

Beckman®



MODELS 24/25 SPECTROPHOTOMETERS

INSTALLATION

The Models 24 and 25 Spectrophotometers and recorder are customer installed.

WARRANTY

This instrument is covered by the Beckman standard warranty but does not include the following:

1. Replacement and installation of tungsten and deuterium source lamps.
2. Replacement and installation of control panel indicator lamps.
3. Warranty against contamination of mirrors and other optical elements.

Service of the above is available at the current labor rates. A complete list of Beckman Service Offices is provided on the back page of this instruction manual.

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ADDENDUM TO BECKMAN INSTRUCTIONS 015-082245-A MODELS 24/25 SPECTROPHOTOMETERS

This addendum, alone, should not be considered to be operating instructions for the Models 24/25 Spectrophotometers. Rather, it provides additional information now required by Federal Food and Drug Administration Regulations about the use of either device.

*Scientific Instruments Division
BECKMAN INSTRUMENTS, INC.
Fullerton, CA 92634*

MODEL 24 SPECTROPHOTOMETER

A Visible, Digital Reading And/Or
Recording Spectrophotometer

MODEL 25 SPECTROPHOTOMETER

An Ultraviolet-Visible, Digital Reading And/Or
Recording Spectrophotometer

INTENDED USE

These devices are intended for use by operators experienced with the methodology during those parts of in vitro diagnostic enzymatic or quantitative or qualitative analysis procedures which require measurement of light transmission through reagents at wavelengths in the visible and ultraviolet regions of the electromagnetic spectrum.

The Model 24 is capable of measuring light at wavelengths from 340 to 700 nm (190 to 700 nm with optional deuterium source). The Model 25 is capable of measuring light from 190 to 700 nm.

INSTALLATION

These devices may be installed by a customer, following the procedures outlined in Section Two, Installation and Check-out, of Beckman Instructions 015-082245-A.

PRINCIPLES OF OPERATION

The Beckman Models 24/25 Spectrophotometers are double-beam, digital reading and/or recording instruments for measuring light levels at specified wavelengths. The Model 24 is capable of measuring light at wavelengths from 340 to 700 nm (190 to 700 nm with optional deuterium source). The Model 25 is capable of measuring light at wavelengths from 190 to 700 nm. In their operation, these devices employ a tungsten light source in the visible range of the spectrum (340 to 700 nm) and a deuterium source (standard on Model 25 and optional on Model 24) in the ultraviolet region of the spectrum (190 to 360 nm). Light from either source is directed to the single-grating monochromator, which has 1200 lines per millimeter and is blazed at 250 nanometers. Constructive interference of a certain wavelength, other than light of the first order, is removed via a filter wheel which blocks second and higher-order light from reaching the sample. The monochromatic light is then directed to the sample and reference via a vibrating mirror bridge which displaces horizontally at a certain frequency. Therefore, this bridge allows light to pass into the sample and reference cell holders with a frequency equal to the displacement frequency of the bridge.

Light transmission through the cells is detected by a side-on multiplier phototube. This tube traps the photons of light passing through the sample and reference at a photocathode. The light energy absorbed by the photocathode increases the kinetic energy of electrons from the photocathode material. Electrons, in proportion to the light energy absorbed, escape the photocathode and are trapped by a dynode which has a high potential. The electrons and their kinetic energy captured by the dynode are multiplied into a release of more electrons, which pass to a second dynode having a still higher

potential than the first dynode. This dynode, in turn, increases the number of electrons passing to a successive dynode until the multiplication of electrons is quite large. Finally, the electrons generated in the tube are captured at an anode which generates a voltage pulse proportional to the total number of electrons received.

The current pulses generated by the sample and reference light levels to the multiplier phototube are then amplified and analyzed in an electronic log converter. The log converter transforms the light transmission current values into optical density values, where absorbance is equal to the logarithm $\frac{1}{\text{transmission}}$. The output from the log converter

is then displayed by a digital display and/or recorder.

**PERFORMANCE CHARACTERISTICS
AND SPECIFICATIONS**

The quoted specifications listed on the frontis of Beckman Instructions 015-082245-A apply to a properly operating device. Should device performance deteriorate to such an extent that it does not conform to the quoted specifications, with the exception of stray light, its use for in vitro diagnostic measurements should be discontinued and corrective action taken.

OPERATING INSTRUCTIONS

The operating instructions for the Models 24/25 Spectrophotometers are found in Section Three of Beckman Instructions 015-082245-A.

CALIBRATION PROCEDURES

The following procedures will enable the operator to verify the performance specifications of the Models 24/25 Spectrophotometers.

Some of the procedures are found in Beckman Instructions 015-082245-A, and will be so referenced when applicable.

Before proceeding with the verifications of the performance specifications, read Paragraphs 2.6 and 2.6.1 of Beckman Instructions 015-082245-A.

MATERIALS AND EQUIPMENT NEEDED

The following materials and equipment are needed to perform the calibration procedures:

1. 40 mg/l of K_2CrO_4 in 0.05N KOH for measuring stray light at 370 nm (not supplied with device).
2. 0.25 g/l of K_2CrO_4 in 0.05N KOH for measuring stray light at 370 nm (not supplied with device).
3. 0.011 g/l of NaI (aqueous) for measuring stray light at 220 nm (not supplied with device).
4. 10 g/l of NaI (aqueous) for measuring stray light at 220 nm (not supplied with device).
5. The 579081 Recorder to measure resolution of the Model 25 Spectrophotometer (supplied if purchased).
6. Holmium oxide filter for measuring resolution of the Model 24 Spectrophotometer (supplied with device).

**WAVELENGTH ACCURACY AND
REPEATABILITY CALIBRATION**

Use procedures outlined in Paragraph 2.6.2 of Beckman Instructions 015-082245-A to verify this specification.

STRAY LIGHT CALIBRATION

The procedure outlined in Paragraph 2.6.5 in Beckman Instructions 015-082245-A, allows the operator to check for less than 1% stray light. In order to check for stray light of less than 0.1% stated in the specifications, use the following procedure:

1. Depress ABS Pushbutton and place Slit Program Selector in the NORMAL position (Model 25 only).
2. Adjust Wavelength Selector Knob until WAVELENGTH Indicator reads wavelength where measurements are being made.
3. Select tungsten source for check at 370 nm. Select deuterium source for check at 220 nm.
4. With both the sample and reference beams clear, adjust BASELINE ADJUST Control until the Digital Display reads 0.000.
5. For measurement at 370 nm:
 - a. Place a 1-cm cuvette containing a solution of 40 mg/l of K_2CrO_4 in 0.05N KOH in the reference beam. This will provide a baseline, which means the sample range on the spectrophotometer is approximately 1 to 3 A at 370 nm while the Digital Display and recorder range is still 0 to 2 A.
 - b. Place a 1-cm cell containing a solution of 0.25 g/l of K_2CrO_4 in 0.05N KOH in the sample beam.
 - c. Proceed to Step 7.
6. For measurement at 220 nm:
 - a. Place a 1-cm cell containing 0.011 g/l NaI (aqueous) in the reference beam. This will provide an approximate 1 to 3 A (at 220 nm) sample range on the spectrophotometer while the recorder and Digital Display are still 0 to 2 A.
 - b. Place a 1-cm cell containing a solution of 10 g/l NaI (aqueous) in the sample beam.
 - c. Proceed to Step 7.
7. Record the absorbance reading.
8. The absorbance reading for both of the samples should be above 2.000; corresponding to less than 0.1% stray light at each wavelength. If the device does not display a value above 1.7 A, contact your local Beckman Field Service Representative for assistance. A reading of 1.7 A corresponds to 0.2% stray light which is considered the limit for serious degradation of performance for most in vitro diagnostic assays.

RESOLUTION

Model 25 Only

The following procedure is for verification of the resolution specification for the Model 25. (A recorder is required to obtain verification.)

1. Place UV Source Switch in the ON position.
2. Place the Source Selector in the UV position.
3. Depress the ABS Pushbutton.
4. Place the Mode Switch in the SB position.
5. Adjust the Wavelength Adjust Knob until the WAVELENGTH Indicator reads 656 nm.
6. Place Slit Program Selector in MANUAL position and adjust MANUAL ADJUST Knob to obtain a slitwidth of 0.075 nm.

7. Very slowly adjust the Wavelength Selector Knob between 657 and 655 nm until the lowest possible absorbance level, without going below 0.000 A, can be obtained. Repeat this operation until satisfied that the lowest absorbance level has been reached.
8. If 0.000 A (or approximately 0 A) cannot be obtained initially, adjust the SB BASELINE Control to obtain a dynode voltage where 0 A can be obtained as described in Step 7, immediately preceding.
9. Depress 2 A SPAN Pushbutton, 5 nm/min WAVELENGTH SCAN Pushbutton, and 5 in/min CHART Speed Pushbutton on recorder. Also depress the Wavelength DRIVE Pushbutton.
10. Scan the 660 to 655 nm range. The absorbance profile will be a reverse absorbance curve with a peak at approximately 656 nm. At a point 0.3 absorbance unit (50% transmittance) from the minimum absorbance, measure the bandwidth. This bandwidth must be less than 0.2 nm to meet specifications. If a value larger than 0.2 nm is obtained, contact your local Beckman Field Service Representative for assistance.

Model 24 Only

The following procedure is used for verification of the resolution specification of the Model 24:

1. Place a holmium oxide filter in the sample beam.
2. Place Source Selector in VISIBLE position.
3. Depress ABS Pushbutton.
4. Place Mode Switch in the DB position.
5. Adjust Wavelength Adjust Knob until WAVELENGTH Indicator reads 470 nm.
6. Depress 2 A SPAN Pushbutton, 5 nm/min WAVELENGTH SCAN Pushbutton, and 1 in/min CHART Speed Pushbutton. Also depress Wavelength DRIVE Pushbutton.
7. Scan from 470 to 446 nm. In order for the resolution to be better than 4 nm, the peak at approximately 453 nm must be two small divisions (on 0 to 100-division chart paper or 0.02 A on absorbance chart paper) higher than the trough which occurs at a slightly higher wavelength. If the specification cannot be verified, contact your local Beckman Field Service Representative for assistance.

PHOTOMETRIC ACCURACY AND REPEATABILITY

Use the procedure outlined in Paragraphs 2.6.3 and 2.6.4 of Beckman Instructions 015-082245-A for verification of these specifications.

0 A LINE

Use the following procedure to verify the 0 A line specification:

(Maximum deviation ± 0.009 A throughout operating range, including noise.)

1. Depress ABS Pushbutton.
2. Place Slit Program Selector in the NORMAL position (Model 25 only).
3. Scan the wavelength range of the instrument either manually or automatically from the high to low wavelength. Remember that with the Model 25 there is a source change at 350 nm. If the spectrophotometer does not perform as

specified, contact your local Beckman Field Service Representative for assistance.

BASELINE STABILITY

This specification (double-beam better than 0.004 A per hour) is verified as follows:

(A recorder is required to obtain verification.)

1. Depress Δ Pushbutton.
2. Place Slit Program Selector in NORMAL position (Model 25 only).
3. Turn on tungsten source and place Source Selector in VISIBLE position. Allow source to warm up for two hours.
4. Adjust Wavelength Selector Knob until WAVELENGTH Indicator reads 543 nm.
5. Depress 0.1 A SPAN Pushbutton and 0.1 in/min CHART Speed Pushbutton on recorder.
6. Depress START Pushbutton and allow recorder to run for one hour or more at 0.1 in/min. If the spectrophotometer does not perform as specified, contact your local Beckman Field Service Representative for assistance.

OPERATIONAL PRECAUTIONS AND LIMITATIONS

The following operational precautions and limitations are cited so that the operator may avoid those actions which can damage either device, disrupt an automated series of determinations, or adversely affect the validity of a quantitative or qualitative determination.

1. READ BECKMAN INSTRUCTIONS 015-082245-A BEFORE OPERATING DEVICE.

It is also recommended that Beckman Instructions 015-082058, "An Introduction to Ultraviolet Spectrophotometry," by Robert J. Manning, be read.

2. Do not subject either spectrophotometer or recorder to mechanical shock.
3. Use only Beckman-recommended spectrophotometer cells, or equivalent, and use procedures found on Pages 10 and 11 of Beckman Bulletin 7685, and Paragraph 4.1 of Beckman Instructions 015-082245-A, for selection, care, and cleaning of cells.

4. When selecting a wavelength, always approach the wavelength from the high side of the scale. This will prevent backlash in the Wavelength Adjust Knob and, thereby, inaccurate wavelength readings.

5. There is no substitute for careful adherence to the procedures indicated by the test kit manufacturer.

The operator should check *all* outputs from the Models 24/25 Spectrophotometers with expected results.

HAZARDS

The following hazards are identified for maximum safety of the operator.

1. Do not remove any covers from the Models 24/25 Spectrophotometers, or recorder, other than the lamp housing cover as described in Paragraphs 4.3 and 4.4 of Beckman Instructions 015-082245-A. These paragraphs describe the replacement of the tungsten and deuterium sources. The equipment inside the other covered areas of the devices is not operator serviceable and presents electrical shock hazard. Refer servicing to a qualified Beckman Field Service Representative.
2. Paragraphs 2.2.1, 2.2.2, and 2.2.3 are not intended for operator use. When a change from one voltage to another is required as stated in these paragraphs, it must be performed by a qualified Beckman Field Service Representative.
3. When replacing and aligning the deuterium lamp, the operator is reminded that ultraviolet radiation from this lamp is harmful to the eyes. Protective (not necessarily safety) glasses must be worn during the alignment procedure.

SERVICE

No operator service other than the replacement of the tungsten and deuterium sources (Paragraphs 4.3 and 4.4 of Beckman Instructions 015-082245-A), and front panel indicator lamps, is recommended. Refer service to a Beckman Field Service Representative. Offices are listed on the inside back cover of Beckman Instructions 015-082245-A.

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MODELS 24/25 SPECTROPHOTOMETERS

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CONTENTS

SECTION	TITLE	PAGE
ONE	INTRODUCTION	1
1.1	Model 25 Spectrophotometer	1
1.2	Model 24 Spectrophotometer	1
1.3	Recorder	1
TWO	INSTALLATION AND CHECKOUT	1
2.1	Location	1
2.2	Instrument Setup	1
2.2.1	Hookup For 120 and 240 Operation of the Spectrophotometer	2
2.2.2	Hookup For 120 and 240 Volt Operation of Recorder	2
2.2.3	Hookup of Recorder For 50 and 60 Hz Operation	2
2.3	Recorder Connection	3
2.4	BCD Connector	3
2.5	Sample Compartment	3
2.5.1	Installation of Dual-Cell Holder	3
2.5.2	Installation of Silica Windows (Optional Accessory)	3
2.6	Checkout	3
2.6.1	Startup	4
2.6.2	Wavelength Calibration Checkout	4
2.6.3	Absorbance Checkout	4
2.6.4	Photometric Linearity Checkout	5

SECTION	TITLE	PAGE
2.6.5	Stray Light Checkout	5
THREE	OPERATION	6
3.1	Operating Controls	6
3.1.1	Spectrophotometer Operating Controls	6
3.1.2	Recorder Operating Controls	6
3.2	Startup	7
3.3	Absorbance Measurements	8
3.3.1	Manual Measurements of Absorbance	8
3.3.2	Recording Absorbance Versus Wavelength ...	8
3.3.3	Recording Absorbance Versus Time	9
3.4	Measurement of Concentration	10
3.4.1	Manual Concentration Measurement	10
3.4.2	Recording Concentration Measurements	10
3.5	Differential Absorbance Measurements	11
3.6	Operation With Recorder Zero Supp Control ..	12
3.7	Proper Slit Width Selection	12
FOUR	MAINTENANCE	13
4.1	Care and Cleaning of Silica Windows	13
4.2	Chart Paper Replacement	13
4.3	Replacing and Aligning Tungsten Lamp	13
4.4	Replacing and Aligning Deuterium Lamp	15
4.5	Replacing Front-Panel Indicator Lamps	15
FIVE	SELECTED REPLACEMENT PARTS LIST ..	16

ILLUSTRATIONS

FIGURE	TITLE	PAGE
1	Model 25 Spectrophotometer	1
2	Model 24 Spectrophotometer	1
3	Recorder	1
4	Hookup of Spectrophotometer for 120 Volts or 240 Volts	2
5	Hookup of Recorder For 120 Volts or 240 Volts	2
6	579054 Chart Logic and Clock Board	2
7	Electrical Connections	2
8	Silica Windows Installed in Sample Compartment	3
9	Holmium Oxide Filter Wavelength Peaks and Tolerances	3
10	Holmium Oxide Filter Spectrum	3
11	Absorbance Values for SRM 930 Filters	4
12	Net Absorbance Values for SRM 931 Filters .	4
13	Chromate Linearity Check	5
14	Stray Light Test Samples	5

FIGURE	TITLE	PAGE
15	Spectrophotometer Operating Controls	6
16	Recorder Operating Controls	7
17	Sample Compartment	8
18	Chart Expansions in nm/in	8
19	Concentration Ranges Available on Digital Display for a Given Absorbance Value	10
20	Concentration Ranges for Various Span Settings on the Recorder	10
21	Differential Spectrum	11
22	Fullscale Settings Obtainable with Each Span Pushbutton and the Δ Pushbutton Depressed ..	12
23	Chart Paper Installation	13
24	Source Compartment-Sample Compartment Cover	14
25	Rear View of Source Compartment	14
26	Front View of Source Lamps	15

SPECIFICATIONS

MONOCHROMATOR

Single monochromator, filter-grating, 1200 lines/mm and blazed at 250 nm.

OPTICAL PRINCIPLE

Double-beam and single-beam.

WAVELENGTH RANGE

Model 24 Spectrophotometer: 340 to 700 nm.

Model 25 Spectrophotometer: 190 to 700 nm.

WAVELENGTH PRESENTATION

Linear, digital counter.

WAVELENGTH ACCURACY

± 0.5 nm.

WAVELENGTH REPEATABILITY

Better than 0.25 nm.

STRAY LIGHT

Model 24 Spectrophotometer: less than 0.1% at 370 nm.

Model 25 Spectrophotometer: less than 0.1% at 220 nm.

RESOLUTION

Model 24 Spectrophotometer: 4 nm.

Model 25 Spectrophotometer: 0.2 nm.

SLITS

Model 24 Spectrophotometer: Fixed, 0.8 mm.

Model 25 Spectrophotometer: Two programs for double-beam operation, and manually adjustable 0.05 mm to 2.0 mm.

PHOTOMETRIC READOUT

Digital Display

4-digit digital display, 0 to 2 A, or 0 to 8000 counts in concentration.

Recorder

100-millivolt per absorbance unit.

Differential

-0.3 A to +0.7 A with 1 A Pushbutton depressed.

BCD Connector

0 to 2 A or 0 to 8000 counts in concentration through 36-pin connector.

PHOTOMETRIC ACCURACY

0.5% of reading or 0.001 A, whichever is larger.

PHOTOMETRIC REPEATABILITY

0.25% of reading or 0.001 A, whichever is larger.

RECORDER SCALES

0.1 A, 0.25 A, 0.5 A, 1.0 A, and 2.0 A fullscale.

SCANNING SPEEDS

100, 50, 20, and 5 nm/min selectable on accessory recorder unit.

0 A LINE

Maximum deviation ± 0.009 A throughout operating range, including noise.

BASELINE STABILITY

Double-beam better than 0.0004 A per hour.

APPROXIMATE WEIGHT

Normal: 70 pounds net.

Shipping: 85 pounds.

OVERALL DIMENSIONS

24 inches long x 16 inches deep x 14 inches high.

POWER REQUIREMENTS

120/240 volts ($\pm 10\%$), 50/60 Hz, 2 amperes.

SECTION ONE INTRODUCTION

The Models 24 and 25 Spectrophotometers are double-beam, digital reading or recording instruments which can make absorbance, concentration, and differential absorbance measurements. Quantitative and qualitative analysis and reaction rate studies are easily accomplished. Specialized techniques, such as automatic sampling and batch sampling, are possible with addition of easily mounted accessories.

1.1 MODEL 25 SPECTROPHOTOMETER

The Model 25 Spectrophotometer, Figure 1, is mounted in a compact case. Major design features include: two sources (tungsten and deuterium), power supplies, monochromator, detector, large sample compartment, variable slit control, slit program selector, solid-state circuitry, and a built-in aperture system.

1.2 MODEL 24 SPECTROPHOTOMETER

The Model 24 Spectrophotometer, Figure 2, is housed in a compact case with front and rear panel controls, and a four-digit display. Major design features of the instrument include: one source (tungsten), power supplies, monochromator, detector, large sample compartment, fixed slit, solid-state circuitry, and a fixed aperture.

1.3 RECORDER

The Recorder, Figure 3, is a single-pen, strip-chart, potentiometric recorder with fullscale sensitivity from 0.1 A to 2.0 A fullscale. The instrument has four scanning speeds (100, 50, 20, and 5 nm/min) and the recorder has seven chart speeds (10, 5, 2, 1, 0.5, 0.2, and 0.1 inch/min).

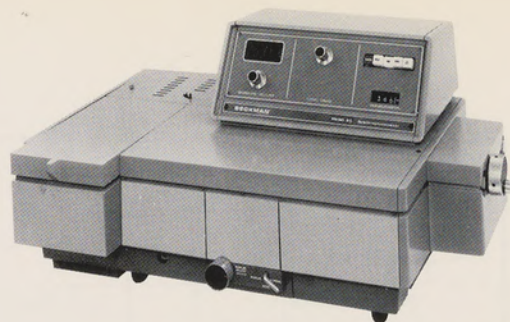


Figure 1. Model 25 Spectrophotometer

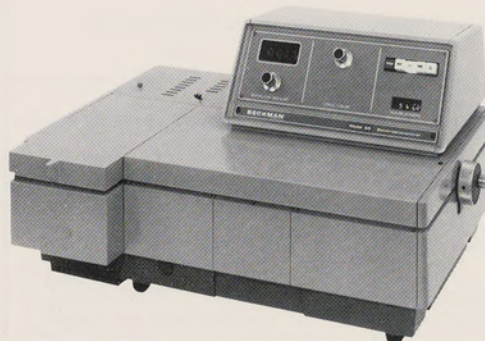


Figure 2. Model 24 Spectrophotometer



Figure 3. Recorder

SECTION TWO INSTALLATION AND CHECKOUT

The Models 24 and 25 Spectrophotometers, recorder, and sipper system are customer installed.

This section contains the necessary information to install and check out the operation of the instruments.

2.1 LOCATION

The instruments should be placed in a semi-protected area that is reasonably free of dust and excessive moisture, and not subject to significant temperature variations. As in any sensitive instruments, performance may be affected by strong electromagnetic fields, as would exist in proximity to large electric motors or diathermy machines. Disturbances of this nature should be avoided when determining location.

2.2 INSTRUMENT SETUP

The spectrophotometer and the recorder are packaged with

the care given all Beckman precision instruments and should be unpacked with equal consideration.

Remove the protective shipping tape from the Wavelength Adjust Knob, Manual Adjust Knob, sample compartment, and Source Selector.

Place the instrument and the recorder in their operating positions and connect their power cords to an electrical outlet with the proper power output. The power cord line is a three-conductor cable with a three-prong plug that affords an earth ground. When the electrical outlet used does not have a third (ground) contact, a proper outlet must be installed.

When the instruments leave the factory they have been built to the specified power requirements (120 or 240 volts, 50 or 60 Hz) of the customer. However, if the instruments are operated at a different power rating, it is necessary to make certain internal wiring changes. The following paragraphs describe the wiring changes.

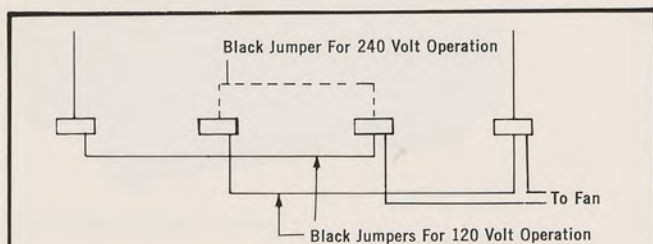


Figure 4. Hookup of Spectrophotometer for 120 Volts or 240 Volts

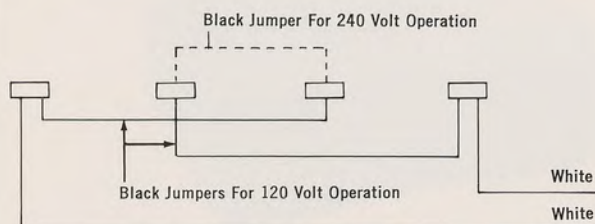


Figure 5. Hookup of Recorder for 120 Volts or 240 Volts

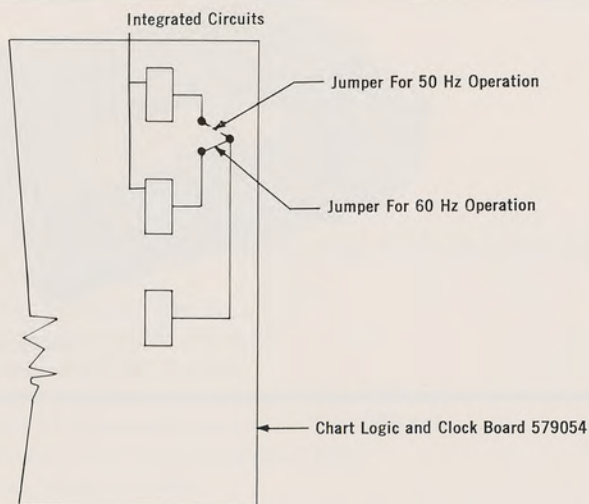


Figure 6. 579054 Chart Logic and Clock Board Integrated Circuits

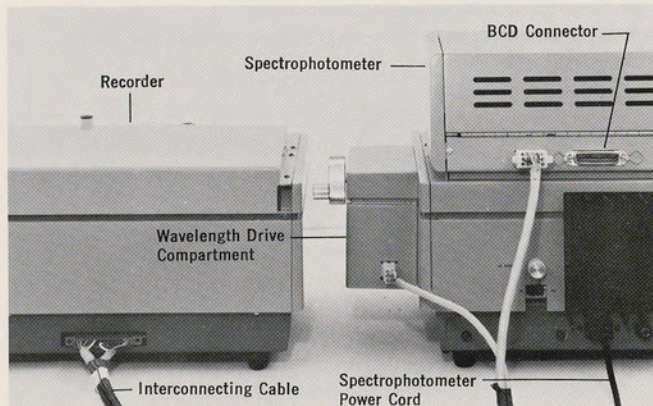


Figure 7. Electrical Connections

2.2.1 HOOKUP FOR 120 AND 240 VOLT OPERATION OF THE SPECTROPHOTOMETER

The following procedure and Figure 4 explain the hookup for 120 and 240 volt operation:

1. Turn off instrument and disconnect power cord from electrical outlet.
2. Remove two screws that secure control panel and then lift up panel.
3. Locate cardboard cover labeled, "DANGER HIGH VOLTAGE." Lift cover to expose terminal strip, Figure 4.
4. If the instrument is hooked-up for 120 volts and operation on 240 volts is desired:
 - a. Remove two black jumpers shown in Figure 4.
 - b. Replace one jumper as shown in Figure 4, for operation on 240 volts.
 - c. Proceed to Step 6.
5. If the instrument is hooked up for 240 volts, and operation on 120 volts is desired:
 - a. Remove single, black jumper shown in Figure 4.
 - b. Place two, black jumpers between terminals, as shown in Figure 4.
 - c. Proceed to Step 6.
6. Also, it is necessary to change fuses when power sources are changed:
 - a. 120 volt operation requires 2 ampere SLO-BLO fuse.
 - b. 240 volt operation requires 1 ampere SLO-BLO fuse.

2.2.2 HOOKUP FOR 120 AND 240 VOLT OPERATION OF RECORDER

The following procedure and Figure 5 explain the hookup of the recorder for 120 and 240 volt operation:

1. Turn off recorder and disconnect power cord from electrical outlet.
2. Remove two screws that secure top panel of recorder and then lift panel straight up.
3. The a.c. terminals are located behind the chart-drive mechanism, on the corner of the recorder main board, Figure 5.
4. If recorder is hooked up for 120 volt operation and operation on 240 volts is desired:
 - a. Remove two black jumpers shown in Figure 5.
 - b. Replace with single, black jumper for 240 volt operation, Figure 5.
 - c. Proceed to Step 6.
5. If the recorder is hooked up for 240 volt operation and operation on 120 volts is desired:
 - a. Remove single black jumper shown in Figure 5.
 - b. Replace with two black jumpers for 120 volt operation, Figure 5.
 - c. Proceed to Step 6.
6. Also, it will be necessary to change fuses as follows:
 - a. 120 volt operation requires 1 ampere SLO-BLO fuse.
 - b. 240 volt operation requires 1/2 ampere SLO-BLO fuse.

2.2.3 HOOKUP OF RECORDER FOR 50 AND 60 HZ OPERATION

The recorder chart logic and clock board is strapped to match the frequency of the supply voltage. If the recorder frequency must be changed:

1. Turn off recorder and disconnect power cord from electrical outlet.
2. Remove ZERO Control Knob. This is done by loosening the setscrew in the knob (requires 0.05-inch hex wrench).

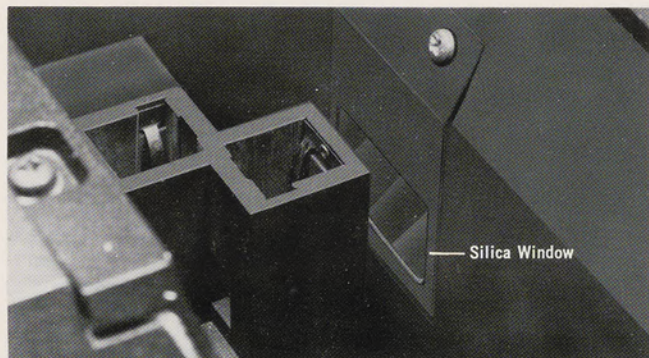


Figure 8. Silica Windows Installed in Sample Compartment

3. Remove control panel by first removing screw in upper left-hand corner of panel and two screws from the blue strip on the front of the instrument, and then lifting panel straight up.
4. In order to expose the 579054 Chart Logic and Clock Board, it is necessary to remove the board from the recorder. The board is one of three boards that are attached together in a module (the logic board is the front board). The entire module is removed by pulling it straight up.
5. Locate area shown in Figure 6 (upper right-hand corner of board). Change jumper as indicated in Figure 6.

2.3 RECORDER CONNECTION

The recorder is connected to the spectrophotometer, as shown in Figure 7, using the interconnecting cable supplied with the recorder.

2.4 BCD CONNECTOR

The BCD connector, shown in Figure 7, provides a BCD output equivalent to the Digital Display on the front panel of the instrument.

The BCD connector is designed primarily for use with the Beckman Model 3109 Inter coupler or the Beckman Model 3115 Printer, but also may be used with any system capable of processing data in a binary-coded decimal format. The outputs provided are capable of driving one medium-power RTL gate, two DTL gates, or one T²L (+) gate.

2.5 SAMPLE COMPARTMENT

The dual-cell holder supplied with the spectrophotometer must be installed in the instrument as directed in Paragraph 2.5.1. If the silica windows (optional accessory) are supplied with the instrument, they must be installed as directed in Paragraph 2.5.2.

2.5.1 INSTALLATION OF DUAL-CELL HOLDER

Remove the dual-cell holder from the shipping kit. Be sure that the vertical-adjustment screws are adjusted so that the top surfaces of the screws are at or below the bottom of the cell compartment. Open the cell compartment cover. Place the two hooks on the bottom of the dual-cell holder over the rear rail of the sample compartment and then push down on the front of the dual-cell holder until it snaps in place over the front rail.

WAVELENGTH (nm)	TOLERANCE (nm)
279.3	± 1.0
287.6	± 1.0
360.8	± 1.0
418.5	± 1.0
453.4	± 1.0
536.4	± 1.0
637.5	± 1.0

Figure 9. Holmium Oxide Filter Wavelength Peaks and Tolerances

2.5.2 INSTALLATION OF SILICA WINDOWS (Optional Accessory)

The silica windows are installed in the sample compartment as follows:

1. Open sample compartment cover and locate four screw holes on each side of the sample compartment.
2. Attach windows to sample compartment walls, as shown in Figure 8, using screws provided.

2.6 CHECKOUT

The following checkout procedure is recommended to assure adherence to quality control requirements of the American Association of Clinical Pathologists – College of American Pathologists (ASCP – CAP), and the National Bureau of Standards (NBS). Checkout of the instrument consists of wavelength calibration checkout (Paragraph 2.6.2), which should be performed daily; absorbance checkout (Paragraph 2.6.3), which should be checked weekly; photometric linearity checkout (Paragraph 2.6.4), which should be checked

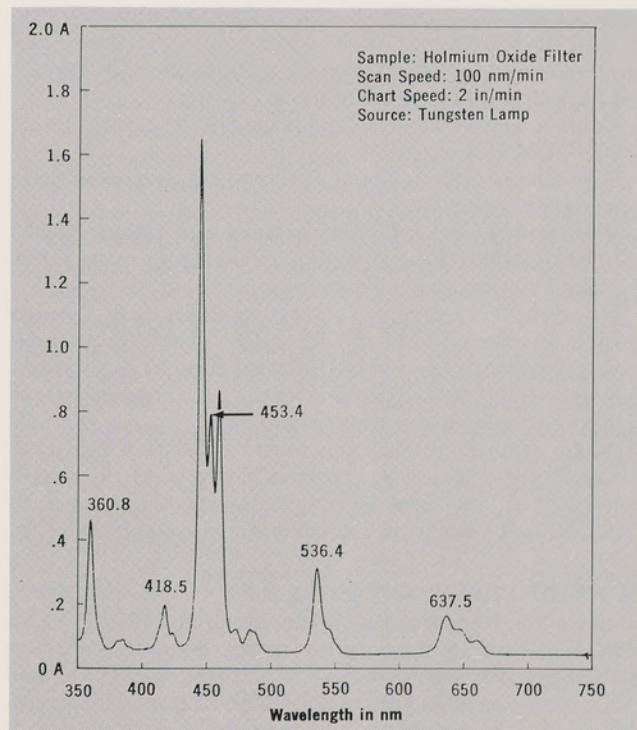


Figure 10. Holmium Oxide Filter Spectrum

weekly; and, stray light checkout (Paragraph 2.6.5), which should be checked when data warrant it. If the spectrophotometer does not perform as specified, contact your local Beckman Service Representative for assistance.

2.6.1 STARTUP

Before starting instrument, read Paragraphs 2.2.1, 2.2.2, and 2.2.3. Start instrument as follows:

1. Connect power cord on rear of instrument to proper power supply outlet.
2. Depress POWER Pushbutton (light behind button should come on).
3. If the recorder is to be used, connect power cord on rear of recorder to proper power supply outlet and depress POWER Pushbutton (light behind button should come on).
4. For operation in UV range (190 to 360 nm), proceed as follows:
 - a. Place Source Selector in UV position:
 - b. Place UV Source Switch in ON position.
5. For operation in the visible range (340 to 700 nm), place the Source Selector in VISIBLE position.
6. Place Mode Switch, located on rear of instrument, in DB position.

2.6.2 WAVELENGTH CALIBRATION CHECKOUT

Wavelength calibration can be checked by using a holmium oxide filter as a wavelength standard. Holmium oxide glass has a number of sharp absorption bands which occur at precisely known wavelengths in the visible and ultraviolet regions of the spectrum. Use the wavelengths in Figure 9 and the spectrum in Figure 10 to check wavelength calibration.

To check wavelength calibration:

1. Depress ABS Pushbutton and place Slit Program Selector in NORMAL position.
2. Select tungsten lamp above 350 nm, and deuterium lamp below 350 nm.
3. With sample and reference beams clear, adjust BASELINE ADJUST Control until Digital Display reads 0.000.
4. Place holmium oxide filter in sample beam.
5. Using the Wavelength Control, manually scan through each wavelength until the absorption peak is found. Always approach each point from the long to short wavelength; i.e., 360.7 from 365 nm. Check wavelength in accordance with the wavelengths in Figure 9. The holmium oxide filter spectrum may be recorded using the recorder parameters as stated in Figure 10. If the instrument does not meet specifications, do not attempt to adjust; call your local Beckman Service Representative.

2.6.3 ABSORBANCE CHECKOUT

A series of Standard Reference Materials (SRM's) has been developed to standardize various aspects of spectrophotometric measurements. Two of these, SRM 930 and 931, are designed to test absorbance accuracy of spectrophotometers in the visible and near-ultraviolet regions.

SRM 930 is a set of stable neutral-density filters for which

absorbance certification is provided by the National Bureau of Standards (NBS). Supplied in sets of three, these filters have nominal absorbances of 0.550, 0.750 and 1.000 at four specified wavelengths between 440 and 650 nm. Absorbance values for a typical set of SRM 930 filters are shown in Figure 11.

Filter and Set Identification Number	ABSORBANCE (A)*			
	Wavelength and (Bandpass) nm			
	440.0 (2.2)	465.0 (2.7)	590.0 (5.4)	635.0 (6.0)
1-227	0.563	0.523	0.574	0.548
2-227	0.745	0.693	0.764	0.730
3-227	1.026	0.953	1.054	1.006

*Values shown pertain only to set indicated. Each filter set is supplied with its own set of certified values.

Figure 11. Absorbance Values for SRM 930 Filters

Although relatively expensive, absorbance standards of this type have many advantages, especially stability and ease of handling. They may be purchased from the Division of Analytical Chemistry NBS, Washington, DC. Liquid standards are also available.

SRM 931 Liquid Filter Set consists of a set of four sealed vials. Three of the four vials contain solutions of Cobalt and Nickel in a mixture of nitric and perchloric acids. The fourth vial contains a blank solution. The absorbances of the solutions are certified by NBS at 302, 395, 512, and 678 nm, and cover an absorbance range of 0.1 to 0.9. The set is intended, as is the SRM 930, to check the photometric scale and to provide interlaboratory comparison of spectrophotometers.

Use the following procedure:

1. Depress ABS Pushbutton and place Slit Program Selector in NORMAL position.
2. Place Source Selector Lever in either VISIBLE or UV position.
3. Select desired wavelength.
4. With nothing in the sample and reference beams, adjust BASELINE ADJUST Control until a reading of 0.000 is obtained.
5. Insert a cuvette containing the blank solution into the reference beam and insert a matched cuvette containing the standard solution into the sample beam. Now read the absorbance value.
6. Repeat Steps 3 through 5 for each wavelength. The absorbance readings should be within the range as listed in Figure 12.

Filter	Wavelength and (Bandpass), nm			
	302(1.0)	395(1.7)	512(2.0)	678(6.5)
"A"—"Blank"	0.307 ± 0.003	0.304 ± 0.003	0.303 ± 0.003	0.115 ± 0.002
"B"—"Blank"	0.608 ± 0.005	0.605 ± 0.005	0.606 ± 0.005	0.229 ± 0.003
"C"—"Blank"	0.906 ± 0.007	0.907 ± 0.007	0.911 ± 0.007	0.345 ± 0.003

NOTE: Net absorbances ("A"—"Blank," "B"—"Blank," and "C"—"Blank") were determined using 10.00 mm cuvettes at 25.0°C.

Figure 12. Net Absorbance Values for SRM 931 Filters

If the SRM 930 or 931 Filters are not available, a standard test solution of potassium chromate in 0.05 N KOH, at a concentration of 40 mg/l, may be used as a substitute (recommended by NBS).

2.6.4 PHOTOMETRIC LINEARITY CHECKOUT

Photometric linearity can be checked by plotting absorbance versus concentration, using a series of special solutions of known concentration. Acid and alkaline chromate, nitrophenol, and cobalt ammonium sulfate of various concentrations are used as photometric linearity test solutions. However, alkaline potassium chromate, one of the substances proposed by NBS for testing absorbance accuracy, is also used as a check for photometric linearity. Use the following procedure to check photometric linearity.

1. Prepare the following solutions:
 - a. 0.05 N KOH—3.3 gm AR grade KOH/liter distilled water
 - b. 40 mg/l potassium chromate solution (stock)—20 mg AR grade K_2CrO_4 /500 ml of 0.05 N KOH
 - c. To four 50 ml volumetric flasks, pipette as follows:
 - 10 ml stock K_2CrO_4 solution (a)
 - 20 ml stock K_2CrO_4 solution (b)
 - 30 ml stock K_2CrO_4 solution (c)
 - 40 ml stock K_2CrO_4 solution (d)

Dilute each to 50 ml with 0.05 N KOH.

Solutions will now contain 8 mg/l, 16 mg/l, 24 mg/l, and 32 mg/l of potassium chromate standard solution.

2. Depress ABS Pushbutton.
3. Place Slit Program Selector in NORMAL position.
4. Turn on tungsten lamp and place Source Selector Lever in VISIBLE position.
5. Turn Wavelength Selector Knob until 370 nm is indicated by Wavelength Indicator.
6. Place cuvette containing 0.05 N KOH in the sample beam.
7. Adjust BASELINE ADJUST Control to obtain 0.000 reading.
8. Remove cuvette from instrument and rinse twice with solution (a); i.e., 8 mg/l K_2CrO_4 standard solution, then fill the cuvette with solution (a) and replace the cuvette in the sample compartment.
9. Record the absorbance reading.
10. Repeat Steps 8 and 9 using solutions (b), (c), (d), and the stock K_2CrO_4 solution.
11. Plot absorbance versus K_2CrO_4 concentration (mg/l) on linear graph paper. A typical linearity curve is given in Figure 13.

2.6.5 STRAY LIGHT CHECKOUT

Stray light is light striking the detector at wavelengths other than that indicated by the Wavelength Indicator.

The usual effect of stray light is to reduce the apparent absorbance. In any given analysis, this appears as a gradual departure from linearity of an established chemistry at the high end of the concentration range. If this is observed, a stray light check should be made at a wavelength near that at which the measurement is being made.

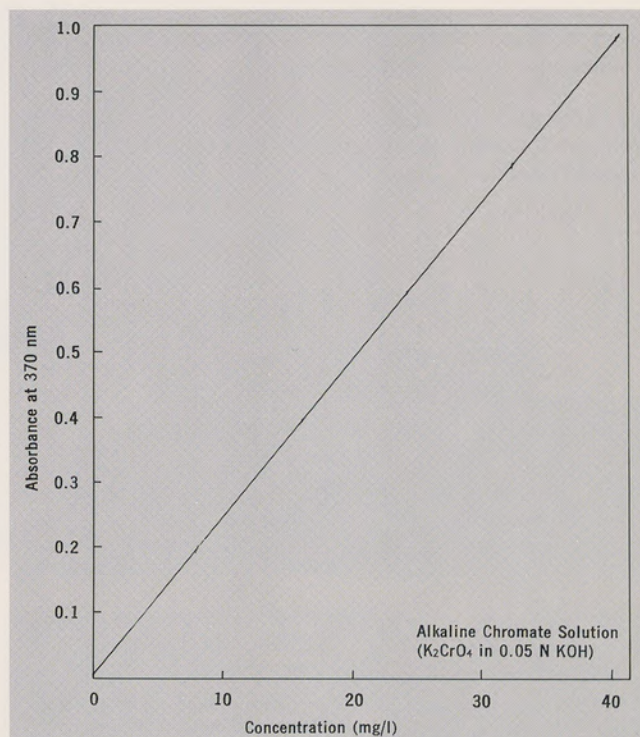


Figure 13. Chromate Linearity Check

Stray light is measured by selecting a sample which has a high absorbance at the test wavelength so that any light that passes through is stray light. Several stray light test materials are listed in Figure 14.

Use the following procedure to measure stray light:

1. Depress ABS Pushbutton and place Slit Program Selector in the NORMAL position.
2. Select tungsten source lamp.
3. Select the desired test wavelength.
4. With both the sample and reference beams clear, adjust the BASELINE ADJUST Control until 0.000 is obtained.
5. Place cuvette containing distilled water in the reference beam. Place a matched cuvette containing the test solution in the sample beam.
6. Record the absorbance reading.
7. The absorbance readings for all of the samples in Figure 14 at the corresponding wavelengths should be above 2.000, corresponding to less than 1% stray light at each wavelength.

If the instrument does not meet the stray light specifications, contact your local Beckman Field Service Representative for assistance.

WAVELENGTH (nm)	SAMPLE	REFERENCE
220	NaI (aqueous) 10 g/l, 1 cm	H ₂ O
370	K_2CrO_4 , 0.25 g/l, 0.05 N KOH, 1 cm	H ₂ O
400	K_2CrO_4 , 0.5 g/l, 0.05 N KOH, 1 cm	H ₂ O
680	$CuSO_4$, 350 g/l, H ₂ O, 1 cm	H ₂ O

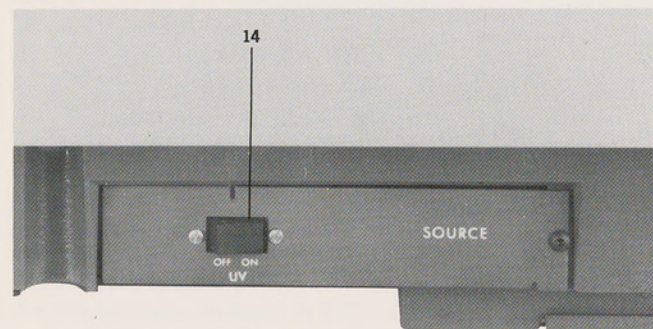
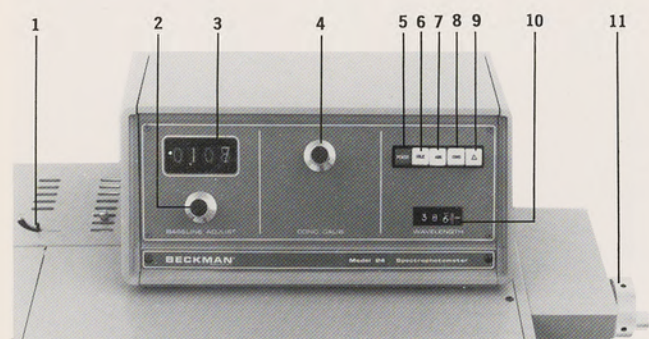
Figure 14. Stray Light Test Samples

SECTION THREE OPERATION

The information contained in this section is essential to proper operation of the instrument and should be thoroughly studied prior to starting the instrument.

3.1 OPERATING CONTROLS

The two following paragraphs describe the spectrophotometer and recorder controls.



B. SIDE-PANEL CONTROLS

A. FRONT-PANEL CONTROLS

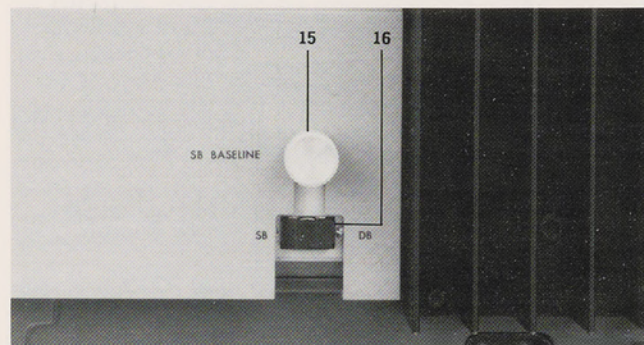
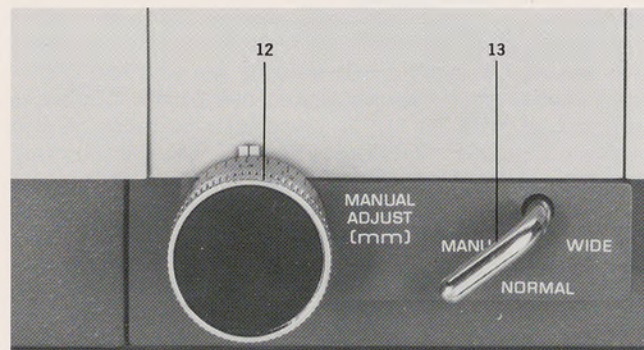
- Source Selector** Mechanically positions source mirror to select either tungsten lamp (VISIBLE position 340 to 700 nm) or deuterium lamp (UV position 190 to 340 nm).
- BASLINE ADJUST Control** This control adjusts the Digital Display or recorder to read zero absorbance or zero concentration, depending upon which mode pushbutton is depressed.
- Digital Display** Indicates numerical values of absorbance (0.000 to 2.000) or concentration (0 to 8000), depending upon position of CONC. CALIB. Control. No decimal point in concentration.
- CONC. CALIB. Control** When CONC. Pushbutton is depressed, this control is used to adjust Digital Display or recorder to read desired concentration value.
- POWER Pushbutton** Turns on and off all power to the instrument. When pushbutton is depressed, light behind pushbutton comes on, and when the pushbutton is depressed again, the light goes out (power off). Also turns on and off power to the tungsten lamp.
- IDLE Pushbutton** When this pushbutton is depressed, power to the detector and the tungsten lamp is turned off, but power to the rest of the instrument remains on. This mode of operation is used for short-term shutdowns.
- ABS Pushbutton** When this pushbutton is depressed, the Digital Display and recorder indicate absorbance values (0.000 to 2.000).
- CONC. Pushbutton** When this pushbutton is depressed, the Digital Display and recorder indicate concentration values (0000 to 8000).
- Δ Pushbutton** If the instrument has been zeroed with the BASELINE ADJUST Control and the Δ Pushbutton is depressed (1 A SPAN Pushbutton and ZERO SUPP OFF Pushbutton depressed), the recorder scale will be minus (—) 0.3 A to plus (+) 0.7 A, with zero at 30 divisions on chart. It is recommended that the ZERO SUPP Control not be used when the Δ Pushbutton is depressed. The Digital Display is turned off in this mode. (See Paragraph 3.5.)

3.1.1 SPECTROPHOTOMETER OPERATING CONTROLS

All of the operating controls on the front panel, rear panel, and side panel are shown and described in Figure 12.

3.1.2 RECORDER OPERATING CONTROLS

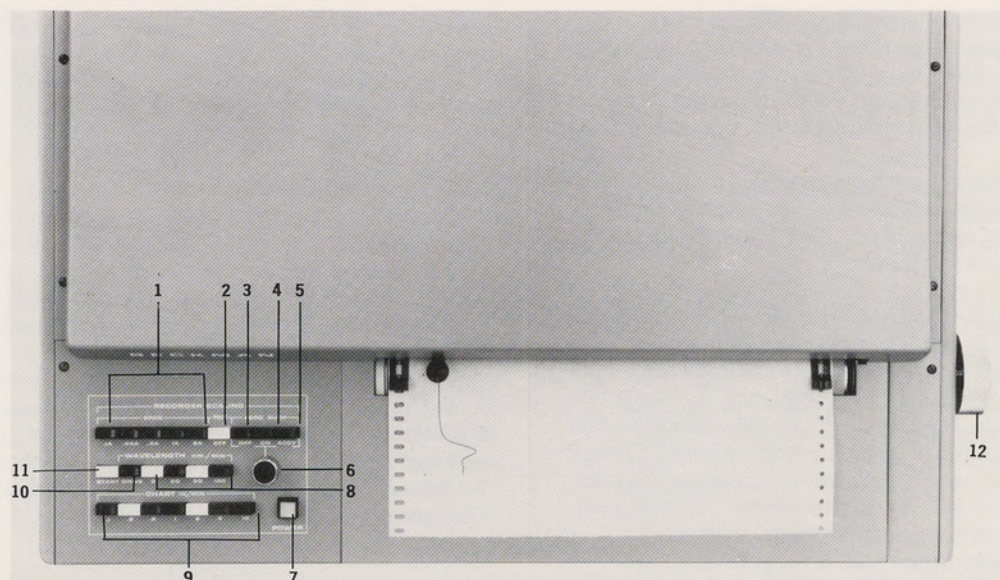
All of the recorder operating controls are shown in Figure 13.



C. REAR-PANEL CONTROLS

- WAVELENGTH Indicator** Indicates wavelength in nanometers (nm). Each division is equal to 0.2 nm. The chosen wavelength should always be approached from the high side of the scale.
- Wavelength Adjust Knob** Rotation of this knob determines the central wavelength of the band of light passing through the exit slit and is displayed by the WAVELENGTH Indicator.
- MANUAL ADJUST Knob (Model 25 Only)** This knob opens and closes the slit manually. The scale is calibrated 0 to 2.0 mm, and each division is equal to 0.05 mm. (See Paragraph 3.7.)
- Slit Program Selector (Model 25 Only)** In the MANUAL position, the MANUAL ADJUST Knob is used to select the desired slit width. In the WIDE and NORMAL positions, the slits are programmed automatically. In the WIDE position, the energy is approximately nine times that in the NORMAL program. NORMAL is used for general routine analysis. WIDE is used for microcells or highly absorbing samples.
- B. SIDE-PANEL CONTROLS**
- UV Source Switch** Turns on and off power to the deuterium lamp. (Automatic control of the lamp power supply causes an approximate 2½ minute delay in lamp ignition.)
- C. REAR-PANEL CONTROLS**
- SB BASELINE Control** Adjusts voltage to the dynodes of the detector tube, thus determining gain of the system in single-beam operation. It is not functional in double-beam mode, since dynode voltage then is controlled automatically. Always start with control in fully counterclockwise position (as viewed from rear of instrument).
- Mode Switch** Two-position switch used to select either single-beam (SB) or double-beam (DB) operation.

Figure 15. Spectrophotometer Operating Controls



1. **SPAN Pushbuttons** Select fullscale span of recorder (0 to 0.1 A, 0 to 0.25 A, 0 to 0.5 A, 0 to 1 A, and 0 to 2 A) with zero suppression off.
2. **PEN OFF Pushbutton** When this pushbutton is depressed, the pen becomes inactive but the recorder remains on. This pushbutton can be used when it is desired to use the spectrophotometer but not the recorder and yet not turn the recorder off. Also it can be used when the spectrophotometer is being set up. This avoids having the pen go back and forth unnecessarily.
3. **ZERO SUPP OFF Pushbutton** When this pushbutton is depressed, zero suppression is off and the recorder reads the span as selected by the SPAN Pushbuttons.
4. **ZERO SUPP ON Pushbutton** When this pushbutton is depressed, the ZERO SUPP Control is active and may be used to suppress up to 1.9 absorbance units.
5. **ZERO SUPP ACCY Pushbutton** When this pushbutton is depressed, the individual Cell Zero Suppression Controls on the Automatic Sample Changer (optional accessory) become active.
6. **ZERO SUPP Control** This control is used (ZERO SUPP ON Pushbutton depressed) to suppress the recorder reading by as much as 1.9 absorbance units. It is recommended that this control not be used when the Δ Pushbutton is depressed. However, in special cases of differential absorbance, this control may be used

with the Δ Pushbutton in the up position. This control is held in position by a friction lock.

7. **POWER Pushbutton** When depressed (light comes on) power is on to the recorder and when depressed again (light goes out) power is off to the recorder.
8. **WAVELENGTH Scan Pushbuttons** Selects one of four scanning speeds (5, 20, 50, and 100 nm/min).
9. **CHART Speed Pushbuttons** Selects one of seven speeds (.1, .2, .5, 1, 2, 5, and 10 in/min).
10. **Wavelength DRIVE Pushbutton** When this pushbutton is depressed, it engages the wavelength drive motor, but does not start recorder. This pushbutton is depressed when a wavelength scan is going to be made, and is left in the up position for absorbance-versus-time recordings.
11. **START Pushbutton** When depressed, starts recorder and wavelength-drive motor (if Wavelength DRIVE Pushbutton is depressed), chart-drive, and pen-drive motor. When the pushbutton is depressed again, it stops the recorder and wavelength-drive motor.
12. **Manual Chart Drive Knob** Permits manual operation of recorder chart-drive mechanism independent of wavelength-drive mechanism.

Figure 16. Recorder Operating Controls

3.2 STARTUP

Before starting instrument, read Paragraphs 2.2.1, 2.2.2, and 2.2.3. Start instrument as follows:

1. Connect power cord on rear of instrument to proper power supply outlet.
2. Depress POWER Pushbutton (light behind button should come on).
3. If the recorder is to be used, connect power cord on rear of recorder to proper power supply outlet and depress

POWER Pushbutton (light behind button should come on).

4. For operation in UV range (190 to 360 nm), proceed as follows:
 - a. Place Source Selector in UV position.
 - b. Place UV Source Switch in ON position.
5. For operation in the visible range (340 to 700 nm), place the Source Selector in VISIBLE position.
6. Place Mode Switch in DB position.

3.3 ABSORBANCE MEASUREMENTS

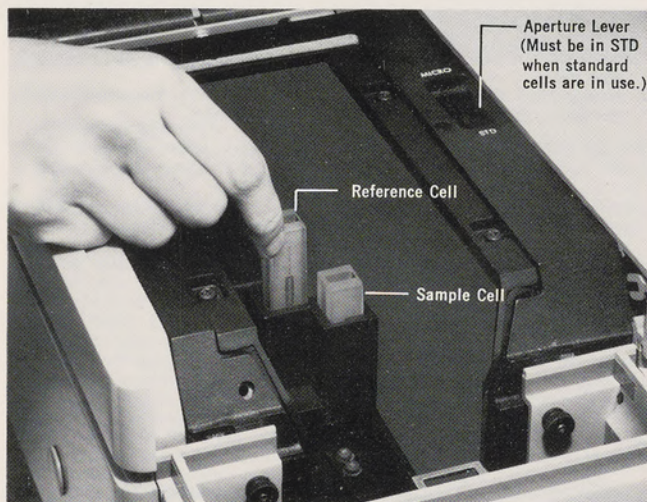
This mode has the advantage that the absorbance value is displayed directly on the digital display or on the recorder. This simplifies preparation of a calibration graph. Absorbance is a linear function of sample concentration over a fixed range.

For quantitative analysis, where possible, the concentration of the sample solution should be such that the absorbance reading is in the range of 0.3 A to 1.2 A. In this range the analytical accuracy is optimized.

3.3.1 MANUAL MEASUREMENT OF ABSORBANCE

After the instrument is started, proceed as follows:

1. Rotate Wavelength Adjust Knob until desired wavelength is indicated by WAVELENGTH Indicator.
2. Place Slit Program Selector in NORMAL position and place MANUAL ADJUST Knob at zero (Model 25 only).
3. Fill reference and sample cells with appropriate solvent.
4. Seat cells in proper cell holders in sample compartment, Figure 17. Close sample compartment cover.
5. Depress ABS Pushbutton.
6. Adjust BASELINE ADJUST Control until Digital Display reads zero (0.000).
7. Discard solvent in sample cell. Refill cell with sample, and replace cell in cell holder, seating cell in the same orientation as in the preceding step when zero absorbance was established.
8. Close sample compartment cover.
9. Read absorbance value on Digital Display. Some samples may require fine adjustment of wavelength to find the center of the bands.



Install cells in proper section of cell holder. Cells are held firmly in place by springs. Optical surfaces of cells must face to either side of instrument. Avoid touching optical surfaces of cells. Never touch surfaces that will be in light beam. Establish reproducible procedure.

1. Make certain cells are dry before placing in holder.
2. Always place cells in compartment, with decal to the same side.
3. Always use the same cells for the run that were used for calibration.

Figure 17. Sample Compartment

3.3.2 RECORDING ABSORBANCE VERSUS WAVELENGTH

After the instrument is started, proceed as follows:

1. Rotate Wavelength Adjust Knob until longest (highest) wavelength of range to be scanned is reached; i.e., if the wavelength range to be scanned is 600 nm to 400 nm, select 600 nm.
2. Place Slit Program Selector in NORMAL position and place MANUAL ADJUST Knob at zero (Model 25 only). Occasionally, the operator may desire to use a fixed slit width when scanning a short wavelength range. (For proper slit width selection see Paragraph 3.7.)
3. Depress ABS Pushbutton.
4. Depress desired SPAN Pushbutton on recorder.
5. Fill reference and sample cells with appropriate solvent.
6. Seat cells in proper cell holders in sample compartment, Figure 14, and close sample compartment cover.
7. Adjust BASELINE ADJUST Control until recorder reads zero.

NOTE

Be sure PEN OFF Pushbutton is in UP position.

8. If the formatted chart paper is being used, the following combinations of scan speed and chart speed may be used to obtain the proper chart presentation:
 - a. Scanning visible region
100 WAVELENGTH Scan Pushbutton and 2 CHART Speed Pushbutton, or 5 WAVELENGTH Scan Pushbutton and .1 CHART Speed Pushbutton
 - b. Scanning ultraviolet region
100 WAVELENGTH Scan Pushbutton and 5 CHART Speed Pushbutton, or 20 WAVELENGTH Scan Pushbutton and 1 CHART Speed PushbuttonIf unformatted chart paper is being used, refer to Figure 18 to obtain proper chart expansions.
9. After depressing desired WAVELENGTH Scan and CHART Speed Pushbuttons, depress WAVELENGTH Drive Pushbutton.

SCAN SPEED (nm/min)	CHART SPEED (in/min)						
	10	5	2	1	0.5	0.2	0.1
100	10	20	50	100	200	500	1000
50	5	10	25	50	100	250	500
20	2	4	10	20	40	100	200
5	0.5	1	2.5	5	10	25	50

Figure 18. Chart Expansions in nm/in

10. In order to set the chart prior to starting wavelength scan, complete the following:
 - a. Determine scan starting point on chart paper.
 - b. Rotate Manual Chart Drive Knob to advance paper until the starting point is passed, and then return the chart paper to desired starting point.

11. To calibrate for differences in cells, run a 0 A line over the area to be scanned. This line establishes a reference for corrections on the recorded curve.
12. Start 0 A line by depressing START Pushbutton. When scan is finished, depress START Pushbutton to stop scan.
13. Remove sample cell, rinse, and fill with sample. Reinsert cell in sample compartment in the same orientation as in Step 6, preceding. Close sample compartment cover.
14. Use Wavelength Adjust Knob to return the WAVELENGTH Indicator to starting wavelength.
15. Return chart paper, using Manual Chart Drive Knob, so that the pen position coincides with the beginning of the 0 A line run.
16. To start scan, depress START Pushbutton. Scan will be continuous from long to short wavelength. When scan is finished, depress START Pushbutton to stop scan.
17. If recorder scale expansion is desired, refer to Paragraph 3.6.

3.3.3 RECORDING ABSORBANCE VERSUS TIME

After the instrument is started, proceed as follows:

1. Rotate Wavelength Adjust Knob until desired wavelength is reached.

NOTE

When selecting a wavelength, always approach the wavelength from the high side of the scale; i.e., approach 305 nm from 310 nm.

2. Place Slit Program Selector in NORMAL position and MANUAL ADJUST Knob at zero (Model 25 only).
3. Depress desired SPAN Pushbutton on recorder.
4. Fill reference and sample cells with appropriate solvent.
5. Seat cells in proper cell holders in sample compartment, Figure 17, and close sample compartment cover.
6. Adjust BASELINE ADJUST Control until recorder reads zero.

NOTE

Be sure PEN OFF Pushbutton is in UP position.

7. Depress desired CHART Speed Pushbutton. A speed of 1 inch per minute is recommended for kinetic studies and most other measurements except for column chromatography. For column chromatography, a speed of 0.1 inch per minute is recommended.

NOTE

DO NOT depress Wavelength DRIVE Pushbutton.

8. In order to set chart prior to starting recorder, complete the following:
 - a. Determine starting point on chart paper.
 - b. Rotate Manual Chart Drive Knob to advance paper until the starting point is passed, and then return the chart paper to desired starting point.
9. Remove sample cell, rinse, and fill with sample. Reinsert cell in sample compartment in the same orientation as in Step 6 preceding. Close sample compartment cover.
10. To start recorder, depress START Pushbutton. When recording is finished, depress START Pushbutton to stop recorder.
11. If recorder scale expansion is desired, refer to Paragraph 3.6.

3.4 MEASUREMENT OF CONCENTRATION

The concentration mode is extremely useful for quantitative analysis (of samples obeying Beer's Law,) because it eliminates computation. Analytical results are read from the instrument directly in the desired units. This mode is used with the digital readout, and also can be used with a recorder, within limitations of the chart format.

Precision will be improved if the calibration is made at the high end of the scale. For example, if samples are expected to lie in the range 50 to 200 g/liter, a calibration sample should be made up at about 200 g/liter rather than 50 g/liter, so that more digits are used in the calibration. When there is a choice, the readout should be set to use the greatest number of digits. For example, when possible, the 200 g/liter calibration sample should be set to read 2000 rather than 200.

3.4.1 MANUAL CONCENTRATION MEASUREMENTS

After the instrument is started, proceed as follows:

1. Rotate Wavelength Adjust Knob until desired wavelength is indicated by WAVELENGTH Indicator.
2. Place Slit Program Selector in NORMAL position and set MANUAL ADJUST Knob at zero (Model 25 only).
3. Prepare a zero-concentration standard solution and a high-concentration standard solution.
4. Place cells containing zero-concentration standard solution in the reference and sample cell holders, Figure 17, and close the sample compartment cover.
5. Depress CONC Pushbutton.
6. Adjust BASELINE ADJUST Control until Digital Display reads zero (0000).
7. Remove sample cell from sample compartment and discard zero-concentration standard solution. Place high-concentration standard solution in sample cell and place cell in sample compartment, orientating cell in the same manner as in Step 4, preceding.
8. Adjust CONC. CALIB. Control until Digital Display reads concentration of high-concentration standard solution. Figure 19 shows the concentration ranges available for absorbance value of sample.
9. Remove sample cell containing high-concentration standard solution and refill cell with unknown. Replace sample cell in sample compartment and read concentration value.

3.4.2 RECORDING CONCENTRATION MEASUREMENTS

Use the following procedure to measure concentration with the recorder:

ABSORBANCE OF HIGH-CONCENTRATION STANDARD SOLUTION	DISPLAY RANGE
2.0	0 to 8000
1.6	0 to 6400
1.2	0 to 4800
1.0	0 to 4000
0.7	0 to 2800
0.3	0 to 1200
0.1	0 to 400

Figure 19. Concentration Ranges Available on Digital Display for a Given Absorbance Value

1. Rotate Wavelength Adjust Knob until desired wavelength is reached.

NOTE

When selecting a wavelength, always approach the wavelength from the high side of the scale; i.e., approach 305 from 310 nm.

2. Place Slit Program Selector in the NORMAL position and MANUAL ADJUST Knob at zero (Model 25 only).
3. Depress the desired SPAN Pushbutton on the recorder. It is recommended that, when operating in concentration mode, only the 1 A Pushbutton be used. With the 1 A Pushbutton depressed, the recorder will read 100 divisions (on 100-division paper) when the Digital Display reads 1000. The maximum concentration reading possible with the recorder is 2000. With the recorder in 2 A range and the Digital Display adjusted for a concentration of 2000, the recorder will read 100 fullscale. See Figure 20 for the corresponding recorder readings for the five span settings.

SPAN SETTING	CONCENTRATION RANGE ON THE RECORDER
.1 A	0 to 100
.25 A	0 to 250
.5 A	0 to 500
1 A	0 to 1000
2 A	0 to 2000

Figure 20. Concentration Ranges for Various Span Settings on the Recorder

4. Prepare a zero-concentration standard solution and a high-concentration standard solution.
5. Place the cells containing the zero-concentration standard solution in the reference and sample beams, Figure 17, and close the sample compartment cover.
6. Be sure that ZERO SUPP OFF Pushbutton is depressed.
7. Turn CONC. CALIB. Control fully counterclockwise.
8. Depress the CONC. Pushbutton.
9. Adjust the BASELINE ADJUST Control until the Digital Display reads zero.

NOTE

Be sure PEN OFF Pushbutton is in UP position.

10. Remove the sample cell and discard the zero-concentration standard solution. Rinse the cell and place the high-concentration standard solution in the cell. Replace cell in sample beam and close sample compartment cover.
11. Turn CONC. CALIB. Control clockwise until recorder reads concentration of high-concentration standard solution. Depending upon the concentration of the sample, it may be necessary to renumber the recorder chart paper or use an un-numbered chart paper. (See Section Five for chart paper part numbers.)

EXAMPLE: If the concentration of the high-concentration standard solution is 78 g/liter, the CONC. CALIB. Control would be adjusted until the recorder reads 78 divisions on the 100-division chart paper.

EXAMPLE: If the concentration of the high-concentration standard solution is 25 g/liter and 100-division chart paper is being used, it would be best to renumber the chart paper and then adjust the CONC. CALIB. Control accordingly.

12. Depress desired CHART Speed Pushbutton. A speed of 1 inch per minute is recommended for kinetic studies and most routine work except for column chromatography. Use 0.1 inch per minute for column chromatography.

NOTE

DO NOT depress Wavelength DRIVE Pushbutton.

13. Remove high-concentration standard from sample beam and discard solution. Place unknown sample in cell and replace cell in sample beam.
14. In order to set the chart prior to starting recorder scan, complete the following:
 - a. Determine starting point on chart paper.
 - b. Rotate Manual Chart Drive Knob to advance paper until the starting point is passed and then return the chart paper to desired starting point.
15. To start recorder, depress START Pushbutton. When recording is finished, depress START Pushbutton again to stop recorder.

3.5 DIFFERENTIAL ABSORBANCE MEASUREMENTS

The differential mode of operation allows adjustment of 0 A between 0 and 100 on the chart paper, depending upon which SPAN Pushbutton is depressed (it is recommended that differential measurements be made with the 1 A span) and the position of the ZERO SUPP Control. This feature makes it possible to analyze two samples of similar composition by placing one sample in the reference beam and the other sample in the sample beam and then recording the differential spectrum, Figure 21.

Before starting the actual procedure, it will be helpful to make several statements about differential measurements.

1. When the Δ Pushbutton is depressed, the 1 A SPAN Pushbutton is depressed, and the ZERO SUPP Control is off, the recorder will read minus (−) 0.3 A to plus (+) 0.7 A, with zero at 30 divisions on 100-division chart paper. See Figure 22 for fullscale settings obtainable with each SPAN Pushbutton.
2. In special cases of differential absorbance where .1 A range fullscale is desired, the ZERO SUPP Control (Δ Pushbutton in UP position) may be used; i.e., if absorbance of sample is zero and .1 A SPAN Pushbutton is depressed, pen may be positioned to a convenient location on chart. If pen is set at midscale, chart will read −0.05 to +0.05.

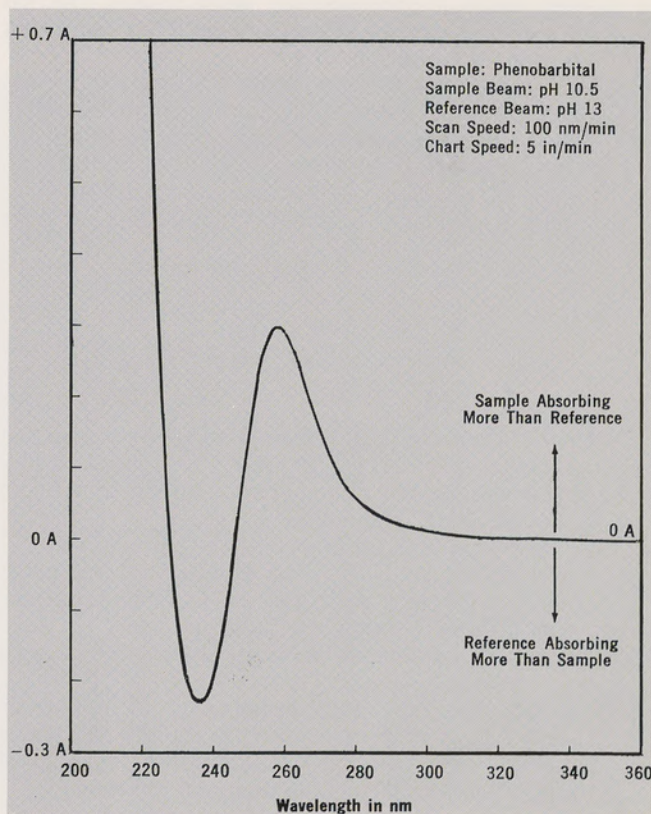


Figure 21. Differential Spectrum

RECORDER SPAN	RECORDER FULLSCALE* READING	ZERO A ON CHART PAPER
0.1	-0.3 to -0.2	Offscale**
0.25	-0.3 to -0.05	Offscale**
0.5	-0.3 to +0.2	60
1.0	-0.3 to +0.7	30
2.0	-0.3 to +1.7	15

*Readings taken with zero suppression off.

**Not useful in differential mode.

Figure 22. Fullscale Settings Obtainable with Each Span Pushbutton and the Δ Pushbutton Depressed

To operate in differential mode, complete the following:

1. Rotate Wavelength Adjust Knob until longest (highest) wavelength of the range to be scanned is reached. For example, if the wavelength range to be scanned is 600 nm to 400 nm, select 600 nm.

NOTE

When selecting a wavelength, always approach the wavelength from the high side of the scale. For example, approach 305 nm from 310 nm.

2. Place Slit Program Selector in NORMAL position and set MANUAL ADJUST Knob at zero (Model 25 only). Occasionally, the operator may desire to use a fixed slit for scanning short wavelength range. (For proper slit selection see Paragraph 3.7.)
3. Depress ABS Pushbutton.
4. Depress desired SPAN Pushbutton on recorder.
5. Fill reference and sample cells with appropriate solvent.
6. Seat cells in proper cell holders in sample compartment, Figure 17, and close sample compartment cover.
7. Adjust BASELINE ADJUST Control until recorder reads zero.

NOTE

Be sure PEN OFF Pushbutton is in UP position.

8. Depress desired CHART Speed Pushbutton. A speed of 1 inch per minute is recommended for kinetic studies.
9. In order to set the chart prior to starting wavelength scan, complete the following:
 - a. Determine scan starting point on chart paper.
 - b. Rotate Manual Chart Drive Knob to advance paper until the starting point is passed and then return the chart paper to desired starting point.
10. Depress Δ Pushbutton. Recorder scale is now minus (-) 0.3 A to plus (+) 0.7 A.
11. If a recorder scale other than minus (-) 0.3 A to plus (+) 0.7 A is desired, depress the ZERO SUPP ON Pushbutton and adjust the ZERO SUPP Control until the 0 A line is in the desired position on the chart.
12. Now remove cells from sample compartment and discard solvent. Place one sample in reference cell and one

sample in sample cell and replace in sample compartment.

13. To start scan, depress START Pushbutton. When scan is finished, depress START Pushbutton to stop scan. A curve similar to the one in Figure 21 should be obtained.

3.6 OPERATION WITH RECORDER ZERO SUPP CONTROL

The recorder is provided with five fixed spans of 0 to 0.1 A, 0 to 0.25 A, 0 to 0.5 A, 0 to 1.0 A, and 0 to 2.0 A (with zero suppression off). The ZERO SUPP Control can be used to suppress up to 1.9 absorbance units. The following two examples show how the ZERO SUPP Control is used to suppress zero on the recorder.

EXAMPLE NO. 1

If a recorder scale of 0.3 to 0.4 A on 100-division chart paper is desired, and the sample has an absorbance of 0.364, the following procedure is used:

1. Depress 0.1 A SPAN Pushbutton.
2. Place sample in sample beam and close sample compartment cover.
3. Depress ZERO SUPP ON Pushbutton.
4. Adjust ZERO SUPP Control until recorder pen reads 64 divisions (Digital Display reads 0.364). Sixty-four divisions is equal to an absorbance of 0.364 on 100-division chart paper with a scale of 0.3 to 0.4 A. The scale is now set for a range of 0.3 to 0.4 A.

EXAMPLE NO. 2

If a recorder scale of 0.7 to 1.2 A on 100-division chart paper is desired, and the sample has an absorbance of 1.05, the following procedure is used:

1. Depress 0.5 A SPAN Pushbutton.
2. Place sample in sample beam and close sample compartment cover.
3. Depress ZERO SUPP ON Pushbutton.
4. Adjust ZERO SUPP Control until recorder pen reads 70-divisions (Digital Display reads 0.700). Seventy divisions is equal to an absorbance of 1.05 on 100-division chart paper with a scale of 0.7 to 1.2 A. The recorder scale is now set for a range of 0.7 to 1.2 A.

3.7 PROPER SLIT WIDTH SELECTION

Occasionally, the operator may desire to use a fixed slit width when scanning a narrow wavelength range. The slit is selected manually by placing the Slit Program Selector in the MANUAL position and then turning the MANUAL ADJUST Knob until the desired slit is reached.

The slit width selected should be such that the resultant spectral slit width is approximately 1/10 of the observed bandwidth of the sample; i.e., if the absorption band is 25 nm wide at half of its height, the spectral slit width should be 2.5 nm. This would mean that the slit width set on the instrument should be 1.0 mm. This is calculated from the dispersion data. Since the Models 24 and 25 are grating instruments, the dispersion is essentially constant with wavelength. The actual dispersion is approximately 2.5 nm/mm slit width. The Model 24 has a fixed slit width of 0.8 mm.

SECTION FOUR MAINTENANCE

Operator maintenance of the instrument includes: care and cleaning of cells and windows, chart paper installation, replacing and aligning tungsten lamp, replacing and aligning the deuterium lamp, and replacement of front-panel indicator lamps.

4.1 CARE AND CLEANING OF SILICA CELLS AND WINDOWS

Silica cells and windows should be cleaned periodically whenever corrosive materials are used, to prevent deterioration and to maintain energy and double-beam balance. Deterioration can be caused by: films deposited on the cell walls or on the window surfaces, etching, or contamination.

Clean cells and windows as follows:

1. Remove windows from sample compartment.
2. Always start with distilled water for aqueous solutions, or use any suitable organic solvent for organic materials.
3. Mild non-ionic detergents may be used if it is certain that they are true solutions and do not contain particulate matter or abrasives.
4. For hard-to-remove deposits, use a solution made from equal parts by volume ethanol and 3 N HCl.
5. Remember that organic reagents may leave a deposit on the windows or cells after evaporation. NEVER use any brush or instrument that might scratch the cells or windows. NEVER use alkali, abrasives, etching materials, or hot, concentrated acids.

4.2 CHART PAPER REPLACEMENT

Replace the chart paper as shown and described in Figure 23.

4.3 REPLACING AND ALIGNING TUNGSTEN LAMP

The 32-candlepower, 6-volt tungsten lamp emits radiation in the wavelength region between 340 nm and 3500 nm. Below 340 nm, the glass envelope begins to absorb so that the lamp cannot be used in the ultraviolet region of the spectrum. The useful life of the tungsten lamp is estimated at 1200 hours when operated at potential of 5.4 volts, with a current of 4.5 amperes. The life of the lamp is markedly decreased by an increase in the operating voltage.

With time, the evaporation of tungsten produces a deposit on the inner surface of the tungsten lamp and reduces emission of energy. Dark areas on the bulb indicate this condition. It is recommended that the tungsten lamp be replaced periodically according to a regular schedule. Replace the tungsten lamp with a new one as follows:

1. Turn off instrument and disconnect power cord from electrical outlet.
2. Expose source compartment by using a screwdriver to loosen two screws on top of source compartment, Figure 24, and then lifting off the sample compartment-source compartment cover.
3. Replacement of the lamps is much easier if the lamp mounting bracket is turned to make lamps more accessible. This is accomplished by loosening the two bracket attachment screws, Figure 25, and then twisting the lamp mounting bracket to expose the lamps.
4. Loosen screws that secure tungsten lamp wires to terminals, Figure 25, enough so that pigtail leads can be slipped from behind the screws.

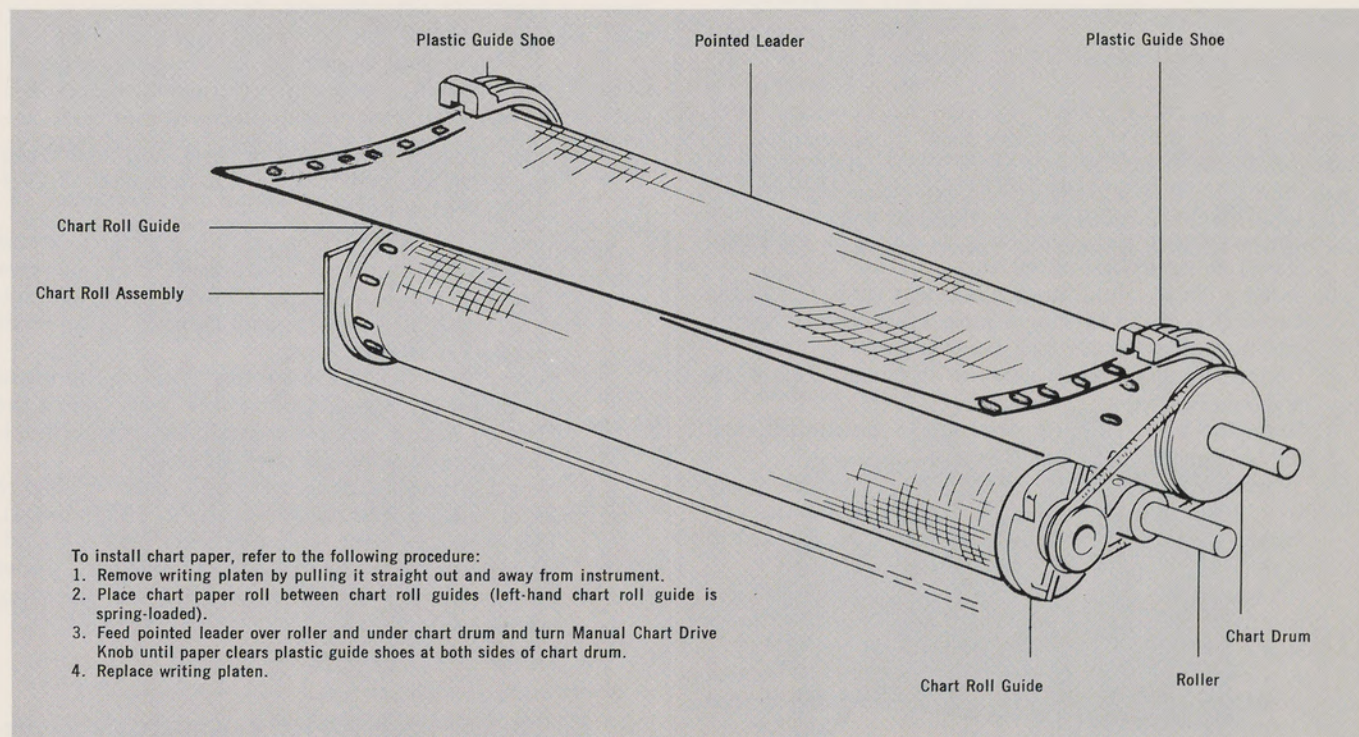


Figure 23. Chart Paper Installation

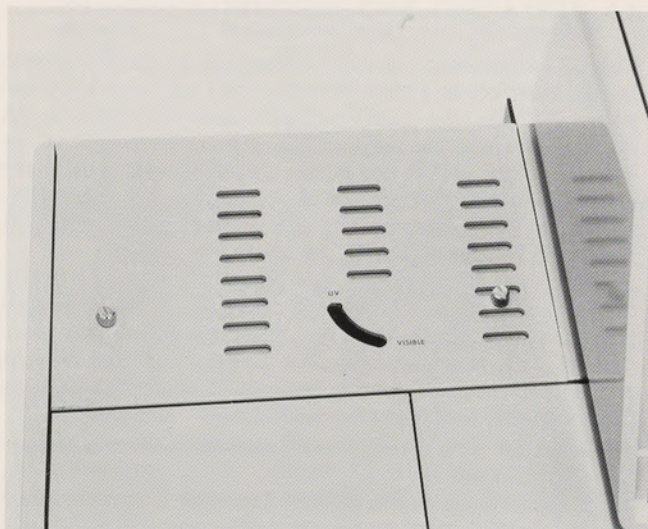


Figure 24. Source Compartment-Sample Compartment Cover

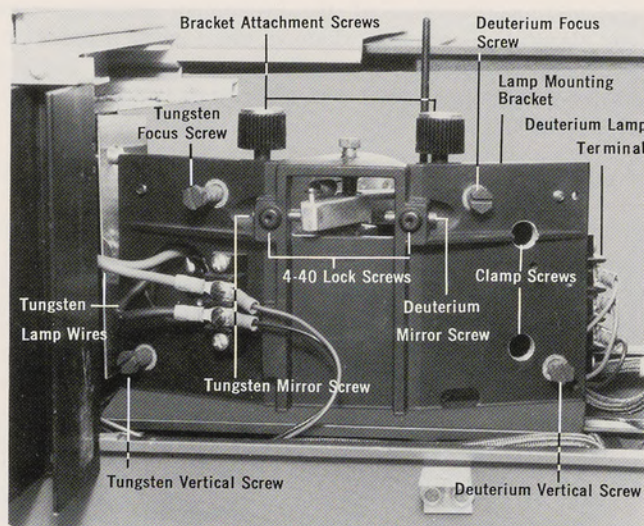


Figure 25. Rear View of Source Compartment

5. For protection, wrap a cloth around the glass bulb of the tungsten lamp. **DO NOT TOUCH MIRROR OR LAMPS.** Gently push in the lamp and twist it counterclockwise until the alignment pins slide past the keyed slots, freeing the base of the lamp.
6. Wrap a cloth around the replacement lamp. Orient the keyed slots to fit alignment pins. Press lamp in gently and turn clockwise to lock lamp in position.
7. Connect tungsten lamp wires to terminals, Figure 25, and tighten screws.
8. Remount lamp mounting bracket in instrument, and tighten bracket attachment screws.
9. When tungsten lamps are substituted, or changed in any way, major adjustments normally are not necessary. However, alignment of the lamp should be checked to make sure it is delivering maximum energy.
 - Operators will find it helpful to cover the sample compartment with a folded cloth to prevent light leaks when following alignment procedure.
 - a. Connect instrument power cord to outlet and depress **POWER** Pushbutton and **ABS** Pushbutton. This also turns on tungsten lamp.
 - b. Cover sample compartment with folded cloth.
 - c. Place Mode Switch in SB position, and turn Wave-length Selector until 500 nm is indicated on **WAVE-LENGTH** Indicator.
 - d. Turn **SB BASELINE** Control fully counterclockwise (as viewed from rear of instrument). Now, increase dynode voltage by adjusting **SB BASELINE** Control, or slit width, if necessary, until Digital Display reads near 0.05 A after each step.
 - e. Loosen the 4-40 lock screw that holds the tungsten mirror screw, Figure 25, in place. Now adjust the tungsten mirror screw for maximum energy (minimum A) reading on the Digital Display.
 - f. Now the tungsten focus and vertical screws, Figure 25, are adjusted for maximum energy (minimum A). This should be done with care since only slight adjustment of these screws should be necessary. These adjustments are not independent of the preceding mirror adjustment.
 - g. Tighten 4-40 lock screw.
10. Remove folded cloth and replace sample compartment-source compartment cover.

4.4 REPLACING AND ALIGNING DEUTERIUM LAMP

The useful operating life of the deuterium lamp is 500 hours under normal conditions. The end of the useful life of this lamp is denoted by failure to start or by a rapidly decreasing energy output. Ionization may occur inside the anode (internal firing) rather than in a concentrated path in front of the window. Usually, this occurs when the lamp is turned on while still hot from previous operation. If this occurs, the lamp must be turned off and allowed to cool before re-starting.

1. Turn off instrument and disconnect power cord from electrical outlet.

WARNING
PLACING THE UV SOURCE SWITCH IN THE OFF POSITION DOES NOT REMOVE ALL VOLTAGES FROM THE DEUTERIUM LAMP TERMINAL STRIP. THE POWER CORD MUST BE DISCONNECTED FROM THE ELECTRICAL OUTLET BEFORE SERVICING.

2. Expose source compartment by loosening two screws on top of source compartment, Figure 24, and then lifting off the sample compartment-source compartment cover.
3. Replacement of deuterium lamp is much easier if the lamp mounting bracket is turned to expose lamps. This is accomplished by loosening the two bracket attachment screws, Figure 25, and then twisting the lamp mounting bracket to expose lamps.
4. Locate deuterium lamp wires, Figure 25, and notice how they are connected to terminal strip; blue on top, red in middle, and black on bottom. Now loosen screws enough so that pigtail connectors of lamp wires can be removed from behind screws.
5. Note how deuterium lamp is positioned in clamps, with the arc opening facing the source mirror, Figure 27.
6. For protection, wrap a cloth around the lamp and then loosen the clamp screws, Figure 25, until the lamp can be removed from between the clamps.
7. Wrap the lamp with a cloth and place it between the clamps, orienting the arc opening toward the source mirror, Figure 26. Adjust the lamp vertically so that the arc opening is centered on the mirror. Now tighten the clamp screws but do not overtighten.
8. Connect deuterium lamp wires to terminals, as described in Step 5 preceding.
9. Replace lamp mounting bracket in instrument and tighten bracket attachment screws.
10. Align deuterium lamp as follows:

WARNING
Ultraviolet radiation from the deuterium lamp is harmful to the eyes. Protective (not necessarily safety) glasses must be worn during the alignment procedure.

- a. Connect instrument power cord to electrical outlet and depress POWER Pushbutton.
 - b. Place UV Source Switch in the ON position (2½ minutes are required before lamp fires).
 - c. Cover sample compartment with folded cloth.
 - d. Place Mode Switch in SB position, and turn Wavelength Adjust Knob until 250 nm is indicated on WAVELENGTH Indicator.
 - e. Turn BASELINE Control fully counterclockwise (as viewed from rear of instrument). Now, increase dynode voltage by adjusting SB BASELINE Control, or slit width, if necessary, until Digital Display reads near 0.05 A after each step.
 - f. Loosen the 4-40 lockcrew that holds the deuterium mirror screw, Figure 25, in place. Now adjust the deuterium mirror screw for maximum energy (minimum A) reading on the Digital Display.
 - g. Now the deuterium focus and vertical, Figure 25, are adjusted for maximum energy (minimum A). This should be done with care since only a slight adjustment of these screws should be necessary. These adjustments are not independent of the preceding mirror adjustment.
 - h. Tighten 4-40 lockcrew.
11. Remove folded cloth from sample compartment and replace sample compartment-source compartment cover.

4.5 REPLACING FRONT-PANEL INDICATOR LAMPS

The POWER, IDLE, ABS, CONC, and Δ Pushbutton lamps are replaced as follows:

1. Turn off instrument and disconnect power cord from electrical outlet.
2. Remove plastic button by pulling it straight out.
3. Remove lamp from socket by grasping lamp very carefully with a pair of needle nose pliers, then pushing down on lamp and pulling it straight out. Replace it with a new lamp. (See Section Five for replacement part numbers).
4. Replace plastic button and push it in until it snaps into place.

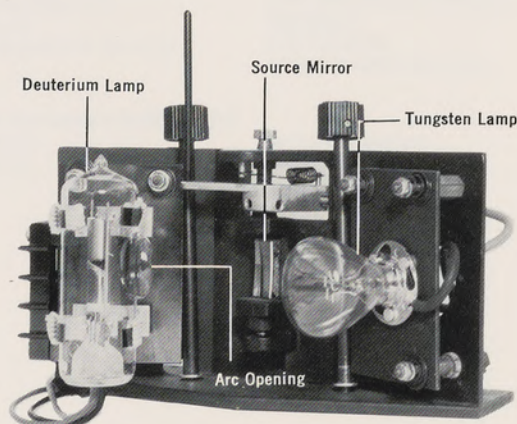


Figure 26. Front View of Source Lamps

SECTION FIVE SELECTED REPLACEMENT PARTS LIST

Beckman

Part No.	Description
189311	Deuterium Source Lamp
564333	Tungsten Source Lamp
873611	Front-Panel Indicator Lamps

Preprinted Repeating Format
Chart Paper With Wavelength
Markings (8-inch x 10-inch
grid area)

	Abscissa	Ordinate
579354	Ultraviolet (200 to 360 nm)	0 to 2 A
579356	Ultraviolet (200 to 360 nm)	-0.3 to +0.7 A
579358	Ultraviolet (200 to 360 nm)	100 lines unmarked
579357	Visible (350 to 750 nm)	0 to 2 A
579359	Visible (350 to 750 nm)	-0.3 to +0.7 A
579361	Visible (350 to 750 nm)	100 lines unmarked
580215	Visible-UV (200 to 360 and 350 to 750 nm)	-0.3 to +0.7 A
580216	Visible-UV (200 to 360 and 350 to 750 nm)	0 to 2 A
580217	Visible-UV (200 to 360 and 350 to 750 nm)	100 division unmarked
568415	Pen, Disposable, Red, Pkg of 5	
568416	Pen, Disposable, Blue, Pkg of 5	
568417	Pen, Disposable, Black, Pkg of 5	
568418	Pen, Disposable, Green, Pkg of 5	
915894	Pen, Disposable, Fine Line, Red, Pkg of 5	
915285	Pen, Disposable, Fine Line, Green, Pkg of 5	
915286	Pen, Disposable, Fine Line, Blue, Pkg of 5	
915287	Pen, Disposable, Fine Line, Black, Pkg of 5	
015-082245	Instruction Manual	

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