WinFLOW V4 Operator's Manual

Advanced Technology in Ion Analysis

ALPKEM™

O·I·Analytical

151 Graham Road · P.O. Box 9010 · College Station, Texas 77842-9010
Telephone (409) 690-1711 · FAX (409) 690-0440

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Chapter 1
Introduction

Thank you for choosing ALPKEM™ WinFLOW V4 Software. WinFLOW V4 is a Windows®-based program designed to offer a high level of automation when used in combination with any ALPKEM™ Flow Solution ion analyzer as well as many other ion analyzers. We are confident that you will find WinFLOW V4 to be an intuitive, easy, and highly productive software package.

About this Manual

This manual should be regarded as an integral part of the Flow Solution system and the WinFLOW V4 software. It provides tools for mastering the use of WinFLOW V4 during routine analysis as well as an in-depth discussion of the many specialized features available for more advanced work.

Features

- Automatic startup and shutdown enhances system productivity.*
- Pause option permits run and sample table modification anytime during a run.
- Sensitivity drift correction eliminates the need to fully recalibrate after ICV/CCV failure.
- User-defined dilution factors add user flexibility in autodilution mode.*
- Sample dilution before the run reduces analysis time.
- Automatic working calibration standard preparation from stock solutions* reduces operator workload.
- Manual injection mode permits system operation without an autosampler.
- Peak editing improves peak height and peak area marking.
- Post-run peak height/area and digital signal processing algorithms enhance analyte detectability.
- Mean and RSD values add important QA/QC data to reports.
- Complete French, German, and Spanish language support.

***Note**: This software feature requires specific hardware for full-system functionality.

The WinFLOW V4 software package includes:

- CD-ROM containing:
  - WinFLOW V4 Software
  - WinFLOW V4 Software Manual
  - ALPKEM Instrument Manuals
  - ALPKEM Consumables Catalog
  - Adobe Acrobat Reader Program
- Postage paid software registartion card
- WinFLOW V4 software serial number (labeled on the outside of the CD-ROM case)

Computer Requirements

The following table contains the recommended and minimum computer requirements for successful installation and use of the WinFLOW V4 software.

Table 2.1. Computer Requirements

Parameter	Recommended
Type	IBM compatible
Speed	Pentium 133 or greater
Memory	32 MB or greater
Hard Drive Capacity	1 GB Hard Drive
Operating System	Windows 95/98/NT
Mouse	Bus Mouse
Graphics	VGA graphic card 1 MB RAM on-board
Com Port	1 free high speed COMM port

Note: Working knowledge of the Windows 95/98 or NT operating system is required.

Note: Recommended computer requirements are mandatory for Flow Solution III and Flow Solution IV systems larger than three channels and Flow Solution 3000 systems larger than two channels.

Note: WinFLOW V4 will **not** operate with x386 processor-based computers. This is due to the requirement for a math co-processor. WinFLOW V3 is the only version that will operate using x386 processor-based computers.

Safety Information

Operator Precautions

For operator safety, pay attention to ***WARNING*** and ***CAUTION*** statements throughout the manual.

- A ***WARNING*** indicates a condition or possible situation that could result in physical injury to the operator.

- A ***CAUTION*** indicates a condition or possible situation that could damage or destroy the product or the operator's work.

Warnings and precautions in this manual or on the instrument must be followed during operation, service, and repair of the instrument. Failure to follow these warnings and precautions violates the safety design standards and intended use of the instrument. OI Analytical will not be liable for the operator's failure to comply with these warnings and precautions.

WinFLOW V4 Quick Start

Note: The following instructions assume that all modules have been installed and all hydraulic connections have been made.

1. Turn on all Flow Solution modules and the computer.
2. Open the WinFLOW V4 software program.
3. Verify that all tubing connections are made, then initiate pump(s) rotation.
4. Select the **Method Editor** button  and verify that all settings are correct.
5. Select the **Sample Table** button  and alter an existing table or create a new sample table. Print the completed sample table.
6. Select the **Data Collect** button  and select a Method and Table in Run Setup.
7. Enter an **Operator ID** and a **Filename** for the run results.
8. Click on the **Play** button  and monitor the baseline. Once the baseline is stable with start-up solution, switch to reagents and again monitor the baseline until it stabilizes.
9. While the baseline is stabilizing, use the Sample Table printout from step 4 to load the sampler trays.
10. Select the **fast forward** button  when the baseline is stable.
11. Monitor the run until the SYNC peak has eluted and successfully marked. Allow the run to continue.
12. If the run completed automatically, the data will be saved automatically to the result file defined in step 7.

Note: Select the **stop** button  to stop the run. Be sure to save results once the completed run is displayed.

13. Review the results.

Button Ribbon

 Opens the Data Collect screen.

 Opens the Sample Table screen.

 Opens the Method Edit screen.

 Opens the Data Analysis screen.

 Play button. Initiates data display.

 Rewind button.

 Fast forward button. Initiates data collection.

 Pause data collection button.

 Stop data collection button.

 Baseline signal zero button.

 Grid on/off button.

 Peak plot update lock button.

 Peak editor cursor button.

 Delete/reprocess peaks button.

 Recalculate button.

Notes

Chapter 2
Installation

This chapter includes step-by-step procedures for properly installing WinFLOW V4 software.

Installing from the CD-ROM

The WinFLOW V4 software program, the WinFLOW V4 software manual, and all instrument operation manuals are contained on the single CD-ROM. WinFLOW V4 can be run with either Windows 95/98 or Windows NT as the operating system. Locate the CD-ROM and follow the instructions below.

1. From the Windows environment, insert the WinFLOW V4 CD-ROM into the available CD-ROM drive. The ALPKEM WinFLOW Setup Disc screen will appear on the desktop (see Figure 2.1).

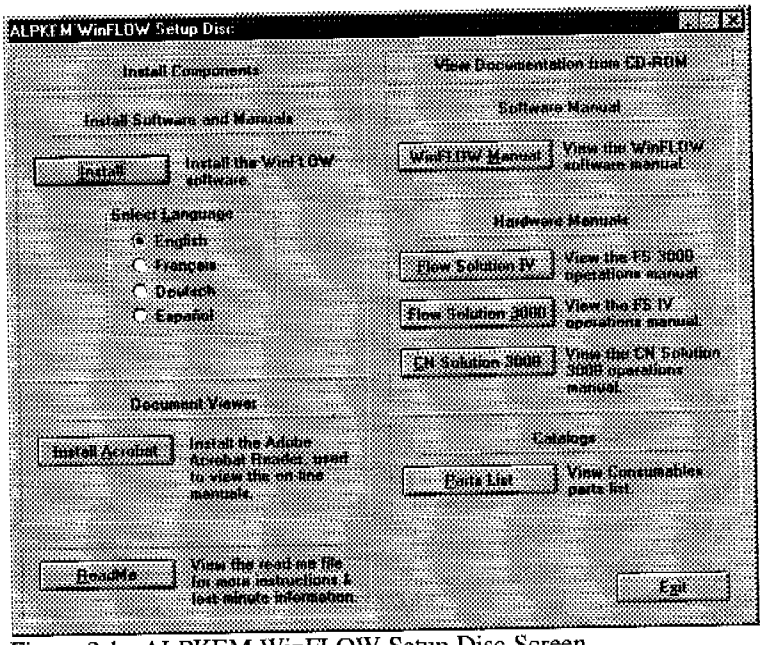


Figure 2.1. ALPKEM WinFLOW Setup Disc Screen

Note: If the ALPKEM WinFLOW Setup Disc screen does **not** automatically appear after inserting the CD-ROM disk:

a. Click on the Windows **Start** button.

b. Select **Run**.

c. Type *x:/setup* where *x* represents the letter assigned to the CD drive.

d. Click on **OK**. This will start the ALPKEM WinFLOW Setup Disc screen.

e. See Figure 2.1 and continue with step 2 below to install WinFLOW V4.

2. Select the appropriate language and click **Install**.

3. The installation process will begin. Follow the instructions on the screen.

4. At the Setup Type screen, choose the setup type to install the options below:
   Typical: Program files
   AlpCOM
   WinFLOW V4 Manual
   Compact: Program files
   Custom: defined by user (see Figure 2.2)

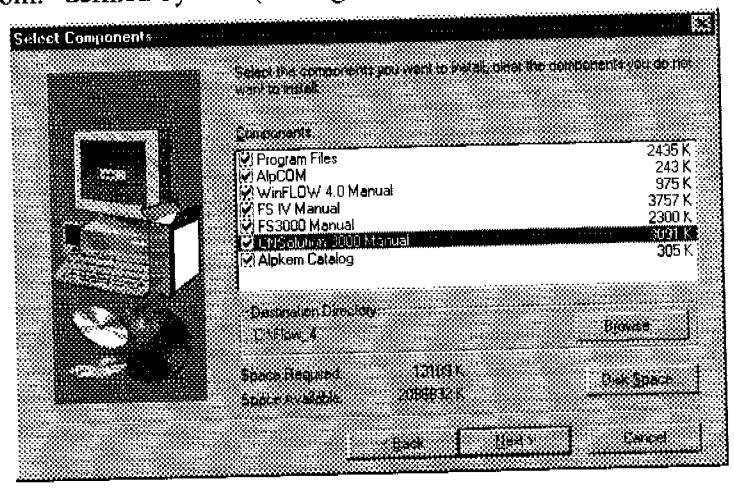


Figure 2.2 Install ALPKEM WinFLOW Screen

5. Once the WinFLOW V4 program is installed, click on **OK**.

6. The Configure WinFLOW screen will appear (see Figure 3.1). Proceed to Chapter 3, "Configuration," for details on how to configure WinFLOW V4.

Installing from the 3.5" Diskette

1. From the Windows 95/98 desktop, click on the **Start** button and select **Run**. The Run dialog box will appear.

2. Type A:\Setup (or appropriate drive) and click on **OK**.

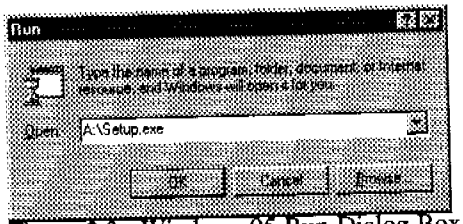


Figure 2.3. Windows 95 Run Dialog Box

3. Continue installation from step two under "Installing from the CD-ROM."

Chapter 3
Configuration

Opening WinFLOW Configuration

Instructions for configuring WinFLOW V4 software are provided for the Microsoft Windows 95 operating system.

1. From the **Start** button, choose the Programs folder.

2. Choose the WinFLOW V4 folder.

3. Click on the FLOW Config 4.00 icon. The Configure WinFLOW screen will appear.

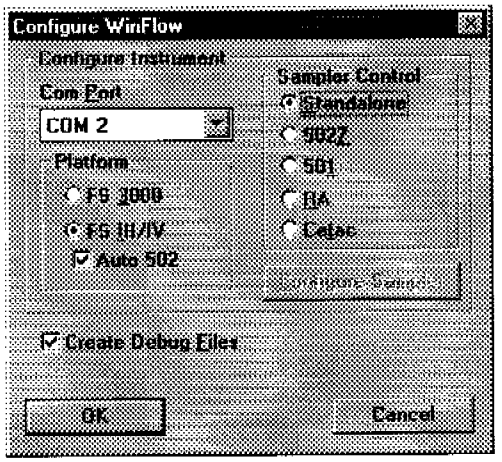


Figure 3.1. Configure WinFLOW Screen

4. Select the Communication Port (**Com Port**), **Sampler**, and the type of Flow Solution System (**Platform**) to be used. Only available communication ports will be listed as choices.

Most Flow Solution 3000 systems use a 5027 Sampler. Flow Solution III and IV systems use a 501 or RA Sampler. Upgraded systems that do not have one of the listed samplers will run in the stand-alone sampler control mode.

The RA Sampler must be selected as the **Sampler Control** to be able to use the vials and vial racks that are provided with the sampler.

Configuration is also required if an autodilutor (such as the OI Analytical ALKEM Model 511 or MicroSTEP dilutor) is used in conjunction with a 501 or RA Sampler.

Note: It is not necessary to configure WinFLOW V4 every time the software is opened. WinFLOW V4 configuration changes are only necessary if there is a change in instrument configuration.

Configuring the 501 Sampler

This step is only neccssary if the 511 dilutor is used with the 501 sampler.

After selecting the 501 Sampler in thc Confirgure WinFLOW screen, click on the **Configure Sampler** button. The 501 Sampler Configuration screen will appear. Check thc **Use Dilutor** box and click on **OK**.

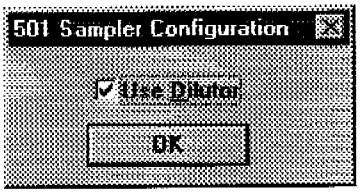


Figure 3.2. 501 Sampler Configuration Window

Configuring the RA Sampler

After selecting the RA sampler in the Configuration WinFLOW screen, click on thc **Configure Sampler** button. This will bring up the RA Configuration screen.

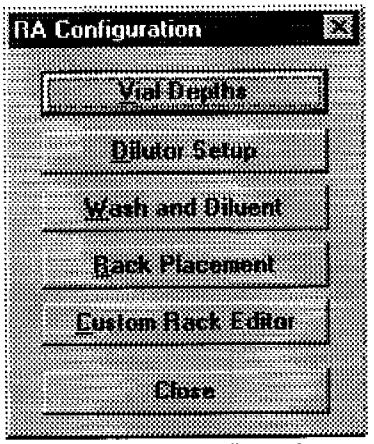


Figure 3.3. RA Configuration Screen

Vial Depths

The depth of sample probe travel into a given sample cup can be adjusted by selecting the **Vial Depths** button. Adjust the vial depth by clicking in the vial depth boxes.

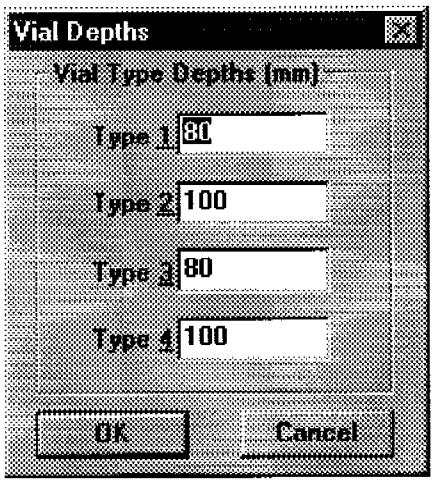


Figure 3.4. RA Sampler Vial Depths Configuration Screen

If a TWR (traveling wash reservoir) is used, the maximum depth of the probe in the sample vial is approximately 100 mm. If a static wash reservoir (no TWR) is being used, the range will depend on the set-up position of the probe in the sampler arm and the type of wash reservoir being used. The range will generally be larger than 100 mm. See Tables 5.2 and 6.3 for specific vial types and volumes used for this sampler.

Dilutor Setup

If the MicroSTEP dilutor is to be used with the RA sampler, click on the **Dilutor Setup** button. The following screen will appear:

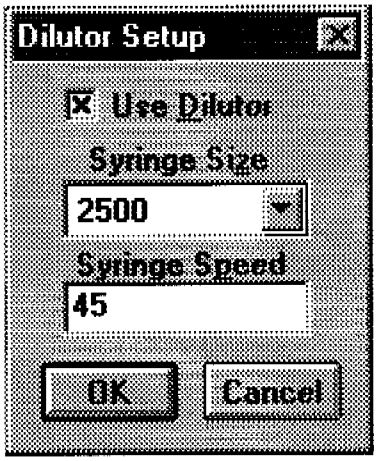


Figure 3.5. RA Sampler Dilutor Configuration Screen

By checking the **Use Dilutor** box, the **Syringe Size** and **Syringe Speed** settings will become available for input. There are three syringe sizes available: 1000 µL, 2500 µL, and 5000 µL. Select the 2500-µL syringe (standard volume) in the pull down menu. Select the proper speed based on syringe size and application. The speed ranges from 25 for the larger syringes to 50 for the smaller syringes. The default value for the 2500-µL syringe is 45.

Wash and Diluent

There are two types of RA Sampler probe arm configurations available. In order to specify which type to be used, click the **Wash and Diluent** button. The following screen will appear:

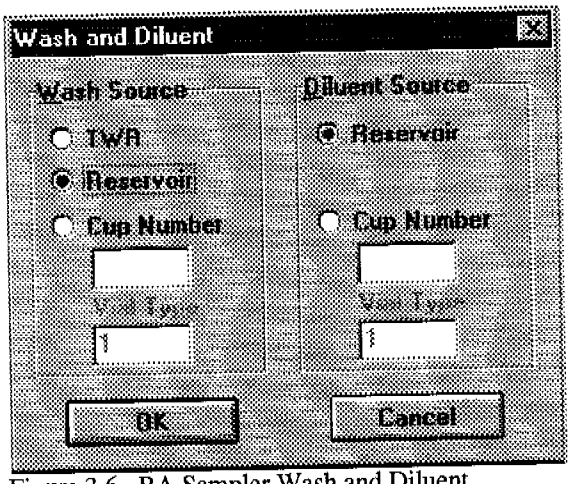


Figure 3.6. RA Sampler Wash and Diluent Configuration Screen

TWR If a TWR (traveling wash reservoir) is to be used with the RA sampler, select **TWR** under **Wash Source**.

When a dilutor is used with the RA sampler, select **Reservoir** under **Diluent Source** column. If a dilutor is not present, **Diluent Source** is not used. If a dilutor is present, it is also possible to use the TWR module and draw the diluent from any specified location on the RA sampler tray.

If a diluent source location other than the reservoir provided with the dilutor is to be used, select **TWR** under **Wash Source** and **Cup Number** under **Diluent Source** to specify its location.

Reservoir If a stationary wash reservoir is to be used with the RA Sampler, select **Reservoir** item under **Wash Source**.

If a dilutor is not present, **Diluent Source** is not used. When a dilutor is used with the RA sampler, select **Reservoir** under **Diluent Source**. Alternatively, it is possible to draw the diluent from any specified location on the RA sampler tray. If another diluent source location (other than the reservoir provided with the dilutor) is to be used, select **Cup number** under **Diluent Source** to be able to specify its location.

Cup Number It is also possible to draw both wash and diluent from any specified location on the RA sampler tray. Select **Cup number** under **Wash Source** and/or **Cup number** under **Diluent Source** to specify various locations. These two functions can be used in any combination with the TWR or the Reservoir.

Rack Placement

To set up the racks being used with the RA sampler click on **Rack Placement**. The following screen will appear:

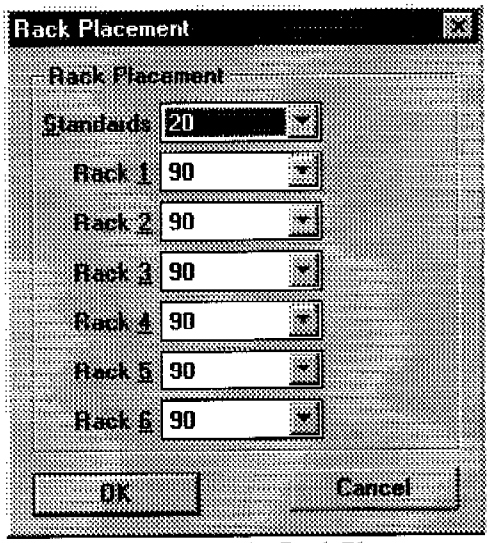


Figure 3.7. RA Sampler Rack Placement Screen

The normal configuration of the RA sampler allows for three sample racks and one standards rack. The preprogrammed sample rack sizes are 21, 24, 40, 60, and 90, the default value being 90 for each sample run. The standards rack sizes available on this screen are 10 and 20, the default rack size being 20.

Custom Rack Editor

The Custom Rack Editor allows the RA sampler to use virtually any size or type of rack. Click on **Custom Rack Editor** and the following screen will appear:

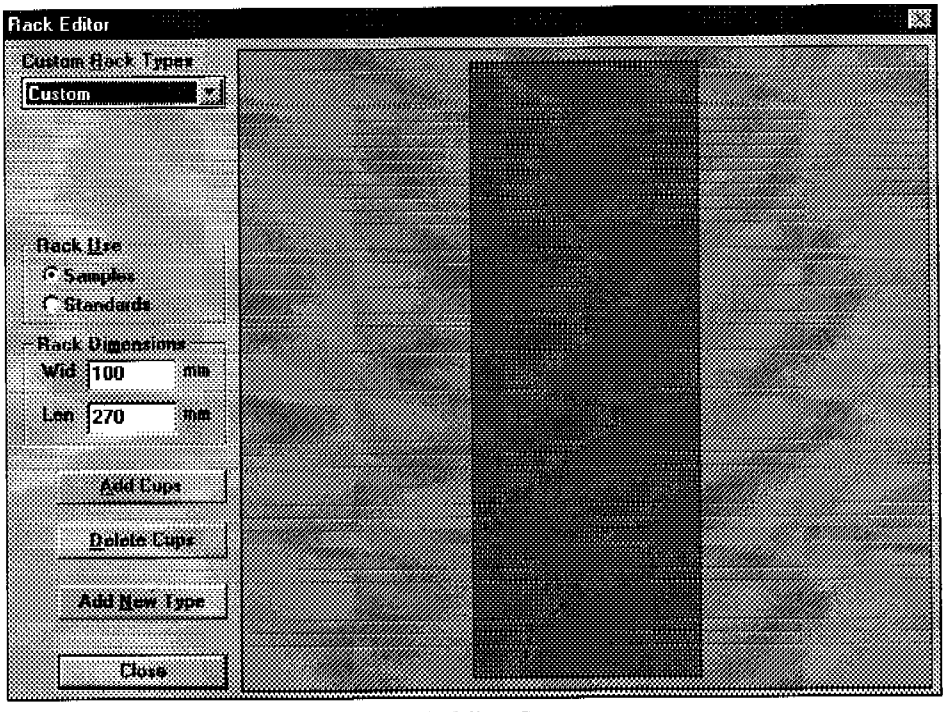


Figure 3.8 RA Sampler Custom Rack Editor Screen

To use racks other than those provided with the RA sampler, take measurements of the rack, vial slot dimensions, and distance between each vial slot. Input these measurements into the corresponding locations of the Custom Rack Editor.

When saved, custom racks will be displayed as an option in the Rack Placement screen. Both sample and standard racks can be created in this way. Please refer to the example below for assistance.

1. From the Rack Editor screen (Figure 3.8), click on **Add New Type**. Type the name of the custom rack and click on **OK**. Again, from the Rack Editor screen, click on **Add Cups**. The following window will appear:

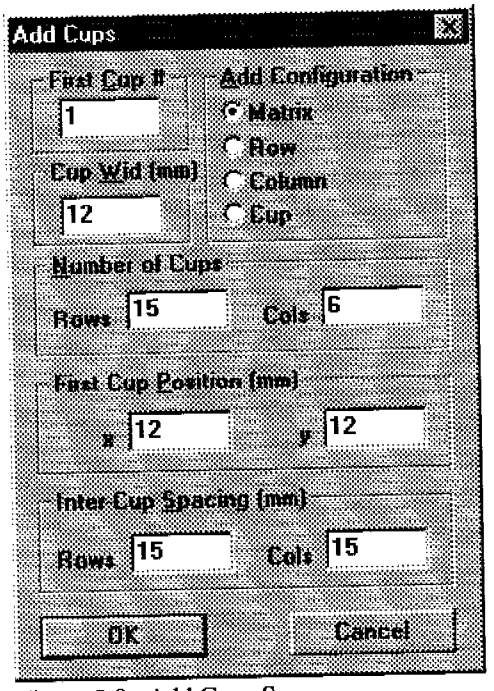


Figure 3.9. Add Cups Screen

2. Enter the following data in the corresponding fields:

First Cup #	number between 1 and 99
Cup Wid (mm)	width of the cup in millimeters
Number of Cups	number of rows (left to right) and number of columns (back to front)
First Cup Position (mm)	distance from standard tray upper-left corner (closest to the sampler body) to middle of the 1st cup in millimeters
Inter-Cup Spacing (mm)	distance from cup-to-cup center in millimeters

3. When all data has been entered click on **OK**. Then click on **Close** in the Rack Editor screen. In the RA Sampler Configuration screen, click on **Rack Placement** (see Figure 3.7).

4. Select the custom rack name that you have just created for the rack placement desired. After selecting a custom rack, the Align Rack screen appears.

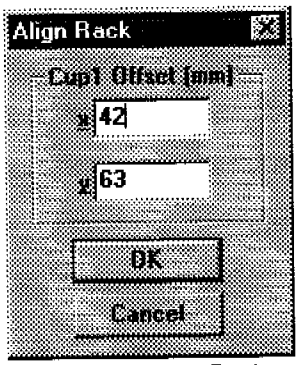


Figure 3.10. Align Rack Screen

5. Click on **OK** to accept the default settings. The RA sampler rack is now ready and displayed for use.

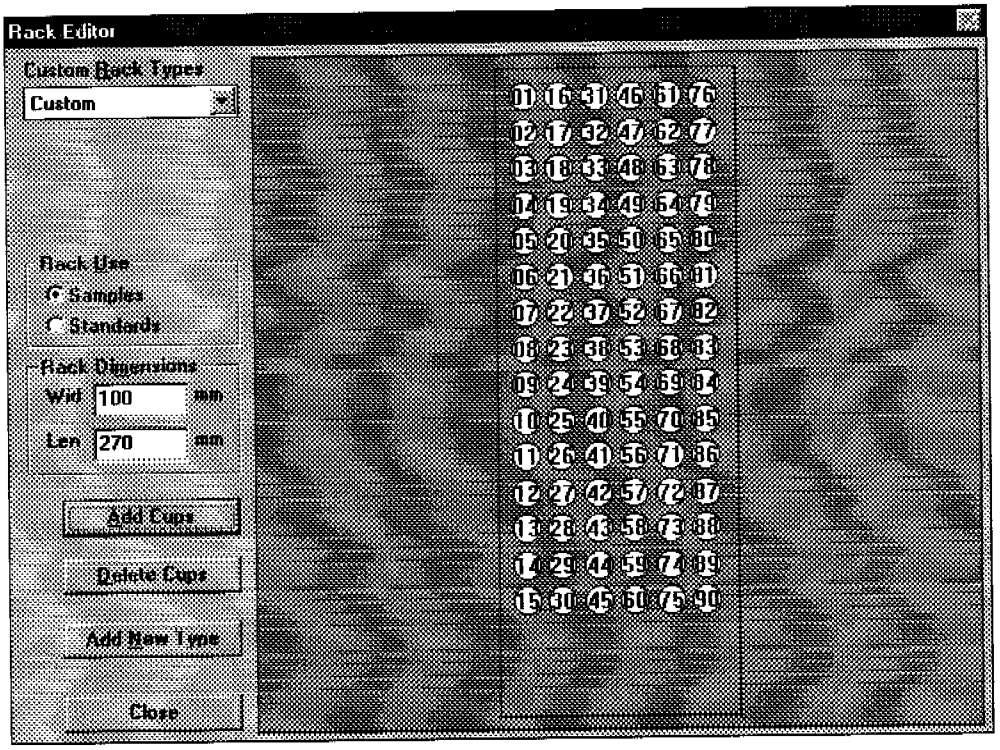


Figure 3.11. RA Sampler Rack Ready for Use

Notes

Chapter 4
Structural Overview

Opening WinFLOW V4

Follow the instructions below to open the WinFLOW V4 software in Microsoft Windows 95 and Windows 98 operating systems.

1. Click on the **Start** button and choose the Programs folder.
2. From the Programs folder, choose the WinFLOW V4 folder.
3. Click on the WinFLOW V4 icon.
4. Enter the serial number included in the WinFLOW documentation.

Note: The serial number will only be requested the first time the WinFLOW V4 is opened.

Select View Screen

Upon opening the WinFLOW V4 program, the Select View screen is displayed.

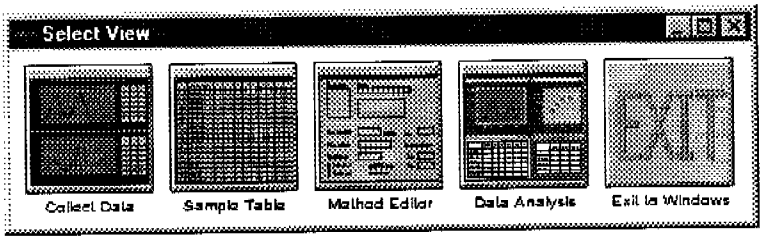


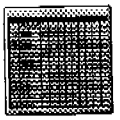
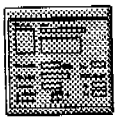
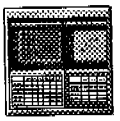
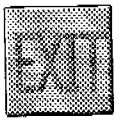
Figure 4.1. Select View Screen

The Select View screen allows easy single-click access to the four sub-programs in WinFLOW V4. It also provides a button to exit the WinFLOW V4 program. The four principal sub-programs within WinFLOW V4 are outlined in Table 4.1.

When the Select View screen is active the main menu bar displays the **File**, **Setup**, **Window**, and **Help** menus.

The **Window** and **Help** menus are available from all of the WinFLOW V4 screens. The **File** menu also provides the **Exit** option.

Table 4.1. WinFLOW V4 Windows

Program	Select View Button	Shortcut Key	Button Ribbon	Function
Collect Data		F2		Initiates data collection process following selection of a sample table and method.
Sample Table		F3		Opens a table which displays: cup #, name, type, replicate, dil. And wt. factors for each sample.
Method Editor		F4		Data collection parameters are set here, as well as calibrant and QC information.
Data Analysis		F5		Allows access to results from any previously saved run. Contains peak plot, peak table, calibration plot, and calibration data tables.
Exit		F12		Exits WinFLOW V4 software.

Setup Menu

The options from the **Setup** menu are **Preferences**, **Configuration**, and **Auto Event Scheduler**. These options access their corresponding screens.

Preferences

In the Preferences screen, the operator can set global WinFLOW V4 preferences such as default views and languages.

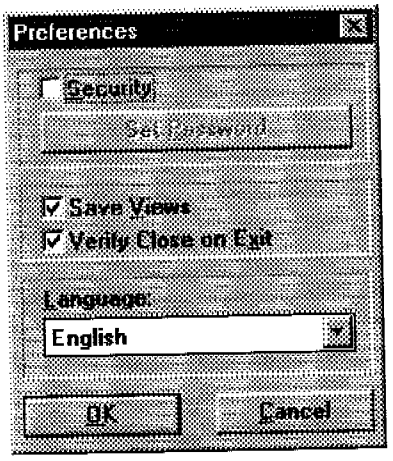


Figure 4.2. Preferences Screen

Security Allows for the input of a password that will be requested each time WinFLOW V4 is opened.

Save Views Any screen remaining open when WinFLOW V4 is exited will be displayed when WinFLOW V4 is opened again.

Note: Save Views will not bring up Results and Analysis files left open when exiting.

Verify Close Allows the opeartor to select whether to have an automatic prompt verifying the operator's intention to leave the program when exiting WinFLOW V4.

Language WinFLOW V4 has multilanguage capability. Select from English, French, German, and Spanish. Contact your local OI Analytical distributor for more information about other available languages.

Configuration

The Configuration Screen provides information about the instrument platform, such as the number of communicating detectors (channels), valves, pumps, the type of sampler installed with the system, and the firmware version.

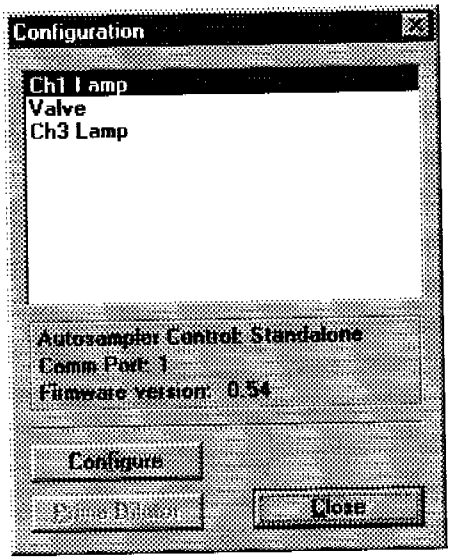


Figure 4.3. Configuration Screen

The Configuration screen allows the operator to determine if the analyzer, as well as each installed component, is communicating with the computer. Selecting **Configure** when the analyzer or a component loses communication will reestablish a link between it and the computer.

Auto Events Scheduler

The Auto Events Scheduler allows the operator to select what action(s) the pump(s) will take when any of the specified events occur.

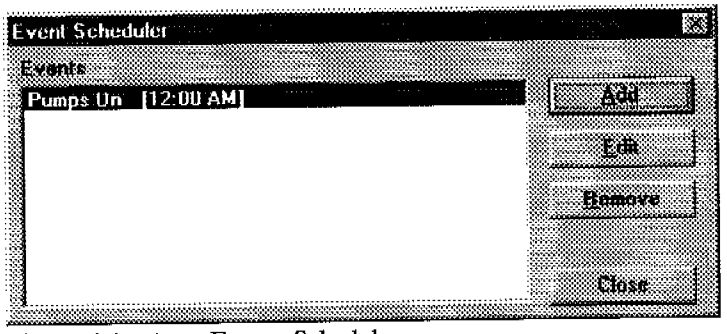


Figure 4.4. Auto Events Scheduler

Add Selects the type of event, time and action.
Edit Edit any previously chosen event.
Remove Remove any previously chosen event.

Pumps Menu

The **Pumps** menu contains **Pump Timer**, **Pumps On**, **Pumps Off**, and **Pumps Slow** options.

Pump Timer Indicates the number of hours the pumps have been in use. This feature is useful for determining when pump tubes should be replaced, membranes changed, etc...
Pumps On Allows the operator to turn on all pumps.
Pumps Off Allows the operator to turn off all pumps.
Pumps Slow Allows the operator to slow all pumps.

Window Menu

The **Window** menu contains options for displaying the open windows within WinFLOW V4. Options to **Cascade**, **Tile**, and **Arrange Icons** are provided. All open windows are listed in and can be accessed from the **Window** menu by selecting the name or by typing the number associated with the window.

About Menu

The ***About*** menu contains information about the software and firmware versions as well as the technical support access numbers.

Shortcut Keys

WinFLOW V4 contains several keyboard shortcut keys. The function keys provide an additional method for locating and enabling various software functions. The available function keys are described in Table 4.2.

Table 4.2. Function Keys

Function Key	Description
F2	Opens the Data Collect screen.
F3	Opens the Sample Table screen.
F4	Opens the Method Editor screen.
F5	Opens the Data Analysis screen.
F12	Exits WinFLOW V4.

These function keys duplicate the Select View buttons, which are also available from the button ribbon or the pull down menu. Therefore, the operator has multiple avenues for accessing the most frequently used features of the program.

System Security

System security is provided to reduce unauthorized use of the WinFLOW software. Password protection is accomplished using a single, operator-specified password.

If the system password is ever lost, contact OI Analytical Customer Service at (800) 336-1911 or (409) 690-1711.

1. Under the **Setup** menu, select the **Preferences** option to open the Preferences screen (Figure 4.2). Select the box next to **Security**. The password dialog box will appear (see Figure 4.6).

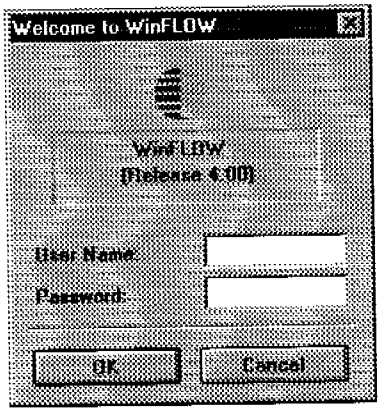


Figure 4.6. Welcome to WinFLOW screen.

Note: The first time the Security option is exercised, the default user name and password will be used. The default user name is **system** and the default password is **manager**.

Only after exiting and reopening WinFLOW will the user name and password be changeable.

2. Enter user name and password. Select **OK**.

3. To edit, delete, or add a user name and password, click on **Set Preferences** from the Preferences screen (Figure 4.2). The Security screen will appear (see Figure 4.7).

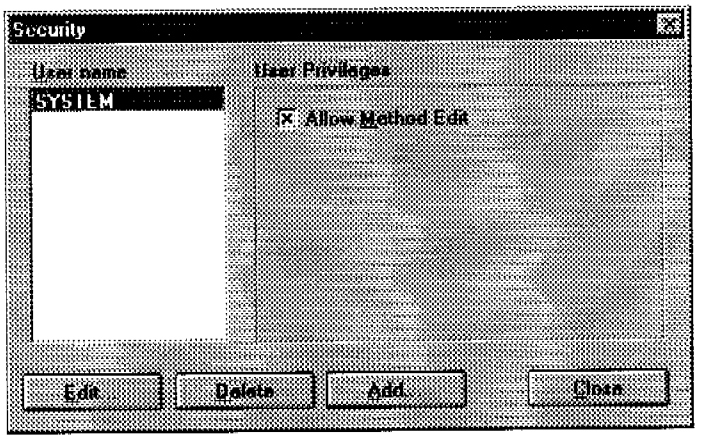


Figure 4.7. Security Screen

4. Choose either **Edit**, **Delete** or **Add** and follow the instructions presented to edit, delete, or add a user name and password.

Chapter 5
Method Editor

The WinFLOW V4 Method Editor controls how data is collected and interpreted.

General Description

Opening the Method Editor

There are three ways to open the Method Editor:

- Click on the **Method Editor** button  on the Select View Bar,
- Click on the **Method Editor** button  on the Main Button Ribbon, or
- Press the [F4] key.

Dialog Boxes

The Method Editor contains six screens that are used to modify all of the method parameters necessary for running an analysis.

- Primary Options screen
- Channel Options screen
- Calibration/Quantification Options screen
- Automatic Options screen
- Timed Events screen
- Calibrants Table screen

Each of these screens and their associated menus are discussed in this chapter. Once properly set, these screens should not require modification during future runs.

The title of each screen is located at the top of the window. The window bar also contains the name of the open method. There are three ways to access each screen.

- The Method Editor button ribbon,
- The Method Editor button ribbon advance and reverse arrows, and
- The Methods menu lists each of the screens separately along with their associated quick key functions.

Method Editor Button Ribbon

 Advance arrow

 Primary options

 Channel options

 Calibration/Quantitation options

 Automatic options

 Timed events

 Calibrants table

 Reverse arrow

Creating a Method

The fastest way to create a method is to alter an existing method and then use the **Save As** option. WinFLOW V4 is installed with two methods: a single channel method and a dual channel method. Use these methods as templates for creating new methods. Most of the parameters in the installed methods will not need to be changed when creating a new method for a given chemistry.

Saving a Method

When a method is changed and the changes have not been previously saved, the **Save As** dialog box will be presented when another method is opened.

CAUTION:
Always use the ***Save As*** *function when altering an existing method so the original will not be overwritten.*

Primary Options Screen

The Primary Options screen (see Figure 5.1) allows the operator to activate channels, enable the baseline verification function, set the sampling mode, set the default vial type (only when the 501 or RA Sampler is used), and define pump speeds.

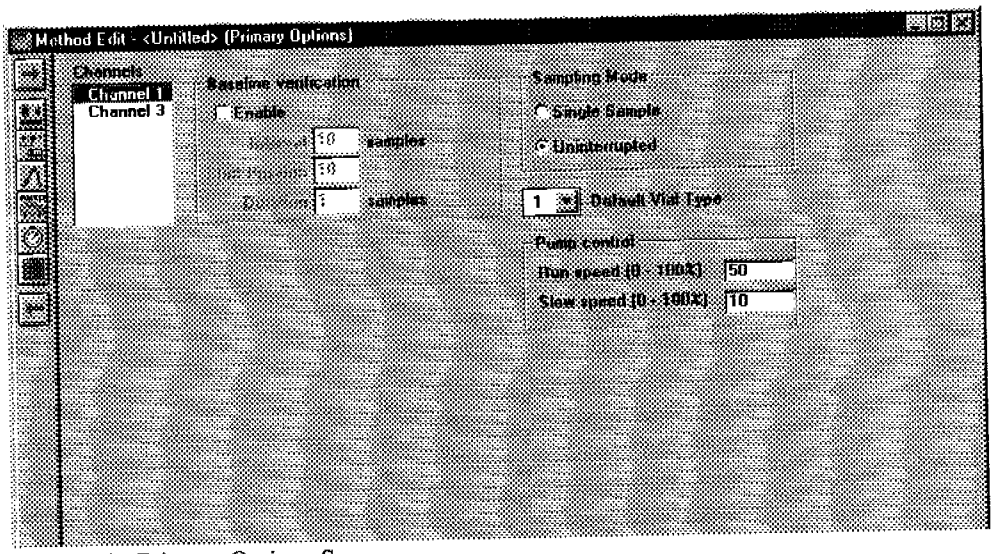


Figure 5.1. Primary Options Screen

Channels

The Channels listbox displays all of the available channels. The number of available channels depends on the number of installed detectors. A channel is presented as available following successful communication with a detector. To activate a channel, double-click on the channel name. A "#" sign will appear to the left of the activated channel name (see Figure 5.1). To deactivate a channel, double-click on the channel name again. Method parameters will only apply to active channels; thus, channels must be activated before moving on to other screens in the Method Editor.

Note: If a method is opened that designates an unavailable channel, an "X" will appear to the left of the channel name. To re-establish contact, go to the Main Menu, select Setup and Configure. If this is unsuccessful, restart WinFLOW V4.

Baseline Verification

Baseline Verification allows the operator to insert wash baseline (WBL) points at regular intervals. WBL points force the baseline through the point. When used with the RA and 501 Sampler in a segmented system, the WBL is taken from the wash reservoir instead of a sample cup. For an FIA system, the sample probe stays in the wash, but the valve does not inject. Thus, the baseline is determined using the carrier solution. To enable baseline verification, click on the **Enable** box then set the desired interval, duration, and start position. See Table 5.1 for further explanation.

Table 5.1. Baseline Verification Specifications

Specification	Explanation
Interval	Number of samples between baseline verifications. (2–50)
Start Position	Sample table location where first baseline verification point will occur. Check the Sequence Table Preview in the Data Collection screen in order to ensure that this placement is correct.
Duration	Number of baseline verifications to run in a row. (1–5)

When enabled, the baseline verification operates on all the activated channels. Baseline verification points, which are given the type **WBL**, are not entered into the Sample Table, but they do appear in the Sequence Table Preview.

Default Vial Type

Default Vial Type is used in conjunction with the 501 and RA samplers. There are four default types based on the volume or depth dimensions of the vials used. Use table 5.2 to determine the vial type. The Default Vial Type should be set to represent the majority of the sample cups in a given run. For cups that do not match the chosen default, a vial type can be selected for that cup in the sample table.

Table 5.2. Vial Types for the 501 and RA Sampler

Vial Type	Sample Cup Volumes for 501 Sampler	Sample Cup Depths for RA Sampler
1	0.5 mL	80 mm
2	2.0 mL and 4.0 mL	100 mm
3	12 x 85 cm	Input by operator
4	13 x 100 cm	Input by operator

Sampling Mode

Sampling Mode can be set to one of two sampling modes: **Single Sample** or **Uninterrupted**.

Uninterrupted

All samples in a sample table will be analyzed in a single, continuous run. This feature functions identically to previous WinFLOW versions.

Single Sample

The single sample mode is a new WinFLOW V4 feature that allows FIA instruments such as the Flow Solution/CNSolution 3000 Series units to operate without an autosampler. In Single Sample mode, each sample will be loaded and injected when the operator chooses. While Single Sample mode was not specifically designed for use in continuous flow analysis, this option is also active if a Flow Solution III or Flow Solution IV is used. In this case, the sampler will aspirate a single sample and wait for the operator to manually select when each successive sample will be aspirated.

1. Select **Single Sample** under the **Sampling Mode** in the Primary Options screen.

2. Proceed with data collection as described in Chapter 7.

3. At the point of data collection, the following screen will appear:

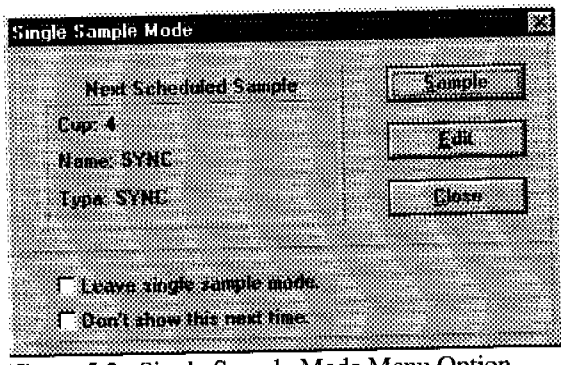


Figure 5.2. Single Sample Mode Menu Option

4. Place the probe in the sample/standard vial and click on **Sample** when the proper amount of sample/standard has been aspirated.

5. Figure 5.2 will be displayed at the completion of each sample cycle time until all the samples of the sample table have been completed.

6. The operator can also use a blank table each time a sample/standard is aspirated. In this case, the **Generate Blank Table** box is selected when data collection is initiated (Figure 5.3). When the sample is ready to be aspirated, click on **Edit** (Figure 5.2), and a blank table will be presented. Fill in the next sample/standard and close the window. A prompt to save the table will be displayed. Saving the table will automatically begin the analysis mode.

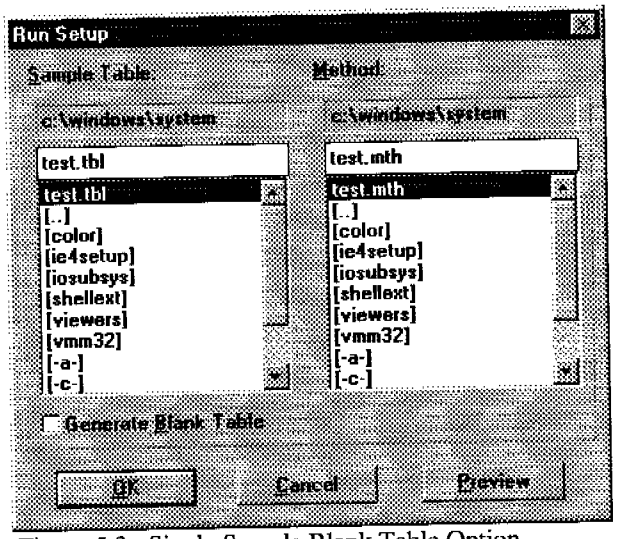


Figure 5.3. Single Sample Blank Table Option

Pump Control

Pump Control is used in conjunction with the Flow Solution 3500 Series, FSIII, or FSIV's Model 502 Peristaltic Pump or the FS3000 and CNSolution 3000 Peristaltic Pump to control pump speed during operation.

Automated Model 502 Control Options

During normal operation, the 502 pump operates at 50% speed in a counterclockwise direction. This default value, -50, is automatically set in the software if FSIV/III and Auto 502 are selected in the WinFLOW Configuration Setup screen (see Figure 5.4 and Chapter 3, "Configuration," for details). If the pump is used in slow mode, the default value is set to -10. The operator can edit the default values in increments of 1%.

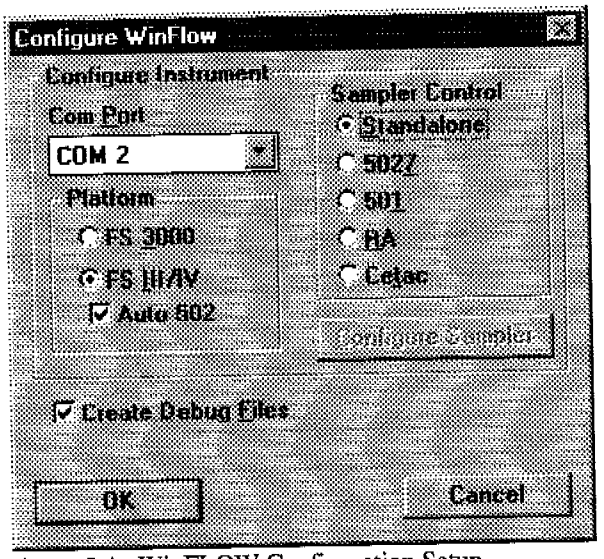


Figure 5.4. WinFLOW Configuration Setup Screen with Auto 502 Pump Setting

Automated FS3000/CNSolution 3000 Pump Control Options

During normal operation, the FS3000/CNSolution 3000 pump operates at 100% speed in a clockwise direction. This default valve, 100, is automatically set in the software if FS3000 is selected in the WinFLOW Configuration Setup screen (see

Figure 5.4). If the pump is used in slow mode, the default value is 50, which means the pump will turn at full speed 50% of the time and will be off 50% of the time. The cycle time of this calculation is 30 seconds; therefore, a 50% selection will operate the pump for 15 seconds and then stop the pump for 15 seconds. The operator can edit the default values to any integer between 0 and 100. A selection of 0 will stop the pump continuously.

Channel Options Screen

Channel Options (see Figure 5.5) are set for each detector channel individually. To update information for a particular channel, click on each channel line under the **Channels** box. The resultant information on the screen will pertain only to the highlighted channel. Only active channels are listed in the Channel Options screen.

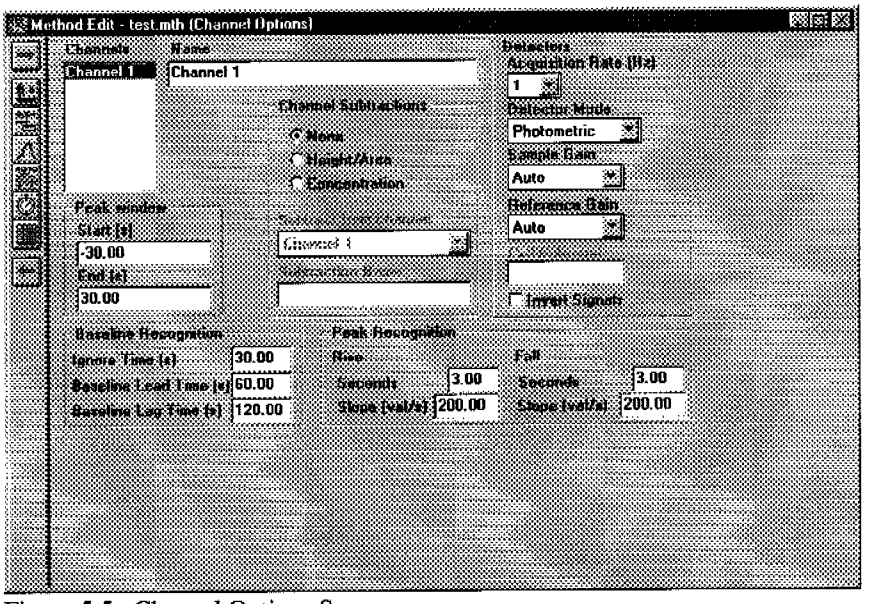


Figure 5.5. Channel Options Screen

Name

Name can be used to describe the chemistry being run. The default is the channel designation (e.g., Channel 1).

Acquisition Rate

The **Acquisition Rate** is determined in Hz or points/second. The default value of 1 Hz is usually sufficient. When running very low levels, an increase in acquisition rate may result in an increase in sensitivity. When operating multiple channels, an acquisition rate of 1 Hz is recommended so as not to overload the computer resurces with data.

Detector Mode

Detector Mode can be set for either a photometric or an amperometric detector. Select the **Photometric** mode for the Expanded Range (ER)™ digital photometer.

Select **Amperometric** mode for the amperometric detector and for the analog-to-digital (A/D) converter.

Sample Gain

Sample Gain should be set to **Auto** for all photometric detectors. For the photometric detector the gain is automatically set to the optimum based on the amount of light reaching the photodiode. The amperometric detector has a fixed gain of 1.

Reference Gain

Reference Gain should be set to **Auto** for all photometric detectors. For the photometric detector the gain is automatically set to the optimum for the amount of light reaching the photodiode. The amperometric detector has no reference gain.

Cell Potential

The **Cell Potential** in amperometric mode is set by the 10-turn precision potentiometer on the detector. **Cell Potential** is not set via software.

Invert Signals

Invert Signals is used when the chemistry being run is measuring a decrease in response instead of the usual increase. This should only be selected if the analytical methodology for the chemistry being run states that it is an inverse chemistry.

Peak Screen

Peak screen defines the peak area integration times (in seconds) and is specified by a **Start** and **End** time. Proper peak quantitation depends on the accurate designation of a window within the overall peak zone. The default values are -30s and +30s, resulting in a window 60 seconds wide.

WinFLOW V4 can quantitate using peak area or peak height. When using peak area for quantitation, both the start and end markers are used. To set the start and end markers it is necessary to know 0.00 is the middle of the peak. Therefore, the start marker will be a negative number and the end marker will be a positive number.

Channel Subtraction

Channel Subtraction is used to subtract peak concentration or peak height/area in one channel from peak concentration or peak height/area in another channel. The addition of subtracting peak height/area is a new feature in WinFLOW V4.

For example, nitrate+nitrite is typically analyzed on one channel, and nitrite is analyzed on another. This function can be used for automated determination of nitrate concentrations by subtraction of the nitrite concentrations from the nitrate+nitrite concentrations. Channel subtraction is only enabled on one of the two channels, because the peak concentrations in one channel are being subtracted

from peak concentrations in another channel. For example, (see Figure 5.5) peak concentrations in Channel 1 (Nitrite) are being subtracted from those in Channel 2 (nitrate+nitrite); therefore, channel subtraction is enabled on Channel 1 and not on Channel 2. Subtracted data (nitrate) is published with other data in the Peak Results Table.

Channel Subtractions consists of the two parameters described below.

Subtracted From Channel

In the nitrate/nitrite example cited above, the Subtract From Channel is the Nitrate+Nitrite channel (Channel 2).

Subtraction Name

Subtracted data must be given a name. For example, if nitrite peak concentrations are being subtracted from nitrate+nitrite peak concentrations, the subtracted data would be given the name "Nitrate."

Baseline Recognition

Baseline Recognition consists of the three parameters described below.

Ignore Time

Used to ensure that the program does not mistakenly recognize a spurious peak or noise spike in the baseline prior to the appearance of a SYNC peak. It should be set to approximately 30 seconds less than the actual dwell time of the sample from sampler to detector. During the Ignore Time no peaks will be marked whether or not they satisfy the Peak Recognition parameters. For this reason it is important that Ignore Time be set for less time (in seconds) than it takes for the SYNC peak to reach the detector (see "Synchronization Peaks" in Chapter 6). The default value is 30 seconds.

Baseline Lead Time

The time (in seconds), before the SYNC peak, that will be used as the initial baseline region. The baseline lead is determined after the SYNC peak has been marked by counting backward from the SYNC peak marker. It is important that the baseline lead time be longer than half the width of the SYNC peak so that WinFLOW V4 does not mark the baseline on the rising slope of the SYNC peak itself. A general rule is to set the baseline lead time to 15 seconds more than the peak width. This designation will be used as the first baseline point.

Baseline Lag Time

The time (in seconds) after the elution of the last peak used for final baseline determination. The default is 120 seconds. It should not require modification (i.e., the baseline lag is marked by counting 120 seconds from the last peak marked). The default value is 60 seconds.

Peak Recognition

Peak Recognition parameters allow the operator to control peak finding. The **Rise** and **Fall** are set by a requirement of x seconds with a vertical difference of y (response in micro absorbance units, µAU, or picoAmperes, pA).

The peak recognition parameters are used to find a peak based on shape. The software attempts to find all peaks except baseline types using the peak finding parameters. Once the SYNC is marked, the software forecasts location in the data trace to look for the next peak using the Cycle Duration Time. The actual peak is then located in the forecast region using the peak marking parameters. If no peak meeting the peak marking parameters is found, the peak is marked using less stringent parameters. Baseline and blank type peaks are marked by using only the Cycle Duration Time. (see "Timed Events" in this chapter).

Table 5.3. Peak Recognition Parameters

Parameter	Low Range Settings	Default Settings	High Range Settings
Rise Threshold (seconds)	2	3	5
Rise Slope (val/pt)	50	200	500
Fall Threshold (seconds)	2	3	5
Fall Slope (val/pt)	50	200	500

When running a noisy chemistry such as sulfate or a high range chemistry, it may be necessary to optimize peak recognition by increasing the number of points used in the rise and fall. When running low-level chemistries, it may be necessary to optimize peak recognition by decreasing the number of points used in the rise and fall and decreasing the slope values. The default values are usually appropriate for the majority of analysis conditions.

Calibration/Quantification Options Screen

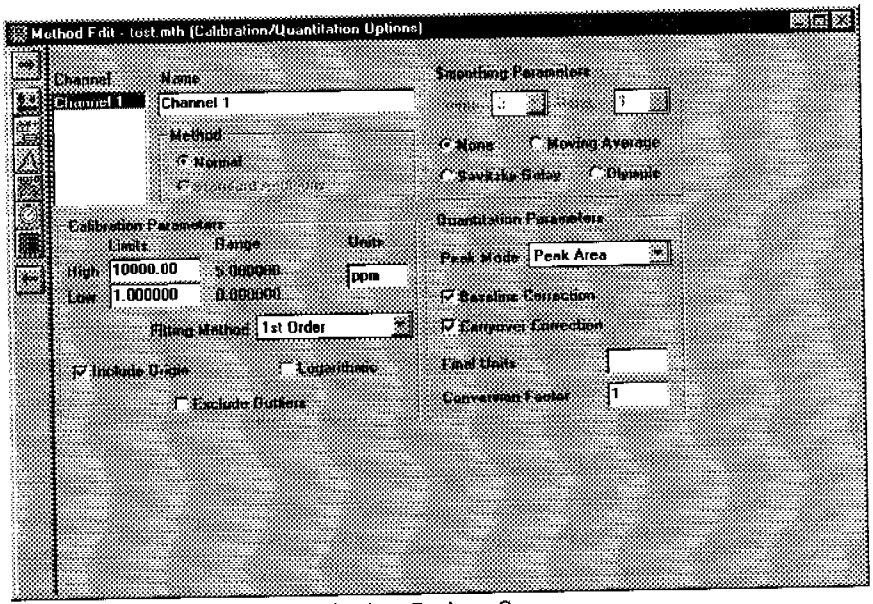


Figure 5.6. Calibration/Quantitation Options Screen

Calibration/Quantitation options (see Figure 5.6) are unique to each data collection channel. Therefore, the operator must first select the channel for which the options will be specified. The channel names can also be modified in this dialog box.

Method

Method is always set to **Normal**. The **Standard Additions** choice is not currently available.

Calibration Parameters

Calibration Parameters define the high and low limits of the calibration curve (the algorithm used to calculate the calibration curve and the analyte units).

Limits

Set by the operator to define the top and bottom range of the calibration curve. The recommended limit is 5% to 10% above the high calibrant and below the low calibrant. If a sample concentration exceeds either the high or low limit, a corresponding flag will be displayed in the results table for that sample. If **Autodilution** is enabled, these limits set the criteria for autodilution (see "Automatic Options" in this chapter).

Range

The displayed values are based on the high and low calibrants listed in the calibrants table (see "Calibrants Table" in this chapter).

Units

Set to the concentration units of the calibrants. The default is ppm.

Fitting Method

Permits the selection of either a first order (linear), second order, third order, or weighted linear calibration algorithm. The WinFLOW V4 default is first order. Second and third order curves are often useful when operating over an expanded range. The weighted linear fitting method applies an additional statistical analysis to the calibration data, enhancing low-level results when operating over an expanded range with a linear chemistry. The method gives greater statistical weight to low concentration measurements compared to its linear model.

Include Origin

Allows the origin to be set as an additional point when the regression is performed. This does not force the regression to zero; rather, it includes the point (0,0) in the calculations.

Logarithmic

Chosen if the calibration concentrations are in a logarithmic progression.

Exclude Outliers

Allows the operator to automatically remove replicate outlier data points that will not be used in the curve fit. The outlier's will be identified in the final report.

Quantitation Parameters

Quantitation Parameters include peak mode, baseline correction, carryover correction, final units, and a conversion factor. Several parameters govern how the raw data are treated.

Peak Mode

Peak mode can be set to **Peak Height** or **Peak Area**. Peak height is the most commonly used method for quantitation in flow analysis.

Baseline Correction

Baseline correction should always be selected but will not be implemented unless baseline points are inserted throughout the run. Baseline correction allows for adjustment due to baseline drift throughout the run.

Carryover Correction

Carryover correction should always be selected but will only function if the sample table contains a line with a replicate of two CO types in a row from the same cup. Carryover correction quantitates the interaction between samples. It is recommended that this be a blank following a mid-to-high-range calibrant. The SYNC peak often serves this function well.

Final Units

Final units is only required if a conversion factor is entered to change the units. For instance, final units can be used to convert from ppm to µM. The postconversion units should be listed here. All results will be reported in the converted units.

Conversion Factor

When a conversion factor is necessary for a particular analyte, the operator must specify a conversion factor as well as different units. The value and new units are to be entered in the **Conversion Factor** and **Final Units** areas, respectively. The conversion factor will be multiplied by the concentration for all values in the results table.

Smoothing Parameters

Smoothing Parameters are used to set several software-based signal processing algorithms to reduce noise, improve precision, and ease integration of difficult peaks (small size relative to the noise). These algorithms include Moving Average, Olympic, and Savitzky-Golay. Each of the real-time smoothing features can be processed by selecting the appropriate algorithm and value in the Method Editor. Alternatively, if **None** is selected, these features can be accessed postrun in the *.rst file.

Moving Average

A simple algorithm for filtering where the average of an input set is used to generate one output value. The tendency in this algorithm is to reduce peak height maxima due to the lower values on either side. Peak area measurements are largely unaffected. This filtering algorithm is new in WinFLOW V4.

Olympic

A variation of the Moving Average algorithm. In this model, the high and low values are thrown out, and the remaining values are averaged. This algorithm is very effective at screening out spikes (high frequency noise). Like the Moving Average algorithm, it still has a tendency to reduce peak height. Peak area measurements are largely unaffected. This filtering algorithm is new in WinFLOW V4.

Savitzky-Golay

Savitzky-Golay multiplies each point by a weighting factor and then averages the points in a data set. The factors, which are derived from second- and third-degree polynominals, weights the center of the data point set and progressively lessens the weighting to the points further out. Unlike the other algorithms, peak height maxima distortions are minimized. Peak area measurements are unaffected.

Points

Points defines the number of input data points that are processed to generate each output data point. It is equivalent to the filter size. This parameter allows the operator to optimize the filter for the peak width and noise frequency of the data set so that noise is properly filtered without adding peak distortion. The filter size is measured in points (data points). As a result, the data collection rate is a very important parameter in determining the optimum number of points for a given peak width.

Iterat.

An abbreviation for iterations. Iterations function by treating the output of one operation (filtering algorithm) as the input for the next operation. This process can be repeated as many times as necessary. Iterations allow the operator to increase the degree of filtering (smoothing) while keeping the filter width (points) optimized for the peak width.

Automatic Options Screens

The Automatic Options screen contains two main option groups: Autorecalibration Options and Autodilution Options.

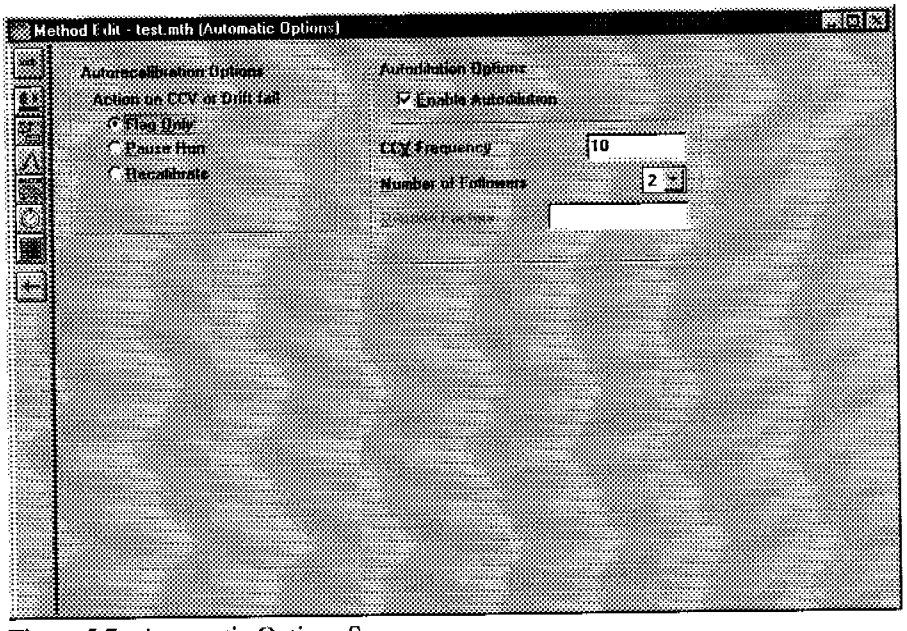


Figure 5.7. Automatic Options Screen

Autorecalibration Options

Autorecalibration Options

Autorecalibration options require a decision concerning system action following a failed Continuing Calibration Verification (CCV). For more information on CCVs see "Continuing Calibration Verification" in this chapter.

Flag Only This will place an "F" in the flag column of the Results Table.

Stop Run If a CCV fails it will be flagged and the run will be aborted.

Recalibrate If a CCV fails it will be flagged and the system will rerun the calibration section. Sampling will resume at the cup after the last successful CCV.

Autodilution Options

Autodilution options are only available when the RA or 501 sampler and the 511 or MicroSTEP autodilutor are in use. To enable autodilution, click in the **Enable Autodilution** box. Once enabled, the other options become available.

CCV Frequency Choose the frequency of the CCV to be run with the diluted samples (i.e., every 10 samples run a CCV). The CCV to be run is designated in the sample table after the dilution cups (refer to rows 26 through 28 in Figure 6.1 for an example).

Number of Followers Select the number of followers to be run. A follower is defined as the sample(s) after an overrange sample. The recommended protocol is two followers.

Dilution Factors Whereas the Model 511 Dilutor has hardware-fixed dilution factors, the MicroSTEP (MS) Autodilutor gives the operator the option to select a continuously variable set of dilution factors. Enter up to five dilution factors separated by commas. The MS Autodilutor will use the first entered dilution factor for any sample that goes over the highest calibration standard value. Subsequent operator-entered dilution factors will be used if the initial dilution is still too low. While the MS Autodilutor has 1:24,000 step resolution, dilution factors in excess of 1000 are not recommended.

Timed Events Screen

The Timed Events screen is a list of devices and their associated actions including operating states, timing intervals, and timing types. The timed events table sets the timing for the run to begin (Cycle Start), for the peak marking (Cycle Duration), and for the sampler motions to and from sample and wash. For the FS3000, pump and valve commands are also automated.

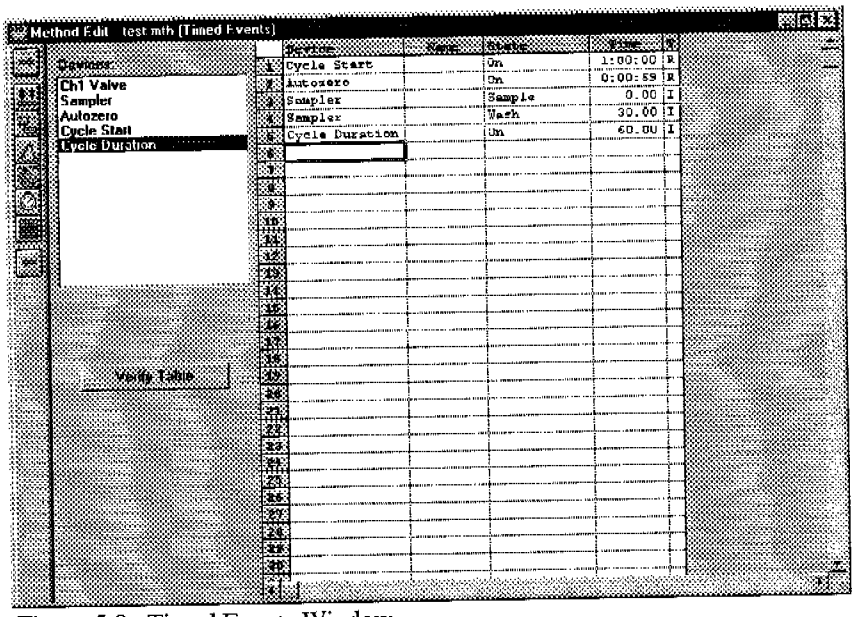


Figure 5.8. Timed Events Window

Devices include modules such as pumps, valves, and samplers, as well as software functions that require a timing schedule. To add a device, click on the next open cell in the Device column of the spreadsheet, and then click on the desired device in the Device menu on the left.

Cycle Start The time at which data collection begins. The detectors and display will be reset at this point, and the data collected will be saved but is generally used as a baseline monitoring period. The cycle start time is always run-based and can be set long to allow for baseline stabilization before the run. The cycle will begin immediately if the **Data Collect** button is selected during the display time.

Cycle Duration Determined by the chemistry, carryover, and desired throughput as specified in each methodology. It sets the timing for the analysis cycle and is always injection-based (I).

Autozero Time Autozero time should be set for one minute less than the cycle start time in order to autozero upon initiation of data collection. Always set as a run-based (R) event.

Valve For Flow Injection Analysis (FIA) only. The injection valve switches between load and inject throughout the run, thus it is an injection-based event. The valve should load at time 0 and inject when enough time has passed to allow the sample loop to fill completely. This time is entered by the operator. The valve will stay in the inject position from the time it was switched to the end of the cycle duration time. At the end of the inject cycle it will return to the load position to begin another cycle.

Pump Only the FS3000 pumps are controlled in the Timed Events Table. Generally, the pump is turned ON at time 0. Pump timing is run-based (R). To have the pump automatically turn OFF at the completion of an analysis, add a second pump line that turns the pump OFF and is shutdown-based (S).

Sampler Switches between sample cups and the wash reservoir throughout the run; thus, it is an injection-based (I) event. The sampler should be in SAMPLE at time 0 then switch to WASH. This timing is method dependent, refer to the methodology for recommended values. When a valve is used, the sampler and valve times must coincide.

Name

Name can be any alphanumeric value.

State

State defines the status of an instrument variable such as the injection valve. Examples of variable states are; On, Off, Load, Inject, Sampler, Wash, etc.

Time

Time column specifies a time for an event to occur. When the cell is in the Time column, the Time box to the left becomes an edit box, allowing only numeric time entries specific to the represented device. Below the Time box in bold type will appear the format for the time. Format choices are as follows:

HH:MM:SS ..... Hour:Minute:Seconds
SS ..... Seconds

The time must be entered in the format displayed above. If the format is HH:MM:SS, the colons must be included in the time. For the SS format only the number of seconds needs to be entered.

Type

When the cell is in the **Type** column, the **Timing Type** box changes into a list of available timing types. There are four available timing types. They are listed below.

Injection-Based (I) Timing is based on the Cycle Duration time. Injection-based timing is used for events that happen throughout a run. Timing is set in SS.

Run-Based (R) Items that occur one time during the run are timed using run-based timing. The timing is set in HH:MM:SS.

Clock Time (C) The computer's internal clock time governs the event. The timing is set in HH:MM:SS.

Shutdown (S) Occurs at the end of a run. Shutdown events begin after the baseline lag point has been marked. Timing is set in HH:MM:SS.

Verify Table

The Verify Table function will identify any "illegal" requests or errors in the Method Editor table. This function verifies that the entries are correct relative to the events table parameters, not that the settings are a valid sequence of events. Select the **Verify Table** button after completing the Timed Events table.

Calibrants Table Screen

The Calibrants Table is used to identify the Calibrants (C), Initial Calibration Verification (ICV), Continuing Calibration Verification (CCV), and Spikes (SPK) in conjunction with the Sample Table (see Chapter 6). Space is provided in the table to list the name, type (T), concentration, and percent acceptable deviation (%) for each standard. One column is presented for each active channel in the method as defined in the channels list in Primary Options.

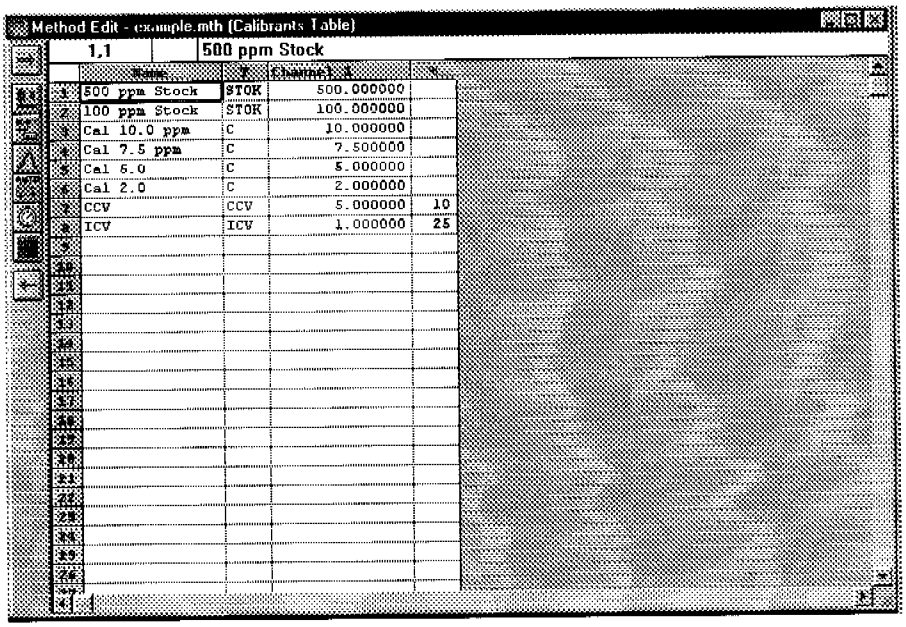


Figure 5.9. Calibrants Table Screen

Calibrants

Calibrants are standards of known concentration used in the creation of the calibration curve. The calibration curve is the mechanism for determining the concentration of all samples. Calibrants should be prepared to cover the expected concentration range of the unknown samples.

Calibrant names can be no longer than 15 characters. They must exactly match the calibrant name typed into the sample table as the software distinguishes between calibrants only by their name. If an undesignated calibrant is entered in the sample table, WinFLOW V4 will prompt the operator to fix the problem. Enter a name, type [C], and a concentration for each calibrant. Calibrants do not use the % column.

Initial Calibration Verification (ICV)

ICVs are outside standards used solely for checking the performance of the instrument and the validity of the prepared standards. If an ICV fails, the run is automatically aborted. Like calibrants, ICVs are recognized both by name and type. Provide a unique name, the type (ICV), the concentration, and the percent acceptable deviation (%) for each ICV. ICV names can be no longer than 15 characters. They must exactly match the name typed into the sample table, as the software distinguishes between ICVs only by their name. If an undesignated ICV is entered in the sample table, WinFLOW V4 will prompt the operator to fix the problem.

Continuing Calibration Verification (CCV)

The CCV is used to reconfirm that the calibration curve remains valid over the course of a run. Typically, one calibration standard is used as the CCV. The CCV determines if a recalibration is necessary. The action taken in response to a CCV failure is designated in the Automatic Options screen (see "Automatic Options" screen in this chapter). CCV names may be no longer than 15 characters. They must match the CCV name typed into the sample table, as the software distinguishes between CCVs only by their name. If an undesignated CCV is entered in the sample table, WinFLOW V4 will prompt the operator to fix the problem.

Spike (SPK)

A spiked sample is used to determine the effect of a sample's matrix upon the sample results. This function will calculate the % recovery of a known concentration addition made to a sample by comparing the spiked sample value to the sample immediately before it in the sample table. In the calibrants table, enter the known added analyte concentration (e.g., 1 mg/L). Spike names can be no longer than 15 characters. They must match the spike name typed into the sample table, as the software distinguishes between spikes only by their name. If an undesignated spike is entered in the sample table, WinFLOW V4 will prompt the operator to fix the problem.

On-line Calibrant Preparation from Stock Calibrants (STOK)

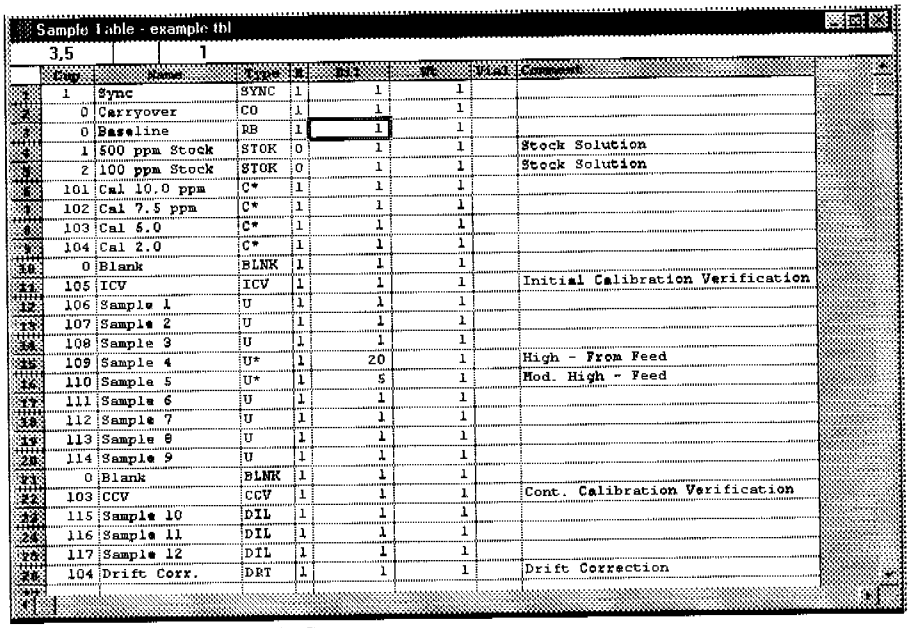
When used in conjunction with the ALPKEM MicroSTEP Dilutor, WinFLOW V4 has the added capability of allowing preparation of working calibration standards from pre-defined stock calibration solutions (STOK). Once the stocks are defined, the working calibration standards are added to the calibrant table. In order for the instrument to prepare the desired working calibration standards, the stocks and calibrants must also be listed in the sample table (like standards prepared by hand). Instrument-prepared working standards will be identified in the sample table with the C* notation, whereas the operator-prepared standards will be identified with the normal C notation.

Notes

Chapter 6
Sample Table

Sample Table Fields

The main purpose of the Sample Table is to map the position of the samples and standards in the sampler. It also defines sample types and sample calculation paramcters for each entry in the table. Every row in the sample table corresponds to a specific position on the sampler.



	Flag	Name	Type	#	Rep	Wt	Vial	Comment
1	1	Sync	SYNC	1	1	1		
2	0	Carryover	CO	1	1	1		
3	0	Baseline	BB	1	1	1		
4	1	500 ppm Stock	STOK	0	1	1		Stock Solution
5	2	100 ppm Stock	STOK	0	1	1		Stock Solution
6	101	Cal 10.0 ppm	C*	1	1	1		
7	102	Cal 7.5 ppm	C*	1	1	1		
8	103	Cal 5.0	C*	1	1	1		
9	104	Cal 2.0	C*	1	1	1		
10	0	Blank	BLNK	1	1	1		
11	105	ICV	ICV	1	1	1		Initial Calibration Verification
12	106	Sample 1	U	1	1	1		
13	107	Sample 2	U	1	1	1		
14	108	Sample 3	U	1	1	1		
15	109	Sample 4	U*	1	20	1		High - From Feed
16	110	Sample 5	U*	1	5	1		Mod. High - Feed
17	111	Sample 6	U	1	1	1		
18	112	Sample 7	U	1	1	1		
19	113	Sample 8	U	1	1	1		
20	114	Sample 9	U	1	1	1		
21	0	Blank	BLNK	1	1	1		
22	103	CCV	CCV	1	1	1		Cont. Calibration Verification
23	115	Sample 10	DIL	1	1	1		
24	116	Sample 11	DIL	1	1	1		
25	117	Sample 12	DIL	1	1	1		
26	104	Drift Corr.	DRT	1	1	1		Drift Correction

Figure 6.1. Sample Table Screen

The 5027 (120-place), 501 (300-place), and the RA (290-place) Samplers are all true random access samplers when used with WinFLOW V4. This means the sampler probe can be sent to any cup at any time throughout a run. Table 6.1 describes the available fields in the sample table.

Table 6.1. Sample Table Fields

Item Abbreviation	Item Description	Use
Cup	Cup Number	Corresponds to cups on sampler. Determines sequence for sampler movement.
Name	Sample Name	Operator input, 20-character limit. (For Calibrants, ICVs, CCVs, and Spikes, names must correspond exactly to those listed in the Calibrants Table of the Method Editor.)
Type	Sample Type	SYNC, STOK, C*, C, DRI, DRT, ICV, CCV, BLNK, RB, U*, U, CO, DIL, NULL, SPK
R	Replicate Count	Allows up to 5 replicates from one sample cup.
DIL	Dilution Factor	Multiplies final concentration by the factor input.
Wt	Weight Factor	Divides final concentration by the factor input.
Vial	Vial Type	501 and RA Samplers only. Insert vial type for a specific sample. Default is set in Method Editor.
Comment	Cup Comment	Extra information about the sample. Notes kept here do not carry over to the final report.

The procedure for filling in the table begins by clicking on an empty cell in the **Name** column and then typing in the sample name. Default values will automatically be filled in if no operator-supplied values are entered.

Cup Number

The **Cup Number** corresponds to the position numbers on the sampler. As stated above, all autosamplers supplied by OI Analytical (5027, 501, and RA) are true random access samplers when used with WinFLOW V4.

WinFLOW V4 recognizes cup 0 as the wash position. This number can be used with any type designation (BLNK, U, CCV, etc). Several wash and diluent source possibilities are available (see "Configuring the RA Sampler" in Chapter 3 for details). When using a 5027 Sampler, the home or wash position can be set as cup 121.

The RA sampler cup numbering scheme is different from that of the other samplers. The cup positions in the standards rack are numbered 1–10 or 1–20. The sample racks are defined by three digit numbers. The first digit indicates the rack number and the second two digits indicate the cup position within that rack. For example, when a 90-place tray is used, the first sample rack is numbered 101–190, the second sample rack 201–290, and the third sample rack 301–390. Custom racks follow this numbering system as well.

Sample Names

Sample names are limited to 20 characters. Names of calibrants (STOK, C), check calibrants (CCV, ICV, DRI, DRT), and spikes (SPK) must correspond to those given in the calibration table of the method to be used. When proceeding with analysis, if any of these names do not correspond, an on-screen warning will appear describing the faulty entries. This error must be corrected before proceeding with analysis of samples.

Sample Type

The sample type determines how a sample will be treated in connection with the rest of the samples in a run. WinFLOW V4 contains 15 sample types.

Table 6.2. Sample Types and their Functions

Code	Type	Function
BLNK	Blank	Allows for a blank, which is treated as an unknown sample.
STOK	Stock Calibrant	Used as the stock calibrant solution when using Automatic Standard Preparation.
C*	Calibrant (to be prepared)	Instructs software to prepare this calibrant. C* changes to C after calibrant is diluted.
C	Calibrant	Correlates response to concentration.
DRI	Sensitivity Drift Correction (Incremental)	Reslopes the most recent full calibration to account for any measured sensitivity drift. Value calculated in increments during run.
DRT	Sensitivity Drift Correction (Total)	Reslopes the most recent full calibration to account for any measured sensitivity drift. Value calculated for entire (total) run.
CCV	Continuing Calibration Verification	Verifies continued calibration validity throughout the run and determines if recalibration is necessary.
CO	Carryover	Corrects carryover due to highly concentrated samples.
DIL	Dilution	Placed at the end of the table to designate cup as a dilution cup. Only used when the 501 or RA sampler and 511 or MicroSTEP Autodilutor are enabled.
ICV	Independent Calibration Verification	Made with an outside standard to verify validity of initial calibration curve.
NULL	Null	Cup inserted by WinFLOW at the end of a run to allow for all calculations to be completed before the ending run.
RB	Read Baseline	Baseline correction allows adjustment due to baseline drift. Forces the baseline through the point.
SPK	Spike	Allows a sample to have a known concentration added to it.
SYNC	Synchronization sample	First sample in run, initiates sample cycle timing. Unknown sample.
U	Unknown sample	Dilutes the unknown by the specified in the Dil column prior to data collection.
U*	Unknown (to be prepared)	Baseline correction allows adjustment due to baseline drift. Forces the Baseline through the point. Baseline taken from wash when using FSIII and FSIV systems. Taken from the carrier solution when used on an FS3000. This item is not entered in the sample table. Its placement is determined on the channel options screen of the Method Editor.
WBL	Wash Baseline	

Replicate

The **Replicate** column defines the number of times a specific sample cup will be aspirated/injected. The Replicate column will accept replicate values between one and nine. Another line in the sample table is required to draw from the same cup more than nine times. Zero replicates can be specified to deliberately skip the sample on that line. This is useful when running U* samples when only the dilution is to be analyzed and not the original. Also, STOK cups always force the replicate value to zero.

A dilution of that sample will be performed if the dilution function is enabled and the concentration of a sample is outside the range specified in the Calibration/ Quantitation options of the Method Editor. Furthermore, if a sample has replicates, there will only be one dilution performed by the dilutor and then that number of replicates will be taken from the single dilution cup.

Dilution and Weight Factors

If samples are diluted manually before the run, the dilution and/or weight factors can be entered into the Sample Table for automatic calculation of the original sample concentration. If an autodilution occurs during the run, the dilution factor will be automatically entered into the Results and Analysis Table. If a sample is out of range and is manually diluted during a run, the operator must type in the dilution factor and enter it into the add-on screen of the Sample Table.

Vial Type

The **Vial Type** column is only used in conjunction with the 501 and RA Samplers. Table 6.3 shows the standard cup definitions used with the 501 and RA Samplers. The default vial type is set in the Primary Options screen of the Method Editor; thus, the vial types should only be listed separately for types other than the default.

Table 6.3. Vial Types

Vial Type	RA Sampler	501 Sampler
1	2.0 mL cup	0.5 mL
2	13 × 100 mm	2.0 mL and 4.0 mL
3	Input by Operator	12 × 85 mm
4	Input by Operator	13 × 100 mm

Comments

The **Comments** section is provided for the operator to record notes concerning the sample. This field will not appear in the Results and Analysis Table. If the comments information is desired as part of the results file, a printout of the Sample Table must be made.

Edit Options in the Sample Table

The Sample Table format is much like a standard spreadsheet. The desired cell must be active to input information. To activate, click on the cell so the cell is highlighted. Now the cell is active and ready to accept typed information.

Cells are designated as alpha cells, numeric cells, and or alphanumeric cells. If an entry is incorrect, an error message will appear pointing the operator to the wrong entry in the table.

The Sample Table menu bar contains three menus: File, Edit, and Window. Table 6.4 describes the available options in the **File** menu.

Table 6.4. File Menu Options

Menu Item	Function
New	Creates a new table. Closes existing table and prompts to save changes.
Open	Allows operator to open an existing saved table. Closes existing table and prompts operator to save changes.
From Analysis	Allows access to the sample table when an analysis is loaded or running.
Import	Allows operator to import a table from another program. Tables should be imported in text format.
Export	Allows operator to export a table to another Windows program. Table should be exported in text format.
Save	Saves changes to current file name. Overwrites original table.
Save As	Allows operator to rename a table and save. Prevents overwriting original table if altering an existing table.
Print	Prints the table.
Print Setup	Allows operator to make choices concerning the layout of the table for printing.

The **Edit** menu contains the standard editing functions for working with text. Many of these features will allow the operator to copy the Current Sample table to their normal spreadsheet or word processor if they have need to do so. The editing tools use the standard Windows clipboard in a similar fashion as other Windows programs. Table 6.5 describes the available options under the **Edit** menu.

Table 6.5. Sample Table Edit Menu

Menu Item	Function
Cut	Removes the highlighted text from the current location. Temporarily places text on clipboard for use elsewhere.
Copy	Copies highlighted text and places on clipboard for use elsewhere.
Paste	Places the clipboard contents into the desired location.
Clear	Removes/deletes the currently highlighted text or values without storing anything in the clipboard.
Insert Rows	Inserts a row above the highlighted row.
Delete Rows	Deletes the selected rows. The entire row is actually removed from the table.
Go To	Operator specifies desired column and row of a particular cell to be viewed.
Sort	Allows operator to sort by numeric or alpha order. Click on one cell in the desired field for sorting, then select sort.

Creating a Sample Table

Throughout this section, refer to Figure 6.1 for an example of a typical sample table.

The Synchronization Peak (SYNC)

The first entry in a Sample Table is the SYNC peak. The SYNC peak initiates the peak recognition process. The height of the SYNC peak must be above the minimum peak recognition parameters set for each channel in the method.

Carryover Correction (CO)

The carryover correction feature calculates the percent carryover. This correction is then applied to all samples in a run. The carryover group must contain two samples taken from the same cup. The carryover correction must be performed immediately following the SYNC so the correction is applied real-time to the rest of the run. The %CO value is displayed in the Calibrants Results Table screen.

Calibrants (C)

The calibration curve is created using a series of calibrants of known concentration. Calibrant concentrations should be chosen to bracket the expected concentrations of samples. When multiple aspirations of a single calibrant are taken, each point is used in the calculation of the curve; WinFLOW V4 does not average calibrant points. The curve order defines the minimum number of calibrants. First order curves require two calibrants, second order curves require three calibrants, and third order curves require four calibrants. Weighted linear curves require triplicate aspirations of each calibrant, minimum two.

Unknowns—To Be Diluted (U*)

Unknowns designated by U* will be diluted before the run starts. This feature is for samples that are known to be above the calibration range of the analysis. Enter the desired dilution factor in the Dil column. Place an empty sample cup in the sampler location defined by U*. The source unknown with the U designation must be placed in the sample table and at the cup location immediately prior to the U* location. Source unknowns must have a replicate value of 0 entered to insure that these unknowns will not be entered in the sample table, the source name will be reported in the Peak Table.

Stock Solutions (STOK)

Stock Solutions are used to define the concentrations of solutions being used for automatic standards preparation. Concentrations are specified and sample type identified in the Calibrant Table.

Calibrants —To Be Diluted (C*)

Calibrants designated by C* are the same as the Calibrants above; however, the C* identifies those calibrants that need to be prepared by the system before they are aspirated. Once the calibrant is prepared, the sample type changes to C.

Sensitivity Drift Correction—Incremental (DRI)

The Incremental Sensitivity Drift Correction can be used to adjust a calibration that has become invalid due to sensitivity drift. A standard is chosen from the calibration to act as a drift correction cup. The drift correction will be applied to all peaks following the first drift cup to the next drift cup. If the incremental drift is not within an operator-specified range, the same action will be taken as with a failed CCV. The DRI may also be used to perform a run without running a calibration, using the DRI to adjust a previously run calibration curve.

Sensitivity Drift Correction—Total (DRT)

The Total Sensitivity Drift Correction can be used to adjust a calibration that has become invalid due to sensitivity drift. A standard is chosen from the calibration to act as a drift correction cup. The drift correction will be applied to all peaks following the drift cup. If the total run drift is not within the operator-specified range, the same action will be taken as with a failed CCV. The DRT may also be used to perform a run without running a calibration, using the DRT to update a previously saved calibration curve.

Initial Calibration Verification (ICV)

ICVs are samples of known concentration that are used to verify the validity of the calibration curve. An ICV should be sampled immediately following the calibration curve. ICV samples should be made from a separate stock or from an outside source. If an ICV fails, the run is automatically aborted. If more than one consecutive ICV sample is drawn from the same vial, the run will not be aborted unless all the ICVs in the group fail. If two separate ICVs are run, the failure of either will result in an aborted run. ICVs should always be followed by a baseline marker.

Continuing Calibration Verification (CCV)

CCVs are samples of known concentration used to verify that the calibration remains valid throughout the sample run. It is recommended that the CCV samples be made from mid-range calibrants. CCVs are placed at regular intervals throughout the series of unknowns; every 10 samples is the recommended frequency. CCVs should always end with a baseline marker. The three responses to CCV failure as determined in the Automatic Options of the Method Editor are:

- Flag the CCV as failed (F) and continue on with the run,
- Abort the run, and
- Recalibrate and begin sampling from the last good CCV.

If more than one consecutive CCV sample is drawn from the same vial, the group will not fail unless all CCVs in the group fail.

Spiked Samples (SPK)

A spiked sample is used to determine the effect of a matrix condition that can alter a sample's results. This function will calculate the % recovery of a known concentration addition made to a sample. To use this feature, place the spiked sample immediately after the unspiked sample.

Blanks and Baseline Samples (BLNK, RB, WBL)

Blanks are samples drawn from the wash position defined in the configuration of the sampler. They are often used to minimize tailing of calibrants or unknowns into the CCV or ICV. They are typically placed throughout the sample run preceding CCVs and ICVs.

Baseline samples are also drawn from the wash position defined in the configuration of the sampler. Baseline samples force the baseline through that point and are used to correct for baseline drift. They are typically placed before a series of unknowns and before the calibration series. They are usually drawn in duplicate to allow for maximum flexibility in adjusting the baseline.

Adding Samples to a Current Run

Once a run has begun, it is possible to add more samples to the run. The add-on function is accessed when data collection has begun by opening the sample table, adding samples to the nongrayed-out area and closing the sample table. See "Adding Samples to a Current Run" in Chapter 7 for more information.

Note: It is a good idea to check the sample table preview function in the Data Collect screen (see "Modifying Sample Table During a Run" in Chapter 7) before beginning analysis. This will allow the actual sample sequence to be viewed, highlighting any errors in replications or placement of automatic entries (WBL).

Autodilution

The autodilution function requires use of the 501 sampler and the 511 autodilutor or the RA sampler and the MicroSTEP autodilutor. This feature allows over-range samples to be diluted and re-sampled in a single run. Any sample concentration that falls above the limits set in the Calibration/Quantitation Options screen of the Method Editor is marked high and will be diluted. Follow the instructions below to ensure proper use of the autodilution feature.

1. Prior to opening WinFLOW V4, ensure that the 501 or RA sampler and autodilutor options are selected in WinFLOW Configuration (see Chapter 3).

2. Go to the Automatic Options screen in the Method Editor and select the **Enable Autodilution** box.

3. Select the frequency of the **CCV Group**. It is best to run a CCV group every

10 samples. The CCV group to be run should be entered in the sample table at the end of the DIL cups.

4. Select the number of followers to be run. A follower is defined as the sample(s) after an over-range sample. The most common choice is two.

5. After the last sample in the sample table, enter several cups with the type DIL. No names are required.

6. At the end of the DIL cups, enter the CCV group. This is the CCV group that will be used throughout the dilution samples.

7. The 511 autodilutor will always begin with a dilution factor of 10. If that sample is still over range, it will be diluted by 100. The MicroSTEP autodilutor will use the dilution sequence specified in the Dilution Factors (in the Method Editor under Automatic Options and Autodilution Options).

8. All dilutions are performed at the end of the regular run.

Import and Export Functions

Exporting a Table

To export a sample table, go to the Sample Table **File** menu and select **Export**. A dialog box will appear requesting the name and location for the exported file. All tables export as text files (*.txt). The sample table can then be opened in any program that can read (*.txt) files.

Importing a Table

To import a table from an external program, go to the Sample Table **File** menu and select **Import**. A dialog box will appear requesting the name and location of the desired file. All tables must be in text file (*.txt) format to be imported. Importing is much easier if an actual WinFLOW V4 sample table is exported to the program of choice first. That table can then be used as a template for creating new tables for import.

Notes

Chapter 7
Data Collection

Initiating WinFLOW V4 instrument control and data collection is an easy four-step operation. After launching the program, click on the Data Collection screen. WinFLOW V4 will prompt the operator for input in these areas:

1. Enter Operator ID.
2. Define which Sample Table to use.
3. Define which Method to use.
4. Define a Results file name.

Initiating Data Collection

1. Click on the **Data Collect** button  . The Operator ID dialog box will appear.

2. Enter the Operator ID and click on **OK**. The Run Setup dialog box will appear.

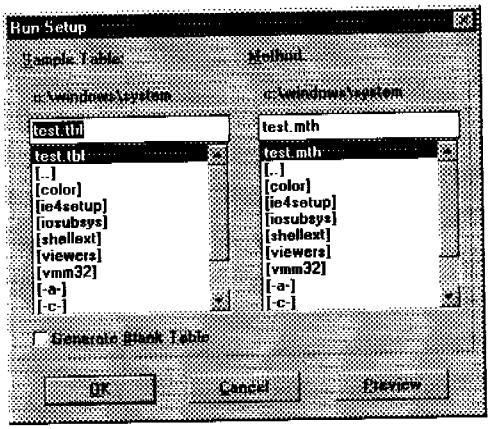


Figure 7.1. Run Setup Dialog Box

3. The program will request a file name under which to store the final results.

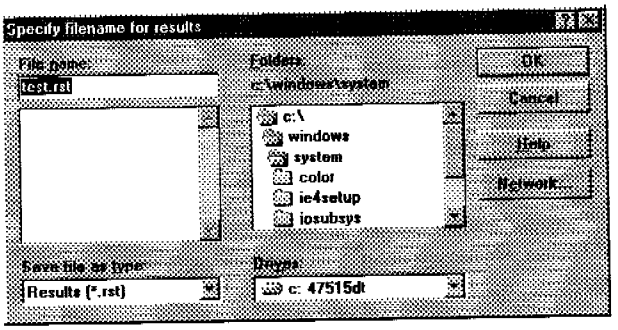


Figure 7.2. Results Filename Dialog Box

4. Enter a suitable Results file name and click on **OK**. The Data Collection screen will appear. If **Preview** was selected, the Run Sequence Table will appear as the main window. After viewing the Run Sequence Table, close the window, and the Data Collection screen will become accessible. The Data Collection screen will display the number of channels selected in the method chosen for this run.

   If a single channel is being used, the four aspects of the Data Collection screen will automatically be displayed (see Figure 7.3).

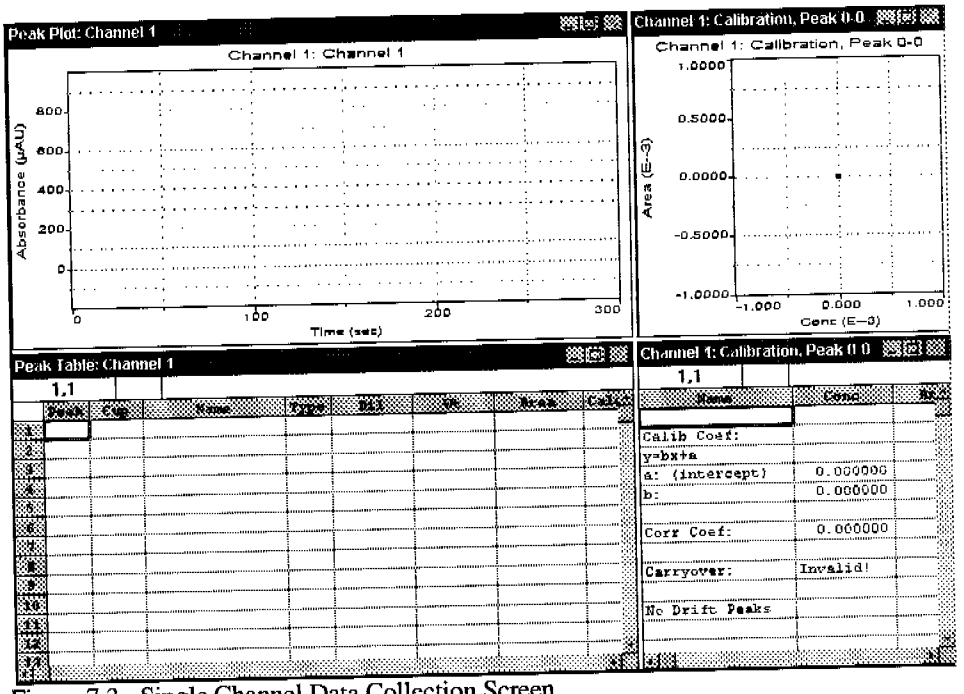


Figure 7.3. Single Channel Data Collection Screen

If more than one channel is being used, only the peak plot window of each channel will be displayed (see Figure 7.4). In order to access the Data Collection window for each individual channel, place the pointer on a peak plot window and double-click. The four aspects of that channel will be displayed. This action can also be accomplished by using the **View** menu and selecting the required channel.

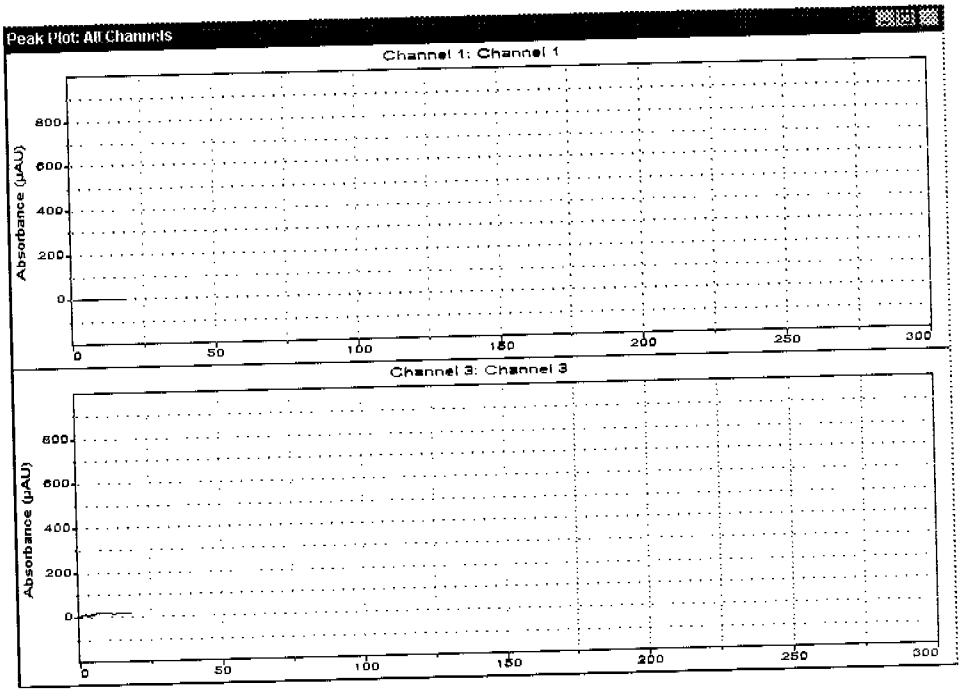


Figure 7.4. Two Channel Data Collection Screen

5. Go to the **Pumps** menu and select the appropriate pump(s) to turn on.

6. Initiate baseline data collection from the detector by clicking the **play** button. This will begin a baseline monitoring period. The length of this monitoring period is defined in the cycle start time set in the Timed Events Table of the Method Editor. This defined time period allows the operator to monitor the baseline and make sure the system is stable prior to beginning the run. The status bar in the bottom left corner of the screen will display "Start-Up" during this period.

Monitoring the Baseline

1. A real-time signal will now begin to display in the Peak Plot window. The Fast Forward, Stop, and Zero buttons will now become active on the Data Collection Button Ribbon. The Peak Results Table, Calibration Plot window, and Calibration Results Table will become active once the sample run is initiated.

Note: Percent full scale and gain values of the detector for the reference and sample can be obtained by going to the Window menu and selecting **Serial Communication**. This is a convenient way to determine if the lamp is functioning properly.

2. During the monitoring period the signal produced by a start-up solution will be displayed and should meet the criteria in Table 7.1.

Table 7.1. Start-up Solution Baseline Criteria

	Photometric Detector	Amperometric Detector
Vertical Noise	<100 µAU	<1000 pA
Drift	<200 µAU/300 seconds	<1000 pA /300 seconds

The software scaling occurs in real time and is dynamic. The scale is set by the highest point on the screen at any one time. Therefore, when monitoring the baseline, it is important to note the scale on the vertical axis when determining whether or not it is stable. Figure 7.5 shows an example of a quiet baseline for a photometric detector.

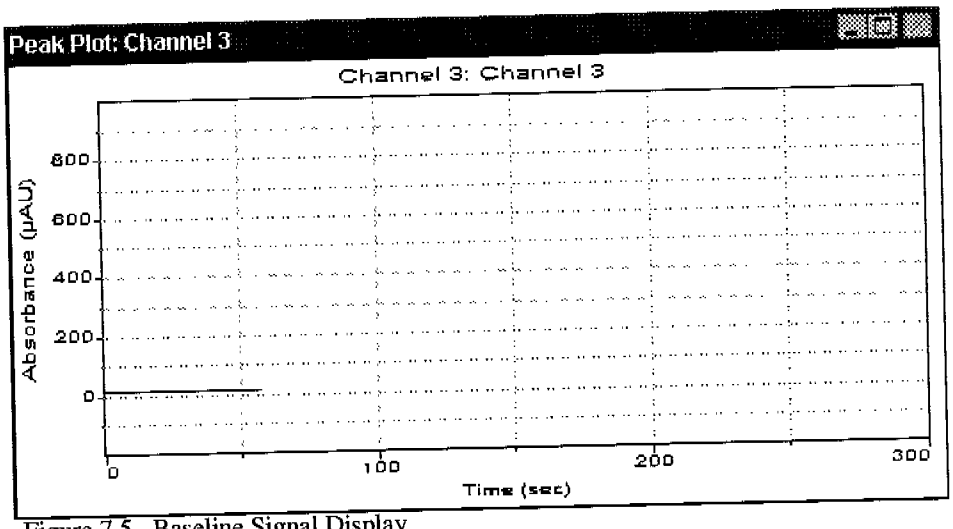


Figure 7.5. Baseline Signal Display

Establish a stable baseline on a start-up solution then switch to reagents.

If baseline collection is discontinued by selecting the **stop** button, the **rewind** button becomes available.Selecting the **rewind** button will clear the screen and reset WinFLOW V4 to collect baseline data.

3. Once a stable baseline has been established with reagents, and all appropriate standards, blanks, and samples are correctly placed in the sampler, the system is ready for the initiation the sampling sequence.

Starting a Sample Run

To initiate the sampling sequence, select the **fast forward** button from the select view bar or from the Data Collect menu. At this time, the sampler will proceed to the vials as defined in the sample table specified for this run. Because the operator has real time access to the data as it is being collected, all four screens of the data collection window are available during the run. Each of the screens is described below. There are many editing functions available in these screens during data collection. For a detailed description please refer to Chapter 8, Results and Analysis.

Peak Plot Screen

This is the main peak monitoring screen. As each peak is detected, its signal is

displayed throughout the run. Based upon the SYNC peak, each subsequent peak maximum (apex) is marked according to the parameters defined in the method and by the sample sequence defined in the sample table. A peak marker is placed at the apex of each peak according to the peak-finding parameters set by the operator in the method used for the run. This peak marker is used to measure the peak height from the established baseline. For peak area, a mark will be placed in front and behind the apex according to the parameters set by the operator in the Method Editor. The area of integration is shaded for easy recognition.

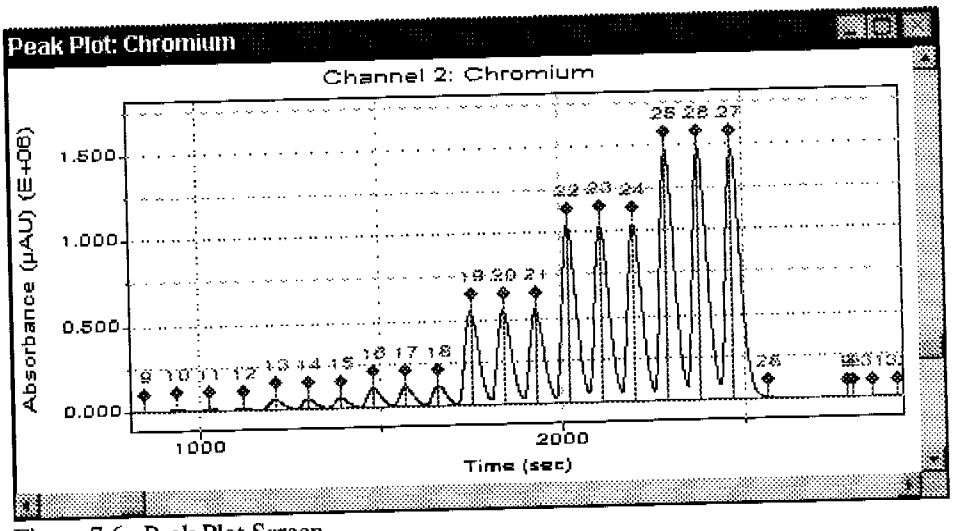


Figure 7.6. Peak Plot Screen

Calibration Plot Screen

This screen displays the calibration curve as produced by the standards during the run. If a calibration point is considered undesirable, it can be removed while data is being collected. This can be performed by double-clicking on any point with the Peak Editor cross or by changing the sample type of the standard from C to U in the Peak Table Results screen (see Figure 7.9). By making these changes, this data point is removed from the calculations of the standard curve. The point can be reinserted by reversing the steps above.

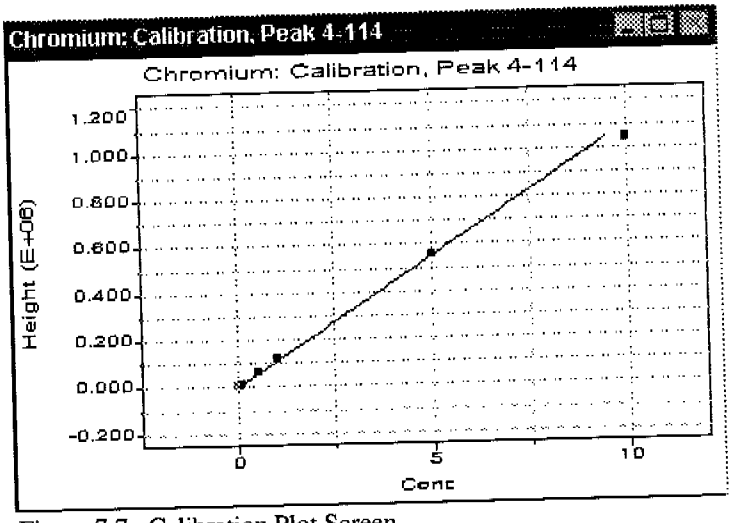


Figure 7.7. Calibration Plot Screen

Calibration Results Screen

This screen displays the calibrants as entered in the method, the corresponding peak heights or areas, and the details of the calibration curve such as the correlation coefficient, and the carryover percentage.

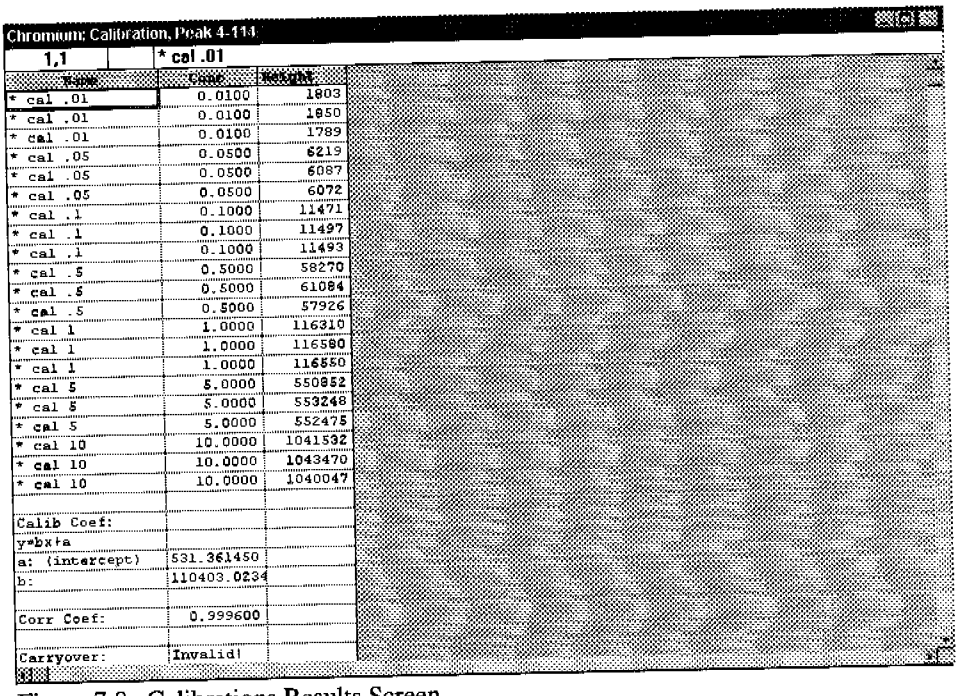


Figure 7.8. Calibrations Results Screen

Peak Table Results Screen

This screen displays information such as peak heights, calculated concentrations, informational flags, and any sample specific information previously entered into the sample table used for that run. Changing the **Type**, **Dil**, or **Wt** will produce a corresponding change in calculated results both while the data is being collected and during post-run analysis.

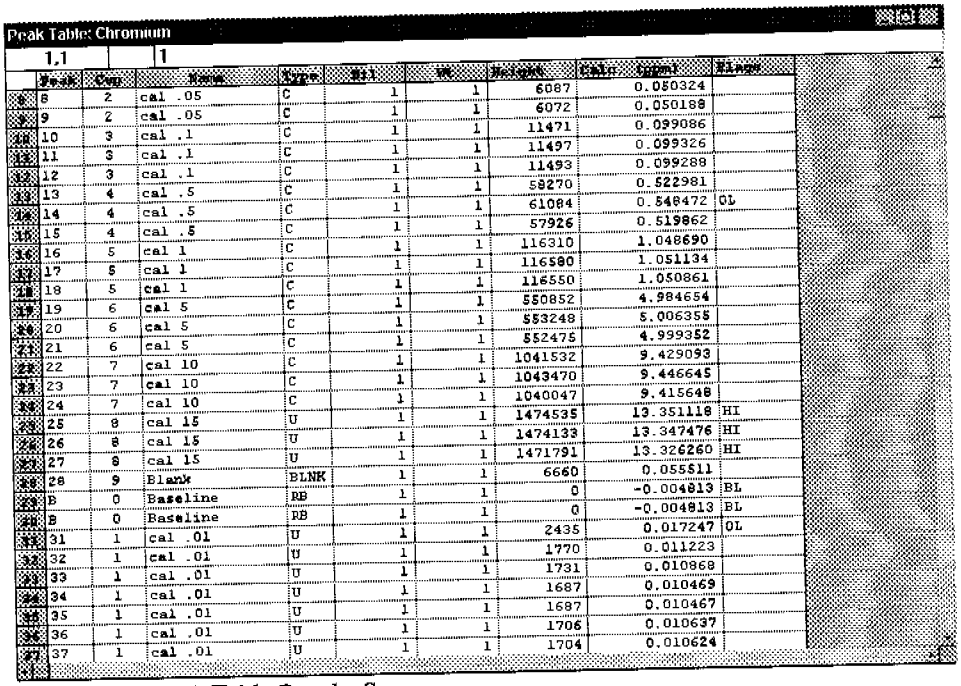


Figure 7.9. Peak Table Results Screen

Append Samples to a Sample Table

Adding samples to the end of a sample table that is already initiated has been made easier in WinFLOW V4. To add additional samples, click on the **Sample Table** button. The active sample table will be displayed with all current samples grayed-out indicationg that these samples cannot be edited (see Figure 7.10).

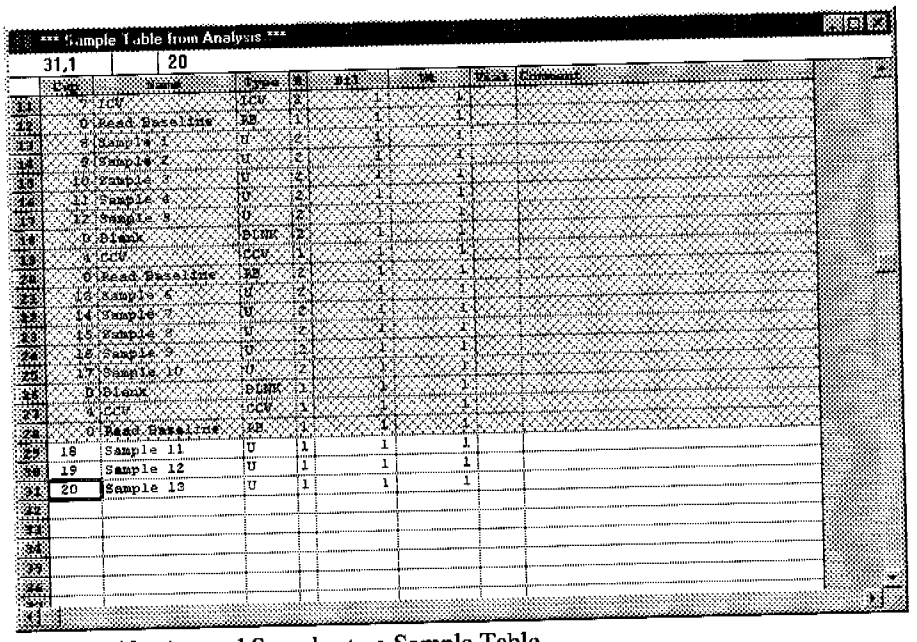


Figure 7.10. Append Samples to a Sample Table

The remainder of the table will be available for adding samples. Type in the cup number, name, type, number of replicates, etc. When completed, the sample table must be closed and saved. A dialog box will ask if you want to save the revised table. Click on **Yes**. The additional samples have been successfully added to the end of the sample table.

Modifying the Sample Table During a Run

Pause Button

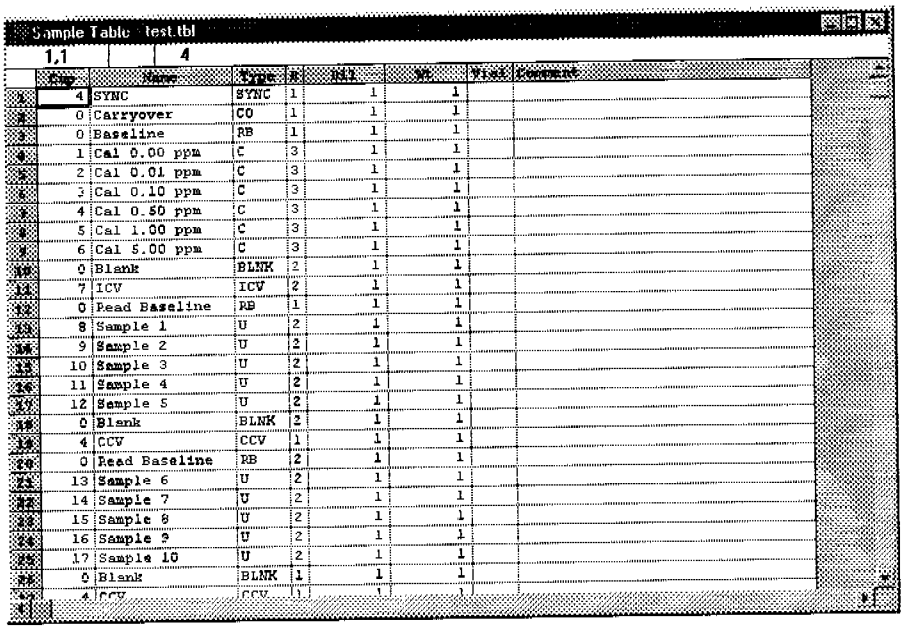
Once data collection has begun the run can be paused so that the current sample table can be edited in any way as required. To pause the run, click on the **pause** button.

At this point the following will occur:

1. Any single group of replicates that are currently being injected will be completely injected and marked.

2. The software will wait for the sampling pass to end, during which time baseline lag points (Null peaks) will be marked. Usually two or three Ns will be displayed during this lag period. At the end of the lag period the Sample Table will become available for editing. Those samples that have already been analyzed will be grayed out and are not available for editing.

3. At this point, select the **Sample Table** button and perform any editing as required. All normal parameters of the sample table are available.



	Amp	Name	Type	R	Inj	Wt	Flag	Comment
1	4	SYNC	SYNC	1	1	1		
2	0	Carryover	CO	1	1	1		
3	0	Baseline	RB	1	1	1		
4	1	Cal 0.00 ppm	C	3	1	1		
5	2	Cal 0.01 ppm	C	3	1	1		
6	3	Cal 0.10 ppm	C	3	1	1		
7	4	Cal 0.50 ppm	C	3	1	1		
8	5	Cal 1.00 ppm	C	3	1	1		
9	6	Cal 5.00 ppm	C	3	1	1		
10	0	Blank	BLNK	2	1	1		
11	7	ICV	ICV	2	1	1		
12	0	Read Baseline	RB	1	1	1		
13	8	Sample 1	U	2	1	1		
14	9	Sample 2	U	2	1	1		
15	10	Sample 3	U	2	1	1		
16	11	Sample 4	U	2	1	1		
17	12	Sample 5	U	2	1	1		
18	0	Blank	BLNK	2	1	1		
19	4	CCV	CCV	1	1	1		
20	0	Read Baseline	RB	2	1	1		
21	13	Sample 6	U	2	1	1		
22	14	Sample 7	U	2	1	1		
23	15	Sample 8	U	2	1	1		
24	16	Sample 9	U	2	1	1		
25	17	Sample 10	U	2	1	1		
26	0	Blank	BLNK	1	1	1		
27	4	CCV	CCV	1	1	1		

Figure 7.11. Sample Table Screen

4. After the editing process is complete, close the sample table. By selecting **Yes**, the changes made will be saved and incorporated into the run once it is restarted.

5. To restart the run, select the **play** button.

Stopping a Sample Run

Sample runs stop in several ways. The most common is for the run to come to completion on its own. Other software-controlled stops include the failure of an ICV or a second failure of a CCV standard. A run can also be stopped by the operator at any point by selecting the Stop button from the collecting window.

Operator-Stopped Run

If a run is stopped manually by selecting the **stop** button, WinFLOW V4 will verify that command.

If **Cancel** is selected, data collection will continue; if **OK** is selected, the data file can be handled under the following choices:

- If the operator does not wish to save any previously displayed data but wants to immediately reinitiate data collection by selecting the **rewind** button, the following message will be presented:

  If **Yes** is selected, all previously displayed data will be cleared and WinFLOW V4 will be reset to collect baseline data.

If **No** is selected, previously displayed data will remain until the operator takes further action.

- If the data is to be saved, go to the **File** menu and select **Save**. This will automatically save it under the file name previously given when data collection was initiated (see "Initiating Data Collection" in this chapter).

  Close the Data Collection screen and reinitiate the data collection process.

WinFLOW-Stopped Run

If the run is allowed to proceed to completion or is stopped due to a failed CCV or ICV, the data will automatically be saved under the operator-defined filename and will then proceed directly to the Results and Analysis screen.

In the case of a sudden loss of power, WinFLOW V4 will automatically preserve all data collected. When WinFLOW V4 is restarted, the data file will be displayed and the operator can choose to save it.

Notes

Chapter 8
Results and Analysis

This chapter discusses data processing following a completed run. Since the WinFLOW V4 software has on-line editing capabilities, most of the processes described in this chapter are also available during data collection (see Chapter 7).

A single data file will be used throughout this chapter as an example. By thoroughly exploring one data file we hope the operator will develop a better understanding of the data processing capabilities of WinFLOW V4 and ultimately enable the operator to process acquired data more quickly, efficiently, and accurately.

Loading A Data File

To load a saved data file, either select the **Data Analysis** button  from the button ribbon or from the Select View Bar, or, if already in Result and Analysis screen, go to the **File** menu and select **Open**. The Open Results dialog box will appear.

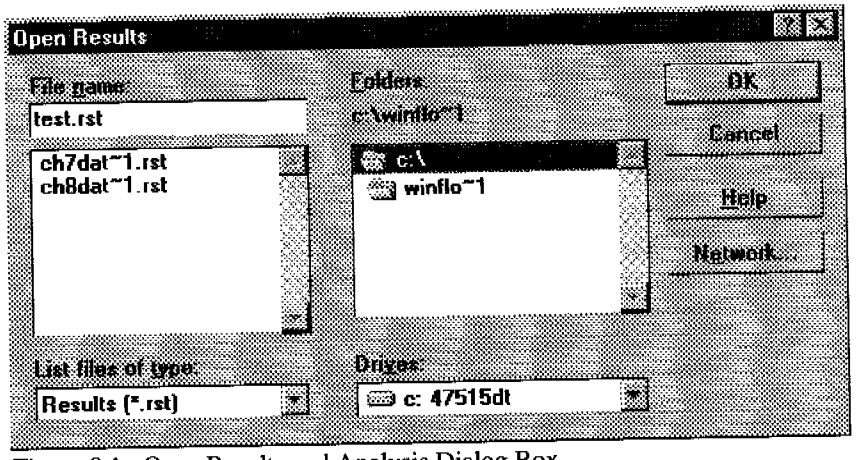


Figure 8.1. Open Results and Analysis Dialog Box

A file may be loaded from any active drive on the computer. During processing, a data file can be opened from any drive, processed, and saved to any location.

Data Processing Tools

Results and Analysis Screen

Each channel of the Results and Analysis section of the WinFLOW V4 software is split into four windows: Peak Plot, Calibration Plot, Peak Results Table, and Calibration Results Table.

Each of the four windows in the Results and Analysis screen are available for post-run processing. To access an individual window, click the **Maximize** button in the top right corner; the **Minimize** button will return the operator to the main windows.

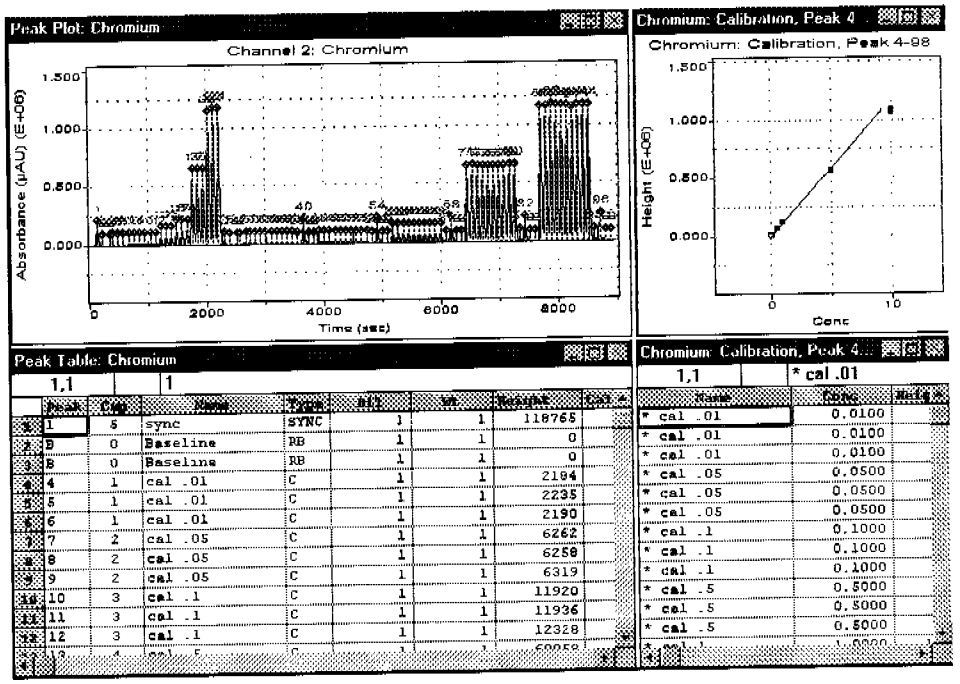


Figure 8.2. Results and Analysis Screen

Data Processing Tools and Menus

This section contains an overview of the editing tools available in the Results and Analysis screen. Functions will be described in detail throughout the rest of the chapter.

The Peak Editor Button

Selecting the **peak editor** button  toggles the mouse cursor between the standard pointer and the peak editor cross.

Grid On/Off Button

Selecting the **grid on/off** button  removes or restores the grid lines in the Peak Plot and the Calibration Plot. Removing the grid lines increases the printout speed.

Recalculate Button

Selecting the **recalculate** button  recalculates results according to changes made by the operator (such as moving a peak marker or moving a baseline point). The **recalculate** button becomes available after any data editing that could result in a change in the calculated values. Once selected, the edited data is applied to all four windows of the Results and Analysis screen. Results will also be recalculated automatically when exiting or entering another window.

Delete/Reprocess Peaks Button 

This button is used primarily to reanalyze peaks to guard against any peak mismarking that may have occurred. Most frequently, this will occur with the SYNC peak. For example, if the SYNC peak is marked in the wrong place, all

subsequent peaks will be marked incorrectly. In this case, delete the incorrectly placed SYNC peak mark, remark at the apex of the proper sync peak, and reanalyze.

When the **delete/reprocess peaks** button is selected, a new cursor appears.

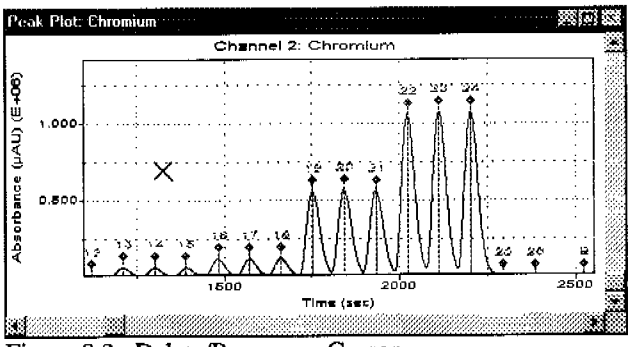


Figure 8.3. Delete/Reprocess Cursor

When the new cursor is placed over a peak and the left mouse button is released, the Delete All Peaks Warning Dialog Box will appear. By selecting **Yes**, the operator chooses to delete the selected peak and all peaks to the right of the selected peak.

Once the peak markers have been deleted, add a peak marker to the first available non-marked peak using the **peak editor cursor** button . This will now be the peak that determines how all of the peaks to the right will be reanalyzed. The factors for this reprocessing will be the information previously saved in the Method Editor, such as cycle time and peak finding parameters.

Once the first peak in the series of deleted peaks has been placed, select the **recalculate** button and the rest of the peaks will be marked. If these changes have been made after results have been collected, be sure to save any changes made.

Menu Options

The Results and Analysis pull-down menus contain the standard menu options as well as options specific to the Results and Analysis screen. (See Chapter 4 for a discussion of the standard menu options.) Many of the operations discussed throughout this chapter are duplicated in mouse and menu options.

File Menu

The File menu contains file-related options.

Open	Opens a new results file.
Save	Saves the current results file.
Save As	Saves the current results file under another file name.
Export	Exports Peak Table or Calibration Table to the operator-specified location as a text (.txt) file. Only available when the Peak Table or Calibration Table is maximized.

Update Method	Updates Method Editor file with the changes made post run (e.g., first order changed to second order curve fit).
Print Setup	Standard Windows print setup options.
Print	Prints current window. Must have one of the four windows maximized to print.
Exit	Exits the Results and Analysis screen.

Data Collect Menu

The Data Collect menu provides options for data collection.

Run Setup	Initiates the data collection process.
Single Sample Mode	This feature is only functional during data collection.

Analysis Menu

The Analysis menu contains options for post-run data analysis.

Smoothing	See Chapter 5 for a description of the different smoothing choices.
Recalculate	Updates results according to changes made by the operator.
Delete Peaks	See "Recalculate Button" in this chapter.
Include Origin	Includes the point (0,0) in the calibration curve.
Calculation Mode	Gives the operator the choice of measuring peaks by height or area. The current selection will be indicated with a check mark. See Figure 8.4 and "Recalculate Button" in this chapter.

Figure 8.4. Calculation Mode

Fitting Model	Applies first order, second order, third order, or weighted linear curve fit to results. See Chapter 5 for a discription of this function.
Logarithmic	Allows for selection of logarithmic curve fit.

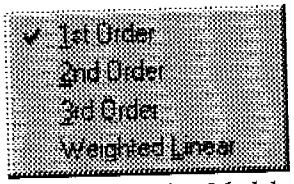


Figure 8.5. Fitting Model Choices Menu

Calibration Menu

The **Calibration** menu allows calibration curves to be imported and exported. The menu also displays a list of the calibration curves used in the run.

Load Calibrations	Allows a previously saved calibration curve to be imported into the current data file.
Save Calibrations	Saves the current calibration curve to a separate file so that it may be used in another results file.
Use Loaded	Applies the loaded calibration curve to the current data file.

View Menu

The bottom portion of this menu displays the number of channels that were used to collect the data of the current open file. The channels will be listed by the name (if any) given by the operator in the Channel Options screen of the Method Editor (see Chapter 5). By selecting one of these channels, the operator can move directly to another channel without having to close the windows each time.

Show Baseline	Allows the operator to view or hide the baseline on the Peak Plot.
Peak Labels on Printout	Displays the sample name and the sample concentration above each peak on the printout.
Format	Allows the operator to select the color schemes for the Peak Plot.
Analysis Format	Allows the operator to select the color schemes for the Calibration Plot.
Calibration Reports	Displays results information from all of the channels used to collect data in one table.
Preview	Displays the Run Sequence Table of the current file.
Scroll Lock	Allows the operator to freeze the baseline update used during data collection.
Grid	Displays or hides the grid lines in the Peak Plot and the Calibration Plot.

Peak Plot Screen

Magnifying the Peak Plot

To magnify any portion of the Peak Plot, place the cursor near the point to be magnified, hold down the right mouse button, and drag the box that will appear around the area to be enlarged. When the right mouse button is released, the highlighted area will fill the entire Peak Plot screen. Several levels of magnification can be produced by sequentially repeating this process.

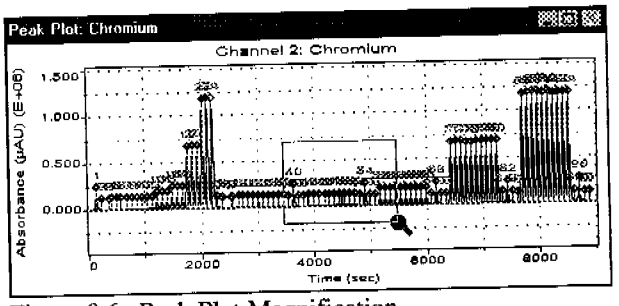


Figure 8.6. Peak Plot Magnification

A single click on the right mouse button will display a menu (see Figure 8.7) that will allow the zoom to be reversed step-by-step or by an immediate return to the original full scale plot size. The Peak Plots in Figures 8.6 and 8.7 clearly demonstrate the differences in scale that the magnification function produces.

Figure 8.7. Peak Plot Magnification

Using Cursor For Peak Identification

This ID/Information tool provides information about the Peak Plot or Calibration Plot points when the cursor is placed on a peak number and the left mouse button is pressed and held. The Peak Plot screen displays the peak number, type, height, cup number, and concentration of the selected point. The Calibration Plot screen displays the identification name, theoretical concentration, and height of the selected point (see Figure 8.14).

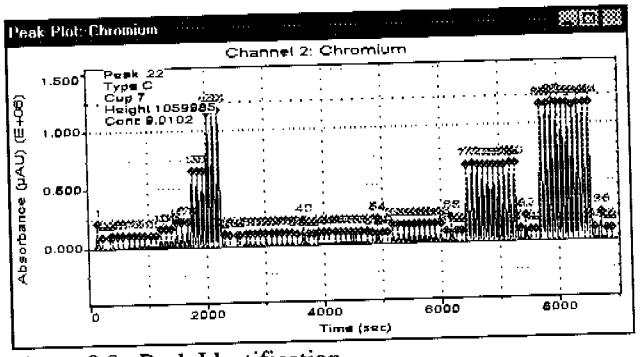


Figure 8.8. Peak Identification

Moving a Peak Marker

To move a peak marker, place the peak editor cross on the marker to be moved. Click and hold the left mouse button, drag the marker to the desired location, and release the left mouse button.

Changing a Peak State (Add, Delete, Toggle Baseline Point)

To change the peak state, place the peak editor cross on the desired peak and click the right mouse button. The peak state options will be presented (see Figure 8.9). To add or remove a peak, place the cursor at the baseline point, click the right mouse button, and select **Add Peak Mark** or **Delete Peak Mark**. To toggle a baseline point (B), right click on the peak with the peak editor cross and select "Toggle Baseline Point" in the menu that appears.

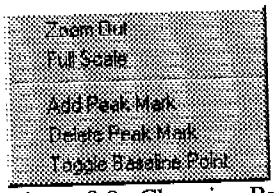


Figure 8.9. Changing Peak State Options

Calibration Plot Screen

Magnifying the Calibration Plot

To magnify any portion of the Calibration Plot, place the cursor near the point to be magnificd, hold down the right mouse button, and drag the box that will appear to enclosc the area to be enlarged (see Figure 8.10). When the right mouse key is released, the highlighted area will fill the entire Calibration Plot screen. Several levels of magnification can be produced by repeating this process. A single click on the right mouse button will return the display to the previous level of magnification. The Peak Plots in Figures 8.6 and 8.7 clearly demonstrate the differences in scale that the magnification function produces.

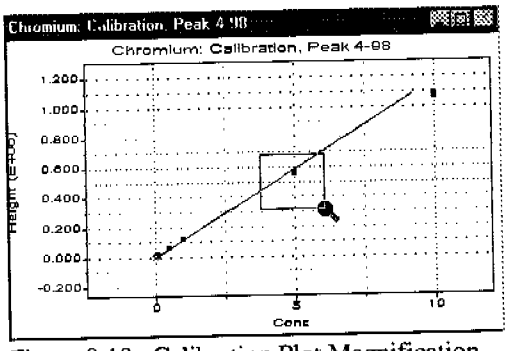


Figure 8.10. Calibration Plot Magnification

Using the Cursor for Calibration Point Identification

This ID/Information tool provides information about the Calibration Plot points when the cursor is placed on a peak number and the left mouse button held down. The information displayed is the identification name, theoretical concentration, and height of the selected point (see Figure 8.11).

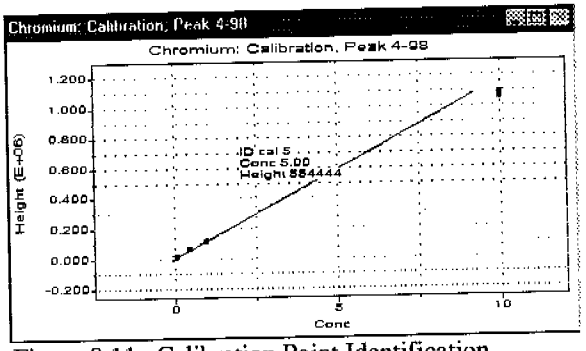


Figure 8.11. Calibration Point Identification

Removing Outlier Calibration Points

To remove an undesired point, place the peak editor cross on the point to be removed and double-click with the left mouse button. To return the point to the plot, repeat the process. The removed value will not be used in the calculations for the standard curve. Calibration points that have been removed are shaded gray.

Peak Results Table

Peak Table: Chromium

1,1 | 1

	Peak	Cup	Name	Type	Wt	Wt	Height	Calc (ppm)	Flags
1	1	5	sync	SYNC	1	1	118765	1.003561	
2	0	0	Baseline	RB	1	1	0	-0.006736	BL
3	0	0	Baseline	RB	1	1	0	-0.006736	BL
4	4	1	cal .01	C	1	1	2184	0.011845	
5	5	1	cal .01	C	1	1	2235	0.012273	
6	6	1	cal .01	C	1	1	2190	0.011894	
7	7	2	cal .05	C	1	1	6262	0.046529	
8	8	2	cal .05	C	1	1	6258	0.046497	
9	9	2	cal .05	C	1	1	6319	0.047015	
10	10	3	cal .1	C	1	1	11920	0.094663	
11	11	3	cal .1	C	1	1	11936	0.094802	
12	12	3	cal .1	C	1	1	12328	0.098135	OL
13	13	4	cal .5	C	1	1	60058	0.504160	
14	14	4	cal .5	C	1	1	59847	0.502360	
15	15	4	cal .5	C	1	1	59740	0.501449	
16	16	5	cal 1	C	1	1	118509	1.001376	
17	17	5	cal 1	C	1	1	118620	1.002322	
18	18	5	cal 1	C	1	1	118445	1.000835	
19	19	6	cal 5	C	1	1	554444	4.709733	
20	20	6	cal 5	C	1	1	563877	4.789979	
21	21	6	cal 5	C	1	1	560256	4.759169	
22	22	7	cal 10	C	1	1	1059985	9.010196	
23	23	7	cal 10	C	1	1	1080820	9.187434	
24	24	7	cal 10	C	1	1	1079185	9.173528	
25	25	0	Blank	BLNK	1	1	4034	0.027581	
26	26	0	Blank	BLNK	1	1	518	-0.002334	LO
27	0	0	Baseline	RB	1	1	0	-0.006736	BL
28	0	0	Baseline	RB	1	1	0	-0.006736	BL
29	29	1	cal .01	U	1	1	2064	0.010818	OL
30	30	1	cal .01	U	1	1	1890	0.009338	

Figure 8.12. Peak Results Table

Peak Results Table Fields

Peak Number Identifies the peak ID number or baseline designation.

Cup Number Displays the location of the sample source in the sample tray.

Name Displays the name of the sample.

Type Displays the sample type. (See Table 6.2.)

Dilution Displays the factor by which a sample has been diluted.

Weight Accounts for weight factors in soil and plant analysis.

Height Displays the peak height as measured from the baseline to the peak marker.

Calculated Concentration Displays the calculated value relating height to concentration based on the calibration curve.

Flags Provides details about a particular point in the run. For a list of defined flags and their functions, see Table 8.1.

Peak Results Table Flags

The Peak Results Table contains a column of Flags.

Table 8.1. Peak Table Flags

Flag	Name	Function
BL	Baseline	Used for all baseline markers. RB type and baseline verification.
HI	High	Marks peaks that are above specified limits.
LO	Low	Marks peaks that are below specified limits.
UM	User Modified	Marks all samples that have had peak markers moved post run.
F	Failed	Marks all CCVs and ICVs that do not meet percent deviation requirements.
I	Invalid	Marks all peaks before a failed CCV section. More peaks will appear later in report with the same name.
PE	Preempted	Shows when CCV fails on one channel but not the other. Second channel peaks will be marked as preempted.
FL	Follower	Used to specify that peaks will be run again after an over scale sample which gets diluted.
D	Diluted	Marks overlimit peaks to show that they were diluted later in the run.
XD	No DIL Cup	Shows when more samples need dilution than cups provided for dilution. System will dilute as many samples as there are cups and flag others.

Calibration Results Table

The Calibration Results Table provides the user with important information regarding the efficiency of the calibration process. Important information such as the calibration coefficient (e.g., slope, y-intercept) and correlation coefficient. Carryover calculations are also displayed in these tables.

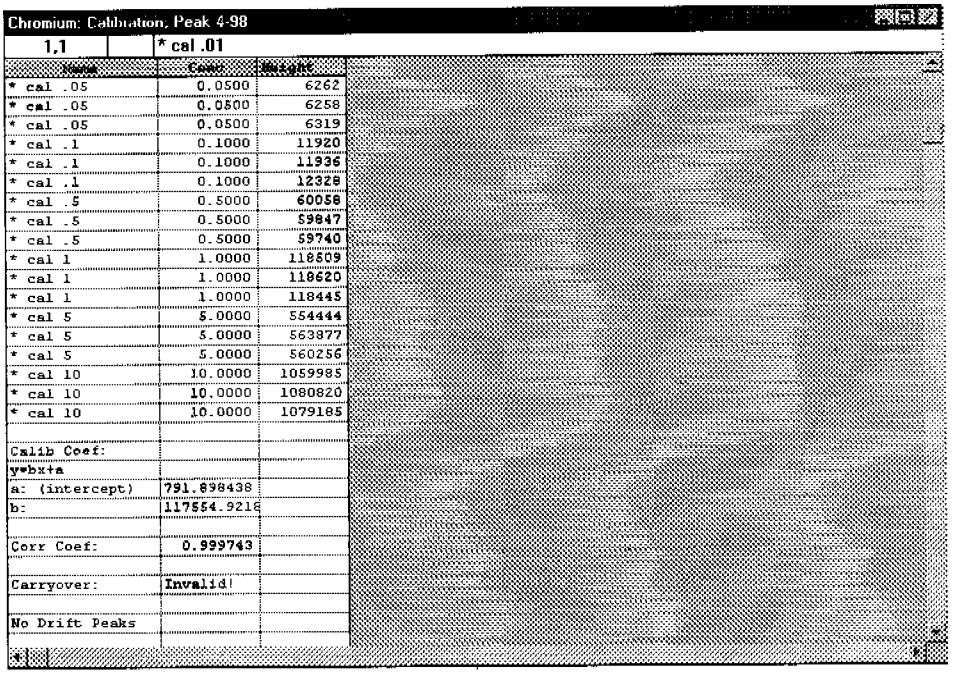


Figure 8.13. Calibration Results Table

Calibrant ID

The first section of the calibration table lists the calibrant names, theoretical concentrations, and peak heights. The * to the left of the calibrant name indicates that it is being used in the calibration curve (i.e., the data point has not been removed by the operator). The concentration values are editable. Therefore, if the cups were mispoured or if the concentration value was labeled incorrectly before the run, it can be corrected post-run.

Calibration Coefficients

The second section lists the calibration coefficients. The number of coefficients depends on the order of the curve. First order has two, second order has three, and third order has four.

Correlation Coefficient

The correlation coefficient is a mathematical indication of the strength of the correlation between two data sets (in this case, peak height or peak area and calibrant concentrations). A perfect correlation would result in a correlation coefficient of 1.00. A correlation coefficient greater than 0.995 represents a correlation strong enough to consider a calibration curve valid according to USEPA standards.

Standard Error

The standard error is an average of the residuals over the entire curve.

Carryover

The %CO is the number used to correct for the interaction between peaks. If the carryover correction was not implemented correctly, or not used at all, the %CO will read "n/a".

Final Report

WinFLOW V4 data can be viewed in the Final Report.

Within the Peak Results Table, go to the **View** menu and select **Reports**. The WinFLOW V4 Final Report will be displayed. This report (see Figure 8.14) differs from the Peak Results Table in that it contains the time stamp for each analysis as well as mean and RSD calculations for any sample or standard run in triplicate or greater.

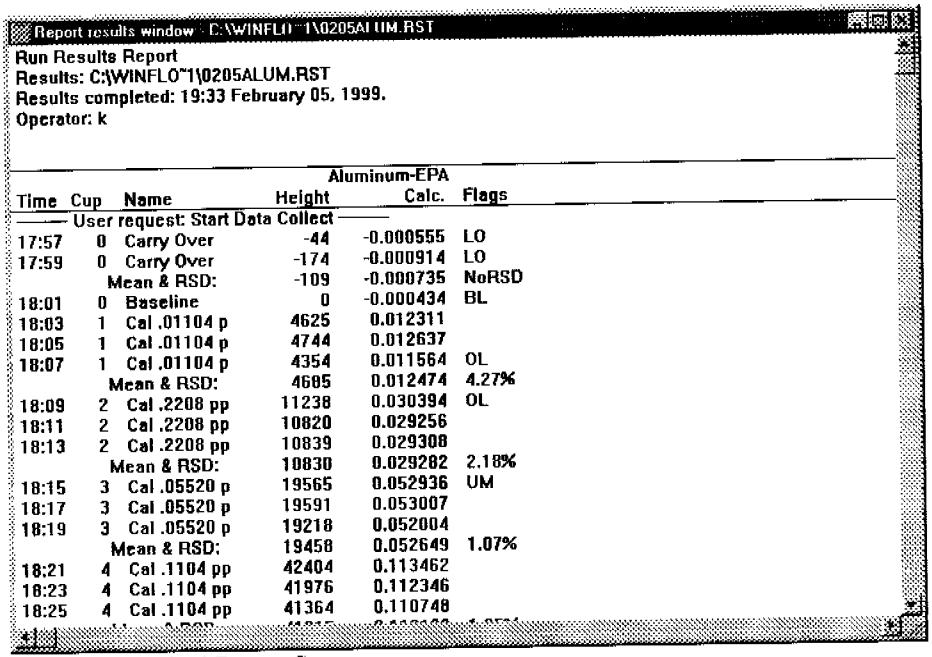


Figure 8.14. Final Report Screen

Notes

Appendix
Error List

Instrument Errors

The following is a list of the errors that the Flow Solution/CN Solution can generate along with a short description of each. These errors are reported to the operator in a WinFLOW V4 message box that identifies "instrument error encountered:" and then lists the exact text received from the instrument. See the notes below this table for more information on what the cause could be.

Code	Error	Description
01	Syntax error	Unrecognized command, or parameters invalid.
02	Out of memory	Most likely, the sample table is too large.
03	Invalid mode	Command sent is not valid in this mode.
04	Label not found	The label # specified in a GTL command does not exist.
05	Invalid parameter	Tried to execute invalid parameter in stored sequence. Tried to set illegal baud rate in a PBR or DBR command
41	Detector timeout	Detector did not respond within a certain time. Resetting the instrument may be required.
42	Detector timeout or overflow	Sent command before last command finished. Resetting the instrument may be required.
43	Invalid detector data	Detector sent incorrect data. Resetting the instrument may be required.
44	Bad detector command	Received "?" from detector. Resetting the instrument may be required.
45	Serial channel Rx buffer full	Resetting of the instrument may be required. Possibly an Octal UART board problem.
46	Serial channel Tx buffer full	Resetting of the instrument may be required. Possibly an Octal UART board problem.
60	Autosampler fault	No response from autosampler; reset.
71	PC RHR overrun	A character was not removed from UART channel in time
72	PC framing error	UART communication error.
73	PC parity error	UART communication error.
74	PC Rx buffer full	Analyzer mode set to invalid value.
75	PC Tx buffer full	Characters are not being sent to PC fast enough.
81	EXCEPTION	Stack frame error.
82	EXCEPTION	Invalid command code generated.
83	EXCEPTION	Analyzer mode set to invalid value.
84	EXCEPTION	Impossible code passed to GET-PARAM-RANGE.
C1	EXCEPTION	Data received from inactive detector (AVG=0).
C2	EXCEPTION	Value received from detector in an invalid mode (not A or P).
C3	EXCEPTION	DUART input ready flag generated, but no delimiter found. PC input ready flag generated, but no delimiter found.
F1	EXCEPTION	

Additional information about the above errors:

Errors	Descriptions
01, 03–06	These errors are likely (but not necessarily) software programming errors. Contact OI Analytical technical support at (800)336-1911.
02	The sample table may be too long or there may be too many timed events.
41–60	Most likely a hardware problem such as a defective Octal UART board or bad detector. If one channel in particular generates a disproportionate number of errors compared to the other channels, suspect the detector on that channel.
71–75	Most likely a hardware problem on the TDS-2020 board such as a chip going bad.
81–F1	Most likely a hardware problem on the TDS-2020 board such as a memory chip going bad. If the problem cannot be identified, contact OI Analytical at (800)336-1911.
C1–C3	In addition to the above, could also indicate a problem with either the Octal UART board or a detector board.

Even the most robust electronic device will occasionally malfunction due to environmental problems such as a static charge, current or voltage spikes, brown-out, moisture, or even a physical shock (such as a hit or drop) to the system. In these cases, the error that is generated may have little to do with what caused the problem in the first place and may be impossible to trace. This type of error generally occurs infrequently and is not repeatable. If an error occurs consistently or repeatedly, it usually indicates a hardware problem.

WinFLOW V4 Errors

There are a number of possible software-based errors that could be generated while running WinFLOW V4. They range from fatal (e.g., General Protection Fault) to inconvenient (e.g., problem initializing autosampler) to helpful (e.g., must have three data points for second order curves). There is no simple error list as there is for the instrument because of the scope of the software. The best thing to do is to keep a log of reported errors and what was done to fix them.

General Protection Fault (GPF): All Windows software will occasionally experience a GPF. Many of these are actually caused by the Windows operating system itself. However, if a certain area of the software or the performance of certain activities causes GPFs consistently, this usually indicates a problem with the WinFLOW V4 software.

Assert: WinFLOW V4 was programmed with what are known as "asserts." These are put in the software where certain conditions must be true (e.g., the analysis window has to exist in order to draw the curve trace). These asserts assist in debugging the software to a point where the assert condition to be false. Asserts are considered to be very similar to GPFs; however, they usually offer more information about what went wrong and where in the program the problem occurred.

Reporting the Errors

The most important thing about tracking down an error is its reproducibility. If the problem cannot be reproduced, chances are that the factory will not be able to find it. A good tool for finding errors is to use a standard questionnaire, including questions such as in the following example.

Error Reporting Form

Use another sheet if necessary.

Explain the error in as much detail as possible.
List the exact text of the error message.
What was the software doing when the error occurred?
What did you expect to happen instead of the error?
Can you cause it to happen again? If yes, explain how.
How many times has this exact error occurred?
What revision of the software was being used? (This is noted under the Help/About menu item.)
What firmware revision was being used? (This is noted under the Help/About menu item.)
How many channels were being used?
List computer type (486DX, Pentium, etc.) and processor speed.
How many MB of RAM?
Operating system (e.g., Windows 95, Windows 98)
Comments
In order for OI Analytical to track the error reported, please submit copies of the following files: • the sample table (*.tbl) the run is based on • the method (*.mtd) the run is based on • the result file (*.rst) that was generated or being used • WinFLOW.ini file • all files with this type of name: debugXX.txt

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O·I·Analytical

Methodology

Post-Distillation Cyanide by Segmented Flow Analysis (SFA)

(Cartridge Part #A002692)

1.0 Scope and Application

1.1 This method is used for the determination of cyanide in distilled samples that includes water, wastewater, soil, and sludge.

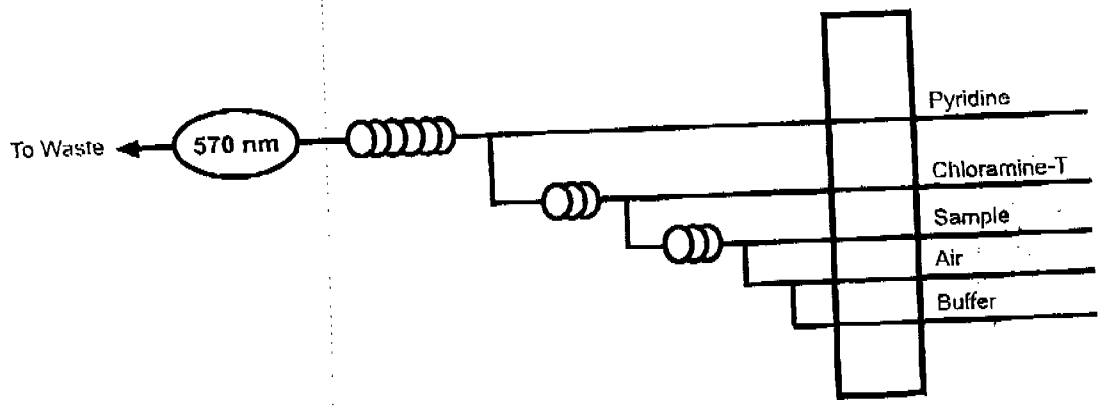
1.2 The Method Detection Limit (MDL) for this method is 2.6 µg/L. The applicable range of this method is 5.0–500 µg/L. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

2.1 Cyanide is released from cyanide complexes by an off-line manual distillation and collected in a sodium hydroxide receiver solution. Sodium cyanide is converted to cyanogen chloride by reaction with chloramine-T at a pH less than 8. The cyanogen chloride then reacts with the pyridine-barbituric acid reagent to form a red colored complex. The complex is measured at 570 nm (References 15.2 and 15.5). For manual distillation procedures, see Reference 15.2.

2.2 The quality of the analysis is assured through reproducible calibration and testing of the SFA system.

2.3 A general flow diagram of the SFA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

4.0 Contaminations and Interferences

4.1 Several interferences are encountered with this method. Some of the known interferences are aldehydes, nitrate/nitrite, and oxidizing agents such as chlorine, thiocyanate, thiosulfate and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. Some interferences are eliminated or reduced by the distillation.

4.2 Sulfides adversely affect the procedure by producing hydrogen sulfide during distillation. If a drop of the sample on lead acetate test paper indicates the presence of sulfide, treat 25 mL more of the stabilized sample (pH ≥ 12) than that required for the cyanide determination with lead carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of lead and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

4.3 High results may be obtained for samples that contain nitrate and/or nitrite. During the distillation nitrate and nitrite will form nitrous acid that will react with some organic compounds to form oximes. These oximes will decompose under test conditions to generate HCN. The interference of nitrate and nitrite is eliminated by pretreatment with sulfamic acid.

4.4 Oxidizing agents, such as chlorine, decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch paper (KI-starch paper) at the time of collection; a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper; then add an additional 0.06 g of ascorbic acid for each liter of sample volume. Sodium arsenite has also been employed to remove oxidizing agents.

4.5 Other compatible procedures for the removal or suppression of interferences may be employed provided they do not adversely affect the overall performance of the method.

4.6 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Barbituric Acid ($C_4H_4N_2O_3$)

5.3.2 Chloramine-T ($CH_3C_6H_4SO_2NNaCl{\cdot}3H_2O$)

5.3.3 Hydrochloric Acid, concentrated (HCl)

5.3.4 Potassium Cyanide (KCN)

5.3.5 Potassium Hydroxide(KOH)

5.3.6 Pyridine (C_5H_5N)

5.3.7 Sodium Hydroxide (NaOH)

5.3.8 Sodium Phosphate, monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$)

Warning: The cyanide ion, hydrocyanic acid, all cyanide salts, and most metal-cyanide complexes are extremely dangerous (Reference 15.4). As a contact poison, cyanide need not be ingested to produce toxicity. Also, cyanide solutions produce fatally toxic hydrogen cyanide gas when acidified. For these reasons, it is mandatory that work with cyanide be carried out in a well-ventilated hood by properly trained personnel wearing adequate protective equipment.

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

6.1 Segmented Flow Analysis (SFA) System (OI Analytical Flow Solution IV) consisting of the following:

6.1.1 Model 502 Multichannel Peristaltic Pump

6.1.2 Random Access (RA) Autosampler

6.1.3 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 570-nm optical filter

6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software

6.1.5 Post-Distillation Cyanide Cartridge (OI Analytical Part #A002692)

6.2 Sampling equipment — Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Barbituric Acid ($C_4H_4N_2O_3$)

7.1.2 Chloramine-T ($CH_3C_6H_4SO_2NNaCl{\cdot}3H_2O$)

7.1.3 Hydrochloric Acid, concentrated (HCl)

7.1.4 Potassium Cyanide (KCN)

7.1.5 Potassium Hydroxide (KOH)

7.1.6 Pyridine (C_5H_5N)

7.1.7 Sodium Hydroxide (NaOH)

7.1.8 Sodium Phosphate, monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$)

7.1.9 Brij®-35, 30% w/v (OI Analytical Part #A21-0110-33)

7.2 Reagent Preparation

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparation of degassed reagent water, protect it from re-absorption of atmospheric gases by storing it in a tightly sealed container. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Start-up Solution (1 L)—Add 4 mL of Brij-35 to 2 L of deionized water and mix gently.

7.2.3 Sodium Hydroxide, 10 N (250 mL)

7.2.3.1 While continuously stirring, cautiously add 100 g sodium hydroxide to approximately 175 mL deionized water in a 250-mL volumetric flask.

7.2.3.2 When the solution is cool, dilute to 250 mL with deionized water and mix well.

7.2.3.3 Store tightly capped in a plastic container. Storage is limited to one month.

Caution: The dissolution of sodium hydroxide in water releases a great amount of heat.

7.2.4 Phosphate Buffer (1 L)

7.2.4.1 Dissolve 138 g of sodium phosphate monobasic monohydrate in approximately 800 mL of deionized water in a 1,000-mL volumetric flask.

7.2.4.2 Dilute the solution to 1,000 mL with deionized water.

7.2.4.3 Add 4 mL of Brij-35 to this solution and mix gently.

7.2.4.4 Store at 4°C.

7.2.5 Chloramine-T (500 mL)

7.2.5.1 Dissolve 2 g of chloramine-T in approximately 400 mL of deionized water in a 500-mL volumetric flask.

7.2.5.2 Dilute the solution to 500 mL with deionized water and mix well.

7.2.6 Pyridine-Barbituric Acid Reagent (500 mL)

Warning: This reagent must be prepared in a hood. Barbituric acid is an acute irritant and is toxic if inhaled or digested (Reference 15.4). Pyridine has a Threshold Limit Value (TLV) of 5 ppm or 15 mg/M^3 (Reference 15.4).

7.2.6.1 In a ventilation hood, place 7.5 g of barbituric acid in a 500-mL beaker.

7.2.6.2 Add 50 mL of deionized water, rinsing down the sides of the beaker.

7.2.6.3 Place the beaker on a magnetic stirrer and insert a magnetic stirring bar.

7.2.6.4 While stirring the solution, add 37.5 mL of pyridine and then 7.5 mL of hydrochloric acid.

7.2.6.5 Add 300 mL more of deionized water, cover the beaker, and continue stirring until the barbituric acid is completely dissolved.

7.2.6.6 Transfer the solution to a 500-mL volumetric flask, dilute to the mark with deionized water and mix well.

7.2.6.7 Filter the reagent through a 0.45-µ filter.

7.2.6.8 Prepare this reagent weekly.

7.3 Calibrant Preparation

7.3.1 Stock Calibrant, 100 mg/L CN (1,000 mL)

7.3.1.1 Dissolve 2 g of potassium hydroxide in approximately 800 mL of deionized water in a 1-L volumetric flask.

7.3.1.2 Add 0.2505 g of potassium cyanide, and stir until dissolved.

Warning: Potassium cyanide is a poison (Reference 15.4). It is toxic if inhaled or ingested and is also absorbed through the skin. Contact with acidic solutions will cause evolution of deadly hydrogen cyanide gas.

7.3.1.3 Dilute the solution to 1,000 mL with deionized water and mix well.

7.3.2 Intermediate Calibrant, 10mg/L CN (100 mL)

7.3.2.1 Use a volumetric pipet to add 10 mL of stock calibrant to approximately 80 mL of deionized water in a 100-mL volumetric flask.

7.3.2.2 Dilute the solution to 100 mL with deionized water and mix well.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Working calibrants may be prepared to cover the desired range by adding the appropriate amounts of stock (or intermediate) standard to 100 mL volumetric flasks that contain approximately 80 mL of 0.25 N NaOH.

7.3.3.2 Dilute the solutions to 100 mL with 0.25 N NaOH.

EQUATION I

$$C_1V_1 = C_2V_2$$

Where:
C_1 = *Desired concentration (in mg/L) of working calibrant to be prepared*
V_1 = *Final volume (in L) of working calibrant to be prepared*
C_2 = *Concentration (in mg/L) of stock solution (or calibrant)*
V_2 = *Volume (in L) of stock solution (or calibrant) to be used*

By solving this equation for the volume of stock solution to be used (V_2), the following equation is obtained:

$$V_2 = \frac{C_1V_1}{C_2}$$

Since the desired concentration (C_1), the final volume (V_1), and the concentration of the stock solution (C_2) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_2) is easily calculated.

7.3.3.3 Alternately, standard curves in desired ranges can be derived from the tables below:

Final Concentration (µg/L)	Vol. of Stock Cal. (µL)	Conc. of Stock Cal (mg/L)	Final Volume (mL)
50	50	100	1,000
100	100	100	1,000
200	200	100	1,000
300	300	100	1,000
400	400	100	1,000
500	500	100	1,000

Final Concentration (µg/L)	Vol. of Stock Cal. (µL)	Conc. of Stock Cal (mg/L)	Final Volume (mL)
5.0	50	10	100
10.0	100	10	100

8.0 Sample Collection, Handling, and Preservation

8.1 Add approximately 2 mL of 10 N sodium hydroxide per liter of sample to obtain a pH greater than 12 and cool to 4°C.

8.2 If residual chlorine is present, add 1.2 g of ascorbic acid to each liter of sample.

8.3 The maximum holding time for samples without any sulfide present is 14 days.

Note: Samples may be tested for sulfide with lead acetate paper before adding sodium hydroxide.

8.4 With sulfide present the holding time is 24 hours. If sulfide is present it can be removed as follows:

8.4.1 Add lead carbonate powder.

8.4.2 After the addition of lead carbonate, filter the sample immediately.

8.4.3 Add 2 mL of 10 N NaOH per liter of sample.

8.4.5 The samples may be held for 14 days following removal of the sulfide (Reference 15.3). A more complete description of sample handling can be found in Reference 15.3.

9.0 Quality Control

Note: The following QC procedures are provided for reference purposes only and are not a substitute for any QC procedures that may be required for regulatory compliance.

9.1 It is recommended that each laboratory that uses this method operate a formal quality control program. The minimum requirements of such a program should consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance should be compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.2 Method Detection Limit (MDL) — To establish the ability to detect cyanide at low levels, the analyst should determine the MDL using the apparatus, reagents, and calibrants that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 should be achieved prior to practice of this method.

9.2.1 An MDL is calculated by analyzing a matrix spike at a concentration of two to three times the expected detection limit of the analyzer. Seven consecutive replicate analyses of this matrix spike should be analyzed, and the MDL should be calculated using Equation 2:

EQUATION 2

$$MDL = (t) \times (S)$$

Where:
t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = Standard deviation of the replicate analyses

9.2.2 It is recommended that the MDL be calculated after every six months of operation, when a new operator begins work, or whenever there is any significant change in the instrument response.

9.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix).

9.3.1 Matrix Spike/Matrix Spike Duplicate (MS/MSD) — The laboratory should spike, in duplicate, a minimum of 10% of all samples (one sample in duplicate in each batch of ten samples) from a given sampling site.

9.3.2 The concentration of the spike in the sample shall be determined as follows:

9.3.2.1 If, as in compliance monitoring, the concentration of cyanide in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit.

9.3.2.2 If the concentration of cyanide in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.4 Analyses of Laboratory Reagent Blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis.

9.5 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained.

9.5.1 An LCS should be analyzed with every sample batch, and the mean (m) and the standard deviation (S) should be recorded. After multiple analyses, the mean should be plotted with limits of $m+2S$ and $m-2S$. The mean and the limits should be recalculated after every 5–10 new measurements.

9.5.2 If the LCS measurement falls outside the range calculated in Section 9.5, then the problem should be addressed, and that sample batch should be reanalyzed if necessary.

9.6 Reference Sample — To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institutes of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-Up

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical Flow Solution IV Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Cyanide Post-Distillation Cartridge (Part #A002692) is configured as illustrated in the flow diagram in Section 17.0.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their respective reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.2). Start the pump at 40% speed, allowing the start-up solution to flow through the entire system.

10.2.2 Make sure that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging.

10.2.3 Once a stable flow is achieved, connect the reagent pump tubes to their respective reagent bottles. Allow the reagents to flow through the entire system, then, once again, verify that the flowcell of each detector is purged of all bubbles.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.6) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected cyanide concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.6).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Verify that the baseline does not drift and is free from large fluctuations. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the cyanide intermediate calibrant (Section 7.3.2) according to Equation 1, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of decreasing concentration and analyze each calibrant according to Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.5). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Analysis

11.1.1 Place all reagents on-line. Pump 5–10 minutes. Obtain a stable baseline at 570 nm.

11.1.3 Load the sampler tray with calibrants, blanks, samples, and QC samples. The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.1.4 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the "Fast Forward" button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.

11.1.5 When analysis is complete, pump start-up solution through the system and stop the pump. Release the tension on all pump tubes, and power off the system.

11.2 Operating Notes

11.2.1 Operate the system under a ventilation hood or in a well ventilated area.

Warning: Cyanogen chloride is a very toxic gas (Reference 15.4). Use care in operating the system to ensure that complete color formation occurs.

11.2.2 Add sodium hydroxide to the waste container to ensure that the wastes do not become acidic and evolve hydrogen cyanide gas.

11.2.3 This procedure is for 0.25 N NaOH distillates. If the normality of the distillates varies, it may be necessary to adjust the buffer. After the addition of buffer and distillate, the pH must be less than 8.

11.2.4 If the standards are not distilled, it is advisable to check the efficiency of the distillation process by taking at least one standard through the distillation procedure.

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the cyanide concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Method Performance

Range:	5.0–500µg/L CN
Rate:	72 samples/hr
Precision (100µg/L):	<1.5% RSD
Precision (400µg/L):	<1.0% RSD
Method Detection Limit:	2.6 µg/L
Percent Recovery:	90–110%

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.1).

15.0 References

15.1 *Less is Better: Laboratory Chemical Management for Waste Reduction.* Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.2 Cyanide. *Methods for Chemical Analysis of Water and Wastewater:* EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 335.3.

15.3 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii, xix, xx.

15.4. Sax, N.I. *Dangerous Properties of Industrial Materials*, 4th ed.; Van Nostrand Reinhold Company: New York, 1975.

15.5 *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, D.C., 1989; pp 4–178.

15.6 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

°C	degrees Celsius
%	percent
±	plus or minus
≥	greater than or equal to

16.1.2 Alphabetical characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
µg	microgram
µg/L	microgram per liter
mL	milliliter
ppm	parts per million
ppb	parts per billion
M	molar solution
N	normal solution

16.1 Calibration Blank—A 100-mL volume of CO_2-free reagent water analyzed using the FIA procedure.

16.2 Calibration Standard (CAL)—A solution prepared from the dilution of stock standard solutions. A 100-mL aliquot of each of the CALs are subjected to the analysis procedure. The resulting observations are used to calibrate the instrument response with respect to the analyte concentration.

16.3 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.4 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.5 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

16.6 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.

16.7 Minimum Level (ML)—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.

16.8 Ongoing Precision and Recovery (OPR)—See Section 16.4, "Laboratory Control Sample."

17.0 Figures

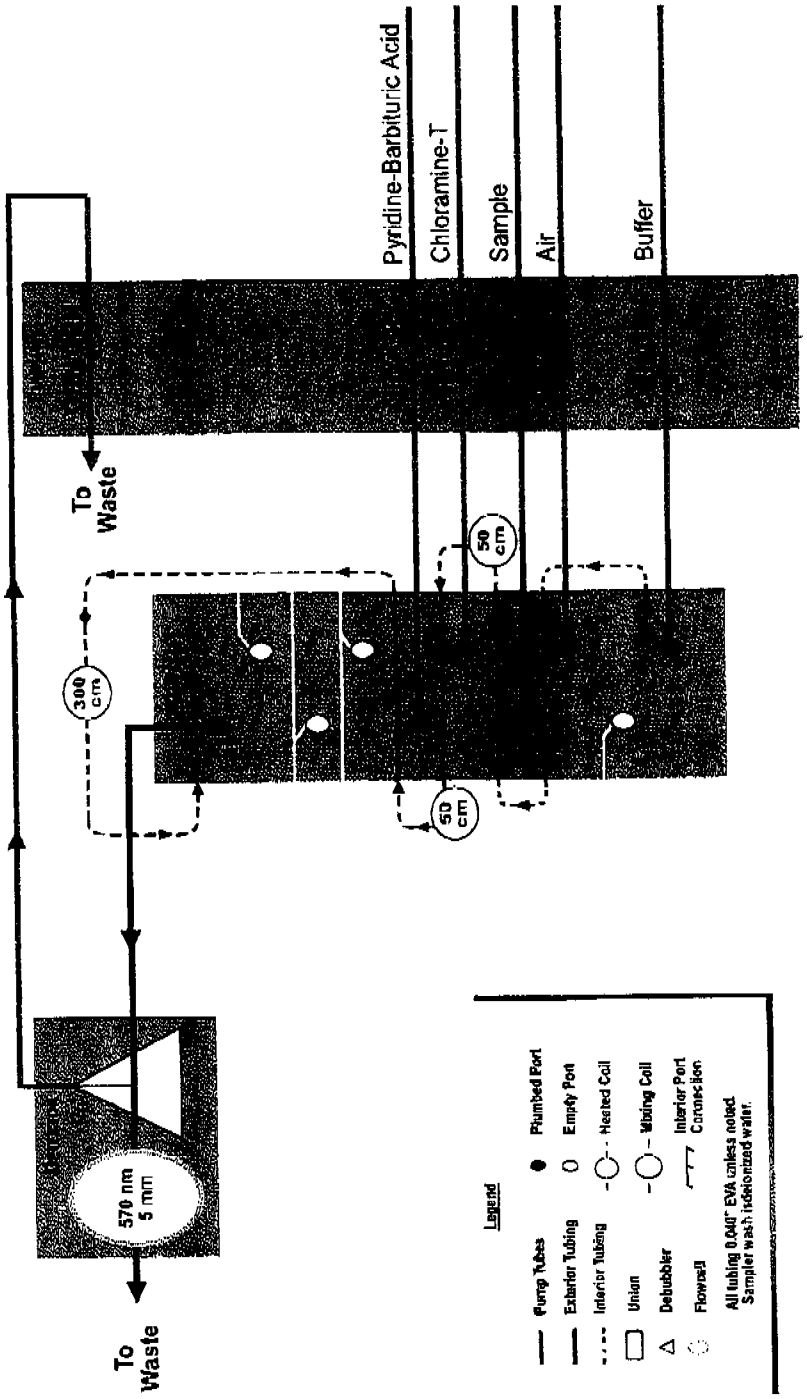


Figure 1. Detailed Flow Diagram for Post-Distillation Cyanide by SFA on a Flow Solution IV, Carridge Part #A002692

Sample Table - oitest.tbl

File name: C:\FLOW_4\OITEST.TBL
Date: October 31, 2000

Cup	Name	Type	R	Dil	Wt	Vial
6	Sync	SYNC	1	1	1	
0	Carryover	CO	1	1	1	
0	Baseline	RB	1	1	1	
1	Cal 0.00 ppm	C	1	1	1	
2	Cal 0.05 ppm	C	1	1	1	
3	Cal 0.10 ppm	C	1	1	1	
4	Cal 0.20 ppm	C	1	1	1	
5	Cal 0.50 ppm	C	1	1	1	
6	Cal 1.00 ppm	C	1	1	1	
0	Blank	BLNK	1	1	1	
5	CCV	CCV	1	1	1	
0	Read Baseline	RB	1	1	1	

Cup	Name	Comment
6	Sync	
0	Carryover	
0	Baseline	
1	Cal 0.00 ppm	
2	Cal 0.05 ppm	
3	Cal 0.10 ppm	
4	Cal 0.20 ppm	
5	Cal 0.50 ppm	
6	Cal 1.00 ppm	
0	Blank	
5	CCV	
0	Read Baseline	

Method Settings Dump: C:\FLOW_4\0ITEST1.MTH
Date: October 31, 2000
Channel Options (Channel 2)

Name: Channel 2
Acquisition Rate (Hz): 1hz
Detector Mode: Photo
Sample Gain: Auto
Reference Gain: Auto
Cell Potential: 0
Invert Signals: No
Channel Subtractions: None
Subtract from channel: 1
Subtraction Name:
Peak window Start (s): -10.000000
Peak window End (s): 10.000000
Ignore Time (s): 30.000000
Baseline Lead Time (s): 60.000000
Baseline Lag Time (s): 120.000000
Peak Recognition:
  Rise Seconds: 3.000000
  Rise Slope (val/s): 200.000000
  Fall Seconds: 3.000000
  Fall Slope (val/s): 200.000000
Smoothing Parameters:
  Mode: Savitzky Golay
  Points: 5
  Iterations: 1
Peak Mode: Height
Units: ppm
Final Units:
Conversion Factor: 1.000000
Carryover Correction: Disabled
Baseline Correction: Enabled
High Concentration limit: 1.100000
Low Concentration limit: 0.000000
Fitting Method: 1st Order
Include Origin: No
Logarithmic: No
Exclude Outliers: No

Method Settings Dump: C:\FLOW_4\OITEST1.MTH
Date: October 31, 2000

Baseline verification: Disabled
Interval: 10
Start Position: 10
Duration: 1
Default Vial Type: 1
Run speed (0 - 100%): 50
Slow speed (0 - 100%): 10
Action on CCV or Drift fail: Flag Only
&Enable Autodilution: No
CC&V Frequency: 10
&Number of Followers: 2
&Dilution Factors:

Sample Table - sample.tbl

File name: C:\FLOW_4\SAMPLE.TBL
Date: October 31, 2000

Cup	Name	Type	R	Dil	Wt	Vial
4	Sync	SYNC	1	1	1	
0	Carryover	CO	2	1	1	
0	Baseline	RB	1	1	1	
1	Cal 0.00 ppm	C	3	1	1	
2	Cal 0.01 ppm	C	3	1	1	
3	Cal 0.10 ppm	C	3	1	1	
4	Cal 0.50 ppm	C	3	1	1	
5	Cal 1.00 ppm	C	3	1	1	
6	Cal 5.00 ppm	C	3	1	1	
0	Blank	BLNK	2	1	1	
7	ICV	ICV	1	1	1	
0	Read Baseline	RB	2	1	1	
8	Sample 1	U	2	1	1	
9	Sample 2	U	2	1	1	
10	Sample 3	U	2	1	1	
11	Sample 4	U	2	1	1	
12	Sample 5	U	2	1	1	
0	Blank	BLNK	2	1	1	
4	CCV	CCV	1	1	1	
0	Read Baseline	RB	2	1	1	
13	Sample 6	U	2	1	1	
14	Sample 7	U	2	1	1	
14	Sample 8	U	2	1	1	
16	Sample 9	U	2	1	1	
17	Sample 10	U	2	1	1	
0	Blank	BLNK	2	1	1	
4	CCV	CCV	1	1	1	
0	Read Baseline	RB	2	1	1	

Cup	Name	Comment
4	Sync	
0	Carryover	
0	Baseline	
1	Cal 0.00 ppm	
2	Cal 0.01 ppm	
3	Cal 0.10 ppm	
4	Cal 0.50 ppm	
5	Cal 1.00 ppm	
6	Cal 5.00 ppm	
0	Blank	
7	ICV	
0	Read Baseline	
8	Sample 1	
9	Sample 2	
10	Sample 3	
11	Sample 4	
12	Sample 5	
0	Blank	

Cup	Name	Comment
4	CCV	
0	Read Baseline	
13	Sample 6	
14	Sample 7	
14	Sample 8	
16	Sample 9	
17	Sample 10	
0	Blank	
4	CCV	
0	Read Baseline	

O·I·Analytical **Methodology**

Total Kjeldahl Nitrogen (TKN), USEPA by Segemented Flow Analysis (SFA)

(Cartridge Part #A002597)

1.0 Scope and Application

1.1 This method is used for the determination of Total Kjeldahl Nitrogen (TKN) in drinking, surface, and saline waters; as well as domestic and industrial wastes according to USEPA Method 351.2 (Reference15.4). During the digestion, amino acids, proteins, peptides and other nitrogen compounds of biological origin are converted to ammonium sulfate. Nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some tertiary amines may not be converted.

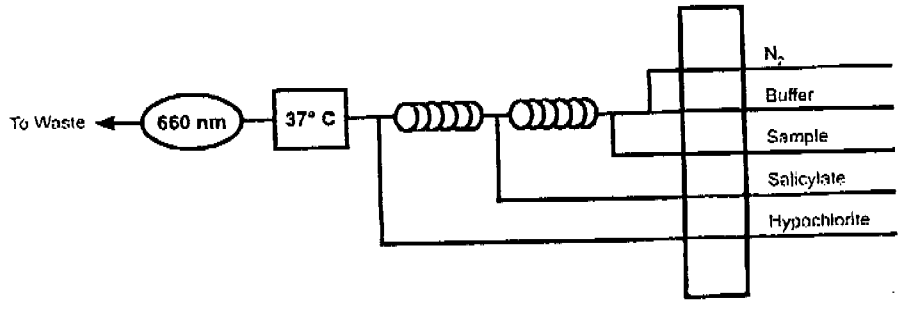
1.2 The Method Detection Limit (MDL) of this method is 0.2 mg/L TKN. The applicable range of this method is 0.1–20 mg/L TKN. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

2.1 The sample is digested prior to analysis by heating in the presence of sulfuric acid, potassium sulfate and mercury catalyst to a final temperature of 380°C. Free ammonia and organic nitrogen compounds are converted to ammonium sulfate under the conditions of this digestion.

2.2 The ammonium is then reacted with salicylate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide (pH 12.8–13) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm (Reference 15.4).

2.3 A general flow diagram of the SFA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

4.0 Contamination and Interferences

4.1 Precipitation of calcium and magnesium hydroxides is eliminated by potassium sodium tartrate in the working buffer.

4.2 Digestates that are turbid must be filtered or centrifuged prior to determination.

4.3 Digestates with background absorbance at the analytical wavelength may interfere with analysis.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Ammonium Sulfate ($(NH_4)_2SO_4$)

5.3.2 Chloroform ($CHCl_3$)

5.3.3 Potassium Sodium Tartrate, tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$)

5.3.4 Potassium Sulfate (K_2SO_4)

5.3.5 Red Mercuric Oxide (HgO)

5.3.6 Sodium Hydroxide (NaOH)

5.3.7 Sodium Hypochlorite, 5.25% solution (NaOCl, household bleach)

5.3.8 Sodium Nitroferricyanide, dihydrate ($Na_2Fe(CN)_5NO \cdot 2H_2O$)

5.3.9 Sodium Phosphate, dibasic (Na_2HPO_4)

5.3.10 Sodium Salicylate ($NaC_7H_5O_3$)

5.3.11 Sulfuric Acid, concentrated (H_2SO_4)

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

6.1 Segmented Flow Analysis (SFA) System (OI Analytical Flow Solution IV) consisting of the following:

6.1.1 Model 502 Multichannel Peristaltic Pump

6.1.2 Random Access (RA) Autosampler

6.1.3 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 660-nm optical filter

6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software

6.1.5 Ammonia Nitrogen/TKN, USEPA Cartridge (OI Analytical Part #A002597)

6.2 Sampling equipment - Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°-150°C for a minimum of one hour.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Ammonium Sulfate ($(NH_4)_2SO_4$)

7.1.2 Brij-35®, 30% solution (OI Analytical Part #A21-0110-33)

7.1.3 Chloroform ($CHCl_3$)

7.1.4 Deionized Water, ammonia-free (ASTM Type I or II)

7.1.5 Potassium Sodium Tartrate, tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$)

7.1.6 Potassium Sulfate (K_2SO_4)

7.1.7 Red Mercuric Oxide (HgO)

7.1.8 Sodium Hydroxide (NaOH)

7.1.9 Sodium Hypochlorite, 5.25% solution (NaOCl, household bleach)

7.1.10 Sodium Nitroferricyanide, dihydrate ($Na_2Fe(CN)_5NO \cdot 2H_2O$)

7.1.11 Sodium Phosphate, dibasic (Na_2HPO_4)

7.1.12 Sodium Salicylate ($NaC_7H_5O_3$)

7.1.12 Sulfuric Acid, concentrated (H_2SO_4)

7.1.13 Teflon® or glass boiling stones. Chemware TFE stones (Teflon) are available from Markson Science, Inc., Phoenix, Arizona.

7.2 Reagent Preparation

Note: For best results, filter and degas all reagents prior to use.

Note: All reagents need to be made with ammonia-free deionized water. Ammonia-free water can be prepared by passing distilled water through a mixture of strongly acidic cation and strongly basic anion exchange resins (Reference 15.4).

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15-20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15-20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparation of degassed reagent water, protect it from re-absorption of atmospheric gases by storing it in a tightly sealed container. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Digestion Reagents

Note: For best results, filter all reagents prior to use.

7.2.2.1 Sulfuric Acid, 1:5 (50 mL) — Carefully add 10 mL sulfuric acid to approximately 40 mL of deionized water while stirring.

Caution: The mixing of sulfuric acid with water releases a great amount of heat.

7.2.2.2 Mercuric Sulfate (100 mL)

7.2.2.2.1 Dissolve 8 g of red mercuric oxide in 80 mL 1:5 H2SO4 (Section 7.2.2.1).

7.2.2.2.2 Dilute to 100 mL with deionized water in a volumetric flask and mix well.

Warning: Mercury compounds are highly toxic.

7.2.2.3 Digestion Solution (1 L)

7.2.2.3.1 Carefully add 200 mL of sulfuric acid to about 800 mL of deionized water.

7.2.2.3.2 Add 133 g of potassium sulfate and stir to dissolve.

7.2.2.3.3 Add 25 mL of mercuric sulfate solution (Section 7.2.2.2) and allow to cool.

7.2.2.3.4 Transfer the mixture to a 1-L volumetric flask, dilute to 1,000 mL with deionized water, and mix well.

Cautioin: The mixing of sulfuric acid with water releases a great amount of heat.

7.2.3 Colorimetric Analysis Reagents

Note: For best results, filter all reagents prior to use.

7.2.3.1 Sodium Hydroxide, 10 N (250 mL)

7.2.3.1.1 While continuously stirring, cautiously add 100 g of sodium hydroxide to approximately 175 mL of deionized water in a 250-mL volumetric flask.

7.2.3.1.2 When the solution is cool, dilute to 250 mL with deionized water and mix well.

7.2.3.1.3 Store tightly capped in a plastic container. Storage is limited to one month.

Caution: The dissolution of sodium hydroxide in water releases a great amount of heat.

7.2.3.2 Stock Potassium Sodium Tartrate Solution (1 L)

7.2.3.2.1 Dissolve 200 g of potassium sodium tartrate in approximately 800 mL of deionized water in a 1-L volumetric flask.

7.2.3.2.2 Dilute the solution to 1,000 mL with deionized water and mix well.

7.2.3.3 Stock Buffer, Sodium Phosphate, dibasic (1 L)

7.2.3.3.1 Dissolve 134 gof sodium phosphate, dibasic in approximately 800 mL of deionized water in a 1-L volumetric flask.

7.2.3.3.2 Add 50 mL of 10 N sodium hydroxide and dilute the solution to 1,000 mL with deionized water and mix well.

7.2.3.4 Working Buffer (1 L)

7.2.3.4.1 Mix 200 mL of stock buffer and 200 mL of deionized water in a 1-L volumetric flask.

7.2.3.4.2 While stirring, add 250 mL of stock potassium sodium tartrate.

7.2.3.4.3 Continue stirring and slowly add 60 mL of 10 N sodium hydroxide.

7.2.3.4.4 Dilute the solution to 1,000 mL with deionized water and mix well.

7.2.3.4.5 Filter the solution. Add 0.5 mL of Brij-35, 30% and mix gently to prevent foaming.

7.2.3.4.6 Prepare the working buffer daily.

7.2.3.5 Salicylate/Nitroferricyanide Solution (500 mL)

7.2.3.5.1 Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroferricyanide in approximately 300 mL of deionized water.

7.2.3.5.2 Filter the solution through fast filter paper into a 500-mL volumetric flask.

7.2.3.5.3 Dilute the solution to 500 mL with deionized water and mix well.

7.2.3.5.4 Store the solution in a dark bottle.

7.2.3.6 Sodium Hypochlorite (200 mL)

7.2.3.6.1 Add 12 mL of sodium hypochlorite solution to approximately 150 mL of deionized water in a 200-mL volumetric flask.

7.2.3.6.2 Dilute the solution to 200 mL with deionized water and mix well.

7.2.3.6.3 Place the hypochlorite solution in an amber bottle.

7.2.4.6.4 Prepare the sodium hypochlorite reagent daily.

7.2.3.7 Sampler Wash, 4% H_2SO_4 (2 L)

7.2.3.7.1 Carefully add 80 mL of sulfuric acid with stirring to approximately 1,800 mL of deionized water in a 2-L volumetric flask.

7.2.3.7.2 Cool the solution and dilute to 2,000 mL with deionized water and mix well.

Caution: The mixing of sulfuric acid with water releases a great amount of heat.

Note: If the acid content of the digestates is different than 4%, prepare the sampler wash at that concentration.

7.2.3.8 Start-up Solution (500 mL)—Add 1.0 mL Brij-35, 30%, to 500 mL deionized water and mix gently.

7.3 Calibrants

7.3.1 Stock 1,000 mg/L Ammonia Nitrogen (1 L)

7.3.1.1 Dissolve 4.717 g of dry ammonium sulfate in approximately 800 mL of deionized water in a 1-L volumetric flask.

7.3.1.2 Dilute the solution to 1,000 mL with deionized water.

7.3.1.3 Preserve the stock calibrant with two drops of chloroform and refrigerate at approximately 4°C.

7.3.2 Intermediate Calibrant 100 mg/L Ammonia Nitrogen (100 mL)

7.3.2.1 Use a volumetric pipette to add 10 mL of stock calibrant to approximately 80 mL of deionized water contaned in a 100-mL volumetric flask.

7.3.2.2 Dilute the solution to 100 mL with deionized water and mix well.

7.3.2.3 Prepare the intermediate calibrant fresh daily.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Add the designated volumes of stock calibrant (see Equation 1), to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of deionized water.

7.3.3.2 Dilute each solution to the mark with deionized water and mix well.

EQUATION 1

$$C_1V_1 = C_2V_2$$

where:
C_1 = *desired concentration (in mg/L) of working calibrant to be prepared*
V_1 = *final volume (in mL) of working calibrant to be prepared (generally 100 mL)*
C_2 = *concentration (in mg/L) of stock (or intermediate) calibrant*
V_2 = volume (in mL) of stock (or intermediate) calibrant to be used

By solving this equation for the volume of stock solution to be used (V_2), the following equation is obtained:

$$V_2 = \frac{C_1V_1}{C_2}$$

Since the desired concentration (C_1), the final volume (V_1), and the concentration of the stock solution (C_2) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_2) is easily calculated.

7.3.4 Alternately, standard curves in the desired ranges can be derived from the table below:

Final Concentration (mg/L)	Volume of Stock Cal. (µL)	Conc. of Stock Cal. (mg/L)	Final Volume (mL)
0.1	10	1,000	100
0.2	20	1,000	100
2.0	200	1,000	100
4.0	400	1,000	100
6.0	600	1,000	100
8.0	800	1,000	100
10.0	1000	1,000	100
12.0	1200	1,000	100
16.0	1600	1,000	100
20.0	2000	1,000	100

8.0 Sample Collection, Handling, and Preservation

8.1 Samples should be analyzed as soon as possible to reduce loss of analyte.

8.2 Samples may be preserved with 2 mL of concentrated sulfuric acid per liter of sample and refrigerated at 4°C.

8.3 The holding time for preserved, undigested samples is 28 days (Reference 15.5).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.2). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.2. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

a) calibration (Section 10.4)

b) calibration verification (Section 9.5)

c) initial precision and recovery (Section 9.2.2)

d) analysis of blanks (Section 9.4)

e) ongoing precision and recovery (Section 9.6)

(f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) sample numbers and other identifiers

b) analysis dates and times

c) analysis sequence/run chronology

d) sample weight or volume

e) sample volume prior to each cleanup step, if applicable

f) sample volume after each cleanup step, if applicable

g) final sample volume prior to injection

h) injection volume

i) dilution data, differentiating between dilution of a sample or modified sample

j) instrument and operating conditions

k) other operating conditions

l) detector

m) printer tapes, disks, and other recording of raw data

n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.

9.1.7 Accompanying QC for the determination of TKN is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Method Detection Limit (MDL) — To establish the ability to detect TKN at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.1) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 must be achieved prior to practice of this method.

9.2.2 Initial Precision and Recovery (IPR) — To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.

9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (s) for TKN. Use Equation 2 for the calculation of the standard deviation of the percent recovery(s).

EQUATION 2

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where:
s = Standard deviation
n = Number of samples
x = Percent recovery in each sample

9.2.2.3 Compare *s* and *x* with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of *s* exceeds the precision limit or the value of *x* falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD) — The laboratory shall spike, in duplicate, a minimum of 10 percent of all samples (one sample in duplicate in each batch of ten samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of TKN in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of TKN in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of TKN.

9.3.2.1 Spike this sample with the amount of TKN stock solution (Section 7.3.1) to produce an TKN concentration in the sample of 5 mg/L. If necessary, prepare another stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of TKN (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of TKN in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{A - B}{T} \times 100$$

Where:
P = Percent recovery
A = Measured concentration of TKN after spiking (Section 9.3.2.2)
B = Measured background concentration of TKN (Section 9.3.2)
T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \left[\frac{|D_1 - D_2|}{(D_1 + D_2)/2}\right] \times 100$$

Where:
RPD = Relative percent difference
D_1 = Concentration of TKN in the spiked sample
D_2 = Concentration of TKN in the spiked duplicate sample

9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.

9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_{μ}) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from P_{μ}-$2s_p$ to P_{μ} + $2s_p$. For example, if P_{μ} = 90% and s_p = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

9.4 Laboratory Reagent Blanks (LRB) — Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.

9.4.2 If TKN is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.

9.5 Calibration Verification — Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the mid-range calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).

9.6 Laboratory Control Sample (LCS) — To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.

9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for TKN by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if R = 95% and s_r = 5%, the accuracy is 85% to 105%.

9.7 Reference Sample — To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institutes of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Calibration and Standardization

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical Flow Solution IV Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Ammonia Nitrogen/TKN, USEPA Cartridge (OI Analytical Part #A002597) is configured as illustrated in the flow diagram illustrated in Section 17.0.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Connect the buffer and color reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.3.8). Start the pump at 40% speed, allowing the start-up solution to flow through the entire system.

10.2.2 Make sure that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging.

10.2.3 Once a stable flow is achieved, connect the reagent pump tubes to their appropriate reagent bottles. Allow these reagents to flow through the entire system, then, once again, verify that the flowcell of each detector is purged of all bubbles.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.6) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected TKN concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.6).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Verify that the baseline does not drift and is free from large fluctuations. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the ammonia stock solutions (Section 7.3.1) according to Equation 1, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of decreasing concentration and analyze each calibrant according to Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.6). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Digestion

11.1.1 Blanks, calibrants, samples, QC samples

Note: The final diluted volume after digestion will be 25 mL.

11.1.1.1 For blanks, add 25 mL of deionized water to each tube.

11.1.1.2 For calibrants, pipet the appropriate amount of stock or intermediate calibrant to each tube and rinse it down the side well with deionized water to an approximate total volume of 25 mL. Use adjustable microliter pipets or class A volumetric pipets. Mix well.

11.1.1.3 For samples, add exactly 25 mL to the tube and mix well. If samples are known to be out of range, smaller volumes can be used and the result multiplied by the appropriate dilution factor.

11.1.1.3.1 For example, if 10 mL of sample is digested. After digestion and analysis, multiply the result by 2.5.

11.1.1.3.2 For example, if 5 mL of sample is digested. After digestion and analysis, multiply the result by 5.

11.1.1.4 For QC samples, prepare the sample according to its instructions and add exactly 25 mL to the tube. Mix well.

11.1.2 Add 5 mL of digestion reagent (Section 7.2.2.3) to each tube and mix all tubes well.

Note: The 5 mL of digestion reagent should be measured accurately to help ensure uniform acid content.

11.1.3 Add 4 -6 glass or Teflon boiling stones to each tube.

Caution: Too many stones will cause the sample to boil over.

11.1.4 Place the tubes in the block digestor and set the temperature for 160°C. Allow the volume to be reduced to about 5 mL in each tube (approximately 1 hour).

11.15 Increase the temperature to 380°C for 2 1/2 hours. When the digestion is complete, the final volume will be about 2–3 mL, and the liquid will be clear or straw colored.

Caution: The purpose of this evaporation step is to reduce the volume of liquid in the tube before increasing the temperature for digestion. If during the digestion, any amount of liquid boils out of a tube, the result will be in error.

Note: If an exhaust system or scrubber is placed on top of the digestion tubes during the digestion, care must be taken not to draw off significant amounts of the fumes during the high temperature stage. Variable acid concentrations may result. The digestion can be carried out without an exhaust system if the digestion apparatus is placed in a properly operating fume hood.

11.1.6 Remove the tubes from the block and allow to cool. It is preferable not to allow the digestate to crystallize while cooling. A few milliliters of ammonia-free water can be added and mixed with the digest after cooling the tubes for a few minutes to prevent the crystallization.

Caution: If the water is added too soon and the acid is still too hot, the contents of the tube may react vigorously and some material may be lost from the tube.

11.2.7 Dilute the digestate to 25 mL with deionized water. The digestates must be accurately diluted to a known volume in a calibrated container. This is important to help ensure a consistent acid content in the final solutions which will affect the accuracy of the measurement. If the contents of the tube are transferred to a separate container during this step, ensure that all of the digestate is transferred.

Note: Use ammonia-free water for all dilutions.

11.2 Analysis

Note: Pay special attention to Sections 11.2.2 and 11.2.6 to avoid precipitation of the salicylate reagent.

11.2.1 Set up the cartridge as shown in the flow diagram in Section 17.0. Set cartridge temperature to 37°C.

11.2.2 When starting up the cartridge, leave the salicylate line in deionized water while pumping all other reagents for at least 5 minutes. Then put the salicylate line in reagent. If a precipitate forms after addition of the salicylate, it was added too soon or the pH of the stream is too low. Immediately stop the pump and flush the cartridge with water.

11.2.3 When the system has stabilized, load the sampler tray with calibrants, blanks, samples and QC samples.

11.2.4 Place all reagents on-line. Pump 5–10 minutes. Obtain a stable baseline at 660 nm and autozero before the start of sample run.

11.2.5 Load the sampler tray with calibrants, blanks, samples, and QC samples. The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.2.6 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the "Fast Forward" button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the sample table.

11.2.7 Before shutting down the system, remove the salicylate line from reagent first and put it in deionized water for at least five minutes. Then place the other lines in deionized water.

11.2.8 When analyzsis is complete, turn the pump "OFF", release the tension on all pump tubes, and power off the system.

11.3 Operating Notes

11.3.1 Variations in acid concentration from sample to sample can cause problems in the analysis of the digests. Titrate several aliquots from different tubes (after diluting to volume) to determine that a consistent acid content is being achieved.

11.3.1.1 If the acid content determined by titration is consistent, but different from the sampler wash solution, remake the sampler wash solution to the same concentration.

11.3.1.2 Raise or lower the amount of NaOH in the working buffer proportionately.

Note: It is possible that with some samples (e.g. those high in organics, color etc.) the final acid concentration may not end up as 4%. This is also true of samples that are highly acidic or basic or contain substantial amounts of carbonates or bicarbonates (alkalinity).

11.3.2 Problems can be isolated to either the cartridge or the digestion by preparing undigested calibrants in sampler wash solution and analyzing them according to the procedure in Section 11.2.

11.3.3 If digestates give an off-scale response during the analysis:

11.3.3.1 Dilute the digestate with blank solution having the same acid content before re-analysis.

Note: Use blank solution only. Do not dilute with water.

11.3.3.2 Multiply the result by the appropriate dilution factor. As each sample source becomes known, the proper sample amount can be used for digestion to effectively dilute it. For example, a sample becomes known to always need a factor of 10 dilution, 2.50 mL can be pipetted to the digestion tube. (In this case, a few mL of water may also be added to facilitate mixing if it has been determined that the water has no background.) A final dilution to 25 mL will then be a 10x dilution of the sample. Multiply the analysis result by 10.

11.3.4 If a stable baseline on reagents cannot be achieved:

11.3.4.1 Filter the working reagents if not previously done. Make sure the reagent containers are free of any debris or other contamination.

11.3.4.2 Disconnect the debubbler from the flowcell by disconnecting the transmission tubing from the debubbler. Use a syringe fitted with a union to flush the flowcell with strong surfactant water followed by deionized water. Reconnect the debubbler.

11.3.4.3 Remove reagents from the cartridge and flush surfactant water through the cartridge for about 10 minutes. Pump surfactant water through the lines and see if a stable baseline is observed on surfactant water.

11.3.4.4 Clean the cartridge with 1 N HCl. Remove the salicylate line to deionized water for five minutes before putting reagent lines in 1 N HCl to prevent precipitation in the cartridge.

11.3.5 If poor peak shape or carryover is observed:

11.3.5.1 Check that proper sample and wash times are set on the sampler.

11.3.5.2 Verify that the bubbles from the sampler travel smoothly through the tubing to the cartridge. If not, replace the pump tube and transmission tubing.

11.3.5.3 Verify that the flow on the cartridge is smooth and consistent. Check for loose fittings.

11.3.5.4 Remake all reagents, including stock reagents. Sodium hypochlorite must be fresh.

11.3.6 To prevent ammonia contamination from the air, segment the analytical stream with nitrogen or draw air through a 5N sulfuric acid solution. Add 35mL of concentrated H_2SO_4 to 200 mL of deionized water, and dilute to 250 mL with deionized water.

Caution: The mixing of sulfuric acid with water releases a great amount of heat.

11.3.7 If a high background or blank values are observed, carefully evaluate the source of water, cleaning procedure for the digestion glassware, and laboratory environment.

11.3.8 If poor sensitivity and nonlinearity are present:

11.3.8.1 Check for excessive pump tube wear or a flow restriction. Replace pump tubing and verify that all lines are pumping freely.

11.3.8.2 Verify that the heatbath is on and the temperature is not set too low.

11.3.8.3 Deteriorated reagents or mismatched acid content of the digestates compared to the sampler wash and the hydroxide in the buffer. It is important to match the acid content of the digestates with that of the sampler wash.

11.3.9 The pH of the stream coming out of the flowcell should be approximately pH 13.

11.3.9.1 Verify that the pH of the stream coming out of the flowcell is 13 before pumping the salicylate reagent.

11.3.9.1 If salicylate does precipitate, immediately turn off the pump, disconnect the manifold from the debubbler, and place all reagent lines in 1 N NaOH. Flush the air port (port #3 on the manifold) with deionized water. Turn the pump on and flush the system for at least 10 minutes or until all of the precipitate has been flushed from the system. Rinse for five minutes with deionized water. Return the reagent lines, except for the salicylate, to their bottles and continue as in Section 11.2.2.

11.3.10 If a problem is encountered with peak heights being shorter than expected, ensure that the heater is at 37°C.

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the TKN concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Performance Specifications

Range:	0.1–20 mg/L TKN
Rate:	90 samples/hr
Carryover:	0.3%
Precision (2 mg/L):	1.0% RSD
Precision (16 mg/L):	0.8% RSD
Method Detection Limit:	0.02 mg/L TKN
Percent Recovery:	90–110%

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, Section 13.6 (Reference 15.4).

15.0 References

15.1 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.

15.2 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.

15.3 *Less is Better: Laboratory Chemical Management for Waste Reduction.* Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.4 Nitrogen, Kjeldahl, Total, All Forms. *Methods for Chemical Analysis of Water and Wastewater;* EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 351.2.

15.5 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.

15.6 *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, D.C., 1989; 4--178.

15.7 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

°C	degrees Celsius
%	percent
±	plus or minus
≥	greater than or equal to

16.1.2 Alphabetical characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
µg	microgram
µg/L	microgram per liter

mL	milliliter
ppm	parts per million
ppb	parts per billion
M	molar solution
N	normal solution

16.1 Calibration Blank—A 100-mL volume of CO_2-free reagent water analyzed using the SFA procedure.

16.2 Calibration Standard (CAL)—A solution prepared from the dilution of stock standard solutions. A 100-mL aliquot of each of the CALs are subjected to the analysis procedure. The resulting observations are used to calibrate the instrument response with respect to the analyte concentration.

16.3 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.4 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.5 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

16.6 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.

16.7 Minimum Level (ML)—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.

16.8 Ongoing Precision and Recovery (OPR)—See Section 16.4, "Laboratory Control Sample."

17.0 Figures

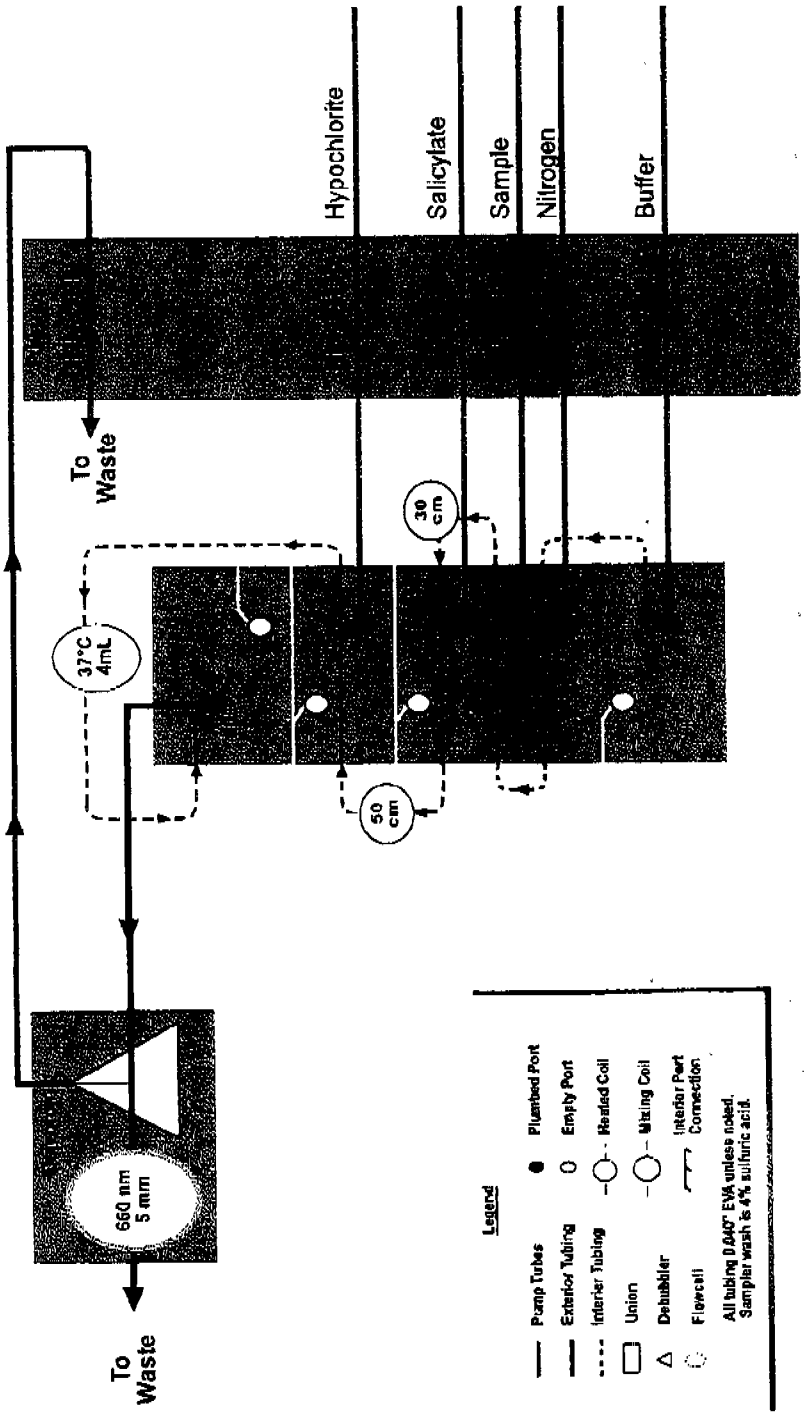


Figure 1. Detailed Flow Diagram for Total Kjeldahl Nitrogen (TKN) by SFA on a Flow Solution IV Cartridge Part #A0002597

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity, operator skill, and maintenance of instruments.

O·I·Analytical
P.O. Box 9010
College Station, Texas 77842-9010
Tel: (979) 690-1711 · FAX: (979) 690-0440

O·I·Analytical **Methodology**

Nitrate plus Nitrite Nitrogen and Nitrite Nitrogen, USEPA by Segmented Flow Analysis (SFA) or Flow Injection Analysis (FIA)

(Cartridge Part #A002670)

1.0 Scope and Application

1.1 This method is used to determine the concentration of nitrate (NO_3^-) plus nitrite (NO_2^-) or nitrite singly in drinking, ground, and surface waters, as well as domestic and industrial wastes according to USEPA Method 353.2 (Reference 15.5).

1.2 The Method Detection Limit (MDL) for this method is 1.0 µg/L (ppb), and the Minimum Level (ML) is 5.0 µg/L (ppb). The dynamic range of this method is 5.0 µg/L (ppb) to 10.0 mg/L (ppm) nitrate plus nitrite and nitrite nitrogen. The range may be extended above 10.0 mg/L by applying a higher order calibration curve fit or by sample dilution prior to analysis.

1.3 This method is for use by analysts experienced with segmented flow analysis (SFA) and/or flow injection analysis (FIA) equipment or under close supervision of such qualified persons.

1.4 The laboratory is permitted to modify this method to overcome interferences or to lower the cost of measurements provided that all performance criteria in this method are met. Requirements for establishing method equivalency are given in Section 9.1.2.

2.0 Summary of Method

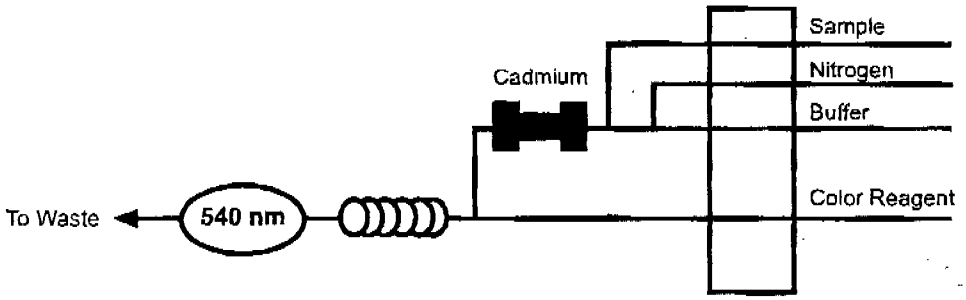
Note: This methodology is designed specifically for SFA; however, it may be used for FIA. Refer to Section 18.0 for specific instructions on running FIA.

2.1 Nitrate is reduced quantitatively to nitrite by cadmium metal. Nydahl (Reference 15.8) provides a good discussion of nitrate reduction by cadmium metal. The nitrite thus formed, in addition to any nitrite originally present in the sample, is colorimetrically detected as a highly colored azo dye at 540 nm following its diazotization with sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride (Reference 15.11). A calibration curve allows for accurate quantitation of the detected nitrite.

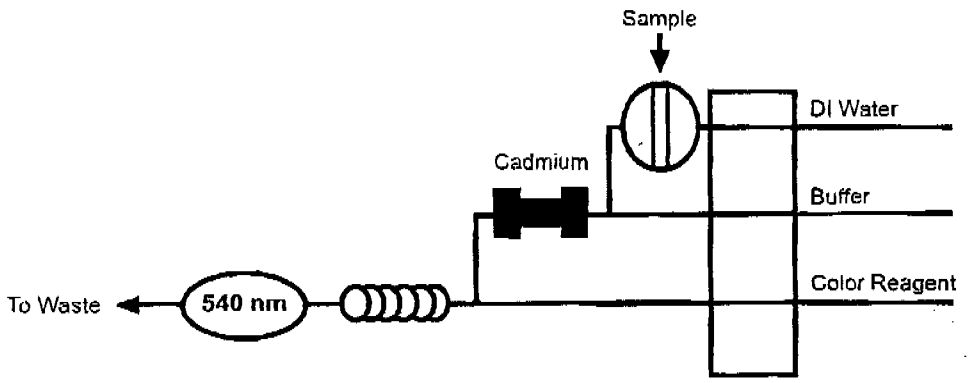
2.2 Nitrite singly may be measured by performing the same analysis mentioned in Section 2.1 without the cadmium reduction. Without the cadmium, nitrate is not reduced to nitrite, and, therefore, nitrate is not detected since only nitrite forms the azo dye.

2.3 Both nitrate and nitrite may be measured simultaneously by using a two-channel flow analyzer. One channel is used to measure nitrate plus nitrite (Section 2.1), while the second channel is used to measure nitrite only (Section 2.2). Using WinFLOW™ software, the results of the nitrite analysis may be subtracted from the results of the nitrate plus nitrite analysis, thus providing quantitative nitrate results.

2.4 A general flow diagram of the SFA system used in development of this method is shown below:



2.5 Alternately, a FIA system may be used as detailed in Section 18.0. A general flow diagram is shown below:



2.6 For additional reference, Patton (Reference 15.9) and Fox (Reference 15.2) provide discussions of the mechanisms and kinetics of the color forming reactions used in this method.

3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

4.0 Contamination and Interferences

4.1 Turbid samples may interfere with the photometric detector's ability to measure the true absorbance of the sample. Turbid samples should be filtered prior to analysis to reduce the possibility of such bias.

4.2 Iron, copper, and other metals may interfere with the accurate analysis of nitrate and nitrite by binding with the nitrate and/or nitrite in the sample, thus blocking the color formation reaction. Ethylene diaminetetraacetic acid (EDTA) is used in the buffer solution to eliminate this interference.

4.3 Samples with a pH that are outside the functional range of the ammonium chloride buffer used in this method may affect the results obtained from this method. Such samples should be adjusted within a pH range of 5 to 9 using either concentrated hydrochloric acid (HCl) or ammonium hydroxide (NH_4OH).

4.4 Oil and grease will coat the cadmium surface, thus reducing its reduction efficiency. Therefore, samples containing large concentrations of oil and grease must be extracted with an appropriate organic solvent (Reference 15.5).

4.5 Sulfide in the presence of cadmium will form cadmium sulfide (CdS) which will precipitate from solution. Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts (Reference 15.10).

4.6 Chlorine may reduce the reduction efficiency of the cadmium reactor. Samples that may contain residual chlorine should be tested for reduction efficiency through the analysis of Matrix Spike/Matrix Spike Duplicate (MS/MSD) samples (Section 9.3). When necessary, dechlorinate samples with sodium thiosulfate ($Na_2S_2O_3$).

4.7 Method interferences may be caused by contaminants in the reagents, reagent water, glassware, etc., which may bias the results. Care should be taken to keep all such items free of contaminants.

4.8 Norwitz and Keliher (References 15.6 and 15.7) have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrite.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Cadmium (Cd)

5.3.2 Phosphoric Acid (H_3PO_4)

5.3.3 Chloroform ($CHCl_3$)

5.3.4 N-1-naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)

5.3.5 Sulfanilamide ($C_6H_8N_2O_2S$)

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

6.1 Segmented Flow Analysis (SFA) System (OI Analytical Flow Solution IV) consisting of the following:

6.1.1 Model 502 Multichannel Peristaltic Pump

6.1.2 Random Access (RA) Autosampler

6.1.3 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 540-nm optical filter

6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW software

6.1.5 Nitrate/Nitrite, USEPA Cartridge (OI Analytical Part #A002670)

6.1.6 For FIA, Flow Solution IV must be equipped with the FIA option. See Section 2.0 and 18.0 for further information on FIA.

6.2 Sampling equipment — Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Ammonium Chloride, NH_4Cl (CAS 12125-02-9, Sigma-Aldrich #A4514)

7.1.2 Ammonium Hydroxide, NH_4OH (CAS 13360-21-6, Sigma-Aldrich #A6899)

7.1.3 Brij®-35, 30% w/v (OI Analytical Part #A21-0110-33)

7.1.4 Chloroform, $CHCl_3$ (CAS 67-66-3, Sigma-Aldrich #320242)

7.1.5 Deionized Water (ASTM Type I or II)

7.1.6 Ethylene Diamine Tetraacetic Acid, disodium salt dihydrate (EDTA), $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (CAS 6381-92-6, Sigma-Aldrich #E5134)

7.1.7 N-1-naphthylethylenediamine Dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$ (CAS 1465-25-4, Sigma-Aldrich #N9125)

7.1.8 Phosphoric Acid, Concentrated (85%), H_3PO_4 (CAS 7664-38-2, Sigma-Aldrich #P6560)

7.1.9 Potassium Nitrate, KNO_3 (CAS 7757-79-1, Sigma-Aldrich #P8394)

7.1.10 Potassium Nitrite, KNO_2 (CAS 7758-09-0, Sigma-Aldrich #P7391)

7.1.11 Sulfanilamide, $C_6H_8N_2O_2S$ (CAS 63-74-1, Sigma-Aldrich #S9251)

7.2 Reagent Preparation

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15-20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15-20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparation of degassed reagent water, protect it from re-absorption of atmospheric gases by storing it in a tightly sealed container. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Start-up Solution (1 L) — Add 2 mL of Brij-35, 30% w/v, to approximately 800 mL of reagent water in a cleaned 1-L Erlenmeyer flask and mix well. Dilute to 1 L with reagent water (Section 7.2.1).

7.2.3 Stock Ammonium Chloride/EDTA Buffer, pH 8.5 (1 L) — Dissolve 85 g of ammonium chloride and 0.1 g of disodium EDTA in approximately 900 mL of reagent water in a 1-L beaker. Adjust the pH to 8.5 with concentrated ammonium hydroxide. Transfer the solution to a 1-L volumetric flask and dilute to 1 L with reagent water. For best results, filter this solution through a 0.45-µm filter before use.

7.2.4 Working Buffer (1 L) — Add 2 mL of Brij-35, 30% w/v, to 1 L of stock ammonium chloride-EDTA buffer (Section 7.2.3) and mix gently.

7.2.5 Color Reagent (500 mL) — Cautiously add 50 mL of concentrated phosphoric acid to approximately 400 mL of reagent water in a 500-mL volumetric flask while stirring. Dissolve 20 g of sulfanilamide and 1 g of N-1-naphthylethylene-diamine dihydrochloride in the phosphoric acid solution (continue stirring until completely dissolved, which may take 2-3 hours). Dilute to 500 mL with reagent water. For best results, filter this solution through a 0.45-µm filter before use. Store in an amber bottle and keep in the dark when not in use. If stored properly, this color reagent is typically stable for 2–3 months.

7.2.6 Sampler Wash — Reagent water (Section 7.2.1)

7.2.7 Open Tubular Cadmium Reactor (OTCR), (OI Analytical Part #A000897)—Refer to Section 18.0 for FIA.

7.2.8 Reagents for OTCR Activation:

7.2.8.1 Stock Ammonium Chloride/EDTA Buffer (Section 7.2.3)

7.2.8.2 Reagent Water (Section 7.2.1)

7.2.8.3 Cupric Sulfate Pentahydrate, $CuSO_4 \cdot 5H_2O$, 2% Solution, (1 L) — Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ in approximately 900 mL of reagent water in a 1-L volumetric flask. Dilute the solution to 1 L with reagent water and mix well.

7.2.8.4 Hydrochloric Acid, HCl, 0.5-N Solution (100 mL) — Add 4.15 mL of concentrated HCl to approximately 70 mL of reagent water in a 100-mL volumetric flask. Dilute to 100 mL with reagent water and mix well.

7.2.9 Activation of the OTCR:

7.2.9.1 Using a 10-mL luer-lock syringe and ¼" 28 luer female fitting (OI Analytical, Part #A000543), slowly flush the OTCR with 10 mL of reagent water. If any debris is seen exiting the OTCR, then continue to flush with reagent water until all debris is removed.

7.2.9.2 Slowly flush the OTCR with 10 mL of the 0.5-N HCl solution (Section 7.2.8.4). Quickly proceed to the next step as the HCl solution can cause damage to the cadmium surface if left in the OTCR for more than a few seconds.

7.2.9.3 Flush the OTCR with 10 mL of reagent water to remove the HCl solution.

7.2.9.4 Slowly flush the OTCR with 10 mL of 2% cupric sulfate solution (Section 7.2.8.3). Leave this solution in the OTCR for approximately 5–10 minutes.

7.2.9.5 Forcefully flush the OTCR with 10 mL of stock buffer solution (Section 7.2.3) to remove any loose copper that may have formed within the reactor. Continue to flush until all debris is removed.

7.2.9.6 The OTCR should be stored filled with reagent water when not in use. Buffer containing Brij-35 should not be used when flushing or storing the OTCR.

7.2.10 Reduction Efficiency of the OTCR

7.2.10.1 In the OTCR, nitrate is reduced to nitrite. However, under some conditions, reduction may proceed further with nitrite being reduced to hydroxylamine and ammonium ion. These reactions are pH dependent:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \quad (1)$$

$$NO_2^- + 6H^+ + 6e^- \rightarrow H_3NOH^+ + H_2O \quad (2)$$

$$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O \quad (3)$$

7.2.10.2 At the buffered pH of this method, reaction (1) predominates. However, if the cadmium surface is overly active, reactions (2) and (3) will proceed sufficiently to give low results for nitrite.

7.2.10.3 Also, if the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite (Reference 15.2). This condition is defined as poor reduction efficiency.

7.2.10.4 To determine the reduction efficiency, run a high-level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. The reduction efficiency is calculated as shown in Equation 1.

EQUATION 1

$$PR = \frac{|N3 - N2|}{N2} \times 100$$

Where:
PR = Percent reduction efficiency
N3 = Nitrate peak height
N2 = Nitrite peak height

7.2.10.5 If the response of the nitrite is as expected but the reduction efficiency is less than 90%, then the OTCR may need to be reactivated (Section 7.2.9).

7.2.10.6 If the response of the nitrite is lower than expected and/or the reduction efficiency is greater than 110%, then the cadmium may need to be stabilized. To stabilize the reactor, analyze fifteen replicates of a high-level concentration nitrate calibrant followed by three replicates of an equivalent nitrite calibrant. The high-level nitrate calibrant will allow the cadmium reactor to stabilize by slightly reducing its activity to a more consistent level. The high-level nitrite calibrant should be compared to the

nitrate calibrant to ensure that a reduction efficiency between 90% and 110% is being obtained.

7.3 Calibrant Preparation

7.3.1 Stock 1,000 mg/L Nitrate Solution (1 L) — Dissolve 7.218 g of potassium nitrate (KNO_3) in approximately 800 mL of reagent water (Section 7.2.1) in a 1-L volumetric flask. Dilute this solution with reagent water to 1 L and preserve it by adding 2 mL of chloroform (per liter). Keep refrigerated. This solution should be stable for approximately 6 months.

7.3.2 Stock 1,000 mg/L Nitrite Solution (1 L) — Dissolve 6.076 g of potassium nitrite (KNO_2) in approximately 800 mL of reagent water (Section 7.2.1) in a 1-L volumetric flask. Dilute this solution with reagent water to 1 L and preserve it by adding 2 mL of chloroform (per liter). Keep refrigerated. This solution should be stable for approximately 6 months.

7.3.3 Working calibrants can be prepared from the 1,000 mg/L nitrate (Section 7.3.1) and nitrite (Section 7.3.2) stock solutions as follows (all dilutions should be made in cleaned volumetric flasks of the appropriate volume) using Equation 2.

EQUATION 2

$$C_1V_1 = C_2V_2$$

Where:
C_1 = *Desired concentration (in mg/L) of working calibrant to be prepared*
V_1 = *Final volume (in L) of working calibrant to be prepared*
C_2 = *Concentration (in mg/L) of stock solution (or calibrant)*
V_2 = *Volume (in L) of stock solution (or calibrant) to be used*

By solving this equation for the volume of stock solution to be used (V_2), the following equation is obtained:

$$V_2 = \frac{C_1V_1}{C_2}$$

Since the desired concentration (C_1), the final volume (V_1), and the concentration of the stock solution (C_2) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_2) is easily calculated.

7.3.4 Alternately, standard curves in the desired ranges can be derived from the table below:

Final Concentration (mg/L)	Volume of Stock Cal.	Conc. of Stock Cal. (mg/L)	Final Volume (mL)
10.0	1 mL	1,000	100
5.0	1 mL	1,000	200
0.5	100 µL	1,000	200
0.05	10 uL	1,000	200
0.01	100 µL	10	100
0.005	100 µL	10	200

8.0 Sample Collection, Preservation, and Storage

8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).

8.2 The volume of sample collected should be sufficient to ensure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.

8.3 Preserve samples with concentrated sulfuric acid (H_2SO_4) to a pH of less than 2 and keep refrigerated to 4°C from the time of collection. Do not preserve samples with mercuric chloride.

8.4 Sample analysis should be performed as soon as possible to eliminate loss of analyte. Should storage be required, preserved samples (Section 8.3) that have been refrigerated at 4° C may be held for a maximum of 28 days from the time of collection (Reference 15.7).

8.5 For analysis of nitrate or nitrite singly, samples should be refrigerated at 4°C and analyzed within 48 hours from the time of collection.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.3). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method perfor-

mance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.2. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

a) calibration (Section 10.4)

b) calibration verification (Section 9.5)

c) initial precision and recovery (Section 9.2.2)

d) analysis of blanks (Section 9.4)

e) ongoing precision and recovery (Section 9.6)

(f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) sample numbers and other identifiers

b) analysis dates and times

c) analysis sequence/run chronology

d) sample weight or volume

e) sample volume prior to each cleanup step, if applicable

f) sample volume after each cleanup step, if applicable

g) final sample volume prior to injection

h) injection volume

i) dilution data, differentiating between dilution of a sample or modified sample

j) instrument and operating conditions

k) other operating conditions

l) detector

m) printer tapes, disks, and other recording of raw data

n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.

9.1.7 Accompanying QC for the determination of nitrate and/or nitrite is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Method Detection Limit (MDL) — To establish the ability to detect nitrate and/or nitrite at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B

(Reference 15.1) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 must be achieved prior to practice of this method.

9.2.2 Initial Precision and Recovery (IPR) — To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.

9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (s) for nitrate and/or nitrite. Use Equation 3 below for the calculation of the standard deviation of the percent recovery(s).

EQUATION 3

$$S = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where:
s = *Standard deviation*
n = *Number of samples*
x = *Percent recovery in each sample*

9.2.2.3 Compare s and x with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of s exceeds the precision limit or the value of x falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD) — The laboratory shall spike, in duplicate, a minimum of 10 percent of all samples (one sample in duplicate in each batch of ten samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of nitrate and/or nitrite in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of nitrate and/or nitrite in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of nitrate and/or nitrite.

9.3.2.1 Spike this sample with the amount of nitrate and/or nitrite stock solution (Section 7.3.1 or 7.3.2) to produce a nitrate and/or nitrite concentration in the sample of 5 mg/L. If necessary, prepare another stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of nitrate and/or nitrite (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of nitrate and/or nitrite in each aliquot using Equation 4.

EQUATION 4

$$P = \frac{A - B}{T} \times 100$$

Where:
P = Percent recovery
A = Measured concentration of nitrate and/or nitrite after spiking (Section 9.3.2.2)
B = Measured background concentration of nitrate and/or nitrite (Section 9.3.2)
T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 5:

EQUATION 5

$$RPD = \left[\frac{|D_1 - D_2|}{(D_1 + D_2)/2}\right] \times 100$$

Where:
RPD = Relative percent difference
D_1 = Concentration of nitrate and/or nitrite in the spiked sample
D_2 = Concentration of nitrate and/or nitrite in the spiked duplicate sample

9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.

9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P_a - 2s_p$ to $P_a + 2s_p$. For example, if P_a = 90% and s_p = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

9.4 Laboratory Reagent Blanks (LRB) — Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.

9.4.2 If nitrate and/or nitrite is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.

9.5 Calibration Verification — Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the mid-range calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).

9.6 Laboratory Control Sample (LCS) — To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.

9.6.2 If the precision and recovery results for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for nitrate and/or nitrite by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85% to 105%.

9.7 Reference Sample — To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institutes of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-up

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical Flow Solution IV Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Nitrate/Nitrite, USEPA Cartridge is configured as illustrated in the flow diagram.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Connect the buffer and color reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.2). Start the pump at 40% speed, allowing the start-up solution to flow through the entire system.

10.2.2 Make sure that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging.

10.2.3 Once a stable flow is achieved, connect the reagent pump tubes to the appropriate reagent bottles. Allow these reagents to flow through the entire system, then, once again, verify that the flowcell of each detector is purged of all bubbles.

10.2.4 If nitrate plus nitrite is to be determined, install an activated OTCR (Section 7.2.7) onto the cartridge manifold as shown in the flow diagram. If nitrite is to be determined singly, do not install the OTCR as in the flow diagram. If operating a two-channel system, it is possible to configure one cartridge for nitrate plus nitrite and one cartridge for nitrite only.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.12) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected nitrate and/or nitrite concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.12).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Verify that the baseline does not drift and is free from large fluctuations (greater than ±50 µAu). Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the nitrate and/or nitrite stock solutions (Section 7.3.1 and/or 7.3.2) according to Equation 2, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of decreasing concentration and analyze each calibrant according to Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.6). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Load the sampler tray with calibrants, blanks, samples, and QC samples. The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.2 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the "Fast Forward" button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.

11.3 After the analysis is complete, the OTCR must be removed prior to removing the buffer solution. Remove the reactor and reconnect the tubing for the cartridge in the nitrite analysis configuration. The reactor should be flushed and stored with reagent water (Section 7.2.1).

Note: Do not allow any solutions other than reagent water and working reagents to flow through the OTCR. Some solutions may cause irreversible damage to the reactor.

11.4 After removing the OTCR, connect the buffer and color reagent pump tubes to the reagent bottle containing the start-up solution. Allow the start-up solution to flow through the complete system for a few minutes to completely flush all tubing.

11.5 Turn the pump "OFF", release the tension on all pump tubes, and power off the system when analysis is complete.

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the nitrate and/or nitrite concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve along with the relative standard deviation (RSD) of each replicate analysis.

13.0 Method Performance

Range (mg/L):	0.005–10.0 mg/L (ppm)
Method Detection Limit (MDL):	1.0 µg/L (ppb)
Calibration Curve Fit:	Weighted Linear
Sample Throughput:	60 samples/hr
Pump Speed:	40%
Sample Time:	20 seconds
Wash Time:	40 seconds
Carryover:	< 0.1%
Precision (0.005 mg/L):	< 5.0% RSD
Precision (0.05 mg/L):	< 3.0% RSD
Precision (10.0 mg/L):	< 1.0% RSD
Percent Recovery:	90–110%

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, Section 13.6 (Reference 15.4).

15.0 References

15.1 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.

15.2 Fox, J.B. *J. Anal. Chem.* **1979**, 51, 1493.

15.3 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.

15.4 *Less is Better: Laboratory Chemical Management for Waste Reduction.* Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.5 *Methods for the Determination of Inorganic Substances in Environmental Samples*; EPA/600/R-93/100; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1993; Method 353.2.

15.6 Norwitz, G.; Keliher, P.N. Study of Interferences in the Spectrophotometric Determination of Nitrite Using Composite Diazotization-Coupling Reagents. *Analyst* **1985**, *110*, 689–694.

15.7 Norwitz, G.; Keliher, P.N. Study of Interferences in the Spectrophotometric Determination of Nitrite Using Composite Diazotization-Coupling Reagents. *Analyst* **1986**, *111*, 1033-1037.

15.8 Nydahl, F. *Talanta* **1976**, 23, 349–357.

15.9 Patton, C.J. Doctoral Dissertation, Michigan State University, 1982.

15.10 *Standard Methods for the Examination of Water and Wastewater*, 14th ed.; APHA-AWWA-WPCF: Washington, D.C., 1975, 365.

15.11 *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, D.C., 1989, 4–137.

15.12 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes listed below are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols:

°C	degree Celsius
%	percent
±	plus or minus
<	less than

16.1.2 Alphabetical Characters:

g	gram
mg	milligram
µg	microgram
L	liter
mL	milliliter
µL	microliter
mg/L	milligram per liter
µg/L	microgram per liter
ppm	parts per million
ppb	parts per billion
M	molar solution
N	normal solution

16.2 Definitions:

16.2.1 Method Detection Limit (MDL) – The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

16.2.2 Minimum Level (ML) – The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.

16.2.3 Dynamic Range – The range over which the analytical system responds in any manner (not necessarily linearly) to changes in analyte concentration.

16.2.4 Interferences – Species other than the intended analyte that produce a response by the analytical system.

16.2.5 Initial Precision and Recovery (IPR) – The analysis of four aliquots of the laboratory reagent blank (LRB) spiked with the analyte(s) of interest that is used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or analytical system is modified.

16.2.6 Matrix Spike/Matrix Spike Duplicate (MS/MSD) – An aliquot of an environmental sample to which a quantity of the method analyte(s) is added in the laboratory. The MS/MSDs are analyzed using the same procedure for sample analysis, and their purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured values in the MS/MSD corrected for the background concentration.

16.2.7 Laboratory Control Sample (LCS) – An aliquot of the LRB to which a known quantity of the method analyte(s) is added in the laboratory. The LCS is analyzed using the same procedure for sample analysis, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.2.8 Laboratory Reagent Blanks (LRB) – An aliquot of reagent water or other blank matrix that is treated like a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

16.2.9 Reference Sample – Sample containing a certified quantity of the method analyte that has been prepared by an external source such as the National Institute of Standards and Technology (NIST). Analysis of a reference sample is recommended to ensure that the analytical system is in control and the results being obtained are accurate.

17.0 Figures

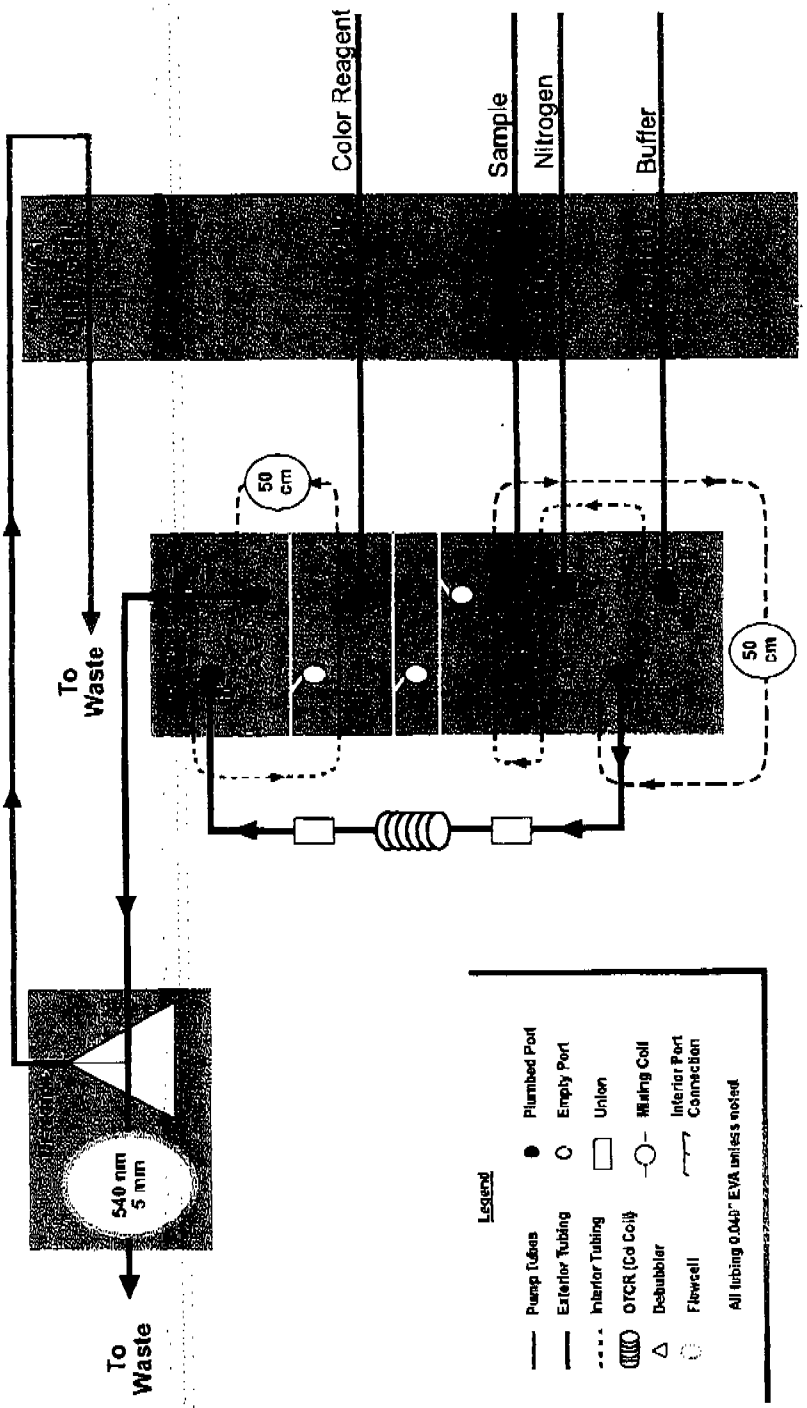


Figure 1. Detailed Flow Diagram for Nitrate+Nitrite and Nitrite Nitrogen by SFA on a Flow Solution IV, Cartridge Part #A002670

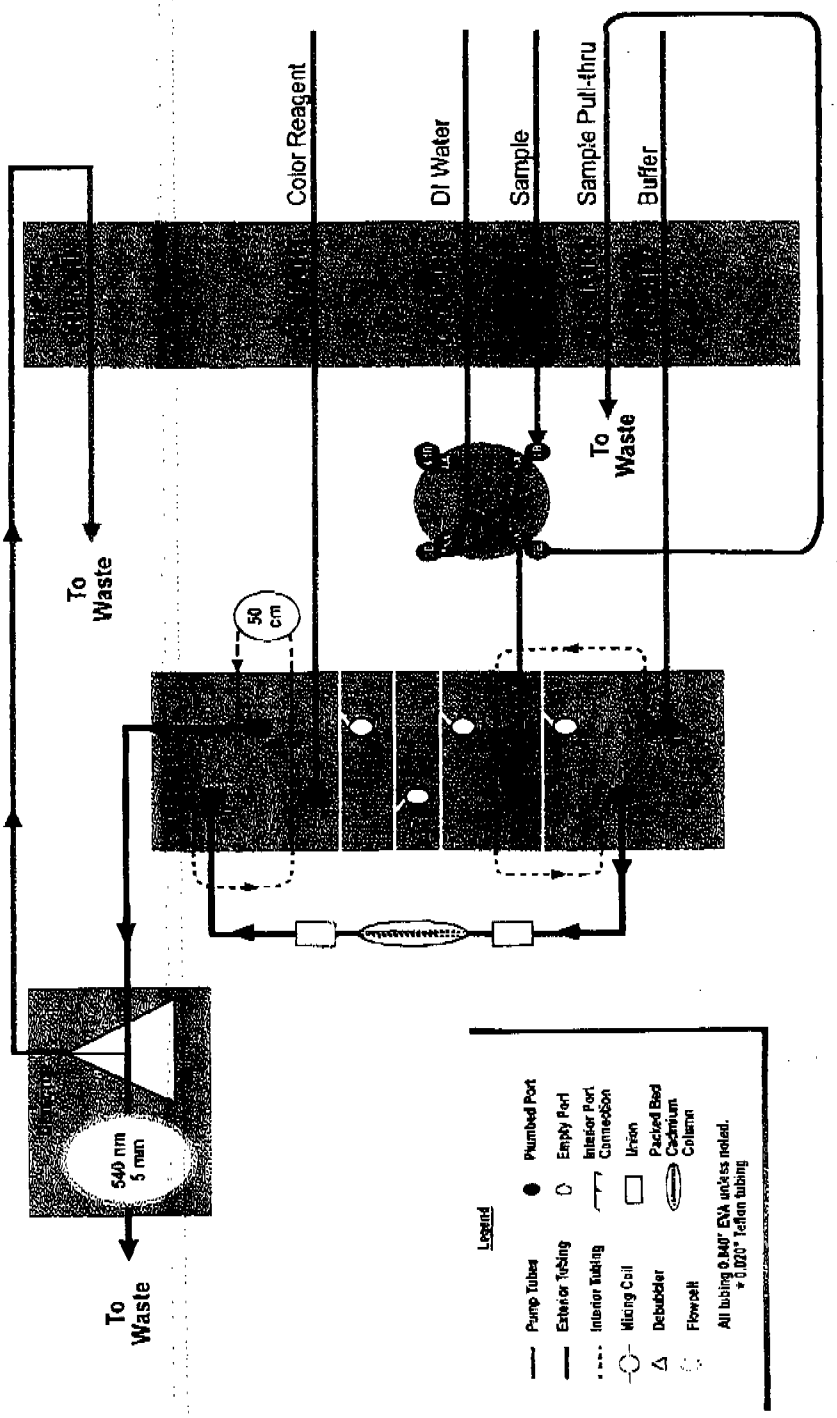


Figure 2. Detailed Flow Diagram for Nitrate+Nitrite and Nitrite Nitrogen by FIA on a Flow Solution IV, Cartridge Part #A002670

18.0 Appendix A – FIA Procedure

To use the preceding method for analysis via flow injection analysis (FIA), replace the corresponding numbered steps in Sections 7.0 and 10.0 with the following modifications:

18.1 FIA Modifications to Section 7.0

7.2.7 Granular Cadmium Column (OI Analytical Part #A21-0651-1)

7.2.7.1 In the cadmium column, nitrate is reduced to nitrite. However, under some conditions, reduction may proceed further with nitrite being reduced to hydroxylamine and ammonium ion. These reactions are pH dependent:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \quad (1)$$

$$NO_2^- + 6H^+ + 6e^- \rightarrow H_3NOH^- + H_2O \quad (2)$$

$$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O \quad (3)$$

7.2.7.2 At the buffered pH of this method, reaction (1) predominates. However, if the cadmium surface is overly active, reaction (2) and (3) will proceed sufficiently to give low results for nitrite.

7.2.7.3 Also, if the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite (Reference 15.2). This condition is defined as poor reduction efficiency.

7.2.7.4 To determine the reduction efficiency, run a high-level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. The reduction efficiency is calculated as shown below in Equation 6:

EQUATION 6

$$PR = \frac{\overline{|N3 - N2|}}{N2} \times 100$$

Where:
PR = Percent reduction efficiency
N3 = Nitrate peak height
N2 = Nitrite peak height

7.2.7.5 If the response of the nitrite is as expected but the reduction efficiency is less than 90%, then the column may need to be replaced.