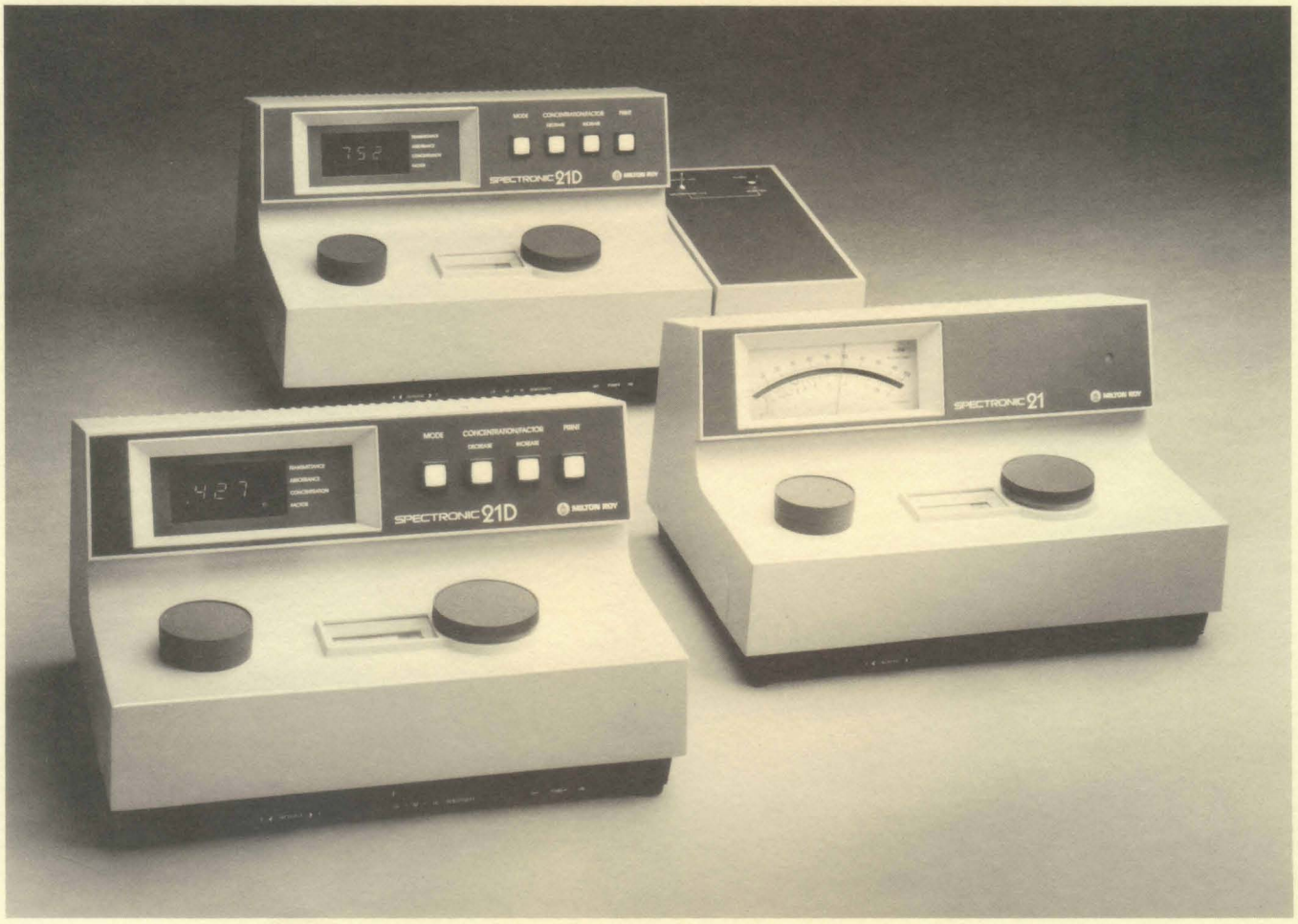
MILTON ROY

SPECTRONIC® 21

SPECTROPHOTOMETERS



OPERATOR'S MANUAL

332278-10062 • P9A21881 • 0891

LIMITED WARRANTY

Milton Roy Company Analytical Products Division instrumentation and related accessories are warranted against defects in material and workmanship for a period of one (1) year from date of delivery. This warranty is provided only if the warranty registration card is returned to Milton Roy within fifteen (15) days after delivery.

This warranty covers all parts (except those specified below) and applies only to equipment which has been installed and operated in accordance with the operator's instruction manual and which has been serviced only by authorized Milton Roy dealers or service personnel. This warranty does not apply to equipment and accessories that have been modified or tampered with in any way, misused, or damaged by accident, neglect or conditions beyond Milton Roy's control.

This warranty does not apply to lamps, glassware and similar expendable components. However, such parts and components may be warranted by their manufacturer.

Milton Roy is not responsible under this warranty for loss in operating performance due to environmental conditions.

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FCC COMPLIANCE STATEMENT FOR AMERICAN USERS

This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instruction manual, may cause interference to radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference in which case the user at his own expense will be required to take whatever measures may be required to correct the interference.

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NOTE

This Operator's Instruction Manual contains information, instructions and specifications for the SPECTRONIC®21 series of spectrophotometers that were believed accurate at the time this manual was written. However, as part of Milton Roy's on-going program of product development, the specifications and operating instructions for the spectrophotometers may be modified or changed from time-to-time. Milton Roy reserves the right to change such operating instructions and specifications. Under no circumstances shall Milton Roy be obligated to notify purchasers of any future changes in either this Operator's Instruction Manual or any other instructions or specifications relating to SPECTRONIC 21 spectrophotometers, nor shall Milton Roy be liable in any way for its failure to notify purchasers of such changes.

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INTRODUCTION

This manual provides information on the operation and maintenance of the SPECTRONIC® 21 series of spectrophotometers, a list of available accessories, and a brief description of basic spectrophotometry.

Please read this manual carefully and return the prepaid warranty registration card.

DESCRIPTION

In the SPECTRONIC 21 series of spectrophotometers, three models are available in the 115-volt and the 220-volt configurations:

Model MV: Meter readout and Visible wavelength range, Cat. No. 332242 (Domestic) and 332282 (European)

Model DV: Digital readout and Visible wavelength range, Cat. No. 332278 (Domestic) and 332284 (European)

Model DUV: Digital readout and UV/Visible wavelength range, Cat. No. 332279 (Domestic) and 332285 (European)

The major differences among these instruments involve the wavelength range and the type of readout used; however, the three models have many features in common:

- Ridges around the sample well and wavelength dial prevent spilled liquids from reaching the electronics or optics.
- The sample compartment contains a universal test tube holder which accommodates a wide variety of test tubes and cuvettes.
- The modulated optical system makes routine setting of 0% transmittance unnecessary.
- A tungsten light source is used in the visible range to 1000 nm. A deuterium light source is used for ultraviolet capability to 200 nm on the UV model.
- The 10 nm spectral slitwidth provides the sensitivity required for almost any application.
- One solid-state silicon detector covers the entire wavelength range, eliminating the need to change detectors between analysis.

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- Automatic filter insertion makes manual insertion of filters unnecessary.
- An analog output in percent transmittance allows the use of recording devices. On the two digital models, an RS-232C output connector permits the use of a data printer or computer.
- For added versatility, an exterior General Purpose Sample Compartment module is available as an accessory to accommodate long-path cells or multiple cuvettes.

SPECIFICATIONS

Wavelength Range	(MV,DV) 340 to 1000 nm (DUV) 200 to 1000 nm
Wavelength Accuracy	Better than 2 nm at 365 nm and 546 nm
Wavelength Readability	Better than 1.0 nm
Wavelength Repeatability	Better than 1 nm
Spectral Slitwidth	10 nm
Stray Radiant Energy	0.05% (typical) at 340 nm
Photometric Readout	(MV) 5-1/2-inch meter, linear transmittance (T) non-linear absorbance (A), mirrored scale. (DV,DUV) Digital display, selectable for transmittance (T), absorbance (A), or concentration (C), and A to C conversion factor (concentration factor).
Photometric Range	(MV) 0-100%T, 0 to 2A (DV,DUV) 00.0 to 100.0 in T 0.000 to 1.980 in A 0.000 to 1980 in C (.1 to 1000 in F)
Photometric Noise	Less than 0.1%T near 100%T 0.001 near 0.00A 0.002 near 1.00A
Photometric Linearity	(MV) Better than 1.0%T at meter and 0.2%T at analog output. (DV,DUV) Better than 0.2%T at display and analog output.

Photometric Accuracy	(MV) Better than 0.3%T at analog output. (DV,DUV) Better than 0.3%T (0.003A at 0.4A) at digital display.
Long-term Drift	0.003A per hour
Zero Drift	Less than 0.1%T per day
Accessory Output	Analog in %T, 1 volt (adjustable), RS-232C
Power Consumption	(MV) 40 Watts (DV) 45 Watts (DUV) 60 Watts
Voltage Requirements	Selectable: 100V, 115V, 220V, 240V; 50, 60 Hz
Size	(MV,DV) 14-1/2" W x 10-1/2" D x 8-1/2" H (36.8 cm x 26.7 cm x 21.6 cm) (DUV) 20" W x 10-1/2" D x 8-1/2" H (50.8 cm x 26.7 cm x 21.6 cm) Add 5-1/2" (14 cm) to width for General Purpose Sample Compartment
Weight	(MV) 16 lbs. (7.3 kg) (DV) 16-1/2 lbs. (7.5 kg) (DUV) 22-1/2 lbs. (10.2 kg) Add 3-1/2 lbs. (1.6 kg) for General Purpose Sample Compartment.

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After carefully unpacking the contents, check the materials against the packing list to ensure that you have received everything in good condition.

If any part is missing or damaged, or if you find any other defect, contact your dealer or Milton Roy sales representative immediately.

NOTE

Do not discard the shipping carton or packing material. This material has been designed to protect your unit during shipping and should be reused to protect your unit if it needs to be shipped in the future.

Included with your SPECTRONIC 21 spectrophotometer are:

Operator's Manual
Box of 12 10-mm cuvettes
Universal test-tube holder (built into sample compartment)
Square cuvette adapter
Dust cover
Occluder
Screwdriver
Spare tungsten lamp

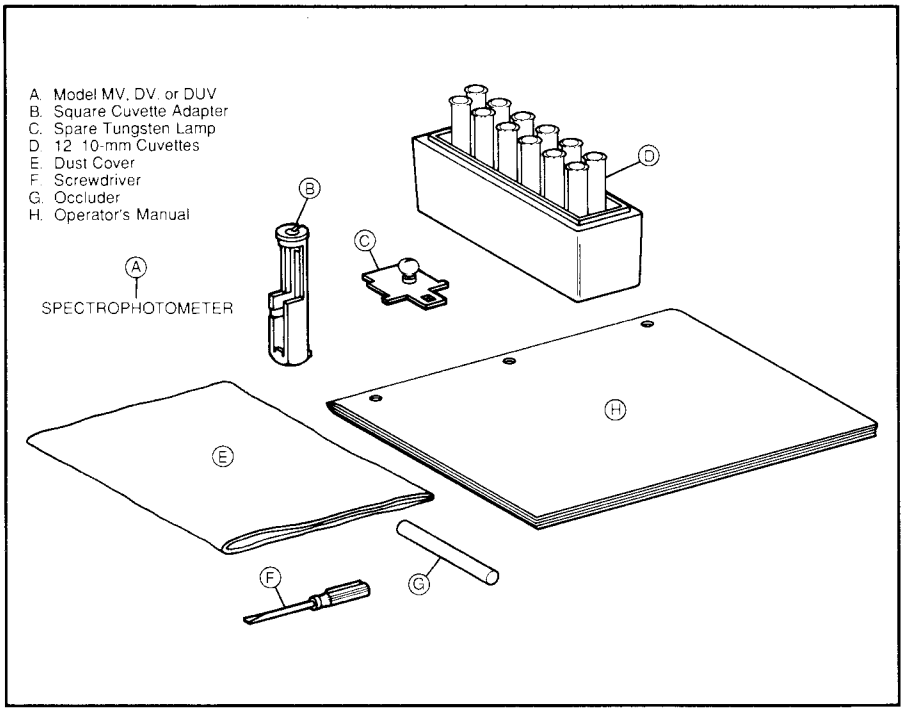

FIGURE 1.
Visual Checklist for SPECTRONIC 21 Spectrophotometer

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Place the spectrophotometer in an appropriate location near a power outlet. Plug the three-pronged cord attached to the instrument into the appropriate power outlet.

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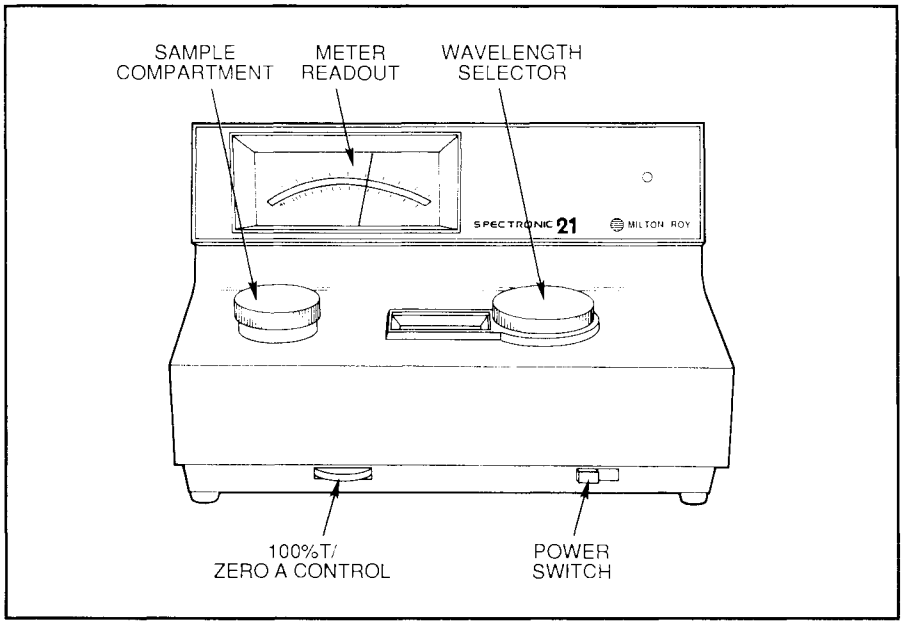

FIGURE 2.
SPECTRONIC 21 MV Operating Controls

OPERATING FEATURES

Figure 2 shows the key features of your spectrophotometer.

WAVELENGTH SELECTOR.
This control knob is used to select the desired analytical wavelength of the instrument. The selected wavelength is indicated by the graduated dial to the left of the knob.

100%T/ZERO A CONTROL.
This control is used to set the display to 100%T (0A). **IT MUST BE RESET WHENEVER THE ANALYTICAL WAVELENGTH HAS BEEN CHANGED.** Also, when operating at a fixed wavelength for an extended period of time, check the 100%T readout and readjust if necessary.

METER READOUT.
The 5-1/2" meter, mirrored to prevent parallax error, is linear in transmittance and also calibrated in absorbance. The meter scale reads from 00.0 to 100% transmittance with linear scale divisions at every 1% transmittance. For most analytical purposes absorbance values are more linear than transmittance values and can be more conveniently utilized. An analog output connector permits the use of recording devices to record results in percent transmittance.

SPECTRONIC 21 OPERATING PROCEDURES

NOTE

The instrument is set to operate on a 115 VAC line. If this voltage is not correct for your line, see the Voltage Conversion instructions in the Maintenance section.

Operating Controls are shown in Figure 2.

1. Plug the instrument into a grounded outlet.

2. Turn the instrument on, using the POWER switch on the right side of the lower control panel. Although the instrument can be used almost as soon as it is turned on, best performance is achieved following a warmup period of 15 to 30 minutes.

3. Select the desired wavelength with the wavelength selector. Wavelength is indicated on the dial located to the left of the wavelength selector.

4 Choose matched cuvettes of the appropriate pathlength for the analytical method. Use the same pathlength cuvettes for all blanks, standards, and samples.

5. Open the sample compartment cover.

6. Fill one cuvette with a blank solution and insert it into the sample compartment. Fill the cuvette with enough solution to completely cover the light beam passing through the sample compartment. Milton Roy test tube cuvettes are provided with a horizontal fiducial mark to indicate the proper fill level. A vertical fiducial mark is also provided for alignment with the mark on the sample compartment.

NOTE

a. *Solution level must be at least 20 mm high in a standard cuvette (10 mm square; used with cuvette adaptor).*

b. *Solution level must be at least 32 mm high in a test tube cuvette (used with universal test tube holder).*

7. Close the sample compartment cover.
8. Set 000A or 100%T for the blank using the 100%T/zero A control located on the left side of the lower control panel.
9. Remove the blank.
10. Fill the matched cuvettes, each with a standard solution or sample to be measured.
11. Insert each sample or standard solution, in turn, in the sample compartment.
12. Read and record the value.
13. Construct a standard curve by plotting the absorbance on the y-axis vs. the concentration of each standard solution on the x-axis.
14. Determine the concentration of each sample by finding its absorbance value on the y-axis of the standard curve and reading the corresponding concentration value off the x-axis.
15. Correct for any sample blank or interference effects as necessary.

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SPECTRONIC 21 DV AND DUV OPERATION

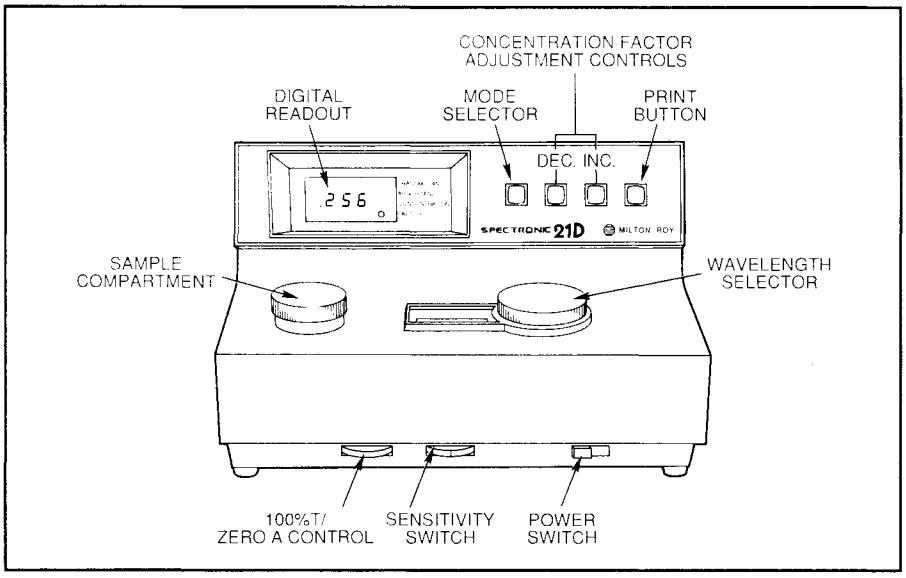
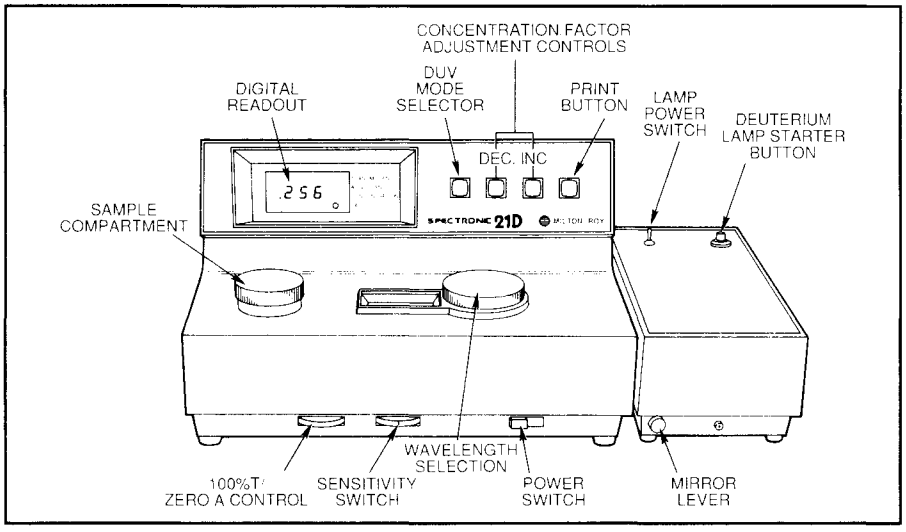
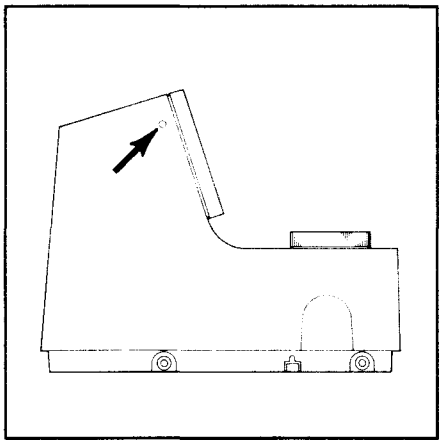


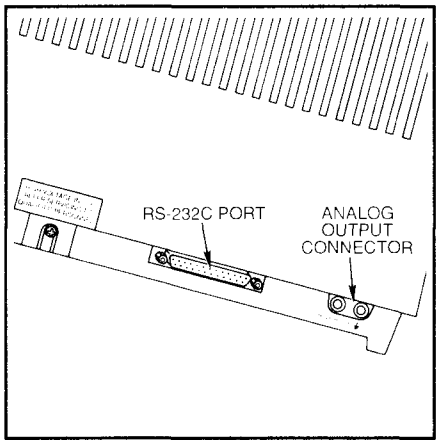
FIGURE 3.
SPECTRONIC 21 DV and DUV Operating Controls

Front View, SPECTRONIC 21 DV



Front View, SPECTRONIC 21 DUV





LEFT: Left side view showing location of calibration adjustment

RIGHT: Back view showing location of RS-232 Port/ Serial Port and Analog Output Connector.

Figure 3 shows the key features of your spectrophotometer.

WAVELENGTH SELECTOR
This control knob is used to select the desired analytical wavelength of the instrument. The selected wavelength is indicated by the graduated dial to the left of the knob.

100%T/ZERO A CONTROL
This control is used to set the display to 100%T (0A). **IT MUST BE RESET WHENEVER THE ANALYTICAL WAVELENGTH HAS BEEN CHANGED.** Also, when operating at a fixed wavelength for an extended period of time, check the 100%T readout and readjust if necessary.

SENSITIVITY SWITCH
This control is a coarse adjustment for the 100%T/Zero A control. If you cannot set 100%T, 0A or 0C on the blank, move the Sensitivity Switch to the M (medium) or the HI (high) position. Any position at which 100%T can be set is acceptable. However, **BE SURE TO RESET THE 100%T/ZERO A CONTROL EACH TIME THAT YOU RESET THE SENSITIVITY SWITCH.** Also, all blank, standard solution, and sample measurements should be made at the same sensitivity setting.

DIGITAL READOUT
The Digital Readout displays the data readings. The four LED status indicators, next to the labels TRANSMITTANCE, ABSORBANCE, CONCENTRATION, and FACTOR, indicate the currently active mode.

MODE SELECTOR
This control selects the TRANSMITTANCE, ABSORBANCE, CONCENTRATION, or FACTOR mode.

CONCENTRATION/FACTOR ADJUST CONTROLS
The buttons labeled INCREASE and DECREASE are used in CONCENTRATION and FACTOR modes. To set a lower CONCENTRATION or FACTOR value, press and hold down the DECREASE button until the desired value is displayed. To set a higher value, press and hold down the INCREASE button until the desired value is displayed.

PRINT BUTTON
Press this button to send displayed data to a printer or computer connected to the serial port.

RS-232C PORT
This is the DB25P connector located on the back of the instrument. The pin functions for this connector are listed below:

1 - Chassis ground.
2 - SPEC 21 transmits data.
3 - SPEC 21 receives data.
5 - Clear to send signal.
7 - Signal ground.

ANALOG OUTPUT
The analog output connector is a double-banana jack outlet located on the back of the instrument. The analog output signal level is factory-set to approximately 1.0 VDC at 100%T. The output is in %T only. This allows you to interface a Y-T recorder to obtain a permanent record of photometric values during sample analysis, or to monitor changing values during time-rate enzyme procedures.

OPERATING PROCEDURES

NOTE

Check the instrument voltage printed on the serial plate. If this voltage is not correct for your line, see the Voltage Conversion instructions in the Maintenance section.

1. Plug the instrument into a grounded outlet.

2. Turn the instrument on, using the POWER switch on the right side of the lower control panel. Although the instrument can be used almost as soon as it is turned on, best performance is achieved following a warmup period of 15 to 30 minutes.

3. Select the desired wavelength with the wavelength selector. Wavelength is indicated on the dial located to the left of the wavelength selector.

IF YOU HAVE A SPECTRONIC 21 DUV, DO ALL REMAINING STEPS. FOR MODEL DV, CONTINUE AT STEP 7.

4. Adjust the mirror position for the lamp required by pulling the mirror lever out when using the deuterium lamp (UV) and pushing the mirror lever in when using the tungsten lamp (VIS).

5. Choose the correct lamp for the selected wavelength by flipping the lamp power switch to TUNGSTEN-VIS or DEUTERIUM-UV.

6. For UV wavelength analyses, ignite the deuterium lamp by pushing the STARTER button for approximately 2 seconds and releasing. The deuterium lamp requires a warm-up of at least 10 minutes before readings are taken. Maximum lamp stability takes longer.

NOTE

The portion of the wavelength dial marked with a red line indicates that the tungsten lamp should be used. The portion of the wavelength dial marked with a blue line indicates that the deuterium lamp should be used. The portion of the wavelength dial marked with alternate red and blue marks indicates that the lamp which provides the most energy (gives the highest %T when no sample is in place) should be used.

7. Choose matched cuvettes of the appropriate pathlength for the analytical method. Use the same pathlength cuvettes for all blanks, standards, and samples.

NOTE

The glass test tubes supplied with the instrument are useable only above 325 nm. Quartz or "fused silica" cuvettes must be used below 325 nm.

8. Open the sample compartment cover.

9. Fill one cuvette with a blank solution and insert it in the sample compartment. Fill the cuvette with enough solution to completely cover the light beam passing through the sample compartment. Milton Roy test tube cuvettes are provided with a horizontal fiducial mark to indicate the proper fill level. A vertical fiducial mark is also provided for alignment with the mark on the sample compartment.

NOTE

a. Solution level must be at least 20 mm high in a standard cuvette (10 mm square; used with cuvette adaptor).

b. Solution level must be at least 32 mm high in a test tube cuvette (used with universal test tube holder).

10. Close the sample compartment cover.

11. Select the operating mode - TRANSMITTANCE, ABSORBANCE, or CONCENTRATION - using the mode selector.

12. Set the SENSITIVITY switch located in the center of the lower control panel to LO.

13. Set 100%T, 000A, or 000C for the blank using the 100%T/zero A control located on the left side of the lower control panel. If there is not enough energy to set 100%T, 000A, or 000C on the blank, move the SENSITIVITY switch to the M (medium) or, if necessary, to the HI position. Be sure to reset the 100%T/zero A control each time that you reset the SENSITIVITY switch.

14. Remove the blank.

15. Fill each of the matched cuvettes with a standard solution or sample to be measured. Then proceed to step 16 under the appropriate heading of the mode you are using.

ABSORBANCE or TRANSMITTANCE MODE

16. Press MODE SELECTOR for the appropriate mode.

17. Construct a standard curve by plotting absorbance/transmittance on the y-axis vs. the concentration of each standard solution on the x-axis.

18. Determine the concentration of each sample by finding its data value on the y-axis and reading the corresponding concentration value off the x-axis.

19. Correct for any sample blank or interference effects as necessary.

CONCENTRATION MODE (to be used only if the linearity of the standard curve has been verified)

16. Press MODE SELECTOR until the CONCENTRATION LED is lit. Place the standard solution in the sample compartment.

17. Using the CONCENTRATION/FACTOR ADJUST controls, set the concentration value of the standard on the digital display. Repeat this step with other standards for verification.

18. Insert samples in the sample compartment and read results directly in concentration units.

NOTE

If the factor is already known for your chemistry, you can enter this value by pressing MODE until the FACTOR LED is lit, and use the CONCENTRATION/FACTOR ADJUST controls to set the display to the desired factor value.

SENDING DATA TO THE SERIAL PORT

Normally, the spectrophotometer is in the print mode when it is first turned on, and operates at a rate of 1200 baud. Other baud rates may be selected when the spectrophotometer is connected to a computer. These rates include 110, 300, 1200, 2400, 4800, and 9600 baud.

If the SPECTRONIC 21 DV or DUV spectrophotometer is turned on while the PRINT button is pressed, the serial port adjusts to the computer's baud rate upon receipt of either the letter E (Enter) or a CR (carriage return) character from the computer.

An external device may send the following commands to the SPECTRONIC 21 DV or DUV spectrophotometer:

P - "Print"
A - Set the data mode to absorbance.
T - Set the data mode to transmittance.
C - Set the data mode to concentration.
F - Set the data mode to factor.

CONTROL-X - Reset spectrophotometer to initial power-up condition.

E (or an ASCII carriage return, CR) - Sets spectrophotometer's baud rate to the rate used by a computer connected to the serial outputpor

DATA FORMAT. (See Table 1. Serial I/O Data Conventions.) Data from the instrument to a printer or other remote device is sent as shown in the following examples:

Photometric Data (three digits, with decimal point)	A, T, C, or F
-01.5	A CR & LF
1.58	A
90.0	T
1.080	C
-.004	C

Table 1. Serial I/O Data Conventions

WORD LENGTH	8 data bits, 1 stop bit. Most significant bit set to 0.
PARITY	None.
ECHO	None.
TERMINATORS	Ignores all carriage returns or line feed characters sent by external device (except for CR in Auto Baud Rate mode). Transmits ASCII CR or LF after every data line.

SPECTRONIC 21 PROGRAMMING EXAMPLES:

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The programs are written in IBM BASICA language using IBM DOS, Version 3.3.

These programs are intended only as hypothetical examples to demonstrate the interface capability of the spectrophotometer and how to obtain data. Although these examples have performed their functions correctly using IBM AT equipment available to the Milton Roy Applications Laboratory, Milton Roy Company is not responsible for the execution of these programs on equipment at other locations or for the accuracy of the results obtained using these programs.

EXAMPLE A

```
1000 '  Spec 21-Digital Program
1010 '  Changes measurement modes and prints data to CRT
1020 '
1030 '  Uses Default 21-D Baud Rate of 1200
1040 '
1050 '
1060 OPEN "COM1:1200,N,8,1" AS #1     :'  Set IBM Serial Port
1070 '
1080 PRINT #1, "A"                    :'  Set 21 to Absorbance
                                          mode
1090 FOR I = 1 TO 10000:NEXT I        :'  Wait loop for comple-
                                          tion of command
1100 GOSUB 1250                       :'  Input and print data
1110 '
1120 PRINT #1, "T"                    :'  Set 21 to Transmit-
                                          tance mode
1130 FOR I = 1 TO 10000:NEXT I
1140 GOSUB 1250
1150 '
1160 PRINT #1, "C"                    :'  Set 21 to Concentra-
                                          tion mode
1170 FOR I = 1 TO 10000:NEXT I
1180 GOSUB 1250
1190 '
1200 PRINT #1, "F"                    :'  Set 21 to Factor
1210 FOR I - 1 TO 10000:NEXT I
1220 GOSUB 1250
1230 END
1240 '
1250 PRINT #1, "P"                    :'  Cause 21 to output
                                          data
1260 INPUT #1, DATUM$                 :'  Cause computer to
                                          input data string
1270 PRINT DATUM$                     :'  Print data string on
                                          CRT
1280 RETURN
```

EXAMPLE B

```
100 '   Program to automatically set the 21-D Baud Rate
110 '
120 OPEN "COM1:9600,N,8" AS #1      :'  Set up IBM Serial
                                        Port
130 '
140 PRINT #1,"E";                   :'  Send character for
                                        Auto Baud Rate
150 FOR I = 1 TO 500                :'  Wait loop for comple-
                                        tion of command
160 NEXT I
165 '
170 ON ERROR GOTO 310               :'  Error trap for full
                                        input buffer
180 PRINT #1,"P";                   :'  Clear input buffer
190 INPUT #1,A$
200 '
210 ON ERROR GOTO 0                 :'  IBM command to
                                        enable error response
220 '
230 'Obtain data at new baud rate
240 PRINT #1,"A";                   :'  Set 21 to Absorbance
                                        mode
250 PRINT #1,"P";                   :'  Cause 21 to send data
260 INPUT #1,A$                     :'  Cause computer to
                                        input data string
270 PRINT A$                        :'  Print data string on
                                        CRT
280 '
290 CLOSE                           :'  Close communica-
                                        tions file
300 END
305 '
310 CLOSE                           :'  Close communica-
                                        tions file
320 OPEN "COM1:9600,N,8" AS #1      :'  Re-open communica-
                                        tions file
330 RESUME
```

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SCOPE OF OPERATOR MAINTENANCE

This section describes all maintenance procedures that should be performed by the operator. All other maintenance, troubleshooting, or repair tasks should be performed only by an authorized Milton Roy service representative.

TROUBLESHOOTING

Table 2 presents the probable causes and remedies for most operating and instrument problems. If you encounter a problem that the table does not enable you to diagnose, call an authorized Milton Roy service representative.

Table 2. Operator Troubleshooting Chart

PROBLEM	POSSIBLE CAUSE	REMEDY
Instrument inoperative.	Line cord not connected to outlet.	Plug instrument in.
	Dead power outlet.	Change to different outlet.
	Internal fuse blown.	Call an authorized service representative.
	Defective electronic component.	Call an authorized service representative.
Cannot set 100%T (.000A) with no sample in sample holder.	Light beam blocked.	Check sample compartment.
	Wrong source lamp for wavelength being used (UV model).	Check lever position for lamp being used. Lever should be pushed in for visible range and pulled out for UV range.
	Sensitivity Switch setting is too low.	Set to M or HI position.
	Source lamp not adjusted (UV model).	Refer to Lamp Alignment procedures.

Table 2. (Cont.)

PROBLEM	POSSIBLE CAUSE	REMEDY
Cannot set 100%T (.000A)–continued	Source lamp defective or old.	Refer to Lamp Replacement procedures.
	Defective electronic component.	Call an authorized service representative.
T cannot be set to 00.0% with occluder block in sample holder.	Sample compartment drain hole or port cover not closed.	Close covers.
	Instrument out of electronic calibration.	Refer to Electronic Calibration procedure.
	Defective electronic component.	Call an authorized service representative.
	Light leaks around detector access cover.	Call an authorized service representative.
Instrument drift and noise.	Excessive line voltage variation.	Check line voltage and grounding.
	Incorrect voltage selector switch setting.	Refer to Voltage Conversion procedure.
	Source lamp defective or old.	Replace with appropriate lamp.
	Bubbles or particles in solution.	Check sample preparation and analytical procedure.
	Source lamp not adjusted (UV model).	Refer to Lamp Alignment procedures.
	Defective or dirty detector.	Refer to Detector Maintenance procedures.
	Defective electronic component.	Call an authorized service representative.
Incorrect transmittance-to-absorbance correlation.	Defective electronic component.	Call an authorized service representative.

Table 2. (Cont.)

PROBLEM	POSSIBLE CAUSE	REMEDY
Digital display does not change regardless of instrument settings.	Lamp burned out.	Replace lamp.
	Defective electronic component.	Call an authorized service representative.
Incorrect reading obtained.	Stray sample preparation vapors.	Prepare samples away from instruments; use proper ventilation.
	Insufficient sample volume.	Fill cuvette with greater sample volume.
	Bubbles or particles in solution.	Check sample preparation and analytical procedure.
	Wrong wavelength setting.	Check analytical procedure and wavelength setting.
	Instrument out of electronic calibration.	Refer to Electronic Calibration procedure.
Cannot send data to printer or computer.	Cable disconnected.	Connect cable.
	Wrong baud rate.	Change baud rate to correct value, using procedure on Page 20.

SPECTRONIC 21 VOLTAGE CONVERSION

1. Turn off and unplug the instrument.
2. Tilt the instrument onto its back for access to the voltage selector switch, located on the underside of the instrument base.
3. Refer to Figure 4. Remove the voltage selector switch cover (mounted with two holding screws).
4. Refer to Figure 5. Loosen the locking nuts one turn each.
5. Set the voltage selector switch at the desired voltage (100, 115, 220, or 240 VAC).
6. Retighten the locking nuts.
7. Replace the voltage selector switch cover and tighten the two holding screws.

FIGURE 4. Location of Voltage Selector Switch

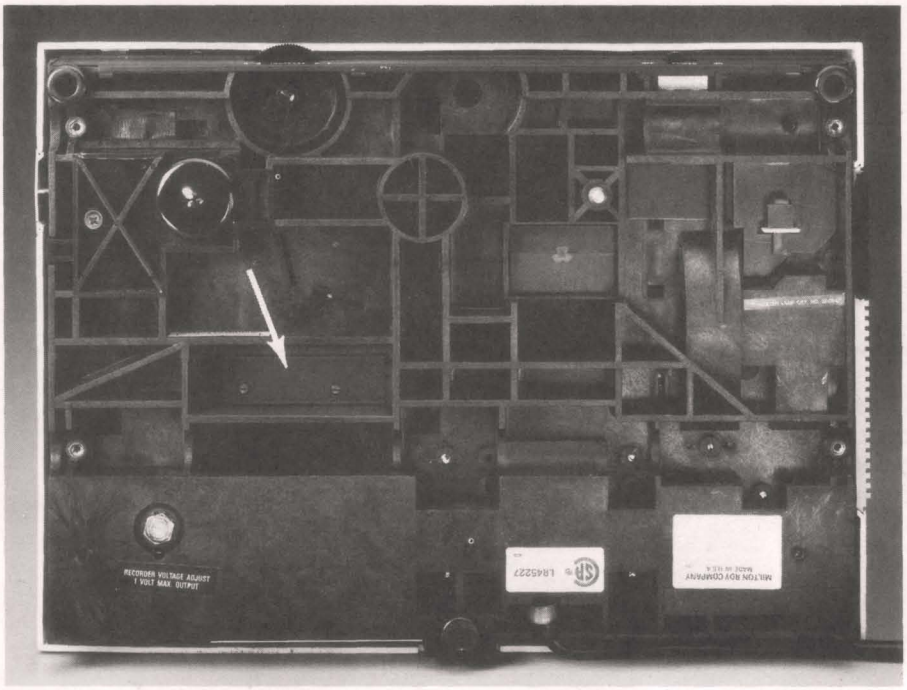
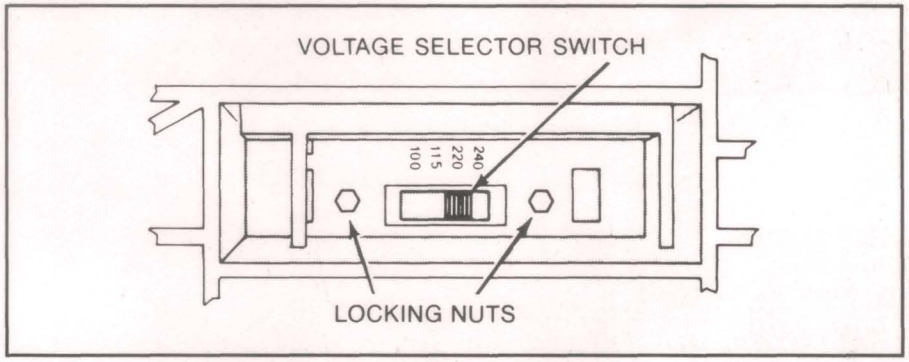


FIGURE 5. Voltage Selector Switch

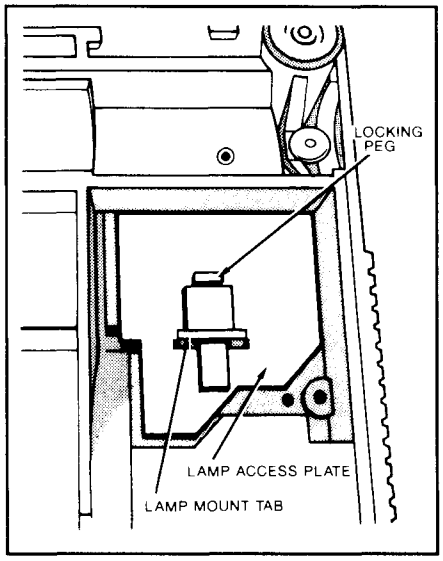


TUNGSTEN LAMP REPLACEMENT

Models MV and DV

1. Turn off and unplug the instrument.

2. Tilt the instrument onto its back for access to the lamp housing, located in the upper right corner of the instrument base.



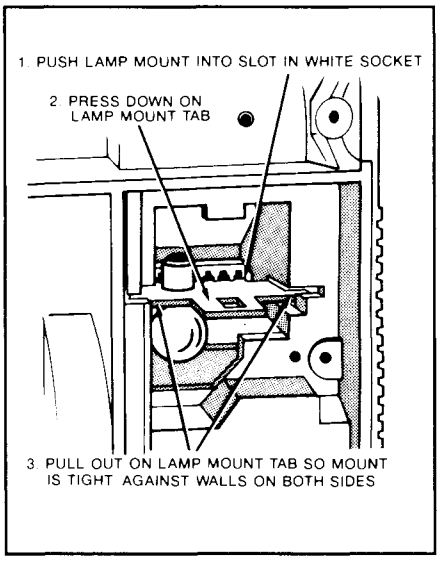


FIGURE 6. (Left) Exterior of Lamp Housing on Model MV or DV

FIGURE 7. (Right) Installation of Lamp in Model MV or DV

3. Refer to Figure 6. Lift the locking peg out of the lamp mount tab (pry gently with a screwdriver, if necessary). Pull the lamp access plate off.

4. Grasp the lamp mount tab and raise the lamp mount so its edges are aligned with the slots in the base. Pull the lamp mount out through the slots.

5. Check the new lamp for cleanliness and wipe if necessary. Avoid touching the lamp during installation.

6. Refer to Figure 7. Slide the new lamp assembly into the housing and push the plated end of the lamp mount into the socket at the rear of the lamp housing.

7. Press the lamp mount tab down, then pull forward on the tab until the front edge of the mount is tight against the walls on both sides.

8. To replace the access plate, hook the tab which is on the inner surface of the access plate under the tab at the top of the housing, and press the plate into place. Slide the plate as far to the right as possible.

9. Insert the locking peg into the eye on the lamp access plate. Slide the peg down through the lamp mount tab until the peg is snug.

NOTE

When properly installed, the tip of the peg is visible on the outside of the lamp access plate. If not, remove the plate and go back to step 5.

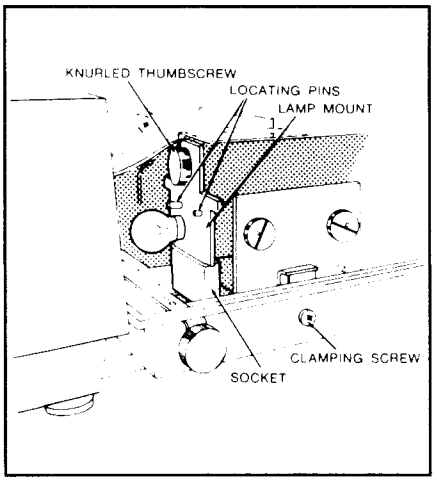
Model DUV

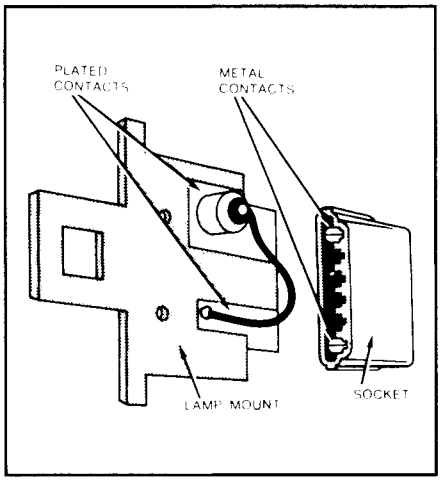
1. Turn off and unplug the instrument.

2. Refer to Figure 8. Loosen the clamping screw on the front of the lamp housing base two full turns. Lift the lamp housing cover open.

FIGURE 8. (Left) Tungsten Mounting in Model DUV

FIGURE 9. (Right) Alignment of Tungsten Lamp Mount and Socket in Model DUV





3. Unscrew and remove the knurled thumbscrew securing the tungsten lamp mount. Slide the lamp mount off the two locating pins. Grasp the socket and pull the lamp mount to remove the old lamp assembly.

CAUTION
Do not pull on the wires.

4. Check the new lamp for cleanliness and wipe if necessary. Avoid touching the lamp during installation.

5. With the contact aligned as shown in Figure 9, plug the new lamp into the socket.

6. Slide the lamp mount onto the two locating pins. Replace the knurled thumbscrew.

7. Perform the Tungsten Lamp Alignment procedure in this section.

8. Close the lamp housing cover and tighten the clamping screw.

DEUTERIUM LAMP REPLACEMENT

(MODEL DUV ONLY)

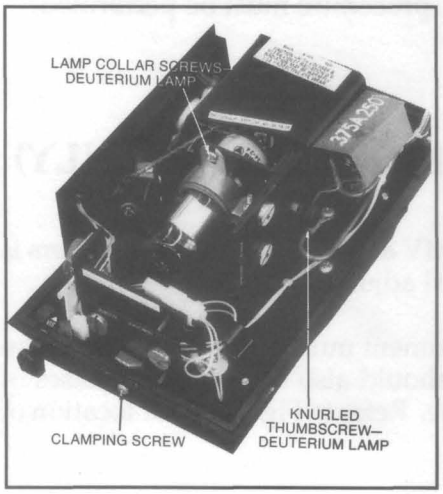
1. Turn off and unplug the instrument.

2. Refer to Figure 10. Loosen the clamping screw on the front base of the lamp house cover two full turns and open cover.

3. Pull the lamp wires off the spade connectors located behind the lamp mounting plate (see Figure 11).

4. Unscrew the knurled thumbscrew, shown in Figure 10, to release the lamp mounting plate from the adjusting bracket and lift out the lamp bracket assembly.

CAUTION
Do not touch the lamp envelope. Any fingerprints on the lamp should be removed before ignition of the lamp or they will be baked on.



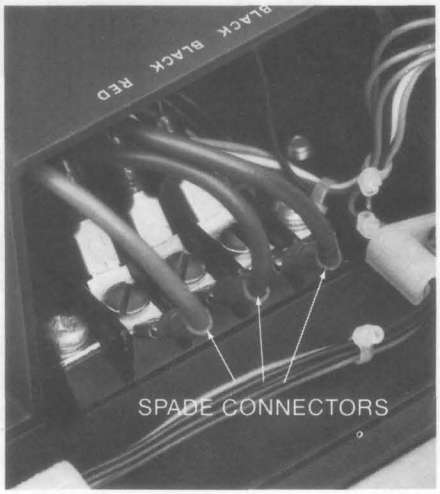


FIGURE 10. (Left)
Deuterium Lamp Mounting

FIGURE 11. (Right)
Deuterium Lamp
Spade Connectors

5. Unscrew the lamp collar screws to remove the lamp assembly from the mounting plate.

6. Place the new lamp assembly on the lamp mounting plate and secure with the lamp collar screws.

7. Place the lamp mounting plate against the adjusting bracket, making sure the bottom aligning post is in the slot, and the top aligning post is through the hole provided on the mounting plate.

8. Replace and tighten the knurled thumbscrew.

9. Place the lamp wires over the spade connectors located behind the mounting plate, matching colors as indicated on the label.

10. Be sure the lamp is properly aligned (see Lamp Alignment procedure in this section) and completely free of dust and fingerprints.

11. Close the lamp house cover and tighten the clamping screw.

EXTENDED LIFE TUNGSTEN LAMP

(CAT. NO. 333308)

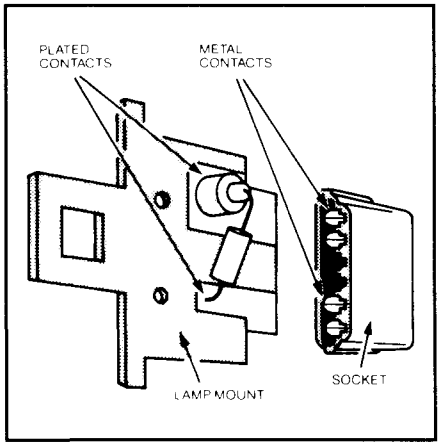


FIGURE 12. Alignment of Extended Life Lamp Mount and Socket in Model DUV

This 380-to-1000-nm lamp can be used in all models of the SPECTRONIC 21 spectrophotometer. Installation and replacement procedures are exactly the same as given for Tungsten Lamp 333307.

If an Extended Life Tungsten Lamp is installed in Model DUV, the Tungsten Lamp Alignment procedure must be performed.

LAMP ALIGNMENT (MODEL DUV ONLY)

The lamp in the SPECTRONIC 21 MV and DV spectrophotometers is self-aligning and requires no special adjustment.

In a Model DUV, lamp alignment must always be done when a lamp assembly is replaced, and should also be checked in cases of reduced lamp energy or excess noise. Refer to Figure 13 for location of adjustments.

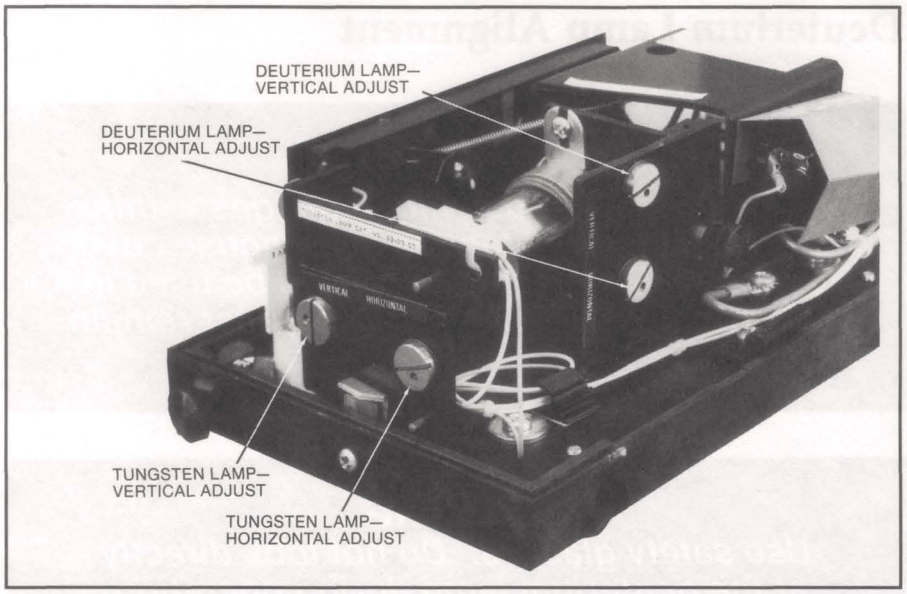


FIGURE 13.
Lamp Alignment Adjustments

Tungsten Lamp Alignment

1. Turn the instrument on.
2. Set the lamp power switch to TUNGSTEN-VIS.
3. Push the mirror lever all the way in for the visible range.
4. Loosen the clamping screw on the front base of the lamp house cover two full turns and open the cover.
5. Using the wavelength selector, set the wavelength to 450 nm.
6. Set the mode selector to TRANSMITTANCE.
7. Close the sample compartment door.
8. Rotate the 100%T/zero A control to set a convenient transmittance reading around 50%T.
9. Using the eccentric screws on the tungsten lamp adjusting bracket, adjust the position of the lamp to obtain a maximum transmittance reading as follows:

   Turn the left screw to move the lamp assembly vertically to obtain a maximum transmittance readout. Turn the right screw to move the lamp assembly horizontally to obtain a maximum transmittance readout.
10. Repeat left and right screw adjustments until a maximum transmittance value is obtained. It may be necessary during this procedure to adjust the 100%T/zero A control (step 8) to reset a convenient, observable transmittance reading.
11. Close the lamp house cover and tighten the clamping screw.

SPECTRONIC 21 Deuterium Lamp Alignment

WARNING
There are dangerous high voltages within this equipment. When the lamp house cover is removed, utmost care should be used in this procedure since the power must remain on.

WARNING
Use safety glasses. Do not look directly at the deuterium lamp during operation. Eye damage may result.

1. Turn the instrument on.
2. Set the lamp power switch to DEUTERIUM-UV.
3. Ignite the deuterium lamp by depressing the starter button for approximately two seconds.
4. Set the mirror lever to the UV position.
5. Loosen the clamping screw on the front base of the lamp house cover two full turns and open cover.
6. Using the wavelength selector, set the wavelength to 240 nm.
7. Set the mode selector to TRANSMITTANCE.
8. Close the sample compartment cover.
9. Rotate the 100%T/zero A control to set a convenient transmittance reading around 50%T.
10. Using the eccentric screws on the deuterium lamp adjusting bracket, adjust the position of the lamp to obtain a maximum transmittance value as follows:

    Adjust the screws to the position shown in Figure 13. By turning the upper screw, move the lamp assembly vertically to obtain a maximum transmittance readout. By turning the lower screw, move the lamp assembly horizontally to obtain a maximum transmittance readout.

11. Repeat upper and lower screw adjustments until a maximum transmittance value is obtained. It may be necessary during the procedure to adjust the 100%T/zero A control (Step 9) to reset a convenient, observable transmittance reading.

12. Close the lamp house cover and tighten the clamping screw.

DETECTOR MAINTENANCE

Low energy or the inability to set 100%T or zero A may indicate the need for cleaning or replacing the detector.

1. Turn off and unplug the instrument.

2. Tilt the instrument onto its back for access to the bottom.

3. Remove the detector access plate (shown in Figure 14) by removing the holding screw and lifting the tab on the left.

4. Grasp the detector mounting board and pull it out through the aligning slots. If pliers are used, pull directly below the socket and only contact the mounting board on the very edge to avoid damaging metal runs or components.

5. Inspect the silicon chip for dirt and fingerprints. If necessary, clean gently with a cotton swab which has been dipped in methyl alcohol.

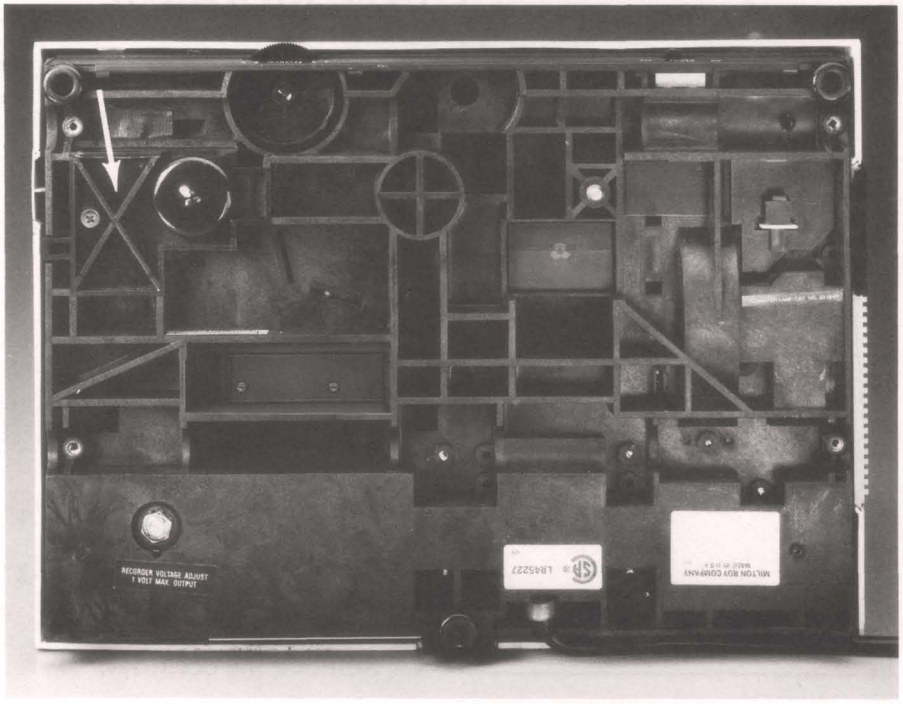


FIGURE 14.
Location of
Detector Access Plate

6. Insert the cleaned detector or insert a new detector by grasping the mounting board and inserting it through the aligning slot (as shown in Figure 15). Be sure the silicon detector faces toward the light source and that the white plastic terminal properly engages the connectors located in the instrument.

7. Replace the detector access plate and secure with the holding screw.

FIGURE 15. Detector Replacement

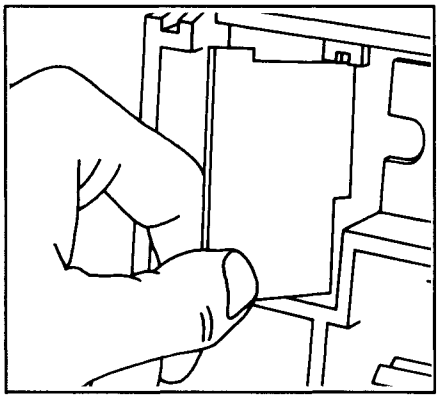


FIGURE 16. Location of 0%T Calibration Adjustment for Model MV.

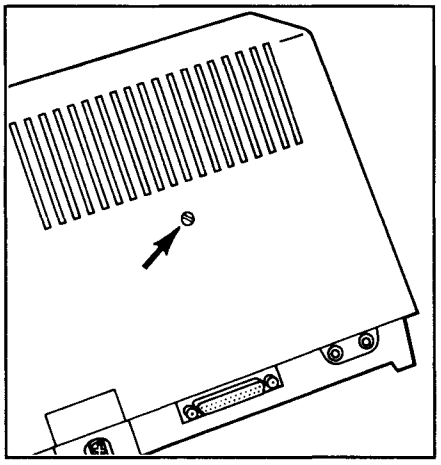
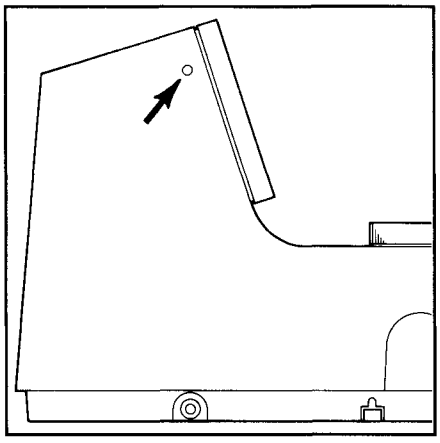


FIGURE 17. Location of 0%T Calibration Adjustment for Models DV and DUV.



ELECTRONIC CALIBRATION

The spectrophotometer has been factory-calibrated and does not require periodic calibration. Calibration should be performed only when results lead you to believe it is necessary.

In the following procedures, an accuracy of ±1 digit is adequate for most applications.

Before calibrating, turn the spectrophotometer on and let it warm up for at least 30 minutes.

0%T CALIBRATION (ALL MODELS)

1. Set the wavelength dial to 450 nm. On digital models, set the mode selector to TRANSMITTANCE. With the 100%T/zero A control, set the display to read 100.0.

2. Install the occluder in the sample well and close the cover.

3. Turn the 0%T calibration adjustment (shown in Figures 16 and 17) until the meter reads "zero" or the digital display reads exactly 00.0.

4. Remove the occluder.

NOTE

Log-linear recorders such as those available from Houston Instrument Co. will permit direct recording in T, A, or C.

40-COLUMN PRINTER

110V, Cat. No. 335488
220V, Cat. No. 335489

SAMPLE-HANDLING ACCESSORIES

General Purpose Sample Compartment, Cat. No. 332244

Comes with lens system, multiple sample holder, and connection hardware for all models.

For Use with General Purpose Sample Compartment

SEMI-MICRO FLOW-THRU CUVETTE HOLDER, Cat. No. 332233

Required for using 10 mm pathlength semi-micro or flow-thru cuvettes 45 to 48 mm tall.

THERMO-REGULATED CUVETTE HOLDER, Cat. No. 333014

Water-jacketed multiple sample holder with three-position thermo-block maintains 10 mm square cuvettes at temperatures between 5 and 55°C while measurements are being made on sample requiring a temperature-controlled environment.

SPECTRONIC 21

For Use with Main Instrument Sample Compartment

CUVETTE ADAPTER, Cat. No.. 332208
Required for short cuvettes, 45 to 48 mm tall.

SAMPLE COMPARTMENT LIGHT SHIELD, Cat. No. 335410
For tall test tubes or cuvettes up to 150 mm tall.

SPACER, Cat. No. 333528
For use with 2 mm pathlength cuvettes, 45 mm tall.

SPACER, Cat. No. 333527
For use with 5 mm pathlength cuvettes, 45 mm tall.

SPACER, Cat. No. 333529
For use with 1 mm pathlength cuvettes, 45 mm tall.

CUVETTES

Selected, 10 mm pathlength, optical glass, box of 12.
Cat. No. 331775.

Selected, 20 mm pathlength, optical glass, box of 12.
Cat. No. 331777.

Square cuvettes, 10 mm pathlength, optical glass, matched set of 2.
Cat. No. 331709.

Square cuvettes, 10 mm pathlength, silica, matched set of 2.
Cat. No. 331742.

Cylindrical cells, 50 mm pathlength, optical glass, matched set of 2.
Cat. No. 331731.

Cylindrical cells, 50 mm pathlength, silica, matched set of 2.
Cat. No. 331768.

Cylindrical cells, 100 mm pathlength, optical glass, matched set of 2.
Cat. No. 331732.

Cylindrical cells, 100 mm pathlength, silica, matched set of 2.
Cat. No. 331769.

SPECTRONIC® STANDARDS, Cat. No. 333150

SPECTRONIC standards let you check spectrophotometer performance quickly, accurately, as part of normal laboratory quality control program. They make it as easy as loading a cuvette to check 0%T, wavelength accuracy, stray radiant energy, optical alignment, and photometric accuracy, linearity, and precision. The standards are individually tested and certified by Milton Roy and traceable to NBS wherever applicable.

NULL MODEM CABLE

Cat. No. 335243

The cable is used to connect the SPECTRONIC 21DV or DUV and the IBM PC/XT or PS/2 computers. The IBM PC/AT requires 335243 and 345002-111.

REPLACEMENT ITEMS

CAT. NO.	DESCRIPTION
333307	Tungsten lamp assembly, 10 watts, 6.7 volts, for all SPECTRONIC 21 models, package of 2, prealigned
333308	Extended-life visible lamp assembly for all SPECTRONIC 21 models, package of 2, prealigned, usable between 380-1000 nm
343424	Deuterium lamp for Model DUV
332224	Detector assembly for Models MV and DV
332223	Detector assembly for Models DUV and General Purpose Sample Compartment
332239	Sample compartment cover, hinged, brown
332221	Lens system, entrance, for General Purpose Sample Compartment
333152	Sensitized paper for SPECTRONIC Standards

SPECTRONIC 21

40

APPENDIX A
BASIC SPECTROPHOTOMETRY

Spectrophotometry refers to the relative measurement of transmitted light as a function of wavelength. The measurements are relative because the intensity of light transmitted by the sample material (I_S) is related to the intensity of light transmitted by the reference material (I_R) or "blank."

The transmittance, then, is the ratio I_S/I_R. By Beer's Law, the transmittance (T) is inversely related to the concentration (C) of the absorbing species and the pathlength (b) of sample through which the light passes.

$$T = \frac{I_S}{I_R} = 10^{-abc}$$

A.1 WAVELENGTH

The term nanometer (nm = 10^{-9}meters = 10 Angstroms) is the wavelength unit used and is preferred over the older, but equivalent term, millimicron (mμ). A deuterium lamp in the spectrophotometer emits wavelengths in the ultraviolet region, and a tungsten lamp is used for wavelengths in the visible region.

WAVELENGTH regions:	200 to 360 UV
	360 to 700 VISIBLE
	700 to 1000 near IR

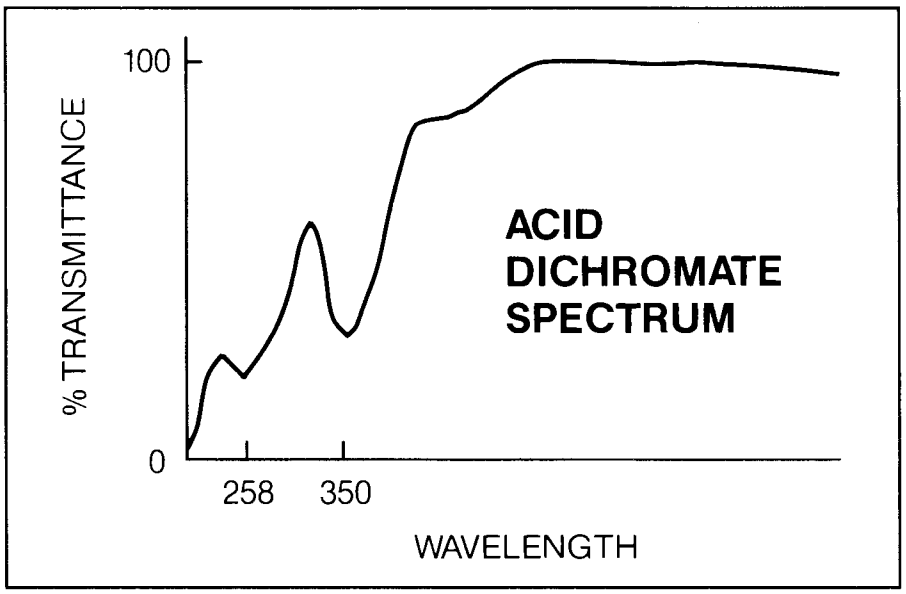
A monochromator is used to isolate from the emission of either lamp, the particular wavelengths required for analytical purposes.

A.2 SPECTRUM

The term "spectrum" refers not only to the range of wavelengths available, but also to the light-absorbing profile of a material as a function of wavelength. Typically, a transmittance minimum is chosen as the analytical wavelength in order to relate the material's light-absorbing properties to its concentration. The transmittance minimum is used both for sensitivity and because many materials are more likely to obey Beer's Law at this wavelength. In the spectrum of dichromate (See Figure A.1), 350 nm is often the analytical wavelength of choice when ultraviolet instrumentation is not

available. If ultraviolet instrumentation is being used, the more sensitive 258 nm peak may be used.

FIGURE A.1
SPECTRUM



A.3 ABSORBANCE

Although the spectrophotometer measures the relative amount of light transmitted, this value is usually converted to absorbance by a table, meter, or electronic conversion to yield results in absorbance ($-\log_{10}T$), which is directly proportional to concentration, and hence, more useful to the operator than transmittance.

$$A = abc$$

This equation holds only for true solutions. Particles in suspension cause an error as they also absorb and scatter light but not in a manner related to Beer's Law.

A.3.1 Absorptivity (a) and Pathlength (b)

The constants (a) and (b) which relate absorbance to the concentration of the absorbing species are pathlength (b) of the solution in the cuvette through which the light passes, and the absorptivity of chemical species (a).

The absorptivity is a physical constant and unique to each chemical species. It is a function of wavelength and is, therefore, usually specified at one or more absorbance maxima (transmittance minima).

When an endpoint analysis is carried out at the wavelength of maximum absorbance, the concentration of the unknown is related to the absorbance by the equation $c = A/ab$, if the absorptivity is known.

A.3.2 Standard Curve

More usually, however, a standard curve is constructed by plotting the measured absorbance of chemical standards on the y-axis versus the known concentrations of those standards on the x-axis. Unknown concentrations are determined from the standard curve by finding the "C" corresponding to the measured A of the unknown.

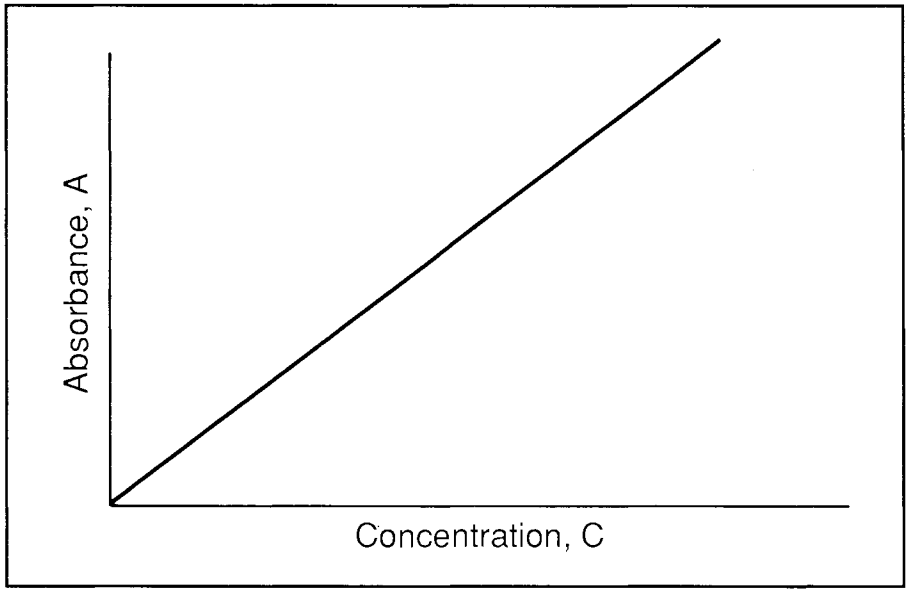


FIGURE A.2
STANDARD CURVE

The standard curve is also a check on the linearity of the chemical method and a check on the validity of Beer's Law for a particular chemical species. Furthermore, if either the absorptivity or the slope of a linear standard curve is known, a multiplier ($^1/ab$) can be used to yield results directly in concentration units on the spectrophotometer.

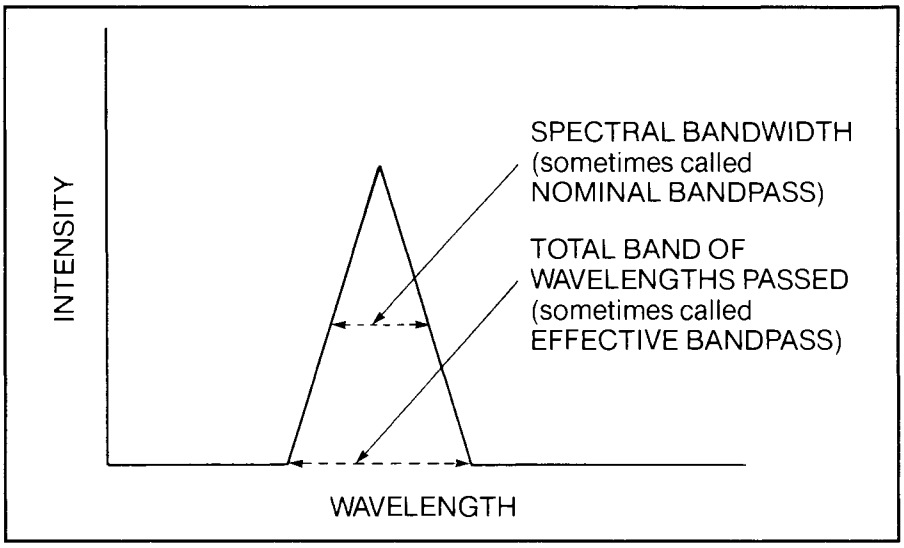
A.3.3 Spectral Bandwidth

The spectral bandwidth or "bandpass" of a spectrophotometer describes the spectral purity of the light passed through the sample. In practice it is not possible to pass a single wavelength, but rather a band of wavelengths. Assuming a triangular distribution of the energy of the light passed, the spectral bandwidth is that wavelength range corresponding to half the peak height. With such a triangular distribution of light, the spectral bandwidth also corresponds to three quarters of the energy passed thru the sample.

As the bandwidth of an instrument decreases, three effects are noticeable on chemical determinations. The number and sharpness of peaks (i.e., resolution) in the spectrum of a chemical species increases down to some bandpass beyond which narrowing the bandpass only increases the noise. Secondly, as the resolution

improves, the wavelength absorbance maximum may shift as the integration of side peaks diminishes. Thirdly, the absorptivity of the chemical species also increases; approaching its maximum value at a sufficiently narrow bandwidth. In practice, this absorptivity increase of a chemical species at narrower instrument bandwidth is responsible for increased sensitivity (slope) of the standard curve.

FIGURE A.3
SPECTRAL BANDWIDTH



What constitutes "sufficiently narrow bandwidth," however, depends on the chemical species being measured, and which absorbance peak of that species is used as the analytical wavelength. Thus, a compound of very narrow natural bandwidth, such as benzene vapor, requires a narrower instrument bandwidth than hemoglobin, for example, to completely resolve the peaks and to obtain the true absorptivity. Hemoglobin, correspondingly, requires a narrower instrument spectral bandwidth than the tungsten blue complex of uric acid which has a wide natural absorbance band.

A.4 ACCURACY

Although the true or limiting absorptivity corresponds to the maximum slope of the standard curve, it is possible to obtain adequate analytical results at an instrument bandwidth which does not fully resolve the peaks or yield the theoretical absorptivity. If a standard curve can be constructed, the sensitivity or slope of the curve is decreased at larger bandwidths, provided the bandwidth is not too large (shown by a non-linear standard curve), unknown sample concentrations can be easily determined from the standard curve. The accuracy of the chemical standards, therefore, determines the accuracy of the analytical result. In enzyme determinations, however, where there are no standards to use for reference, it is far more critical that a bandwidth of 8 nm or less be used so that 6.2 cm^2 /μmole, the true micromolar absorptivity of reduced NAD, be obtained.§

A.5 PRECISION

The limiting factor in most chemical methods is the precision of pipetting and the reliability of reagents. In enzyme determinations, temperature fluctuations may also be a source of imprecision. Aside from these considerations, however, precision of spectrophotometric tests can be improved by working in the optimal transmittance/absorbance range of the spectrophotometer. The plot shown in Figure A.4 is the Twyman-Lothian curve showing percent error in concentration as a function of percent transmittance. This curve shows that at either extreme of the scale, absorbance values above 1.0 A or below 0.15 A (10% to 70%T), any spectrophotometer will yield less precise results than will be obtained in the optimal spectrophotometric range of 0.1 to 0.7A (50% to 20%T).

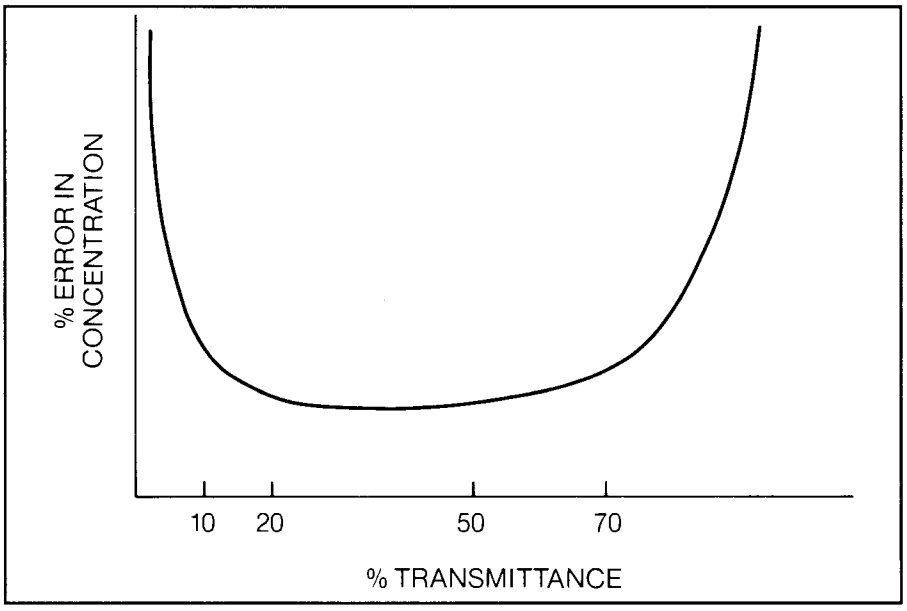
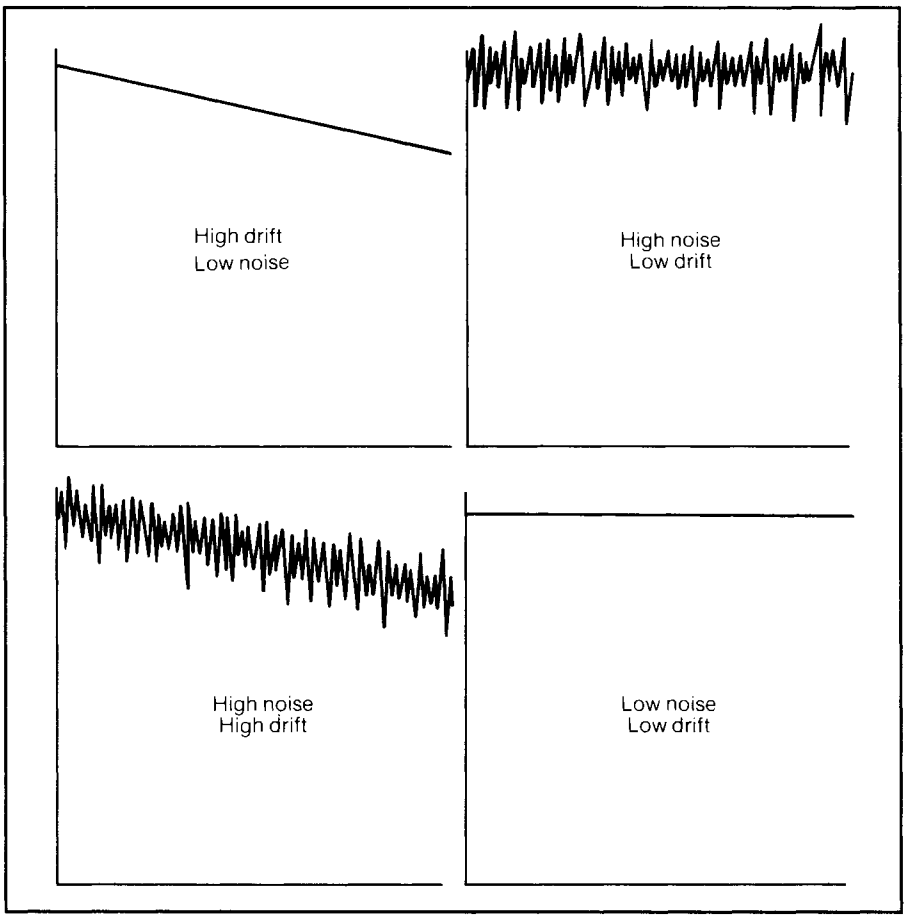


FIGURE A.4
TWYMAN-LOTHIAN CURVE

It is well, therefore, in clinical tests to have the diagnostically significant upper end of the normal range fall within 0.3 to 0.7 A on the standard curve.

Instrument noise (See Figure A.5) is another important factor determining the precision obtained on spectrophotometric assays. Whereas drift refers to a trend in readings over a prolonged period of time, noise refers to the quality of short term stability. Noise is of greater importance than drift in enzyme assays, since each reaction is its own blank. Instrument stability (noise) at the high starting absorbances expected in enzyme tests (and at the wavelength to be used) is a critical factor in evaluation of spectrophotometric instrumentation for enzyme assays. Drift, on the other hand, represents a matter of operator convenience in reducing the frequency of resetting blanks and standards in endpoint testing.

FIGURE A.5
INSTRUMENT NOISE



A.6 CALIBRATION

There are a number of checks that can be performed on a spectrophotometer to verify that it is operating properly. Perhaps one of the most useful checks is careful inspection of a standard curve of a chemical species that is known to obey Beer's Law. Linearity of the standard curve is a good indication of instrument linearity and low stray light levels.

A convenient solution to use for instrument checks is a carefully prepared solution of potassium dichromate ($K_2Cr_2O_7$) in 0.01N H_2SO_4.

The potassium dichromate should be pure (NBS standard preferred) and dried to constant weight at about 110°C for two to four hours before weighing out 1.0000 gram into a liter volumetric flask. Dilute to volume with 0.01N H_2SO_4. Careful dilutions of this solution may be used to check linearity: dilute 0 (BLANK), 25, 50, 75, 100, 125, 150, and 175 ml of the stock solution each to one liter with 0.01N H_2SO_4. This yields dichromate solutions with concentrations (C) = 0, .025, .050, .075, .125, .150, and .175g/liter, respectively.

A.6.1 Spectrophotometric Linearity

Spectrophotometric Linearity is indicated by the linearity of the dichromate standard curve (A vs C) at 350 nm using the 0.01N H_2SO_4 to set .000A on the reagent blank.

A.6.2 Spectrophotometric Accuracy

Spectrophotometric Accuracy is indicated by obtaining the true absorbance of each dichromate dilution that would be predicted from the well-documented absorptivity of dichromate, a = 10.72 l/g-cm, A = 10.72 X pathlength in cm X c.

A.6.3 Stray Light

Stray light may be checked by suitable filters: 100%T is set at the wavelength of interest, and a filter is inserted to block all wavelengths above this wavelength of interest. For example, if 100%T is set at 400 nm, a filter may be used that blocks all light below 450 nm. A convenient filter is the Corning #3389 for use at 400 nm. (Even a dark solution that absorbs light at 400 nm can be used to block the 400 nm light, providing the solution transmits all light over 450 nm.)

After the filter is inserted, the remaining %T indicates the stray light level. To assure the filter itself is not passing any of the 400 nm light, a duplicate filter may be inserted with the first filter. If all the remaining %T really is stray light, it remains at essentially the same %T reading. If the filter was passing light near 400 nm, the %T reading now drops essentially to zero %T. To test stray light at 340 nm, the Corning #3060 filter is useful.

A.6.4 Wavelength Accuracy

Wavelength accuracy may be checked using a filter with very sharp absorbance peaks. By constructing a point-by-point wavelength plot of %T of the filter (vs 100%T set with an air blank) the wavelengths of minimum %T may be identified. With a 20 nm bandpass, the 585 nm absorbance peak of a didymium filter is useful. For an 8 or 2 nm bandpass instrument, the 360.1 nm peak of a holmium oxide filter is useful. A transmittance minimum (absorbance maximum) should occur at the wavelength mentioned. Because the wavelengths of minimum % transmittance are a function of bandpass, other wavelengths for calibration must be known at the instrument bandpass to be used. Checks of instrument function should always be related back to the performance expected from the instrument specifications.

A.7 BIBLIOGRAPHY

1. Manual on Recommended Practices in Spectrophotometry, American Society of Testing and Material, Committee E-13, THIRD Edition; ASTM, Philadelphia, September 1969.

2. Spectrophotometer Education Manual, Analytical Products Division, Milton Roy Company.

3. Analytical Absorption Spectroscopy, M. G. Mellon, John Wiley & Sons, Inc., New York, 1950.

§4. Effect of Spectral Bandwidth and Wavelength Accuracy on the Measurement of Reduced NAD, Abstract #038, S. Rains, 25th National AACC meeting.

B.1 TRANSMITTANCE MODE

The transmittance mode is useful for calibration; stray light tests; and filter, glass and lens studies. Also, very low concentrations can be measured with greater sensitivity in the transmittance mode. When the transmittance mode is used, the reagent blank is used to set 100%T, and the results for known standards and unknown samples are obtained as percent transmittance. A standard curve may be constructed on semi-logarithmic paper by plotting the percent transmittance on the logarithmic axis versus the concentration of known standards. The best line is drawn through these points. The concentration of unknown samples may then be determined by locating the concentration value which corresponds to the percent transmittance of the unknown on the standard curves.

B.2 ABSORBANCE MODE

Usually, the operator desires results in absorbance for direct correlation to concentration by Beer's Law, Absorbance = absorptivity x path length x concentration (See Appendix A, Basic Spectrophotometry). When the absorbance mode is used, the reagent blank is normally used to set 0 Abs, and the results for known standards and unknown samples are obtained as absorbance. Results in absorbance may be plotted against the concentration of known standards on linear graph paper. The best line is drawn through these points. The concentration of unknown samples may then be determined by locating the concentration value which corresponds to the absorbance of the unknown on the standard curves.

Absorbance measurements are also useful for kinetic studies and for reaction systems which do not obey Beer's Law, and therefore have non-linear standard curves.

B.3 CONCENTRATION MODE

The instrument's digital readout in Concentration mode offers you a more convenient readout, which eliminates the necessity for constructing a standard curve. The instrument electronically converts absorbance data to concentration units by multiplying the absorbance value by a factor (1/ab or dC/dA). Note that the concentration mode may be used only if the linearity of the standard curve has been verified for the test conditions used. These conditions include wavelength, concentration range of interest, cuvette pathlength, and analytical procedure.