Manual 517300C

SPECTROPHOTOMETER

OPERATING INSTRUCTIONS

BECKMAN

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SECTION ONE

INTRODUCTION

1.1 General Description

The DU® Series 600 Spectrophotometer is a microprocessor controlled spectrophotometer intended for use in quantitative and qualitative biological research and industrial procedures that require spectrophotometric measurements in the UV-visible region of the electromagnetic spectrum. If the instrument is used in a manner other than as described, the safety and performance of the instrument can be impaired.

The DU Series 600 Spectrophotometer operates in the wavelength range of 190 to 1100 nm. Models of the instrument are available with either a monochrome or a color video display. Data storage on a 3½ inch diskette is optional. Various optional accessories are available, to configure the instrument for specific application needs.

The instrument features a graphic video display, which provides operational information using windowing techniques, and a "mouse" for operator control. The mouse is used to position an arrow on the window. When the arrow points to the desired position, the left button on the mouse is pressed to initiate the desired action. In these instructions, the positioning of the arrow and pressing the left mouse button is called "clicking on".

The instrument has two rapid reading modes: RediRead™ for taking readings at a fixed wavelength and RediScan™ for making a wavelength scan.

The instrument has three standard Routine Measurement modes. They include:

Fixed Wavelength - Takes absorbance or transmittance readings at up to 12 wavelengths. Readings at each wavelength can be multiplied by a factor.

Wavelength Scan - Performs wavelength scans in absorbance or transmittance. Data are automatically stored for manipulations including Trace, zoom, overlay and tabulate. Calculations include peak pick,

valley pick, point pick, first to fourth derivative, log of absorbance, scatter correction, spectral addition, subtraction and multiplication, and net absorbance. Repetitive scanning is also performed in this mode.

Kinetics/Time - Calculates the rate of an absorbance versus time reaction with a choice of blank subtraction and graphic display of the data for multiple samples. Data are automatically stored for manipulations including Trace, zoom, overlay and tabulate.

Other Application modes are also available, which include:

Protein Analysis - Calculates the protein concentration using the Bradford, Lowry, Biuret, direct UV method, colloidal gold, and bicinchoninate (BCA). Prepares a standard curve using up to 30 standards. The user can choose to add, delete or rerun individual standards based upon a statistical analysis of the standard curve.

Nucleic Acid - Determines protein impurity in nucleic acid samples based upon the ratio of readings at two wavelengths with a choice of background correction. Protein and nucleic acid concentrations can also be calculated using the Warburg and Christian[1] coefficients.

Fraction Read/Plot - Collects, plots and tabulates data from a set of related fractions. Readings can be corrected for dilution. After data collection, individual fractions can be added, deleted or rerun. Data are plotted versus either fraction number or volume. Other data obtained for the fractions can be input and plotted with the absorbance data.

Single component quantitative analysis - Calculates a standard curve from up to 30 standards using either linear or nonlinear least-squares regression. Performs statistical analysis on the standard data. Allows the user to add, delete or rerun individual standards to optimize the calibration. Calculates the concentration of samples from the calibration data.

Enzyme Mechanism - Guides the operator through the necessary steps to calculate Km and Vmax. Displays the following plot types: Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf. Calculates the Hill constant from the Hill Plot. Graphs inhibitor plots to determine Ki.

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[1]Warburg, O. and Christian, W., Biochem. Z:**310**, p. 384f (1942).

Enzyme Activity - Calculates the enzyme activity of large numbers of samples.

Multicomponent analysis - Uses Full Spectrum Quantitation (FSQ™) to calculate the concentration of up to ten components in a mixture from up to 32 standards which are mixtures. Performs a statistical analysis of the standard data to determine the accuracy of the calibration. Allows individual standards to be added, deleted or rerun to optimize the calibration. Calculates the concentration of samples from the calibration data.

Gel Scan - Collects and plots absorbance data as a function of distance for a sample prepared by electrophoresis. Calculates peak and valley locations, which are used for subsequent area calculation or molecular weight determination.

Performance Validation - Provides a simple procedure to verify the performance of an instrument. Tests which are performed include: wavelength accuracy and repeatability, resolution, baseline flatness, noise and stability.

One model of the instrument also contains a program mode, which allows the user to customize applications by writing programs which blank, collect and store data at the desired wavelengths, calculate results from the data, prompt the operator, format and label the output and control sampling accessories.

The instrument is provided with a parallel output for a Dot Matrix Printer and a bidirectional RS-232 communications port that can be configured for either communications or an X-Y Plotter. The communications port can be used for data transfer to and from the instrument and for remote control of the instrument by an external computer.

A full line of modular sampling accessories is available for the instrument. Included are temperature controlled cell holders, automatic multi-position cell holders, a batch sampler, sipper samplers, and long pathlength cell holders. The automated sampling accessories (sipper samplers, batch sampler, and auto samplers) are designed to operate in the modes described previously to automate analyses. Microsampling capabilities include the use of the 50 µL Microcell, the 100 µL Multi-Microcell, and the 5 µL Ultra-Microcell.

1.2 Principles of Operation

Optical Principle

The optical diagram of the DU Series 600 Spectrophotometer is shown in Figure 1-1.

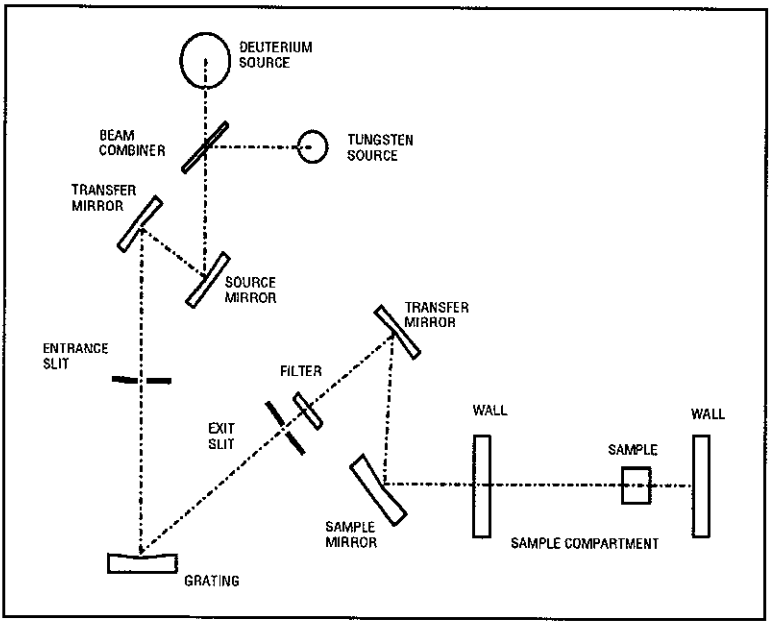

Figure 1-1.
DU Series 600 Spectrophotometer Optical Diagram

The DU Series 600 Spectrophotometer is a single beam spectrophotometer. Light from both sources enters the monochromator, where it is dispersed by the concave holographic grating. Monochromatic light exits the monochromator and illuminates the sample. The amount of light that passes through the sample is measured by the single photodiode detector.

The focal point of the beam in the sample compartment is on the right-hand side. This location permits the maximum amount of transmitted light to reach the detector from scattering samples. All sampling accessories position the sample at the focal point for best performance with microsamples and gels.

Blanking Method

A blank is always required before data collection; any reading taken without a blank is invalid. A blank reading is taken when **<<BLANK>>** (located in the permanent menu bar on the bottom of the window) is clicked on.

NOTICE

In the RediRead™ Mode the blank command is **<ReadBlank>**. In the RediScan™ Mode, the blank command is **<ScanBlank>**.

When the instrument blanks, the following steps are performed:

1. The monochromator is moved to the proper wavelength. This is the specified wavelength for a single wavelength reading.

2. The proper detector gain value is selected automatically. This minimizes the noise level and maximizes photometric accuracy.

3. Dark current is measured and corrected. This compensation assures accurate readings at high absorbance.

4. In the Wavelength Scan mode, only, a background scan is made. The blank (or reference) is automatically scanned over the same range at the same speed that the samples will be scanned, so that the background correction is optimal.

This calibration assures repeatable readings every time the instrument is used.

In all modes, a blank solution should be in the sample compartment during the blank. It is suggested that the solvent used to prepare the samples be used for the blank. However, air (no sample) may be used. A new blank reading should be taken each time the solvent is changed.

NOTICE

Plastic cuvettes, glass (Pyrex) cuvettes, and some solvents have significant absorption in the UV region. Verify that they transmit UV light by scanning them versus air before using them in the UV region.

To re-zero the instrument at any time between samples, insert the same blank solution and click on **<<BLANK>>**.

The instrument stores the blank and uses it until either the sources are turned off or another blank reading is taken. For best results, the instrument should be blanked frequently, allowing the blank reading to be taken shortly before the sample measurement is taken. A new blank should be read if the instrument has not been used for an hour.

Scanning

The background scan, made as part of the Blank procedure, is stored in the instrument and can be reused for an unlimited number of sample scans as long as the range and scan speed remain the same; unless the user blanks, the sources are turned off or the instrument is turned off. (The range can be decreased as long as the scan speed remains constant and no new background is required.) When a new background is required, it is indicated on the display.

A new background scan should be made every time a solvent is changed, because the background spectrum will likely be different. A new background scan should also be made if no scan has been made for over an hour. To rescan the background, click on **<<BLANK>>**, while in the Wavelength Scan mode.

The selected scanning speed determines the distance between each data point that is collected as the instrument scans through the chosen region. At 1200 nm/min, a data point is collected every nanometer. At 600 nm/min, a data point is collected every half nanometer.

As the sample data are collected the background is subtracted and the difference in absorbance (or ratio in transmittance) is plotted on the display.

Read Average Time

The noise level of the instrument, and therefore the uncertainty of a sample reading, is decreased by taking a number of readings and averaging them. The instrument takes a reading every 0.05 second. It takes a series of these readings over a user-selected time and averages them to obtain the blank and sample readings. For example, with a read average time of 0.5 seconds, ten readings are taken and averaged. The operator can specify a read average time from 0.05 to 99.9 seconds in all modes except Wavelength Scan and Multicomponent Analysis.

Read averaging is not used in the RediScan and Wavelength Scan modes. Background and sample scans are collected without averaging. Smoothing is used to improve the appearance of the data.

Smoothing

The displayed wavelength scan can be smoothed using a selectable smoothing function. The calculation, using the Savitzky and Golay[2] coefficients (as modified for end points by Peter A. Gorry[3]), 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 does not rid the scan of noise. Use of too many points can cause real peaks to be combined.

[2]Savitzky, A., and Golay, M., Anal Chem, **36**, 1964, p1627f.

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

SECTION TWO

INSTALLATION

2.1 Installation Instructions

The DU Series 600 Spectrophotometer is user installable. As an option, it can be installed by a qualified Beckman Field Service Engineer. The instructions for user installation are provided in Manual 517315.

Location

The DU Series 600 Spectrophotometer is designed to sit on a lab bench or table, which is level and flat and is capable of supporting its weight and the weight of all accessories.

The instrument is designed to operate in a clean laboratory environment, free from dust, fumes, excessive moisture, and corrosive chemicals. It should not be exposed to drafts from heating and cooling vents, heating elements, open windows or doors. Lab areas that receive direct sunlight should also be avoided.

An ambient room temperature of 15 - 40°C (59 - 104°F) should be maintained. Relative humidity should be 85% or less.

Instrument performance can be affected by strong electromagnetic fields that can exist in the proximity of large electric motors, centrifuges, diathermy machines and microwave sources.

The batch sampler must be placed adjacent to the right-hand side of the instrument. The other accessories can be placed in a convenient place, near the instrument, and within reach of the cables. Additional space is required for air circulation around the instrument and accessories for proper performance. Do not block these air spaces.

2.2 Utility Requirements

Each of the following needs its own electrical outlet: the spectrophotometer, display, dot matrix printer, X-Y Plotter, batch sampler, and Peltier temperature controller. The electrical requirements of each are summarized in Table 1 for 100/120V and Table 2 for 220/240V systems.

The same circuit should be used for the instrument and all accessories. A dedicated circuit is preferred. Do not use a circuit which is also used by equipment that operates intermittently and creates wide fluctuations in power demand, such as refrigerators, water baths and centrifuges.

For 100/120V operation. The power line should provide three-wire single phase power. To provide multiple outlets, grounding type power strips may be used. Extension cords or multiple outlet adapters should not be used.

For 220/240V operation. The power line should provide three-wire single phase power. To provide multiple outlets, grounding type power strips may be used. Extension cords or multiple outlet adapters should not be used.

	Frequency (Hz)	Voltage (VAC)	Current (Amps)
Spectrophotometer	50/60	100/120V±10%	1.8
Display	50/60	110V±10%	0.8
Dot Matrix Printer	50/60	120V±10%	0.6
X-Y Plotter	48 - 66	120V -10%,+5%	0.2
Batch Sampler	50/60	117V±10%	0.8
Peltier Temperature Controller	50/60	120V±10%	1.0

Table 1. Electrical Requirements for 100/120V Systems

	Frequency (Hz)	Voltage (VAC)	Current (Amps)
Spectrophotometer	50/60	220/240V±10%	0.9
Display	50/60	220V±10%	0.4
Dot Matrix Printer	50/60	220/240V±10%	0.3
X-Y Plotter	48 - 66	240V -10%,+5%	0.1
Batch Sampler	50/60	234V±10%	0.4
Peltier Temperature Controller	50/60	220/240V±10%	0.5

Table 2. Electrical Requirements for 220/240V Systems

2.3 Power Up

The DU Series 600 Spectrophotometer is powered up using the following steps.

1. Verify that the voltage on the rating plates on the instrument, display and printer are the same as the power source.

   ⚠ CAUTION

   The voltage indicated on the display, the instrument and the printer must be the same as the power source. If the incorrect voltage is indicated, do not plug in the instrument and contact the local Beckman service office.

   The instrument, display and printer must be plugged into grounded power outlets.

   NOTICE

   If a power cord is not supplied, contact the local Beckman service office.

2. Plug the power cords on the instrument, the display and the printer into grounded, three-prong outlets that are on the same power line.

3. Turn on the display. The indicator light on the front of the display should illuminate.

4. Turn on the instrument. The power switch is located on the right-hand side of the back of the instrument. The instrument fan should turn on.

   If the fan does not turn on, turn off the instrument and check the fuses. Directions for fuse replacement are provided in the Corrective Maintenance section of this manual.

5. The display should illuminate, a message window with "Executing Power Up Diagnostics" should be displayed, and an arrow should appear on the display. The arrow should move when the mouse is moved.

   If the display does not illuminate:

   a. Verify that the indicator light on the display is illuminated, showing that there is power to the display.

b. Verify that the brightness control on the display is adjusted properly.

c. Verify that the cable on the display is connected to the "DISPLAY" port on the back of the instrument.

If the mouse does not move the arrow, verify that the cable attached to the mouse is connected to the "MOUSE" port on the back of the instrument.

NOTICE

If the recommended action does not correct the problem, power down the instrument, then power it up again. If the problem persists, contact the local Beckman service office.

6. Turn on the printer using the switch on the left-hand side of the printer. Verify that the "ON LINE" light is illuminated (or blinking).

7. Verify that the printer paper is loaded and that the top of the first page is aligned properly.

NOTICE

The instructions for loading of the paper and the replacement of the ribbon are located in Manual 514521.

2.4 Configuration

After the DU Series 600 Spectrophotometer has been installed and powered up, the Configuration mode is used to select parameters which setup the instrument, the output devices, and the sampling accessories. It is also used to assign the passwords for method protection. Configuration parameters are generally selected when the instrument is installed and are not changed during operation of the instrument.

The DU Series 600 Spectrophotometer user interface operates on the principle of windows. The "mouse" is used to position an arrow on the window. When the arrow points to the desired position, the left button on the mouse is pressed to initiate the desired action. In these instructions, the positioning of the arrow and pressing the left mouse button is called "clicking on". If the Power Up Diagnostic window is displayed, click on **<Quit>** to remove the window and display the Main window.

The Configuration window, Figure 2-1, is used to select one of seven windows that are used for different configurations. It is displayed when "**CONFIGURATION**" is clicked on from the Main window.

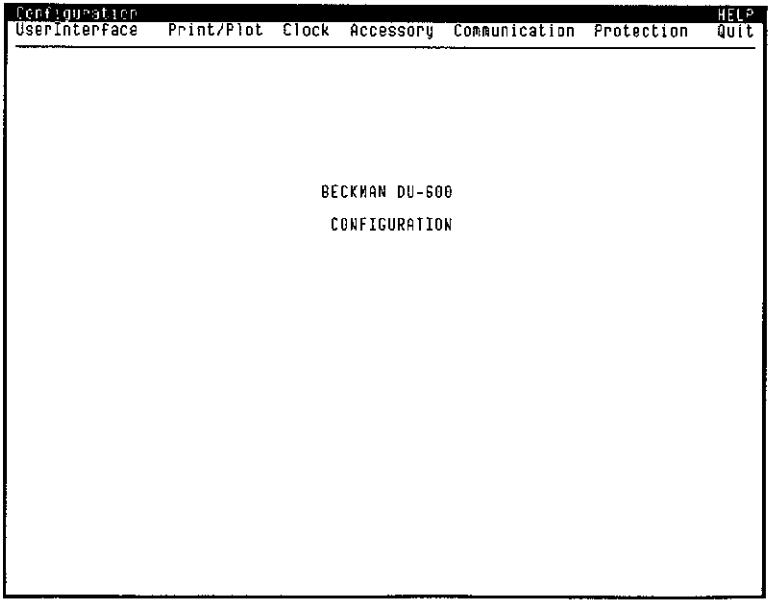

Figure 2-1. Configuration Window

NOTICE

Instructions to configure the instrument and Printer/Plotter, only, are provided in this section. Instructions for the X-Y Plotter are provided in Manual 514523. Instructions for the sampling devices are provided in Manual 517314.

User Interface Configuration

The User Interface Configuration window, Figure 2-2, is displayed when **<UserInterface>** is clicked on from the Configuration window.

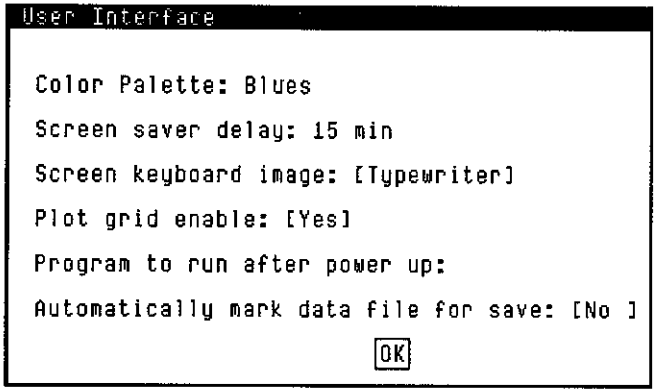

Figure 2-2. User Interface Configuration Window

Set the following:

1. **Color palette** - This determines the set of colors used on the display for a color monitor, or if a monochrome monitor is used. Click on the current selection to display a window with the palette options. Click on the box before the palette of choice, then **[OK]** to remove the window.

2. **Screen saver delay (minutes)** - The instrument dims the display whenever the instrument is not used for the selected amount of time and both sources are turned off. Click on and input the desired amount of time.

3. **Screen keyboard image** - This determines the order of keys on the alphanumeric keypad. Click on to toggle between **[Typewriter]** and **[Alphabetic]**.

4. **Plot grid enable** - This determines whether a grid is displayed on graphs. Click on to toggle between **[Yes]** and **[No]** to enable or disable the grid, respectively.

The input to this selection determines whether the grid is plotted on printouts generated on the Dot Matrix Printer. For printouts on the X-Y Plotter, however, the grid is enabled or disabled on the Printer and Plotter Configuration window.

5. **Program to run after power up** - This allows a user-written program to be executed automatically after power up is completed. The program could turn on the sources, have a delay to allow the sources to warm up, then execute the Performance Validation tests. (The Program mode, which is used to write the program, is not included on all models.)

   To select a program, click on the displayed program name to display the Program directory. Click on one of the program names to select it.

6. **Automatically mark data file for save** - This affects the Save Clear and Quit windows, only. If this is enabled, the box is automatically darkened for each data file when the window is displayed. If this is disabled, the box is darkened when it is clicked on or if the file name is input.

When the desired parameters are selected, click on **[OK]** to store the entries and remove the User Interface Configuration window from the display.

Clock Configuration

The Clock Configuration window, Figure 2-3, is displayed when **<Clock>** is clicked on from the Configuration window.

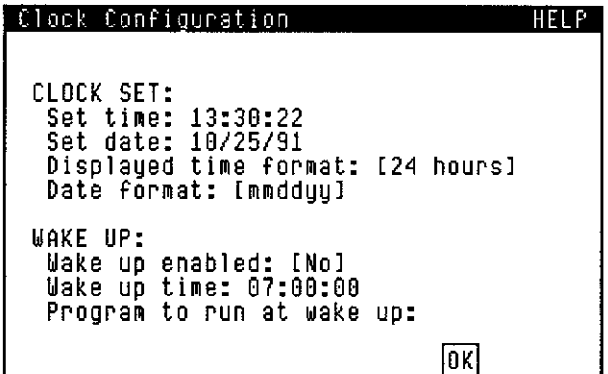

Figure 2-3. Clock Configuration Window

CLOCK SET

Input the following information to set the date and time, displayed in the permanent menu bar.

1. **Set time** - Input the current time, using the 24 hour clock. The clock starts when **[OK]** is clicked on to remove the window.

2. **Set date** - Input the current date.

3. **Time format** - Select format for the time: [12 hours] or [24 hours]. If a 12 hour clock is selected, am and pm indications are *not* given.

4. **Date format** - Select format for the date: [mmddyy], [ddmmyy], or [yymmdd].

WAKE UP

This allows the instrument to turn on the sources at an operator determined time, so that the instrument and the sources can be warmed up and ready to use. The instrument should be left with the Main window displayed and the sources turned off in preparation for Wake Up to execute.

5. **Wake up enabled** - Toggle between **[Yes]** and **[No]** to enable or disable the wake up mode.

6. **Wake up time** - Input the wake up time using the 24-hour clock.

7. **Program to run at wake up** - This allows a user-written program to be executed automatically after the sources are turned on. The program could have a delay to allow the sources to warm up, followed by the Performance Validation mode or some other diagnostic tests. (The Program mode, which is used to write the program, is not included on all models.)

   To select a program, click on the displayed program name to display the Program directory. Click on one of the program names to select it.

When the desired parameters are selected, click on **[OK]** to store the entries, start the clock and remove the Clock Configuration window from the display.

Protection Configuration

The Protection Configuration window is used to identify each user and assign a password. The password prevents methods from being modified by another user. The master access code is required before passwords can be assigned or changed. This prevents unauthorized change of password.

To input or change passwords:

1. Click on **<Protection>** from the Configuration window. The Protection Configuration window, Figure 2-4, is displayed.

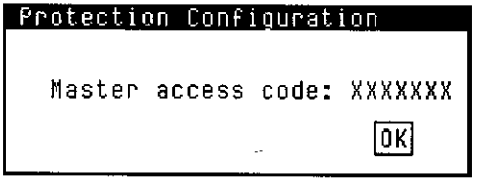

Figure 2-4.
Protection Configuration Window

2. Click on the X's that are displayed for the master access code, input the code, then click on **[OK]**. The Edit User Names and Passwords window, Figure 2-5, is displayed.

NOTICE

The master access code is assigned by Beckman Instruments and cannot be changed.

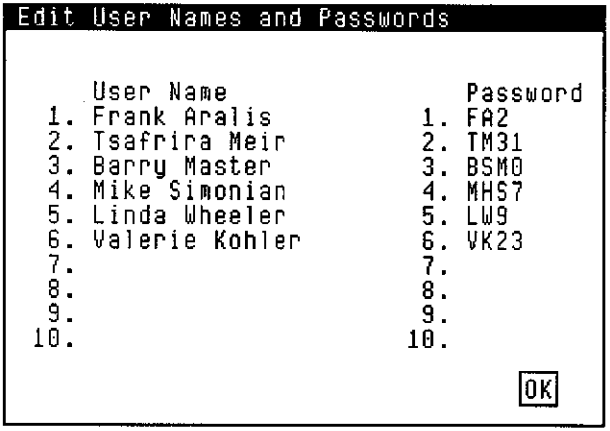

Figure 2-5.
Edit User Names and Passwords Window

3. Click on a position in the table in the "**User Name**" column and input a user name. Then click on the corresponding position in the "**Password**" column and input a password. Repeat for each user.

4. To edit any of the entries, click on it and input the desired information.

5. When all of the information has been input correctly, click on **[OK]** to store the information and remove the Edit User Names and Passwords window from the display. Then click on **[OK]** to remove the Protection Configuration window from the display.

Printer Configuration

The Printer and Plotter Configuration window, Figure 2-6, is displayed when **<Print/Plot>** is clicked on from the Configuration window.

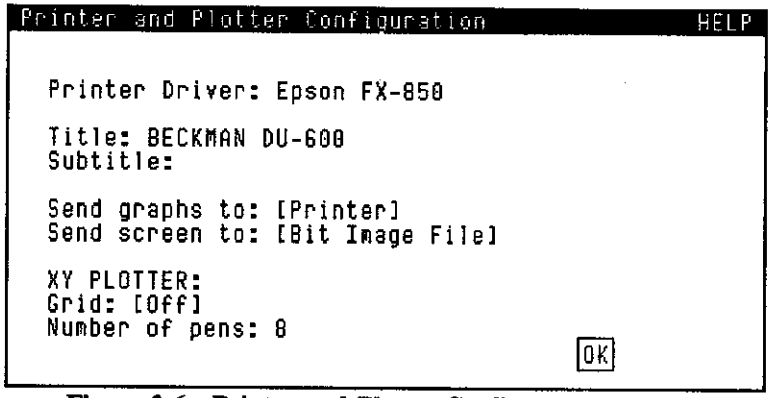

Figure 2-6. Printer and Plotter Configuration Window

Select the following parameters:

1. **Printer Driver** - Selects the printer type being used. If a HP Desk Jet is selected, all text screens with 80 characters or less are printed portrait, and all graphs and all text screens with more than 80 characters are printed landscape, and, if any line has more than 100 characters, the print is condensed. To use the Cannon BJ-200, select the Epson LQ-510 and make sure that the DIP switches are set for the Epson LQ-510 mode.

2. **Title** - Input up to 60 alphanumeric characters which are printed at the top of each printout made with the **<Print>** command. This can be used to identify the institution or department where the data were generated.

3. **Subtitle** - Input up to 60 alphanumeric characters which are printed on the second line of each printout made with the **<Print>** command. This can be used to provide an analysis description or to identify the laboratory where the data were generated.

4. **Send graph to** - If both the printer and plotter are installed, select from **[Printer]** and **[XY Plotter]**. This option determines where a printout, which includes a graph, is printed.

5. **Send screen to** - This determines where a screen copy is sent when **[PrtScrn]** is clicked on. Select from either **[Printer]** or **[Bit Image File]**. If **[Bit Image File]** is selected, the information on the screen is made into a TIFF file. The TIFF file is named by the user and the file is placed in the ASCII directory.

6. **Grid** - This parameter is used for the X-Y Plotter, only. Refer to Manual 514523 for more information.

7. **Number of pens** - This parameter is used for the X-Y Plotter, only. Refer to Manual 514523 for more information.

When the desired parameters are input, click on **[OK]** to store the entries and remove the Printer and Plotter Configuration window from the display, then **<Exit>** to return to the Main window.

2.5 Calibration

There are three calibrations that are performed by the DU Series 600 Spectrophotometer: absorbance (or transmittance), wavelength and scan gain.

Absorbance (or transmittance) - The absorbance reading is calibrated each time a blank reading is taken. Blanking is discussed in section 1.2.

Wavelength - The wavelength is calibrated at the factory prior to shipment. At any time after installation, the wavelength can be recalibrated using the following instructions:

1. Verify that both sources are turned on. If either is off, turn it on by clicking on the appropriate command in the permanent menu bar.

2. With the Main window displayed, click on "**DIAGNOSTICS**" to display the Diagnostics window.

3. Click on **<Calibrate λ>**. The instrument finds the deuterium emission line at 656.1 nm, recalibrates the wavelength and stores the information. (The instrument also checks the scan gain values and adjusts them, if necessary. Scan gain is discussed below.)

4. When the wavelength calibration is complete, click on **<Quit>** to remove the Diagnostics window and display the Main window.

Under normal operating conditions, it should not be necessary to recalibrate the wavelength on a routine basis. However, it may be desirable to recalibrate the wavelength after moving the instrument.

Scan Gain

The scan gain is set at the factory prior to shipment. The gain is checked and adjusted each time that the wavelength is calibrated, as described above. When a wavelength scan is performed using a blank that has significant absorbance in the scanning range, it may be desirable to change the scan gain settings in the instrument to compensate for the absorbance of the blank. This will increase the dynamic range of the instrument when scanning using the absorbing blank. To change the scan gain:

1. With the Main window displayed, click on "**DIAGNOSTICS**" to display the Diagnostics window.

2. Place a cuvette of the blank in the cell holder.

3. Click on **<SetScanGain>**. The instrument adjusts the scan gain values to compensate for the absorbance of the blank.

4. When the scan gain has been set, click on **<Quit>** to remove the Diagnostics window and display the Main window.

NOTICE

The instrument will continue to use the new gain values until the gain is adjusted using either **<Calibrate λ>** or **<SetScanGain>**. It may be desirable to repeat the above procedure with nothing in the sample compartment after changing the gain for a significantly absorbing blank.

2.6 When Not in Use

When the instrument is not in use for more than two hours, turn off both sources. The power to the instrument can, but does not need to, remain turned on. The "Screen Saver Delay" set in the User Interface Configuration Window, dims the display after the selected time. Any movement of the mouse will re-illuminate the display.

Before using the instrument again, turn on either or both sources, depending upon the application, and allow them to warm up for at least 30 minutes before taking readings.