User's Guide

**PA 800 *plus*
Pharmaceutical Analysis
System**

Methods Development

**BECKMAN
COULTER**

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Revision History

Third Revision, A51965AD, October
Change to LIF Detector Test Mix part number

Second Revision, A51965AC, February 2011
32 Karat Software version 9.1 patch
PA 800 *plus* Software version 1.1 patch
PA 800 *plus* Firmware version 9.2
Numerous syntax and grammatical edits

First Revision, A51965AB, February 2010
Revised corporate address.

Initial Issue, A51965AA, April 2009
32 Karat Software version 9.1
PA 800 *plus* Software version 1.1
PA 800 *plus* Firmware version 9.0

Safety Notices

Symbols and Labels

Introduction

The following is a description of symbols and labels used on the Beckman Coulter PA 800 *plus* Pharmaceutical Analysis System or shown in this manual.

> ⚠️ **WARNING**
>
> **If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the instrument may be impaired.**

General Biohazard Symbol

This caution symbol indicates a possible biohazard risk from patient specimen contamination.

A011460L.PNG

Caution, Biohazard Label

This caution symbol indicates a caution to operate only with all covers in position to decrease risk of personal injury or biohazard.


456161-B

CAUTION
TO REDUCE RISK OF PERSONAL INJURY,
OPERATE ONLY WITH ALL COVERS IN PLACE.

A011459L.EPS

Caution, Moving Parts Label

This caution symbol warns the user of moving parts that can pinch or crush.

A015081L.EPS

High Voltage Electric Shock Risk Symbol

This symbol indicates that there is high voltage and there is a risk of electric shock when the user works in this area.

A016352L.EPS
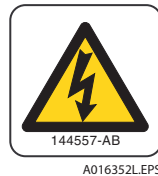
Class 1 Laser Caution Label

A label reading "THIS PRODUCT CONFORMS TO APPLICABLE REQUIREMENTS OF 21 CFR 1040 AT THE DATE OF MANUFACTURE" is found near the Name Rating tag. The laser light beam is not visible.

A016350L.EPS
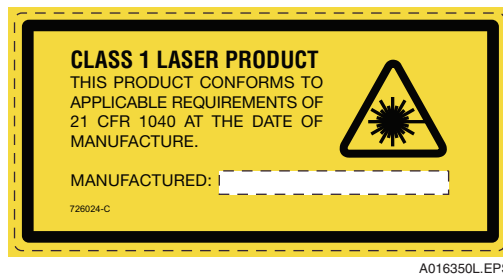
Sharp Object Label

A label reading "CAUTION SHARP OBJECTS" is found on the PA 800 *plus*.

A016351L.EPS

Recycling Label

This symbol is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:

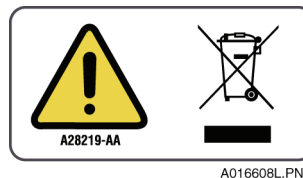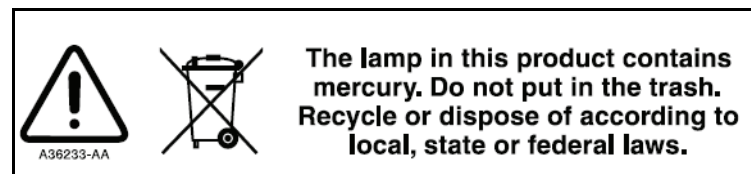1. The device was put on the European Market after August 13, 2005.

2. The device is not to be disposed of via the municipal waste collection system of any member state of the European Union.

A016608L.PNG

It is very important that customers understand and follow all laws regarding the proper decontamination and safe disposal of electrical equipment. For Beckman Coulter products bearing this label, please contact your dealer or local Beckman Coulter office for details on the take back program that facilitates the proper collection, treatment, recovery, recycling, and safe disposal of this device.

Disposal of Devices Containing Mercury Components

The lamp in this product contains mercury. Do not put in the trash. Recycle or dispose of according to local, state or federal laws.

This product contains a mercury-added part. Recycle or dispose of according to local, state, or federal laws. It is very important that you understand and comply with the safe and proper disposal of devices containing mercury components (switch, lamp, battery, relay, or electrode). The mercury component indicator label can vary depending on the type of device.

Restriction of Hazardous Substances (RoHS) Labels

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

RoHS Caution Label

This logo indicates that this electronic information product contains certain toxic or hazardous elements, and can be used safely during its environmental protection use period. The number in the middle of the logo indicates the environmental protection use period for the product. The outer circle indicates that the product can be recycled. The logo also signifies that the product should be recycled immediately after its environmental protection use period has expired. The date on the label indicates the date of manufacture.

RoHS Environmental Label

This logo indicates that the product does not contain any toxic or hazardous substances or elements. The "e" stands for electrical, electronic, and environmental electronic information products. This logo indicates that this electronic information product does not contain any toxic or hazardous substances or elements, and is green and is environmental. The outer circle indicates that the product can be recycled. The logo also signifies that the product can be recycled after being discarded, and should not be casually discarded.

Alerts for Warning, Caution, Important, and Note

⚠️ **WARNING**

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. The warning can be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠️ **CAUTION**

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. The caution can be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT notice adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Contents

Contents

Preface

Overview

The PA 800 *plus* Methods Development User's Guide is a self-paced introduction to the operation of the Beckman Coulter PA 800 *plus* Pharmaceutical Analysis System and the 32 Karat Software, version 9. It contains a brief introduction to the technique of capillary electrophoresis (CE), an overview of the PA 800 *plus* hardware, tutorials for the most frequently used software features, and appendices of useful information.

The PA 800 *plus* Methods Development User's Guide has been designed to assist users who are new to CE or to 32 Karat Software. It is largely built on step-by-step exercises that allow you to create methods, to generate and analyze data, and to report results. It does not attempt to cover every possible feature in the system or every aspect of capillary electrophoresis.

The on-instrument activities contain specific instructions for users with ultraviolet (UV), photodiode array (PDA), and laser-induced fluorescent (LIF) detector systems. The activities are progressive. Each builds on the previous one. To get the most from this manual, work through all the activities in order.

The activities that teach data reprocessing, calibration, and reporting, are all based on data collected with an ultraviolet detector system. The steps in these procedures are very similar regardless of the detector type used, so these exercises are important for users of all detector types. Data files for the post-run exercises are installed with the 32 Karat Software.

Additional information can be found in the 32 Karat Online Help. This can be accessed at any time while using the 32 Karat Software. You can access Help through the Help menu located on the menu bar, by pressing the F1 key while working within the software, or any time you see a Help button displayed within an application window.

This manual was created by a team of technical writers, scientists, and engineers at Beckman Coulter. We value your feedback. Please let us know how we can improve future editions of this manual.

Send your comments to: 32karat@beckmancoulter.com, and include Methods Development User's Guide in the subject line. Questions concerning the exercises can be sent to the same address.

Instructions for Using the Data Samples

In some of the exercises in this manual, you are asked to open specific data and method files. These are located in the data folder, which is a sub-directory of the 32Karat folder created when your software was installed.

IMPORTANT Make sure to read and understand the following before using the sample data files.

The data folder contains the following files:

- CE Data Sample 1.dat
- CE Data Sample 2.dat
- CE Data Sample 3.dat
- CE Level 1.dat
- CE Level 2.dat
- CE Level 3.dat
- CE Level 4.dat
- CE Level 5.dat
- CE Mobility Data.dat
- CE PDA Data Sample.dat
- CE Unknown 1.dat
- ISTD Calib Level 1 Rep 1.dat
- ISTD Calib Level 1 Rep 2.dat
- ISTD Calib Level 2 Rep 1.dat
- ISTD Calib Level 2 Rep 2.dat
- ISTD Calib Level 3 Rep 1.dat
- ISTD Calib Level 3 Rep 2.dat
- ISTD Calib Level 4 Rep 1.dat
- ISTD Calib Level 4 Rep 2.dat
- multi calibration level 1.dat
- multi calibration level 2.dat
- multi calibration level 3.dat
- multi calibration level 4.dat
- multi calibration level 5.dat
- multi calibration level 6.dat

The methods folder contains the following files:

- CE calibrate.met
- multilevelcalibration.met
- pda estd.met

Purpose of These Files

The Data folder contains data and method files to supplement the exercises in this guide. The files were created solely for the purposes of this tutorial. They are intended as training tools only and are not to be used for system validation or any other purpose.

Using the Files

These files are referred to by name in this guide. For some of these exercises, you are asked to open one or more of these files. By using these files, the images you see on the computer screen and the images in the guide are the same.

IMPORTANT Duplicate these files before use.

Processing these data files and methods in the 32 Karat Software causes changes to the files. To keep the original files unaltered and available for later use in these exercises, please follow the steps below.

1 Open Windows Explorer. To open this utility, right-click on the **Start** button in the Windows toolbar and select **Explore**.

2 Navigate to the Data folder. It is a sub-directory of the 32Karat folder.

3 In the left-hand pane of the Explorer window, click on the Data folder to open it.

4 From the menu bar, select **File > New > Folder**.   A new folder is added to the Data folder. The New Folder is highlighted, which indicates the name may be edited. Type a new name. A good choice is the name of the individual who is doing the tutorial exercises.

5 Select all of the data files listed above, but do not select the new folder. There are several ways to do a multiple file select.

Use any one of the following:
- Select the first file in the list by clicking on it. Hold down the Shift key and click on the last file in the list. All the files between these two files are selected.
- Select a file by clicking on it. Hold down the **Ctrl** key and click each individual file in the list.
- Using the mouse, you can click and drag a box around all the files to be selected.

6 When all the files are selected (highlighted), select **Edit > Copy** from the menu bar. Double-click on the new folder to open it. Then select **Edit > Paste** from the menu bar. Repeat steps 1-6 for the methods folder.

You have now created a copy of the sample files in a new folder. When the manual asks you to open a file, open the copy you have just created, not the original file.

⚠ **WARNING**

DO NOT USE THE ORIGINAL FILES!

Save all methods and sequences you create as part of the tutorial in this new folder.

Introduction to CE

Overview

This section describes the common modes of capillary electrophoresis and how these processes are performed by the PA 800 *plus* instrument using the ultraviolet (UV), photodiode array (PDA) and laser-Induced fluorescence (LIF) detectors.

General Description

The PA 800 *plus* instrument separates sample components within a fused-silica capillary, using one of several modes of electrophoresis. All of these modes are generally referred to as Capillary Electrophoresis (CE).

In the PA 800 *plus*, sample is injected into the capillary using either pressure, vacuum, or voltage. Under the influence of an electric field, sample components migrate differentially through the capillary.

There are two basic methods for detecting samples using CE. The first is the absorbance of light. This method is used in UV and PDA detection. As these components pass a window in the capillary, a single wavelength UV detector or a multiwavelength photodiode array detector (PDA) measures absorbance and transmits the signal to the computer. The signal can also be transmitted to an external recorder, integrator, or data system through an analog output. The signal may be plotted graphically in the form of an electropherogram and analyzed.

The second method is to induce the samples to fluorescence and measure the emitted light. This is done by laser-induced fluorescence (LIF). Substances in the capillary that fluoresce at the laser wavelength are detected. The LIF Detector measures and records this fluorescence, which appears as a peak on the computer screen or printed electropherogram.

The PA 800 *plus* instrument may be used to separate many different kinds of samples, including peptides, proteins, nucleic acids, ions, enantiomers, and pharmaceuticals. CE has very low sample requirements relative to other analytical techniques (50 microliters, with the actual injection volume being typically between 5 and 50 nanoliters). It provides a complementary alternative to other separation techniques such as chromatography.

Common Modes of Electrophoresis

Capillary Zone Electrophoresis (CZE)

When voltage is applied to an uncoated fused-silica capillary tube filled with a uniform electrolyte solution, separation of charged species occurs due to differential migration in the electrical field. This process, in which charged particles in solution migrate toward an electrode with an opposite charge, is called electrophoresis. If the inner wall of the capillary is also charged, the fluid in the capillary begins to flow toward the electrode that has the same charge as the capillary wall. This bulk movement of fluid is termed electroosmotic flow or EOF.

Because EOF is usually of higher magnitude than electrophoresis, analytes of both positive and negative charge are ultimately carried in the same direction, although at different rates. Electrophoresis separates like-charged, but otherwise distinct analytes, from one another. In this manner, both positive and negative analyte molecules can be detected as they are swept past the detector. The direction of EOF can be changed by reversing the charge on the electrodes, or by changing the charge on the wall of the capillary by chemical means.

The amount of time required for sample molecules to migrate to the detector depends on the length of the capillary, the electrophoretic mobilities of the particular sample molecules, the specific electrolyte used, the magnitude of the EOF, and the applied voltage. Molecules having different electrophoretic mobilities are detected at different times. Other factors to be considered are the size, shape, and charge of the particles, the electrolyte concentration, the pH of the separation buffer, and the dimensions of the capillary.

Micellar Electrokinetic Capillary Chromatography (MEKC)

In free-zone capillary electrophoresis, neutral molecules travel as a single band. To separate these molecules, micellar additives such as sodium dodecyl sulfate (SDS) can be added to the electrolyte solution. Neutral molecules with differing affinities for the charged micelles separate during electrophoresis under these conditions.

Capillary Gel Electrophoresis (CGE)

In the techniques described above, the analyte molecules are moving in a low viscosity liquid. By increasing the viscosity of this medium to the point where the migration of molecules is physically impeded, it is possible to separate different species based on size and shape, as well as charge. A variety of gel solutions can be employed in this technique.

Capillary Isoelectric Focusing (cIEF)

In cIEF, a mixture of special buffers called ampholytes is used to create a pH gradient along the length of the capillary. During separation, molecules migrate to the point in the pH gradient at which they have no net charge. At the end of the process, the various analytes are located in discrete zones in the capillary. In a separate mobilization step, the bands are moved past the detector for analysis. This technique is most often used for protein and peptide analysis.

Capillary Electrochromatography (CEC)

CEC uses a capillary packed with porous particles similar to those used in HPLC. Driven by EOF, analytes partition between mobile and stationary phases as they migrate down the capillary. Under some conditions, electrophoresis can also participate in the separation. CEC is primarily employed to separate small molecules such as drugs.

System Overview

Instrument

The main components of the PA 800 *plus* Pharmaceutical Analysis System include trays that hold vials of sample, buffer, and other solutions, an interface block, a high-voltage power supply and electrodes, a source optics module and detector, temperature control hardware, and a sample injection mechanism. See Figure 2.1, Figure 2.2, and Figure 2.3.

Figure 2.1 PA 800 *plus* System
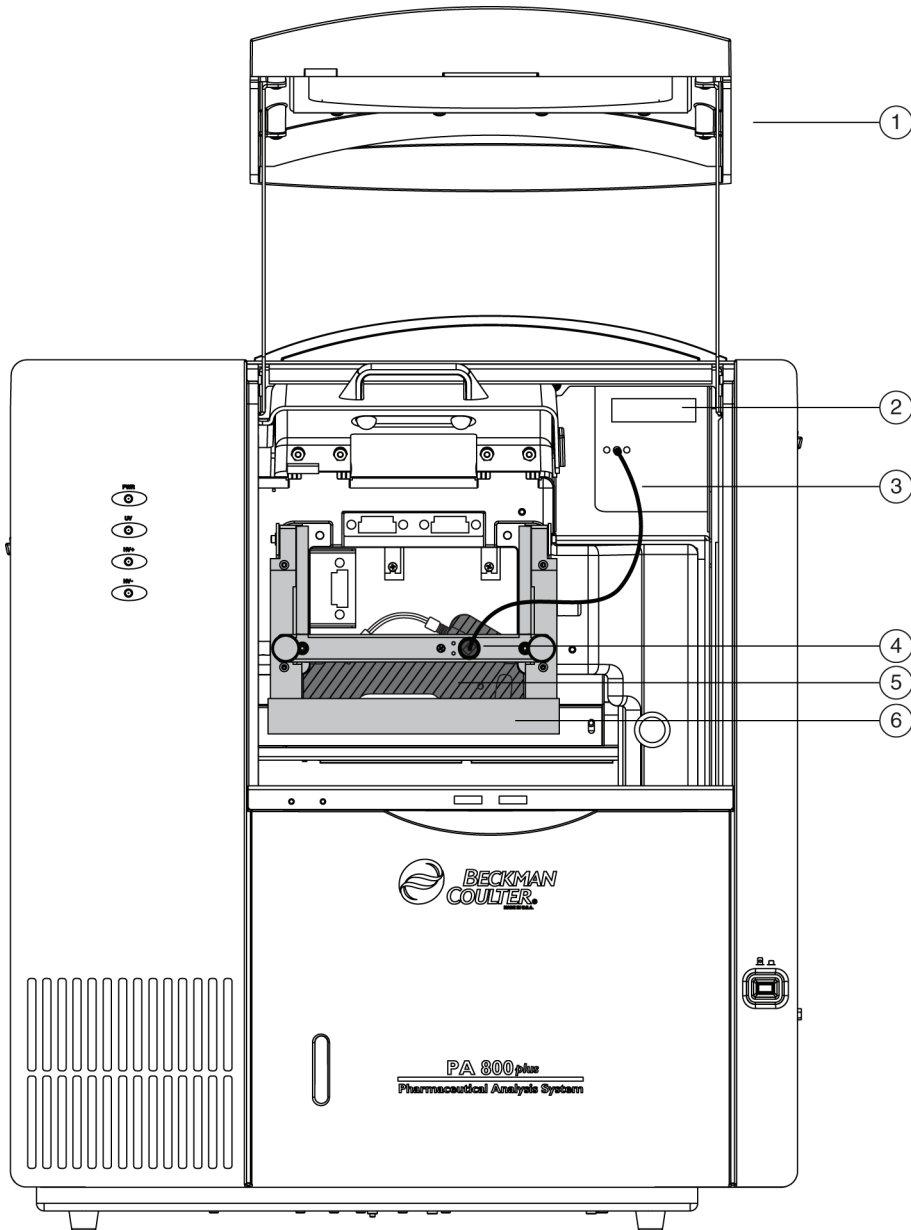
1. Outer Door (open position)
2. UV Detector
3. Two-ended Fiber Optic Cable
4. Clamp Bar and Cable Connection
5. Capillary Cartridge
6. Interface Block

Figure 2.2 PA 800 *plus* System Side View
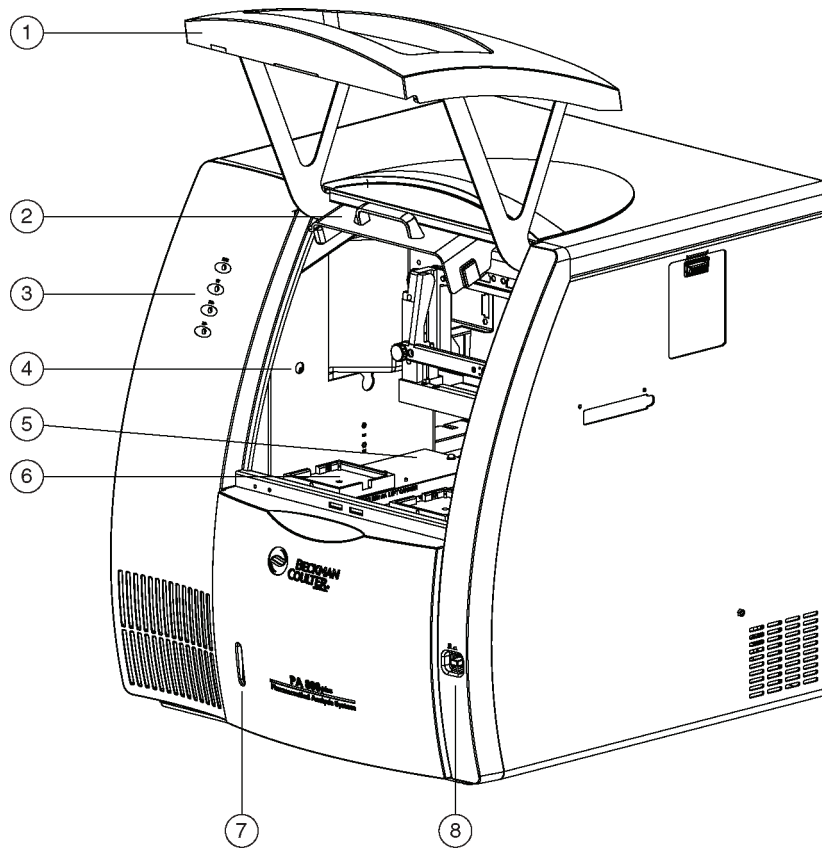
1. Outer Door or Sample Cover (open)
2. Inner Door or Cartridge Cover (open)
3. Indicators
4. Fluid Fill Port
5. Sample Trays
6. Buffer Trays
7. Fluid Bubble Indicator
8. Power Switch

The main power switch is on the lower right side of the front of the instrument. All connections for external system components are on the upper-left side panel of the instrument, except for the AC inlet and the fuse holder. Three fans supply cooling air flow for internal system components. Air is exhausted through the vents at the side and back of the instrument. Keep at least six inches of clearance at each vent to ensure adequate air flow.

The Sample Handling System

The sample handling system holds four trays; two sample trays (inlet and outlet), and two buffer trays (inlet and outlet). The sample trays are primarily used for samples; the buffer trays hold the other solutions required for electrophoresis (for example, buffer and rinse solutions). The trays are on two parallel tracks. Under normal operating conditions, the trays on the left are inlet trays for sample and buffer; the trays on the right are outlet trays for sample and buffer. See Figure 2.3.

Figure 2.3 PA 800 *plus* Trays
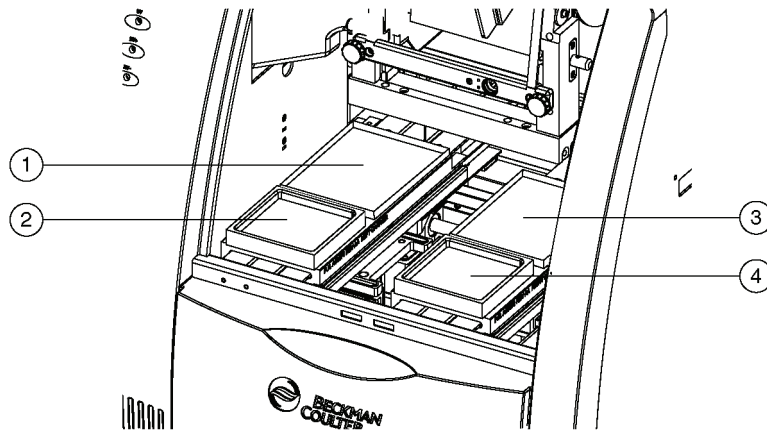
1. Inlet Sample Tray (48) or 96-Well Plate
2. Inlet Buffer Tray (36 Vials)
3. Outlet Sample Tray (48) or 96-Well Plate
4. Outlet Buffer Tray (36 Vials)

Each buffer tray has slots for 36 universal vials. Sample trays hold either 48 vials or a 96-well plate. Each tray is assigned a number from the front to the back, starting with the number 1, and assigned a letter from left to right, starting with the letter A. See Figure 2.4.

Figure 2.4 Sample and Buffer Trays
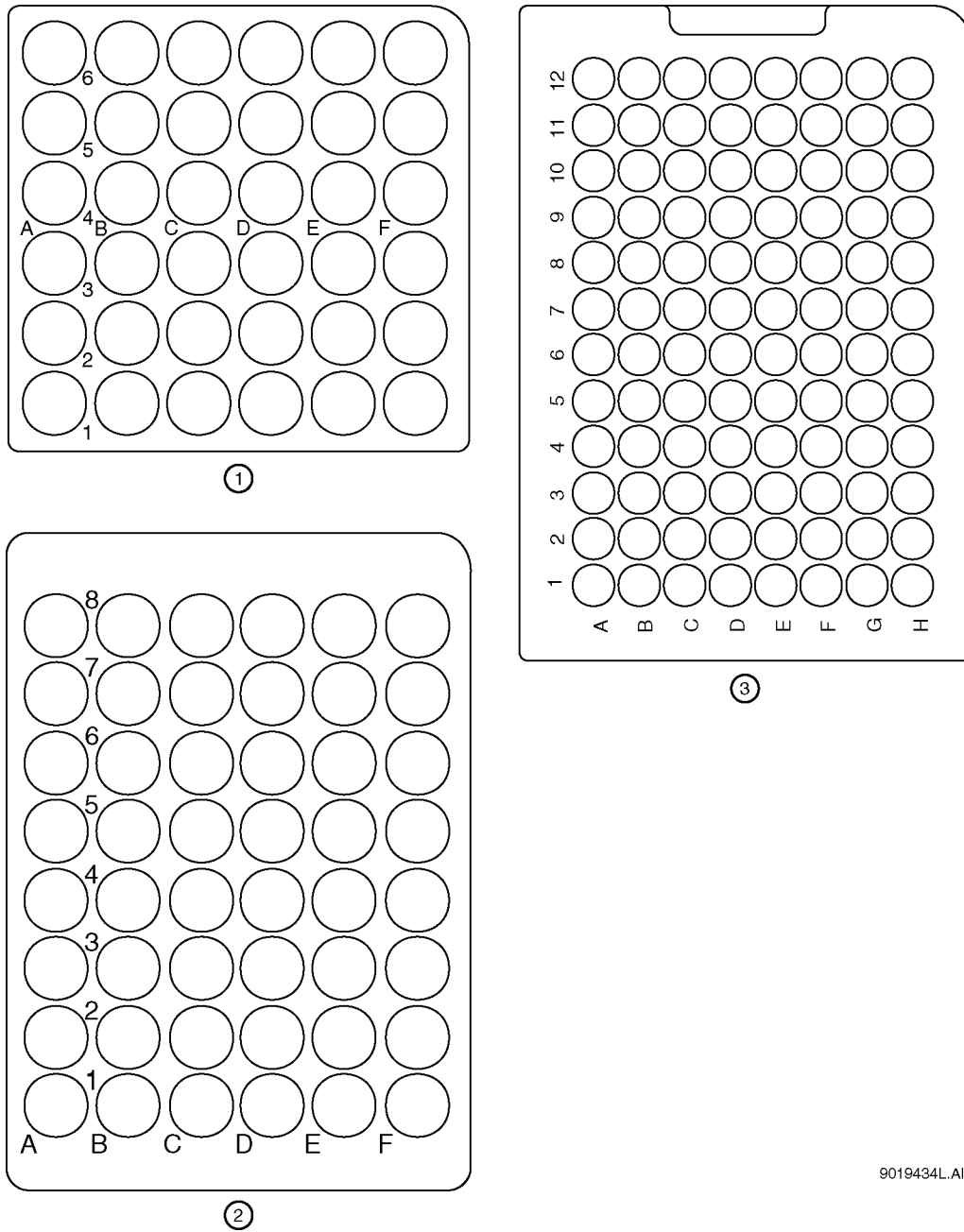
1. Buffer Tray
2. 48-Vial Sample Tray for universal vials or universal vials holding microvials
3. 96-Position Sample Tray

> ⚠️ **WARNING**
>
> **The PA 800 *plus* Pharmaceutical Analysis System is not intended to accommodate volatile materials in 96-well plates. Volatile solvents can release hazardous or flammable vapors leading to a risk of fire or explosion. The solvent vapors can damage the instrument. Do not use volatile solvents in 96-well plates.**

> ⚠️ **WARNING**
>
> **Wear safety glasses when opening the sample cover while the vials are pressurized.**
>
> **The universal vials are pressurized during rinse and separation-with-pressure events. To reduce the risk of breakage and expelled particles, use only Beckman vials PN A62251, and inspect every vial for damage before use. Do not use any vial that appears cracked or damaged in any way.**

Capillary Cartridge

The separation capillary is installed in a cartridge. The cartridge design protects the capillary, supplies a path for liquid coolant, simplifies installation into the instrument, and aligns the detection window in the optics. The components of the cartridge are shown in Figure 2.5.

Figure 2.5 The Capillary Cartridge

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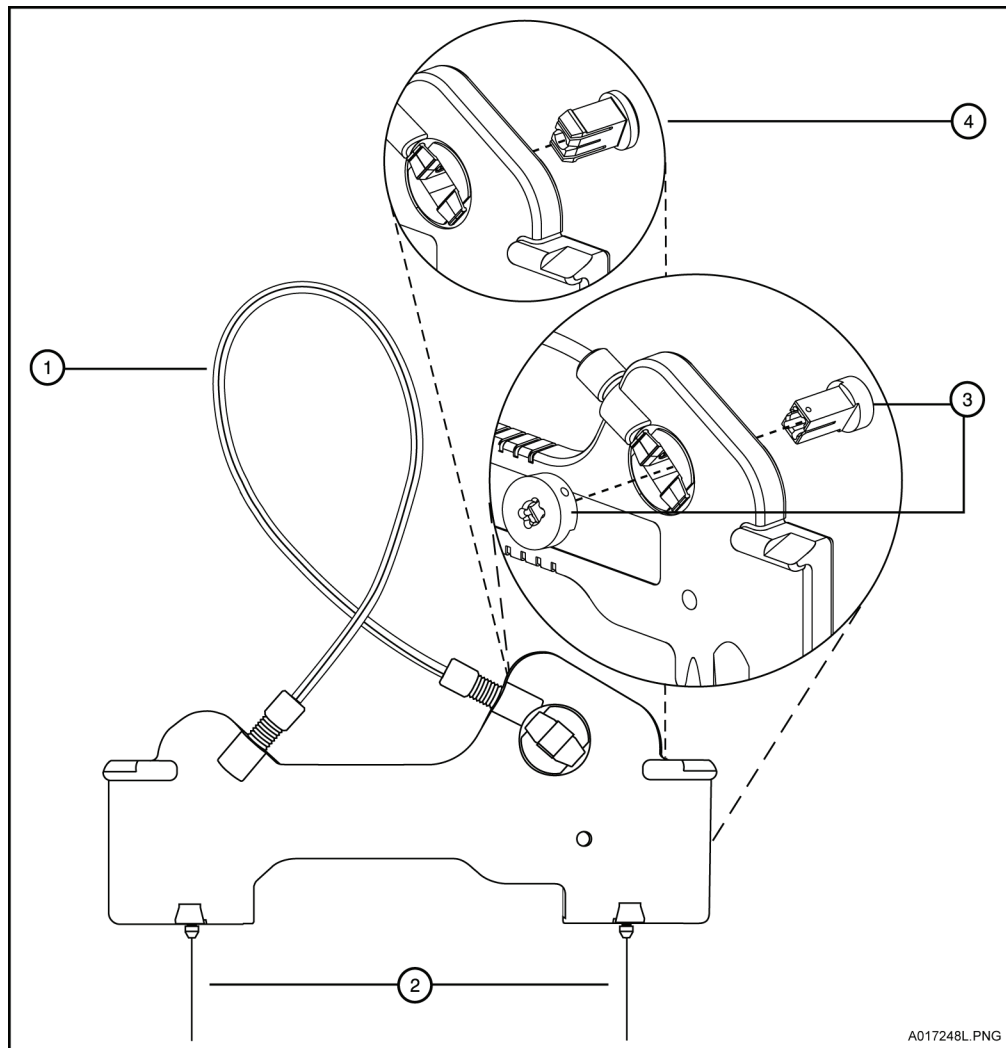
1. Coolant tubing with capillary inside
2. Double seal
3. Detector Window and Aperture - LIF Detector
4. Detector Window and Aperture - UV and PDA Detectors

The detection window is an area of the capillary where the polyimide coating is removed to show the transparent fused-silica. This area of the capillary is put in a part of the cartridge that contains a plug that connects the window to the optical system. One type of plug is used for UV and PDA detectors, and a second type is used for LIF detectors. The procedures to install a capillary in a cartridge are included in the Maintenance section of this manual.

The capillary temperature is controlled with an inert liquid that circulates through the cartridge. The temperature is controlled in a range from 10°C below ambient (with a minimum of 15°C) to 60°C. Coolant flows through the cartridge through two openings in the bottom of the housing (found between the ends of the capillary). This fluid removes the heat generated by electrophoresis.

Fluid Delivery, Power Supply and Interlock

Syringe Pump

The PA 800 *plus* can generate pressures with an internal pump mechanism. This pump can supply 0.1 to 25 psi to perform pressure injections or low-pressure mobilizations. The pump can apply a maximum of 100 psi to move the fluids through the capillary. Vacuum injections can be performed from 0.1 to 5.0 psi. The pressure can be applied to both ends of the capillary at the same time to prevent outgassing of gels.

High Voltage Power Supply

The high-voltage power supply can deliver a maximum of 30 kV with a maximum current of 300 µA. The voltage range is from 1 to 30 kV in 100 V increments. The polarity is configured in the software. The current range is from 3.0 to 300 µA in 0.1 µA increments. The software allows the user to select current, voltage, or power operation. During operation, the system will ramp the voltage or current up to the programmed value. Limits for voltage, current, and power can be entered to protect the capillary. For example, if the user programs a voltage setting for 30 kV but the setting for current is only 3.0 µA, the system can reach the limit set for current before reaching the voltage setting, and control voltage to keep that current.

LED Indicators

The front panel of the instrument contains LED indicators for Power, UV, and High Voltage. See Figure 2.2.

Cartridge and Sample Cover Interlocks

The hinged doors of the PA 800 *plus* have interlock sensors that prevent unsafe access to the inside of the instrument. The first door is called the outer door or sample cover; the second is called the inner door or cartridge cover. See Figure 2.2.

Opening the sample cover:

- Stops any tray movement immediately.
- Prevents the execution of any programmed events that require tray movement.
- Aborts a method when a step that requires tray movement is encountered.

Opening the cartridge cover:

- Turns off the high voltage if it is on.
- Turns off the pump that circulates the capillary coolant.
- Moves the detector filter wheel to the closed position.

UV Detector Optics

The UV optics include an ultraviolet light source, wavelength filters, aperture, capillary, and a photodiode detector, as shown in Figure 2.6.

The UV source is a deuterium lamp with a wavelength range of 190 to 600 nm. Two lenses focus and direct the output of the lamp through one of the wavelength-selecting filters found in a rotating wheel behind the capillary cartridge. The beam continues through the aperture in the cartridge plug and through a section of the capillary that has been treated to remove the polyimide coating (the detection window). The non-absorbed beam then continues through a fiber optic cable to a photodiode. The light signal is converted to an electrical signal, digitized, and sent to the 32 Karat workstation for processing by the software. This signal is also available as an analog output through a connection on the left side of the instrument.

The design of the instrument insures that the optical system stays in alignment. No user alignments are required.

There are eight positions on the UV filter wheel. UV detector systems are shipped with four standard filters: 200 nm, 214 nm, 254 nm, and 280 nm (10 nm bandwidth). The filters are installed in positions 2, 3, 4, and 5 respectively, on the filter wheel. The position 1 is opaque and has the function of a shutter for the detection system.

Additional wavelengths are obtained by placing the appropriate filters in positions 6, 7, and 8. If desired, the standard filters can be replaced. If the instrument is used with a PDA detector, position 8 must be left open (no filter). The filter wheel will accommodate ½ inch (12.7 mm) diameter filters with wavelengths from 190 to 600 nm.

Figure 2.6 UV Optics Layout
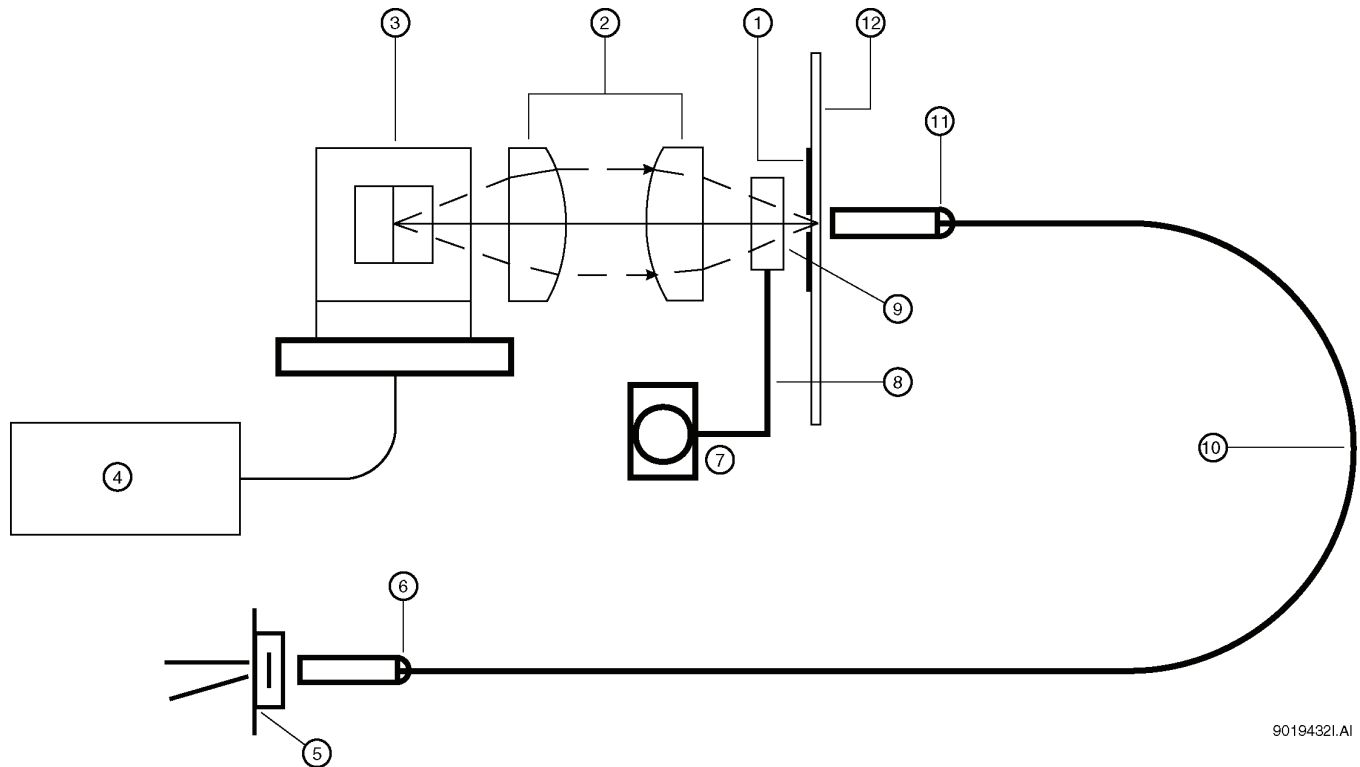
1. Capillary Aperture
2. Lenses
3. Deuterium Lamp
4. Lamp Power Supply
5. Photodiode
6. Fiber Optic Connection
7. Motor
8. Position Filter Wheel
9. Filter Position (e.g., 214 nm)
10. Fiber Optic Cable
11. Fiber Optic Connector
12. Capillary

The Photo Diode Array (PDA) Detector

The Photo Diode Array detector, like the UV detector, uses the absorbance of light to detect if there are samples as they go through the detection window. Unlike the UV detector, the PDA detector can give spectral analysis of samples.

The PDA detector uses the same cartridge configuration as the UV detector. Refer to the Maintenance section of this manual for a description of the cartridge.

In PDA detection, the full spectrum of light from the deuterium lamp illuminates the capillary. See Figure 2.7. Light that is not absorbed by samples is delivered by a fiber optic cable to a grating that breaks the light into a spectrum. This spectrum is projected onto an array of 256 photodiodes. With this design, the absorbance profile of the sample is measured. The PDA detector also allows the simultaneous measurement of light at different discrete wavelengths. The photo diode array converts the light signal into an electrical signal. The electrical signal is digitized and sent to the 32 Karat workstation for processing by the software.

The PDA detector always uses filter wheel position #8. When the PDA is in use, it is essential that no filter is in position #8.

The PDA detector is calibrated using discrete emission wavelength bands generated by a mercury lamp. The mercury lamp is an important part of the detector system. When requested by the user, the calibration is performed automatically.

⚠ **WARNING**

Do not put the mercury lamp into the regular trash. Mercury is a hazardous material and must be disposed of in accordance with local, state, and federal laws.

Figure 2.7 Diode Array Optics Layout

9019433L.AI
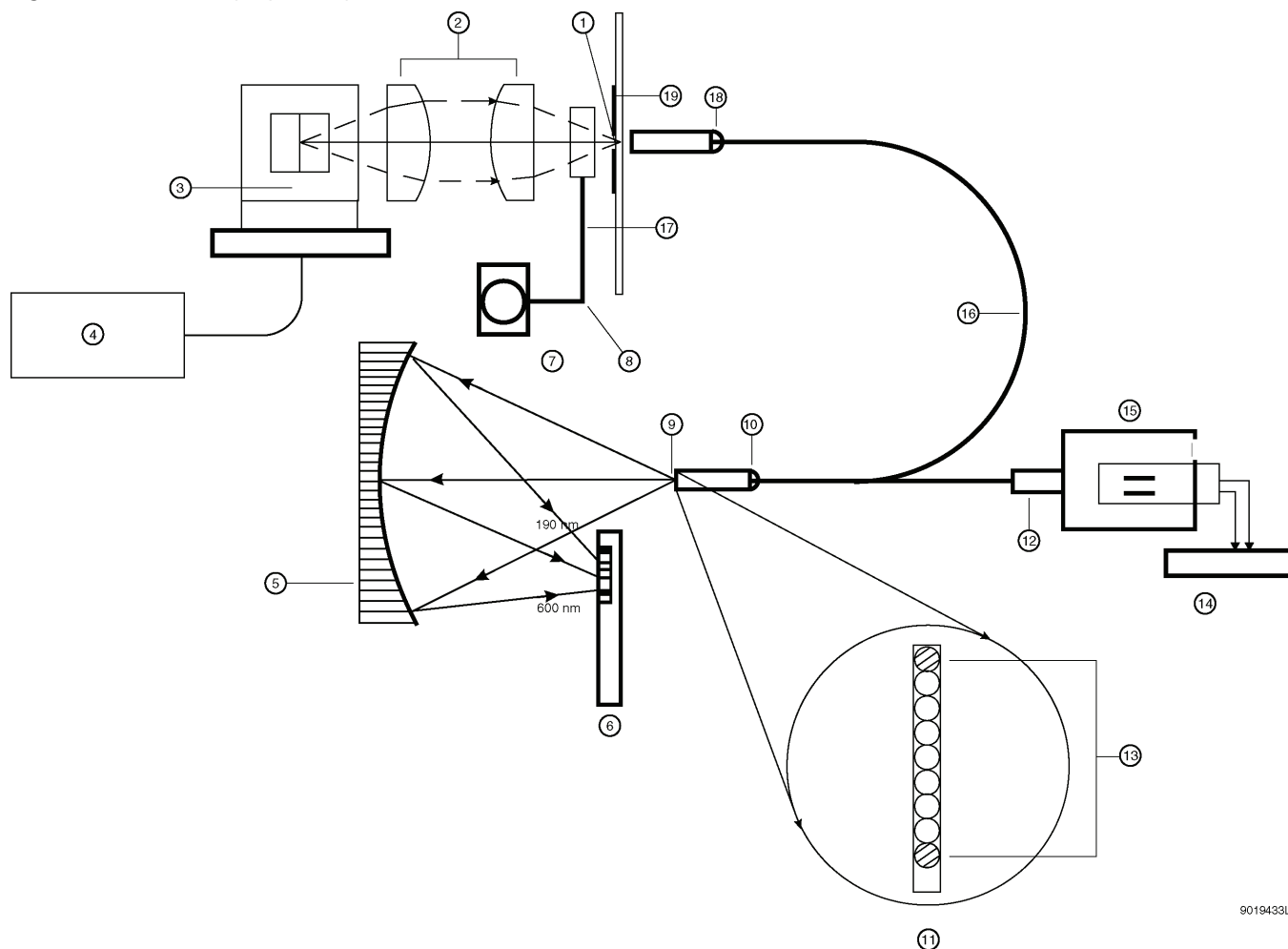
1. Capillary Aperture
2. Lenses
3. Deuterium Lamp
4. Lamp Power Supply
5. Concave Holographic Grating
6. 256 Element Diode Array
7. Motor
8. Position Filter Wheel
9. Monochromator Entrance Slit
10. Fiber Optic Connector
11. 9 by 200 µm Fiber Array (Slit)
12. Fiber Optic Connector
13. Mercury Calibration Fibers
14. Mercury Lamp Power Supply
15. Mercury Lamp
16. Y-fiber Optic Cable
17. Filter wheel in open position 8
18. Fiber Optic Connector
19. Capillary

Laser Induced Fluorescence (LIF) Detector

The LIF Detector consists of the LIF Detector Module and a Laser Module. A PA 800 *plus* capillary cartridge with an LIF Detector plug installed is required for use with this system.

The LIF detector uses an integrated 488 nm laser light source. A fiber cable transmits excitation light from the laser to the capillary in the cartridge. Substances in the capillary that fluoresce at the laser wavelength are detected. The LIF Detector measures and records this fluorescence, which appears as a peak on the electropherogram. For more information on the LIF system, see the PA 800 *plus* System Maintenance manual.

The initial installation of the LIF Detector is performed by a Beckman Coulter Field Service Engineer. The PA 800 *plus* can easily be changed between LIF and UV/PDA modes because the detector components are modular. See Figure 2.8 for a stylized diagram of the LIF Optical System.

Figure 2.8 LIF Optical System

A017254L.PNG
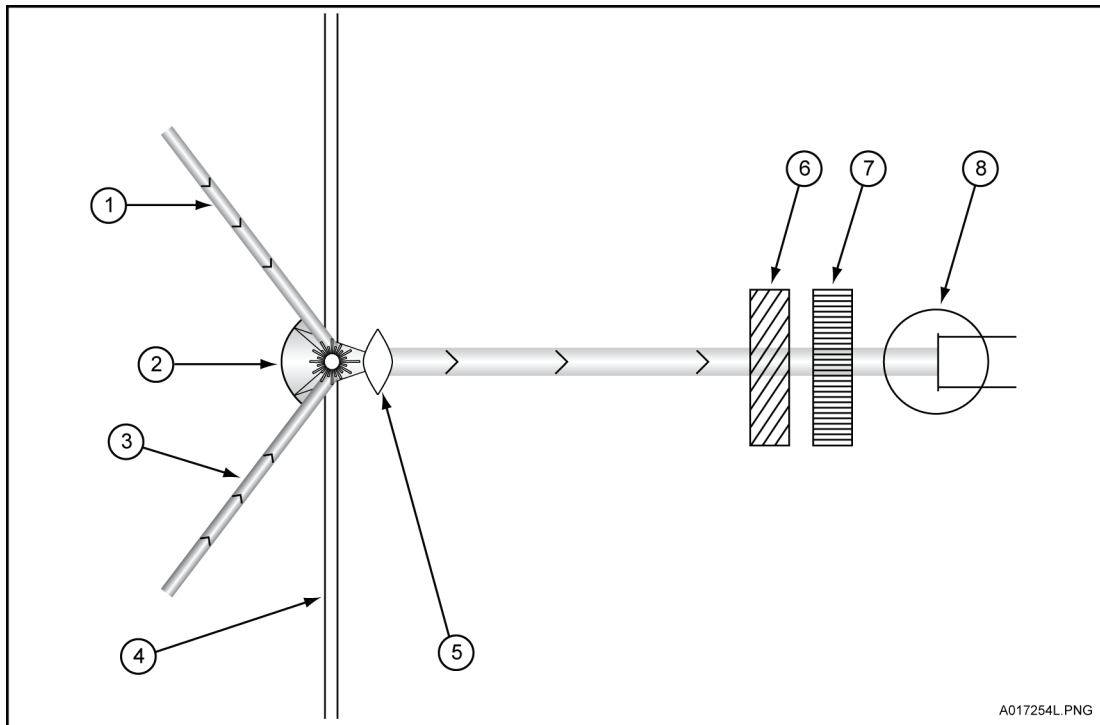
1. Laser 1
2. Spherical Mirror
3. Laser 2
4. Capillary

5. Ball Lens
6. Laser Filters
7. Emission Filters
8. Photo Multiplier Tube

Laser Module

The following section describes the 488 nm laser module and how it interfaces with the PA 800 *plus* instrument and the LIF Detector.

> ⚠️ **WARNING**
>
> **During normal operation of the LIF Detector, laser light is not accessible to the user. To prevent potentially harmful laser light from being emitted from the end of the fiber cable, an interlock mechanism turns off the laser if the laser fiber optic cable is disconnected from the interconnect module or if the cartridge cover is opened.**
>
> **Always turn off PA 800 *plus* instrument before any of the LIF system modules are removed.**

488 nm Laser Module

The Beckman Coulter 488 nm laser is a solid-state laser mounted inside the instrument. It has a fiber connector, and an electrical interlock connector for an external laser connection, that is accessible from the right side of the instrument. This external laser connection accommodates a fiber coupled external laser source for LIF detection.

32 Karat

Starting the Software

To start the 32 Karat Software, select **Programs > 32 Karat > 32 Karat** from the Windows Start Menu button on the Windows Tool Bar.

32 Karat Software opens to the current location / group window that lists the configured instruments.

Figure 3.1 32 Karat Software Enterprise Screen (Main Menu)
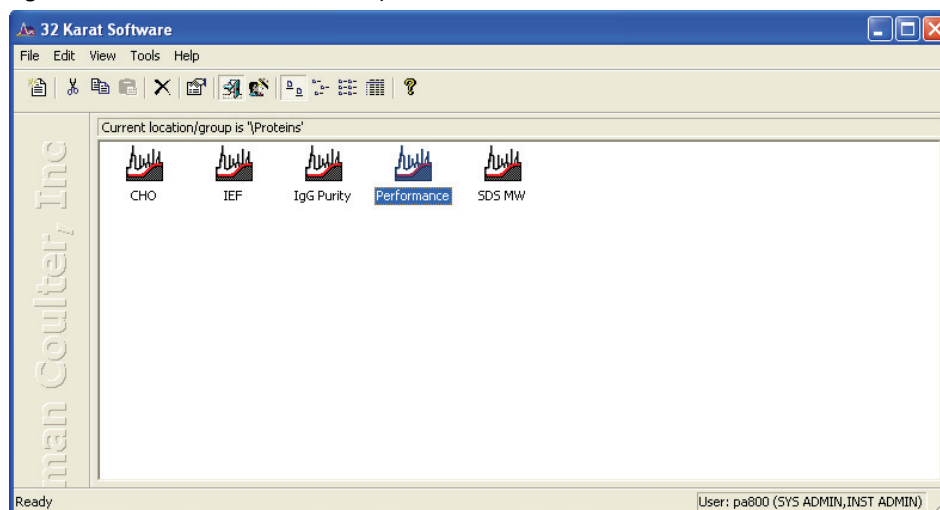
Tool Bar

The Tool Bar appears at the top and, in some cases, the bottom of the active window. The active buttons within the Tool Bar allow for single-click access to many common commands. The following buttons are available from the opening window:

- New
- Cut
- Copy
- Paste
- Delete
- Properties
- Log in or Log out of the Enterprise User
- System Administration Wizard
- Large Icons
- Small Icons
- List
- Details
- Help

Menu Bar

The Menu Bar contains all commands available in 32 Karat Software. The Menu Bar can be accessed by single-click or by holding down the **Alt** key and pressing the underlined letter.

Creating and Configuring an Instrument

In this exercise you will:

- Create a connection to a real instrument
- Create a virtual instrument that can be used for offline data reprocess

NOTE Additional information regarding Instrument Configuration can be found in the 32 Karat Online Help.

IMPORTANT This section assumes that all hardware and interface boards have been installed. If they have not been installed, please refer to the Installation and Maintenance Manual for instructions.

Creating a New Instrument

An unlimited number of offline instruments can be created for offline method editing or data analysis. Use the following exercise to configure a new instrument. Select the detector types appropriate to your hardware configuration. To successfully create a new instrument, you must first log in as the system administrator. Please see the PA 800 *plus* System Administration User's Guide for more details

1 Right-click within the right pane and select **New > Instrument** from the drop-down menu.

2 A new instrument displays with its icon placed in the right pane. Enter the name of the instrument (Instrument 1 in this example) in the highlighted name field. This name is used throughout the 32 Karat Software, in the Instrument window, in data reports, and in the Instrument logs.

Figure 3.2 32 Karat Software Enterprise Screen (Main Menu)
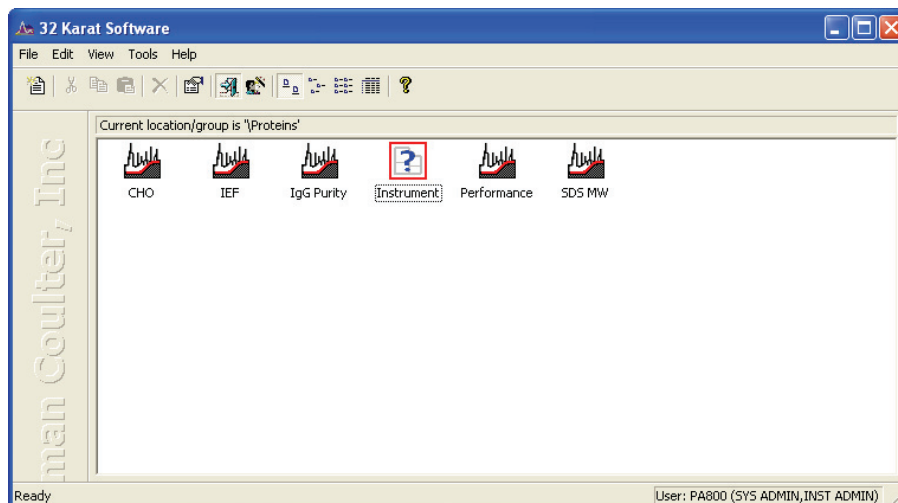
Configuring the New Instrument

Configuring the instrument is a process in which the software is prepared to control the hardware and process the data. It consists of first identifying the PA 800 *plus* as a unique instrument and then identifying the hardware components that are present. Each physical instrument should be represented by at least two icons. One of these is for control and data acquisition; the other is for offline data reprocessing. The following example uses a UV detector as a model.

1 Right-click on the Instrument icon and select **Configure > Instrument** from the drop-down menu.

2 Select PA 800 *plus* from the **Instrument type** drop-down menu and input a name to identify the instrument.

Figure 3.3 Instrument Configuration Dialog Box
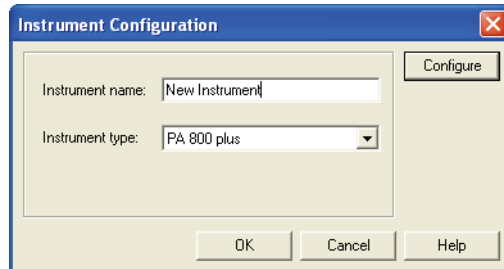
3 Click **Configure**. The PA 800 *plus* Configuration (Module Configuration) dialog box is launched with the modules available for configuration listed in the left pane.

Figure 3.4 PA 800 *plus* Configuration Dialog Box
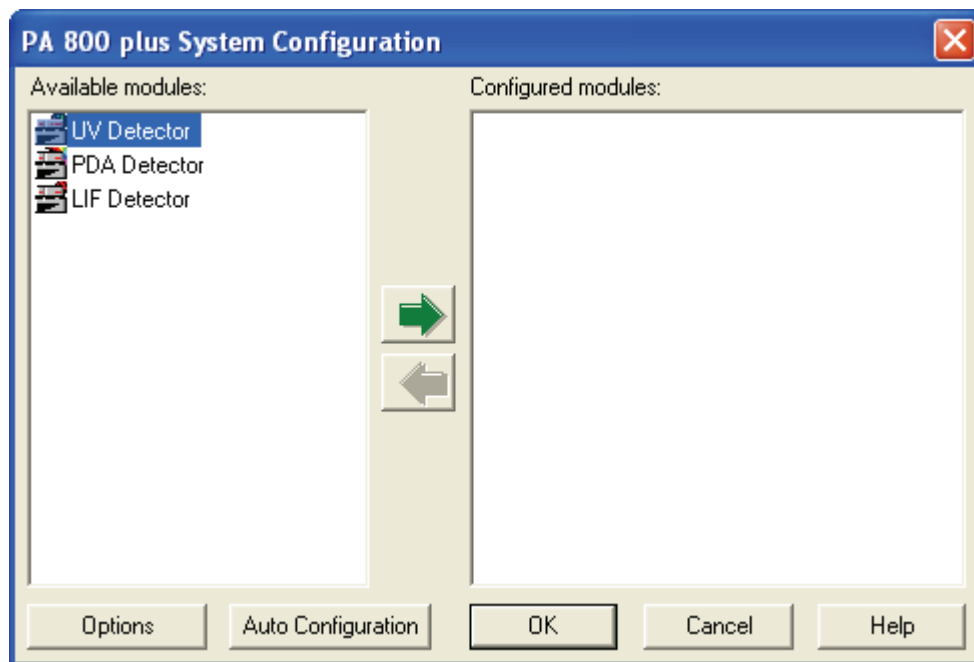
Auto Configuration

32 Karat Software can automatically configure the PA 800 *plus* module in the Instrument. The instrument must be connected to the PC and turned ON.

1 Click the **Auto Configuration** button at the bottom of the dialog box and listen for tray movement within the PA 800 *plus*. This indicates the software is communicating with the instrument. The dialog box below displays once the process completes successfully.

Figure 3.5 Auto Configuration Status
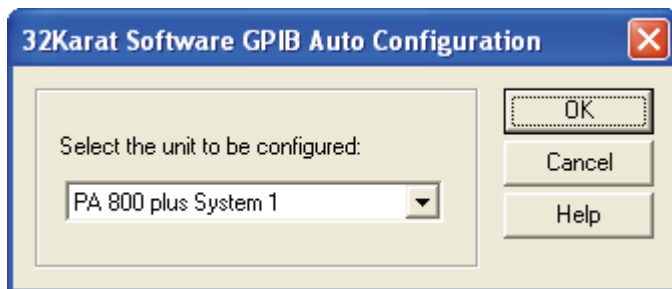
The installed detector displays in the configured modules dialog box.

Figure 3.6 PA 800 *plus* Configuration Dialog Box with Installed UV Detector
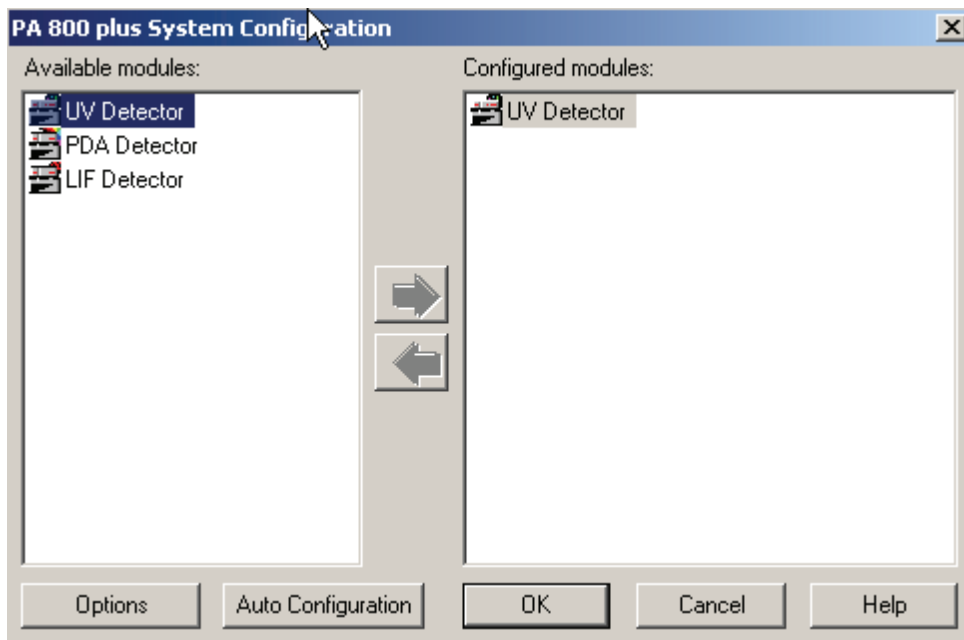
2 Right-click on the new icon and select **Open**. Verify that the instrument was properly detected and configured.

Figure 3.7 PA 800 *plus* Instrument Configuration for UV Detector
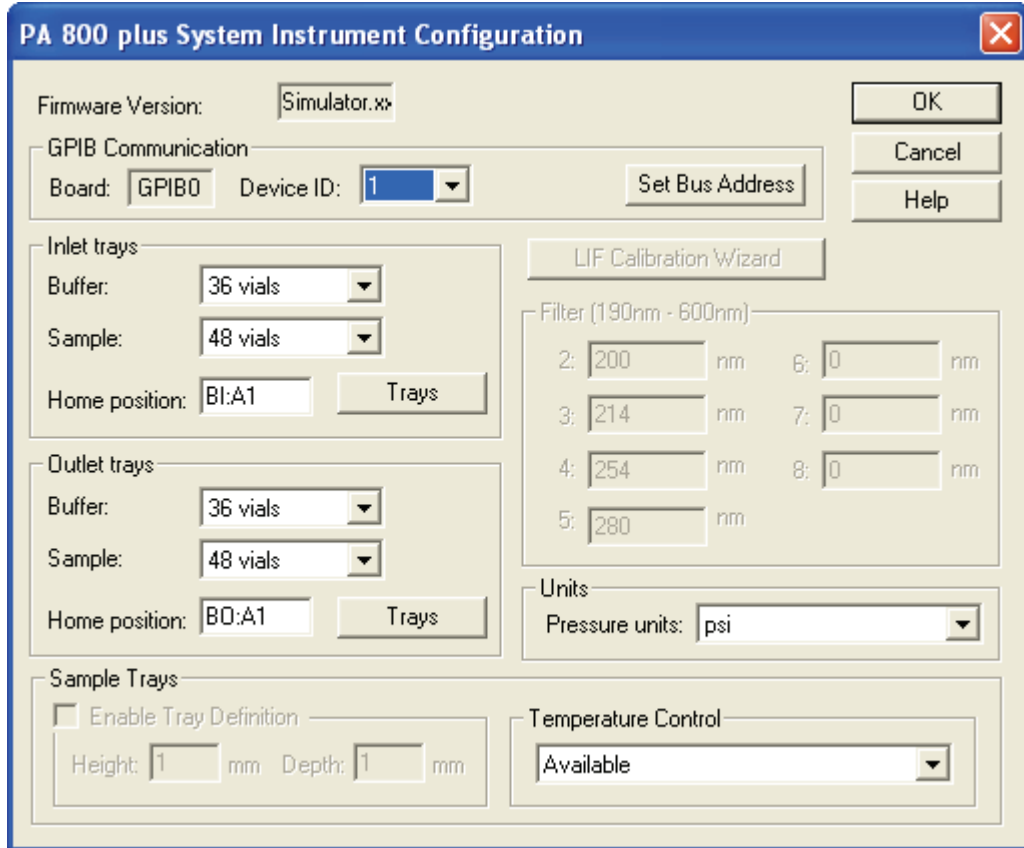
Each PA 800 *plus* connected to the computer must have a unique device ID. The default setting is 1.

3 After the device address is selected, click **Set Bus Address** to send this information to the instrument.

The type of buffer tray and sample tray present is detected at startup. Tray type is detected each time the sample cover is opened and closed. The instrument auto detects two types of trays: 36-vial buffer trays and 48-position sample trays. 96-well plates are not auto detected by the instrument and must be manually configured from this dialog box. Tray configuration can be manually changed at any time.

4 When a 96-well plate is selected as the sample tray type, the **Enable Tray Definition** check box becomes active.
  • If this check box is selected, the user has the option to define the height and depth of the plates installed. This feature allows the use of deep-well and other non-standard plates.

- If this check box is not selected, only standard Beckman Coulter 96-well plates can be used.
- If a Laser Induced Fluorescence Detector has been detected, the LIF Calibration Wizard becomes active. This feature is described in greater detail in the PA 800 *plus* System Maintenance User's Guide.

The Filter dialog box is used to define the specific filters that are installed in a UV detector. The data must be entered manually. It is important for the values entered to match the installed filters exactly. If a PDA detector is installed, position 8 must be empty (no filter installed), indicated by a 0 (zero) value.

Pressure units can be defined in psi (pounds per square inch) or in mbar (millibars). The units selected are only for this instrument.

Temperature control indicates the installation of a sample storage unit. The storage unit may be disabled by selecting **Unavailable** from the drop-down list box.

5   Click **OK** to return to the Instrument Configuration dialog box.

Configuration Options

Clicking the **Options** button will open the **Configuration Option** dialog box. See Figure 3.8.

Analysis Options

Analysis options define what software functions will be available when data is reprocessed.

Figure 3.8 PA 800 *plus* Instrument Configuration Options Dialog Box (Analysis Options)
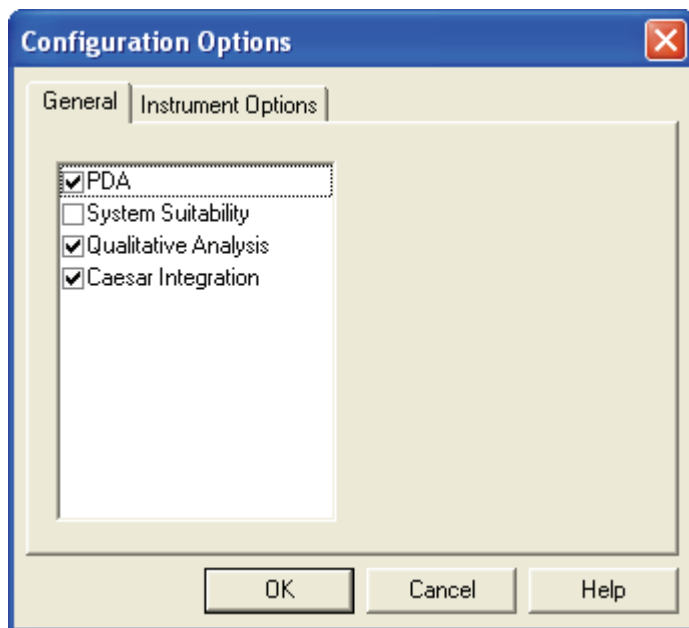
PDA

Allows the analysis of multichannel data from the Photo Diode Array detector.

System Suitability

Enables automatic review of results. Results outside selected ranges can trigger defined responses.

Qualitative Analysis

Enables the identification of peaks by migration time, relative migration time, or mobility.

Caesar Integration

Used to detect peak start and stop. This method is useful for peaks that have abrupt transitions from baseline to peak. It is also useful when S/N (signal noise ratio) is low. Caesar Integration is the preferred method for detecting CE peaks. When un-checked, peak start and stop are based on slope threshold.

Instrument Options

Use the Instrument Options function to select **Standard CE** (default) or **CEC/LC**.

Figure 3.9 PA 800 *plus* Instrument Configuration Options Dialog Box (Instrument Options)
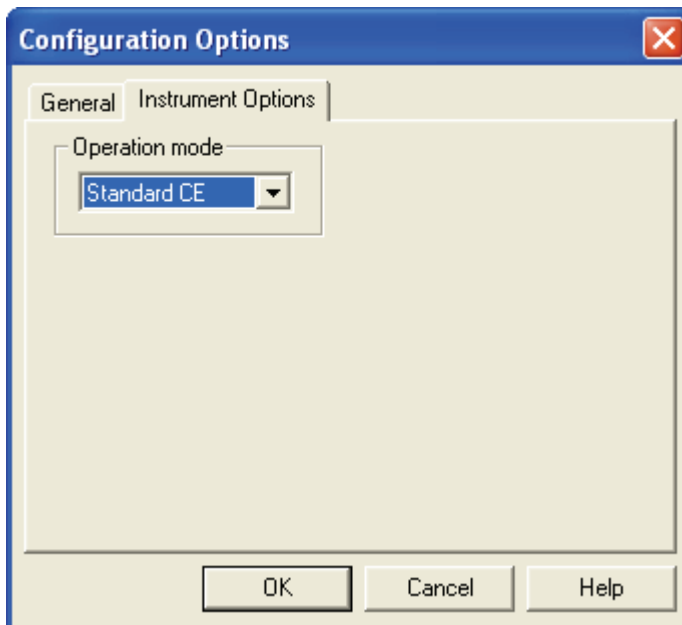
Standard CE

The time for a peak to reach the window is called Migration Time.

CEC/LC

The time for a peak to reach the window is called Retention Time.

This selection is reflected on electropherograms and in reports.

---

1 Click **OK** to exit and save the module configuration.

---

2 Click **OK** to return to the Opening Menu.

Manual Configuration

Manual Configuration of the UV Detector

Manual Configuration may be used to create an instrument when the hardware is offline or in use.

NOTE Many of the exercises in this manual require that a virtual UV instrument be configured. Use this procedure to create a UV instrument, regardless of the type of detector you have installed.

---

1 From the main 32 Karat Software screen, create a new instrument and open the configuration dialog box. Name the instrument UV Detector.

---

2 For this exercise a UV Detector must be configured. Select the **UV Detector** in the left pane and click on the green arrow. The PA 800 *plus* Detector icon is added and must now be configured.

Figure 3.10 UV Detector as Configured Module
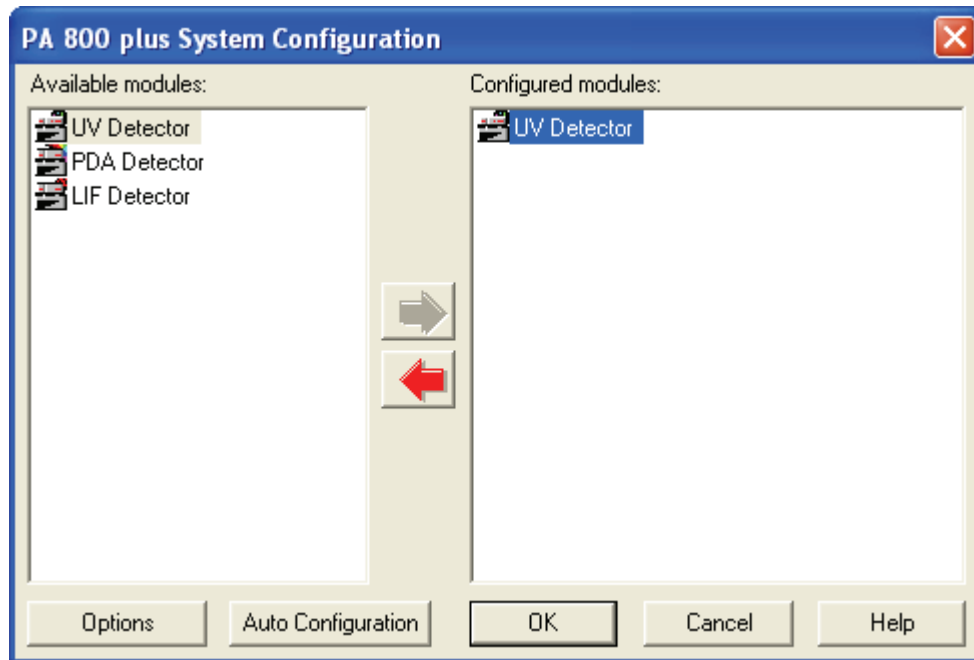
3 Right-click on the new icon and select **Open**. The PA 800 *plus* Instrument Configuration dialog box displays.

Figure 3.11 Instrument Configuration Dialog Box

For information on setting the PA 800 *plus* Instrument Configuration parameters, refer to the 32 Karat Online Help.

---

4 Click **OK** to exit and save the module configuration.

Configuration Options

For information on configuration options, please refer to Configuration Options.

Manual Configuration of the PDA and LIF Detectors

Manual configuration of the PDA and LIF detectors is performed by selecting the appropriate icons in place of the UV detector. Only one internal detector is allowed per instrument.

Direct Control

Introduction

The Direct Control screen is essentially the front panel of the instrument. It allows you to select and change operating parameters with immediate execution. Direct Control is useful in preparing for operations, maintenance, and troubleshooting.

The Direct Control graphical interface is used to control the instrument by clicking on hot areas of the window. These areas either activate the associated dialog box - allowing modification of the instrument settings - or activate the task directly. Direct control displays an animation of current instrument activities and instrument status in real time.

Additional information regarding Direct Control features can be found in the 32 Karat Online Help.

Direct Control Window

To access the Direct Control window, go to the **Control Menu** and select **Direct Control > View**.

Figure 4.1 Hot Spots on the Direct Control window
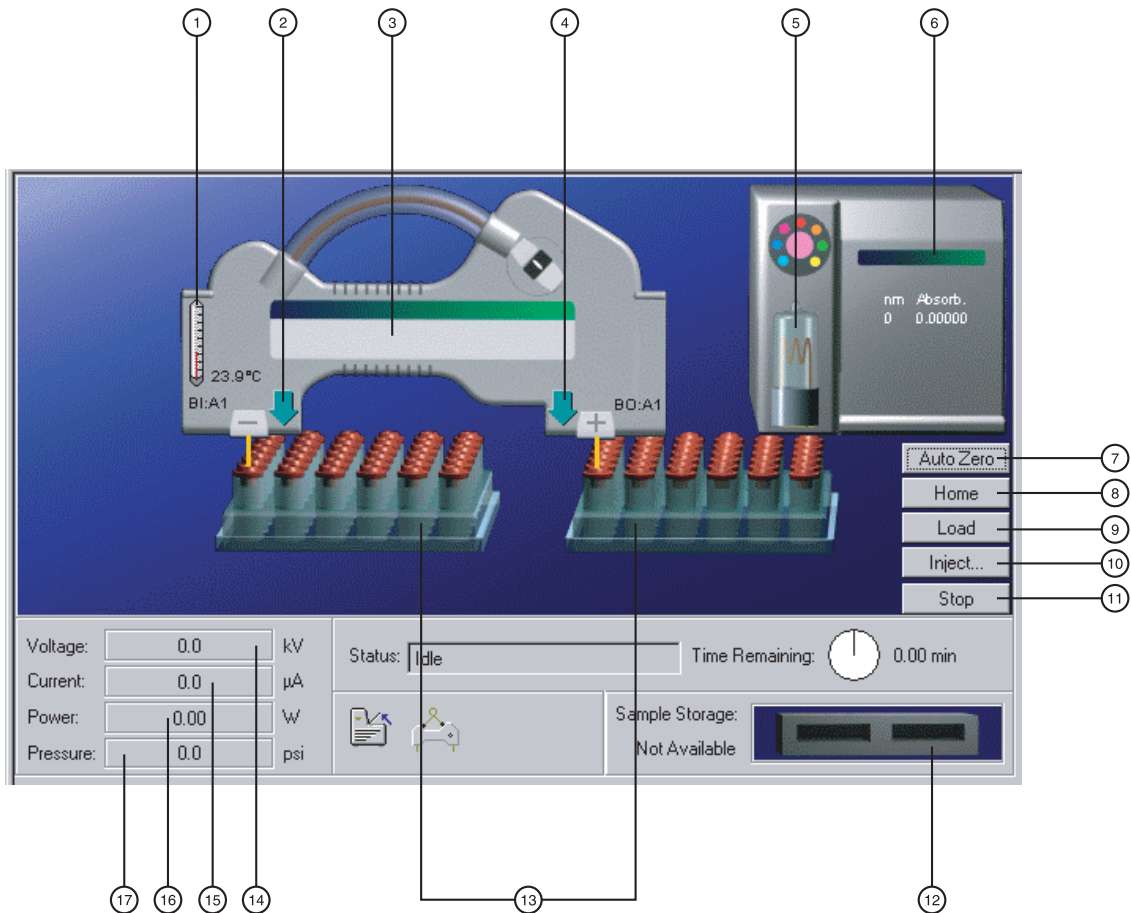
901699L.AI

1. Capillary Temperature
2. Tray Up/Down
3. Label Dialog
4. Tray Up/Down
5. Lamp On/Off
6. Detector Dialog
7. Autozero
8. Trays to Home Position
9. Trays to Load Position
10. Injection Dialog
11. Stop Current Processes
12. Sample Storage Temperature
13. Vial position Dialog
14. Voltage Dialog
15. Current Dialog
16. Power Dialog
17. Pressure Dialog

The UV Detector is shown above. The PDA and LIF Detectors are similar. The functions are defined in Table 4.1.

Table 4.1 Control Functions

	Control Functions in Direct Control		
Group	**Action/Information**	**Button or Hot Spot**	**Description**
Temperature	Capillary Temperature	Hot Spot	Opens a dialog box to set the coolant temperature
	Storage Temperature	Hot Spot	Opens a dialog box to set the sample storage temperature
Tray Motion	Up/Down	Hot Spot	Toggles trays up and down
	Vial Position	Hot Spot	Opens dialog box to move a selected vial to the end of the capillary
	Load	Button	Brings all trays forward for loading
	Home	Button	Returns all trays to their home position
Optics	Detector Control	Hot Spot	Opens a dialog box appropriate for the configured detector
	Lamp/Laser Status	Hot Spot	Opens a dialog box allowing lamp or lasers to be toggled on/off
	Autozero	Button	Adjusts the detector output to zero
Power Controls	Voltage	Button	Opens a dialog box to initiate voltage separation
	Current	Button	Opens a dialog box to initiate current separation
	Power	Button	Opens a dialog box to initiate power separation
	Pressure	Button	Opens a dialog box to initiate pressure separation; also used for capillary rinsing
Other Controls	Inject	Button	Opens a dialog box to initiate injection of sample into the capillary
	Stop	Button	Stops all instrument operations
	Capillary Information	Hot Spot	Opens a dialog box to input capillary/cartridge ID information (used for display only)

Table 4.2 Status Display

	Status Display in Direct Control	
Name	**Display Type**	**Description**
Status	Text	Indicates current instrument function and state
Time Remaining	Text/Graphic	Time remaining for current instrument process
Tray position	Text/Graphic	Indicates the vial or well at which the capillary end is located
Cartridge Status	Graphic	Indicates if a cartridge is installed.
Cover Status	Graphic	Indicates if the door is open or closed
Cartridge Temp	Text/Graphic/Hot Spot	Current coolant temperature is displayed
Storage Temp	Text	Current storage temperature is displayed
Storage Status	Graphic	Indicates presence of trays in the storage unit
Lamp/Laser Status	Graphic/Hot Spot	Indicates Lamp/Lasers on or off
Voltage	Text/Graphic/Button	Indicates present, target, and maximum voltage

Table 4.2 Status Display *(Continued)*

	Status Display in Direct Control	
Name	**Display Type**	**Description**
Current	Text/Graphic/Button	Indicates present, target, and maximum current
Power	Text/Graphic/Button	Indicates present, target, and maximum power
Pressure	Text/Graphic/Button	Indicates present, target and maximum pressure
Pressure	Type/Direction Graphic	Indicates positive pressure or vacuum, and capillary end to which the pressure is applied
Polarity	Graphic	+/- indication of electrode potential

Exercise: Conditioning the Capillary

This exercise uses the Direct Control functions to condition a bare fused-silica (BFS) capillary. This capillary is used for additional exercises in this manual.

NOTE This procedure is intended for bare fused-silica capillaries only. Internal capillary coatings may be destroyed by this procedure.

Materials needed:

- 75 micrometer I.D. capillary, 60 cm total length (50 cm to the detector) installed in cartridge appropriate for detector type configured. See the PA 800 *plus* System Maintenance User's Guide for instructions on building a new cartridge or installing a capillary.
- Methanol (HPLC grade)
- 0.1N HCl in water
- Capillary Regenerator Solution A (0.1N Sodium Hydroxide PN 338424)
- Run Buffer A (PN 338426)
- Distilled or deionized water (HPLC grade)
- Universal vials and Universal caps
- 36-position buffer trays

1 Prepare one vial each of Methanol, HCl, Regenerator, and Water.

2 Prepare two vials of Run Buffer A.

3 Prepare one empty vial.

Vials should be filled to the shoulder as indicated in the PA 800 *plus* System Maintenance User's Guide. All vials (including the empty vial) must be capped.

4 Place the vials in the buffer trays as indicated in the table below:

Solution	Left (Inlet) Buffer Tray	Right (Outlet) Buffer Tray
Methanol	B1	
0.1 N HCl	C1	
Regenerator Solution A	D1	
Water	E1	
Run Buffer A	A1	A1
Empty Vial		B1

5 Check that the capillary cartridge and buffer trays are properly installed. See the PA 800 *plus* System Maintenance User's Guide for more information.

6 Close the door.

NOTE The graphic in Direct Control indicates the presence of the cartridge and the closed door.

You should be able to hear the coolant circulating.

7 Click on the **Pressure** hot spot on the Direct Control screen. The dialog box in Figure 4.2 opens.

Figure 4.2 Pressure Settings Dialog Box
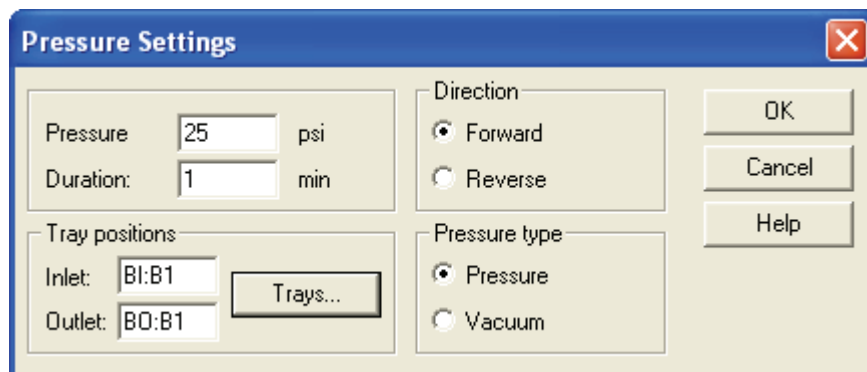
This dialog box is used to set up the conditions for a rinse or a pressure separation. It has the following options:

Pressure Settings

- **Pressure:** Input the desired pressure in psi. The valid range for positive pressure is 0.1 to 100 psi; for vacuum is 0.1 to 5 psi.
- **Duration:** Specifies how long the pressure is applied.

- **Direction:** Designates whether the flow of fluid is Forward (from inlet to outlet or left to right) or Reverse (from outlet to inlet or right to left).

- **Pressure Type:** Specifies whether positive pressure or vacuum is used. This choice defines the valid pressure range.

- **Tray Positions:** Specifies which vials or wells are located at the capillary ends during operations.

8 To select the positions, click the **Trays** button to open the Tray Selection dialog box (see Figure 4.3).

Figure 4.3 Tray Selection Dialog Box
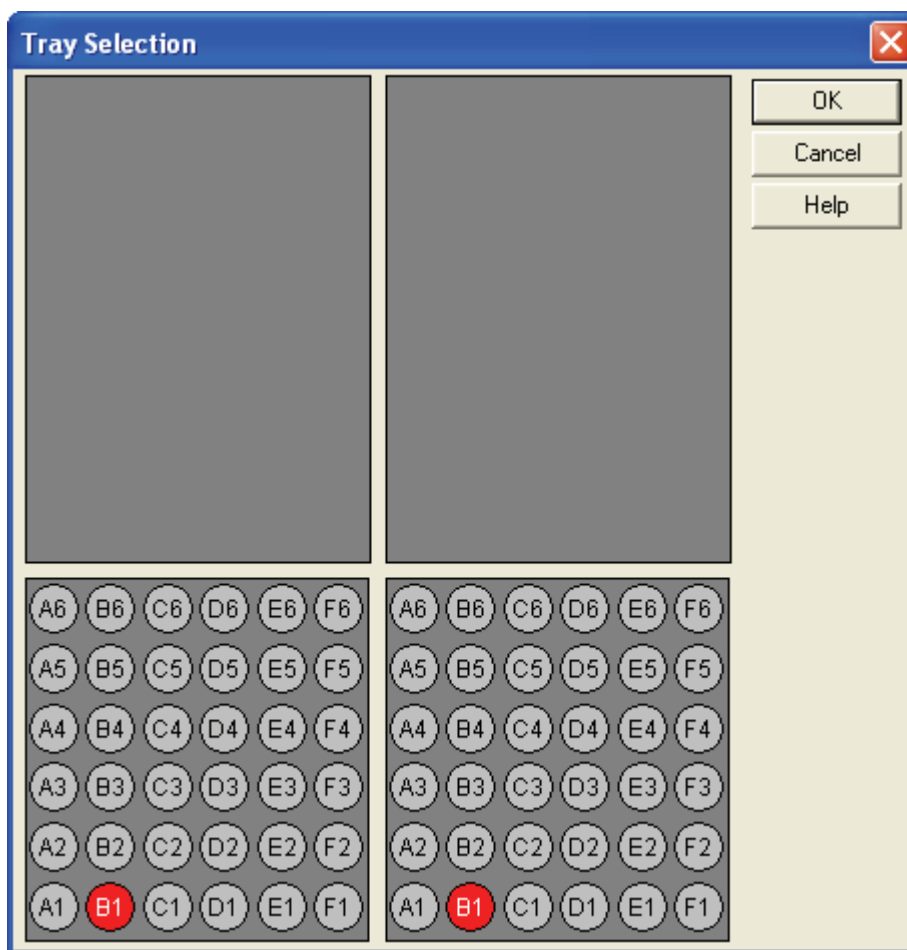
9 Click on the appropriate positions to select them.

For this exercise, click **B1** on the left tray and B1 on the right tray to select the Methanol and Empty vials.

10 Click **OK** to return to the Pressure Settings dialog box. The tray positions area should now indicate Inlet:BI:B1 and Outlet:BO:B1.

11 Type **25** in the Pressure box and **1.0** in the Duration box.

Pressure direction should be Forward and Pressure Type should be Pressure. The dialog box should now look like the one shown above in Figure 4.2.

12 Click **OK**. The trays move to the designated positions and the rinse begins automatically.

13 Observe the Direct Control screen during this process.

14 Perform additional rinse steps according to Table 4.3.

It will only be necessary to change the parameters indicated.

Table 4.3 Rinse Step Parameters

Solution	Inlet (L) Position	Outlet (R) Position	Pressure	Time
Methanol	B1	B1	25	1
Water	E1	B1	25	0.5
O.1 N HCl	C1	B1	20	2
Water	E1	B1	20	0.5
Regenerator A	D1	B1	20	2
Water	E1	B1	20	0.5
Buffer A	A1	B1	20	2

At the end of these steps, the capillary has been regenerated and filled with run buffer. The next step is to test the electrical conductivity of the capillary.

15 Click on the **Voltage** hot spot to open this dialog box. This option is used to configure a voltage separation.

Figure 4.4 Voltage Settings Dialog Box
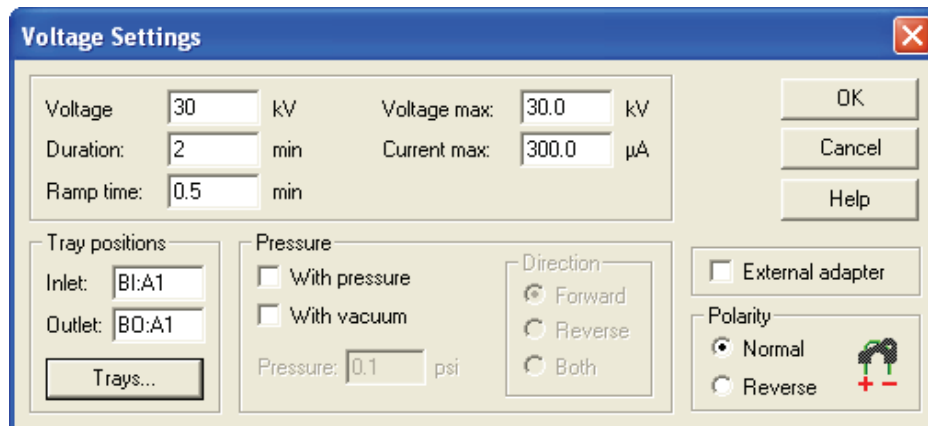
Voltage Setting Parameters

- **Voltage:** The voltage at which the separation is run (0.1 to 30 kV)

- **Duration:** The total time the voltage is applied. (0.1 to 999.9 minutes)

- **Ramp Time:** The amount of time the power supply takes to reach the voltage (0.10 to 999.9 minutes). Ramp time cannot exceed the Duration time.

- **Voltage Max:** The maximum voltage the system is allowed to reach. This must be greater than or equal to the set voltage.

- **Current Max:** Maximum current the system is allowed to reach. If this value is reached at a voltage lower than the set voltage, the set voltage cannot be achieved. Maximum allowed current is 300 μA.

- **Tray Positions:** This is the same as previously described under Pressure.

- **With Pressure:** Allows the simultaneous application of pressure and voltage. When selected, additional parameters of pressure level and direction become available. Maximum pressure is 100 psi. Pressure can be applied to the inlet (Forward), outlet (Reverse), or both ends of the capillary.

- **With vacuum:** Allows the simultaneous application of vacuum and voltage. When selected, additional parameters of vacuum level and direction become available. Maximum vacuum is 5 psi. Vacuum can be applied to the inlet (Forward), or outlet (Reverse), end of the capillary, but not both.

- **External Adapter:** This box must be selected when the external adapter accessory is in use. For additional information, see the PA 800 *plus* System Maintenance User's Guide.

- **Polarity:** Determines the sign of the charge on the electrodes. The graphic indicates the selection.

- For this exercise, use the **Trays** button and select A1 on the inlet side and A1 on the outlet side. Both ends of the capillary are in Run Buffer A. Set the other parameters as follows:
    - Voltage: 30kV
    - Duration: 2 minutes
    - Ramp time: 0.5 minutes

16 Click **OK**.

The trays move to position the selected vials at the ends of the capillary. The voltage begins to ramp up to 30 kV. Observe the Direct Control screen during this process, taking particular note of the Voltage, Current, and Power displays. The Current should reach between 27 and 33 mA when the Voltage is at 30 kV. At the end of two minutes the voltage returns to zero and the Status returns to idle.

This completes the Direct Control exercise. It may be helpful to experiment with the other Direct Control functions before proceeding to the next section.

Creating and Editing a Method

Introduction

In the section on Direct Control the exercise on regenerating a capillary required user input at each step of the process.

A method combines a series of steps into a logical process. The steps in a method are executed automatically by the instrument. This section describes the basic steps in creating an instrument method for data analysis. There are many more features, such as data analysis and reporting, that can be incorporated into a method. Some of these are covered later in this manual. For more information, see APPENDIX A, *Additional Resources*.

Creating and Editing a Method

To create or edit a method, you must be viewing the Instrument Window.

1 To open this window, go to the 32 Karat main screen.

2 Right-click on the icon you created to represent your instrument system and select **Open Offline**.

The instrument window opens after a few seconds. When the window opens, the Instrument Wizard displays. It is possible to open the method editing dialog boxes by selecting **Create or Modify a Method** from this dialog box.

3 For now, just click **OK** to close the Instrument Wizard.

The task is to write a new method to run an instrument test sample.

1 To begin, select **File > Method > New** from the menu bar. The name of the method in the Instrument Window title bar changes to untitled.met.

2 To access the instrument control and data acquisition sections of the method, select **Method > Instrument Setup** from the menu bar. A window opens that contains three or four tabs (depending on your detector type).

3 Select the tab marked **Initial Conditions** to bring it to the front. The dialog box in Figure 5.1 displays.

This dialog box is used to set instrument parameters at the start of a method, before the separation process begins.

Initial Conditions Tab

In the previous section, Direct Control, the exercise on regenerating a capillary required user input at each step of the process. This section shows how to combine a series of these steps into a logical process called a Method. The steps in a method are executed automatically by the instrument. This section will describe the basic steps in creating an instrument method for data acquisition. There are many more features, such as data analysis and reporting, that can be incorporated into a method. Some of these are covered later in this manual. For more information, see APPENDIX C, *Understanding PDA Data*.

In this exercise, you will write a method to run an instrument test mixture.

1 To write a new method, select **File > Method > New** from the menu bar.

2 To access the instrument control and data acquisition parts of the method, select **Method** > **Instrument Setup** from the menu bar. A window opens that contains three or four tabs, depending on the detector configuration.

3 Select **Initial Conditions**.

The dialog box in Figure 5.1 displays.

Figure 5.1 Instrument Setup Dialog Box - Initial Conditions Tab
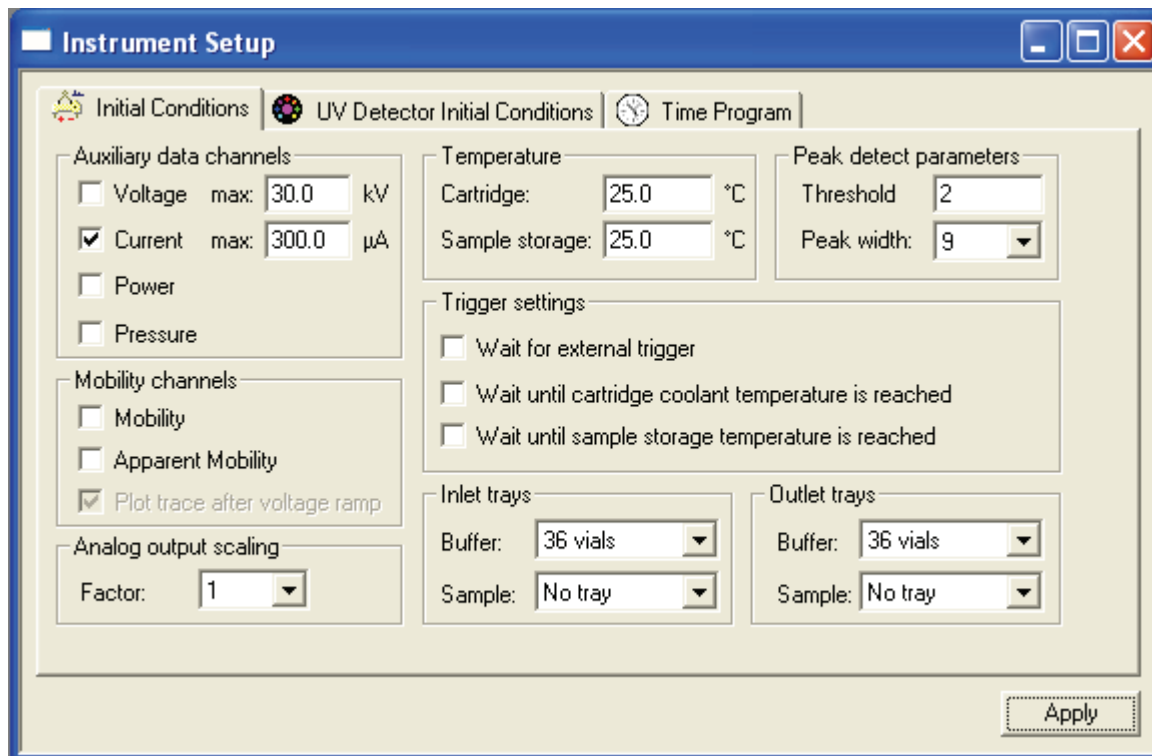
Auxiliary Data Channels

The 32 Karat Software gives you the option to collect any one or all instrument parameters: voltage, current, power, and pressure. Select the channels to record by clicking the appropriate boxes.

The max kV and max µA boxes are used to set the allowable limits for these parameters. Voltage and current are interrelated by the expression V=IR. The system limits both parameters whenever one limit is reached. For example, assume a voltage set at 30kV and a current limit set at 10 µA. With some buffer systems, a voltage of 12 kV generates a current of 10 µA. In this case the voltage does not exceed 12 kV, as the current limit is the determining factor.

Mobility Channels

This topic is covered later in this manual.

Temperature

Sets the initial temperature of the cartridge coolant and the sample storage unit, if installed.

Peak Detect Parameters

These parameters are used to trigger specific events such as fraction collection. They are not used in peak integration or data analysis. Peak integration is discussed later in this manual.

Trigger Settings

The PA 800 *plus* can be forced to wait until certain conditions are met before beginning a run. These are selected by clicking the appropriate box. If **Wait for external trigger** is selected, the PA 800 *plus* becomes a slave device and does not start until an external signal is received. The two **Wait for temperature** options assure that the system has reached the correct operating temperature before beginning a run. These options delay only the start of the time program. Parameters set in initial conditions occur without a wait.

Inlet and Outlet Trays

The types of trays used when a method is run must be specified here. When the method is run, this information is compared to the tray types that are configured in the instrument. If there is a tray type mismatch, the method does not run.

Additional information on these and other parameters of the Instrument Setup window can be found in the 32 Karat Online Help.

Detector Initial Conditions

For this exercise, set conditions in the Initial Conditions tab to match Figure 5.2.

NOTE The next tab sets up the initial conditions for the detector. Select the option below that corresponds to the detector type in your instrument.

UV Detector Initial Conditions

Click on the **UV Detector Initial Conditions** tab.

Figure 5.2 Instrument Setup Dialog Box - UV Detector Initial Conditions tab
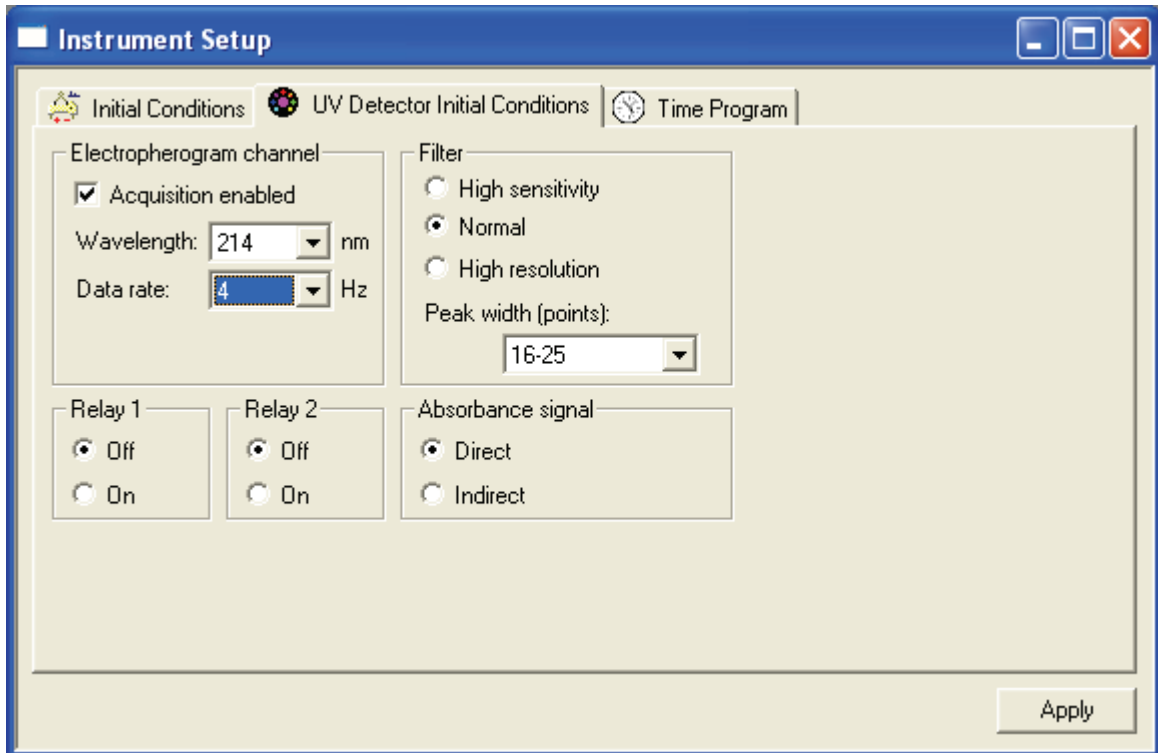
Electropherogram channel

The **Acquisition Enabled** box must be selected for data to be collected and saved.

The **Wavelength** option allows the selection of one of the filters installed in the filter wheel. The available selections are those set up during instrument configuration.

Data rate is selectable from 0.5 to 32 Hz (data points per second). Narrow peaks require a higher data rate. Data rate interacts with the Peak width parameter, set under **Filter Settings** in this dialog box.

Filter

This filter refers to an algorithm for filtering the collected data, and does not refer to the wavelength filters. Data filtering is necessary to remove extraneous noise that might interfere with data analysis. Filter setting requires two steps: the selection of the filter type (High Sensitivity, Normal, or High Resolution) and the setting of the Peak width. High Sensitivity increases signal to noise at the expense of resolution.

Likewise, High Resolution increases resolution at the expense of signal to noise. Normal is a compromise setting that is suitable for most analyses. The filtering algorithm is most efficient if it has information about the number of data points that make up a peak. The optimum number of points is from 16-25. There are also options for **less than 16** and **more than 25** data points per peak. The Data rate setting in the Electropherogram channel dialog box can be adjusted so that a typical peak has from 16-25 data points. The other Peak width settings should be used only if you do not want to change the Data rate.

NOTE Peak Width here is not the same as Peak Width in Data Integration.

Relay 1 and Relay 2

The two relays incorporated into the PA 800 *plus* can be used to control or signal other devices. This dialog box allows you to set the state of the relays at the start of the method to **Off (open)** or **On (closed)**. These relays may also be controlled from the time program.

Absorbance Signal

This parameter has two options, **Direct** and **Indirect**. **Direct** is used when the analytes have higher UV absorbance than does the background electrolyte. In this case, upward deflecting peaks are produced when analytes pass through the detector window. In some modes of CE, the background electrolyte has a higher absorbance than the analytes. In this type of separation, the passage of analytes creates negative peaks (valleys) in the electropherogram. By selecting **Indirect**, the entire electropherogram is inverted, so that these negative peaks appear as positive peaks.

For this exercise, set up the UV Detector Initial Conditions dialog box to match Figure 5.3.

PDA Detector Initial Conditions

Figure 5.3 Instrument Setup Dialog Box - PDA Detector Initial Conditions tab
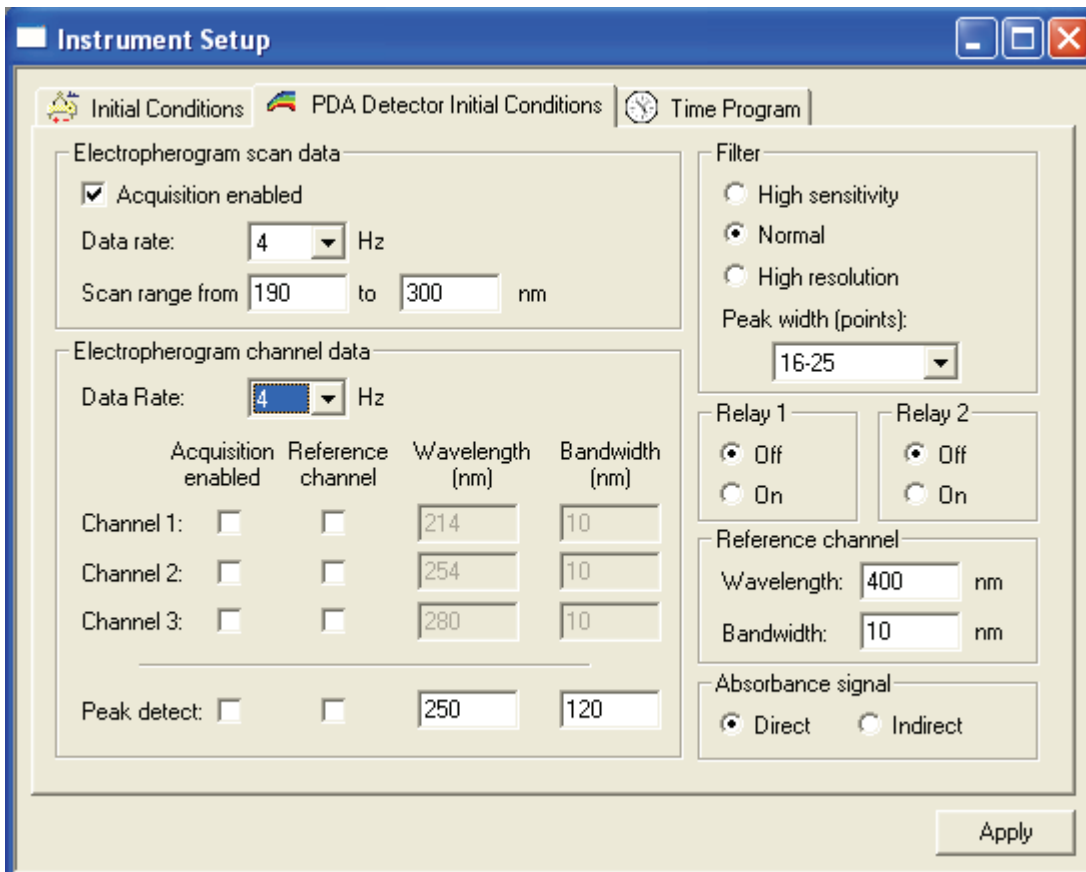
Electropherogram Scan Data

This dialog box controls the parameters for the collection of 3D data. The Acquisition enabled box must be selected for data to be collected and saved. Data rate is selectable from 0.5 to 32 Hz (data points per second). Narrow peaks require a higher data rate. Data rate interacts with the Peak width parameter in Filter settings (below). Scan data collected at high data rates can result in very large data files. Scan range specifies the wavelength range over which data is acquired (limit 190-600 nm).

Filter

See UV Detector Initial Conditions for information on this topic.

Relay 1 and Relay 2

See UV Detector Initial Conditions for information on this topic.

Reference Channel

See the sources in APPENDIX A, *Additional Resources* for information on this feature.

Absorbance Signal

See the UV Detector Initial Conditions for information on this topic.

Electropherogram Channel Data

Up to three independent data channels can be collected simultaneously with the scan data. For each channel the following must be input:

Acquisition enabled: Check this box to enable the collection of data in this channel.

Reference channel: See the sources in APPENDIX A, *Additional Resources* for information on this feature.

Wavelength: This parameter specifies the central wavelength for the data channel.

Bandwidth: This parameter specifies the width, in nanometers, of the data collection channel. Larger values give higher signal to noise ratios than low values. Low values are more specific for molecular structure than are higher values.

Peak detect: See the sources in APPENDIX A, *Additional Resources* for information on this feature.

For this exercise, set up the PDA Detector Initial Conditions to match Figure 5.3.

LIF Detector Initial Conditions

Figure 5.4 Instrument Setup Dialog Box - LIF Detector Initial Conditions tab
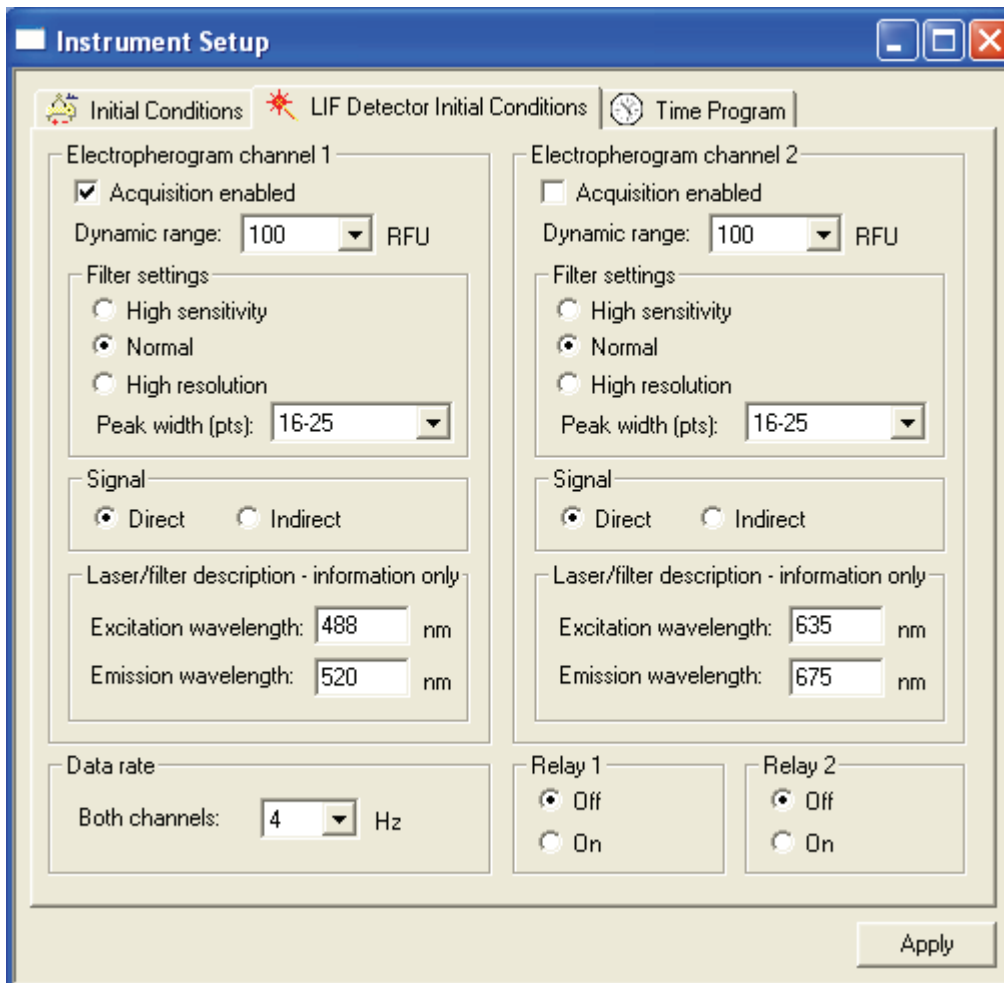
Electropherogram Channel 1 and 2

The LIF Detector is available in one and two channel versions. The programming of these channels is identical. In this exercise use only one channel. If your system is configured with non-Beckman Coulter lasers, the test mixture used in this exercise might not perform as described.

Acquisition enabled: Checked to enable the collection of data in this channel.

Dynamic range: Specifies the upper limit of the signal range. Higher values allow for the collection of larger peaks without truncation but at the cost of sensitivity. Lower values give greater sensitivity, but large peaks may be truncated. This value should be slightly larger than the largest peak expected.

Signal

Select **Direct** when separating fluorescence samples (peaks) in a non-fluorescent background electrolyte. If a fluorescent background electrolyte is used, non-fluorescent samples may be detected. Selecting **Indirect** in this case inverts the signal so that negative peaks appear as positive peaks.

Data Rate

Specifies the rate at which data is acquired. Both LIF data channels have the same data rate. They cannot be specified separately.

Relay 1 and 2

See UV Detector Initial Conditions for information on this topic.

For this exercise, setup the LIF Detector Initial Conditions to match Figure 5.4.

Time Program

The Time Program window is arranged like a spreadsheet. An event is entered into each line. Events are executed in order, top to bottom. Each line in the window contains these columns:

Figure 5.5 Instrument Setup Dialog Box - Time Program tab
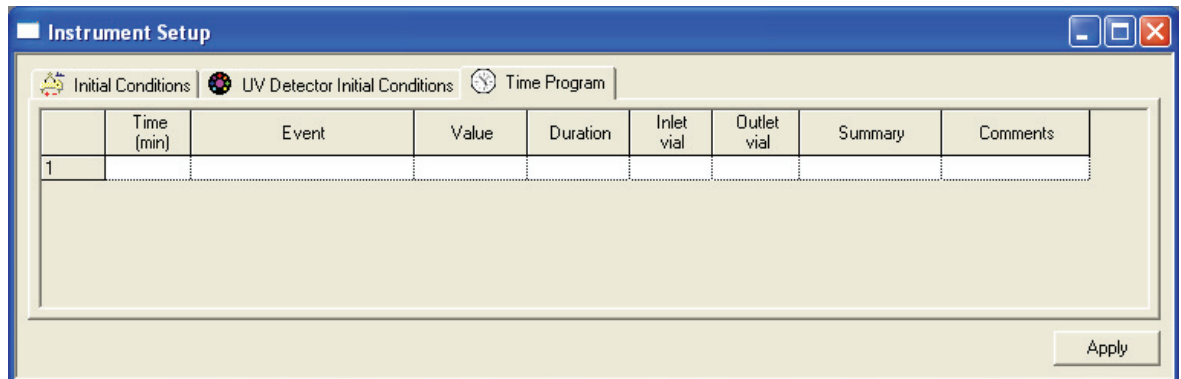
Time: the point after time zero at which the event occurs.

Event: the action that occurs (see below).

Value: this varies depending on the action selected.

Duration: length of time the event lasts.

Inlet and Outlet Vial: where the capillary ends are during the event.

Summary: a system-generated description of the event.

Comments: a user-generated annotation of the event.

Time is not a required event. Events that have no time associated with them are run in the listed order, top to bottom, and each is finished before the next event begins. Timed events must be grouped together; a group of timed events cannot be interrupted by an untimed event. Untimed events can only occur before a group of timed events. Some events do not have a time option, others can be timed or untimed. Data acquisition begins with the first timed event (time 0.00); it ends when the method ends or when a STOP DATA event is reached.

To program a line, click in the **EVENT** box. Click on the down arrow to open a menu of events. Select an event to open a dialog box for that event.

Figure 5.6 Time Program Event List

UV & PDA	LIF
Separate...	Separate...
Rinse...	Rinse...
Inject...	Inject...
Relay on...	Relay on...
Wait...	Wait...
Message...	Message...
Capillary Temperature...	Capillary Temperature...
Sample Storage Temperature...	Sample Storage Temperature...
Fraction Collection...	Fraction Collection...
Timed Fraction Collection...	Timed Fraction Collection...
Wavelength...	Autozero...
Autozero...	Lasers..
Lamp...	Stop Data...
Stop Data...	End...
End...	

The following describes only those events that are used in this exercise. For information on the other event types, see the resources in APPENDIX A, *Additional Resources* or refer to the 32 Karat Online Help.

Separate Dialog Box

The separate dialog box is used to control the conditions under which the separation process takes place. Every method must have at least one separation step. The step at time = 0.00 is usually a separation.

Figure 5.7 Separate Dialog Box
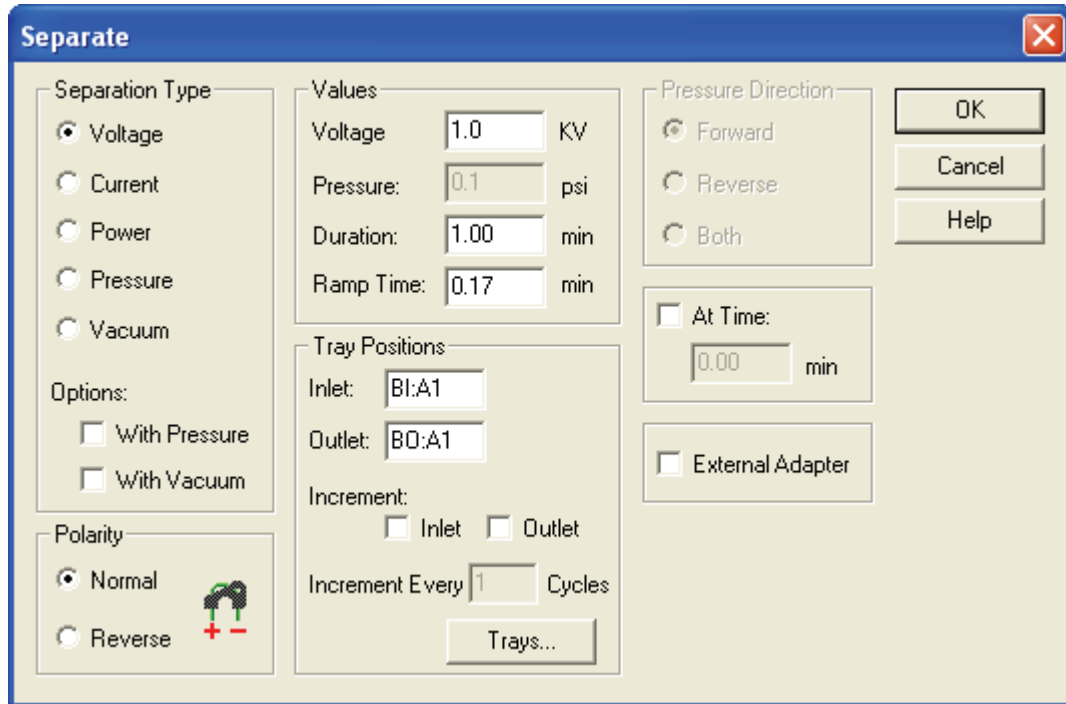
This dialog box has the following parameters:

Separation Type
Electrically driven separations can be done at controlled voltage, current, or power. When one of these is selected, the other two float to a value determined by the resistance of the capillary contents. Voltage and current cannot exceed the limits set in the Initial Conditions window. Separations can also be programmed to use pressure or vacuum to move the fluid in bulk through the capillary. Voltage, current, or power can be combined with Pressure or Vacuum so that two processes are at work simultaneously.

Polarity
Determines the direction of the current. The charge on the electrodes is indicated by the graphic in this dialog box.

Values
Allows for input of the set points for the Separation Type parameters. The available options change depending on the Separation Type selected. Ramp time is only valid for electrical separations. It determines the length of time for the voltage, current or power to change from the present to the programmed level.

Tray Positions
Can be selected graphically by clicking on the Trays button. The type of trays shown are determined by the settings in the Initial Conditions dialog box. When a method is used in a Sequence Table, you might want to change the vial positions after a specified number of cycles. The inlet position, outlet position, or both can be incremented automatically by selecting the appropriate boxes and inputting the desired number of cycles between changes.

Figure 5.8 Tray Selection Dialog Box

Pressure Direction

Specifies whether pressure or vacuum is applied to the inlet or outlet end of the capillary. Pressure (but not vacuum) can also be applied to both ends of the capillary at the same time.

At Time

Specifies this is a timed event at the time specified. Separation is usually a timed event.

External Adapter

Changes the way the instrument manages the power supply. Select this box only if the External Adapter accessory is in use. See the PA 800 *plus* System Maintenance User's Guide for more information.

Rinse Dialog Box

The rinse event is used to clean the capillary and to load fresh buffer or other separation media.

Figure 5.9 Rinse Dialog Box
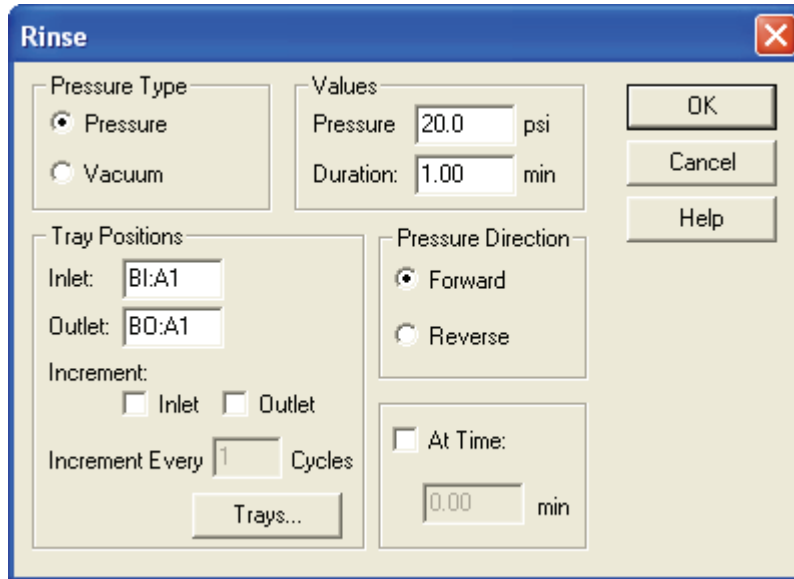
This dialog box has the following parameters:

Pressure Type
Selects the mechanism to be used to move fluid through the capillary.

Tray Positions
Functions exactly as described in the Separation event.

Values
Specifies the magnitude of pressure to be delivered and for how long.

Pressure Direction
Specifies whether pressure is applied to the inlet or the outlet end of the capillary.

At Time
Functions exactly as described in the Separation event.

Inject Dialog Box

The inject event is used to deliver a precisely measured amount of sample into the capillary. This step is always untimed, and usually precedes the first separation step.

Figure 5.10 Inject Dialog Box
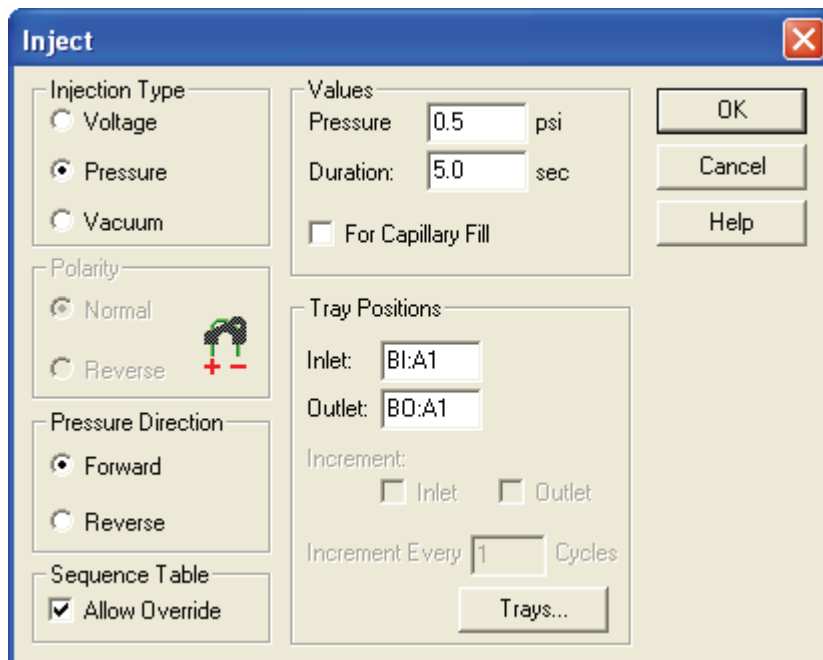
This dialog box has the following parameters:

Injection Type:
Sample can be delivered to the capillary by positive pressure, vacuum, or by the application of voltage (electrokinetic injection).

Polarity
Specifies the charge on the electrodes during a voltage injection.

Pressure Direction
Specifies whether pressure or vacuum are applied to the inlet or the outlet end of the capillary.

Values
Specifies the magnitude of the pressure, vacuum, or voltage and how long it is applied. Higher values inject more sample. The capillary fill option is used for cIEF separation, which requires the capillary to be completely filled with the sample mixture. For more information on cIEF separations please visit www.CELeader.com or see the cIEF Applications Guide for the PA 800 *plus.*

Tray Positions
Functions exactly as described in the Separation event.

Sequence Table:
When a method is used in a Sequence Table, certain parameters in the inject event can be overridden by values input for the Sequence. This parameter determines whether the method Inject event or the Sequence Table has priority. Multiple injection events are allowed. If multiple injections are used, only one injection event can be over ridden or incremented in the sequence table.

Autozero

This event causes the detector output to be reset to zero. It may be timed or untimed.

Figure 5.11 Autozero Dialog Box
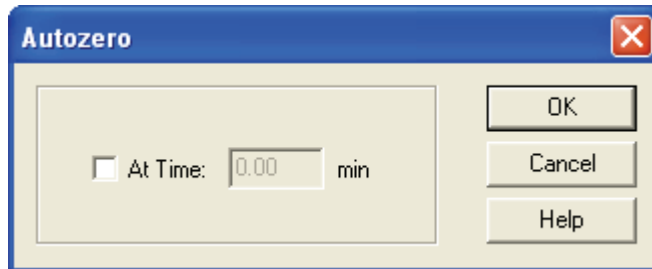
Stop Data

Data collection starts at time = 0.00. Data collection continues until the end of the method unless a STOP DATA event is encountered. This event can be used to avoid data collection during steps such as post-run capillary cleaning. STOP DATA is always a timed event.

Figure 5.12 Stop Data Dialog Box
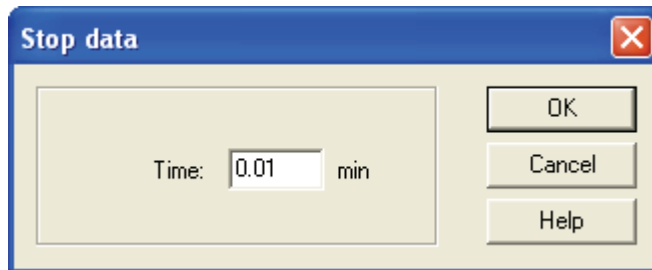
End

End is an optional event. The method does not continue beyond an end event. It is always a timed event.

Figure 5.13 End Dialog Box
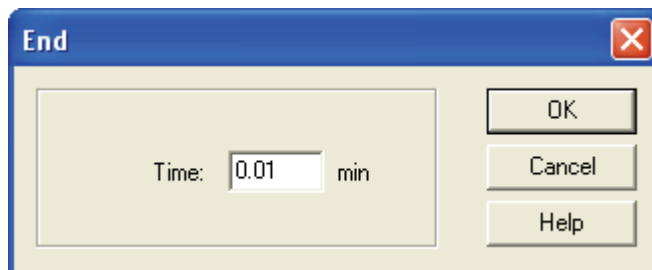
The next part of this exercise creates a time program to go with the initial conditions previously entered.

The example method does the following:

- Performs a mini-regeneration on the capillary.
- Fills the capillary with buffer.
- Injects test mix.
- Separates the test mix.
- Performs a post-run rinse.

This method uses a vial tray setup similar to that used in the section on Direct Control. The vial positions are specified in the table below.

Table 5.1 Vial Positions

Solution	Left (Inlet) Buffer Tray	Right (Outlet) Buffer Tray
Test Mix	C1	
Regenerator Solution A	D1	
Water	E1	
Run Buffer A	A1, A2	A1
Empty Vial		B1

1 Click on the **Time Program** tab to begin. The tab opens with a blank spreadsheet.

2 Click in the **Event** box and use the arrow.

3 From the drop-down menu, select **Rinse**.

4 Click on the **Trays** button.

5 Select **D1** on the Inlet (left) side and **B1** on the Outlet (right) side.

6 Before clicking **OK**, right-click on one of the buffer positions you just selected. A dialog box will open that allows you to identify the contents of that position.

7 Insert the appropriate comments from the table above and repeat for the other vials.

When done, click **OK** to return to the Rinse window.

8 For this step, accept the default values for all other parameters.

Click **OK** to return to the Time Program spreadsheet.

The key parameters have been entered into the table automatically. This rinse is an untimed step, so there is no value in the Time window.

1 In the blank line at the bottom of the table, click in the **Event** column again and select another rinse event. This time, program a rinse with water from position BI:E1 to position BO:B1.

2 Change the rinse time to 0.5 minutes.

This event is added to the table when **OK** is clicked in the dialog box.

3 Click in the **Event** column again and add a step to fill the capillary with buffer rinse from position BI:A1 to BO:B1.

4 This time, use 25 psi for one minute. Click **OK** when done.

5 Click in the **Event** column in the bottom row again.

6 Select **Inject** as the event type.

7 Select the **Trays** option and program the injection from position **BI:C1** to **BO:A1**.

8 Click **OK**.

9 Input 0.5 psi as the pressure and 4.0 seconds as the time.

10 Click **OK**.

At this point in the method you are ready to separate the components in the test mix.

1 Select a new **Event** box, and choose **Separate** as the event type.

2 Use the **Trays** button to select positions **BI:A2** and **BO:A1**.

3 Click **OK**.

4 Select a **Voltage** separation.

5 Set the voltage to 30 kV, ramp time to 0.2 minutes, and duration to six minutes.

6 This will be a timed step, so the **At time** button must be checked.

7 Set the time to 0.00 minutes.

8 Click **OK** to put this event into the table.

Use a final rinse in this method. This requires that you insert a Stop Data event.

1 Select the new **Event** box and choose **Stop Data** as the event type.

2 Enter a value of 6.00 minutes to force data collection to stop at the end of the separation step.

3 Our last step in this process is to rinse with water from the water vial (EI) to the empty vial (BI).
- Program this rinse for one minute at 20 psi.
- Select **At Time** and use a value of 6.10 minutes.

4 It is good practice to Autozero the detector some time after the run starts.

In the new event box, select **Autozero**.

5 Select **At Time** and input a value of 1.00. When this item is entered into the Time Program spreadsheet, it automatically moves to the correct time sequence.

At this point your spreadsheet should look like that in Figure 5.14.

Figure 5.14 Instrument Setup - Time Program

The method can still be edited at this point. Items in the Time, Value, Duration, Inlet, and Outlet columns can be edited by clicking on the existing value and typing in a new value. Alternatively, clicking on the name of any existing event opens the drop-down menu for event types.

6 Select **Current Event** to re-open the event dialog box, or select a **new event** to change the event type.

Saving the Method

IMPORTANT Remember to save your method.

1 Select **File > Method > Save As** from the menu bar.

2 Give the method a name with a title and date.

Example:
Type: **TestMethod_032309**

Click **Save**.

The default path at installation is: C:\32Karat\Projects\Default\Methods.

Your system administrator may have assigned you to a different default path.

The method is ready to run.

In the next chapter, you run the method just created. The resources in APPENDIX A, *Additional Resources* and the 32 Karat Online Help describe events and parameters not covered here.

Running the System

Single Run and Sequence Runs

In the last chapter, you created an instrument method. In this exercise, you will run that method in Single Run mode. In this mode, the method must be manually started prior to every run. Single mode is useful in method development, where the results of one run suggests modifications to the method or other procedures. In the second part of this section, you will create a Sequence to do multiple runs.

You must be online to run samples and acquire data. If you are not online, (offline) appears in the title bar of the instrument window. Close any offline windows before proceeding. From the main 32 Karat Software screen, double click on an instrument icon to go online.

NOTE The detector lamp or lasers must be ON prior to starting a method.

Materials Needed:

- Cartridge prepared in Direct Control exercise (75 micrometer id capillary, 60 cm total length, 50 cm to the detector) installed in a cartridge appropriate for the detector type configured. See the PA 800 *plus* System Maintenance User's Guide for instructions on building a new cartridge or installing a capillary.
- Capillary Regenerator Solution A (0.1N Sodium Hydroxide, PN 338424)
- Run Buffer A (PN 338426)
- Distilled or deionized water (HPLC grade)
- Universal vials and universal caps
- Vial Tray, 36 vial capacity
- For UV or PDA Detection: Test Mix B (PN 501333)
- For LIF detection using a 488 nm solid state laser: LIF Detector Test Mix (PN 726022)

Load the trays as follows:

Table 6.1 Need title

	Left (Inlet) Buffer Tray	Right (Outlet) Buffer Tray
Test Mix[a]	B1	
Regenerator Solution A	D1	
Water	E1	
Run Buffer	A1, A2	A1
Empty Vial		B1

a. PN 501333 for the UV or PDA Detector
PN 726022 for an LIF Detector with a 488 nm laser

Install the loaded trays and the capillary cartridge into the instrument as described in the PA 800 *plus* System Maintenance User's Guide. If the instrument software window is not already open, open it now.

Single Run

Select **Control > Single Run** from the menu bar or click on the **Single Run** icon ▷ to open the Single Run dialog box shown below.

Figure 6.1 Single Run Acquisition Dialog Box

You must provide at least a Sample ID, Method, and Data File. The Data File is stored in the directory identified in the Data Path. Sample ID can be any text string that identifies your sample. This ID appears on all subsequent reports. The method file can be selected by clicking on the Open icon and navigating to the desired method. For this exercise, select the method you created in the Method Editing section.

Data path is selected in the same way as the selection of a method. In this case, you select a directory rather than a file. The data file is entered by typing a unique name in the box. The file name must be one that does not already exist in the data directory; if the name already exists, the run does not start.

The remaining items on this screen are not required to run a method.

When ready to begin, press Start. The system examines the method to verify that it is appropriate for the instrument configuration. The method is then downloaded to the PA 800 *plus.* The instrument first performs a brief start-up check and then executes the method.

If error messages display, or the data does not look like the example shown in Figure 6.2, check the following:

- Are the vials correctly filled with the proper fluids?
- Are the vials in the positions specified by the method?
- Was the correct method selected?
- Was the method written for the current instrument configuration. (For example, a PDA method cannot run on a UV or LIF system)?
- Is the method correct?
- Do the tray types in the method Initial Conditions match the tray types configured for the instrument?

Figure 6.2 Electropherogram of Test Mix
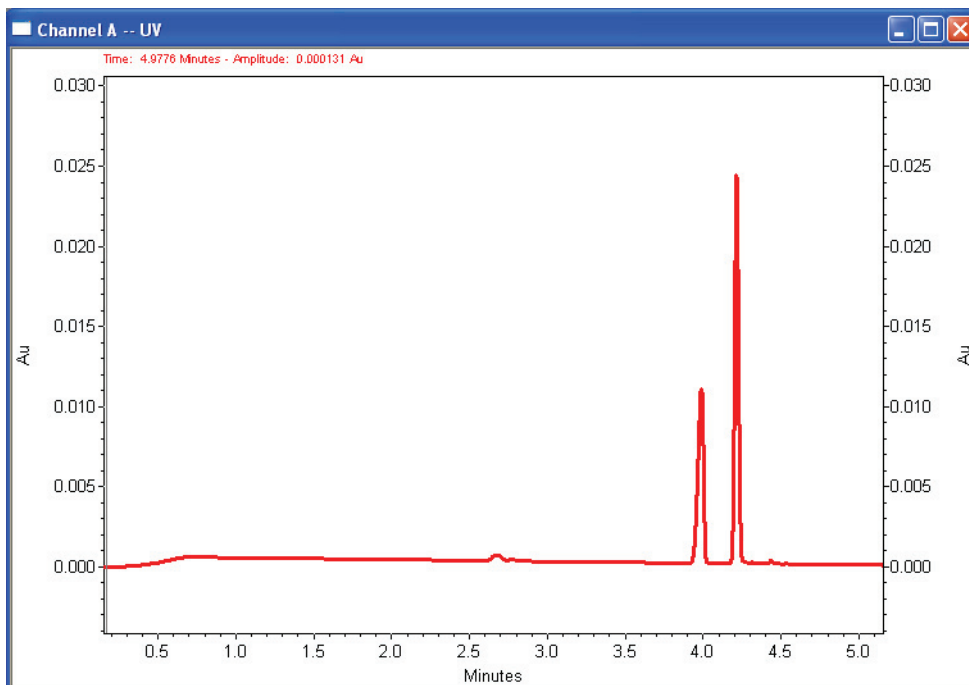
This method is used for additional exercises. Please do not continue to the next section until you have a successful single run.

Programming a Sequence

A Sequence is a list of methods and data files that will be used to run a batch of samples without user intervention. Sequences can be used to acquire data (run the instrument) or to batch reprocess existing data files. In this exercise, you will create a Sequence to acquire data from multiple runs of the test mix method. Later in this manual you will use a sequence to perform batch reprocessing of data. You must be "Online" for this exercise.

1 To create a new sequence, select **File > Sequence > Sequence Wizard** from the Instrument window. This opens the Sequence Wizard dialog box.

The Wizard consists of 5 screens. Not all of them are used for every sequence, and not every feature of each screen is used in this exercise.

Figure 6.3 Sequence Wizard - Method Dialog Box
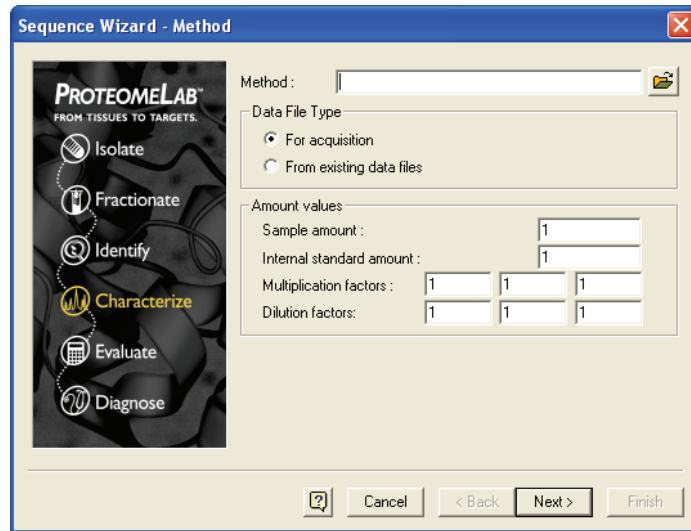
The first screen requires that you select a method.

2 Click on **Open** and navigate to the method file created in the last section.

3 Under **Data File Type** select **For Acquisition**. Amount Values are not used in this exercise.

4 Click **Next**.

Figure 6.4 Sequence Wizard - Unknowns Dialog Box
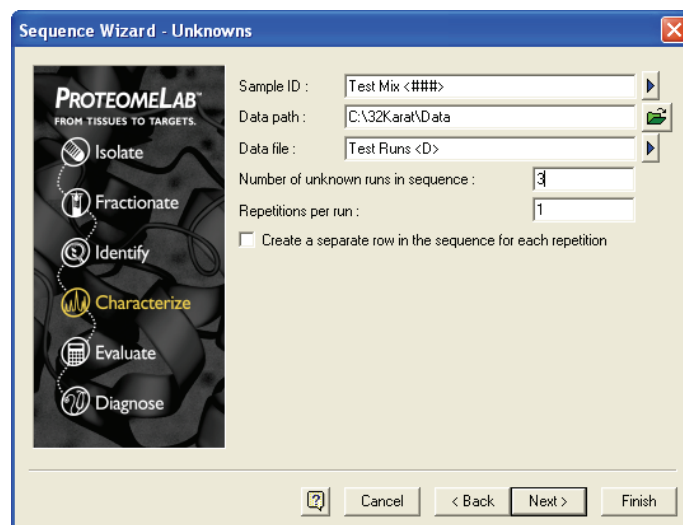
This screen is used to create the Sample ID and Data File ID.

5 Type a text string in the Sample ID box. The blue arrow to the right of the text box opens the following menu:

Figure 6.5 Sample ID text options menu

Line Number
Increment Number
User Name
Method Name
Instrument Name
Date and Time
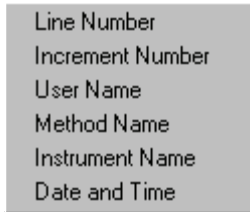
Selecting an item from this menu causes a symbol to be inserted in the Sample ID field. In the example shown, Line Number was selected. This parameter is automatically incorporated into the Sample ID when the sequence is run. You can select any combination of these items. Data Path specifies the directory where the data files are stored.

6 Click **Open**, and select **Data Path**, then navigate to the folder.

7 Type a text string into the Data File box. The blue arrow to the right of the text box opens this menu:

Figure 6.6 Data File Text Options Menu

Line Number
Increment Number
Sample ID
User Name
Method Name
Instrument Name
Date and Time
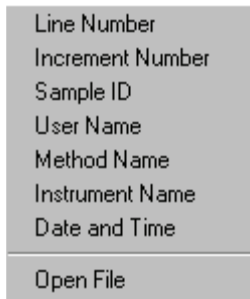
Open File

As described above for the Sample ID, this menu is used to insert codes that are automatically inserted into the filename when the sequence is run. In this example, Date and Time has been selected. (The Open File choice is used to reprocess existing data files, not for data acquisition).

Number of Unknown Runs in Sequence determines how many lines there are in the Sequence Table. For this exercise, input 3.

Repetitions per Run determines the number of times each line in the Sequence Table is run. An identifier is automatically added to the file name for each repetition when this option is used.

1 For this example, input 1.

2 Click **Next**.

Figure 6.7 Sequence Wizard - Vials Dialog Box
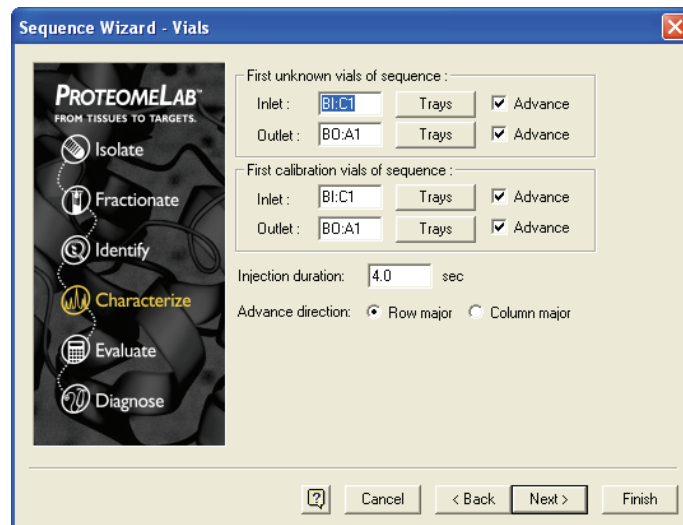
If the method being used allows vial position override (see the Method exercise), the desired starting position can be input here. If override is not allowed, or if auto-incrementing has been set, any input here is ignored. A set of calibration vials can also be identified in this window. See the resources in Appendix 1 for more information on calibrating through the Sequence Table.

Advance Direction enters the advance direction for the sequence.

When the Advance box is enabled for either or both inlet and outlet vials, the software automatically fills down vial position values in the Sample Inject Inlet/Outlet columns of the sequence table. This automatic fill down eliminates the need for manual input, one vial position at a time in the sequence table. If Row Major is selected as the Advance Direction, the software automatically fills down vial positions, incrementing them by rows in the same tray (for example, A1, A2, A3, A4, A5, A6, B1, B2, B3, B4 for buffer tray configuration). If Column Major is selected as the Advance Direction, the software automatically fills down vial positions, incrementing them by columns in the same tray (for example, A1, B1, C1, D1, E1, F1, A2, B2, C2, D2 for buffer tray configuration).

Do not confuse Advancing vial positions with the vial incrementing operation in instrument runs. Advancing vial positions here refers to simplifying sequence table planning and organizing. These vial position values can be changed in the sequence table, and they are not saved with any method. They are saved in the sequence.

Figure 6.8 Sequence Wizard - Calibration Dialog Box
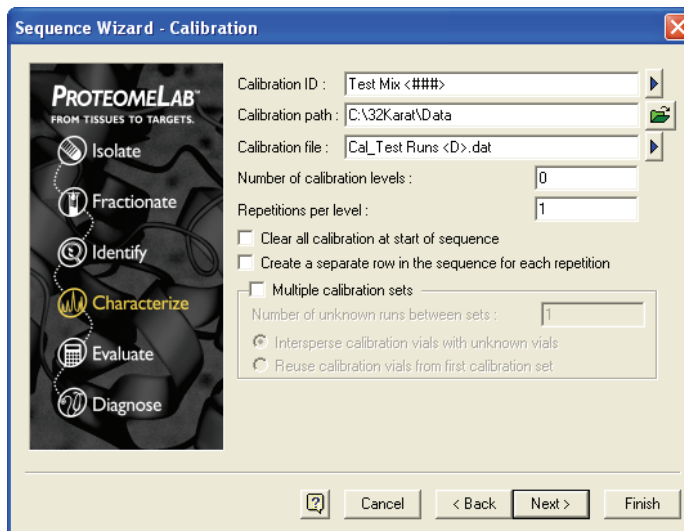
This example is not calibration. Calibration is covered in Appendix 1.

---

1 Input 0 in the Number of calibration levels box, and click **Next** to continue.

Figure 6.9 Sequence Wizard - Reports Dialog Box
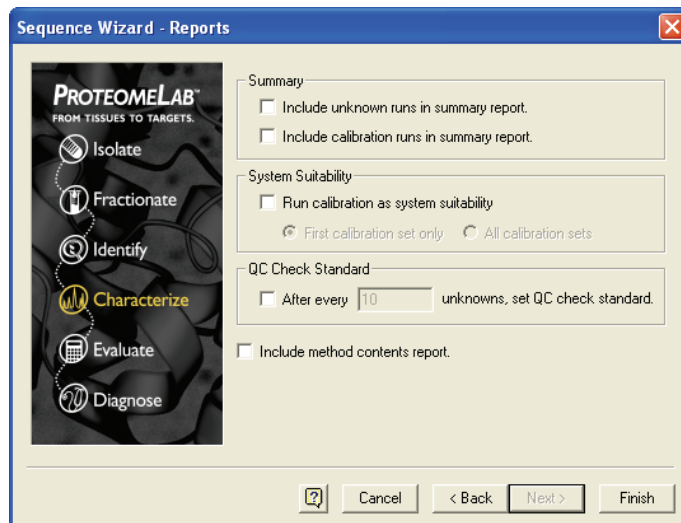
This screen allows you to have reports generated automatically at the end of the sequence run. This exercise does not generate a report. All the other choices should be blank.

---

2 Click **Finish**. A window similar to this one displays.

Figure 6.10 Sequence Table (Part A)

This screen contains many more columns than can be displayed at one time. Most of this information can be edited.

3 Scroll left and right to review the entire screen. Not all of the columns are used in this exercise. The important ones are described below.

Run Type - Because we have not selected any summary reports, all of the runs are of the unknown type.

Reps tells the system how many times to run the line.

4 Click in the **Reps** box for the second line and change the Reps to 2. Line two will now execute twice, giving a total of four runs in this three-line sequence.

5 Scroll to the right until the window resembles the one shown here.

Figure 6.11 Sequence Table (Part B)

Sample ID is based on the Sample ID text you entered in the Wizard. The line number has been added to the Sample ID you input.

6 Click in the **Sample ID** box for Run #2 and change it to read Test Mix 002 Twice.

Method shows the method you entered in the Sequence Wizard.

To change the method, click in the Method box and click on the green icon. (For this exercise, do not change the method.)

Filename is based on the File Name Text you entered in the Sequence Wizard. The filename still shows the Date and Time symbol <D>, because date and time are unknown until the sequence is run.

7 When you are finished editing the table, select **File > Sequence > Save As** from the menu bar. Use a Sequence name that has significance to you, for example, TestSequence_081506.

Running the Sequence

You will now run the sequence. The instrument must be prepared for operation as described under Single Run Mode earlier in this section. Verify through Direct Control that the instrument UV lamp or laser is turned on. The test sample and buffer vials must be loaded and installed. You must be "online" before proceeding.

---

1 Click the ⬛ icon from the Instrument window toolbar. This icon is not available if you are in Offline mode. The dialog box shown here displays:

Figure 6.12 Run Sequence Dialog Box

---
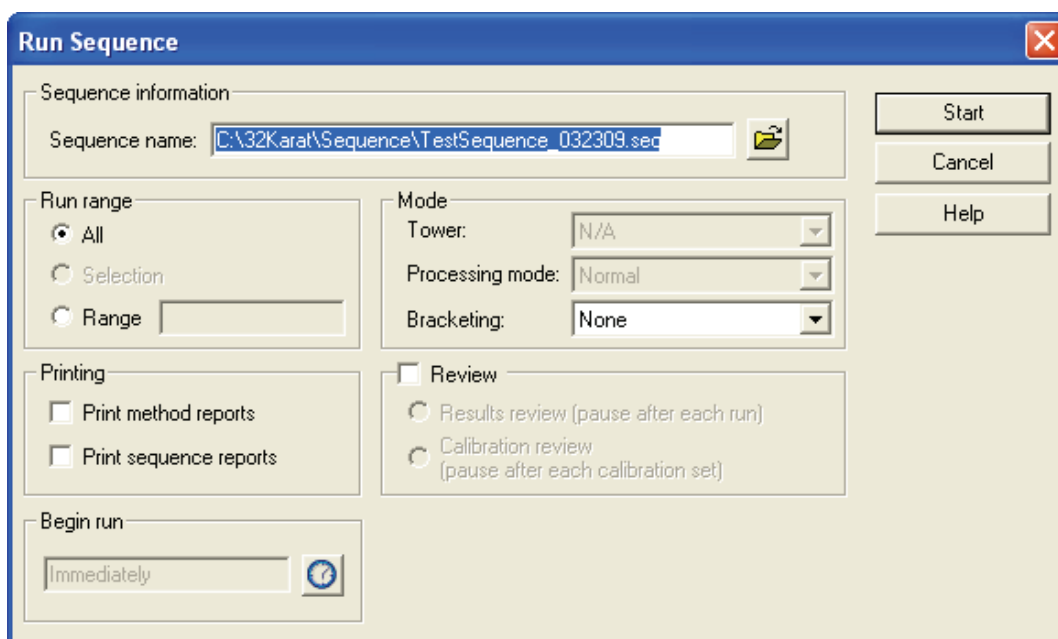
2 Click on the **Open** 📂 icon and select the sequence you just created. The sequence name displays in the text box.

Run Range allows you to run only part of the Sequence.

---

3 For this exercise select **All**.

Printing allows the system to automatically print reports when they are ready. If you do not have a printer connected, leave this box unchecked.

When complete, the dialog box should look like the one shown in Figure 6.12, but with your sequence name in place of the one shown.

4   Verify that the instrument is loaded and ready to run, then click **Start**.

Sequence Validation

The software validates that the methods in the sequence are appropriate for the current instrument configuration. If any problems are detected, a message displays and the sequence does not run. Correct any problems noted and restart the sequence.

After a successful sequence validation, the method in the first line is downloaded to the PA 800 *plus* and the run will begin. Observe the operation of the instrument as a guide to future operations and troubleshooting. You can open the Direct Control window during data acquisition to view real-time information on instrument status. During the run, the data displays in real time in the instrument windows. One window shows the Absorbance (or Fluorescence) signal, and a second window shows the Current signal, as those channels were selected in the method. If you are using a PDA detector, a third window displays a contour plot of the absorbance signal.

At the end of each run, the method for the next run is downloaded before the new run begins. This feature allows you to make changes to a method while a sequence is processing. The version of the method that is current at download (last saved) is the version that runs. You can also edit the Sequence Table during processing. Existing lines that have not been started can be edited or deleted, and additional lines can be added to the table. (Lines that have already completed or are currently executing cannot be edited).

NOTE   When a sequence is built using a method that has **auto-incrementing** enabled, edits that change the auto-generated positions are not allowed. If **allow override** is not selected, relevant sequence table entries are not editable.

When the sequence is complete, you have acquired data from four runs. The two replicate runs from line two have file names to which rep 1 and rep 2 have been added.

It is helpful to experiment with the Sequence Table before proceeding to the next section. Make changes, process the sequence, and observe the effects. You may want to combine these experiments with the writing of new methods. A series of different methods can be entered into the Sequence Table. When processed, the results demonstrate the effects of changes in parameters. For example, you might create a series of methods at different separation temperatures (for example, 20, 22, 24, 26, 28, and 30°C) to examine the effect of that parameter on your separation. Another series of experiments might examine the effects of changing the volume injected. If your method allows override, this can be done with a single method by editing the Sample Inject Duration column in the Sequence Table.

Integration

Description

An electropherogram is a graphical depiction of the passage of molecules through the detector window. The area under the peak is directly related to the amount of the analyte present in the sample. The process of determining this area is known as Integration. In practice, integration includes steps that determine the points at which peaks start and stop and the shape and position of the baseline. If sample components are not fully separated from one another (resolved), it is also necessary to estimate where to divide the area of the two peaks. A very simple data set is shown in the figure below.

Figure 7.1 Typical Data Set
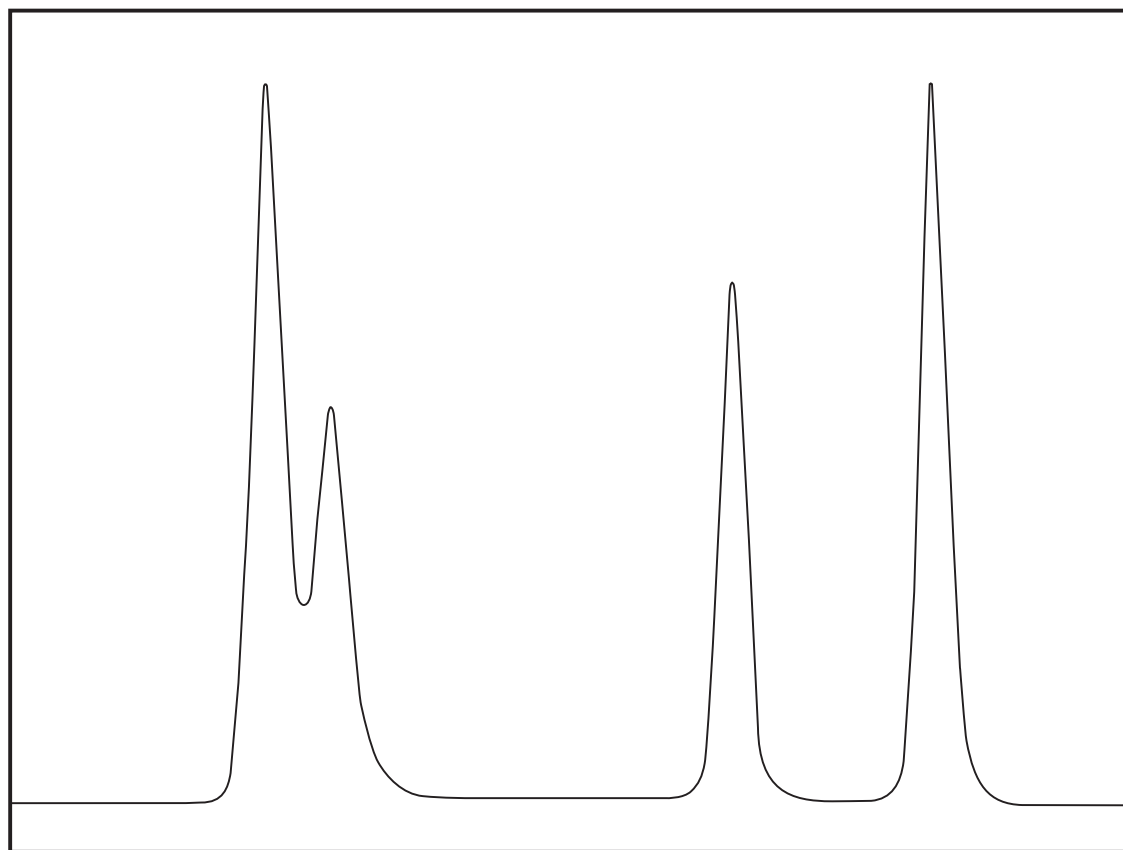
901023L.AI

In this example, there are four components. Two are well resolved from the others. Two other components are only partially resolved. The four components differ in peak area. This may indicate that they are present at different levels, that they produce different responses in the detector, or both. Quantitation is covered in the next section. This exercise focuses on peak detection and baseline construction.

Integrating Data

There are two required parameters for peak integration: width and threshold. More complex separations require the use of more parameters. The test mix separations used in the previous exercises yield very simple electropherograms. In this exercise, the examples are installed with the software. The principles of integration are the same regardless of detector type. Configure a UV instrument for offline analysis.

From the Instrument window select **File > Method > New**. Close the instrument setup window when it appears. It is not needed for this exercise.

NOTE Review the instructions in the *Preface* before using the sample files. Work from a copy of the data files and not the originals.

1 From the Instrument window, select **File > Data > Open**.

2 Navigate to your copy of the Data folder and select the file named CE Data Sample 1.dat. The data opens in the Instrument window.

3 Click the **CHANNEL A WINDOW** to select it, then type Ctrl-Z to zoom the data to maximum size. Only Channel A data is used. Auxiliary data (current, voltage, power, pressure) cannot be integrated.

4 Right-click on the electropherogram and select **Annotations**.

The following dialog box displays:

Figure 7.2 Trace Annotations Dialog Box
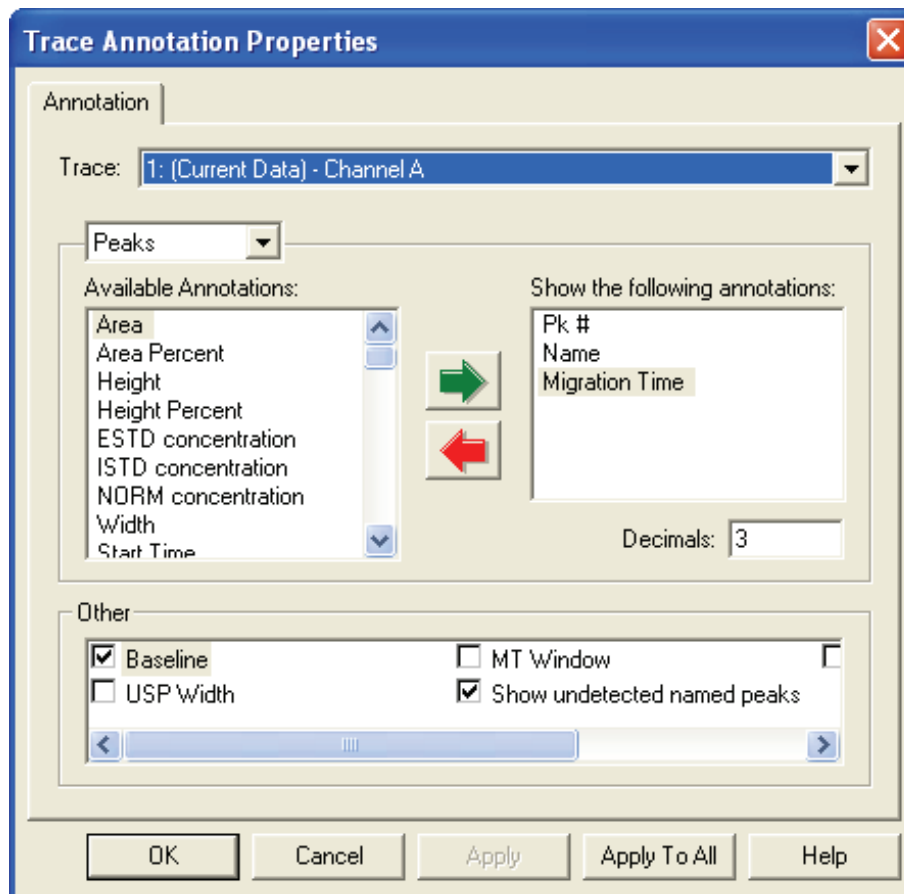
This dialog box allows the selection of items that display in the electropherogram window.

1 Select the desired items (**Peak#**, **Area**, and **Migration Time**) by clicking the item in the left box, then using the green arrow to move the item to the right box.

2 Verify that **Baseline** and **Show undetected peaks** are selected (checked).

3 When the dialog box looks like the one above, click **OK**.

NOTE Some items may not appear on the electropherogram display until the data is integrated.

4 From the Method menu select **Integration Events**. This opens the Integration Events spreadsheet. There are two items in this table by default: Width = 0.2 and Threshold = 50. Every Integration Table must contain at least one Width and one Threshold value.

5 To initially integrate the data, use only the default parameters.

6   Select **Analysis > Analyze** from the menu bar, or click on the **Analyze** button. The integration process takes a few seconds; larger data files, and files with more peaks, take more time to process.

During integration, the Analyze button becomes a Stop button. Clicking the Stop button aborts the analysis.

When integration is complete, approximately 26 peaks display. Above or below each peak the peak number, area, and migration time display. The default parameters have identified major peaks, but also capture a number of baseline artifacts that are not of interest.

To zoom in on the baseline and view integration results in detail, you can use the mouse to rubber box the display. Click and hold anywhere in the image. Drag the mouse to highlight the area you want displayed. When the mouse button is released, the selected area fills the window. Use Ctrl-Z to zoom out.

Examining the area between 3.0 and 3.4 minutes demonstrates that even negative peaks (valleys) are being detected.

Optimizing Integration

1   By selecting **Window > Tile Horizontally** the electropherogram and the Integration events table can be displayed together.

2   Resize the windows as desired by clicking and dragging a window's edge with the mouse.

3   Click in the **electropherogram window**, then use Ctrl-Z to zoom out, if necessary.

4   Click in the **Value** column of the Threshold event in the Integration Events Table.

5   Enter the number **100**.

6   Analyze the data again.

This time there should be about ten peaks visible. Close examination reveals that these are most likely real peaks and not baseline artifacts, although some of them are quite small. Examine the area between 14 and 15 minutes, for example.

Threshold determines how high a peak must rise above the background noise before it is recognized as a peak by the integration software. Increasing the value of Threshold removes background artifacts. If taken to extremes, it can also remove real data.

7 Set the Threshold value to 1000 and analyze the data again.

You should observe that the minor peaks are no longer integrated, and the baseline below the large peaks has shifted dramatically.

8 Before examining the effects of the Width setting, restore the Threshold value to 100.

9 Click **Analyze** again.

10 Zoom in on the base of the major peaks.

11 Change the Width value to 0.1 and Analyze. The tail of the second major peak should now be split off as a separate area.

Change the Width to 0.5 and analyze. The tail is again cut off but in a different place than it was with the smaller value for the window.

The question arises, which is the correct integration? The answer is, it depends on the situation. Your knowledge and judgement determines the correctness of the integration results. In this example, the peak tailing is a separation artifact, and the area of the tails should be included in the area of the peak. To obtain this result, with this data file, a Width of 0.2 and a Threshold of 100 seem to be appropriate.

Integration Parameters

There are many other integration parameters, besides the required Width and Threshold, that can be used to generate an integration result that properly reflects the components of the sample. These are discussed at length in the resources listed in APPENDIX A, *Additional Resources*. In the next part of this exercise, these additional parameters are used to aid in the integration of a somewhat more complex data file.

To begin, return the Width value to 0.2 and the Threshold value to 50 (the default values). Open the data file CE Data Sample 2.dat. Use Ctrl-Z, if necessary, to view the full file. This data file differs from the previous example in several ways. There are multiple major peaks, distributed throughout the run. There is a long stretch, from about 12 to 21 minutes, where there are many features that may be peaks, or may be artifacts. Close examination of the peak between 24 and 25 minutes reveals that it is, in fact, at least three separate peaks that are not fully resolved. The major peaks are also taller and wider than the peaks in the previous example.

After examining this file, zoom out (Ctrl-Z), and analyze with the default Width and Threshold values. A large number of peaks are identified. The big peak at the end of the run is not included. The analysis appears to end at around 28 minutes. Zoom in on the area around 27 to 28 minutes. Many peaks which appear to be quite small have been identified and the last peak is number 150. There is a limit of 150 peaks built into the software, which accounts for the end of the data being ignored.

Not all 150 components are of interest. Some parameters need to be changed to reduce the number that are identified. In this part of the exercise, graphical programming allows you to build the integration table.

Width

1   Zoom out to full size, then zoom in on the first major peak (around 9.5 minutes). This peak is nearly 0.5 minutes wide at the base, and it is significantly wider than the surrounding smaller peaks.

2   Right-click on the electropherogram and select **Graphical Programming**. A new menu of integration parameters displays.

3   Select **Width**. A blue dialog box pops up instructing you to select peak starting point. Click on the start of the Peak.

4   Place the mouse pointer near the left edge of the major peak along the baseline and click.

You are prompted to click on the End of the peak. After the Start and End of the peak have been entered, the following **Width** dialog box displays. Your values might differ slightly from those shown, depending on exactly where you clicked.

Figure 7.3 Determining Width of Peak
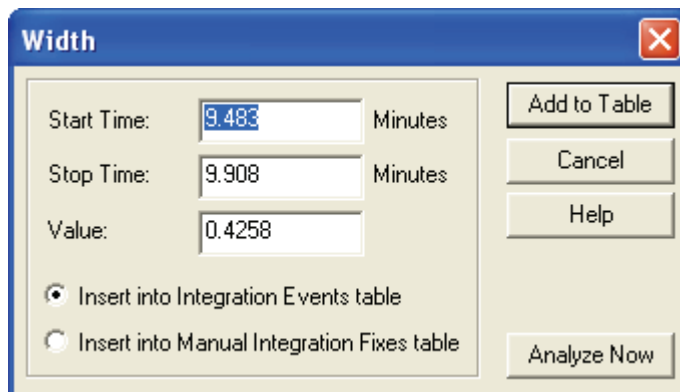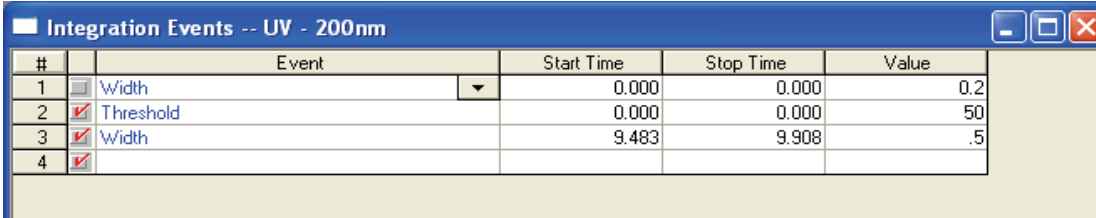
There are several options available in this dialog box. Start and Stop times refer to the points where you clicked. For the Width parameter, the value is the difference in these two time points (the width of the peak). There is an option to add this parameter to the Integration Events table or to the Manual Integration Fixes table. These two choices differ in one critical way. Items added into the Integration Events table become part of the method; they affect any data file that is integrated with that method. The Manual Integration Fixes table becomes part of the data file; items here affect the integration of only the single data file to which they are attached. In this exercise, work only with the Integration Events table. Manual fixes are discussed in the resources in APPENDIX A, *Additional Resources*.

The **Add to Table** option adds the event into the Integration Events table. **Analyze Now** adds the event to the table, but it also causes the integration to be redone with the new parameter in place. Click **Add to Table**.

The new Width item is visible in the Integration Events table. This item is only in effect for the interval between the Start Time and Stop Time. You want this event to be in effect for the entire data set. To have a parameter active over the entire run, set both Start Time and Stop time = 0. Change the Start and Stop times of the new width value to = 0. For purposes of consistency with this manual, change the Value of the new Width event to 0.5. (Your value should already be very close to this.)

Now we have a problem. There are two Width statements with different values, both of which are declared for the entire run. We could delete the old Width statement, but the software offers an easier alternative. At the left of each line is a box with a red check mark. Click on the red mark on line one to de-select it. This line is ignored as long as it is unchecked. Your Integration Events Table should look like this one:

Figure 7.4 Integration Events Table

#		Event	Start Time	Stop Time	Value
1	☐	Width ▼	0.000	0.000	0.2
2	☑	Threshold	0.000	0.000	50
3	☑	Width	9.483	9.908	.5
4	☑				

Integration Events -- UV - 200nm

Threshold

1 Zoom out and click **Analyze**.

There should be a small change in the integration. Changing the Width alone is not sufficient to integrate this data set. We are still finding too many of the very small peaks that are of no interest.

2 Right-click on the electropherogram, select **Graphical Programming**, and then **Threshold**.

3 Following the prompts in the status bar, click near the start of the electropherogram (time 0.0), and at around 8.0 minutes.

4 When the dialog box appears, click **Analyze Now.**

The new threshold value, which should be around 850, has removed many of the small peaks in the 0-8 minute time range. The new threshold value has also been added to the Integration Events table.

5 As with the Width parameter, change the start and stop times of the new Threshold event to 0, and deselect the old Threshold parameter.

6 Again for consistency, adjust the new Threshold value to exactly **850**.

7 Analyze the sample.

You should see that the change in threshold has had dramatic effects. Almost all of the small peaks have dropped out of the integration results. The peaks near the end of the electropherogram have been found. But we now have a problem with the cluster of peaks between 24 and 26 minutes.

8 Zoom into this area and observe that the baseline starts about halfway up the start of the peak.

This demonstrates the fact that global parameters do not always work for every peak in the data set. We need to add new lines that affect only the region of the clustered peak.

Integrating a Peak Cluster

Recall that with the original Width and Threshold values (0.2 and 50, respectively) this cluster was integrated nicely. Perhaps one or both of those values, applied to the region around the cluster, can achieve the desired results.

1 While still zoomed in, select **Width** from the Graphical Programming menu.

2 As prompted, click at about 24 to 25 minutes.

In this case, we are using the graphical function to define Start and Stop times rather than to define Width.

3 In the dialog box that displays, change the Value to 0.2.

4 Click **Analyze Now**. The cluster should appear similar to the one shown here:

Figure 7.5 Well Integrated Peak Cluster
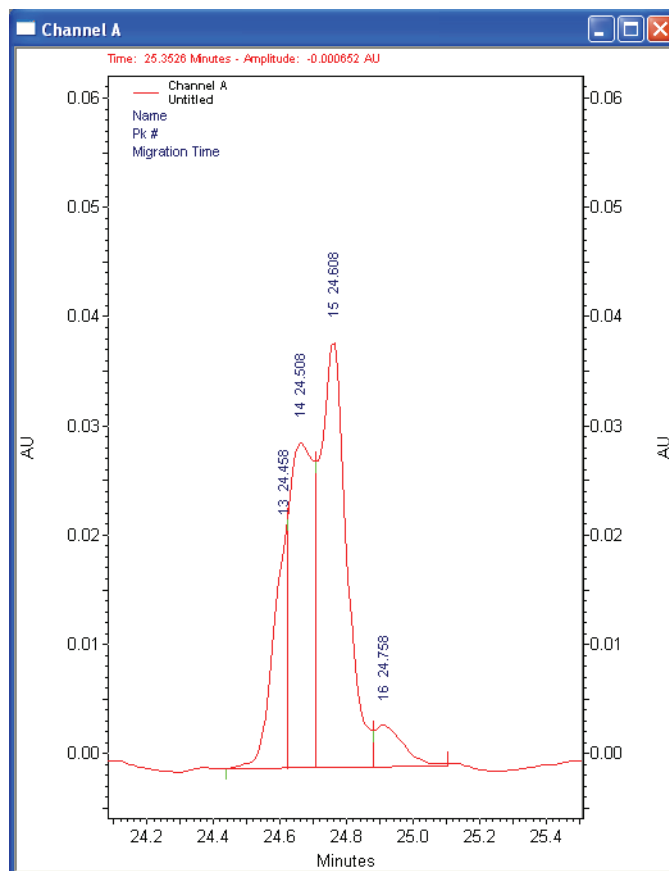
This cluster is well integrated. Even the shoulder on the leading edge of the cluster has been detected. Use Ctrl-Z to zoom out. There are now two Width values in the Integration Events Table. One is global, the other (the new one) is only in effect over the time range specified in the event.

Integration Off

This integration is nearly done, but there is still a problem at the end of the electropherogram. At the end of the run is a baseline shift that is being recognized as a peak.

1 Zoom into the area from about 34 minutes to the end of the run.

2 We can see that there is really nothing of interest beyond the peak at 35.2 minutes.

3 To turn off integration, select **Integration Off** from the Graphical Programming menu (while still zoomed in).

4 Click at about 35.8 minutes, and at the end of the electropherogram, click **Analyze Now**.

The peaks in the specified area do not display from the integration results, and the Integration Off item is added to the table. Like the Width value used to resolve the peak cluster, the Integration Off function is only active over a specific period of time. All of the integration functions except for Shoulder Sensitivity can be used globally or locally. For example, you could use another Integration Off event from Start Time=10 to Stop Time=20 to eliminate the remaining small peaks. The integration now looks acceptable. The Integration Events table should resemble the one below. The Start and Stop times may differ slightly, depending on where you clicked.

Figure 7.6 Integration Events Table

5 Delete lines one and two, since they are unused. Leaving them in and unchecked does no harm.

6 Save the method, or all previous efforts are lost.

7 Select **File > Method > Save As**.

8 Save the method as **Integration Exercise.met**.

NOTE You may see a warning that the Separation Time of the method is 0.0. Select **Yes** to continue with the save. This message appears because, in this exercise, nothing was added to the Instrument Setup window of this method, and this omission has been detected by the Auto-Validation function of the software. In actual practice, Integration Events would be added to the method used to acquire the data.

Integration Results and Reports

The results of your integration efforts go beyond some labels on the electropherogram. These results can be used to generate reports and to identify and quantitate unknowns. These topics are covered in later sections of this manual. For now, use a pre-defined report to capture the current results. From the menu bar select **Reports > View > Area%.** A report displays on the screen showing the Integration results in tabular form. If you want a hard copy of this report (and if a printer connected to your system), right-click on the report and select **Print.**

Calibration

Introduction

The previous exercise demonstrated how the 32 Karat Software can be used to determine peak parameters such as migration time, area, and height. This information is of limited use unless it can be translated into terms that are descriptive of the components in the sample. There are two basic questions that can be asked: what is this component, and how much is present in the sample?

Analysis Components

Qualitative Analysis

The first question, what is this component, refers to QUALITATIVE analysis. It can be answered in two different ways. First, if a known substance is used as a standard, the migration time or mobility of the resulting peak (possibly combined with other information such as a PDA absorbance spectrum), can be taken as evidence that a peak in an unknown sample represents the known substance. Second, if the migration time or mobility varies in some linear way with a molecular property, such as molecular weight or number of base pairs, a standard curve can be created and used to determine the defined quality (such as molecular weight) based on a series of standards. In this method, the unknown need not be present in the standard mixture.

Quantitative Analysis

This exercise deals with the second question, how much is present in this sample, which is a question for quantitative analysis. In quantitative analysis, there are a series of runs with differing concentrations of a known substance or substances. For each concentration of each component a detector response is determined. By generating a graph of detector response versus concentration, it is possible to determine the concentration of the components in an unknown. The 32 Karat Software simplifies this task. It contains tools for collecting the data from calibration runs, generating appropriate curve fits, and using the curve fit data to analyze unknowns. This exercise introduces you to using a sequence for post-run data reprocessing. It uses an External Standard calibration where data from standards and unknowns are acquired in separate runs.

Creating a Calibration

Included in the Data Samples directory are five files named: **CE Level 1.dat**, **CE Level 2.dat**, **CE Level 3.dat**, **CE Level 4.dat**, and **CE Level 5.dat**. These data files are the results of runs of the same two components at five different concentrations as indicated in the table below.

Table 8.1 Data Samples Directory Files

Filename	Component (units/mL)	
	Alpha	**Beta**
CE Level 1.dat	1.0	4.0
CE Level 2.dat	2.0	4.5
CE Level 3.dat	3.0	5.0
CE Level 4.dat	4.0	5.5
CE Level 5.dat	5.0	6.0

The first step in the analysis is to identify the expected migration times of the two peaks. To do this, open an instrument window (continue to use the offline UV instrument from the previous exercise). See CHAPTER 3, *Manual Configuration*.

1 Select **File > Data > Open** and navigate to the **Data Samples** directory.

2 Open your copy of the file **CE Level 3.dat**.

Two channels of data are present: Channel A (UV 214nm) and Channel C (current).

You may minimize the Channel C window; it is not used in this exercise. Channel A is the electropherogram from the analysis of the middle concentration of the calibrators. The smaller of the two peaks between 3.5 and 4.5 minutes is Alpha, and the larger of the two is Beta (previously determined in runs of the individual components).

Developing a Calibration Method

In this exercise, build a new method to calibrate the data set.

1 Select **File > Method > Open**.

2 Navigate to your copy of the Data directory and open the method **CE Calibrate.met**.

3 Select **Analyze** to integrate the data set. (The integration parameters have already been optimized.)

The two large peaks are well integrated with the default parameters.

---

4 Right-click on the electropherogram and select **Graphical Programming**, then select **Define Peaks**.

---

5 Click in the electropherogram to bracket the large peaks (approximately 3.7 and 4.2 minutes).

A dialog box pops up with instructions to click on the beginning and the end of the range of peaks you want to define. After you have chosen the range of peaks, the following screen displays:

NOTE Your time values may differ slightly from those shown.

Figure 8.1 Define Peaks Dialog Box
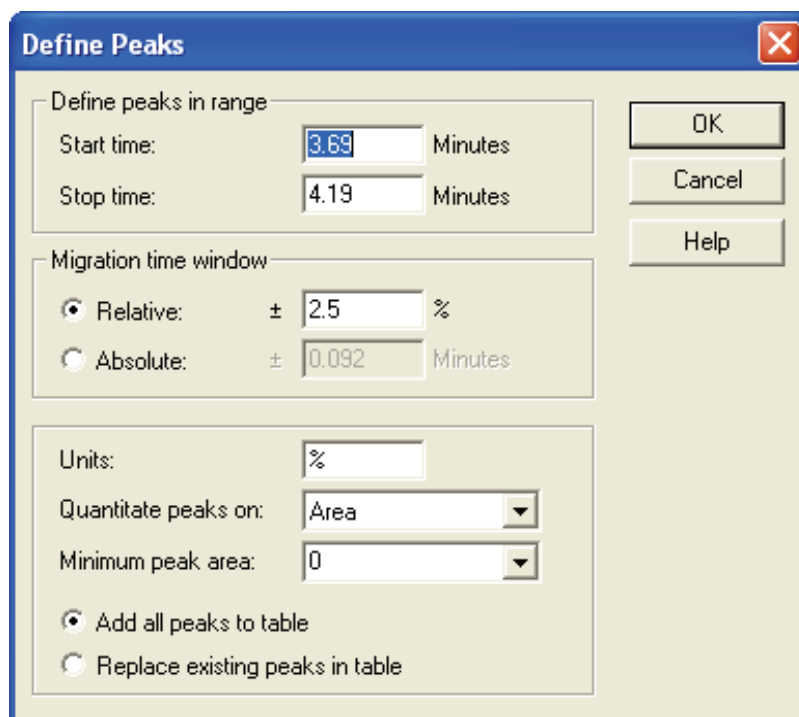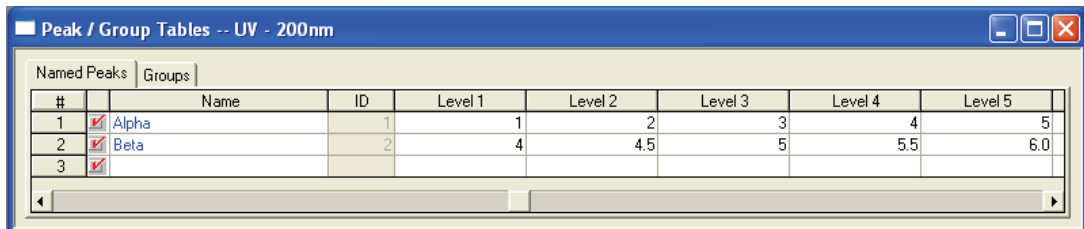
The Define Peaks functions allows you to place any peaks in the specified window into the method's Peaks/Groups table. Only peaks that have been identified during Analysis can be entered into a method. Start and Stop times define the part of the electropherogram that contains the peaks to add. The Migration time window allows us to specify how much variability in migration time is allowed before a peak is no longer considered to be the component of interest. This can be defined as either a percentage of the defined time, or as an absolute time interval. We can add all the peaks in the window to an existing table. If you choose to replace existing peaks, any currently defined peaks that fall within the newly defined time windows are deleted.

For this exercise, accept the defaults by clicking the OK button.

1 From the menu bar select **Method > Peaks / Groups**.

A table opens listing the two peaks just added. As these peaks have not yet been named, the system creates a name based on the migration time.

2 Click in the **Name** column and change the name in line 1 to **Alpha** and the name in line **2** to **Beta**.

3 Use the scroll bar at the bottom of the window to review the column headings in this table.

Some of the columns are used in this exercise. See the resources in APPENDIX A, *Additional Resources* for more information.

The items that can be changed here include:

- Defined migration time and allowed window for a component.
- Data channel to be used (for multichannel detector types).
- Type of curve fit to be used for the calibration data set.
- Up to 10 calibration levels.

You have to input the concentration values from the table above.

4 Scroll right until the columns headed Level 1 through Level 5 are visible.

5 Input the known values for Alpha and Beta. Level 1 is the lowest value, Level 5 is the highest.

Accept the defaults for the other columns. When all the values have been entered, the table should look like Figure 8.2.

Figure 8.2 Peak/Group Tables

6 Save your method.

> **NOTE** You may get a warning message indicating the data collection time of the method is 0.0. Click Yes to proceed with the save.

7 Right-click on the electropherogram and select **Annotations**.

8 Select parameters so that only **Name** and **ESTD concentration** are in the Show box.

9 Click **OK**.

10 Analyze the sample again.

The name of the peak displays on the electropherogram. ESTD concentration is 0.000, because you have not yet completed the calibration process. At this point, you have only used one of the calibration samples to define the peaks. All five calibrators must be used to generate the standard curves for Alpha and Beta.

Generating the Calibration Curve

To generate the curves, you reprocess the data through a Sequence. In an earlier exercise, you used a Sequence to perform multiple runs. Sequences can also be used to do batch reprocessing of data.

1 Select **File > Sequence > Sequence Wizard** to open the sequence wizard.

2 Click on the **File** icon and select the method file you have been editing.

3 In the Data File Type field, select the radio button from existing data files.

4 Click **Next**. The next dialog box allows selection of multiple data files.

5 Click on the **File** icon. This opens a dialog box that allows the opening of multiple files at one time.

6 Locate the file **CE Level 1.dat**.

7 Double-click on the file name. The file name is added to the list at the bottom of the dialog box. Repeat for **CE Level 2**, **CE Level 3**, **CE Level 4**, and **CE Level 5**, so that all five files appear in the list.

8 Click **Open** when done.

9 Verify that the five files are listed in the window, then click **Finish.**

This opens a Sequence table with the method and the five data files listed.

10 Scroll right to the **Filename** column. The five files should be listed in order.

11 Scroll back left to the **Level** column. Change the level in line 1 from **0** to **1**.

12 Use the down arrow to move to the same column in line 2, and change the **0** to **2**. The Level entered in this column of the sequence table corresponds to the Level column in the Peak \ Group table.

13 Continue down the column changing the remaining zeros to 3, 4, and 5, respectively. As you change the level to a non-zero value, the sample type automatically changes to Calibration.

14 Click in the **Run Type** column of line 1. The dialog box shown in Figure 8.3 opens. The items here are the available run types.

15 Click on **Clear All Calibration** and click **OK**. The run type for line one changes to **CAL CCA**.

Selecting this type causes any previous calibrations of this data set to be cleared before the new calibration is performed.

Figure 8.3 Sample Run Types Dialog Box
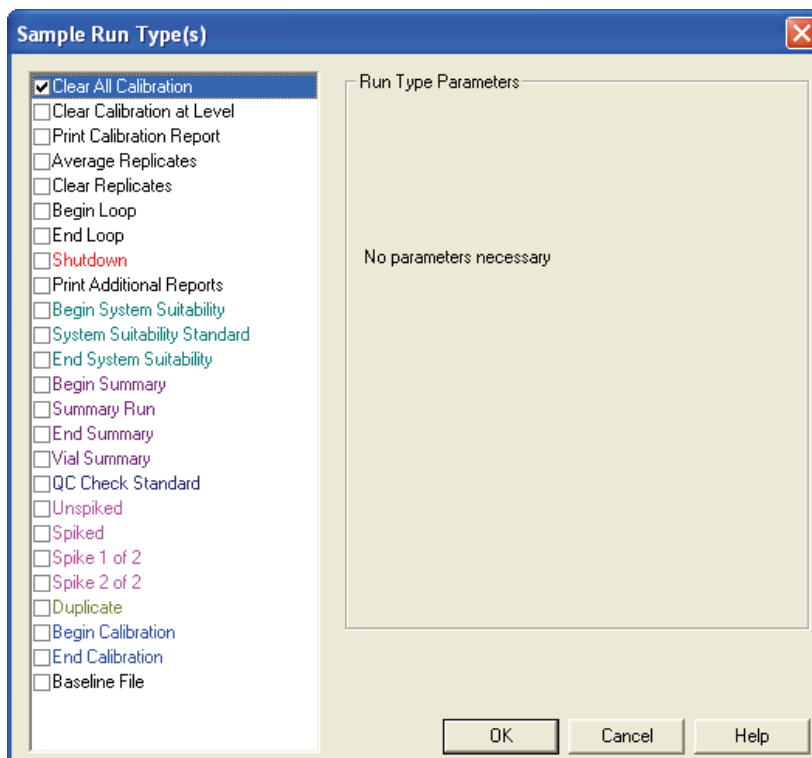
16 Save the sequence with the name CE Calibrate.seq. The right and left sides of the window should look like Figure 8.4.

Figure 8.4 Sequence Table

You are ready to Process the sequence and generate the calibration.

1 Select **Process** from the Sequence menu. The dialog box in Figure 8.5 displays.

2 Run range must be **All**.

3 Processing mode must be set to **Reintegrate**.

4 Click **Start**.

The processing requires a few seconds to complete. As each data file is analyzed, the status column in the Sequence table changes to **Complete**.

Figure 8.5 Process Sequence Dialog Box

5 From the menu bar select **Method > Review Calibration**.

A window opens showing the results of the calibration for the first component (Alpha). The data looks reasonable, but the line fit could be better.

6 Select **Beta** in the upper right corner of the window.

The curve fit here is certainly not acceptable. The lower right corner of the window indicates that this fit has been made point-to-point. Perhaps another type fit would be better.

7 Re-open the **Peaks / Groups** table.

8 Scroll left to the column headed **Fit Type**.

9 For line one (Alpha) select **Quadratic**.

10 For line 2 (Beta) select **Linear**.

11 In line one, select the box in the **Zero** column to force the line through the origin.

12 Do not click Zero in line 2.

13 Save the method and look at the calibration results again.

This time the curve fit should be better for both Alpha and Beta. In practice, you would select and vary the curve fit parameters to determine the most appropriate fit for your data set.

Saving the method saves the calibration. It is not necessary to reprocess the sequence.

Analyzing Unknowns

Creating a standard curve is only useful if it can be used to determine the amount of material in an unknown. Unknowns can be added to the sequence table after calibration lines. When processed, the unknown peaks are compared to the standard curve and the values calculated.

1 Open the **Sequence Window**.

2 In line 6, click in the **Method** column and select **CE Calibrate.met** (the method we have been using).

3 Under **Filenam**e, select CE Unknown1.dat.

4 Save the sequence.

5 Instead of reprocessing the entire sequence, click on the **Range** button in the **Process Sequence** dialog box (Figure 8.5) and enter 6 as the line to be run.

6 Click **Start**.

To see the results of this analysis, display the electropherogram for CE Unknown1.dat or view a report.

To use a default report, select **Reports > View > External Standard** from the menu bar. To print this report to your system's default printer, right-click in the report window and select **Print**.

See Creating a Method Custom Report in CHAPTER 11, *Creating Reports* for more information.

This exercise has covered only the basics of quantitative analysis. For more detail and more options, refer to the resources in APPENDIX A, *Additional Resources*.

Qualitative Analysis

Data Analysis and Reporting

In some modes of CE separation, the order of migration is determined by a molecular quality. The most common applications are separation of proteins by molecular weight and separation of nucleic acids by number of base pairs. The 32 Karat Software allows you to construct a standard curve based on parameters such as these. In the case of CE-SDS for Protein Molecular Weight Determination, for example, a series of fragments of known length are separated in a gel-filled capillary. A plot of migration time versus molecular weight is constructed, and this plot is used to determine the size of an unknown protein.

In this exercise, you create a standard curve based on the test mix containing several molecular weight size standards ranging from 10 to 225 kDa. The test mix is injected into a capillary containing a polymer network. Smaller molecules will move faster through the polymer strands than larger molecules. This results in a separation based on size. The mobility of peaks of known standard sizes is used to create a standard curve that can be used to estimate the size of an unknown fragment.

Figure 9.1 Electropherogram of SDS MW Size Standard
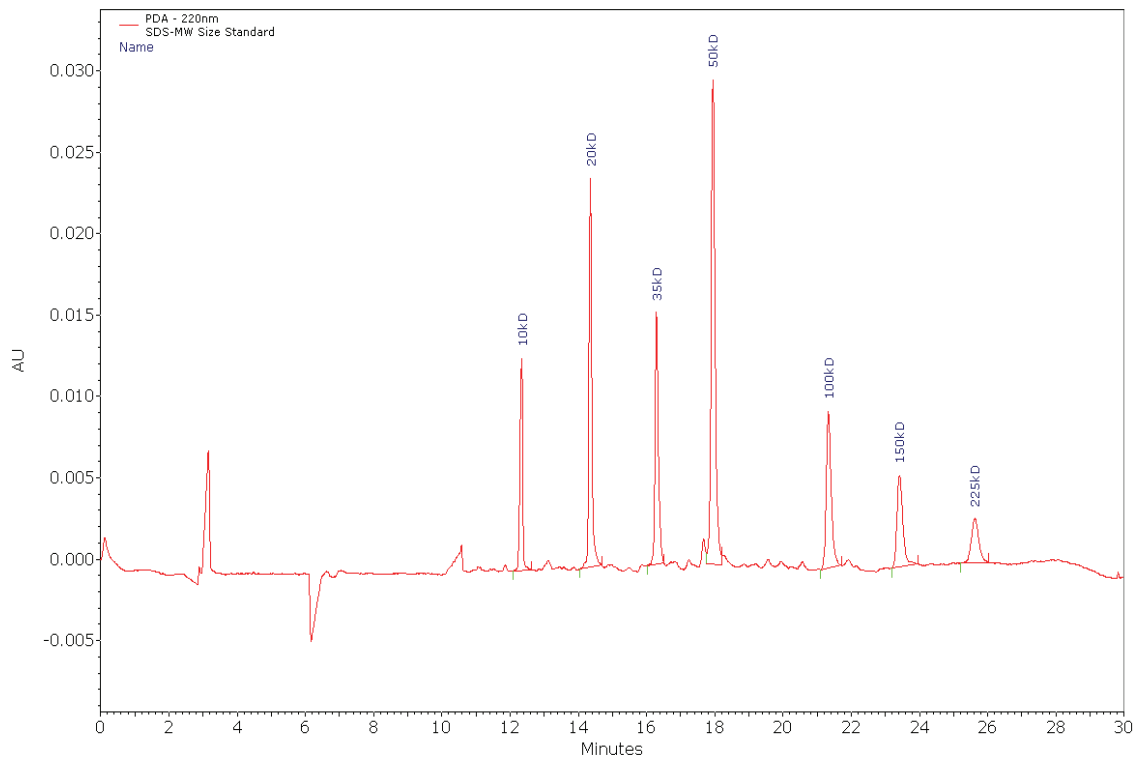
1   Open the SDS MW instrument in off line mode. The 32Karat software prompts you for a user name, password, and project. Choose SDS MW project to log on.

2   From the SDS MW project folder, open the SDS20SizeStandard.dat and SDS MW Separation - PA800 Plus Size Stds.met.

3   Analyze the data.

4   Right-click on the electropherogram and select **Annotations**.

5   Add **Name** and **Mobility** to the list of annotations to display, then click **OK**.

6   From the **Method** menu select **Qualitative analysis** to open the window shown below. Enter the molecular weight (Y) and the mobility (X) into the method.

Figure 9.2 Qualitative Analysis Window
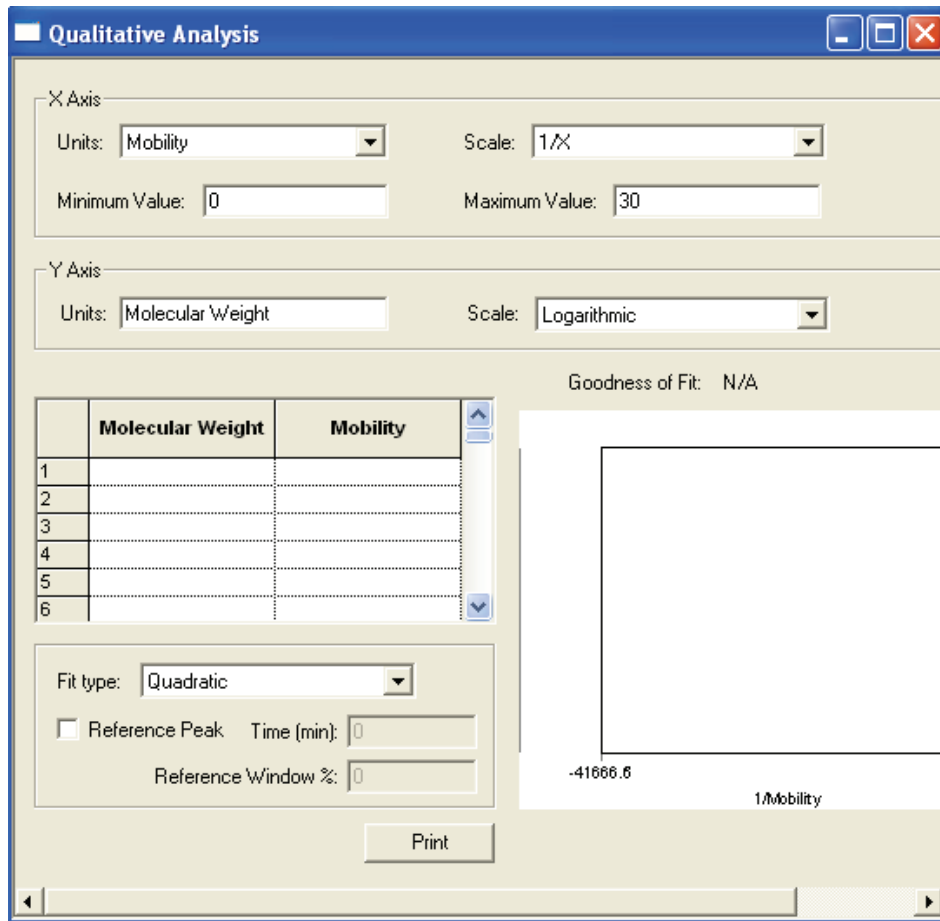
You can select the type of data for the X-axis. In this exercise, use mobility, but you could also use another parameter such as migration time. You can select either a linear or a logarithmic scale for this axis. Minimum and Maximum refer to the X-axis limits over which you allow the interpretation of qualitative data. This limits how far beyond the ends of the data set you can extrapolate to find the quality of an unknown.

For the Y-axis, you can use any name. Because our quality is the molecular weight, enter molecular weight. A Linear Y-axis scale is appropriate.

There is a two column spreadsheet in this window box (Figure 9.2). The Left column contains data for the Y-axis. The heading of this column contains the text entered for the Y-axis label, in this case molecular weight. The Right column contains data for the X-axis. The heading is that selected for the X-axis. In this case, use mobility.

---

1 You must manually enter the X and Y values into the spreadsheet.

For convenience, re-size the electropherogram and qualitative analysis windows.

2 For each peak, enter the molecular weights (from the peak ID table) and the corresponding mobility displayed in the electropherogram screen. Do not forget to type the minus sign (-) prior to the mobility. As you enter the data, it graphically displays in the lower right corner of the window. Make sure the scale for the X Axis and the Y Axis are 1/X and Logarithmic respectively.

3 When all the data has been entered, select **Fit Type** to determine the best curve fit for the data set. In this example, the best **Goodness of Fit** values are obtained using a quadratic fit.

4 When done, do a **Save As**; rename the method **Quality 1.met**.

5 Analyze the data file again.

6 Right-click on the electropherogram and select **Annotations**.

7 Add **Quality** to the right column. Name should already be present.

8 Click on the word **Quality** to highlight it, then set the number of decimals to **2**.

9 Click **OK**.

The electropherogram displays the assigned molecular weight (as Name) and the size calculated using the standard curve (as Quality). The base number is an integer. Because the **Goodness of Fit** is not perfect, there is a small discrepancy between the assigned value and the calculated value.

This method can now be used to estimate the molecular weight of an unknown protein. To do this, the unknown would be analyzed by the same method used to analyze these standards and the data analyzed with this method.

Mobility

Functions of Mobility

Mobility is a parameter that quantifies how a charged particle migrates in an electrical field. The equations for calculating mobility are defined in Cross Reference in Appendix B. Stated briefly, a component with a higher mobility moves more rapidly through the separation medium than does a component with a lower mobility. Because particles may be attracted to either the cathode or the anode, mobility has a vector component — it may be positive or negative. Mobility toward the cathode (negatively charged electrode) is defined as positive, and mobility toward the anode (positively charged electrode) is defined as negative.

Mobility is not a constant like molecular weight, as it can change depending on the separation conditions employed. As an example, consider the amino acid glycine. At alkaline pH, this molecule has a net negative charge (NH2-CH2-COO-) and a negative mobility. At acidic pH, it has a net positive charge (NH3+-CH2-COOH) and a positive mobility. Near pH=7, it has no net charge (NH3+-CH2-COO-) and a mobility of zero.

In practice, mobility is defined for a given molecule under a given set of conditions. Variations in separation conditions that affect all species equally (such as variations in electroosmotic flow or the voltage delivered by the power supply), and variations that have similar effects on closely related groups of compounds (such as the effect of small pH changes on a series of basic drugs), can be accounted for by including a standard of defined mobility in the analytical run.

There are two different mobility values to consider: electrophoretic mobility (μ), which represents the movement of the molecules in the electrical field, and apparent mobility (μapp), which is the algebraic sum of the electrophoretic mobility and any other forces that drive the sample through the capillary. The most common additional force is electroosmotic flow (EOF), sometimes referred to as μeof, although other factors such as pressure-assisted migration could also be considered here. In this discussion, apparent mobility is limited to the sum of electrophoretic mobility and EOF:

$$\mu = \mu_{app} - \mu_{eof}$$

The 32 Karat Software can use mobility as an alternate to migration time for peak identification. Used in this way, mobility increases the robustness of a capillary electrophoretic method by correcting for small changes that can be present from run to run. The software is also capable of displaying electropherograms with the X-axis scaled in terms of mobility rather than time. In this exercise, you review these features of the software.

Mobility Markers

Every run that incorporates mobility calculations must include a mobility marker. This is a component for which the mobility has been defined under a given set of run conditions. Reproducing these run conditions in subsequent runs is critical when using mobility as a parameter for peak identification. Buffer factors, such as ionic strength and pH, are also especially important. See the resources in APPENDIX A, *Additional Resources* for more information on this topic.

Assigning Mobility Values

Assigning a mobility value to the mobility marker involves a series of steps.

NOTE The following assumes that the method development work to create an effective separation technique for the samples has already been done.

1 Create a sample containing the proposed marker and an EOF marker.

The sample matrix should be a close approximation of the unknown samples to be analyzed. The EOF marker is a small, uncharged molecule that can be detected at low concentration. It is used to calculate the contribution of electroosmotic flow (μeof) to the apparent peak mobility. If running under conditions where EOF is minimal (such as a neutral coated capillary) the EOF marker can be omitted, because μeof is approximately equal to zero under these conditions. Standards for other peaks expected in the unknowns should be included in this run.

Figure 10.1 Advanced Method Options Window
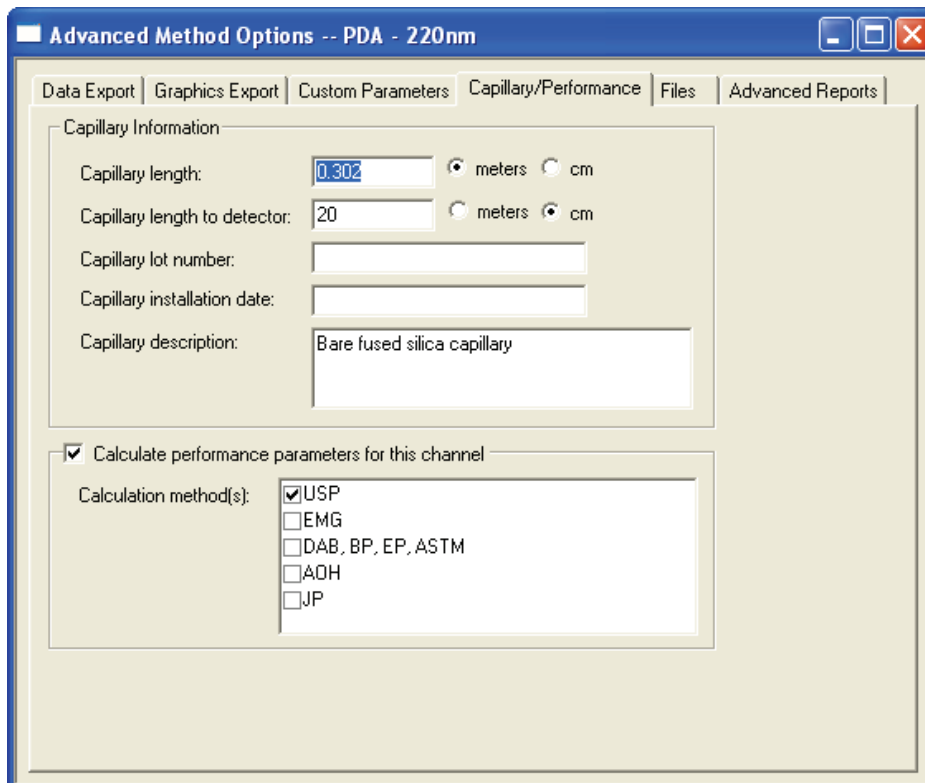
2 Run the separation and integrate the results.

3 Adjust all integration parameters to their final form.

4 Add the mobility marker peak and the EOF marker peak (if used) to the **Peaks** / **Groups** table.

The method must contain the correct dimensions for the capillary length. These are entered in the Capillary/Performance tab of the Advance Methods Options window (accessible from the Method menu). The capillary length and length to the detector are required.

5 Annotate the electropherogram with Apparent Mobility and Mobility.

6 Analyze the data again.

The Apparent Mobility and Mobility values display. At this point, the mobility values should all be zero.

7 Calculate the electrophoretic mobility of the mobility marker according to the following:

$$\mu = \mu_{app} - \mu_{eof}$$

where μapp is the apparent mobility of the reference peak, and μeof is the apparent mobility of the EOF marker.

8 Scroll right in the **Peaks** / **Groups** table to the Mobility column.

9 In the row containing the mobility marker, enter the value of μ determined in step 4.

10 Select it in the **Mobility Marker** option column of that same row.

11 Save the method.

12 Analyze the sample again and examine the electropherogram.

The mobility value of the marker should now be non-zero as should the mobility values for all other peaks in the electropherogram except the EOF marker. The EOF marker should have a value very close to zero. Variation from zero at this stage is the result of rounding errors.

If the results are acceptable, the method can now be edited to allow the generation of an electropherogram with mobility as the X-axis in place of time. This option is selected in the **Initial Conditions** tab of the **Instrument Setup** window:

Figure 10.2 Mobility Options in the Instrument Setup Window
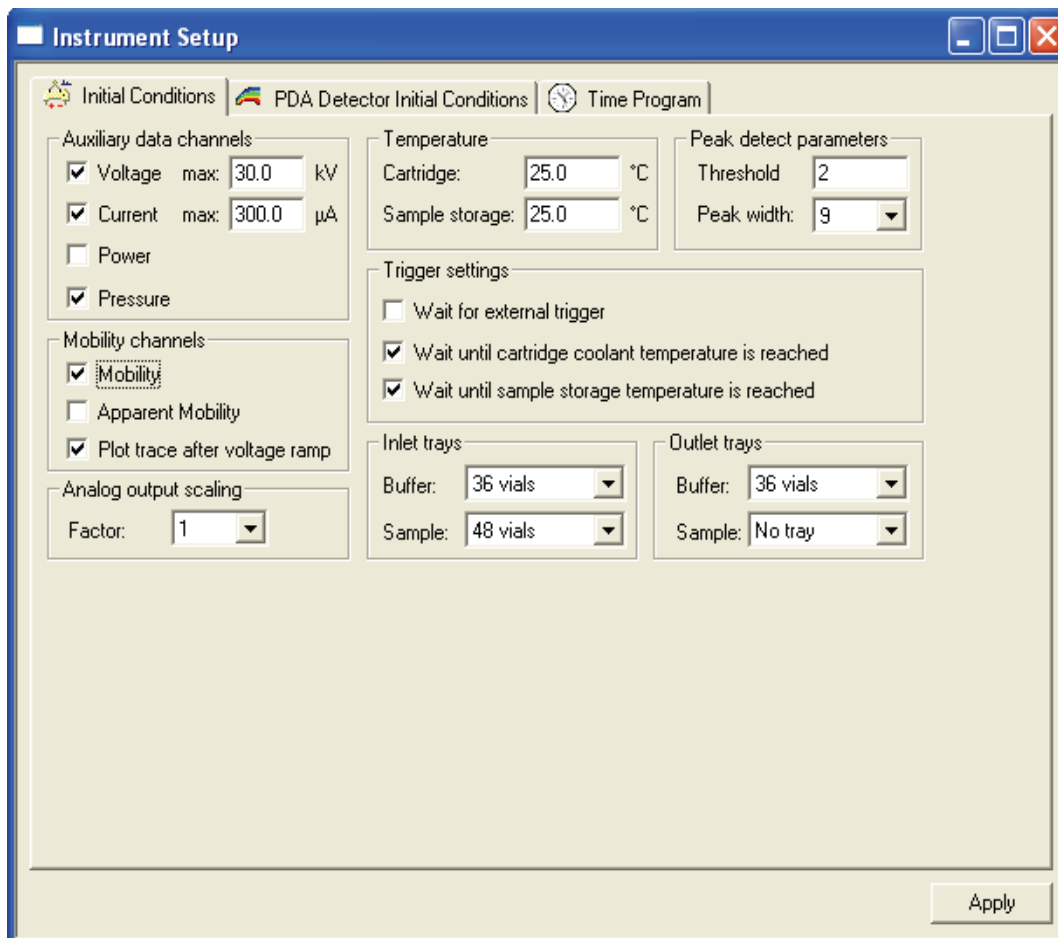
Mobility Plot

Selecting Mobility (or Apparent Mobility) causes the creation of an additional plot after data analysis. This plot shows the electropherogram re-scaled so that the X-axis is displayed in terms of Mobility (or Apparent Mobility) rather than time.

The **Plot trace after voltage ramp** option should be selected unless peaks are detected during the voltage ramp-up phase of the separation. Deselecting this option may result in the voltage ramp interval being over-emphasized in the resulting mobility plot.

NOTE Mobility channels cannot be generated for data files that were created with this option turned off. It must be ON during data acquisition. When using this option, there must be at least one Mobility Marker identified in the Peaks/Groups table of the method.

After selecting the appropriate mobility channel options, save the method.

Run the sample again.

At the end of the run, the mobility trace is generated.

Demonstration: Using the Mobility Features

NOTE The following is not intended to be a hands-on exercise, but rather a step-by-step example of the process described above. This example uses a UV detector and Beckman Coulter Test Mix B (PN 501333).

You previously determined the optimum conditions for the separation of a mixture of two components, ALPHA and BETA. ALPHA has been chosen as the mobility marker. The method has considerable EOF, so you must use an EOF marker.

For this exercise, use Ethanol; it is readily soluble in the sample, creates a detectable peak, and does not interfere with the analysis. All of this has been previously determined. In practice, however, these facts must be shown through experiment. A final concentration of 5% Ethanol has been chosen. The sample is prepared and run. After step 6 (above) the resulting electropherogram looks like the following:

Figure 10.3 Electropherogram for Mobility Features Demonstration
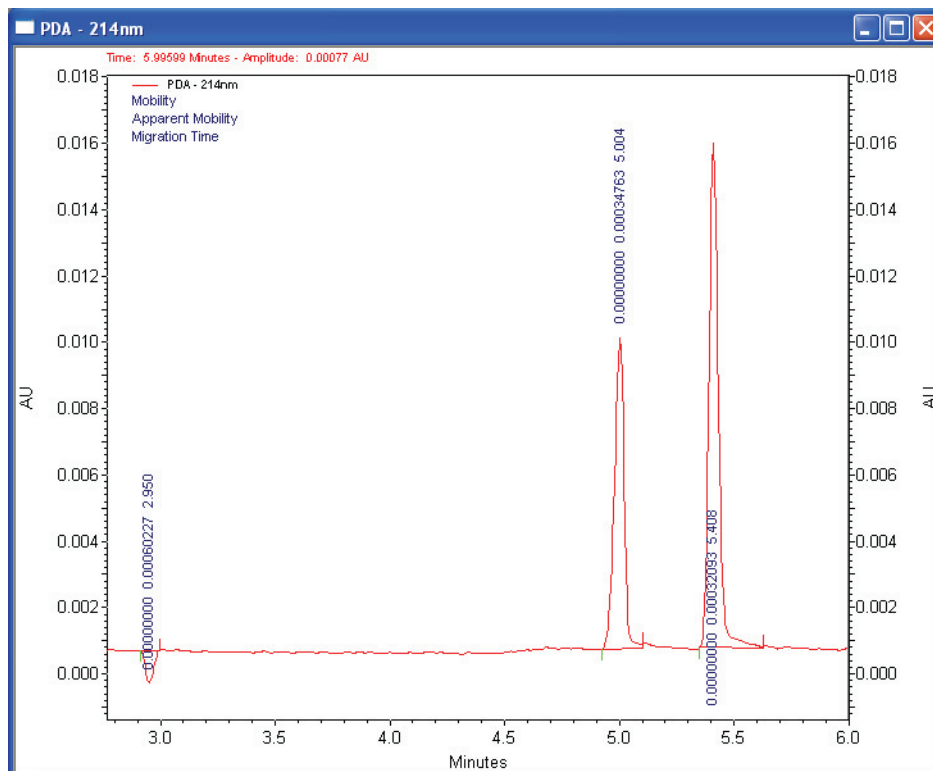
Our ethanol marker gives a small, negative peak, but it is properly detected by the software. The values obtained are listed in Table 10.1:

Table 10.1 Tabulated Values for Peak Name, Apparent Mobility, and Mobility

Peak Name	Apparent Mobility	Mobility
EOF	0.00060227	0.00000000
ALPHA (marker)	0.00034763	0.00000000
BETA	0.00032093	0.00000000

As expected, the faster moving EOF marker has the higher apparent mobility. The units of mobility are: $cm^2V\text{-}1sec^{-1}$

You can calculate the Electrophoretic Mobility of ALPHA according to:

$$\mu = \mu_{app} - \mu_{eof}$$

substituting yields μ = 0.00034763 - 0.00060227 = -0.00025464, the mobility of ALPHA under these separation conditions. The mobility value is a negative number, indicating that ALPHA's electrophoretic migration is toward the anode and is opposite the direction of the electroosmotic flow.

NOTE In practice, the above run should be repeated several times to verify the system is giving consistent results before defining the mobility value for the mobility marker.

Re-open the **Peaks** / **Groups** table and insert the value of the mobility marker as described in steps 9 and 10. The table now looks like the following:

Figure 10.4 Peak/Group Table -- PDA - 214 nm

We save the method and analyze the data file again. The results are now:

Table 10.2 Tabulated Values for Peak Name, Apparent Mobility, and Mobility After Correction for EOF

Peak Name	Apparent Mobility	Mobility
EOF	0.00060227	-0.00000000
ALPHA (marker)	0.00034763	-0.00025464
BETA	0.00032093	-0.00028134

The electrophoretic mobility of the EOF marker is close to zero. The mobility of ALPHA is the value you defined it to be. The mobility of BETA has now been calculated relative to the mobility of ALPHA.

You can now set up to collect data for a mobility plot.

1   Open the **Instrument Setup** window and click on the **Initial Conditions tab**.

2   Select **Mobility**.

After saving the method and running the sample again, you have two electropherograms. One is the UV electropherogram, which looks very similar to the run used to measure the mobility of the marker. The second displays differently, as shown here.

Figure 10.5 Electropherogram Based on Mobility
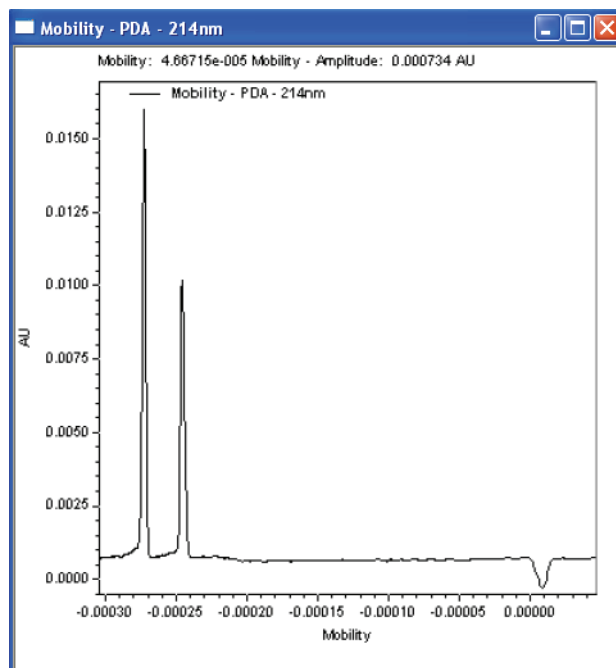
The order of the peaks has been reversed, because the latest eluting peaks have the most negative mobility. The relative spacing between the peaks has also changed.

Peak Identification by Mobility

In the exercise on mobility, you used migration time and a migration time window to establish the identity of peaks in a data file. In the same fashion, you can use mobility and a mobility window to identify peaks based on the mobilities of standards. The choice of migration time or mobility applies to the entire electropherogram; it is not possible to use time for some peaks and mobility for others.

1 The option to use mobility for peak identification must be selected from the Options tab of the Method Properties window (under the Method menu).

2 Select **Mobility based** and click **OK**.

3 Peaks are added to the **Peaks** / **Groups** table (if not already present). Use the **Add Peaks** function from the Graphical Programming menu as described earlier in the chapter on Integration.

4 Open the **Peaks** / **Groups** table and scroll to the Mobility columns.

The assignment of the mobility of a mobility marker has already been described. To identify other peaks by mobility, manually input their mobility value and window.

For the peak BETA in the example, the mobility value was determined to be -0.00028134, so this value is entered into the table for the line defining BETA. The default Mobility Window is zero, which means the peak would only be detected if it had exactly the same mobility value as that defined. Some variation can be expected. The appropriate value is determined by trial and error. A window of 5-10% is a good starting point. For BETA, a 5% window would be 0.00001407. The window is always an unsigned number (not positive or negative). At this point, the Peaks / Groups table looks like this:

Figure 10.6 Peak / Group Tables -- 214 nm

When you click on **Analyze**, the peak identification is displayed by mobility. To verify this, change the Mobility entry for BETA to some other value like -0.00025227 and **Analyze** again.

Because the mobility of the peak is now outside the window, it is integrated, but not associated with the ID BETA.

Creating Reports

Introduction

The purpose of operating a CE or any other instrument system is to answer a question. This answer is only useful if it can be communicated. The 32 Karat Software includes a powerful suite of utilities for creating printed reports. It also allows you to export data and results to other software packages. Full integration into the Windows operating system environment allows you to copy and paste electropherograms and other information from 32 Karat Software directly to a wide variety of other software packages such as word processors. This section of the manual describes how to use the built-in report generating features of the software. For information on other ways of moving data to other applications, see the resources listed in APPENDIX A, *Additional Resources*.

In other chapters of this manual, you have been introduced to some of the default reporting options. Here you learn how to customize a default report to include the information you want to present. You continue to use a UV instrument as a prototype. LIF reports are essentially identical to UV reports. PDA data can be reported in the same formats as UV data. Other PDA-specific report types are available such as PDA Spectrum Library reports.

A simple approach to reporting is to use pre-defined reports. These were used in earlier chapters of this manual. From the menu bar, select **Reports > View** to display the menu shown below, which lists the default set of pre-defined reports.

Figure 11.1 Reports > View Selection
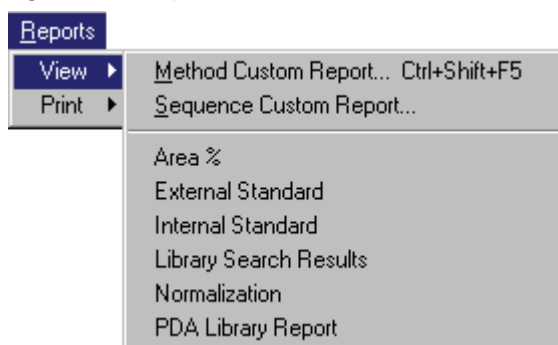
In this exercise, use the data file and method from the exercise on Qualitative Analysis. A review of the reports menu shows there is no default report for qualitative data, so one must be created. You must create a Method Custom Report, which becomes part of the method. Save the report as a standard template that is added to the reports list.

Creating a Method Custom Report

1 Open your Virtual UV instrument from the main 32 Karat window.

2 From your working folder, open the data and method used during the exercise on Qualitative Analysis. You are creating a report based on the analysis results generated during that activity.

3 From the **Method** menu, select **Custom Report**.

4 A blank window opens. (If a method custom report has previously been developed with the method you are using, it automatically opens at this point.)

Modifying a Report Template

Rather than developing a report from nothing, you can modify an existing report template to simplify this task. A report template is a pre-defined set of instructions for the creation of a report. A number of templates are contained in the software. You can add templates by creating a new one or editing an existing one and saving it under a new name. Use the Area% template as the basis for your Qualitative Analysis report. From the file menu select **Report Template > Open** and then select Area%.srp. The template opens with the results from the open data file displayed. The indications that you are looking at a template, and not at a report, are that the header information is highlighted, the table has a broken border, and the ruler is visible.

As a first step, change the title of the report. The report editor works very much like a word processor.

1 Using the mouse, highlight the heading Area% Report.

2 Type **Qualitative Analysis Report**.

3 Highlight the new title, and select **Italic** from the toolbar.

The information below the title that is highlighted in gray represent fields. Fields allow you to insert blocks of information without tedious code writing. Fields and text can be used together. In this template, each field is preceded by a text descriptor. These fields can be easily repositioned.

4 Click after the Acquired field, and press the Delete key. This moves the Print Time field to the same line as the Acquired field.

5 Press the Tab key twice to place space between the two fields.

6 Click in the blank line below these two fields and type User ID:, then press the **Tab** key.

7 Right-click and select **Insert Field**, then **User reporting the data**. A new field displays with the current User Name. (The default name PA800 displays if no user name has been defined.)

8 Use the keyboard just as you would a word processor to align the field and the text in the desired manner.

Below the header information is a graph displaying the electropherogram. By default, the graph is scaled to show the full range of data. In the case of CE Data Sample 3, the area of interest is only at the right end of the electropherogram.

As the next step, adjust the electropherogram display so the peak region is clear.

9 Double-click on the graph to display the **Graph Properties** dialog box.

Figure 11.2 Data Graph Properties
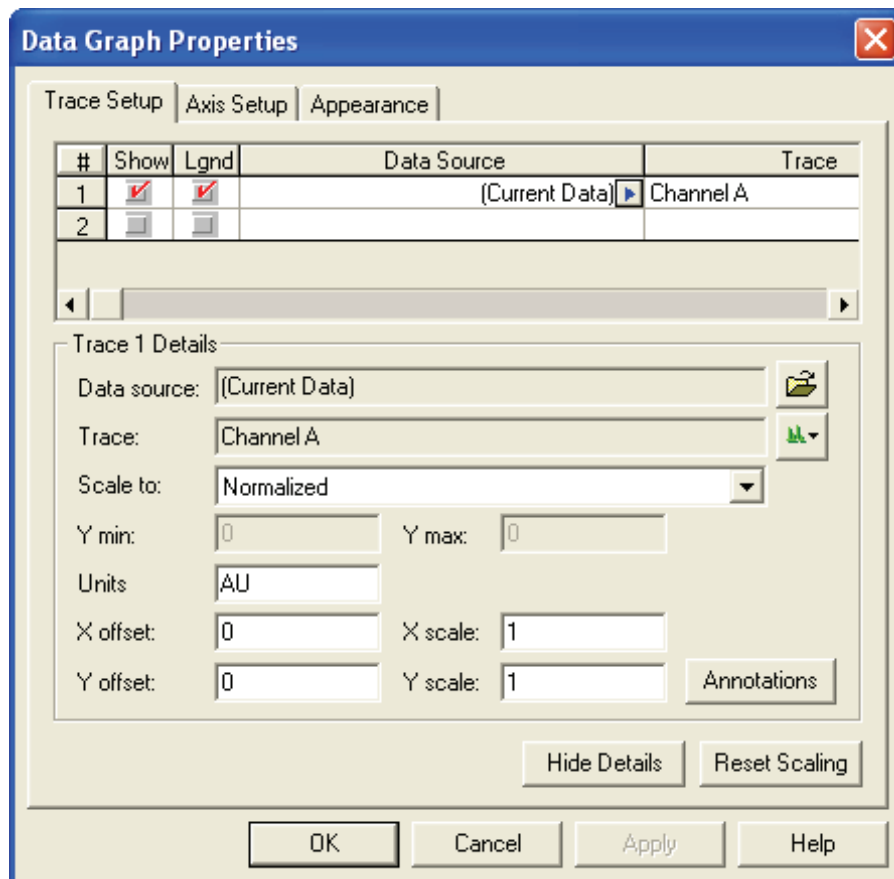
The Trace Setup tab is where you select the data to be displayed. Select the Current Data (the file currently open in the Instrument window) or a data file (or files) stored on the computer.

For example, you might want to display the results from analysis of a standard along with results from an unknown. You can display multiple channels from a common data file by selecting the same Data Source while selecting different channels in the Trace column. For example, the UV and electrical Current traces could be displayed simultaneously. The X and Y offset and scale features can be used to position the traces so that the pertinent details are visible. These features are described in the resources listed in APPENDIX A, *Additional Resources*. Units is a label that can be adjusted to match the data displayed. For an LIF detector, this could be changed to **Fluorescence** by selecting in the **Units** option and entering the text.

10 Click on the **Annotations** button.

11 Because this is a Qualitative report, remove any existing annotations from the right side panel.

12 Add **Quality** and set the number of decimal places to zero.

13 Click **OK** to return to the main dialog box.

Now you can change the X-axis so that the peak area fills the graph window.

14 Click on the Axis Setup tab.

15 With X-axis selected, click on **Use this range**.

16 Enter a minimum of **24** and a maximum of **30** to bracket the 24-30 minute time interval.

17 When done, click **OK**.

The part of the electropherogram containing the 21 peaks should fill the graph, and each peak should be properly labeled with the Quality value.

The graph can now be resized by dragging the handles on the right and bottom edges of the image. Moving the graph requires that it be treated as a text object.

18 To center it on the page, single-click the graph to select it (a narrow dotted border displays) and use the centering icon from the toolbar. Space can be added above and below the graph by inserting blank lines with the Enter key.

Additional charts can be inserted by right-clicking on an empty line and selecting **Insert Graph**. You might, for example, want a second graph showing the full time range of the data set. The new graph is then formatted as described above.

Below the graph is a table with a variety of peak parameters. Some of these must be changed for the new report.

1 Right-click on the column header **Area%** and choose **Change Parameter**.

2 Select **Quality** as the new parameter and change Decimals to zero.

3 In the Column Header field, type Bases.

4 Click **OK**.

You now have a column that contains the number of bases that the software has calculated for each peak.

5 Right-click in the table and select **Report Properties**.

6 Use the red arrow to remove the Height or Height%, since you are not interested in that information.

7 Click **OK**.

Your table is now too wide for the remaining three columns. By clicking on the vertical dividers between columns, you can drag the column widths. At any point during editing, click on the **Print Preview** icon to see how the report looks on your default printer.

Save the method. The new report is now part of the Quality method, and is available whenever the method is used by selecting **Method Custom Report**.

Saving a Report as a Template

To use the report in another method, you can save the it as a template.

1 From the File menu, select **Report Template > Save As**.

2 Type Quality as the filename and click.

By default, the template is saved with the extension *.**rep**.

3   Select **File > New Method**.

4   Open **Method Custom Report** by selecting it from the Method menu (if not already open).

The report is blank, because a new method has no defined Custom Report.

5   From the **File** menu, select **Report Template > Open** and open the Quality.rep template that you created.

When the new method is saved, it contains the Quality.rep as the default Method Custom Report. The Custom Report saved in a method can be edited and changed at any time.

Saving a Report as a Default Report

You can also save this report as one of the default reports in the **Reports** menu.

1   Again select **Report Templates > Save As** from the File menu.

2   This time save the template with the name Quality.srp.

3   Click **Save**.

By saving with the *.srp extension, you have created a new default template. The next time an instrument window is opened, the new report type is available from the Reports menu. This is a handy way to handle reports that are used frequently.

Report Templates

Custom reports are templates for displaying and printing data and objects. 32 Karat Software comes with a suite of standard report templates for a variety of report types. These can be used as is or can be modified using the Method Custom Report or Sequence Custom Report editor and then saved as new templates.

Method Custom Report Templates

A method custom report template is created and saved as part of a method, but it can also be saved as a template file that can be opened from within another method and used or edited.

Sequence Custom Report Templates

Sequence custom report templates are used for reporting data generated during batch sequence operations.

For example, certain Run Types require a report template for reporting the data. When you designate a run as a Begin Summary run, for example, you are required to designate a template name for the summary report.

Other run types requiring templates include Suitability, QC Check Standards, Duplicates, and Sequence Vial Report. Refer to APPENDIX F, *Sequence Vial Report*. Sequence Custom Report templates are created and edited using the Sequence Custom Report editor.

Standard Report Templates

Standard templates for many report types are provided with 32 Karat Software, however, you might want to create your own or edit the templates provided.

Standard report templates (Area%, External Standard, Internal Standard, and Normalization) are located in the C:\32KARAT\TEMPLATE program directory and have the .SRP extension. You can create new standard report templates by saving your report template using the .SRP extension.

Sequence Reports

Sequence reports are only created and saved using the Sequence Custom Report editor. Unlike method custom reports, sequence custom report templates are not saved as part of the sequence file and therefore must be saved as a template file, if you want to use a sequence report template to generate a report.

The following standard sequence report templates are provided with 32 Karat Software:

- Calibration
- Summary
- Duplicate
- QCCheckStd
- Spike
- SysSuit

They are located in the 32 Karat Software/Sequence directory and have the .BRP extension.

Additional Resources

32 Karat Online Help

The Online Help is your primary software reference for the 32 Karat Software. It describes all of the features used in this manual. In addition, it describes all of the advanced features, which are not covered here. To access the Help, select **Help > Contents** from the instrument window menu bar. Context-sensitive help is available by selecting the Help button located in dialog boxes or by pressing the ⌐F1⌐ key.

Installation and Maintenance Manual

The first place to look for answers to questions on hardware and maintenance is the PA 800 *plus* System Maintenance User's Guide. This document includes detailed descriptions of routine operations such as rebuilding a capillary cartridge. An electronic copy is available on the CD-ROM shipped with the 32 Karat Software.

Beckman Coulter Service

If you have determined that a problem exists with the instrument that cannot be fixed by following the procedures in the PA 800 *plus* System Maintenance User's Guide, contact your local Beckman Coulter Service Representative. There may be a charge for this service.

Help by e-mail

A special mailbox has been created for users of 32 Karat Software. Send your questions and comments to 32karat@beckmancoulter.com. A team of specialists reviews your message and contacts you. Messages received outside of regular business hours (Monday through Friday, 8 a.m. to 5 p.m. Pacific time) are reviewed the next business day.

Other References

There are many publications dealing with applications of capillary electrophoresis, methodology, routine operations, and related techniques. Your local research library is the best place to begin.

Beckman Coulter web site, http://www.CEleader.com/ - The online source for the latest information on CE from Beckman Coulter.

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P/ACE Setter Newsletter - A quarterly newsletter for scientists employing capillary electrophoresis. P/ACE Setter features tips and techniques and product updates. Each issue features a specific application for CE. Free subscriptions are available through the Beckman Coulter web site or contact your local Beckman Coulter representative.

Your Local Beckman Coulter Representative - The best source for product information. Contact your representative for information on the latest products or to place an order.

PA 800 *plus* Calculations

Calculations

CE Calculations

Processing of electrophoretic data is similar to the processing of chromatographic data, but there are some differences. Some CE Specific calculations are built into 32 Karat Software. This appendix describes those along with some other useful calculations.

Ohm's Law

$V = IR$

The most important relationship to know in capillary electrophoresis is Ohm's law. This simple relationship defines the interaction between Voltage (V), Current (I), and Resistance (R). For example, if Voltage is held constant, change in Current must be due to a change in Resistance. Information of this type is invaluable in developing and troubleshooting CE methods.

Corrected Migration Time

$$tcorr = t \left(\frac{t'ref}{tref} \right)$$

t=migration time of peak of interest

tref=actual migration time of reference peak

t'ref=expected migration time of reference peak (from peak id table)

Corrected Area

$$Acorr = vA = \frac{LdA}{t}$$

v=velocity
Ld=capillary length to detector
t=migration time
A=uncorrected peak area

In CE, early peaks migrate through the detector window faster than do later peaks. This creates a peak area bias that is eliminated by using corrected peak area.

Mobility

Mobility is calculated as the apparent mobility minus the mobility contribution of electroosmotic flow.

$\mu=\mu_{app} - \mu_{eof}$

Once the mobility of a reference analyte has been determined, the mobility of related analytes can be calculated.

$$\mu = LdLt \left(\frac{1}{Vt} - \frac{1}{Vref\,tref} \right) + \mu ref$$

V=average applied voltage up to the migration time of the peak of interest
Ld=capillary length to detector
Lt=total capillary length
tref=migration time of reference peak in the current run
µref=defined mobility for the reference peak
Vref=average applied voltage up to migration time of reference peak
t=migration time of the peak of interest

Apparent Mobility is the sum of true mobility and the mobility caused by the electroosmotic flow. Apparent mobility can be calculated directly for any peak. The apparent mobility of a neutral marker (one that has a true mobility of zero) is called µeof, the electroosmotic mobility.

$$\mu app = \frac{v}{E} = \frac{LdLt}{Vt} \text{ (apparent mobility)}$$

Ld=capillary length to detector
Lt=capillary total length
V=average voltage up to migration time of peak
t=migration time of the peak
v=velocity

Mobility calculations depend on an accurate measurement of the average applied voltage up to the point that the component passes the detector. The output from the PA 800 *plus* voltage monitor is used to calculate average applied voltage as follows:

$$\overline{V} = \frac{\sum\limits_{i=1}^{n} V_i}{n}$$

n=data point number at peak migration time
Vi=voltage value at data point i

Understanding PDA Data

Overview

Most of the exercises in the manual have used example data from an ultraviolet detector system. LIF data files appear nearly the same as UV data files. From the point of view of data analysis, the Y-axis scale is the primary difference between UV data and LIF data.

PDA data consists of multiple components. These are:

Channel data: each channel is equivalent to a UV trace. Up to three channels can be collected simultaneously.

3D spectral data: the three axes of this data set are time, wavelength, and absorbance.

Multi-Electropherogram data: similar to channel data, this is a trace of absorbance versus time extracted from the 3D spectral data. There are no limits to the number of data sets that can be created.

Channel data and multi-electropherogram data are processed in the same manner as UV data; all the procedures described in this manual apply.

The remainder of this appendix describes some of the features of PDA data. To re-create these displays, you must open an instrument configured for a PDA detector (offline mode is satisfactory). From a copy of the Data Samples directory, open the data file CE PDA Data Sample.dat.

The multiple components of the PDA data set are available by choosing **View > PDA View** from the menu bar.

Figure C.1 View > PDA View
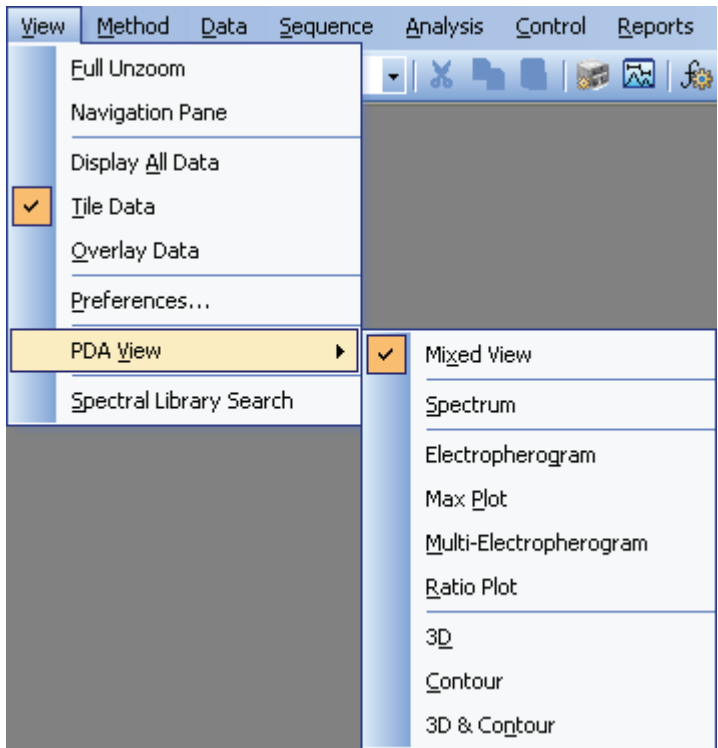
Each item in the menu represents a different way of presenting the data on the screen. Only one of these selections can be made at a time.

Mixed View

This option is shown below. This image illustrates several of the other options as well.

Figure C.2 Multiple Components of the PDA Data Set
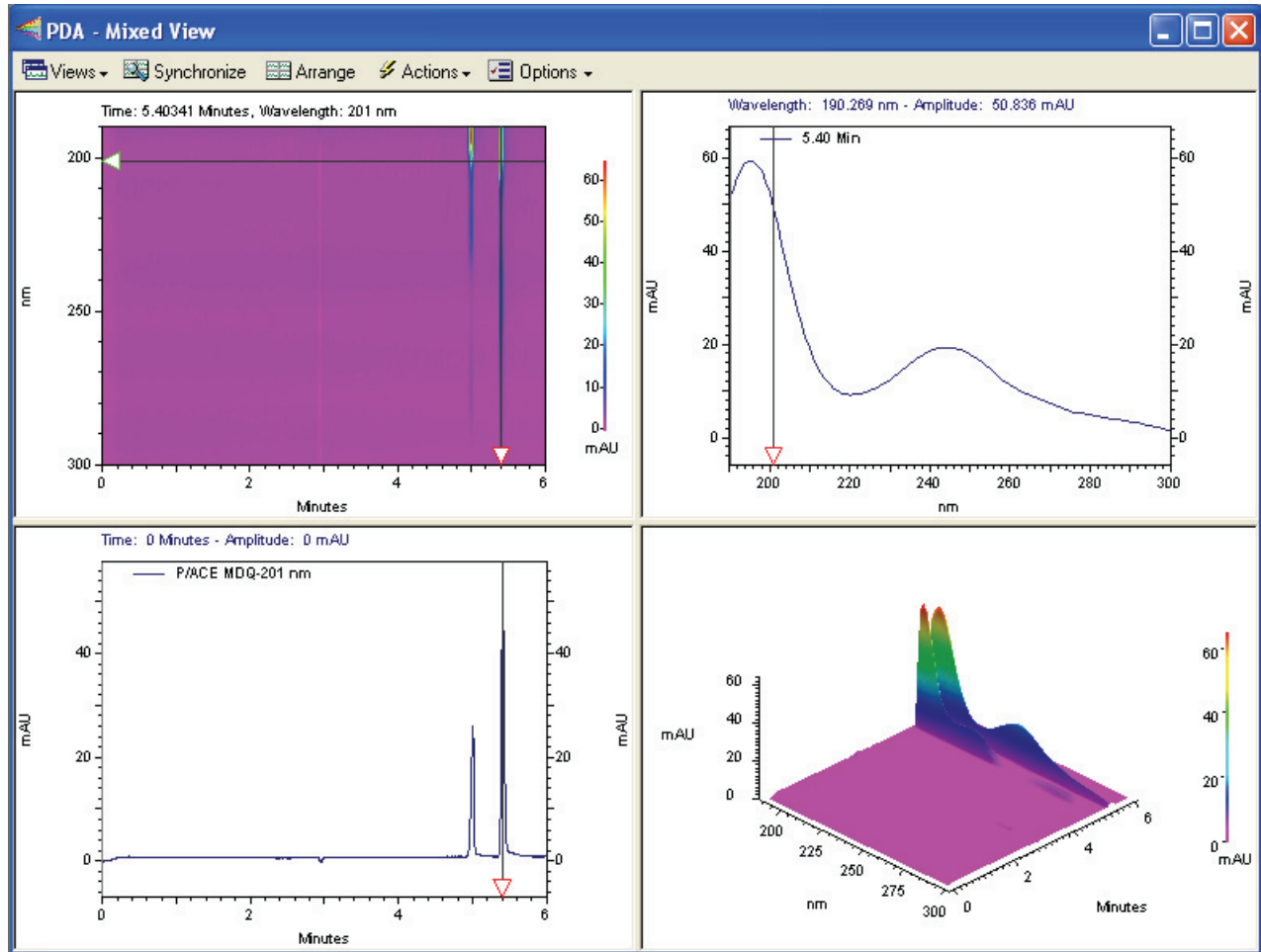
This window shows four different views of the same data set. The sample is the same mixture of Alpha and Beta that has been used throughout this manual. This window is divided into four panes. By clicking and dragging the bars that separate the panes, the relative areas assigned to each pane can be changed. The views available here are:

	Left	Right
Upper	Contour Plot	Spectrum
Lower	Electropherogram	3D Plot

Contour Plot

The Contour plot is an overhead view of a three dimensional data set. The X and Y axes are Time and Wavelength, respectively. Absorbance at any given wavelength and at any given time is indicated by color or by a shade of gray. This plot can be zoomed by using the mouse to rubber box the desired area. Right-click and select **Full Unzoom** to restore the view.

Spectrum

The spectrum detected at a specified point in time. This is selected by moving the horizontal slider in the Contour or Electropherogram plots to the desired time point. It represents a slice through the 3D data. This plot can be zoomed by using the mouse to rubber box the desired area. Right-click and select **Full Unzoom** to restore the view.

Electropherogram

The electropherogram at a specified wavelength. This is selected by moving the vertical slider in the Contour plot or the horizontal slider in the Spectrum view to the desired wavelength. It represents a slice through the 3D data in a direction that is perpendicular to the Spectrum view. This plot can be zoomed by using the mouse to rubber box the desired area. Right-click and select **Full Unzoom** to restore the view.

3D Plot

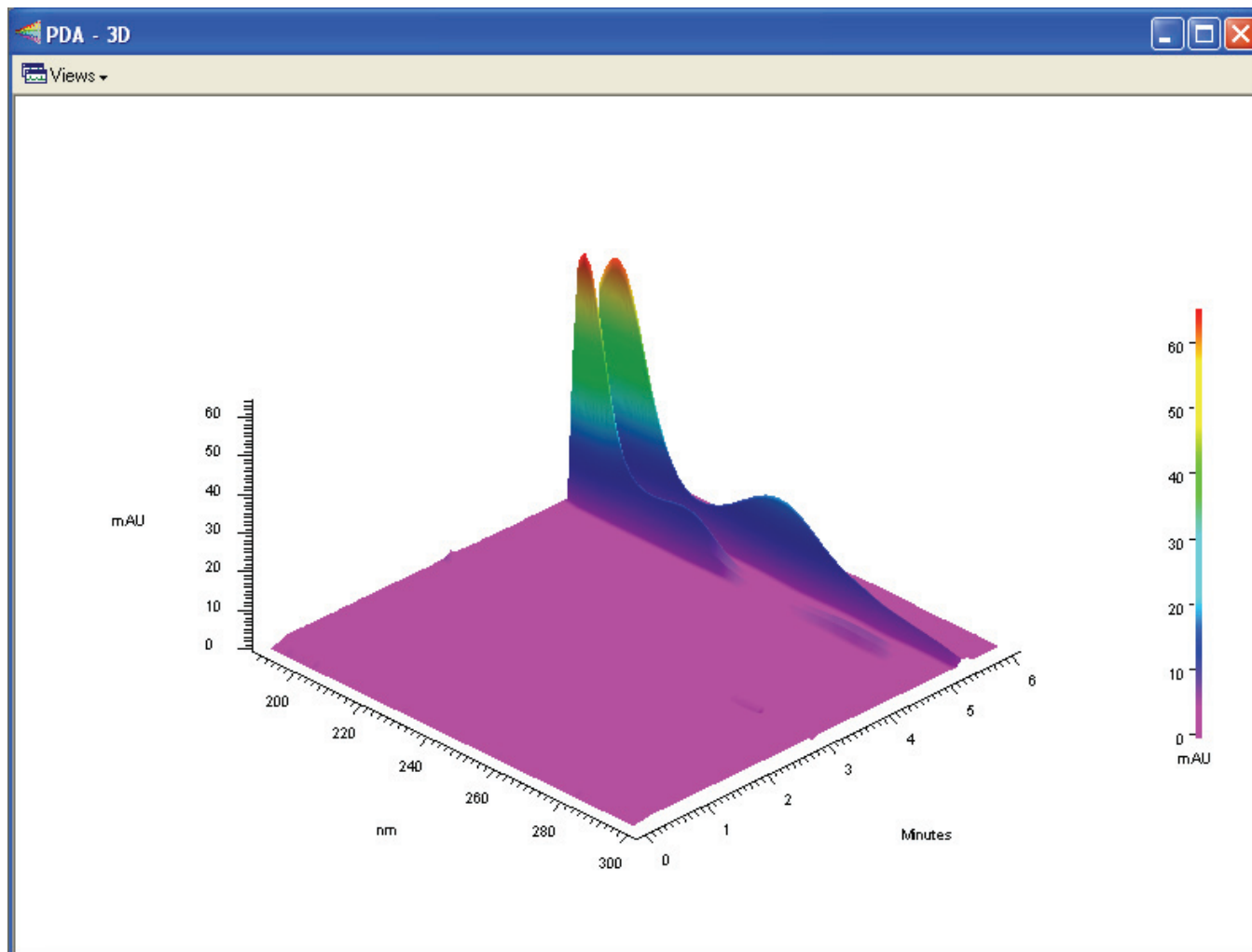A three-dimensional representation of the data. The presentation of this window is not tied to the Contour view. To adjust the display, right-click on the 3D plot and select properties to open the 3D Data Graph Properties window.

Figure C.3 3D Data Graph Properties Window

The plot can be displayed as a one-color surface or color can be used to specify absorbance ranges. Colors are user selectable. The image can be rotated around the X and Y axes by selecting the command from the right-click menu and manipulating the 3D plot with your mouse or by typing in Elevation and Rotation parameters within the 3D Data Graph Properties window. Zooming is accomplished by setting limits of time, wavelength, and absorbance. The Apply button causes the changes to be made to the 3D plot without closing the properties window. This allows you to test changes without having to close and re-open the window.

3D and Contour Plot shows only these two plots in a two-pane window.

The individual **3D**, **Contour**, **Electropherogram**, and **Spectrum** plots display the individual pane as a full window. The limits of the Electropherogram and Spectrum plots are those set on the Contour window even if the Contour window is not being displayed. The remaining choices in the PDA View menu are not related to these four items.

The **Max Plot** view is an electropherogram that does not represent a particular wavelength. It is built by taking the maximum absorbance across the wavelength scan range at each point in time. This plot is useful for finding peaks that might otherwise go undetected. This data can be analyzed and the results reported.

The **Multi-Electropherogram** view is only available if multi-electropherograms are defined in the Instrument Setup component of the method. Like the Max Plot, these traces are synthesized from the 3D data set. To set up these channels, open the PDA Options window from the Method menu and select the Multi-Electropherogram tab. Three items must be specified for each channel. The channel can be checked On or Off. Channels that are Off are not displayed and cannot be analyzed. Wavelength refers to the central wavelength of the data to be incorporated into the multi-electropherogram channel. Bandwidth specifies how many adjacent wavelength bands will be combined to form the new data set. These bands are weighted with bands more distant from the central wavelength contributing less to the final result than do bands closer to the midpoint. Using too narrow a bandwidth results in a noisy data file. Using too wide a bandwidth causes a loss of spectral sensitivity in the data set.

This data can be analyzed and the results reported. If many multi-electropherogram channels have been defined, each occupies a small area of the display. The frame around each channel can be moved by clicking and dragging with the mouse. Channels can also be temporarily disabled by unchecking them in the Multi-Electropherogram tab of the PDA Options window.

Ratio opens a three pane window. Two windows display the electropherograms at the specified wavelength. The third displays an electropherogram that is the arithmetic ratio of the two (Abs#1/Abs#2, on a point-by-point basis). The parameters for this window are set on the X:Y tab of the PDA Options window (available from the Method menu).

When PDA data is selected, the drop-down box in the tool bar contains a list of the available data channels. Before data can be integrated or analyzed, the desired channel must be selected from this list. Each data channel has a unique Peaks \ Groups table and Integration Events table, that are available from the Method menu only when the data channel has been selected.

More information about using PDA data is available in the resources listed in APPENDIX A, Additional Resources.

System Administration

Overview

The System Administration functions include adding and configuring instruments, which has already been discussed in this manual. It also includes features for defining users and projects. Project definitions include: defining default folders in which data and methods are stored; specifying what instruments can be used for a project; defining which users can work on a project and what privilege level each user has. These administrative functions are handled through software wizards that are pre-defined step-by-step processes. It is NOT necessary to implement this function to use the system.

System Administration is managed from the main 32 Karat Software Screen.

1 From the **Tools** menu select **Options**.

2 Click on the Enterprise tab.

3 Clicking on Enable user login and project management activates the system administration function. The effects display the next time the program is started.

⚠ **WARNING**

If the System Administration function is activated, a System Administrator must be defined immediately. If no System Administrator has been defined, there is no user with the privilege to disable the System Administration function, should that be necessary. Depending on the privilege levels assigned to users, not having a System Administrator can make some or all software features inaccessible.

When the **Enable** option is selected, the User list becomes available. Clicking on **Add user** opens a dialog box in which the user's name and password are entered. A password is not required. If a password is used, it must be recorded elsewhere. There is no feature in the software for generating a list of user passwords.

When all the names are entered (or at least the name of the administrator), user privileges must be assigned. New users do not have any privileges until privileges are assigned.

IMPORTANT A user with no privileges cannot use the software.

Click **OK** to close the Options dialog box. From the **Tools menu** select **System Administration Wizard**. The wizard is only available if System Administration Mode has been selected (checked). A screen displays with three options: Users, Instruments, and Projects. If there are secondary administrators, they can only be given access to some of these wizards.

The Users Wizard allows the assignment of privileges. Follow the wizard step-by-step. Each user can be given access to specific areas of the software and to specific instruments. Selection depends on the functions of the individual and the degree of security required. For example, regulated quality control laboratories generally implement tighter controls over instrument and data access than do laboratories engaged in basic research.

The Instrument Wizard allows the administrator to assign users to specific instruments, but not to assign specific privileges on those instruments.

The Project Wizard allows the administrator to create projects. A project is a combination of users, instruments, and folders. Projects are most valuable when instrument systems are used by multiple operators or groups who do not need access to one another's methods and data. Creating a project allows the operations on the instrument to be compartmentalized.

The software is shipped with one project, which is called Default. This is a special project in that it cannot be protected. It should not be used for methods and data that are not intended to be shared.

For more information on System Administration, see the PA 800 *plus* System Administration User's Guide.
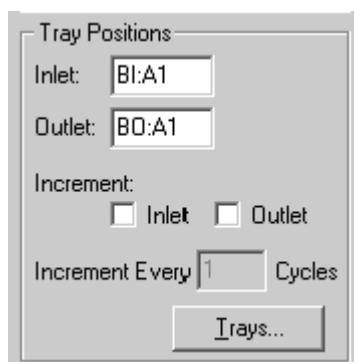
Vial Incrementing

Introduction

Vial incrementing is an automation process in which the inlet or outlet vials can be programmed to advance after a pre-determined number of cycles. This behavior of shifting to adjacent vial positions occurs after the instrument executes a certain number of cycles of a method. Vial incrementing eliminates the need to devise new methods whenever different vial positions are desired during the course of a set of instrument runs. One method can be used to allow the instrument system to change to new vial positions automatically. You can incorporate vial incrementing in your applications as a powerful tool. For example, vial incrementing can be used to perform efficient separation of biological or chemical compounds without constantly replacing or replenishing separation solutions during runs.

Vial incrementing is controlled by the software's method and sequence. Incrementing for either or both of the inlet/outlet vials can be selected under the Tray Positions dialog box for each of the following events in the method time program: Rinse, Inject, Separate, and Wait.

**Figure E.1** Tray Positions Dialog Box

Cycles are the number of repeats of a method before vial incrementing occurs. Selecting **Increment Every 2 Cycles** causes the instrument system to run the method two times before changing vial positions for the specified time program event (for example, rinse or separate).

On the other hand, selecting **Increment Every 5 Cycles** causes the instrument to run the method five times before changing vial positions for the specified time program event.

The Inject event, unlike Rinse, Separate, and Wait, can be programmed through both the method or sequence table. Inject event has the option of allowing you to introduce samples from the vial positions stated in the method time program or in the sequence table. Vial incrementing is enabled

if you choose to inject from the method. If you choose to inject from the sequence table, vial incrementing is disabled, giving sequence table priority through the Allow Override check box.

Figure E.2 Inject Dialog Box
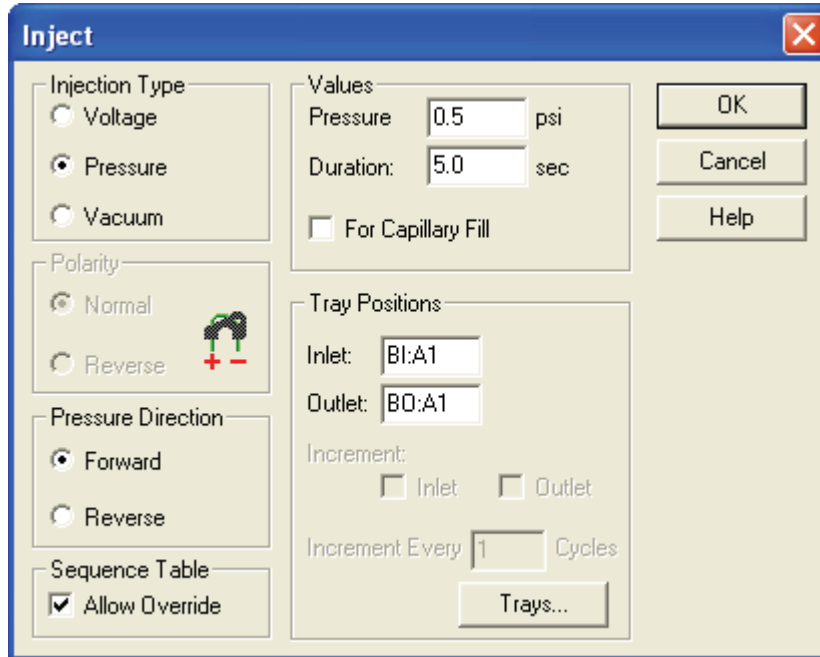
After vial incrementing has been set up in the method, the method can be executed as a single method run using Single Run command. Alternatively, the method can be incorporated into the sequence table using the Sequence Run command. You can also direct the instrument to execute multiple cycles of one or more methods throughout a set of runs. The execution of multiple cycles for the instrument system is called repetitions.

Although the terms are similar, do not confuse repetitions with cycles. The cycles term is used in SETTING UP vial incrementing through the method. The repetitions term is used to describe the EXECUTING of those cycles. For instance, a repetition of 5 for a single method means the instrument executes 5 cycles of the same method. A set of repetitions makes up a run. A set of runs makes up a sequence.

Figure E.3 Cycles, Repetitions, and Runs Make Up a Sequence
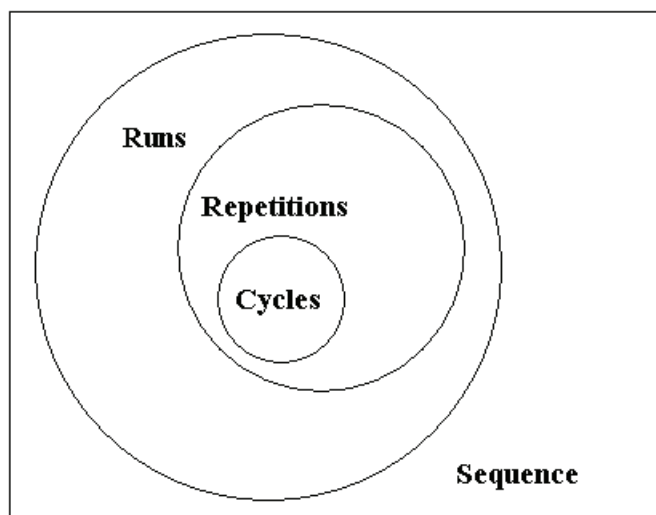
When Single Run (running a single method) is selected, the repetitions term is used. The runs and repetitions terms are used in the sequence table and the Sequence Wizard.

In summary, the method drives vial incrementing. The sequence table plans and organizes the vial incrementing activities for the user.

Examples of Use

Example 1

Figure E.4 Method Time Program B

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary
1		Rinse - Pressure	20.0 psi	0.15 min	BI:A1	BO:A1	forward, In / Out vial inc 1
2		Rinse - Pressure	20.0 psi	0.15 min	BI:B1	BO:A1	forward, In / Out vial inc 1
3		Inject - Pressure	0.5 psi	15.0 sec	SI:A1	BO:A1	No override, forward, In / Out vial inc 1
4		Wait		0.15 min	BI:C1	BO:C1	In / Out vial inc 1
5	0.00	Separate - Voltage	1.0 KV	0.50 min	BI:C1	BO:C1	0.17 Min ramp, normal polarity, In / Out vial inc 1
6							

In Method Time Program B, the following lines explain the incrementing scheme:

1. Rinse 1: Increment inlet and outlet vials after every cycle of the method.
2. Rinse 2: Increment inlet and outlet vials after every cycle of the method.

3. Injection of sample from method: Increment inlet and outlet vials after every cycle of the method.

4. Wait: Increment inlet and outlet vials after every cycle of the method.

5. Separate: Increment inlet and outlet vials after every cycle of the method.

After setting up the Method Time Program B, you can generate the following sequence table (Sequence Table B1) with only 4 repetitions of method B on a single run line:

The results of instrument sequence run of Sequence Table E1 are as follows:

Figure E.5 Results of Sequence Run of Sequence Table E1

Table E.1 Sequence Table - Run Results

| Run #	Repetition #	Event	Actual Vials Used-Inlet	Actual Vials Used-Outlet
1	1	Rinse 1	BI:A1	BO:A1
1	1	Rinse 2	BI:B1	BO:A1
1	1	Inject	SI:A1	BO:A1
1	1	Wait	BI:C1	BO:C1
1	1	Separate	BI:C1	BO:C1
1	2	Rinse 1	BI:A2	BO:A2
1	2	Rinse 2	BI:B2	BO:A2
1	2	Inject	SI:A2	BO:A2
1	2	Wait	BI:C2	BO:C2
1	2	Separate	BI:C2	BO:C2
1	3	Rinse 1	BI:A3	BO:A3
1	3	Rinse 2	BI:B3	BO:A3
1	3	Inject	SI:A3	BO:A3
1	3	Wait	BI:C3	BO:C3
1	3	Separate	BI:C3	BO:C3
1	4	Rinse 1	BI:A4	BO:A4
1	4	Rinse 2	BI:B4	BO:A4
1	4	Inject	SI:A4	BO:A4
1	4	Wait	BI:C4	BO:C4
1	4	Separate	BI:C4	BO:C4

As shown in the Sequence Table run results above, when a method time program event calls for incrementing every one cycle of the method, the vial positions are shifted by one vial after each repetition (for example, from BI:A1 to BI:A2, from BI:A2 to BI:A3, and so on for the Rinse 1 event).

Remember, one vial position is incremented in the same column to the adjacent vial position of the next row. Incrementing is also done from the vial position of the highest row of one column to the lowest row of the next column.

For example, A1, A2, A3, A4, A5, A6, B1, B2, etc.

Alternatively, you can generate the same Sequence Table B1 run results by doing a Single Run of method B using 4 repetitions:

Figure E.6 Single Run of Method B
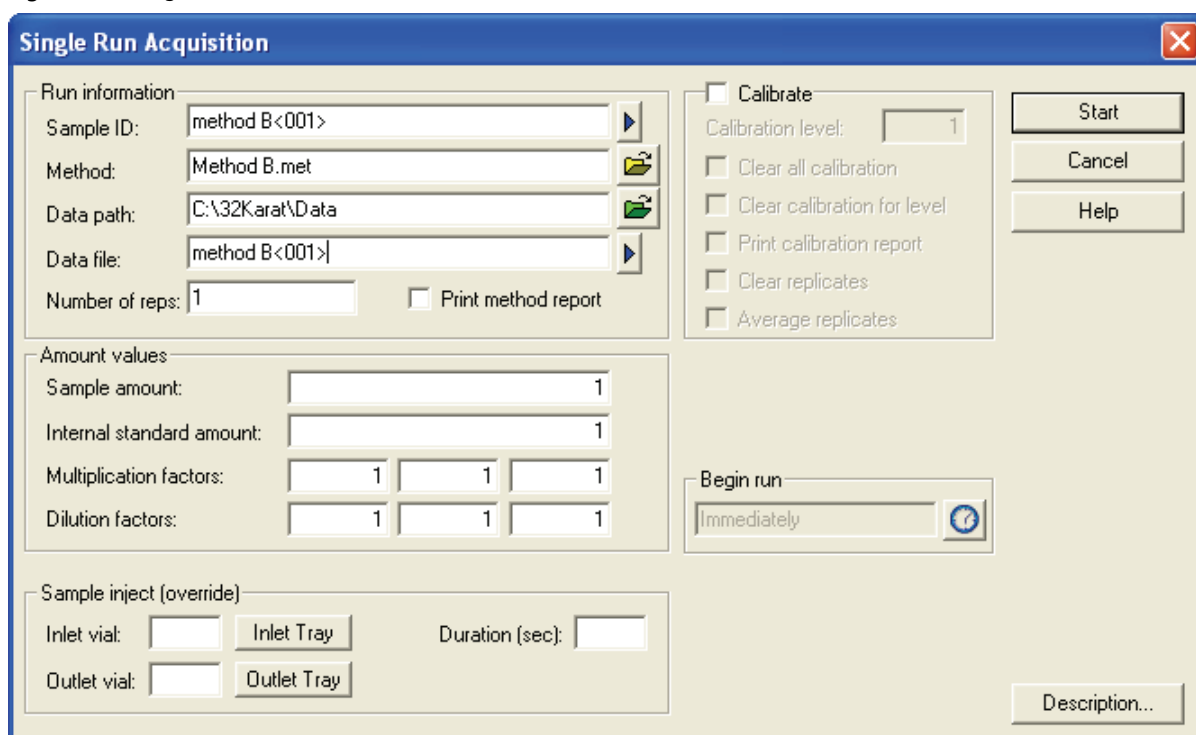
You can then generate another sequence table (Sequence Table B2) with 4 separate runs, each run consisting of only one repetition of the same method B:

Figure E.7 Sequence Table

The results of instrument sequence run of Sequence Table E2 are as follows:

Table E.2 Sequence Table - Run Results

Run #	Repetition #	Event	Actual Vials Used For Inlet	Actual Vials Used for Outlet
1	1	Rinse 1	BI:A1	BO:A1
1	1	Rinse 2	BI:B1	BO:A1
1	1	Inject	SI:A1	BO:A1
1	1	Wait	BI:C1	BO:C1
1	1	Separate	BI:C1	BO:C1
2	1	Rinse 1	BI:A2	BO:A2
2	1	Rinse 2	BI:B2	BO:A2
2	1	Inject	SI:A2	BO:A2
2	1	Wait	BI:C2	BO:C2
2	1	Separate	BI:C2	BO:C2
3	1	Rinse 1	BI:A3	BO:A3
3	1	Rinse 2	BI:B3	BO:A3
3	1	Inject	SI:A3	BO:A3
3	1	Wait	BI:C3	BO:C3
3	1	Separate	BI:C3	BO:C3
4	1	Rinse 1	BI:A4	BO:A4
4	1	Rinse 2	BI:B4	BO:A4
4	1	Inject	SI:A4	BO:A4
4	1	Wait	BI:C4	BO:C4
4	1	Separate	BI:C4	BO:C4

From Sequence Table E2 run results, you can see that those results are identical to results from the Sequence Table E1 run. No matter how the sequence table is organized (multiple repetitions of one method in one run or separate runs, each run consisting of one repetition of the same method), the vial incrementing results are the same.

Example 2

Vial incrementing also works when there are different methods interspersed with one another in the sequence table. Consider 2 methods, methods A and B, that alternate in the sequence table.

Figure E.8 Method Time Program A

In Method Time Program A, line 1 indicates a separation step that increments inlet and outlet vials applied after every second cycle of the method.

By combining Method Time Program A and Method Time Program B (from Example 1), you can generate the following sequence table (Sequence Table AB):

Figure E.9 Sequence Table AB

The results of instrument sequence run of Sequence Table AB are shown below. For simplicity, only the separation event is considered.
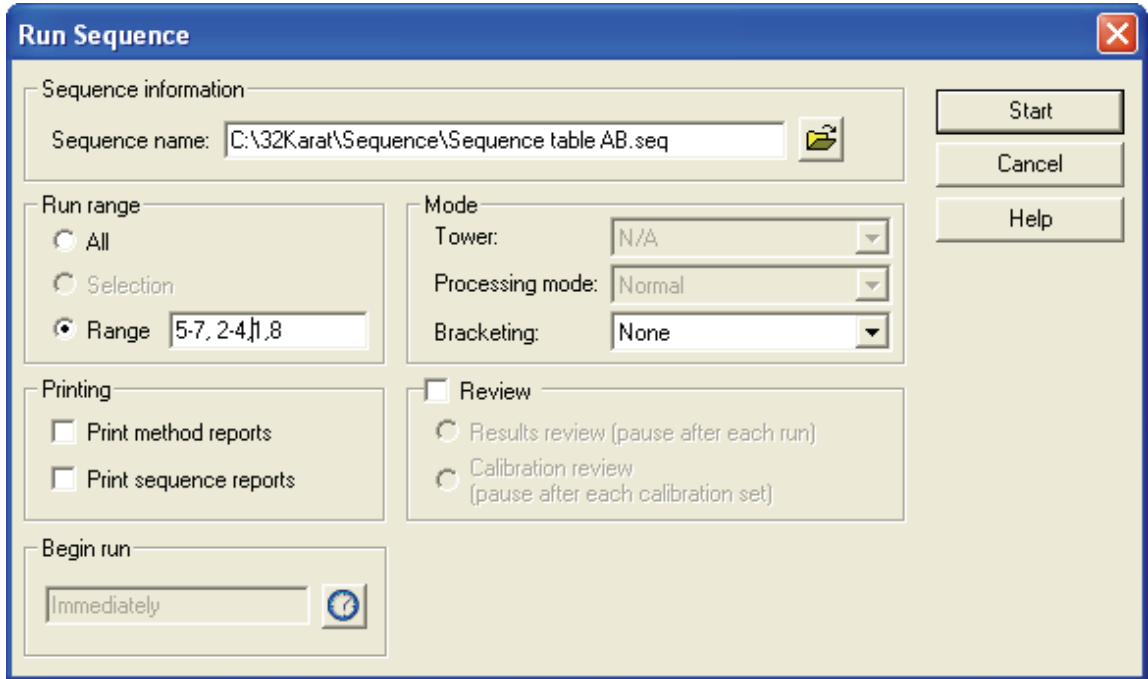
Table E.3 Sequence Table AB

Run #	Repetition #	Method Used	Event	Actual Vials Used-Inlet	Actual Vials Used-Outlet	Cycle # Used
1	1	A	Separate	BI:D1	BO:D1	1
2	1	B	Separate	BI:C1	BO:C1	1
3	1	A	Separate	BI:D1	BO:D1	2
4	1	B	Separate	BI:C2	BO:C2	2
5	1	A	Separate	BI:D2	BO:D2	3
6	1	B	Separate	BI:C3	BO:C3	3
7	1	A	Separate	BI:D2	BO:D2	4
8	1	B	Separate	BI:C4	BO:C4	4

The Sequence Table AB run results show that vial incrementing is followed through for each method even when there are other methods interspersed in between within the sequence table.

In cases where not all of the sequence table run lines are to be executed, vial incrementing follows the number of method cycles executed when the sequence is run. The Run Range dialog box of the Sequence Run command gives you the choice of running only some sequence table run lines:

Figure E.10 Run Range Dialog Box of the Sequence Run Command

In the above run range of run lines 5-7, 2-4, 1, 8 from Sequence Table AB, the sequence run of Sequence Table AB starts on run line 5, continues on to 7, goes back to 2, and continues on to 4, goes back to 1, and finally ends on run line 8.

The results of this instrument sequence run Range of Sequence Table AB are shown below. The results are obtained sequentially in the order that is given here. Again, for simplicity, only the separation event is considered.

Table E.4 Range of Sequence Table AB

Run #	Repetition #	Method Used	Event	Actual Vials Used-Inlet	Actual Vials Used-Outlet	Cycle # Used
5	1	A	Separate	BI:D1	BO:D1	1
6	1	B	Separate	BI:C1	BO:C1	1
7	1	A	Separate	BI:D1	BO:D1	2
2	1	B	Separate	BI:C2	BO:C2	2
3	1	A	Separate	BI:D2	BO:D2	3
4	1	B	Separate	BI:C3	BO:C3	3

Table E.4 Range of Sequence Table AB *(Continued)*

Run #	Repetition #	Method Used	Event	Actual Vials Used-Inlet	Actual Vials Used-Outlet	Cycle # Used
1	1	A	Separate	BI:D2	BO:D2	4
8	1	B	Separate	BI:C4	BO:C4	4

From the above instrument run results of the selected run range, you can see that vial incrementing resets itself when the sequence run is initiated on run line 5 of Sequence Table AB. The vial positions used in the results of the selected run range are the same as those of the sequence run when all lines of the sequence table are executed sequentially from run line 1 to 8 (see Example 2). However, the selected run range now shows that each run line number corresponds to a different set of vial positions from those in the previous sequence run (see Example 2).

IMPORTANT When there is an Injection event in the method, and you choose to inject from the method, vial incrementing is enabled. The vial positions for incrementing in the run range are dictated in the same fashion as the Separate event above.

IMPORTANT If you choose to inject from the sequence table (enabling the Allow Override option in the method time program), vial incrementing is disabled. That being the case, the instrument runs execute and use the vial position values you input in the Sample Inject Inlet/Outlet columns of the sequence table with each run line having specific injection vials.

The following examples illustrate how vial incrementing works:

Summary

The examples show that the method drives vial incrementing, and the sequence table plans and organizes the incrementing for you.

Vial Incrementing in Runs Submitted to Instrument Run Queue:

Vial incrementing is reset whenever you add new runs (single or sequence) to the run queue.

In single runs of one method, the vial positions are incremented within the repetitions of each individual single run. The incrementing is not carried over to the next single run of the same method submitted to the run queue.

In sequence runs, the vial positions are incremented within each individual sequence run. The incrementing is not be carried over to the next sequence run of the same sequence submitted to the run queue.

The following examples illustrate this.

Figure E.11 Single Run

Figure E.12 Sequence Run

In the first example of the run queue that includes single runs of Method Time Program B, the first, second, and third single runs are independent of each other. Vial incrementing is not carried over from the first single run to the second and third single runs. In the second example of run queue that includes sequence runs of Sequence Table B1, the first, second, and third sequence runs are independent of each other. Vial incrementing is not carried over from the first sequence run to the second and third sequence runs.

Sequence Vial Report

Overview

Sequence Vial Report is a pre-defined sequence summary report that shows which vials are used by the instrument system for the method time program events of rinse, separate, and inject. The Sequence Vial Report is a description of the pre-run or post-run of the sequence. There are two forms of the report: Sequence Vial Increment Preview and Sequence Vial Report - Confirmed. Both forms of the report can be viewed and printed.

Sequence Vial Increment Preview

The Sequence Vial Increment Preview report is a preview of which vials the instrument system uses based on what methods and other information are provided in the sequence table. This Preview report can be viewed automatically before executing any sequence runs by selecting Sequence Vials Preview from the Sequence menu:
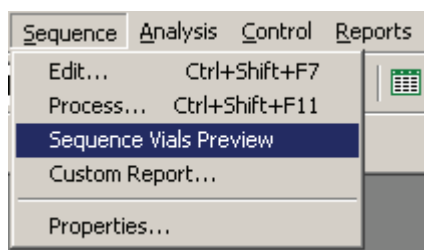
Figure F.1 Sequence Vial Increment Preview Report

Sequence Table: C:\32Karat\projects\SDS MW\Sequence\SDS20.seq
Sequence Vial Report - Projected
Data Path: C:\32Karat\Projects\SDS MW\Data
Method Path: C:\32Karat\Projects\SDS MW\Method
User: PA800
Report Time: 3/22/2009 3:24:42 PM

'*'- indicates that Vial Collision will occur.

Cycle #	Method	Filename	Rep #	Inject In	Inject Out	Inject Time	Other Inject In	Other Inject Out	Rinse Vials In	Rinse Vials Out	Separate Vials In	Separate Vials Out
1	SDS20cmPreConditioning.met		1 of 2						BI:D1, BI:E1, BI:F1, BI:C1	BO:D1, BO:E1, BO:F1, BO:B1	BI:C1	BO:C1
2	SDS20cmPreConditioning.met		2 of 2						BI:D1, BI:E1, BI:F1, BI:C1	BO:D1, BO:E1, BO:F1, BO:B1	BI:C1	BO:C1
3	SDS20cmPreConditioning.met		1 of 2						BI:D2, BI:E2, BI:F2, BI:C2	BO:D2, BO:E2, BO:F2, BO:B2	BI:C2	BO:C2
4	SDS20cmPreConditioning.met		2 of 2						BI:D2, BI:E2, BI:F2, BI:C2	BO:D2, BO:E2, BO:F2, BO:B2	BI:C2	BO:C2
5	SDS20cmPreConditioning.met		1 of 2						BI:D3, BI:E3, BI:F3, BI:C3	BO:D3, BO:E3, BO:F3, BO:B3	BI:C3	BO:C3
6	SDS20cmPreConditioning.met		2 of 2						BI:D3, BI:E3, BI:F3, BI:C3	BO:D3, BO:E3, BO:F3, BO:B3	BI:C3	BO:C3
7	SDS20cmPreConditioning.met		1 of 2						BI:D4, BI:E4, BI:F4, BI:C4	BO:D4, BO:E4, BO:F4, BO:B4	BI:C4	BO:C4
8	SDS20cmPreConditioning.met		2 of 2						BI:D4, BI:E4, BI:F4, BI:C4	BO:D4, BO:E4, BO:F4, BO:B4	BI:C4	BO:C4

At the top right-hand corner of the report, the report is labeled as Sequence Vial Report - Projected to indicate that this report is the Sequence Vial Increment Preview.

The Sequence Vial Increment Preview report reveals the cycles (Cycle # column) of each method to be run as well as the repetitions (Rep # column) from the sequence table to be executed in the run.

The Filename column is left blank by default, because this report is only a preview of a sequence run.

The Inject In/Out columns contain the same inlet/outlet user-entered vial position values as those under the Sample Inject Inlet/Outlet columns in the sequence table. These values are applicable when the user chooses to inject from the sequence table.

The Inject Time column contains the same injection duration time values in seconds as those in the sequence table under Sample Inject Duration column. These values are applicable when the user chooses to inject from the sequence table.

The Inject In/Out and Inject Time columns contain values from the Inject time program in the method by default if the user does not input values in the Sample Inject Inlet/Outlet and Sample Inject Duration columns of the sequence table. This is the case for users who choose to inject from the sequence table. However, the sequence does not run since no values are entered in the Sample Inject Inlet/Outlet and Sample Inject Duration columns of the sequence table.

The Other Inject In/Out columns contain the inlet/outlet vial positions for the injection event when the user chooses to inject from the method. These values do not appear in the sequence table. The Rinse Vials In/Out columns and the Separate Vials In/Out columns contain the inlet/outlet vial

positions for the rinse and separation events programmed in the method. These values do not appear in the sequence table. All the vial position values presented in the report, except the Inject In/Out columns, correspond to each method's time program, including incremented vials to be used in the sequence run if vial incrementing is enabled in the method.

Any possible tray collision (or vial collision) between the inlet tray and the outlet tray, due to invalid vial combinations between the inlet vials and the outlet vials, is indicated by a red asterisk mark next to the specific vial position in the report. See Figure F.2

Figure F.2 Invalid Vial Combinations Indicated by a Red Asterisk

Rinse Vials In	Rinse Vials Out	Separate Vials In	Separate Vials Out
BI:A1, BI:B1	BO:C6, BO:D6	BI:B5	BO:D6
*BI:A2, *BI:B2	*BO:D1, *BO:E1	*BI:B6	*BO:E1
*BI:A3, *BI:B3	*BO:D2, *BO:E2	BI:C1	BO:E2
*BI:A4, *BI:B4	*BO:D3, *BO:E3	BI:C2	BO:E3
*BI:A5, *BI:B5	*BO:D4, *BO:E4	BI:C3	BO:E4

IMPORTANT Users are not aware of possible vial collisions after the sequence table is set up with one or more methods included. The instrument system encounters these invalid vial combinations that lead to vial collisions when the sequence is run. In the course of a sequence run, the instrument system detects invalid vial combinations and aborts execution of any method's repetitions that can lead to collisions. Other repetitions of the same method that have valid vial combinations are successfully executed by the instrument system. It is recommended that the user view the Sequence Vial Increment Preview report before running any sequence.

Sequence Vial Report-Confirmed

The Sequence Vial Report - Confirmed is a sequence summary report generated after running a sequence. It is a confirmation report of the specific vials that have been successfully used by the instrument system in the sequence run. This report is identical to the Sequence Vial Increment Preview report except for the following: 1) Filenames for the sequence run are now included in the Filename column, and 2) Only the vial combinations that have been successfully used in the course of the sequence run by the instrument system are shown.

Figure F.3 Sequence Vial report Confirmation

To generate the Sequence Vial Report - Confirmed, you must change the Run Type of any set of sequence table run lines to be executed from Unknown to Summary. The following steps can be helpful:

1 Begin by clicking on the blue arrow in the **Run Type** box of an initial sequence table run line showing where the sequence run starts. It does not have to be run number 1. The Sample Run Types dialog box opens.

2 Select **Begin Summary** as the run type.

> **IMPORTANT** Do not select Vial Summary as the run type. This run type is not used to generate the Sequence Vial Report - Confirmed.

Figure F.4 Setting Up Run Type Parameters on a Sequence Table
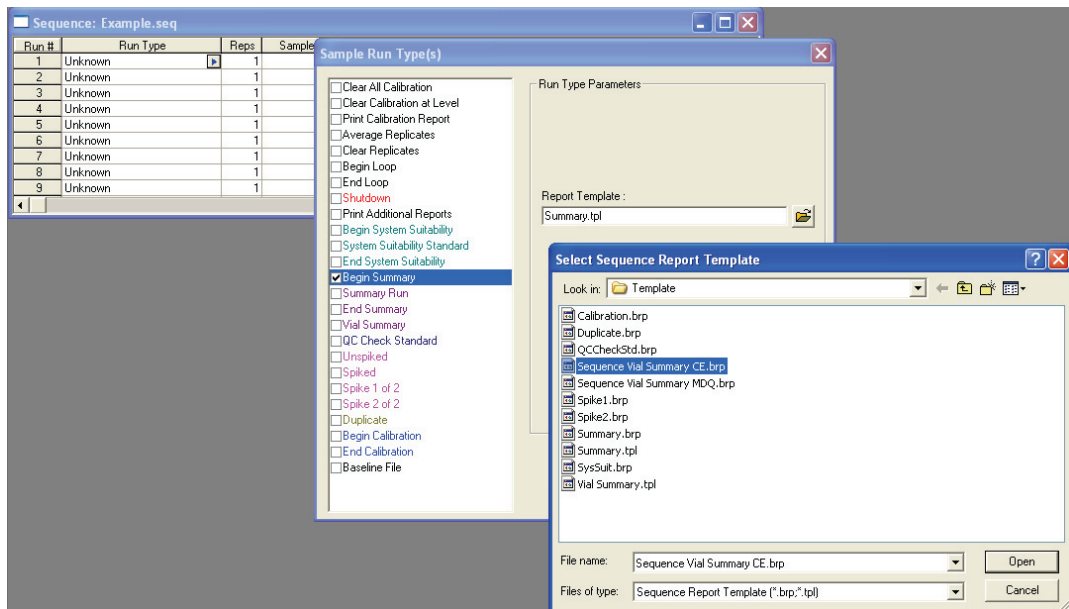
3 Click on the folder icon in the **Run Type Parameters** region to open up a report template.

4 Select Sequence Vial Summary CE.brp as the report template and click **Open**. The report template name should be in the Report Template field after opening.

5 Click **OK** to close the Sample Run Types dialog box and return to the sequence table.

6 The sequence table should display a run type of Summary Begin for the initial sequence run line showing where the sequence run starts.

Figure F.5 Sequence: Example.seq

Run #	Run Type	Reps	Sample Inject Inlet	Sample Inject Outlet	Sample Inject Duration
1	Summary Begin ▶	1	◆	◆	
2	Unknown	1	SI:A1	BO:C1	20.0
3	Unknown	1	SI:A2	BO:C1	20.0
4	Unknown	1	SI:A3	BO:C1	20.0
5	Unknown	1	SI:A4	BO:C1	20.0
6	Unknown	1	SI:A5	BO:C1	20.0
7		1	SI:A6	BO:C1	20.0

7 For subsequent sequence run lines that are to be executed (middle of sequence run), repeat step 1 on those lines, but select Summary Run as the run type. There is no need to open any report template.

8 Click **OK** to return to the sequence table.
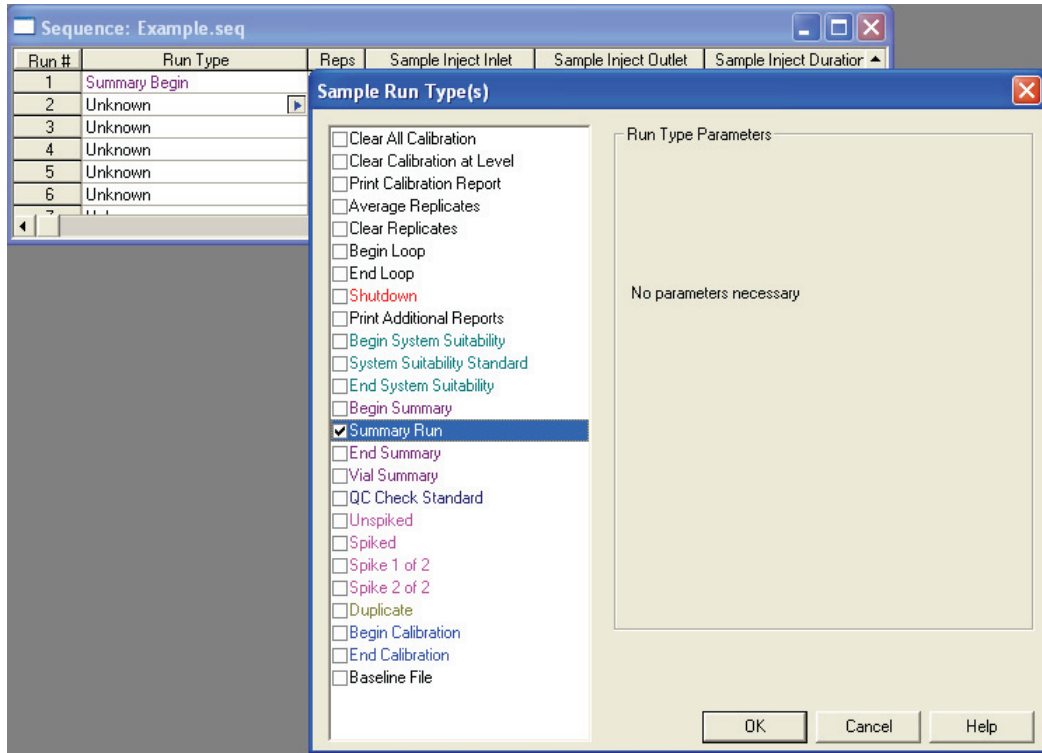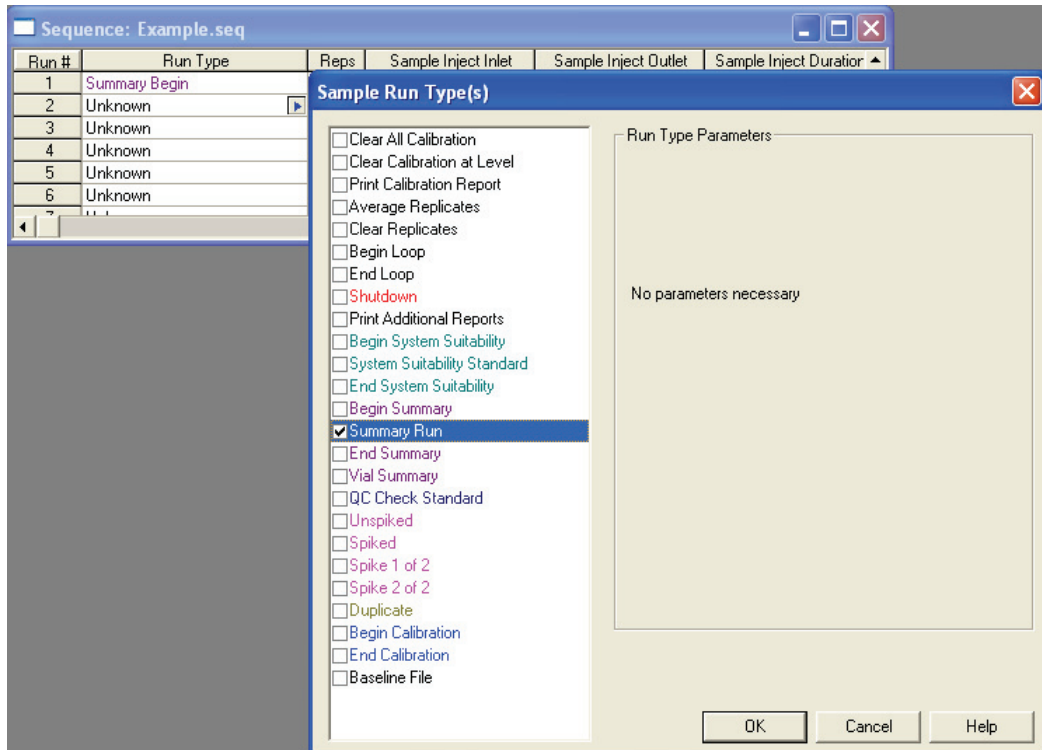
Figure F.6 Return to Sequence Table

Figure F.7 Sample Run Types

9 The sequence table should display Summary Run as the run type for the subsequent sequence run lines (middle of sequence run).

10 For the last sequence run line to be executed (end of sequence run), repeat step 1, but select **End Summary** as the run type. There is no need to open any report template.

11 Click **OK** to return to sequence table.

Figure F.8 Return to Sequence Table
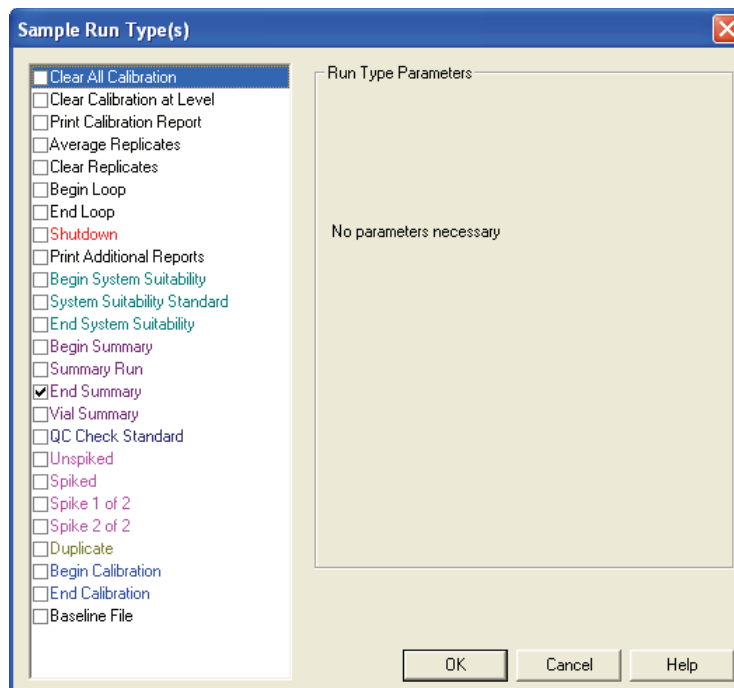
12 The sequence table should display Summary End as the run type for the last sequence run line to be executed (end of sequence run).

Figure F.9 Summary End Run Type

Run #	Run Type	Reps	Sample Inject Inlet	Sample Inject Outlet	Sample Inject Duration ▲
1	Summary Begin	1			
2	Summary Run	1	SI:A1	BO:C1	20.0
3	Summary End	1	SI:A2	BO:C1	20.0
4	Unknown	1	SI:A3	BO:C1	20.0

For users who want to generate the Sequence Vial Report - Confirmed, for any single sequence table run line that consists of one or more repetitions, the process is similar:

13 Begin by clicking on the blue arrow in the **Run Type** box of an initial sequence table run line, showing where the sequence run starts. It does not have to be run number 1. The Sample Run Types dialog box opens.

14 Select **Begin Summary** as the run type.

> **IMPORTANT** Do not select Vial Summary as the run type. This run type is not used to generate the Sequence Vial Report - Confirmed.

15 Click on the folder icon in the **Run Type Parameters** region to open up a report template.

16 Select **Sequence Vial Summary CE.brp** as the report template and click **Open**. The report template name should be in the Report Template field after opening. Do not close the Sample Run Type(s) dialog box yet.

17 Select **End Summary** as the run type in the Sample Run Type(s) dialog box. There is no need to open any report template for End Summary.

There should now be two run types selected: Begin Summary and End Summary.

18 Highlight **Begin Summary** to assure the correct report template has been opened.

19 Click **OK** to close the Sample Run Type(s) dialog box and return to sequence table.

The Sequence table should reveal SMB SME (notations that refer to Summary Begin and Summary End) as the run type for the single sequence table run line that consists of one or more repetitions. The user can repeat steps 1 through 3 for the other sequence table run lines, if Sequence Vial Report - Confirmed is desired for each of the other lines. Sequence table run lines that have Unknown or other run types do not generate the Sequence Vial Report - Confirmed.

Figure F.10 Summary Begin, Summary End Notations

After you have changed the Run Type of any set of sequence table run lines from Unknown to Summary, you can begin the sequence run and have the software collect data for the Sequence Vial

Report - Confirmed. A Complete message in the Status column of the sequence table confirms the end of the sequence run.

Figure F.11 Complete Message in the Status Column

After the sequence run is completed or is terminated, you can activate the Sequence Vial Report - Confirmed by selecting Sequence Custom Report under View from the Reports menu:

Figure F.12 Reports > View > Sequence Custom Report
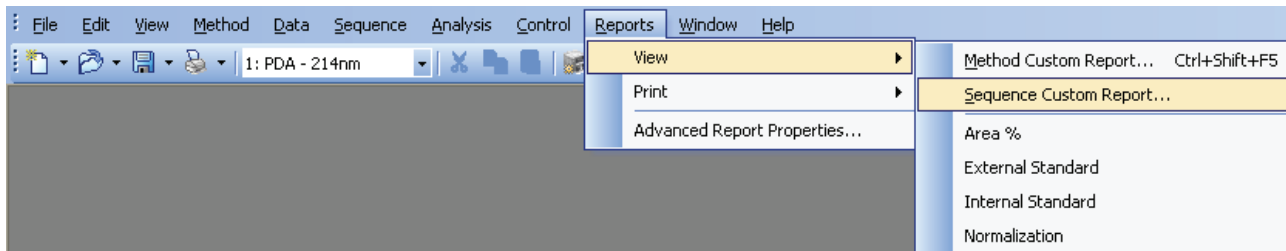
The View Sequence Reports dialog box displays. The listings under Report Type column are the Sequence Vial Reports - Confirmed for each of the sequence table run lines. They are labeled as Sequence Summary reports in the dialog box. The report template used is indicated in the last column.

The numbers under Sequence Records column are the sequence table run line numbers from which the Sequence Vial Reports - Confirmed originate. The number of repeats or iterations (for example. 3,3 or 4,4,4) of the sequence table run lines under Sequence Records reveals the number of repetitions that have been successfully executed for those run lines.

For instance, run line 4 from sequence run of Sequence Table A above has successfully generated a Sequence Vial Report - Confirmed for all three repetitions.

Figure F.13 Sequence Vial Report
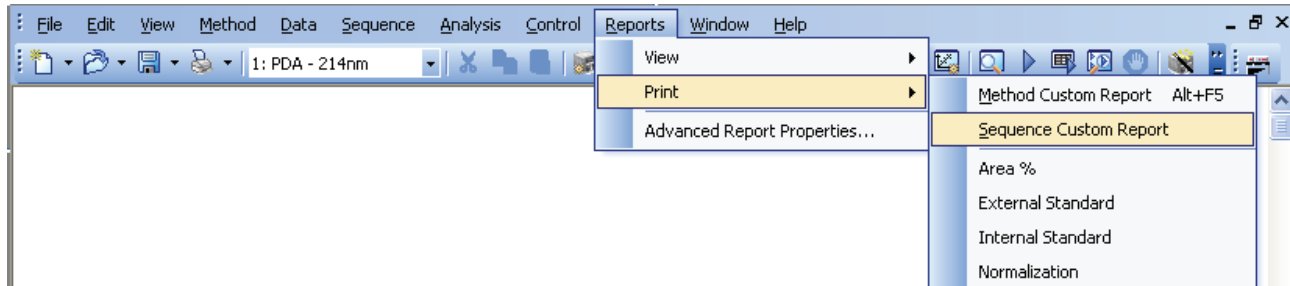
You can highlight any one of the listed Sequence Vial Reports - Confirmed and click View to open it.

To print any Sequence Vial Report - Confirmed, right-click on the report, and then select **Print**.

Alternatively, you can select **Sequence Custom Report** under **Print** from the **Reports menu**:

Figure F.14 Reports > Print > Sequence Custom Report

The Print Sequence Report window displays, and it looks exactly the same as the View Sequence Report dialog box. You can select the desired Sequence Vial Report - Confirmed and click **Print** to print it.

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