SECTION SIX

WAVELENGTH SCAN

Wavelength scans can be collected in either absorbance or transmittance. The spectra are available for formatting changes which include expansion and contraction of either axis, zoom, overlaying of up to twelve spectra, overlaying of multiple functions of the same spectrum, and "Trace" to find the abscissa and ordinate values at any user-selected position.

Calculations can be made on wavelength scan data to find the peaks, valleys and/or specified points, to calculate first to fourth derivative, and to calculate the log of absorbance. Scatter correction can be performed on any spectrum. Spectral addition, subtraction, and multiplication can also be performed on stored spectra. Net absorbance calculations can be made on any stored spectrum using either one or two baseline points.

6.1 Principles of Operation

Blank - Background Data

The background data are collected when the instrument is blanked. A cuvette filled with the solvent used to prepare the samples should be in the sample compartment when the blank is read.

The blank data are stored in the instrument and can be reused for an unlimited number of sample spectra. A new blank should be read every time a solvent is changed. For optimum performance, a new blank should also be read if the instrument has not been used for over an hour.

For very high precision data, the same cuvette should be used for the blank and sample spectra, as even matched cuvettes have a slight mismatch which can be seen by the DU Series 600 Spectrophotometer. The blank should be rerun for every sample.

Sample Data

After the sample data are collected, the blank is subtracted and the difference in absorbance (or ratio in transmittance) is plotted on the display and stored in the instrument memory. The data points are connected.

Repetitive Scanning

Repetitive scans can be made by inputting more than one for the "scans per sample" and setting the "interval time" to the desired value. The interval time is the time between the start of successive scans. The minimum interval time is 0.1 seconds.

Derivative

First to fourth derivative can be calculated and displayed on any absorbance spectra using the Savitzky and Golay[1] coefficients. Two data points before and two data points after the point of interest are used to calculate the derivative. (For the fourth derivative, only, three points before and after the point of interest are used.) This calculation is done for every data point in the spectrum to determine the derivative spectrum.

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[1]Savitzky, A., and Golay, M., *Anal Chem* **36**, p1627f (1964).

Log of Absorbance

The log base 10 is calculated and displayed for absorbance spectra. Because the log of 0 is negative infinity, if some data points in the spectrum are zero or are very close to zero, the log spectrum may not be usable in these regions.

Peak/Valley Pick

The most intense peaks and/or valleys are found and displayed with the spectral data when the peak pick calculation is selected. These are found by calculating the first derivative as described above. When the sign of the derivative changes from positive to negative, a peak is detected. A valley is detected when the sign of the derivative changes from negative to positive. Up to 30 peaks (valleys) can be input. If the instrument finds more than the input number of peaks (valleys), the most intense ones will be tabulated. Each time that the wavelength limits or the smoothing function is changed, the instrument performs the peak pick calculations again.

Point Pick

Point pick is a tabulation of data at up to 12 user-selected wavelengths.

Trace

The actual stored data points are accessed using "Trace".

Smoothing

The displayed wavelength scan can be smoothed using a selectable smoothing function. The calculation, using the Savitzky and Golay[1] method (as modified for end points by Peter A. Gorry[2]), is done for every data point in the scan, using the data points before and after the point of interest. The user selects the total number of data points used for the calculation, from 7 to 25. Use of too few points may not reduce the spectral noise to the desired level, while use of too many points can cause real peaks to be flattened and/or combined.

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[2]Gorry, Peter A., Anal Chem, **62**, 1990, p570f.

Addition/Subtraction/Multiplication - (+-* Scans)

Stored spectra can be added, subtracted or multiplied, using the equation:

$$\text{Scan} = (\text{scan A} \times F1) \; +/-/* \; (\text{scan B} \times F2) \; +/-/* \; F3,$$

where scan A and scan B are stored spectra, F1 and F2 are factors which are multiplied by each data point in scan A and scan B, respectively. F3 is an additional value that is added/subtracted/multiplied to each point in the spectrum.

Scatter Correction (Spectral)

Turbid samples, rough samples or particles in a sample cause light from the source to be scattered, so that it does not reach the detector. In the spectrum of a sample with scattering particles, the scattered light appears as increased absorbance with decreasing wavelength. If two points in the spectrum can be identified, where the observed absorbance is entirely a result of scatter, a spectrum of the scatter can be estimated and subtracted from the sample spectrum to produce a sample spectrum in the absence of scatter.

The equation used to estimate the scatter is:

$$A_s = a \, \lambda^b,$$

where,

A_s is the absorbance due to scatter at a particular wavelength, and a and b are constants.

For the scatter correction to be accurate, it is necessary to correctly specify two wavelengths where the observed absorbance is entirely a result of scatter. These points should be at least 50 to 100 nm apart for the most accurate correction.

The spectrum due to scatter and the corrected sample spectrum are displayed, along with the constants "a" and "b".

Net Absorbance Calculations

It is possible to correct a sample absorbance peak for a raised or sloping baseline, which may be caused by turbidity in the sample, using net absorbance calculations. Net absorbance calculations can also correct for small differences in cuvettes caused by slight uncleanness, as shown in Beckman publication T-1548.

The user can choose either one or two baseline points for the calculation. If one baseline point is chosen, the absorbance at the base wavelength is subtracted from the absorbance at the peak wavelength to provide the net absorbance. The base wavelength can be either to the right or left of the peak wavelength.

If two baseline points are chosen, a straight line is drawn between the absorbance values at two base wavelengths. The absorbance of that line at the peak wavelength is subtracted from the absorbance of the sample at the peak wavelength to provide the net absorbance. The base wavelengths can be on either side of the peak wavelength, or can both be on the same side.

Scan Files

Spectral data are stored in a Scan file. Each Scan file contains the spectral data from one sample. The Scan file is named using up to five characters, allowing the instrument to append the file name with three characters. These characters are numbers, starting with 001. For example, if the Scan file is named "SCANS", the data from the first sample will be stored in file "SCANS001". The data from the second sample will be stored in file "SCANS002". Data from up to 999 samples can be stored with the same file name.

If "Autosave" is enabled, the data for every sample is stored automatically, using the input Scan file name. If "Autosave" is not enabled, the data for each sample is stored in a temporary file named "WORK_00X". When both "Autosave" and "Autoprint" are disabled, scans from up to twelve samples are stored in this temporary manner; then the scans must be cleared or saved before additional scans can be collected.

6.2 Parameter Setup

Click on "**WAVELENGTH SCAN**" from the Main window to start the analysis. The Wavelength Scan window, Figure 6-1, is displayed. The Wavelength Scan window is used to select analysis parameters, collect spectral data and display spectral data. Multiple functions of the sample (i.e. absorbance and first derivative) are displayed in this window. Peak pick, valley pick, and point pick values are also displayed.

NOTICE

Use the RediScan mode to collect and display spectral data with minimum parameter setup.

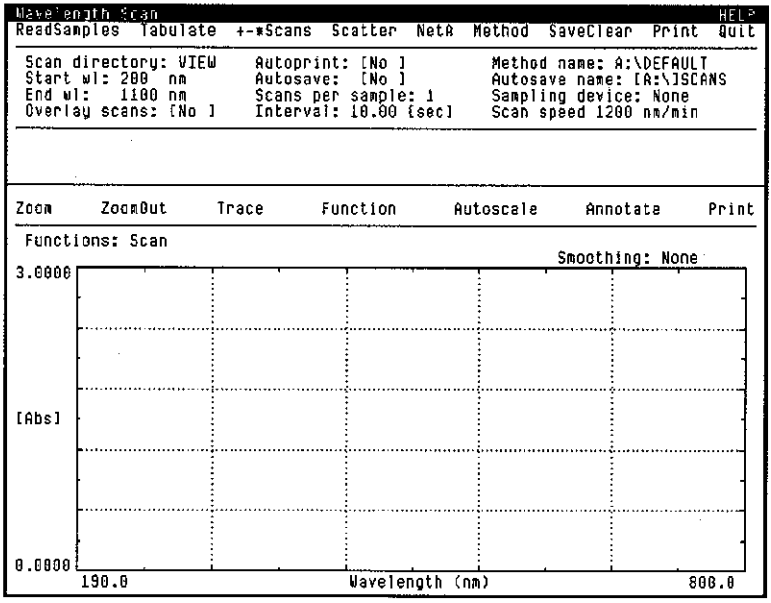

Figure 6-1. Wavelength Scan Window

Use the Method window to setup the analysis parameters:

1. Click on **<Method>** to display the Method window, Figure 6-2. The Method window is used to setup analysis parameters, recall stored methods and create new methods. General information on method windows is provided in section 3.8.

2. To recall a stored method, click on the desired method name in the listing at the top of the Method window.

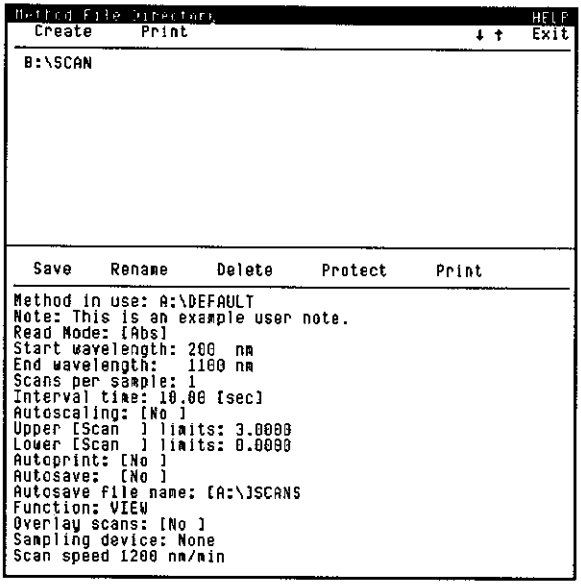

Figure 6-2. Method Window

3. The analysis parameters are displayed on the lower part of the Method window. Input the desired analysis parameters:

   Method in use - This displays the name of the method that has been selected. If the method is protected, **PROTECTED** is displayed following the method name. If the method is protected, the analysis parameters cannot be changed. To input a new method name, click on **<Create>**.

   Note - Click on to input a 40-character message.

   Read mode - Toggle between **[Abs]** and **[%T]** to select readings in absorbance or transmittance, respectively.

   Start / End wavelength - Click on to input the starting (lower), then the ending (higher) wavelength.

   Scans per sample - For repetitive scanning, input the total number of times each sample is to be scanned. If the Auto Cell Holder or Batch Sampler is selected for the "Sampling device", the input for this parameter is set to 1.

   Interval time - For repetitive scanning, input the time between the start of successive scans, in either seconds or minutes. The

minimum interval time is dependant upon the wavelength range and whether "Autosave" and/or "Autoprint" are enabled.

Autoscaling - Autoscaling adjusts the ordinate axis to display the highest peak full-scale. Toggle between **[Yes]** and **[No]** to enable or disable autoscaling.

Upper / Lower limit - Input the upper and lower limits for the ordinate axis display of the spectral data. If autoscale is enabled, the displayed values are ignored.

Autoprint - Toggle between **[Yes]** and **[No]**. If enabled, each spectrum is printed automatically on the Dot Matrix Printer after data collection is complete.

Autosave - Toggle between **[Yes]** and [No]. If it is enabled, each scan is stored automatically as it is collected using the input "Autosave file name".

Autosave file name - If autosave is enabled, input a five-character prefix for the file name.

Function - The Function window is used to select derivative or log spectral display and to select peak, valley and/or point pick tabulation. Click on "**VIEW**" to display the Function window. Refer to section 6.6 for more information. (On the Wavelength Scan window, click on **<Function>** in the menu bar to display the Function window.)

Overlay scans - Toggle between **[Yes]** and **[No]**. If it is enabled, multiple scan files can be overlaid. If it is disabled, only one scan file can be displayed.

Sampling device - Display the Sampling Device window to select the sampling device.

Scan speed - Select from [2400], [1200], [600], [240] and [120] to set the scan speed in nanometers per minute.

3. To store the analysis parameters in the selected method file, click on **<Save>**.

4. Click on **<Exit>** to display the Wavelength Scan window with the parameters from the selected method.

To input the desired parameters on the Wavelength Scan window:

1. The analysis parameters are listed near the top of the window. To change any of these, click on the parameter and input the desired value. A description of the parameters is provided above.

2. The ordinate limits are input by clicking on the displayed values on the graph and inputting the desired values.

3. Calculations (derivative, peak pick, point pick, etc.) performed on the data are selected by clicking on **<Function>** to display the Function window. Refer to section 6.6 for more information.

6.3 Analysis of Single Samples

After the desired parameters are displayed on the Wavelength Scan window, samples can be run.

1. Place a cuvette of solvent in the cell holder. Click on **<<BLANK>>**. The instrument blanks on the solvent.

   Sipper - Aspirate and read the substrate by pressing {**FILL/BLANK**}.

   NOTICE

   If the solvent (blank solution) has significant absorbance in the wavelength region of interest, the dynamic range of the instrument can be increased by adjusting the scan gain for the blank. Instructions are provided in section 2.5.

2. Place a cuvette of sample in the cell holder and click on **<Read-Samples>**. (As an alternative, click on the right mouse button with the cursor in any position.)

   Sipper - Aspirate and read each sample by pressing {**FILL/READ**}. Flush or return each sample before reading the next.

3. The sample is scanned and the data is displayed. The data may be stored or printed automatically, based upon the selections for "Autosave" and "Autoprint". A typical Wavelength Scan window with data from a single sample is shown in Figure 6-3.

4. If "Autoprint" is disabled, click on **<Print>** to print the sample data. The data are plotted on the Dot Matrix Printer unless the X-Y Plotter is installed and selected for "**Send graph to**" in the Printer and Plotter Configuration window.

5. The data can be manipulated using the instructions provided in section 6.5. **<Function>** can be used to display additional functions (derivative, peak pick, point pick, etc.) using the instructions provided in section 6.6.

6. If "Overlay" is enabled, click on **<SaveClear>** to remove the data from previous sample(s) before scanning the next sample, if desired.

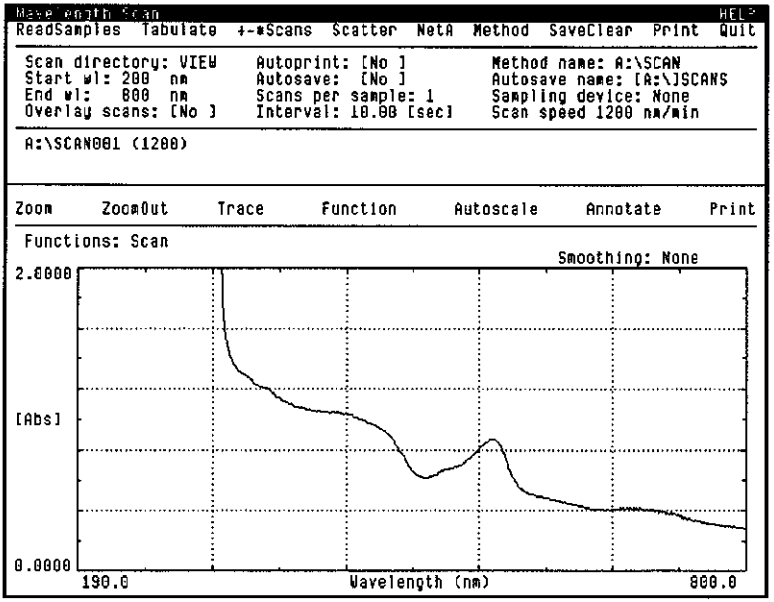

Figure 6-3. Wavelength Scan Window

7. To scan the next sample, repeat steps 2 to 6.

   The Save Clear window is displayed after 12 samples are read, unless "Autosave" or "Autoprint" are enabled. The scan data must be stored or cleared before additional samples can be scanned.

8. To exit the Wavelength Scan mode when all spectra have been collected, click on **<Quit>**. The Quit window is displayed so the method and/or sample data can be stored. Then the Main window is displayed.

6.4 Analysis of Multiple Samples

Wavelength scanning analysis of multiple samples can be automated with the use of a multi-position Auto Cell Holder or one of the batch sampler. When analyzing multiple samples, special considerations should be given to the selection of the optimum analysis parameters.

If an Auto Cell Holder is used, the data can be stored automatically by setting "**Autosave**" to **[Yes]** and inputting a file name, or the user has the option of storing the data after they are collected. Regardless of whether they are stored with a permanent file name, they are stored with a temporary identification and can be manipulated after the analysis is complete.

If the batch sampler is used, "**Autosave**" can also be used to store the data, if sufficient memory exists. However, if "**Autosave**" is not enabled, the scan data are not stored and therefore the data must either be printed or output to the communications port as they are collected.

To print the data as they are collected, set "**Autoprint**" to **[Yes]** and verify that the Dot Matrix Printer is selected for "**Send graph to**" in the Printer and Plotter Configuration window. To format the data in the desired manner, the following should be considered:

If the data are to be plotted individually, set "**Overlay**" to **[No]**. To obtain the optimum plot, set "**Autoscaling**" to **[Yes]**. Peak pick can be selected on the Function window and printed with the spectral data.

If the data are to be overlaid, set "**Overlay**" to **[Yes]**. "**Autoscaling**" can also be set to **[Yes]** to obtain the optimum presentation. Peak, valley, and point pick cannot be selected. Spectra from 12 samples will be overlaid on a single plot, unless less than 12 samples were analyzed.

After the desired parameters are displayed on the Wavelength Scan window, samples can be run.

1. To read the blank:

   Auto Cell Holder - Place the a cuvette of blank in the cell position that is in the light beam, then click on **<<BLANK>>**. The instrument blanks on the solvent.

   Sipper/Batch Sampler - Aspirate and read the blank by pressing **{FILL/BLANK}**. The instrument blanks on the solvent.

NOTICE

If the blank solution has significant absorbance in the wavelength region of interest, the dynamic range of the instrument can be increased by setting the instrument gain for the blank. Instructions are provided in section 2.5.

2. To read the samples:

   Auto Cell Holder - Place the input number of samples in the sampler and click on **<ReadSamples>**.

   ISCO Sampler - Load the samples into the batch sampler, starting with the tube directly under the aspirator arm. Click on **<ReadSamples>**. The samples are read until the last position of the red rack is read.

   Gilson Sampler - Program the Sample Controller with the rack type, number of tubes and orientation of the tubes. Load the samples in the programmed configuration. Click on **<ReadSamples>**. The analysis stops when the programmed number of tubes are read.

3. The samples are scanned and the data are displayed. The data may be stored or printed automatically, based upon the selections for "Autosave" and "Autoprint". A typical Wavelength Scan window with overlaid data from multiple samples is shown in Figure 6-4.

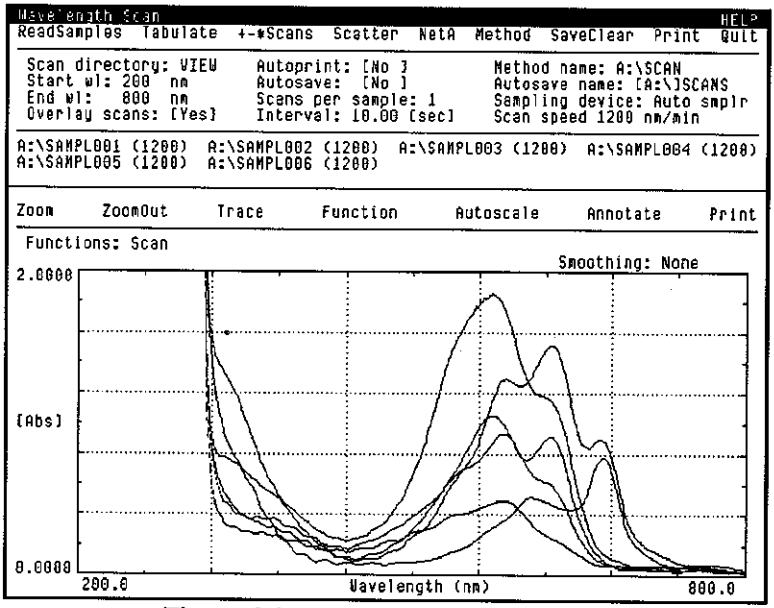

Figure 6-4. Wavelength Scan Window

4. If the data are stored, they can be manipulated using the instructions provided in section 6.5. **<Function>** can be used to display additional functions (derivative, peak pick, point pick, etc.) using the instructions provided in section 6.6.

5. If the Auto Cell Holder is used for the sampling device, and both "Autosave" and "Autoprint" are disabled, the Save Clear window is displayed after 12 samples are read. The scan data must be stored or cleared before additional samples can be scanned.

6. To exit the Wavelength Scan mode when all spectra have been collected, click on **<Quit>**. The Quit window is displayed so the method and/or sample data can be stored. Then the Main window is displayed.

6.5 Data Manipulation

To display stored scan file(s):

1. Before displaying stored scans, clear the display by clicking on **<SaveClear>**.

2. Verify that the desired "**Overlay**" selection is displayed.

3. To select the stored Scan file(s), click on "**VIEW**" following "**Scan directory**" to display the Scan Directory window.

4. Click on to highlight the desired file name(s), then click on **[OK]** to remove the directory window and display the scans. If "Overlay" is enabled, up to 12 file names can be selected. Once the scans are displayed, they can be manipulated using the instructions in this and the following section.

5. The scan speed is displayed following the scan file name on the Scan window.

The presentation of the spectral data can be optimized using the following features:

Ordinate label - The ordinate can be changed from absorbance to transmittance by clicking on the label.

Limit changes - The limits on the ordinate and/or wavelength axes can be changed by clicking on the displayed value and inputting the desired value.

Zoom - The "zoom" feature can be used to expand any portion of the graph. Click on **<Zoom>**, then click on two points on the graph to place crosses at the opposite corners of the area to be enlarged. When the second cross is clicked on, the graph is replotted. This can be repeated as often as desired. To return to the original plot, click on **<ZoomOut>**.

Trace - "Trace" can be used to display the ordinate value and the wavelength at any point on the plot. Click on **<Trace>**. Then move the arrow to the point of interest on the plot and click on the center mouse button to place a vertical line on the spectrum. The values at the place where the vertical line is placed are displayed in the upper right-hand side of the window. To move the vertical line to either the right or left, click on the right or left mouse button, respectively, with the arrow placed anywhere on the plot. To move to a different position on the spectrum, position the

arrow and click on the center mouse button. An asterisk after the reading indicates that it is out of range.

NOTICE

The digital data at all wavelengths are displayed in the Tabulate window.

Autoscale - Automatically scale the ordinate axis. This command does not change the wavelength limits.

Annotate - The data can be annotated by clicking on **<Annotate>**. Then, click on the graph to position a cross and input information from the alphanumeric keypad or keyboard. Up to four annotations can be placed on the graph. The annotations are printed with the window, but are not stored with the data.

Smoothing - Selection of the number of data points to use for smoothing affects the peaks that are picked by the instrument. If too few points are used, extraneous or insignificant peaks are picked. If too many points are used, real peaks are flattened or lost. Click on the displayed smoothing value and input different values, visually examining the data to determine the optimum smoothing value.

6.6 Function Selection

The Function Selection window, Figure 6-5, is used to select derivative or log spectral display and to select peak, valley and/or point pick tabulation. It is displayed when **<Function>** is clicked on from the Wavelength Scan window, or "**VIEW**" from the Method window.

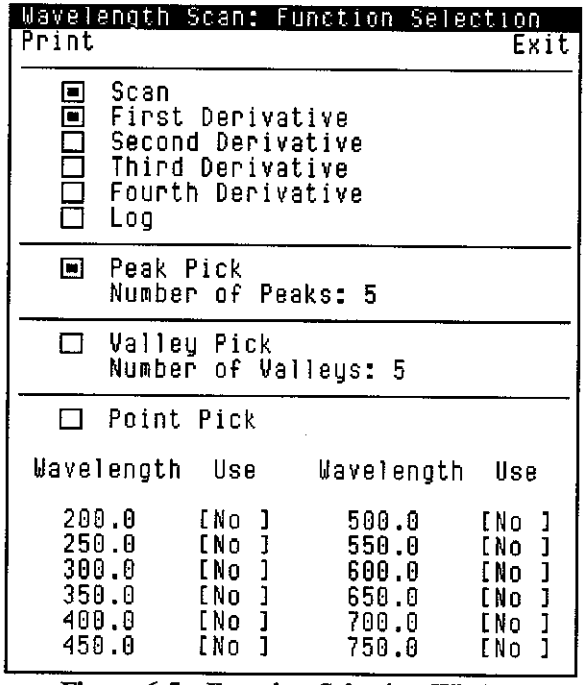

Figure 6-5. Function Selection Window

To select the desired function(s):

1. Verify that the data from the desired sample(s) are displayed on the Wavelength Scan window.

2. Click on **<Function>** to display the Function Selection window.

3. Click on to darken the box to select the desired function(s).

   Derivative and log - Click on to select the desired information to be plotted for the selected scan file. If "Overlay" is disabled, any number of selections can be made. If "Overlay" is enabled, only one selection can be made.

Peak / Valley Pick - Click on to select the feature and input the number of peaks and/or valleys to pick.
Range: 1 to 30 Default: 5

Point Pick - Click on to select the feature. Click on the displayed wavelength value(s) to input the desired value(s) and click on to change the "Use" column to **[Yes]**.

4. When the selections are made correctly, click on **<Exit>** to remove the window from the display.

5. Example data, plotted with the selections in the Function Selection window (Figure 6-5), are shown in Figure 6-6.

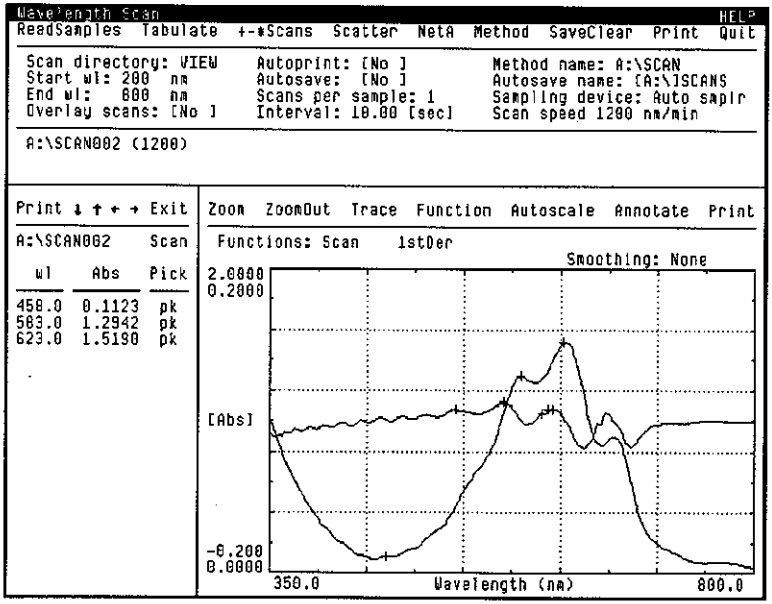

Figure 6-6. Wavelength Scan Window with Multiple Functions

The ordinate axis labels for the different selections are stacked on the left-hand side of the plot. The labels can be changed using the instructions in section 6.5.

The peak pick data for the absorbance scan is displayed. To display the peak pick data for the first or second derivative, click on **<→>**, located in the menu bar above the peak pick data.

6.7 Tabulated Data

To print the absorbance value and corresponding wavelength at each point in the spectrum, click on **<Tabulate>**. The first 11 data points from a typical spectrum are shown in Figure 6-7.

Tabulation for Scan File: A:\SCAN001

wl	Abs
200.0	0.1122
201.0	0.1036
202.0	0.0965
203.0	0.0904
204.0	0.0829
205.0	0.0757
206.0	0.0691
207.0	0.0630
208.0	0.0574
209.0	0.0526
210.0	0.0485

Figure 6-7. Tabulated Scan Data

6.8 Spectral Addition, Subtraction and Multiplication

Spectra that have been stored can be scaled using a factor, then can be added, subtracted or multiplied. The +-* Scans window, Figure 6-8, is used to select these calculations. It is displayed when **<+-*Scans>** is clicked on from the Wavelength Scan window.

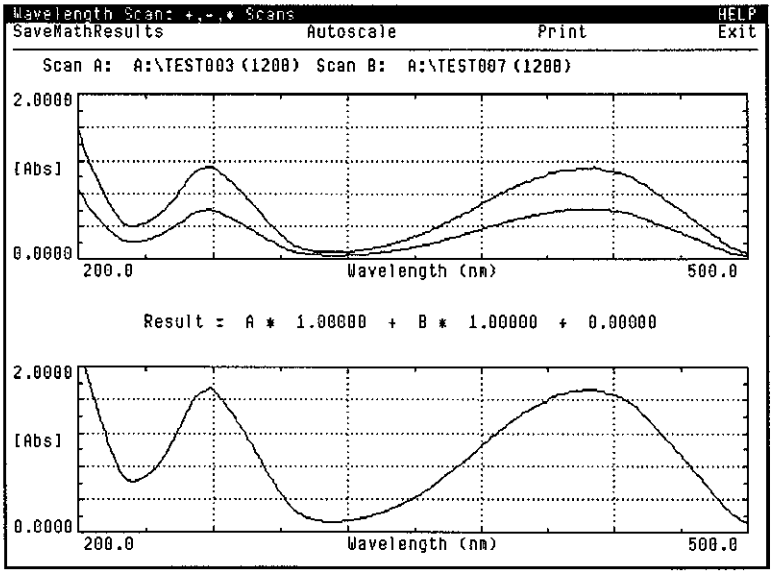

Figure 6-8. +-* Scans Window

The +-* Scans window has two graphic portions. The upper graph is used to display the raw data for the two spectra. The lower graph displays the resultant spectrum.

To perform the manipulation:

1. To input the file name for the first spectrum, A, click on "**Scan A**". The Scan Directory window is displayed. Click on the desired file, then **[OK]**. The spectrum is displayed.

2. Repeat step 1 to select the file for scan B.

3. The format of the spectra can be changed by clicking on the ordinate label and the ordinate and wavelength axis limits, and inputting the desired values.

4. To input the calculation parameters, click on "**Result**" in the middle of the window to display the +-* Parameters window, Figure 6-9.

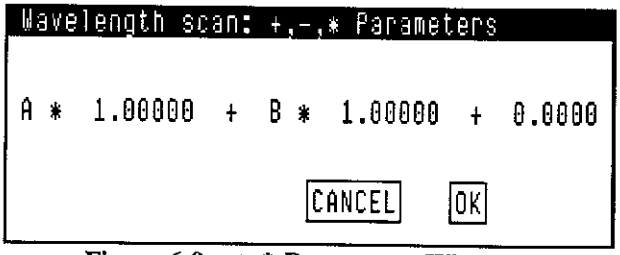

Figure 6-9. +-* Parameters Window

5. Three factors can be input in the window. These are a scaling factor for Scan A, a scaling factor for Scan B, and an additional value that is added to (subtracted from or multiplied by, depending upon sign) the results. Click on each of these factors and input the desired value.

6. Whether the spectra are added, subtracted or multiplied is determined by the sign. Click on to toggle between + (for addition), - (for subtraction), and * (for multiplication).

7. When the desired parameters are displayed, click on **[OK]** to use the equation and remove the +-* Parameters window from the display.

8. The equation and the resultant spectrum are displayed in the lower graph. The format of the spectrum can be changed by clicking on the function, ordinate or wavelength axis limits and inputting the desired values.

9. To store the resultant spectrum, click on **<SaveMathResults>** to display the Save Results window. Input the desired file name, then click on **[OK]** to remove the window and store the data.

10. To print the window, click on **<Print>**.

11. To exit the +-* Scans window and return to the Wavelength Scan window, click on **<Exit>**.

6.9 Scatter Correction

The Scatter window, Figure 6-10, is used to correct a stored spectrum for scatter. It is displayed when **<Scatter>** is clicked on from the Wavelength Scan window.

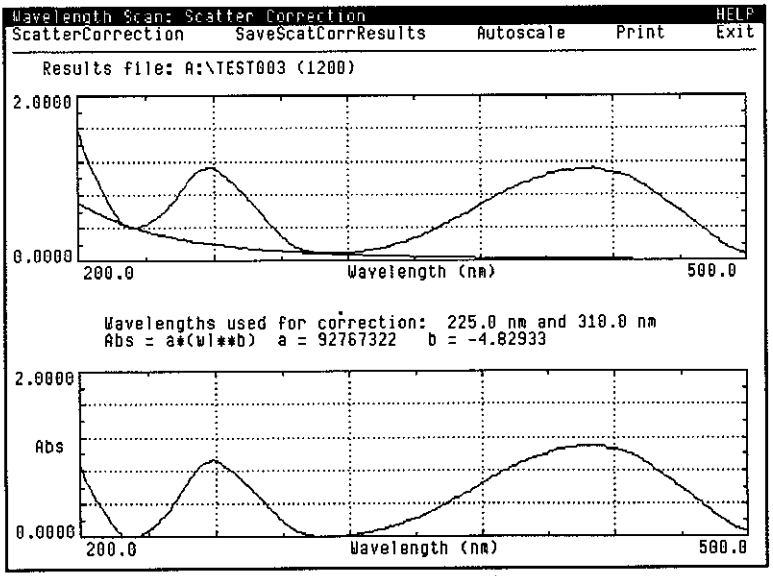

Figure 6-10. Scatter Window

The Scatter window has two graphic portions. The upper graph is used to display the uncorrected spectrum and the scatter component. The lower graph displays the spectrum corrected for scatter.

To correct for scatter:

1. Click on "**Scan file**". The Scan Directory window is displayed. Click on the desired file, then click on **[OK]**. The spectrum is displayed on the upper graph.

2. The format of the spectrum can be changed by clicking on the function, ordinate or wavelength axis limits and inputting the desired values.

3. Click on **<ScatterCorrection>**, then click on two points in the spectrum to place crosses where there is no sample absorption; that is, where all apparent absorption is due to scatter. When the second cross is clicked on, the calculation is performed. The scatter spectrum is displayed in the upper graph, the corrected sample spectrum is

displayed in the lower graph, and the equation for scatter correction is displayed in the middle of the window, along with the constants used to calculate the correction.

As an alternative, the wavelength values in the center of the window can be clicked on and new wavelength values input.

4. The format of the spectrum can be changed by clicking on the function, ordinate or wavelength axis limits and inputting the desired values.

5. To store the resultant spectrum, click on **<SaveScatCorrResults>** to display the Save Results window. Input the desired file name, then click on **[OK]** to remove the directory window and store the data.

6. To print the window, click on **<Print>**.

7. To exit the Scatter Correction window and return to the Wavelength Scan window, click on **<Exit>**.

6.10 Net Absorbance Calculations

The Net Absorbance Calculations window, Figure 6-11, is used to calculate net absorbance from a stored spectrum. Either one or two baseline points can be used. It is displayed when **<NetA>** is clicked on from the Wavelength Scan window.

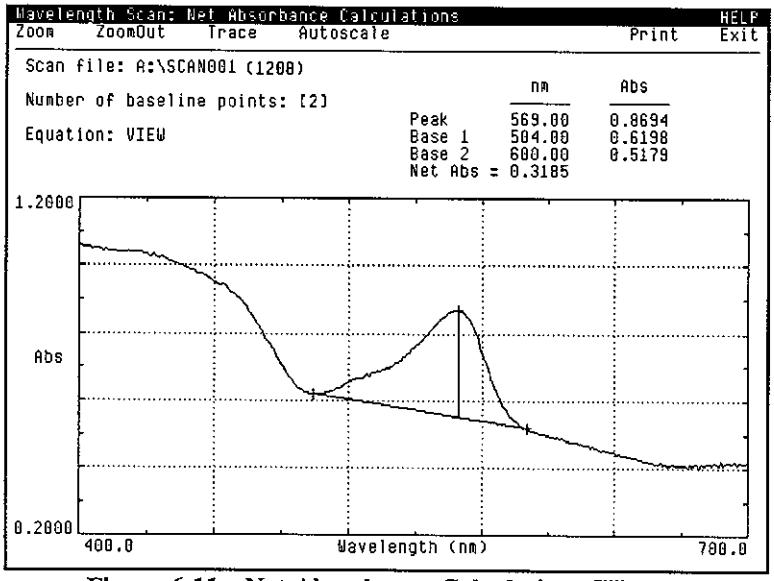

Figure 6-11. Net Absorbance Calculations Window

To calculate net absorbance:

1. Click on "**Scan file**". The Scan Directory window is displayed. Click on the desired file, then click on **[OK]**. The spectrum is displayed.

2. The format of the spectrum can be changed by clicking on the absorbance or wavelength axis limits and inputting the desired values. **<Trace>** and **<Zoom>** can also be used. Refer to section 6.5 for detailed instructions.

3. Select the desired number of baseline points by clicking on the value following "**Number of baseline points**" to change from [1] to [2].

4. If desired, the equation can be displayed by clicking on "VIEW" following "**Equation**".

5. The wavelength values for the peak and the baseline point(s) are displayed in a table at the top of the window. To input the desired wavelengths:

   a. Click on "**Peak**", "**Base 1**", or "**Base 2**" to select the desired point.

   b. Use the mouse buttons to position the "Trace" indicator at the desired wavelength on the graph:

      Center mouse button - move to the data point closest to the position of the arrow.

      Right mouse button - with the arrow positioned anywhere on the graph, move one data point to the right.

      Left mouse button - with the arrow positioned anywhere on the graph, move one data point to the left.

   The position of the "Trace" indicator line is shown in the table. As an alternative, the desired wavelength can be input in the table by clicking on the displayed wavelength value and inputting the desired wavelength value.

   c. Repeat steps a and b until the desired wavelengths are displayed.

   d. Click above the graph on a blank part of the display to remove the trace line and accept the displayed wavelengths. The net absorbance value is calculated, and the lines indicating the points used in the calculation are placed on the spectrum.

6. Examine the data to verify that the desired points were selected. If desired, change any of the points, using the instructions in step 5, above.

7. To print the window, click on **<Print>**.

8. To exit the Net Absorbance Calculations window and return to the Wavelength Scan window, click on **<Exit>**.

6.11 Example Analyses

EXAMPLE 1

Obtain a spectrum of a purified protein sample. Find the absorbance maxima and tabulate the absorbance data at 230, 260, 280, and 320 nm. Store the sample spectrum in file "A:\BSA001".

SOLUTION

Collect the data on the Wavelength Scan window, as shown in Figure 6-12. Use the Function window to select Peak Pick and input the four wavelengths for Point Pick. Be sure to change the "Use" column to **[Yes]**. Use **<<PrtScrn>>** to print the window, so that the point pick data are on the same printout as the spectral data.

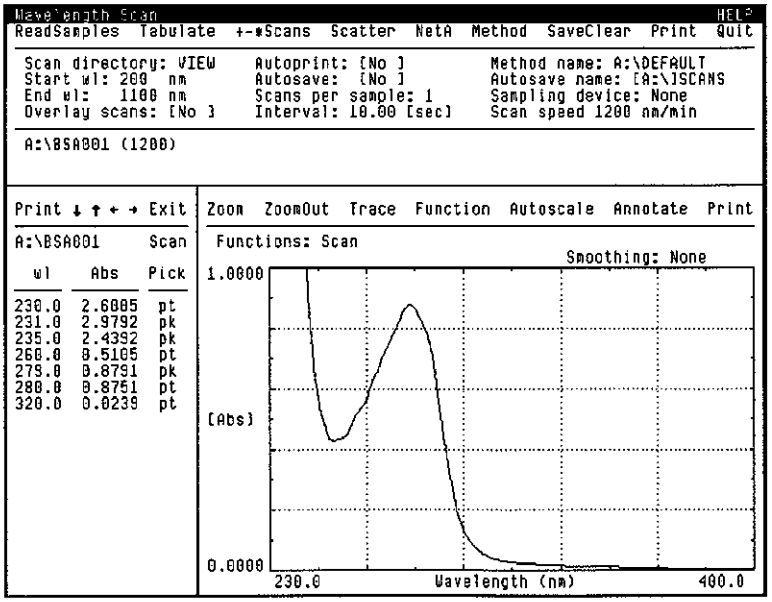

Figure 6-12. Sample Spectral Data with Point Pick

EXAMPLE 2

Display the spectrum shown in Example 1 with the first derivative overlaid.

SOLUTION

With the spectra displayed in Figure 6-12, click on **<Function>** to display the Function window. Click off **"Peak Pick"** and **"Point Pick"**, and click on **"1 Der"**. The overlaid spectra are shown in Figure 6-13. The absorbance axis limits are 0.0 to 1.0. The first derivative axis limits are -0.5 to 0.5.

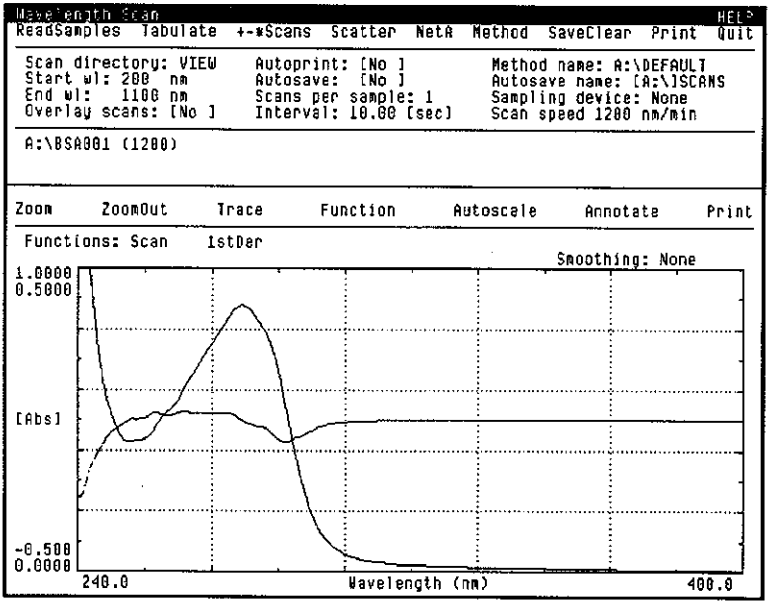

Figure 6-13. Overlaid Multifunction Spectra

EXAMPLE 3

An analgesic sample contains 12.5 µg/mL caffeine. Subtract the caffeine from the sample spectrum. The sample spectrum is file "A:\SAMP001". The caffeine spectrum (25 µg/mL) is file "A:\CAFFN001".

SOLUTION

Use the +-* Window, shown in Figure 6-14. Input Scan A as "A:\SAMP001" and Scan B as "A:\CAFFN001". In the +-* Parameters window, input a factor of 1 for A, a factor of 0.5 for B, add a factor of 0, and set the first sign to "-".

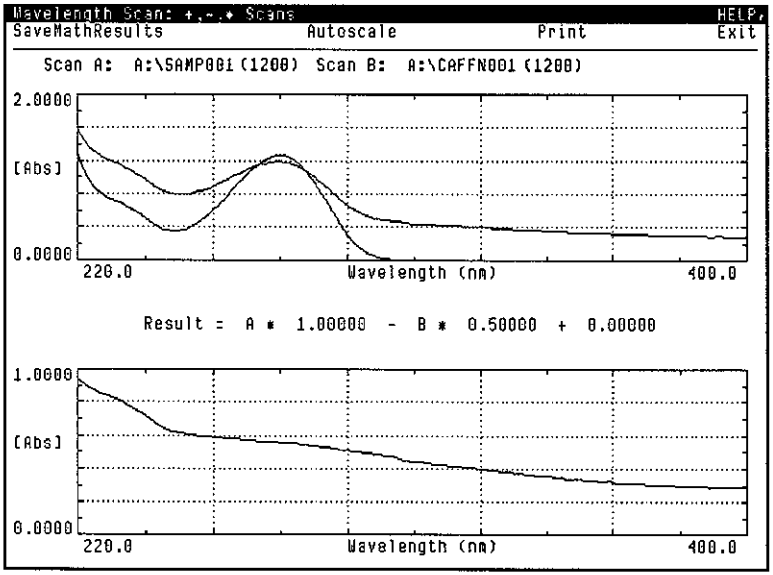

Figure 6-14. Component Subtraction

EXAMPLE 4

The resulting spectrum in Example 3 looks like scatter. Perform scatter correction on the sample spectrum.

SOLUTION

With the +-* Window displayed, click on **<Exit>** to display the Wavelength Scan window. Then click on **<Scatter>** to display the Scatter Correction window shown in Figure 6-15. Input the Scan file "A:\SAMP001". Click on **<ScatterCorrection>** and input wavelengths of 350 and 400 nm. The spectrum corrected for scatter is displayed on the lower part of the window.

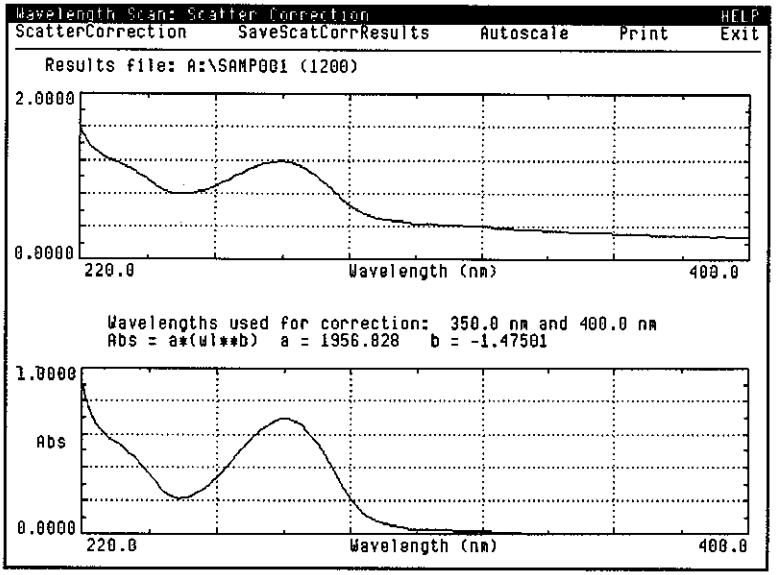

Figure 6-15. Caffeine Sample Corrected for Scatter

6.12 Data Output

Spectral data are output to the Communications port whenever an Output Data Type is selected in the Communications Configuration window. The type of data output is selected: "raw and calculated", "raw", or "calculated". General information on data output is provided in section 9.3.

In the Wavelength Scan mode, the same data are sent, regardless of which output type is selected. The data output is the wavelength, followed by the reading in either absorbance or transmittance, for the entire wavelength range.

The following is the output obtained for a scan from 200 to 210 nm in absorbance. The "User ID" is input in the Communications Configuration window. The "Method ID" is the method name. A <carriage return> and <line feed> are sent at the end of every line.

```
User ID: DU600
Method ID: A:\SCAN
Date: 10\09\91
Time: 10:38
 200.000     0.112
  201.000     0.103
  202.000     0.096
  203.000     0.090
  204.000     0.082
  205.000     0.075
  206.000     0.069
  207.000     0.063
  208.000     0.057
  209.000     0.052
  210.000     0.048
```

6.13 Files

Two file types are created in the Wavelength Scan mode: method files and scan files. The method files are stored in the SCAN_M directory with the .APX extension. Method files are ASCII files and cannot be converted to the Lotus format.

The scan files are stored in the SCAN_D directory with the .SCN extension. They contain scan parameters and data at every wavelength in the scanned range.

6.14 ASCII Format

The ASCII file for a wavelength scan consists of two parts: analysis parameters and sample data.

The sample data include the wavelength, followed by the reading in either absorbance or transmittance, for the entire wavelength range.

The following ASCII file was converted from an absorbance scan from 200 to 210 nm.

```
Start Wavelength:    200.0000
End Wavelength:    210.0000
Read Mode:  ABS
Read Average:      0.1000
Nanometer Interval:      1.0000
Time:      0.0000

  200.0000      0.1122
  201.0000      0.1036
  202.0000      0.0965
  203.0000      0.0904
  204.0000      0.0829
  205.0000      0.0757
  206.0000      0.0691
  207.0000      0.0630
  208.0000      0.0574
  209.0000      0.0526
  210.0000      0.0485
```

6.15 Lotus Format

The Lotus file for a wavelength scan consists of two parts: analysis parameters and sample data.

The sample data start in row 11 and are stored in the following columns:

Column A	Wavelength
Column B	Reading in either absorbance or transmittance

The following Lotus file was converted from an absorbance scan from 200 to 210 nm.

	A	B	C	D	E	F	G	H
1	Start Wavelength:	200						
2	End Wavelength:	210						
3	Read Mode:	ABS						
4	Read Average:	0.1						
5	Nanometer Interval:	1						
6	Time:	0						
7								
8								
	A	B	C	D	E	F	G	H
11	200	0.1122						
12	201	0.1036						
13	202	0.0965						
14	203	0.0904						
15	204	0.0829						
16	205	0.0757						
17	206	0.0691						
18	207	0.0631						
19	208	0.0574						
20	209	0.0526						
21	210	0.0485						

SECTION SEVEN

KINETICS/TIME

Kinetic data are collected in absorbance as a function of time. Multiple samples can be analyzed using an Auto Cell Holder, although the sampler is not required for kinetic analysis.

The data are plotted or tabulated as they are collected and are stored in the instrument memory. Single or multiple blanks can be specified. A second wavelength can be selected for background correction.

After data collection is complete, the user can calculate the rate of the kinetic reaction using a linear regression calculation over the desired time interval for the best fit straight line. Because the data are stored in memory, these calculations can be repeated until the optimum results are obtained.

7.1 Principles of Operation

Temperature Control

If the Temperature Controller is installed, temperature is controlled automatically in this mode. When **<ReadSamples>** is clicked on, the Temperature Controller is turned on and set to the input temperature. This allows time for the cell holder to equilibrate at the reaction temperature before **[START]** is clicked on. The cell holder can be pre-heated, if desired, using the Device Control window to turn on the Temperature Controller.

The cell holder changes temperature quickly. However, it takes about 10 minutes for the Temperature Controller to warm the solution in a standard cuvette from 25 to 37 degrees, because of slow heat transfer through the glass walls of the cuvette. Therefore, it is suggested that the solution be pre-warmed before being placed in the cell holder. The Temperature Controller can efficiently maintain the temperature of pre-warmed solutions.

Instrument Blank

The blank reading for the instrument should be taken on a cuvette filled with solvent (substrate) before the analysis begins. The solvent should be at the same temperature that the analysis will be performed.

Match Correction

When using an Auto Cell Holder, Match can be used to correct for differences in the cell positions by taking a zero reading at an input wavelength on each cell position (with a cuvette filled with blank solution and inserted into the Auto Cell Holder). When Match correction is enabled, all readings taken by the instrument will be corrected by the offsets displayed on the Match window. Match correction should be used with the Micro Auto-6 and the Micro Auto-12 Accessories.

Background Correction - Net Absorbance

It is possible to correct for raised or sloping baselines, which may be caused by turbidity in the sample, using background correction. The user can input one background wavelength for background correction. If a background wavelength is selected, the reading at the background wavelength is subtracted from the reading at the analytical wavelength to calculate a net absorbance reading, A_{na}.

$$A_{na} = A_a - A_{bk}, \quad (1)$$

where, A_a is the reading at the analytical wavelength and A_{bk} is the reading at the background wavelength. Match correction, if enabled, is performed on all cell positions prior to this calculation.

Blank Subtraction - Corrected Absorbance

Any number of blank(s) and sample(s), up to 12 total, can be analyzed and stored in the same Results file. The user specifies which are blanks and which are samples. If more than one blank is specified, the sample(s) that follow each blank will be corrected by the preceding blank.

When rate results are calculated, the blank solution reading is subtracted from the corresponding sample reading at each time point for every sample.

$$A = A_s - A_{bl}, \quad (2)$$

where, A is the corrected absorbance, A_s is the sample reading and A_{bl} is the blank solution reading at each time point. Match correction and/or background correction calculations are performed on both blanks and samples prior to this calculation.

Concentration Calculation

The corrected absorbance (A, equation 2) can be multiplied by a factor to calculate concentration, using the equation:

$$\text{Concentration} = A \times \text{Factor} \quad (3)$$

Rate Calculation

The rate is calculated using linear regression to determine the best fit of a straight line to the data, using the basic rate equation:

$$\text{Rate} = (A_2 - A_1) / (t_2 - t_1) \quad (4)$$

where, the rate is calculated in units of delta A/minute, the corrected absorbance (equation 2) is used for each of the absorbance readings, and the limits of the time interval are selected by the user. The rate is calculated for samples, only. The calculated line is displayed with the actual data so that the user can compare the calculated rate to the actual data. The variance is also calculated and displayed with the rate. The variance gives the user an indication of how well the data fit the calculated line.

Enzyme Activity Calculation

The rate can be multiplied by a user-input factor to convert it to enzyme activity or other concentration units.

$$\text{Enzyme Activity} = \text{Rate} \times \text{Factor}. \quad (5)$$

Data Storage

A single Results file can hold data from up to 12 samples; the number is input by the response to the "number of samples" parameter. Each sample is designated as a sample or a blank, using the "sample assignment" information on the data collection window, or on the Method window.

It is necessary to store data from various samples in the same Results file, if the analysis of the data requires that the data from the samples be compared. Also, data from blanks must be stored in the same file as the associated sample data.

Samples that are analyzed singly are added to the Results file until the input "number of samples" is reached. Then the Results file is complete.

When the Auto Cell Holder is used, "groups" of sample data will be added to the Results file until the input "number of samples" is reached. The size of the "group" is equal to the "number of cells" parameter set for the Auto Cell Holder on the Sampling Device window.

For example, if the "number of cells" is 4 and the number of samples is 12, the Auto Cell Holder will be used three successive times to analyzed four samples. Then the Results file is complete.

7.2 Parameter Setup

Click on "**KINETICS/TIME**" from the Main window to enter the analysis mode. The Plotting window, Figure 7-1, is displayed. The Plotting window is used to setup analysis parameters and display kinetic data graphically as they are collected. (There is another data collection window in the Kinetics/Time mode - the Tabulation window.)

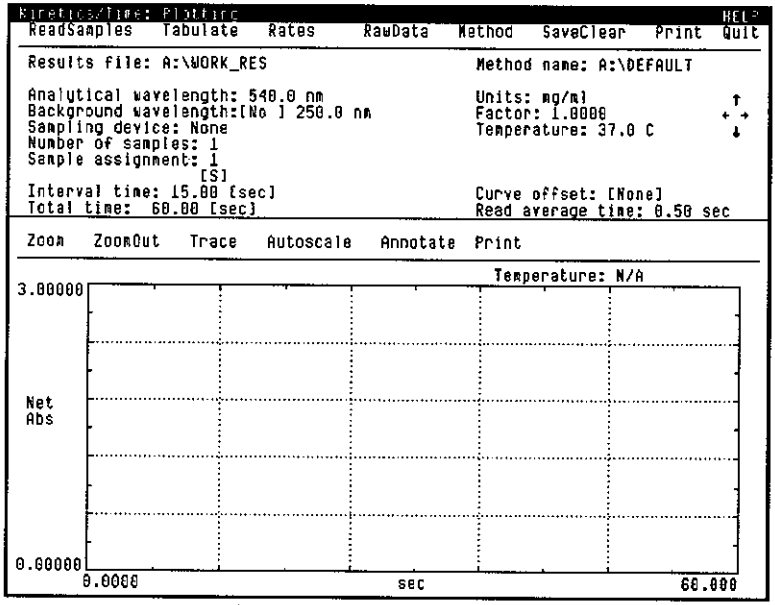

Figure 7-1. Plotting Window

Use the Method window to setup the analysis parameters:

1. Click on **<Method>** to display the Method window, Figure 7-2. The Method window is used to setup analysis parameters, recall stored methods and create new methods. General information on method windows is provided in section 3.8.

2. To recall a stored method, click on the desired method name in the listing at the top of the Method window.

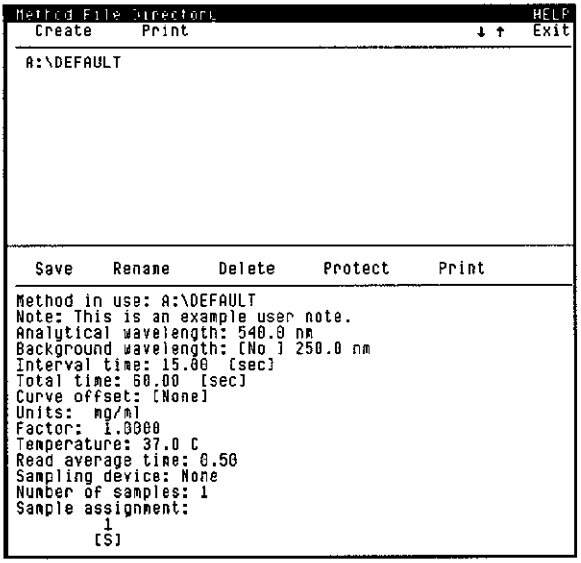

Figure 7-2. Method Window

3. The analysis parameters are displayed on the lower part of the Method window. Input the desired analysis parameters:

   Method in use - This displays the name of the method that has been selected. If the method is protected, **PROTECTED** is displayed following the method name. If the method is protected, the analysis parameters cannot be changed. To input a new method name, click on **<Create>**.

   Note - Click on to input a 40-character message that is used to described the method or procedure.

   Analytical wavelength - The wavelength for data collection.

   Background wavelength - The readings collected at the background wavelength are subtracted from the reading at the analytical wavelength to calculate the net absorbance. Toggle between **[Yes]** and [No] to enable or disable the correction.

   Interval time - The time between successive readings of the same sample. The minimum time is affected by the number of cells and the read average time.

Using a read average time of 0.5 second, the following are the minimum interval times in seconds, based upon the type of Auto Cell Holder and the number of cells.

Auto 6 Cell Holder		Auto 12 Cell Holder			
Cells	Time	Cells	Time	Cells	Time
1	0.05*	1	0.05*	7	9.9
2	3.1	2	2.9	8	11.4
3	4.8	3	4.3	9	12.8
4	6.6	4	5.7	10	14.1
5	8.3	5	7.2	11	15.7
6	10.1	6	8.3	12	17.2

*A read average time of 0.05 second was used.

Total time - The instrument collects data until the total time has elapsed.

Curve offset - This parameter will automatically offset the first reading on each cell, so that the plotted data will not overlay. Toggle between **[Ascending]**, **[Descending]** and [No] to enable or disable the offset. **[Ascending]** is for increasing reactions and **[Descending]** is for decreasing reactions.

Units - Input up to an 8-digit alphanumeric name for the enzyme activity units.

Factor - Input a factor to convert the absorbance to concentration units and the rate to enzyme activity.

Temperature - Input the operating temperature for the Temperature Controller, if used.

Read average time - The time, in seconds, that data are collected and averaged to take a reading. Ten sets of data are collected every second. If the interval time is less than 1 second, a read average time of 0.1 is always used.

Sampling device - Display the Sampling Device window for selection of the sampling device.

Number of samples - The number of sample data to be stored in a single Results file. If using the Auto Cell Holder, the "number of cells" input on the Sampling Device window cannot be greater than the input to this parameter. If it is greater,

then the input to this parameter is used for the "number of cells".

Sample assignment - Click on to identify the samples stored in a single Results file. **[B]** is for blank; **[S]** is for sample. The sample(s) following each blank will be corrected for that blank reading.

3. To store the analysis parameters in the selected method file, click on **<Save>**.

4. Click on **<Exit>** to display the Plotting window with the parameters from the selected method.

To input the desired parameters on the Plotting window:

1. The analysis parameters are listed near the top of the window. To change any of these, click on the parameter and input the desired value. A description of the parameters is provided above.

2. The absorbance limits are input by clicking on the displayed values on the graph and inputting the desired values.

7.3 Data Collection

The data can either be plotted or tabulated as they are collected. If the Plotting window is displayed and tabulation is desired, click on **<Tabulate>**. If the Tabulation window is displayed and a plot is desired, click on **<Plot>**. Either the Plotting or the Tabulation window can be used for data collection.

To collect data using the displayed analysis parameters:

1. Place a cuvette of substrate in the cell holder. Click on **<<BLANK>>**. The instrument blanks on the substrate.

   Auto Cell Holder - Place a cuvette of substrate in the cell position that is in the light beam, then click on **<<BLANK>>**. The instrument blanks on the substrate.

2. If using the Auto Cell Holder, Match can be used to correct for slight differences in the cuvettes. Click on **<<MATCH>>** to display the Match window. Place a cuvette filled with substrate in each of the cell positions that will be occupied with a blank or sample. Take the readings and enable Match correction.

   The cuvettes should not be removed from the instrument before the sample analysis. If the cuvettes are removed, it is necessary to return each cuvette to the same cell position in the same orientation. For most accurate results, Match correction should be repeated each time the cuvettes are removed from the instrument.

3. If the Plotting window is displayed, set the limits on the absorbance axis by clicking on the displayed value and inputting the desired value.

4. Click on **<ReadSamples>**. The Read Samples window is displayed, which is used to read the samples.

   The Temperature Controller turns on, if not already on, and the temperature is controlled to the input temperature parameter. Wait

until the temperature display on the Temperature Controller reaches the desired temperature. (The actual temperature is placed on the Plotting window when **[START]** is pressed.)

5. Place a cuvette of sample in the cell holder and click on **[START]**. The data are plotted or tabulated as they are collected, depending upon the window.

   Auto Cell Holder - Load the blank(s) and samples in the order indicated on the top of the window. Positions are indicated with a "B" for blank and a "S" for sample. Click on **[START]**.

6. The analysis can be terminated at any time by clicking on **[QUIT]**. However, after **[QUIT]** is clicked on, no additional data can be added to the Results file, even if all the samples have not been analyzed.

7. When data collection is complete, if the total "number of samples" has not been analyzed, the window shown in step 4 is displayed. Repeat step 5, until all samples have been analyzed.

NOTICE

Do not change from the Plotting to Tabulation window, or display the Rates window until all samples have been analyzed.

8. When all the samples input for "number of samples" have been analyzed, the window shown in step 4 is removed. The data collection window can be changed from Plotting to Tabulation by clicking on **<Tabulate>**, or from Tabulation to Plotting by clicking on **<Plot>**. A typical Plotting window is shown in Figure 7-3. A typical Tabulation window is shown in Figure 7-4.

   The data on the Plotting window can be reformatted and annotated. Detailed instructions are provided in the next section.

   The net absorbance readings (equation 1) are tabulated on the Tabulation window. These readings have been corrected for background absorbance and Match correction, if used. All the raw data, plus additional calculated data are displayed on the Raw Data window.

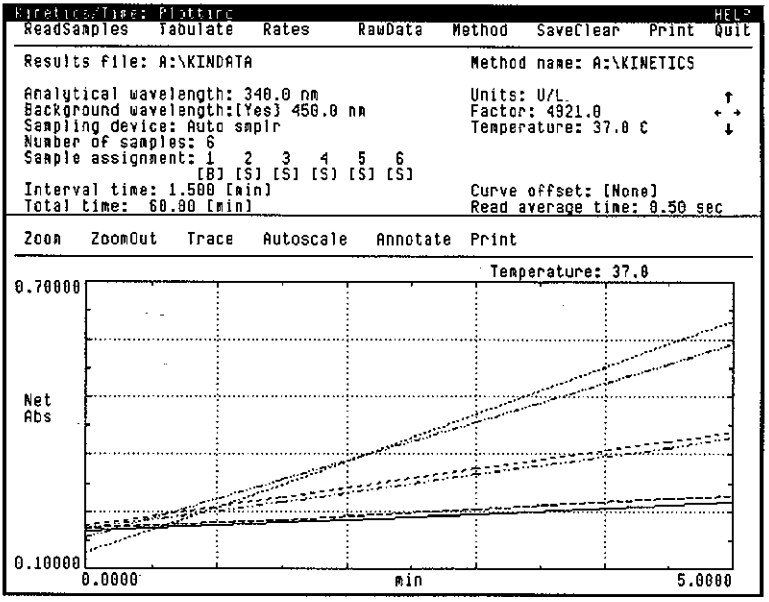

Figure 7-3. Plotting Window

Kinetics/Time: Tabulation HELP

ReadSamples Plot Rates RawData Method SaveClear Print Quit

Results file: A:\KINDATA
Method name: A:\KINETICS

Analytical wavelength: 340.0 nm
Background wavelength:[Yes] 450.0 nm
Sampling device: Auto smplr
Number of samples: 6
Sample assignment: 1 2 3 4 5 6
[B] [S] [S] [S] [S] [S]
Interval time: 1.500 [min]
Total time: 60.00 [min]
Units: U/L
Factor: 4921.0
Temperature: 37.0 C
Curve offset: [None]
Read average time: 0.50 sec

Net Absorbance Readings

Cell # / Sample ID / Time min	1 / 1	2 / 2	3 / 3	4 / 4	5 / 5	6 / 6
0.000	0.1805	0.1857	0.1650	0.1927	0.1340	0.1852
1.500	0.1963	0.2395	0.2867	0.2496	0.2774	0.2023
3.000	0.2149	0.2995	0.4065	0.3108	0.4227	0.2245
4.500	0.2328	0.3540	0.5206	0.3672	0.5706	0.2470
6.000	0.2498	0.4144	0.6517	0.4302	0.7184	0.2675
7.500	0.2679	0.4729	0.7745	0.4901	0.8649	0.2911
9.000	0.2855	0.5306	0.8980	0.5537	1.0082	0.3094
10.500	0.3041	0.5906	1.0200	0.6160	1.1580	0.3296
12.000	0.3201	0.6482	1.1512	0.6776	1.2960	0.3528
13.500	0.3405	0.7064	1.2683	0.7386	1.4573	0.3749
15.000	0.3566	0.7656	1.3898	0.7988	1.5973	0.3966
16.500	0.3774	0.8199	1.5043	0.8577	1.7320	0.4174
18.000	0.3935	0.8772	1.6229	0.9220	1.8931	0.4377
19.500	0.4110	0.9363	1.7520	0.9797	2.0217	0.4581
21.000	0.4291	0.9914	1.8721	1.0418	2.1769	0.4798
22.500	0.4483	1.0519	2.0097	1.1035	2.3721	0.5010

Figure 7-4. Tabulation Window

9. The rates of the kinetic reactions are calculated using the Rates window, described in section 7.5.

10. When all data collection is complete, use the Device Control window to turn off the Temperature Controller. The fan will turn off about 5 minutes after the Temperature Controller is turned off.

11. To clear all sample data from the window or to store the data, then clear the window, click on **<SaveClear>**. The Save Clear window is displayed so the sample data can be stored. If the data are stored, no additional data can be placed in the Results file, even if all the samples have not been analyzed.

NOTICE

Results files can be recalled in the Enzyme Mechanism mode, if they are copied into the "MECH_D" directory. The File Utilities mode is used to copy files.

12. When the analysis is complete, click on **<Quit>**. The Quit window is displayed so the method and/or sample data can be stored. Then the Main window is displayed.

7.4 Plotted Data

The presentation of plotted data on the Plotting and the Rates windows can be optimized using the following features:

Limit changes - The limits on the absorbance and/or time axis can be changed by clicking on the displayed value and inputting the desired value.

Curves offset - If the plotted data for several samples overlay at the zero time point, the data can be offset by enabling the "Curves offset" parameter.

Zoom - The "zoom" feature can be used to expand any portion of the graph. Click on **<Zoom>**, then click on two points on the graph to place crosses at the opposite corners of the area to be enlarged. When the second cross is clicked on, the graph is replotted. This can be repeated as often as desired. To return to the original plot, click on **<ZoomOut>**.

Trace - "Trace" can be used to display the time at any desired position on the plot. Click on **<Trace>**. Then move the arrow to the point of interest in the plot and click on the center mouse button to place a vertical line on the plot. The values at the place where the vertical line is placed are displayed. To move the vertical line to either the right or left, click on the right or left mouse button, respectively, with the arrow placed anywhere on the plot. To move to a different position on the plot, position the arrow and click on the center mouse button. An asterisk after the reading indicates that it is out of range.

NOTICE

The digital data at all wavelengths are displayed on the Raw Data window.

Autoscale - Automatically scale the ordinate axis. This command does not change the time limits.

Annotate - The data can be annotated by clicking on **<Annotate>**. Then, click on the graph to position a cross and input information from the alphanumeric keypad or keyboard. Up to four annotations can be placed on the graph. The annotations are printed with the window, but are not stored with the data.

7.5 Rate Calculation

The Rates window is used for rate calculation. It displays the plotted data, corrected for the blank, and allows the user to input the necessary information to calculate the reaction rate between any two time points on the graph.

The Rates window, Figure 7-5, is displayed when **<Rates>** is clicked on from either of the data collection windows. The Results file cannot be changed on the Rates window, so verify that the desired Results file is displayed on the data collection window before displaying the Rates window. Information about the data in the selected Results file is displayed in the upper portion of the Rates window.

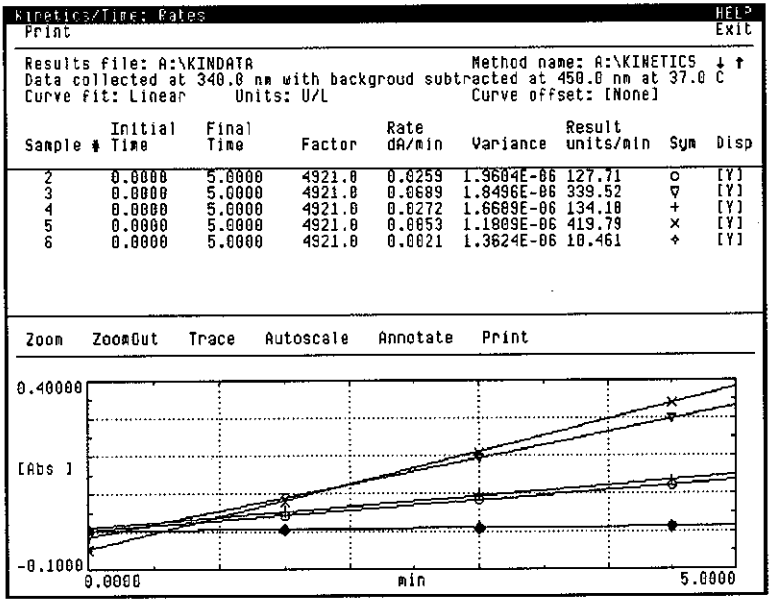

Figure 7-5. Rates Window

To calculate the rate of the kinetic reaction using the Rates window:

1. All sample data stored in the Results file are plotted. (Blanks are subtracted from the corresponding samples and not plotted.) Plotted data for any sample(s) can be deleted from the display by clicking on the [Y] in the "Disp" column in the table, replacing it with an [N] for "no".

2. The presentation of the plotted data can be optimized by using the curves offset, by using "zoom" or by changing the limits individually. Detailed instructions are provided in section 7.4.

3. The label on the ordinate axis can be changed from **[Abs]** to **[Conc]**. Concentration is calculated by multiplying the factor by each absorbance reading (equation 3).

4. The rates are calculated using the parameters input in the "Initial Time", "Final Time", and "Factor" columns. The time points can be found from the plotted data using Trace.

   To input the same value for all cells, click on the column heading. Input the desired value in the displayed window and click on **[OK]**. To input different values for each cell, click on displayed value and input the desired value.

   The line displayed on the graph is the slope of the line calculated from the initial and final time points. These times can be changed to optimize the fit of the calculated line to the collected data.

5. The tabulated results include the rate in delta A per minute (equation 4), the variance calculated for the curve fit, and the enzyme activity in the input units calculated from the factor (equation 5).

6. The table of rate results is printed by clicking on **<Print>**, located in the menu bar above the table. The plotted data are printed by clicking on **<Print>**, located in the menu bar above the graph. The entire display is printed by clicking on **<<PrtScrn>>**.

7. When the rates are calculated, click on **<Exit>** to return to the Plotting window.

7.6 Raw Data

The Raw Data window, Figure 7-6, tabulates the raw data at the analytical and background wavelengths, the net absorbance (background correction), and the corrected absorbance (blank subtraction). This window is displayed when **<RawData>** is clicked on from either of the data collection windows.

The Results file name cannot be changed on the Raw Data window, so verify that the desired Results file is displayed on the data collection window before displaying the Raw Data window. Information about the data in the selected Results file is displayed in the upper portion of the Raw Data window.

Kinetics/Time: Raw data HELP
Print Exit

Results file: A:\KINDATA Method name: A:\KINETICS ↓ ↑
Data collected at 37.0

Sample assignment: 1 [B] 2 [S] 3 [S] 4 [S] 5 [S] 6 [S]

Time (min)	Sample ID	Analyt wl 340.00nm	Bkg wl 450.00nm	Net abs	Corrected Abs
0.000	1	0.2952	0.1147	0.1805	
	2	0.2591	0.0734	0.1857	0.0052
	3	0.4249	0.2599	0.1650	-0.0155
	4	0.3889	0.1882	0.1927	0.0122
	5	0.4837	0.3498	0.1340	-0.0465
	6	0.3076	0.1218	0.1852	0.0047
1.500	1	0.3093	0.1129	0.1963	
	2	0.3147	0.0751	0.2395	0.0432
	3	0.5379	0.2511	0.2867	0.0904
	4	0.4364	0.1869	0.2496	0.0532
	5	0.6280	0.3506	0.2774	0.0810
	6	0.3273	0.1250	0.2023	0.0060
3.000	1	0.3268	0.1119	0.2149	
	2	0.3701	0.0706	0.2995	0.0846
	3	0.6602	0.2537	0.4065	0.1916
	4	0.4978	0.1870	0.3108	0.0959
	5	0.7724	0.3497	0.4227	0.2078
	6	0.3471	0.1226	0.2245	0.0096
4.500	1	0.3451	0.1123	0.2328	
	2	0.4289	0.0749	0.3540	0.1212
	3	0.7794	0.2508	0.5286	0.2958
	4	0.5571	0.1908	0.3672	0.1343
	5	0.9187	0.3481	0.5706	0.3378
	6	0.3671	0.1202	0.2470	0.0141

Figure 7-6. Raw Data Window

To display the raw data and associated information:

1. The cell assignments for the Results file is displayed; they can be changed.

2. Only the first few readings are displayed. The other readings can be displayed by scrolling through the data, using the up and down arrows located in the upper right-hand corner of the window.

3. The tabular data include the net absorbance (equation 1) and the corrected absorbance (equation 2).

4. To print the window, click on **<Print>**. All data, even that which is not displayed because of insufficient room on the display, are printed on the printer.

5. When the Raw Data display is complete, click on **<Exit>** to return to the Plotting window.

7.7 Example Analyses

EXAMPLE 1

Setup the parameters for a kinetic analysis at 340 nm at 37°C. Analyze six samples, each with its own blank, using the Auto 6 Cell Holder. Take readings every 30 seconds for 5 minutes. Store the data in a file named "A:\CK_DATA".

SOLUTION

The analysis parameters are listed on the top of the Tabulation window, shown in Figure 7-7. The Auto 6 Cell Holder must be used twice to analyze all the samples. The data from the first three samples (with blanks) are tabulated on the window. The remainder of the samples will be analyzed subsequently, and stored in the same Results file.

Kinetics/Time: Tabulation HELP

ReadSamples Plot Rates RawData Method SaveClear Print Quit

Results file: A:\CK_DATA
Method name: A:\CKTEST

Analytical wavelength: 340.0 nm
Background wavelength:[No] 450.0 nm
Sampling device: Auto smplr
Number of samples: 12
Sample assignment: 1 2 3 4 5 6 7 8 9 10 11 12
[B] [S] [B] [S] [B] [S] [B] [S] [B] [S] [B] [S]
Interval time: 30.00 [sec]
Total time: 5.000 [min]

Units: U/L
Factor: 4921.0
Temperature: 37.0 C
Curve offset: [None]
Read average time: 0.50 sec

Net Absorbance Readings

Cell # / Sample ID / Time sec	1 / 1	2 / 2	3 / 3	4 / 4	5 / 5	6 / 6
0.000	0.2139	0.2457	0.2283	0.2459	0.2187	0.2558
30.000	0.2108	0.2451	0.2288	0.2429	0.2198	0.2567
60.000	0.2108	0.2455	0.2276	0.2436	0.2194	0.2602
90.000	0.2101	0.2490	0.2281	0.2470	0.2190	0.2654
120.000	0.2107	0.2493	0.2297	0.2495	0.2196	0.2704
150.000	0.2103	0.2525	0.2282	0.2532	0.2196	0.2750
180.000	0.2115	0.2544	0.2294	0.2550	0.2198	0.2805
210.000	0.2100	0.2578	0.2284	0.2584	0.2192	0.2868
240.000	0.2106	0.2604	0.2294	0.2618	0.2195	0.2911
270.000	0.2109	0.2624	0.2292	0.2644	0.2194	0.2979
300.000	0.2113	0.2663	0.2299	0.2662	0.2198	0.3035

Figure 7-7. Example Tabulation Window