Module window. • Wipe the exterior surface of the Detector Module window in similar fashion as described above.

7.2.4 Transmission Checks

The FlowVPE system comes with two tools that are used to verify the accurate performance of the Flow Head. They are the Transmission Tool and the Vessel Holder. As can be seen, the Vessel Holder mimics a Flow Cell, yet it has been designed to accept a sample vessel, which is included in the FlowVPE accessory kit. The Transmission Tool also slides directly onto the Detector Module, and accepts a direct connection of the Delivery Fiber. The transmission check with the Transmission Tool is a quick verification and should be done before every run. The Vessel Holder is a more robust verification of the entire FlowVPE system.



How To: Using the Transmission Tool

1. Slide the Transmission Tool onto the Detector Module.

It will only fit one way. Look for the groove in the Transmission Tool that fits around the pin on the Detector Module. Make sure both sides of the Transmission Tool engage with the dovetail on the Detector Module. If they are not engaged properly, the Transmission Tool will be unsecure and lopsided, and the Captive Fasteners will not fit into the Detector Module.

1. Tighten down the Captive Fasteners to the Detector Module.
2. Connect the Delivery Fiber to the SC bulkhead adapter.

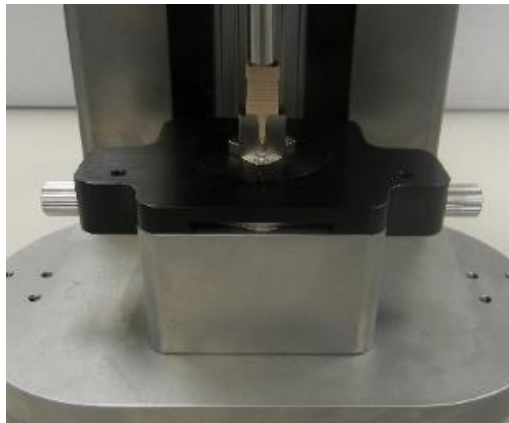


Figure 7-2: Transmission Tool Installed on Detector Module

3. Click on **Load Controls** in the FlowVPE software.
4. Click on the “...” button in the Load Controls Window.
5. The transmission check feature is in the upper right of the box. Input the desired wavelength for a transmission check and click **Read**. The input wavelength will be tested along with 280 nm. The transmission readings will be displayed to the right of the wavelength input box.

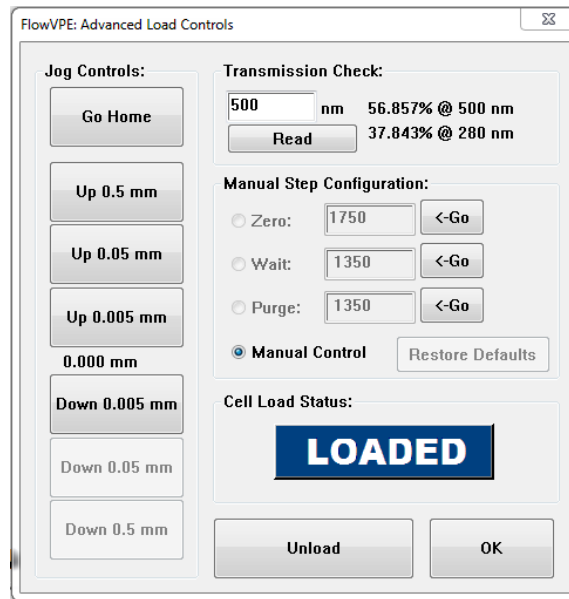


Figure 7-3: Transmission Check in Advanced Load Controls

6. Multiple readings can be taken by changing the wavelength and clicking **Read**. Record any results needed.



How To: Using the Vessel Holder to Verify FlowVPE Functionality

1. Remove the cap of the Vessel Holder by unscrewing it from the Vessel Holder Body.
2. Fill a vessel with 2 mL of Chem013 provided in the FlowVPE Accessory kit. This should fill the vessel with about 10 mm of liquid.
3. Place the filled vessel in to the Vessel Holder.
4. Replace the cap of the Vessel Holder by screwing it down to a hard stop on the Vessel Holder Body.
5. Insert a 10mm Flow Fibrette into the Zero Setting Sleeve of the Vessel Holder. Push the Zero Setting Sleeve all the way up onto the Fibrette
6. Insert the Flow Fibrette with Zero Setting Sleeve into the Vessel Holder Cap. Push down on the Zero Setting Sleeve to seat it into the Vessel Holder Cap, and to straighten the Flow Fibrette.
7. Load the Vessel Holder and Fibrette like a Flow Cell and Fibrette – following the procedure laid out in Section 4.2.

Be careful not to tilt the Vessel Holder unnecessarily as your standard may spill!

Ensure the Vessel Holder is properly engaged with the Detector Module, otherwise it will be tilted and the Flow Fibrette will not fit properly into the Fibrette Clamp and be damaged during clamping.

8. Run the verification test. See Section 6 for detailed instructions on setting up the software for testing.
9. Remove the Vessel Holder using instructions in Section 4.3.

10. Pull out the Flow Fibrette with the Zero Setting Sleeve from the Standard and Vessel Holder Cap. Wipe the tip of the Fibrette before pulling off the Sleeve. Remove the Vessel Holder Cap from the Vessel Holder Body.



Do not let the Chem013 sit in the vessel longer than 1 hour. After 1 hour, replace it before running another test.

11. Dump out the standard and thoroughly clean the glass vessel.
12. Store the Vessel Holder, Flow Fibrette and vessel in a secure location until the next use.

Note: For a Vessel Holder inscribed with a serial number (VAL-XXXX), please reference DOC0095 – FlowVPE Vessel Holder Instructions

7.3 General Troubleshooting

Table 7-3 below describes some undesired behaviors you may see, what causes them and how to resolve it. *If an answer to your problem cannot be found here, please contact the C Technologies Support line for more help.*

Table 7-3: FlowVPE System Troubleshooting

Problem	Recommended Action
Cary 60 unpowered	<ul style="list-style-type: none"> • Make sure the power supply is connected to the Cary 60, and into a powered outlet. • Make sure green light on the AC adapter is lit.
Flow Fibrette Doesn't Fit into Clamp During Load Process	<ul style="list-style-type: none"> • Verify the Flow Cell is seated properly on the Detector Module. • Verify the Fibrette is pushed all the way down to the window of the Flow Cell. • Remove the Flow Cell from the Detector Module completely. Send the Flow Head to the Home Position, then back to the Load Position. • Verify the Fibrette Clamp is open all the way by pushing back on the Clamp Handle.
Leaking Flow Cell at Flow Fibrette Interface	<ul style="list-style-type: none"> • Visually check the overall straightness of the Flow Fibrette. • Ensure the screws holding down the Detector Module are securely fastened.

	<ul style="list-style-type: none"> • Properly reload the Flow Cell and Fibrette. Ensure the screws holding down the Flow Cell are securely fastened to the Detector Module.
Leaking Flow Cell Under Cap	<ul style="list-style-type: none"> • Remove the Flow Cell Cap and check for proper seal seating.
Leaking Flow Cell at Flow Cell to Process Tubing Adapter Interface	<ul style="list-style-type: none"> • Ensure the process tubing adapter is securely fastened to the Flow Cell. • Remove the adapters and check for proper seal seating.
Leaking Flow Cell at Bottom Cover	<ul style="list-style-type: none"> • Tighten bottom cover screws in a star pattern.
No Transmission	<ul style="list-style-type: none"> • Make sure the FlowVPE detector cable is properly connected inside the Cary 60 sample compartment. • Check for and remove any blue tape on the Detector Module window, Flow Cell window, or Coupler mirror. • Check continuity of Delivery Fiber and Flow Fibrette – See Section 7.2.3.
Low Transmission	<ul style="list-style-type: none"> • Restart the Cary 60 for a recalibration. • Check all optical connections and clean all optical surfaces – See Section 7.2.3. • Check continuity of Delivery Fiber and Flow Fibrette – See Section 7.2.3.
FlowVPE Not Moving When It Should Be	<ul style="list-style-type: none"> • Confirm power to Cary 60 and Cary 60 powered on. • Ensure FlowVPE connection with Cary 60 is securely fastened on both ends. • If the “Connect” button is shown on the software window, click on it to connect the Cary 60 to the software control. • If multiple software windows are open, close them all and open only one new window.
Handle Lock not Locking behind Clamp Handle	<ul style="list-style-type: none"> • Ensure the Handle is clamped completely on the Fibrette Flange • Loosen the 2 screws that mount the Handle Lock to the Fibrette Clamp. • Reseat the Handle Lock so the Handle Lock is horizontal and in contact with the Clamp Handle. • Retighten the 2 screws that mount the Handle Lock to the Fibrette Clamp.
Plateau in Section Plot Upper End	<ul style="list-style-type: none"> • Ensure sufficient sample flow through Flow Cell. • Run a Slope Inquiry to determine appropriate Pathlength range for measurements at the current sample concentration.
Plateau in Section Plot Lower End or	<ul style="list-style-type: none"> • Run a Slope Inquiry to determine appropriate Pathlength range for measurements at the current sample concentration. • Ensure the Fibrette Clamp is properly clamped and locked.

Entirely Flat Section Plot	
Noisy Concentration or Section Plot Measurements	<ul style="list-style-type: none"> • Ensure there are no bubbles in the Flow line. • Ensure the Fibrette Clamp is properly clamped and locked.
Noisy Spectra	<ul style="list-style-type: none"> • Check all optical connections and clean all optical surfaces – See Section 7.2.3. • Check for bubbles in the Flow Cell or process tubing.
Missing Concentration Change Data in FlowVPE Plots	<ul style="list-style-type: none"> • Change data collection rate to match concentration change rate. This can be done by changing the Averaging Time of a data point, step size between data points and number of data points in a slope collection.
No Signal From DAQ Hardware	<ul style="list-style-type: none"> • Ensure the hardware is wired properly. • Check for proper setup in the FlowVPE software. See Section 5.1.2.2 for details.

8 Appendices

Additional useful information...

8.1 The Derivation of the Slope Spectroscopy® Equation

The Slope Spectroscopy® Equation is derived from the Beer-Lambert Law and standard equation of a line as follows:

Beer – Lambert Law	Linear Equation
$A = \alpha l c$ <p>where:</p> <ul style="list-style-type: none"> ▪ A is the <u>absorbance</u> of the sample ▪ l is the distance that the light travels through the material (the <u>path length</u>) ▪ α is the <u>absorption coefficient</u> or the <u>molar absorptivity</u> of the absorber ▪ C is the <u>concentration</u> of absorbing species in the material 	$y = mx + b$ <p>where:</p> <ul style="list-style-type: none"> ▪ y is the <u>predicted</u> value of the dependent variable / axis. ▪ m is the slope of the line ▪ X is the given variable. ▪ b is the y-intercept of the line.

Derivation and Version of the Slope Spectroscopy® Equation

$$A = \alpha l c$$

$$\frac{A}{l} = \alpha c$$

We know $\frac{A}{l} = m$

Therefore $m = \alpha c$

and

$$\frac{m}{\alpha} = c \quad \frac{m}{c} = \alpha$$

8.2 How Scatter and Baseline Corrections Work

8.2.1 Baseline Correction

There is an option inside the FlowVPE software applications allowing users to run a Baseline Correction with their sample. While running a Baseline Correction will not cause any issues with data obtained, it may be unnecessary to perform this action. Generally the FlowVPE does not require Baseline Corrections because the user is not looking at absolute absorbance. The FlowVPE instead uses Slope to determine the concentration. If the buffer being used does not have a slope (or little to none), then it should not contribute to the actual slope of the sample. Comparing the corrected slope with a non-corrected slope will most likely show the same results.

With certain buffer types, such as ones containing methanol, Baseline Correction may be needed, most specifically when there is a non-zero slope that contributes to the sample or has Pathlength dependency. Another reason a user might need Baseline Correction is if the sample is very dilute, less than 1 mg/mL. When measuring a sample that is very dilute, it is important to remember that even a small non-zero buffer slope could be significant when measuring a sample that should already have a small slope.

To find out if Baseline Correction is needed, a user may run a Slope Inquiry on the buffer sample as if it were the actual sample. The slope value obtained from running the Slope Inquiry will not be exactly zero. This is due to noise and optical loss from the Cary instrument to the FlowVPE. If the slope value is less than 0.01, Baseline Correction is not needed to run the sample. Another value to check is the R-Squared value. If it is a poor value, well below 0.99, the slope value result is most likely noise. See Figure 6.4.1.1 below.

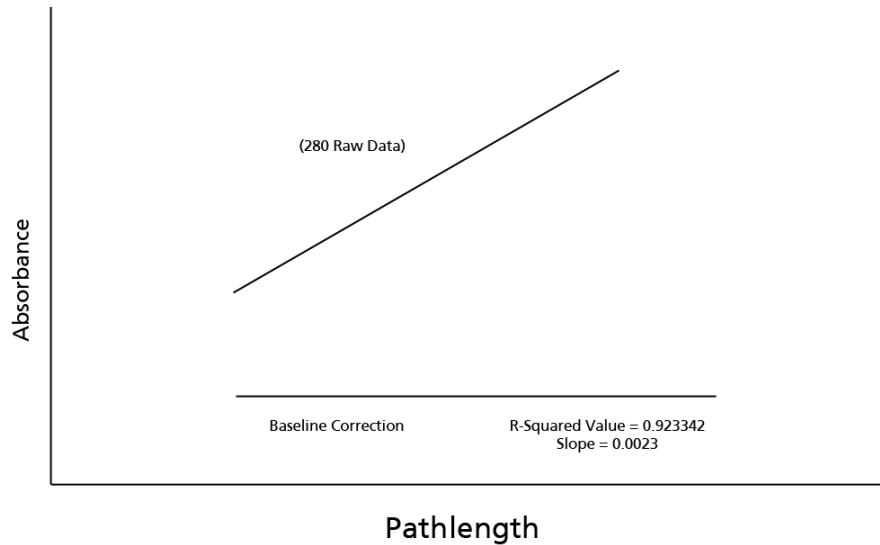


Figure 8-1: Poor R-Squared Value and Low Slope Value

If the value is 0.999 or higher than it is possible that the Pathlength dependence of the buffer is real and Baseline Correction will be needed.

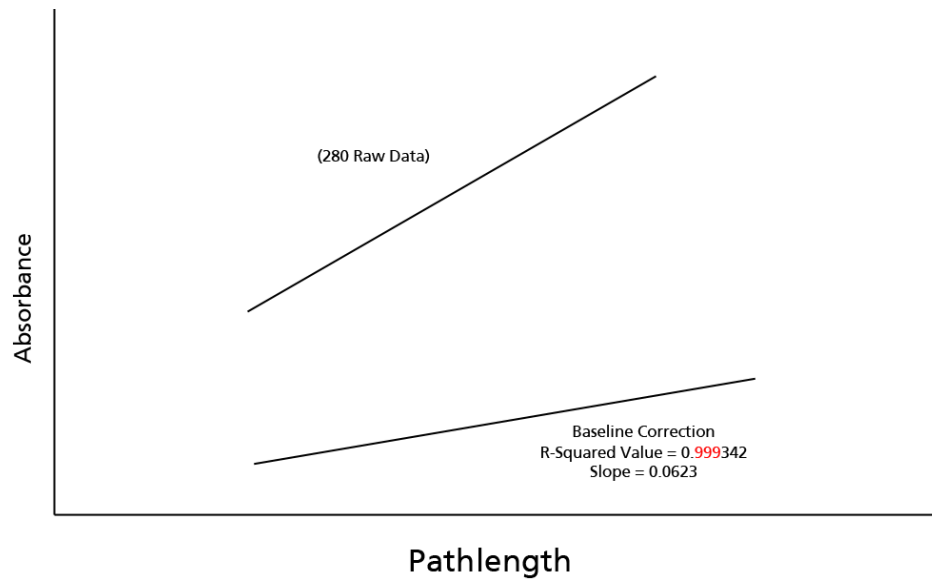


Figure 8-2: Baseline Correction Needed; Good R-Squared Value and High Slope Value

The baseline tests can be run in the FlowVPE Vessel Holder instead of a Flow Cell, to save materials and setup time. Be sure to fill the vessel to at least 10 mm of solution for an adequate sample size, so that the Flow Fibrette is constantly immersed in the solution during measurement.

8.2.2 Single Wavelength (SW) Scatter Correction:

SW Scatter Correction works by taking data points at two different wavelengths at the same Pathlengths. It then subtracts the absorbance at the Scatter Wavelength from the Absorbance at the Method Wavelength to create a Corrected Absorbance at the specified method wavelength. This will isolate the absorbance contribution of the key species being measured at the method wavelength.

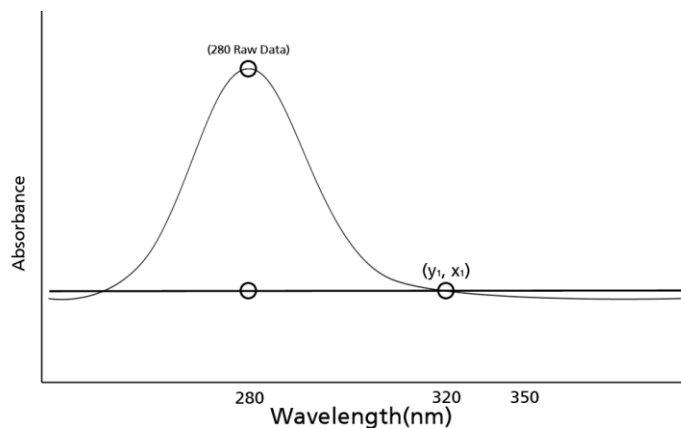


Figure 8-3: Data Collected Before Scatter Correction with SW Scatter

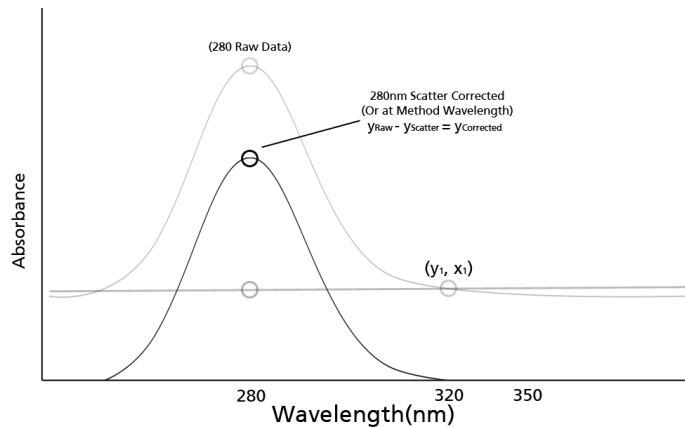


Figure 8-4: The Corrected Absorbance at the Method Wavelength

8.2.3 Dual Wavelength Scatter Correction:

Dual Wavelength Scatter Correction works differently than Single Wavelength Scatter Correction. Instead of subtracting one slope line from the other and shifting the results down, DW will use the two scatter wavelengths to project an absorbance value to be subtracted from your expected wavelength. The dual wavelength feature is similar to a 3-Point drop correct method, which quantifies a sloping scatter contribution line and makes a correction based off of the absorbance values obtained. Each individual Pathlength will be calculated for separately, point for point as the section data is collected.

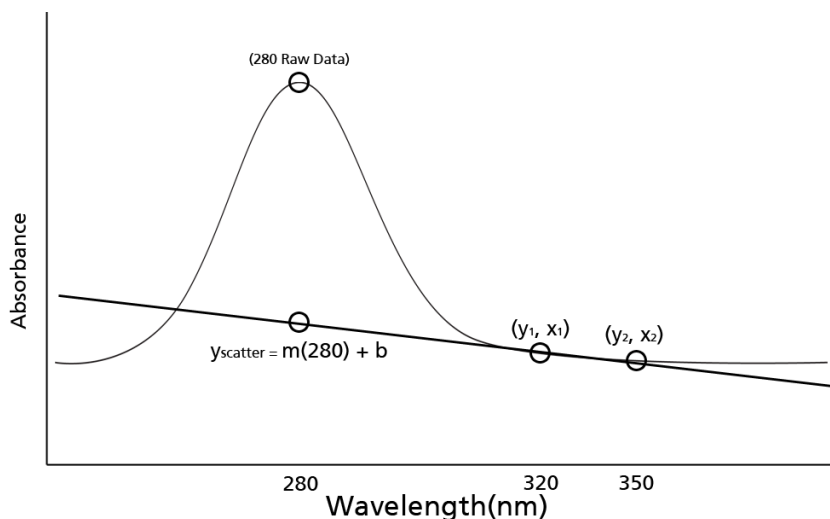


Figure 8-5: Data Collected Before Scatter Correction Occurs

Figure 8-5 shows the raw data taken before any scatter correction is calculated. In the above example the projected points will come from the 320nm and 350nm data points all taken at the same Pathlength. The scatter contribution is determined by defining the line that passes through those two points using the equations below.

$$m = \frac{y_2 - y_1}{x_2 - x_1}$$

Where X and Y are points taken from the 320nm and 350nm wavelengths.

$$y_1 = mx_1 + b$$

After solving for the slope of the projected line, it is plugged in to solve for the y-intercept.

$$b = y_1 - mx_2$$

Once b has been solved for, we are able to calculate the corrected y -abs value by subtracting the absorbance from the scatter value from the absorbance at the method wavelength.

$$y_{Raw} - y_{Scatter} = y_{Corrected}$$

(At the method wavelength)

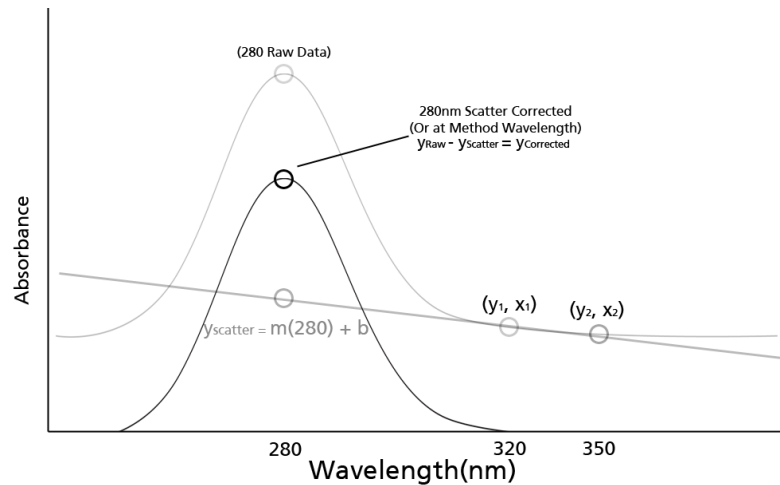


Figure 8-6: Corrected Absorbance at the Method Wavelength

After performing the above calculation for each Pathlength, the slope of the section data will remove the contribution of scatter. Figure 8-6 shows the Corrected Absorbance at the method wavelength after subtraction.

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