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JASCO MODEL CD-1595

**CIRCULAR DICHROISM CHIRAL
DETECTOR**

OPERATION MANUAL



Safety Considerations

To ensure operation safety, this instrument must be operated correctly and maintained regularly according to schedule. Carefully read to fully understand all safety precautions in this manual before operating the instrument. This manual denotes precautions against actions that can result in hazardous situations or equipment damage by using the signal words **WARNING**, **CAUTION**, and *Note*.

(1) Safety symbols



Instruction manual symbol. If the product is marked with this symbol, refer to the instrument manuals to protect the instrument against damage.

WARNING A **WARNING** indicates an potentially hazardous situation which, if not avoided, could result in death or serious injury

CAUTION A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

Do not proceed beyond a **WARNING** or **CAUTION** notice until you understand the hazardous conditions and have taken the appropriate steps.

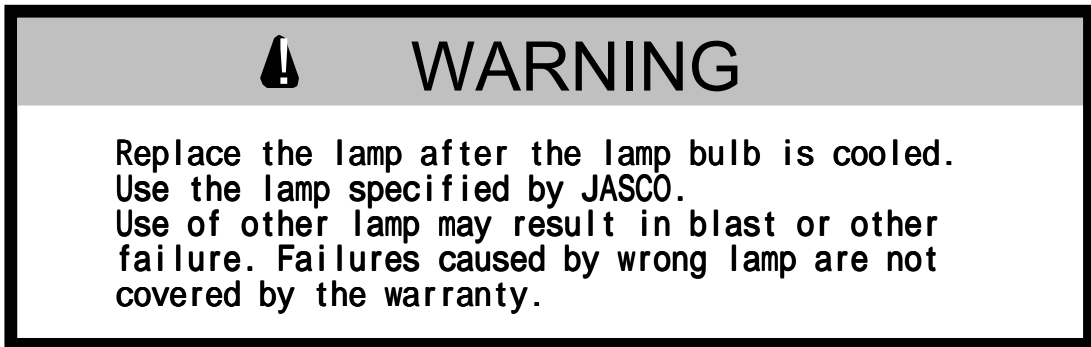
Note A *Note* provides additional information to aid the operator in obtaining optimal instrument performance.

Pressurized, hazardous solvents are used in high-performance liquid chromatography. Always follow the proper laboratory procedures to ensure operator safety. Always wear goggles, gloves and protective clothing when operating the instrument, especially when injecting sample and opening valves.

(2) Warning Labels

Warning labels are attached at several locations on this instrument. Do not remove, deface or damage the warning labels. If a warning label peels off the instrument or becomes illegible, contact your local JASCO distributor (see list on the back cover of this manual) for a replacement label. Be sure to indicate the part number (P/N) on the label.

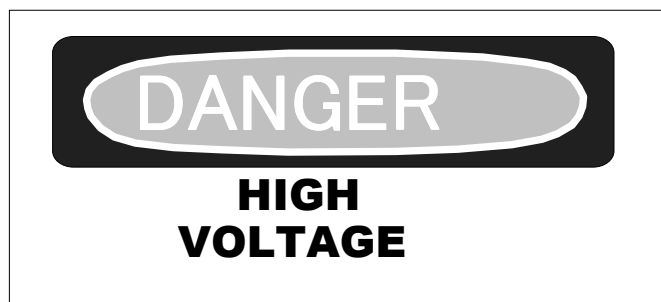
(1) Lamp Replacement Warning Label (P/N: 0822-0078A)



The surface temperature of the Hg-Xe lamp can reach more than 200°C when the lamp is lit. The temperatures of the lamp and the lamp housing do not return to ambient temperature immediately after the lamp is turned off. Therefore, when replacing the lamp, take sufficient care to avoid burn injuries.

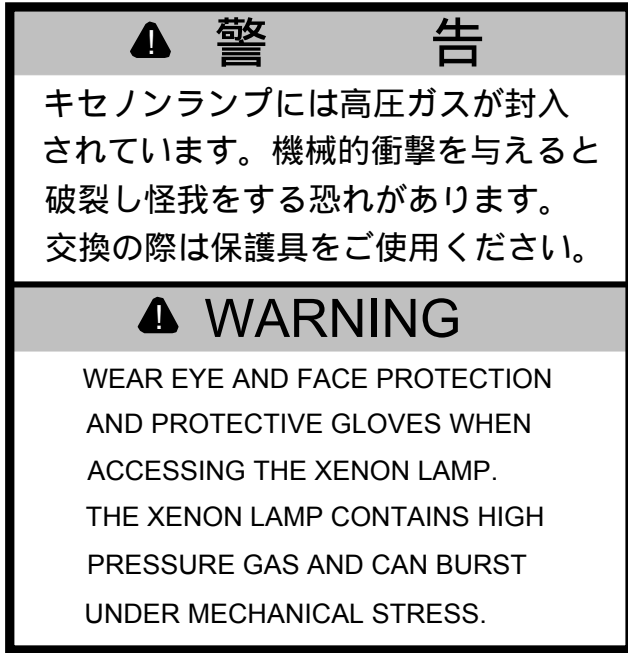
Refer to the maintenance manual for further details regarding lamp replacement. Follow the instructions provided therein and perform all operations carefully.

(2) Danger Warning Label (P/N: 0822-0035A)



This label is located on the high-voltage source used to light the Hg-Xe lamp. A voltage of 200 V or greater is generated during the operation of the detector. Inserting one's hands or other objects into this area may result in electric shock. Be sure to turn off the power and unplug the power cord before performing maintenance in this area.

(3) Xenon Lamp Warning Label (P/N: 0822-0077A)



When replacing the Xenon lamp, refer to the operations manual (P/N 0301-0193A) and follow the instructions carefully.

(4) Fuse Warning Label (P/N: 0822-0120A)

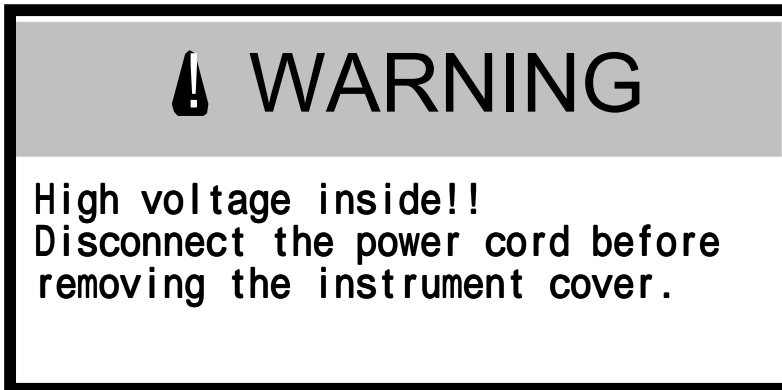


(5) Electric Shock Warning Label (P/N: 0822-0124A)



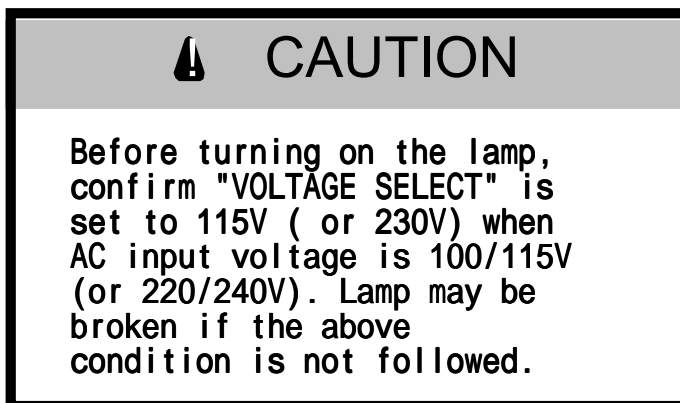
Electric shock may occur if the instrument is not properly grounded.

(6) High voltage inside warning Label (P/N: 0822-0145A)



A voltage of 200 V or greater is generated during the operation of the detector. Be sure to turn off the power and disconnect the power cord before performing maintenance.

(7) Voltage Select Warning Label (P/N: 0822-0097A)



Before turning on the lamp, confirm "VOLTAGE SELECT" is set to 115V when AC input voltage is 100-115V or "VOLTAGE SELECT" is set to 230V when AC input voltage is 220-230V. Lamp may be broken if the above condition is not followed.

(3) Carrying the Instrument

This instrument weighs approximately 21 kg. When carrying the instrument, be sure to hold the instrument as shown in Fig. 3.

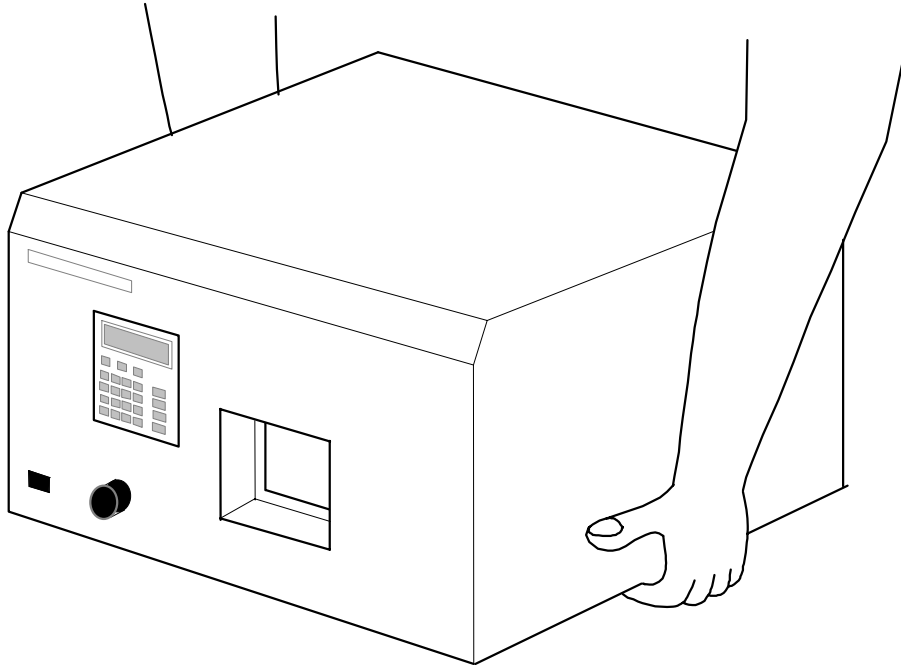


Figure 3 Grip Locations for Carrying the Instrument

Preface

This instruction manual serves as your guidebook for using this instrument. It is intended to instruct first-time users on how to properly use the instrument, and to serve as a reference for experienced users.

Before using the instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual stored in a safe place. Should this instruction manual be lost, order a replacement from your local JASCO distributor.

The manuals for this instrument consist of an operations manual and a maintenance manual. This is the operations manual.

Installation Conditions

To ensure safe operation, the following recommendations should be observed:

- (1) Do not operate the instrument under voltage fluctuations exceeding 10% of the recommended line voltage. Large fluctuations may cause the instrument to fail.
- (2) Use a three-pronged electrical outlet with a ground. When only a two-pronged socket is available, use an adapter and be sure to connect the ground wire of the adapter.
- (3) Operate the instrument under a temperature range of 10 ~ 30°C.
- (4) Operate the instrument under a humidity range of 35 ~ 85% (RH). If ambient humidity exceeds 85% (RH), water vapor may deteriorate optical components. If possible, install the instrument in a location having a humidity of 60% or lower.
- (5) Operate the instrument under an atmospheric pressure of 750 ~ 1060 hPa.
- (6) Avoid strong magnetic fields and sources of high-frequency waves. The instrument may not function properly when near strong magnetic fields or high-frequency wave sources.
- (7) Avoid vibrations caused by vacuum pumps, electric motors, processing equipment and machine tools.
- (8) Avoid dust and corrosive gas. Do not install the instrument in a location where it may be exposed to dust, especially in locations exposed to outside air or ventilation outlets that discharge dust particles.
- (9) Do not install the instrument in a location where it may be exposed to direct sunlight.
- (10) Do not install the instrument in a location where it may be directly exposed to the air current from an air conditioner or heater, as such a location may inhibit stable measurement.

Note: The above conditions do not ensure optimal performance of this instrument.
--

Maintenance

Consult your local JASCO distributor, listed at the back of this manual, regarding maintenance. In addition, contact your local JASCO distributor representative when transporting the instrument.

Replacement parts can be ordered according to part number from local JASCO distributor. When the part number is not known, inform parts name, instrument model name and its serial number.

Notices

- (1) Jasco shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of the use of this product.
- (2) Software prohibitions:
 - Copying of software or related materials for purposes other than backup is prohibited.
 - Transfer or authorization of the use of Jasco software to or by a third party is prohibited.
 - Disclosure of confidential information related to Jasco software is prohibited.
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- (3) The content of this manual is subject to change without notice in accordance with product improvements.
- (4) Unauthorized copying of this manual is prohibited.
- (5) This manual shall not be used to guarantee or copyright industrial rights or other rights.
- (6) Company and product names listed herein are trademarks or registered trademarks of various companies.

Warranty

This product is warranted for a period of one year from the date of delivery. If any defects should occur in the product during this period of warranty, JASCO will repair or replace the defective part(s) or product free of charge.

This warranty does not apply to defects as a result of the following:

- (1) USE FOLLOWING IMPROPER OR INADEQUATE INSTALLATION.
- (2) IMPROPER OPERATION.
- (3) MOVEMENT, MODIFICATION, OR REPAIR BY PERSONS OTHER THAN AUTHORIZED JASCO PERSONNEL.
- (4) USE OF PARTS OTHER THAN THOSE THAT ARE AUTHORIZED BY JASCO.
- (5) INORDINATELY RAPID DETERIORATION DUE TO THE USE OF CORROSIVE SOLVENTS OR SAMPLES.
- (6) NATURAL DISASTERS SUCH AS FIRES, WATER DAMAGE, OR EARTHQUAKES.

In addition, this warranty does not cover:

CONSUMABLE PARTS OR PARTS THAT HAVE A SEPARATE WARRANTY OR A WARRANTY PERIOD THAT IS DIFFERENT THAN THAT SPECIFIED ABOVE.

The warranty period for all parts and repairs supplied under this warranty expires with the warranty period of the original product.

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1. GENERAL DESCRIPTION AND SPECIFICATIONS OF INSTRUMENT

1.1 General Description

JASCO has developed the model CD-1595 chiral detector dedicated to HPLC. It is an innovative detector that offers higher sensitivity for chiral compounds with more information than a conventional optical rotation (OR) detector.

Chiral separation is now one of the most important application areas in HPLC. There is increasing demand for chiral chromatography in various fields, such as drug analysis, drug discovery, biochemical analysis, natural product analysis, organic synthesis, etc. Recent advances in chiral column technology are boosting the use of chiral chromatography in more and more laboratories. Advances in chiral detection technology, however, have not yet met these demands in terms of sensitivity and selectivity. The new CD-1595 detector is expected to meet the requirements.

OR detectors have exclusively been used for detection of chiral compounds, though it is known that a detector based on circular dichroism may offer better sensitivity. Therefore, a lot of efforts have been made to utilize a regular CD spectropolarimeter, which is rather big and expensive, as an HPLC detector. After extensive R&D work, JASCO has successfully developed a compact CD detector dedicated to HPLC that offers simultaneous detection of CD and UV absorption, and even CD and UV spectral measurements by stop-flow scanning.

Most biologically active compounds are chiral. It is well known that chiral isomers often cause totally different biological effects, e.g., one offers therapeutic effect but the other has unwanted one such as teratogenic activity. Most naturally occurring medicines contain only one type of the isomers. However, when such a compound is artificially synthesized, both isomers are, in general, equally produced. Therefore, each isomer must be examined for its biological activity. In such investigation, a conventional CD spectropolarimeter has been effectively used. In order to analyze samples with the CD spectropolarimeter, chiral separation needs to be performed and each isomer must be fractionated. The new CD-1595 detector offers on-line monitoring of CD signals during chiral separation and gives indication which peak components should be collected. In addition, the simultaneous CD and UV monitoring by the detector allows to determine optical purity of unresolved peaks of chiral compounds. This facilitates researchers to speed up their investigation on new drug discovery.

1.2 Specifications

Light source :	Hg-Xe lamp(150W)
Measurement wavelength range :	220-420 nm
Measurement range:	1000 mdeg (300nm) in 12 steps
Spectral bandwidth :	20 nm
Wavelength accuracy :	±5 nm
Sensitivity (S/N=2) :	0.05 mdeg/cm (290 nm), 0.1 mdeg/cm (240 nm) 0.1□g (Camphor-10-Sulfonic acid, 290 nm) 0.01□g (Pantoy Lactone, 240 nm)
Baseline drift :	0.1 mdeg/cm/h (at 290nm)
Gain:	x1, x10
Response :	FAST, STD, SLOW
Flow cell path length :	25 mm(1,5,10 mm optional)
Signal outputs :	Simultaneous outputs of CD (circular dichroism) and UV absorption CD, UV recorder output = 10mV full scale CD integrator output = 1V/deg UV integrator output = 1V/AU and 1V/2AU
Spectral measurement :	Simultaneous measurement of CD and UV spectra
Spectral memory :	220~420 nm(both UV and CD), Memory:10files
Power requirement :	100-240 VAC, 400VA
Dimension:	390(W)x 450(D)x250(H)mm
Weight :	approx. 21kg

The specifications are subjected to change without notice.

2. NAMES AND FUNCTIONS OF PARTS/ASSEMBLY

This chapter contains the names and functions of the parts on the instrument panels.

2.1 Front Panel

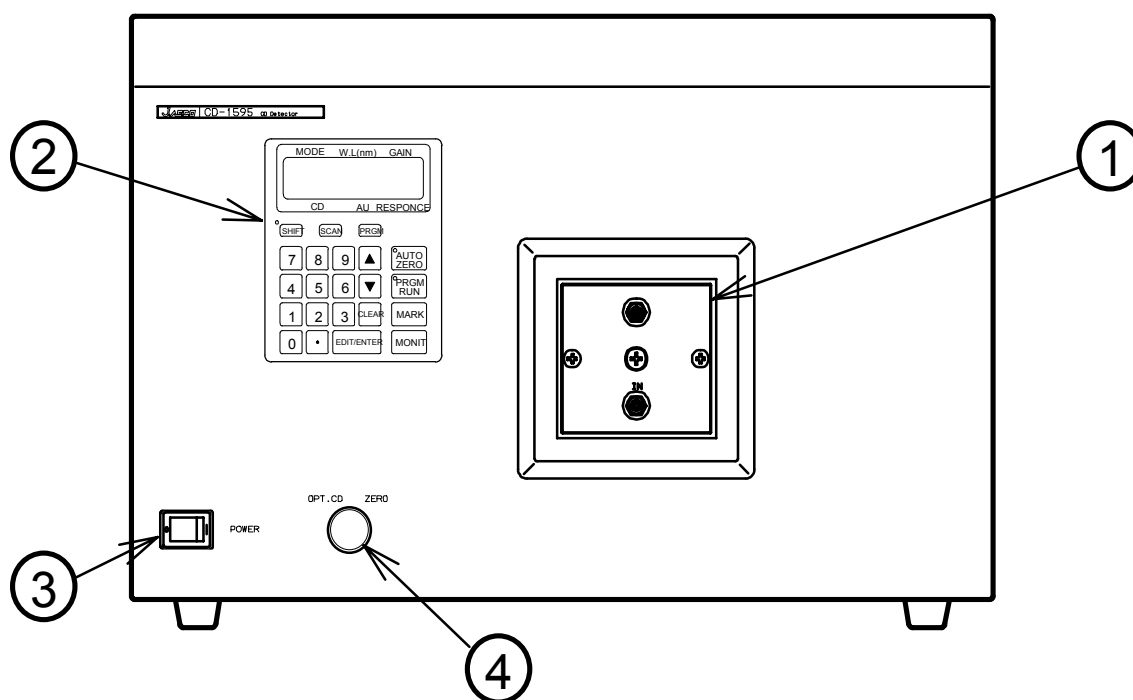


Figure 2.1 Front panel.

Name of part	Description
(1) Flowcell assembly	This is the cassette type flowcell assembly.
(2) LCD display and keypad	Display operating conditions, CD, UV and other signal levels, parameters, and various messages. Parameters necessary for the operation can be entered through the keypad.
(3) "POWER" switch	Used to turn the instrument on or off.
(4) "OPT. CD ZERO" knob	Used to adjust optical zero of the CD signal.

2.2 LCD and Keypad

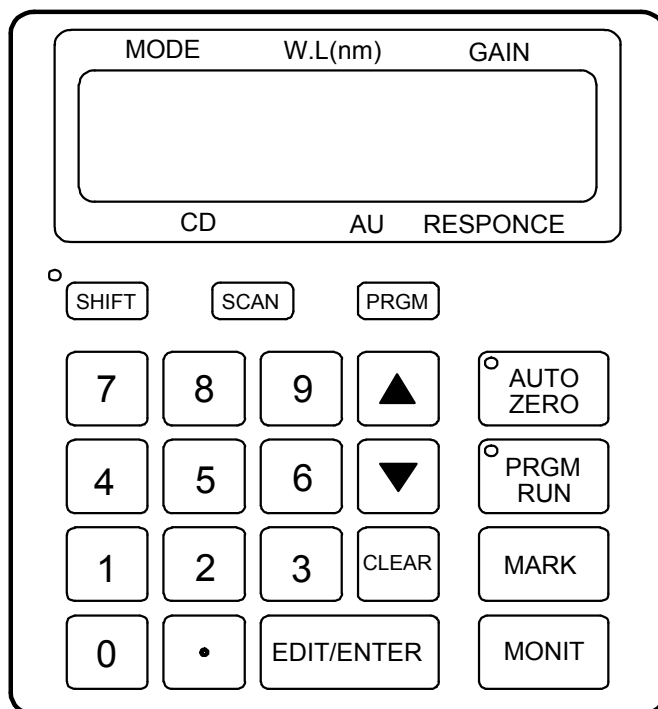


Figure 2.2 LCD and keypad

Name of part	Function
LCD display	Displays the operation status, parameters, and messages.
[AUTO ZERO]	Used to set the integrator and recorder outputs to zero.
[PRGM RUN]	Starts/stops a time program. When the time program is running the lamp on the key lights. It is also used for measuring/outputting CD/UV spectra.
[MARK]	Outputs marker on the recorder output.
[MONIT]	Used to return to or change the monitor mode.
[SHIFT]	Used in combination with the numeric keys to set or display parameters that are less frequently changed.
[SCAN]	Changes to the scan mode.

[PRGM]	Changes to time program mode.
[0] ~ [9][.]	Numeric keys.
[], []	Increment/decrement keys.
[CLEAR]	Clear erroneous entry of parameters. It also clears error messages.
[EDIT/ENTER]	Used to edit/enter parameters.

(NOTE)

Perform optical zero adjustment by turning the CD ZERO knob before activating the Autozero.

2.3 Flowcell Assembly

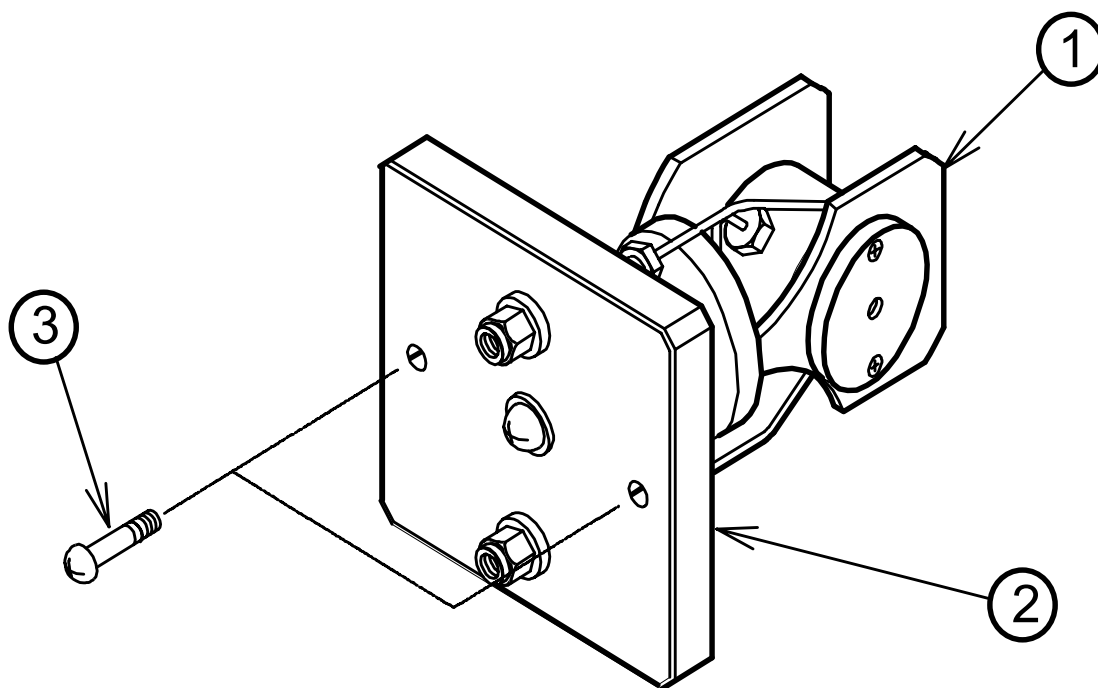


Figure 2.3 Flowcell Assembly

Name of part	Description
(1) Cell body	The light passes through the hole of the cell body.
(2) Cell panel	The cell panel is fixed to the cell body.
(3) Lock screws	Used to secure the flowcell assembly in position to the detector.

(NOTE)

To attach the flowcell assembly to the detector, press the cell panel until the cell body reaches to the bottom, then alternately tighten the lock screws to secure the flowcell assembly.

2.4 Rear Panel

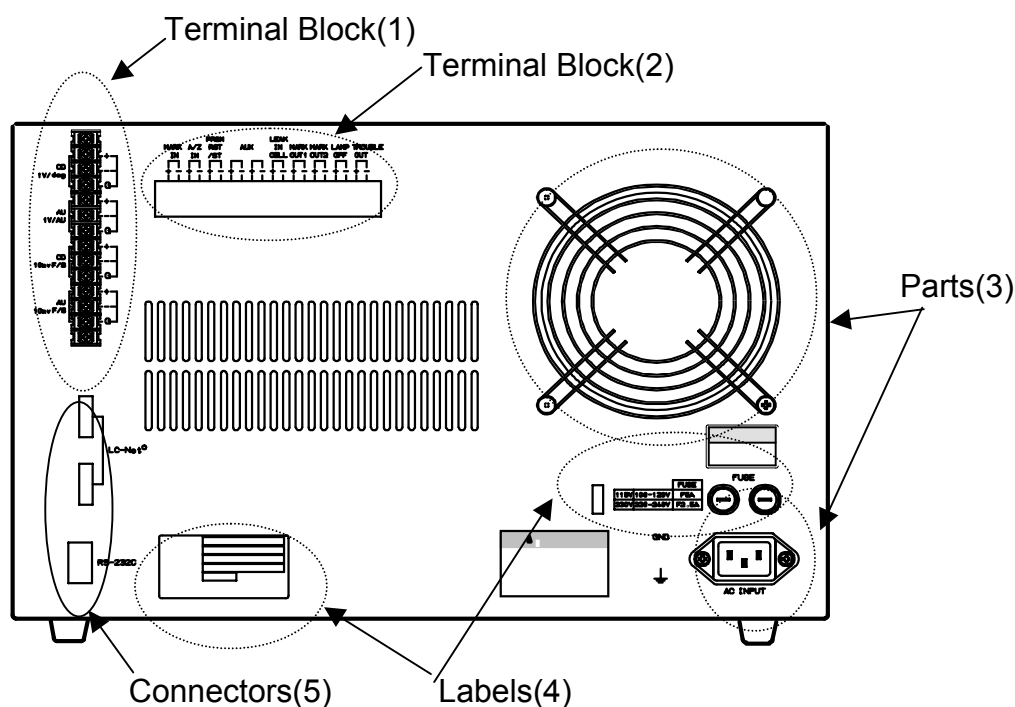


Figure 2.4 Rear Panel

Terminal Block (1)

Terminal	Description
CD 1V/deg + / - / G	CD signal output for integrator. 1V corresponds to 1 deg.
AU 1V/AU + / - / G	UV signal output for integrator. 1V corresponds to 1 AU. The output can be changeable to 1V/2AU by key operation.
CD 10mV/FS + / - / G	CD signal output for recorder.
AU 10mV/FS + / - / G (g FACTOR OUT)	UV signal output for recorder. This output can be switched to g factor output (CD/AU) by the key operation [SHIFT] [4]. See Section 5.6.

Terminal Block (2)

Terminal	Description
MARK IN + / -	Marker input. Shorting + and – terminals generates a tick mark on the recorder output.
A.Z. IN + / -	Autozero input. Shorting + and – terminals activates autozero function.

PRGM RST/ST + / -	Time program reset and start. Shorting + and – terminals resets the current time to zero and start again.
AUX + / -	Not used at present (January 1,2000).
LEAK IN CELL + / -	+ and – terminals are normally open. When liquid leak in the flowcell assembly is detected, the terminals are closed.
MARK OUT1 + / -	Marker outputs. When a marker signal is input, these terminals are momentarily closed.
MARK OUT2 + / -	
LAMP OFF + / -	When lamp is off, + and – terminals are closed.
LAMP TROUBLE OUT + / -	When lamp is erroneously off or the lamp house temperature elevates abnormally, + and – terminals are internally closed.

Parts (3)

Part	Description
Cooling fan	Fan for cooling the lamp house etc.
Power inlet	Power inlet connection.
Fuses	Fuses for power line. Warning: For continued protection against risk of fire, replace only with fuse of the specified type and current ratings.

Labels (4)

Label	Description
Serial No.	Serial number and other important informations are printed.
Fuse	Fuse warning label.
Line voltage	Line voltage selection is described. 115V: 100 – 120V 230V: 220 – 240V

Connectors(5)

Connectors	Description
LC-Net	Used when controlling the detector via Jasco system controller.
RS-232C	Connection for controlling the detector through the RS-232C channel.(Option)

3. Power-On, Self-Diagnostic Test and Power-Off

3.1 Power-on and Self-Diagnostics

Turning the power on will automatically start the power-on self-diagnostics. The following items/functions are checked during the self-diagnostics.

- Memories
 - ROM
 - RAM
 - C-MOS RAM
- DC power supply
- Wavelength drive mechanism
- Light source
- Light source emission
- Shutter

If an error is detected, corresponding error message will appear and the self-diagnostics will be terminated. However, when the light source emission is checked to be poor, the error message “EMISSION POOR” will be shown for several seconds, then resume the diagnostics.

On completion of the self-diagnostics, the following display will appear as shown in Figure 3.1.

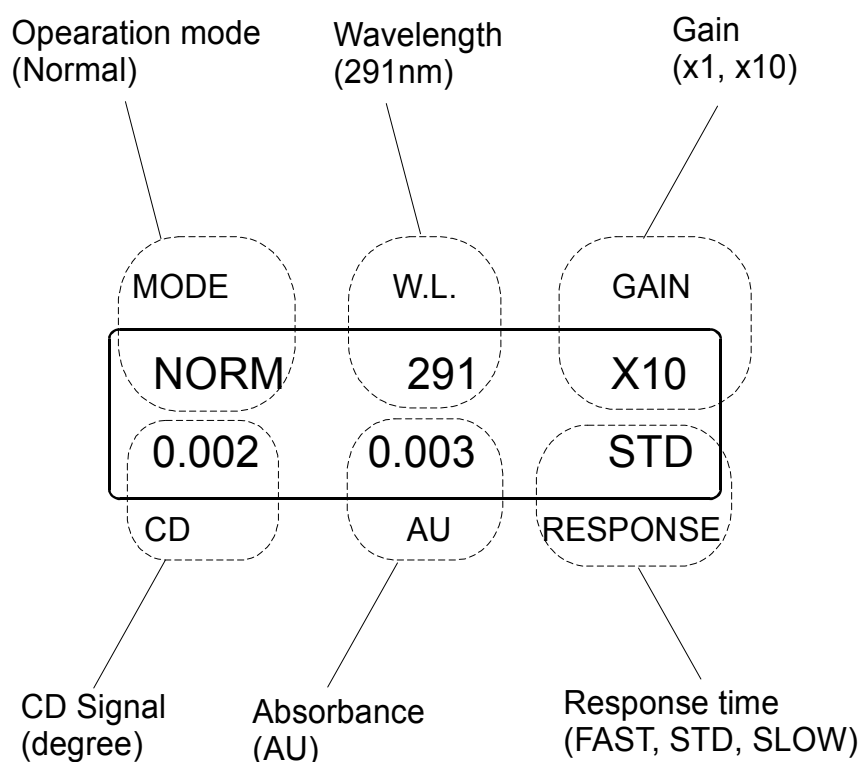


Figure 3.1 Normal mode display

Wavelength, sensitivity range and response shown are those set when the instrument power was turned off in the last use.

3.2 Turning power off

To turn off the power, just turn the POWER switch off. Parameters set during the operation such as wavelength, sensitivity range and response are stored on the C-MOS RAM and these values will be automatically resumed when the instrument power is turn on again.

4. PREPARATION FOR MEASUREMENT AND BASIC OPERATION

This chapter describes the basic operation procedures necessary for a simple measurement.

4.1 Preparation for Measurement

- 1) Connect a strip chart recorder and or an integrator (or a data processing system) to the instrument referring to Chapter 2 "Names and Functions of Parts".

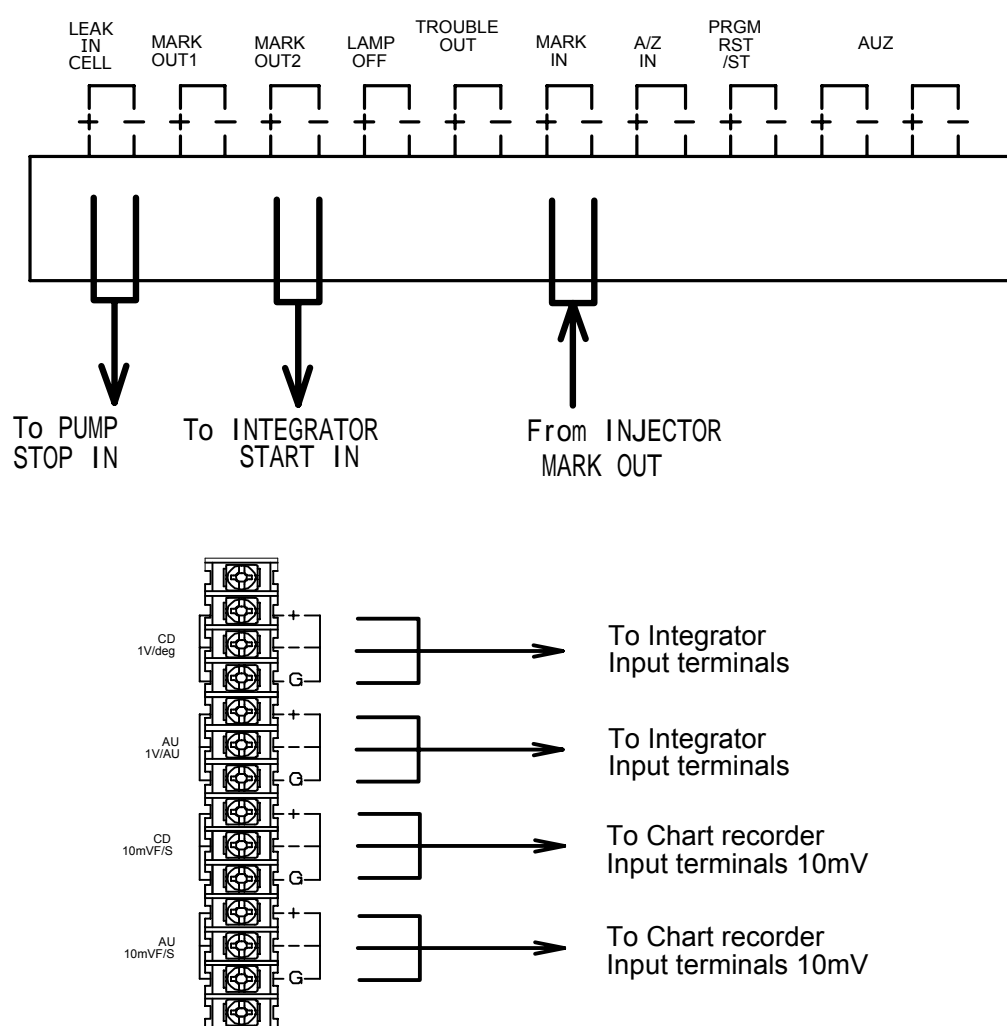


Figure 4.1 Connection of input/output terminals on the rear panel and the other instruments.

- 2) Connect column outlet tubing to the inlet port of the CD-1595 flow cell. Use 1/16 in. OD x 0.25 mm I.D. tube and 1/16 in. fittings.

- 3) Connect a tube (16 in. O.D. x 0.5 mm I.D.) to the outlet port of the flow cell. The total length of the tube should be less than 1m.

<CAUTION>

The maximum allowable pressure of the flow cell is 15 bar (1.5MPa). Do not use a narrow bore tube, e.g. 0.25 mm or less, to avoid excessive pressure that may cause leakage of the flow cell.

- 4) Start the HPLC pump to flow mobile phase solvent and wait for the baseline to be stabilized.

<CAUTION>

The flow cell has been purged with isopropanol before shipping from factory. If a buffer solution containing salt is to be used, be sure to flush the flow cell with water before flowing the solution.

4.2 Setting Parameters

In the normal operation mode, the detector monitors CD and UV signal at a set wavelength and with set sensitivity range and response. These parameters must be set at suitable values to the sample to be measured. This section describes how to set WAVELENGTH, GAIN, RANGE and RESPONSE.

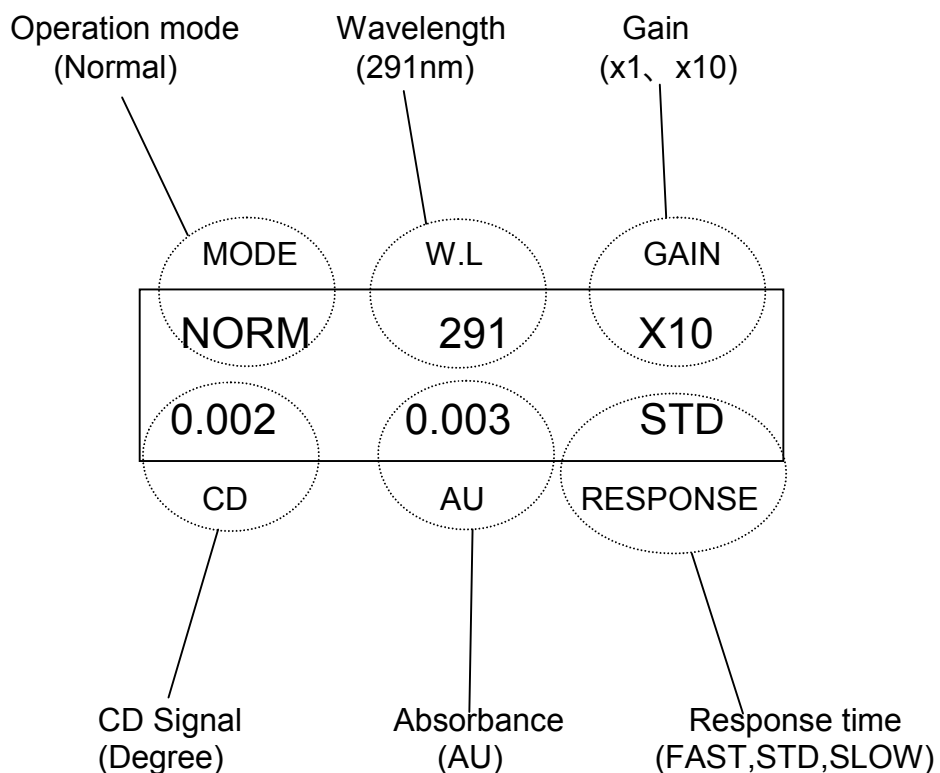


Figure 4.2 Monitor Screen

4.2.1 Changing WAVELENGTH

The wavelength, in general, should be selected so that the CD signal becomes maximum. This wavelength can be found by referring to literature, or to spectra obtained by stop-flow scanning at chromatographic peaks.

Wavelength range: 220 – 420nm

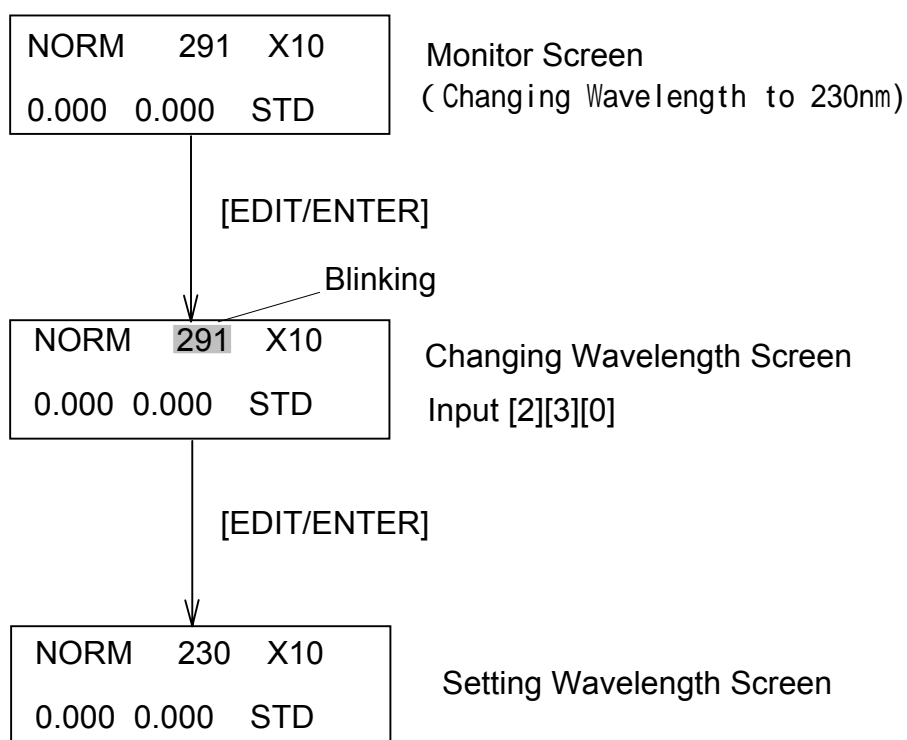


Figure 4.3 Setting WAVELENGTH.

4.2.2 Changing GAIN

The GAIN changes the gain of the CD signal amplifier. Normally, GAIN should be set to "X10". In case a sample compound gives so strong CD signal that may saturate the amplifier and "CD Sig.: 3.9000 V" is displayed(see Fig.5.2) , the setting "X1" should be used to reduce the CD signal to a proper level.

GAIN: X1 or X10

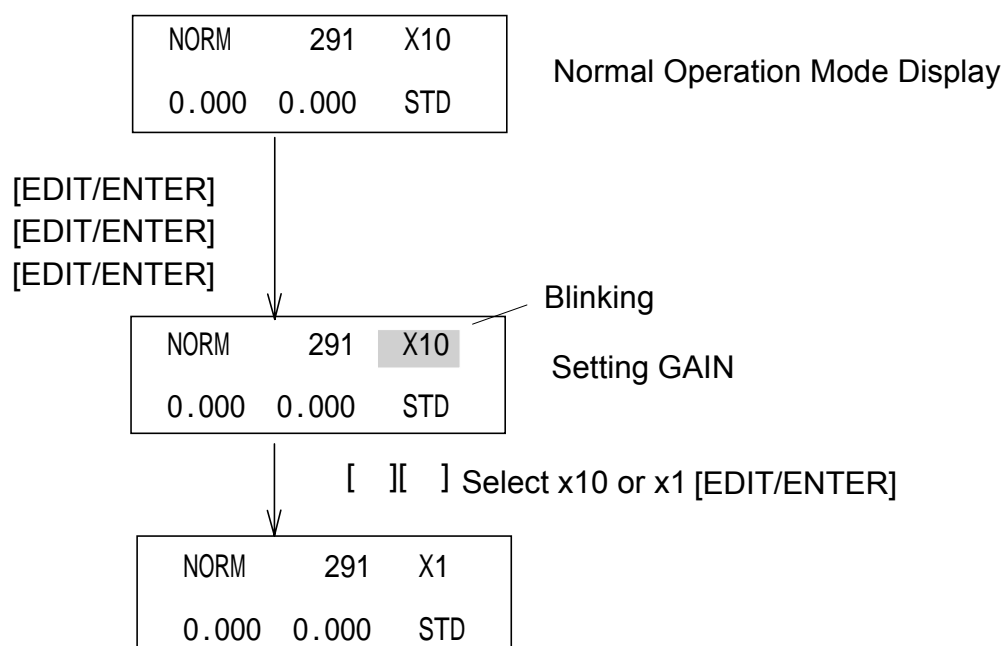


Figure 4.4 Changing GAIN

4.2.3 Changing RANGE

RANGE setting attenuates the output to recorder so that 10mV full scale corresponds to the RANGE parameter value.

Input range: CD RANGE: 0.0005 ~ 1.28 deg, S (Short)

UV RANGE : 0.001 ~ 2.56 AU, S (Short)

(G FACTOR: 1E-5 ~ 2.56E-2, S (Short))

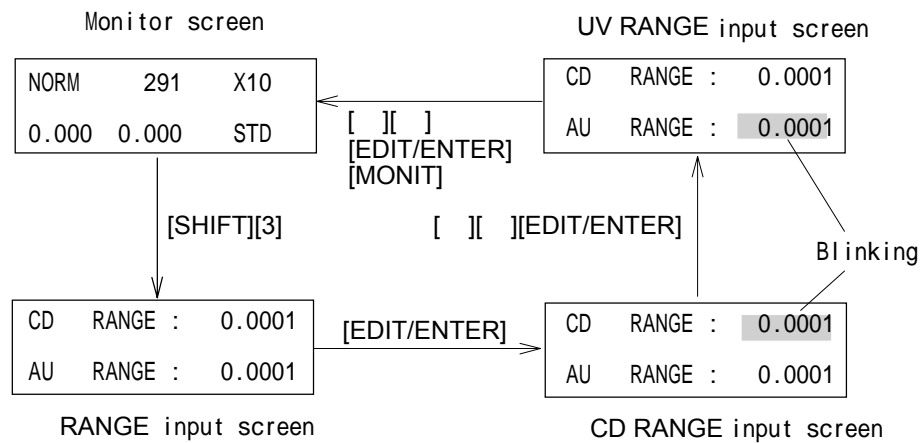


Figure 4.5 Changing RANGE

Note: The value "RANGE" can be changed also with numeric keys.
 Note: AU output can be changeable to g factor output by the key operations.
 (See 「 5.6 Selecting Recorder Output Mode 」)

4.2.4 Changing RESPONSE

The RESPONSE is the parameter that sets the detector response time. The RESPONSE can be selected from FST(fast), STD(standard) and SLW(slow). Normally, it should be set to STD.

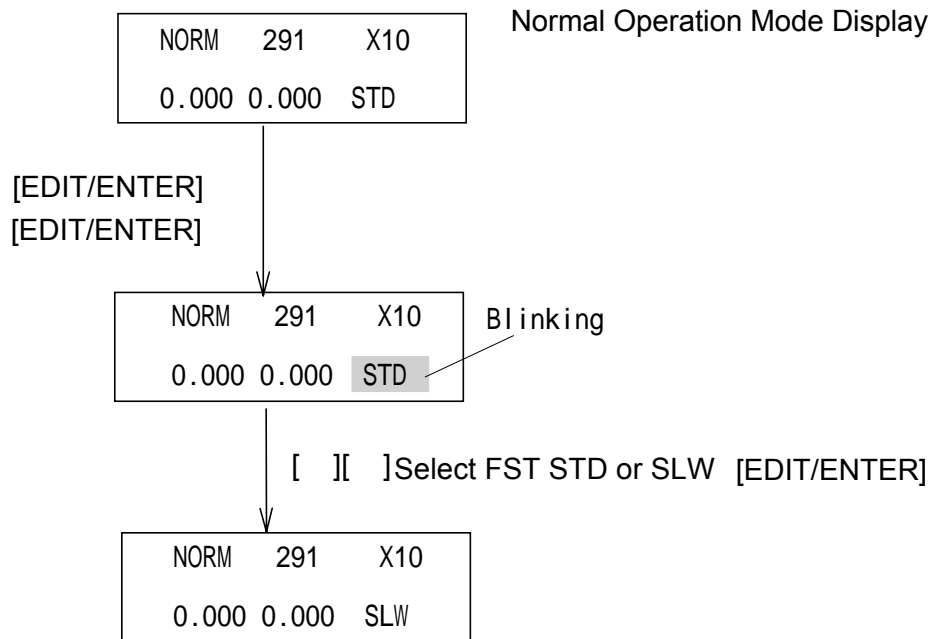


Figure 4.6 Changing RESPONSE.

4.3 Measurement

After the basic parameters setting completed, start measurement.

- 1) After the baseline has been stabilized, press [SHIFT] [1] to display the CD/UV signal screen.

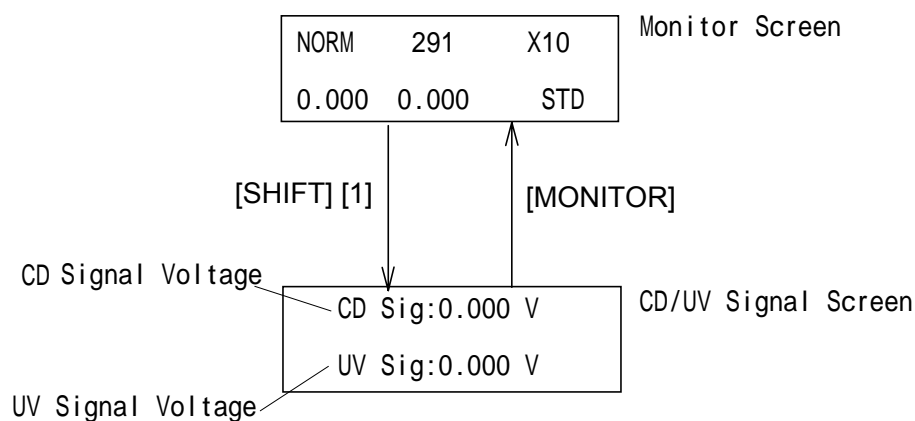


Figure 4.7 Preamplifier Output

- 2) Turn OPT.CD ZERO knob(see Fig.2.1) to adjust the CD signal voltage to become within $0 \pm 0.03V$. After the adjustment finished , press [MONITOR] to return to the normal mode screen.
- 3) After confirming the baseline is stable, press [AUTOZERO] key, then recorder and integrator outputs will be set to zero.
- 4) Inject the sample and immediately push [MARK] to output the marker signal to recorder output.

4.4 Error Messages

Table 4 .1 lists the error messages that can be detected by the CD-1595 during operation. When a problem is detected, a message will appear on the LCD. Press [CLEAR] to return to the monitor screen. If the problem reoccurs, the error message will reappear after approximately ten seconds.

Table 4.1 Error Messages During Operation

Error Message	Meaning
TROUBLE SOLVENT LEAK	The flow cell has a leak.
TROUBLE LAMP OFF	The lamp has gone out.
LAMP OFF TIMER LAMP OFF	The lamp turned off as the lamp off time elapsed.
WARNING CHECK LAMP TIMER	The cumulative lamp operation time has exceeded 1000 hours.
TROUBLE OVER TEMP	Lamp house temperature is unusually high.
TROUBLE DARK ERROR	Dark signal is out of normal range.

When the setting time of the lamp-off timer has elapsed, the LAMP OFF TIMER LAMP OFF message will appear and the lamp will turn off. This is not an error .

To re-light the lamp after it has been turned off by the timer, turn the power off and then on again.

Note: After turning the power off, wait for at least one minute before turning the power on again.
--

5. KEY OPERATION USING [SHIFT] KEY

5.1 Outline of Key Operation

Table 5.1 outlines the key operation using [SHIFT] key.

Key	Function
[SHIFT] [0]	UV Signal output screen for Integrator. 1V/AU or 1V/2AU can be selectable.
[SHIFT] [1]	Preamplifier Output display screen. This function is used to perform optical zeroing as well as checking the conditions of the lamp, other optical elements, presence of air bubbles in the flow cell.
[SHIFT] [2]	Lamp Off Timer screen.
[SHIFT] [3]	Recorder Range setting screen. CD and UV(or G FACTOR) range can be changeable on this screen.
[SHIFT] [4]	Recorder Output Mode setting screen. "AU RE COUT" or "G FACTOR OUT" can be selectable on this screen.
[SHIFT] [5]	Integrator Output Zero Level selection screen. Most integrators can accept an input voltage between -10mv and +1v. This function allows to shift up the voltage on the integrator output terminal , thus preventing the output from going below the -10mv limit even for negative peak.
[SHIFT] [6]	Cumulative Lamp Operation Time display screen. Use this function to determine when the lamp needs to be replaced.
[SHIFT] [7]	Recorder Polarity setting screen. Changing the polarity does not affect the polarity on the Integrator terminal.
[SHIFT] [8]	"Autozero Mode Selection screen. "AUTO","MANUAL" or "HOLD" can be selectable.
[SHIFT] [9]	g FACTOR monitor screen.
[SHIFT] [MARK] [5]	Flowcell Path-length setting screen.
[SHIFT] [MONITOR]	This function will bring up production programs and is not used for usual operation.
[SHIFT] [PROG]	"Program Number Setting" screen.
[SHIFT] [SCAN]	"Spectrum Output" screen.

5.2 [SHIFT] [0]: UV Signal output setting for integrator

1V/AU or 1V/2AU can be selectable.

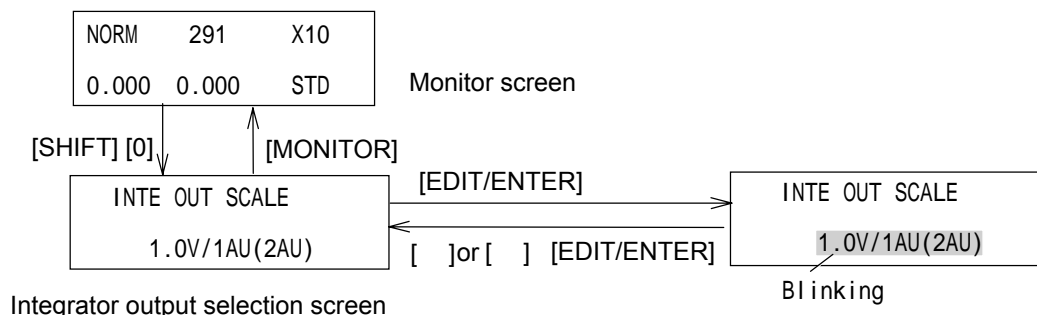


Figure 5.1 Integrator output selection

5.3 [SHIFT] [1]: Performing Optical Zeroing and Checking Preamp Output Level

Pressing [SHIFT] [1] keys will bring up the “preamplifier output level” screen. In order to obtain accurate CD signal, optical zeroing must be performed so that a racemate cancel positive and negative responses each other and gives no peak. To perform optical zeroing, adjust the “Opt. CD Zero” knob (see Fig.2.1) until CD signal shown in Figure 5.2 reads within $0 \pm .003V$ while flowing only mobile phase solvent. This function also provides means to read the preamplifier output signal level that directly shows the UV energy reached the photodiode. Direct reading of preamplifier outputs reflects the conditions of the lamp and other optical elements as well as presence of air bubbles in the flowcell.

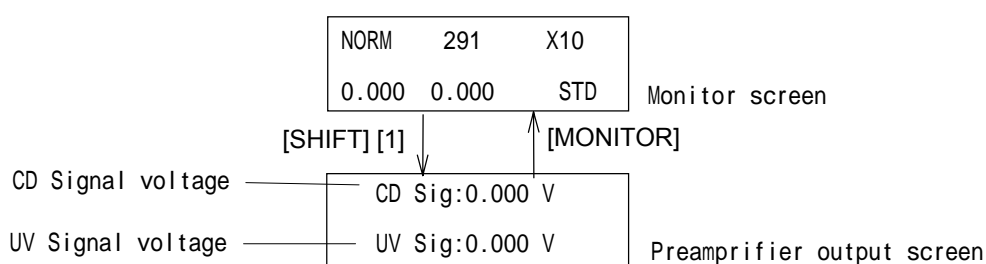


Figure 5.2 Preamplifier outputs

5.4 [SHIFT] [2] : Lamp Off Timer

Pressing [SHIFT] [2] will bring up the “Lamp Off Timer” display. When the detector is turned on, Lamp Off timer is always set to “0.0”. Therefore, to activate this function, the operator needs to set the time when the lamp is to be turned off.

5.4.1 Setting Lamp Off Timer

Input time range: 0(OFF) – 99.9 hours(0.1 hour increment)

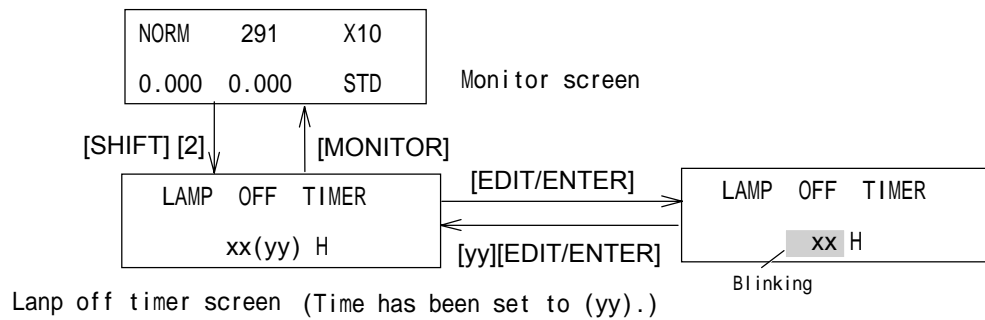


Figure 5.3 Setting Lamp Off Timer

When the Lamp Off Timer is set, the lamp will be automatically turned off when the time is up. Then the following display will appear.

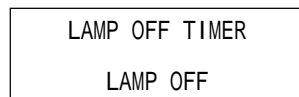


Figure 5.4 Message shown when the Lamp Off Timer turns off the lamp

To set the LAMP OFF TIMER to “OFF”, enter “0.0”.

5.4.2 Turning Lamp On after Lamp Off Timer activated

To turn the lamp on again after Lamp Off Timer activated, turn off the power and then turn on again. Note that while the lamp is hot, the lamp may fail to get lit, wait at least 1 minute after the lamp is turned off until it cools down, then turn the lamp on again.

5.5 [SHIFT] [3]: Setting Recorder Range

Pressing [SHIFT] [3] will bring up the “Recorder Range” display screen.

Note: AU output can be changeable to G FACTOR output by key operations.(Refer to Section [5.6])

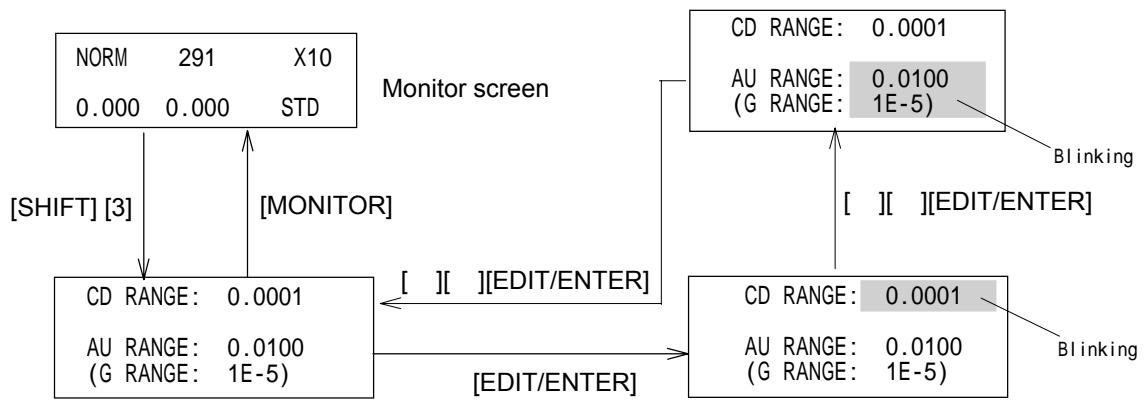


Figure 5. 5 Setting recorder range

Selectable range

CD RANGE: 0.0005 ~ 1.28 Degree and S(Short) 13steps

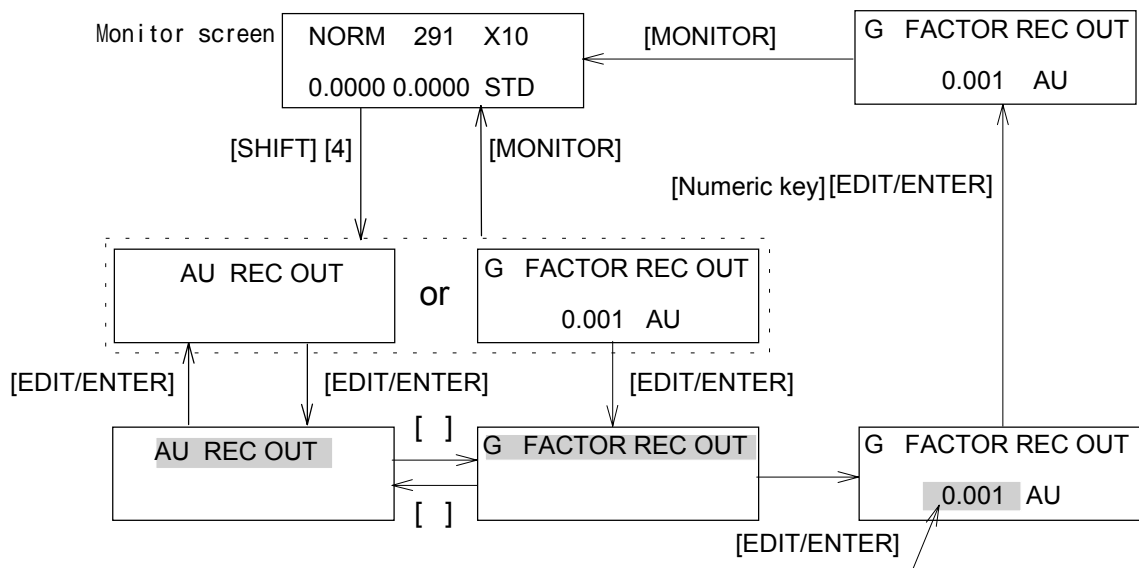
AU RANGE: 0.001 ~ 2.56 AU and S(Short) 13steps

G RANGE: $1E-5(1 \times 10^{-5})$ ~ $2.56E-2(2.56 \times 10^{-2})$ and S(Short) 13steps

5.6 [SHIFT] [4]: Selecting Recorder Output Mode

“AU REC OUT” or “G FACTOR OUT” mode can be selectable on this function.

If “G FACTOR OUT” mode has been selected ,a signal CD/UV (g factor) can be outputted from the UV output terminal for recorder on the reae panel (see Fig.4.1) in place of UV absorbance output.



In case the absorbance value is less than the value setting at here, G FACTOR always outputs 0.00000. (Refer to 「5.11」) This value is settable within the range of 0.001 ~ 1.000.

Figure 5.6 Selecting Recorder Output Mode

5.7 [SHIFT] [5]: Shifting Integrator Output Zero Level

The CD signal usually appears both positive and negative directions by its nature. However most integrators can accept an input voltage between -10mV and $+1\text{V}$. This function allows to shift up the voltage on the integrator output terminal, thus preventing the output from going below the -10mV limit even for negative peak or negative drift.

(NOTE)

This function has no effect on the recorder output.

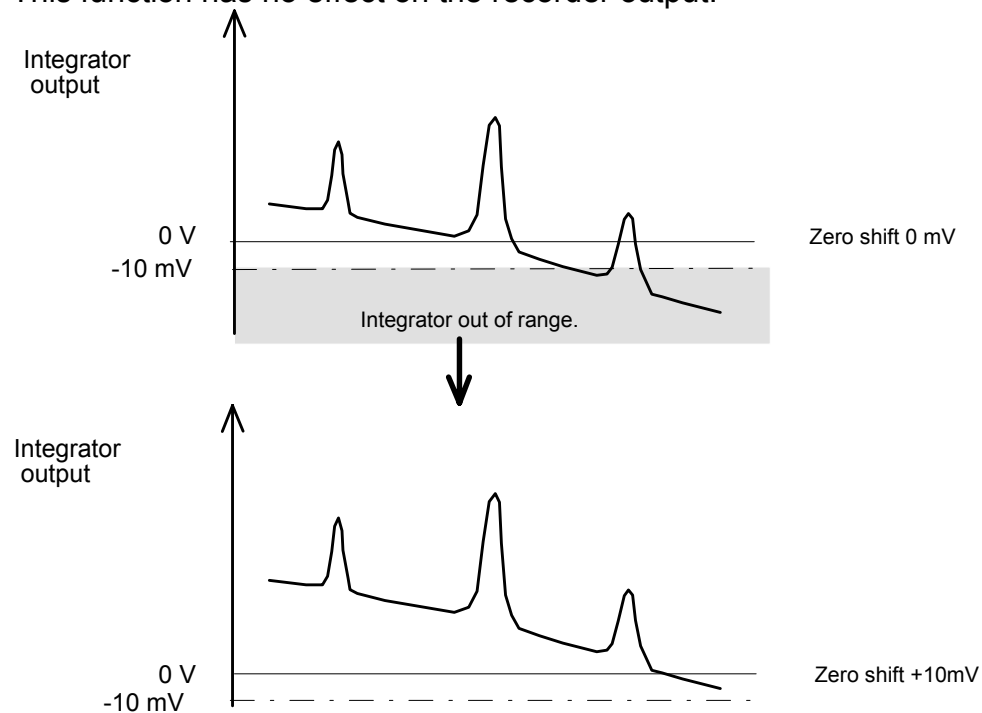


Figure 5.7 Negative drift vs. integrator dynamic range.

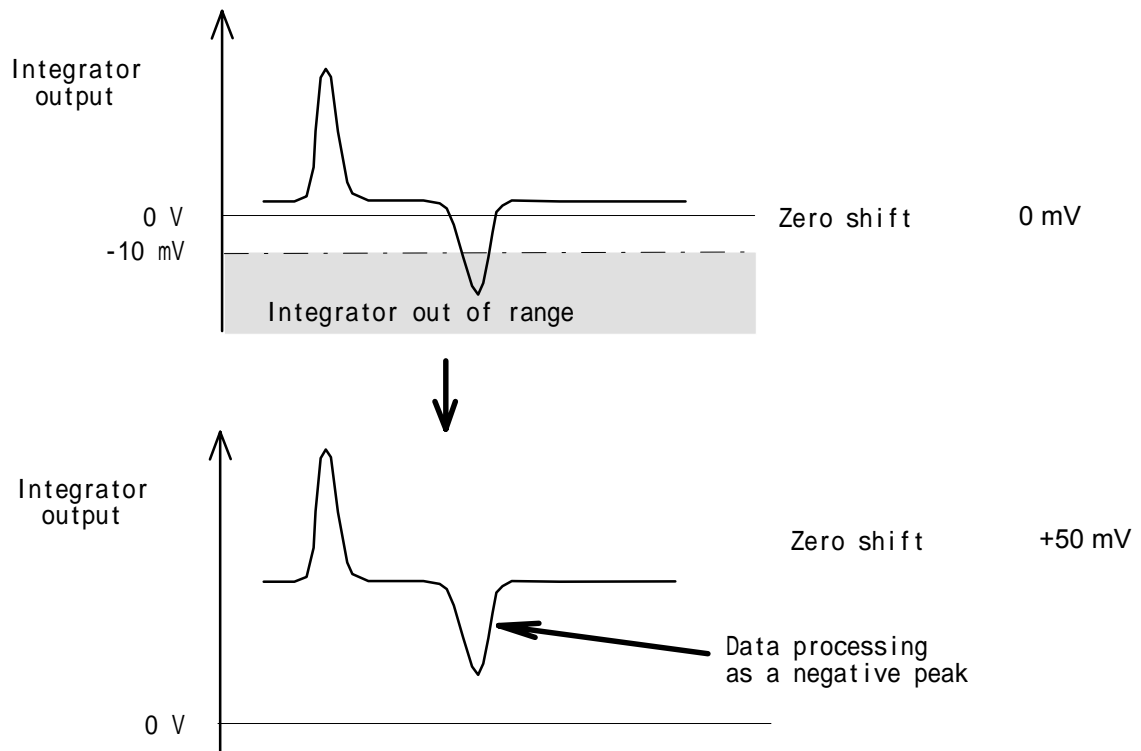


Figure 5.8 Negative peak vs. integrator dynamic range .

5.7.1 Setting integrator output zero level

0,5,10,30,50,100,500,1000mV can be selectable as the integrator output zero level.

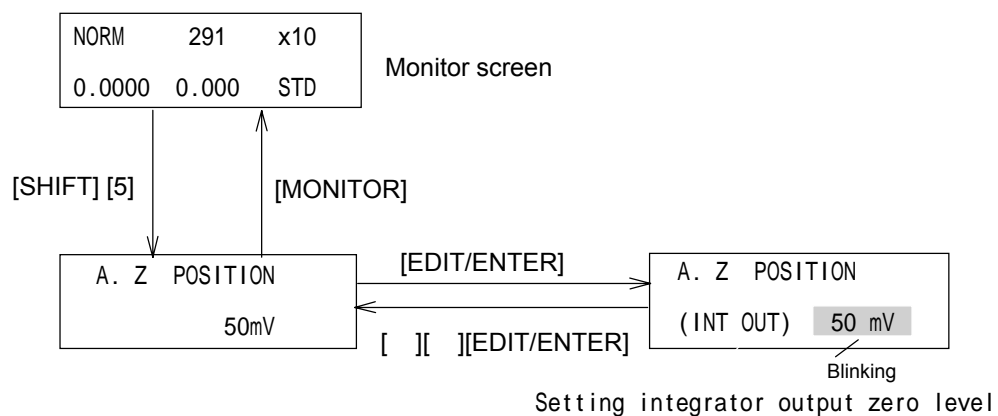


Figure 5.9 Setting integrator output zero level.

5.8 [SHIFT] [6]: Checking Cumulative Lamp Operation Time

The accumulated lamp operation time can be checked to estimate when the lamp should be replaced.

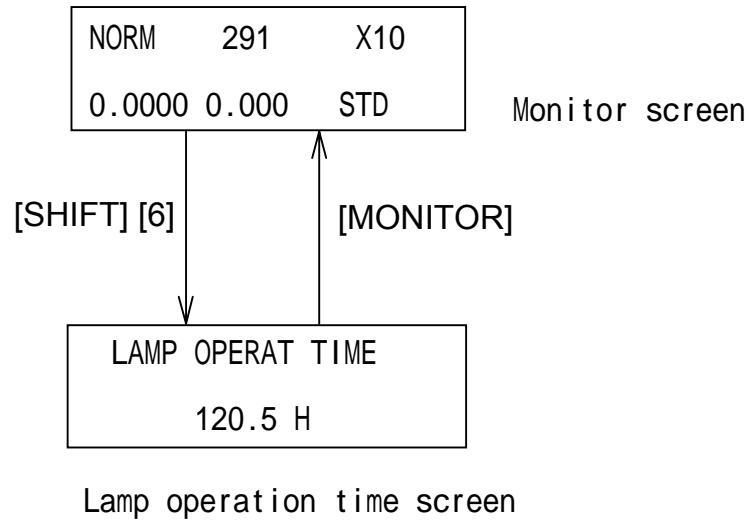


Figure 5.10 Checking cumulative lamp operation time.

5.9 [SHIFT][7]: Changing Recorder Output Polarity

This function has no effect on the integrator output.

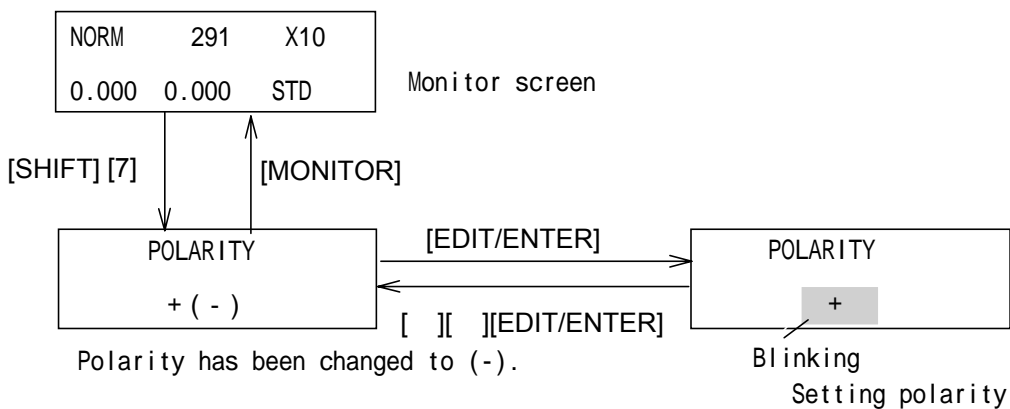


Figure 5.11 Changing recorder output polarity.

5.10 [SHIFT][8]: Selecting Autozero Mode

This function is used to control autozeroing when wavelength or gain parameters is changed or when a signal enters the MARKER IN terminal on the back panel.

AUTO: Autozero is automatically executed and the baseline of the chromatogram is returned to zero when a wavelength settings changed, either in a time program or manually.

When performing repeated analysis using the autosampler, autozero is automatically executed with the addition of a marker on the recorder output when the MARKER IN signal is input.

MANUAL: Autozero is not executed when a wavelength is changed or MARKER IN signal is input

HOLD: The baseline position will not be changed when a wavelength is changed or MARKER IN signal is inputted. Autozero will be executed, but the baseline position will return immediately to the previous position.

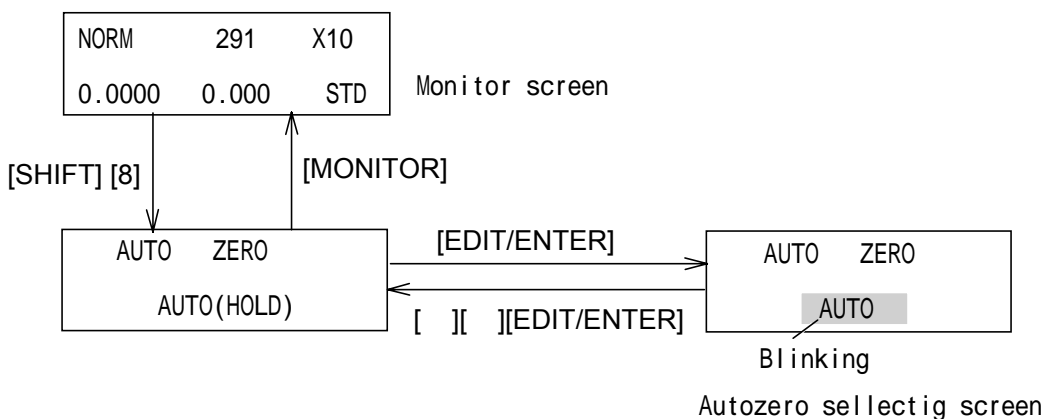


Figure 5.12 Selecting autozero mode

5.11 [SHIFT][9]:

Pressing [SHIFT] [9] keys will bring up the “g factor monitoring screen”. Calculated g factor is displayed on this screen in real time. CD signal and absorbance are also displayed on this screen.

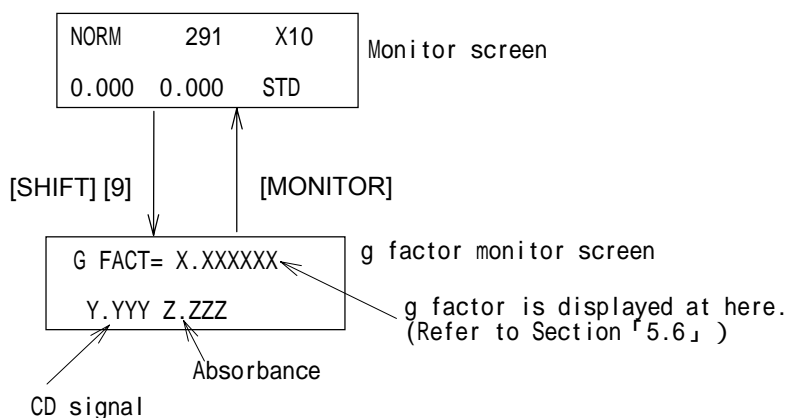


Figure 5.13 Monitoring g FACTOR

5.12 [SHIFT] [MARK][5]: Setting Flowcell Path-length

The CD values described in the material is usually the one measuring with using the cell of 10 mm path length. By setting "25 mm" path length with using standard flowcell (25mm cell path length), the output value is corrected to that with 10-mm cell; that means the CD signal has been corrected to 1/2.5 and outputted as a CD output signal. In case that path length is set to "10 mm", CD signal with no correction is outputted.

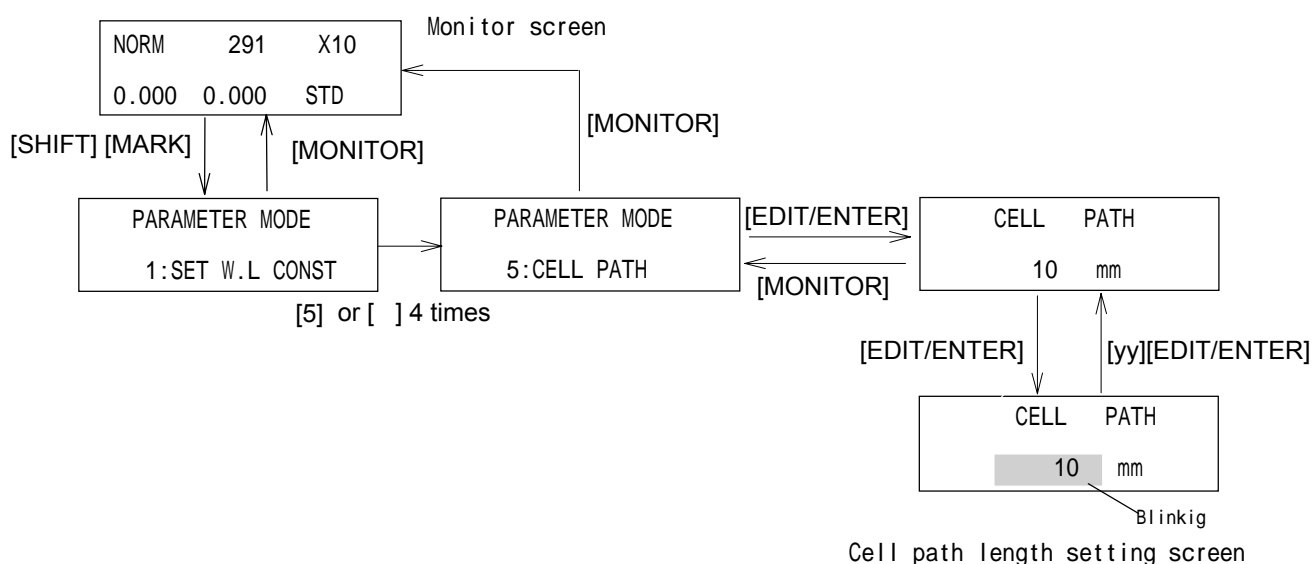


Figure 5.14 Setting FLOWcell Path-length

6. Operations in Program Mode

6.1 Description

In the program mode, the parameters shown below can be changed according to a time program. Time programs can contain up to 64 steps, and up to 10 program files can be stored in the memory of the instrument.

- wavelength
- Range
- Autozero
- Spectrum measurement

In this chapter, the method for setting and performing time programs using the above parameters will be explained.

A description of operations in program mode is provided in the flow chart below.

Set file number	See Section 6.2
Switch to program mode	See Section 6.3
Edit initial parameters	See Section 6.4.1
Edit time program	See Section 6.4.2
Execute	See Section 6.5

6.2 File Number Setting (File Loading)

Up to 10 files (0 ~ 9) can be used. Follow the procedure described below to set the file number.

(1) Changing file numbers: Input range: 0 ~ 9

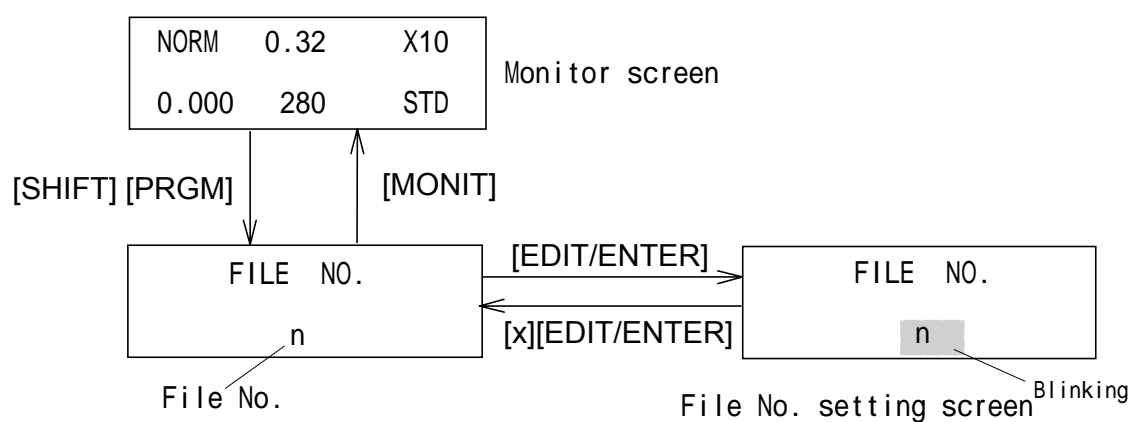


Figure 6.1 Changing the File Number

Note 1: If an incorrect value is entered, press [CLEAR] while the parameter value is blinking, then enter the correct value. Press [EDIT/ENTER] to enter the value.

Note 2: When the file number of a file that already contains a time program is designated, the previous program will be loaded. When a time program is edited after setting the file number, the new file settings are automatically stored. Time programs are stored in C-MOS RAM and therefore are not erased when the power is turned off.

Note 3: File numbers cannot be changed from the program mode monitor screen. If the program mode monitor screen is displayed, press the [PRGM] key to return to the normal operation mode monitor screen.

6.3 Switching Between Normal Operation Mode and Program Mode

Press the [PRGM] key while the normal operation mode screen is displayed. The program mode monitor screen will appear (Fig. 6.2). Press the key again to return to the previous screen.

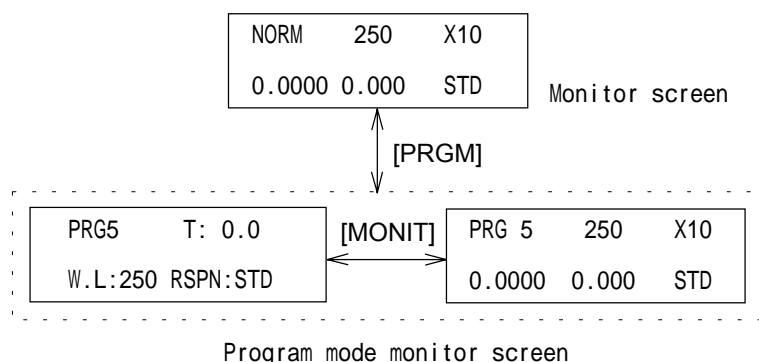


Figure 6.2 Switching Between Normal Operation Mode and Program Mode

The program mode monitor screen consists of a time and wavelength screen and a screen similar to the normal operation mode screen (called the CD · Absorbance display screen). Use the [MONITOR] key to alternate between screens. The various screen areas are described in Fig. 6.3.

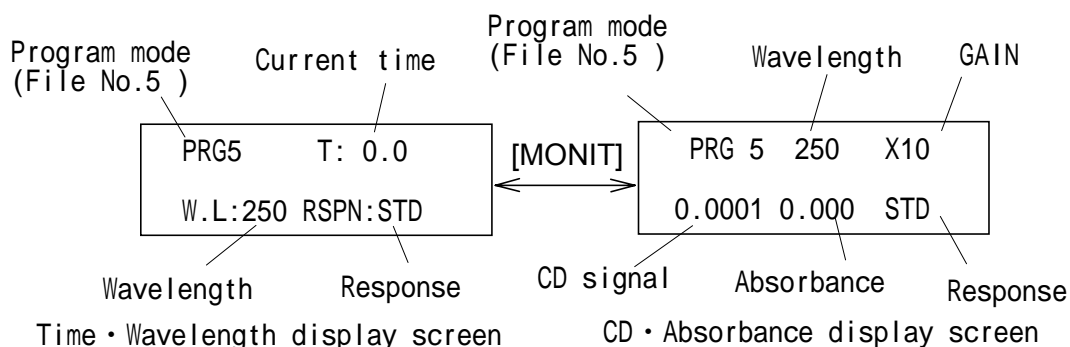


Figure 6.3 Description of the Program Mode Monitor Screens

6.4 Editing Programs

6.4.1 Editing initial parameters

Wavelength and Response are set sequentially as initial parameters.

Note: Gain set in the normal operation mode remains in effect while the instrument is switched to program mode.

Press [] key while the program monitor screen is displayed. The Step 0 screen (Fig. 6.4) will appear. Wavelengths and sensitivity can be changed from this screen using the procedure described in Fig. 6.4.

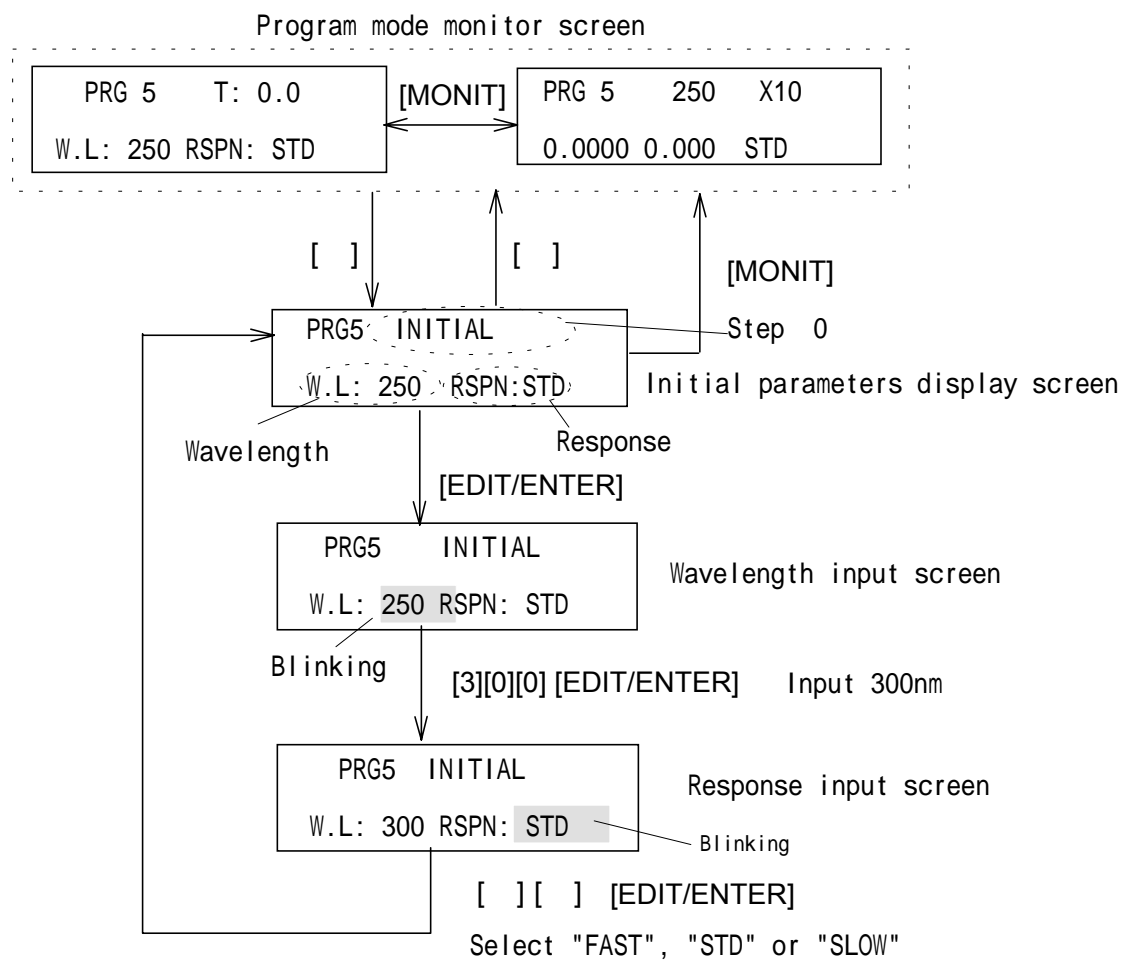


Figure 6.4 Editing the Initial Parameters

6.4.2 Editing the time program

6.4.2.1 Time program input

(1) Time program input example:

As an example, the following time program will be created. First, wavelength will be changed to 250 nm one minute after starting the program under the initial parameters. Next, spectrum measurement will be performed at two minutes after starting the program.

Press the [] key while the initial parameters screen is displayed (Fig. 6.4). The Step 1 screen will appear. Use the procedure described in Fig. 6.5 to complete the time program.

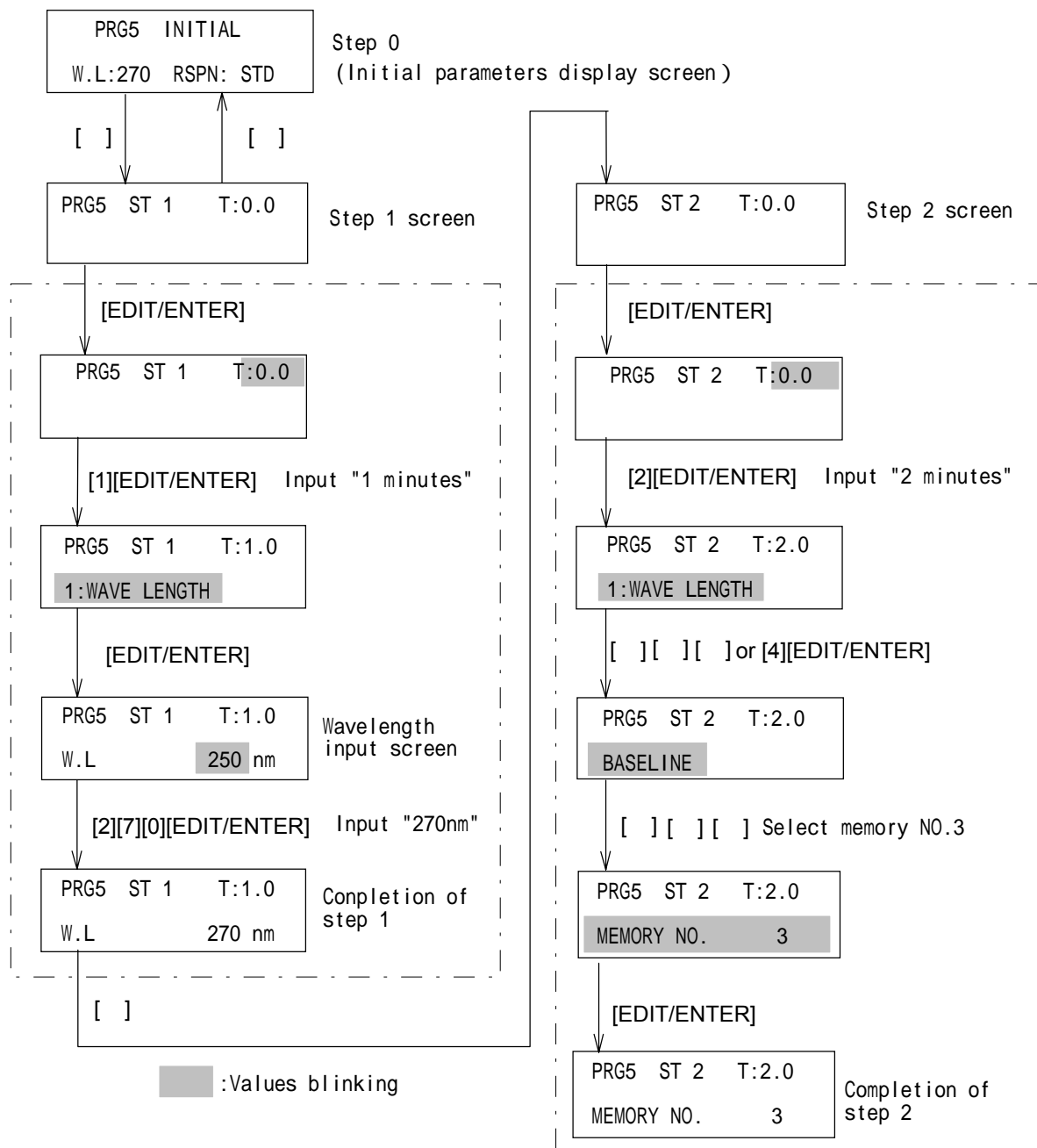


Figure 6.5 Instructions for Creating the Example Time Program

(2) Time program functions and their number

- | | |
|-------------------------|--------------|
| 1) Wavelength | 1:WAVELENGTH |
| 2) CD output range | 2:CD RANGE |
| 3) UV output range | 3:AU RANGE |
| 4) Spectrum measurement | 4:MEASURE |
| 5) Auto zero | 5:AUTO ZERO |

(3) Time program input method:

The input sequence is as follows: time, function, value relative to the function.

- 1) From the initial conditions screen (Step 0 screen), press [] key to advance to the Step 1 screen.
- 2) Press [EDIT/ENTER] (time blinking).
- 3) Enter the time in minutes (minimum increment: 0.1 min).
[xx][EDIT/ENTER]
The function will be blinking.
- 4) Select a function by using numeric key or []. Input the function by pressing [EDIT/ENTER]. The function will appear and the value will blink, awaiting input.
- 5) Input the desired value by using numeric key or [] and press [EDIT/ENTER] to enter the value.
- 6) Press [] to move to the next program step. Repeat this procedure until all steps are entered. After the final step completed, press [MONIT] to go to the program mode monitor screen. (see Fig. 6.4)

Note: Input steps need not be entered in the proper time sequence. When the [MONIT] is pressed in order to return to the monitor screen, steps that are out of time sequence will be rearranged into the proper sequence.

6.4.2.2 Correcting time programs

When times and functions are incorrect:

- 1) Use the [] key to advance to the incorrect step.
- 2) Enter the correct settings, starting with time.

When changing a setting without changing the time or function:

- 1) Use the [] key to advance to the incorrect step.
- 2) Skip correct settings by pressing [EDIT/ENTER] until the desired input screen appears.
- 3) Enter the correct setting. For example, when changing only the wavelength, perform the operations shown in Fig. 6.6. However, if settings are changed starting from time, the function selection screen will appear.

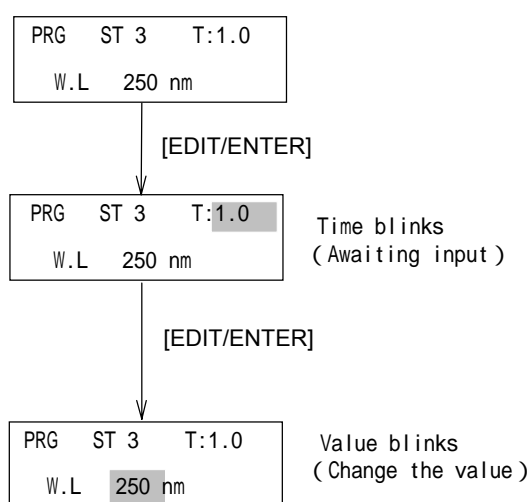


Figure 6.6 Correcting time program

6.4.2.3 Deleting individual steps or several steps from a time program

Deleting individual steps:

- 1) Press the [] key to advance to the step to be deleted.
- 2) Set the time in the step to 0 ([EDIT/ENTER][0][EDIT/ENTER]).
- 3) Press the [MONIT] key to return to the monitor screen. Steps will be rearranged in sequence and the selected step will be deleted.

Deleting all steps following an individual step:

- 1) Press the [] key to advance to the first step of the group of steps to be deleted.
- 2) Press [SHIFT][CLEAR]. The selected step and all subsequent steps will be deleted.

3) Press the [MONIT] key to return to the monitor screen.

6.4.2.4 Inserting steps into a time program

This section describes how to insert a single step into a time program contained in a file.

- 1) Press [SHIFT][] to advance to the final step in the program.
- 2) Press [] to advance to a blank step.
- 3) Enter values according to the instructions provided in Section 6.4.2.1.
- 4) Press the [MONIT] key to return to the monitor screen. Steps will be rearranged in time sequence.

6.4.3 Program example

Figure 6.7 shows an example of analysis performed while changing wavelengths, attenuation, and autozero. Detection conditions for the 5 peaks are shown in Table 6.2.

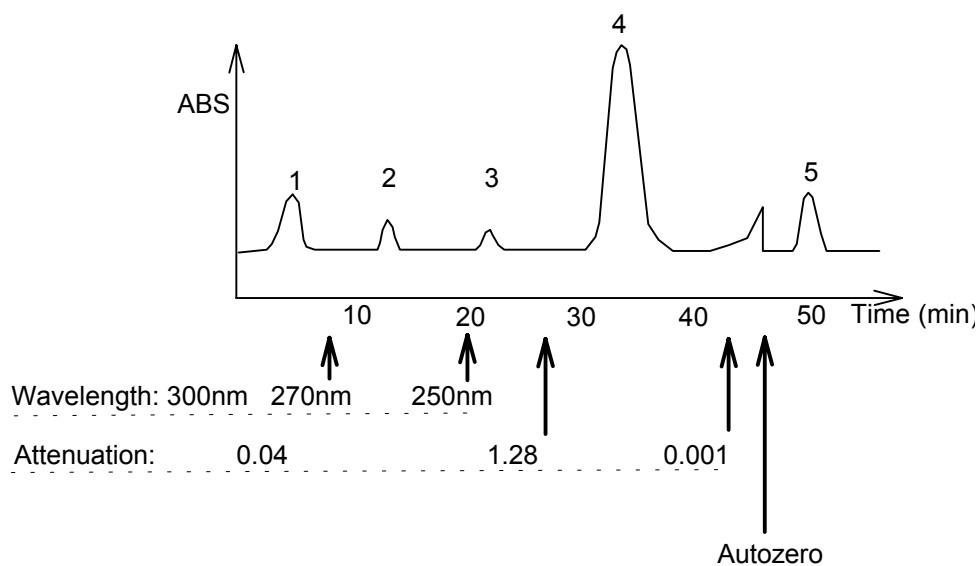


Figure 6.7 Chromatogram(UV absorbance)

Table 6.2 Peak Detection Conditions

Peak number/ Condition	TIME	wavelength (nm)	RANGE	Note
INITIAL		300	0.04	
1	8.0	270	0.04	
2	20.0	250	0.04	
3	27.0	250	1.28	
4	41.0	250	0.001	
5	44.0	250	0.001	*

(*) Immediately following autozeroing

The time program for the above example is shown in Fig. 6.7.

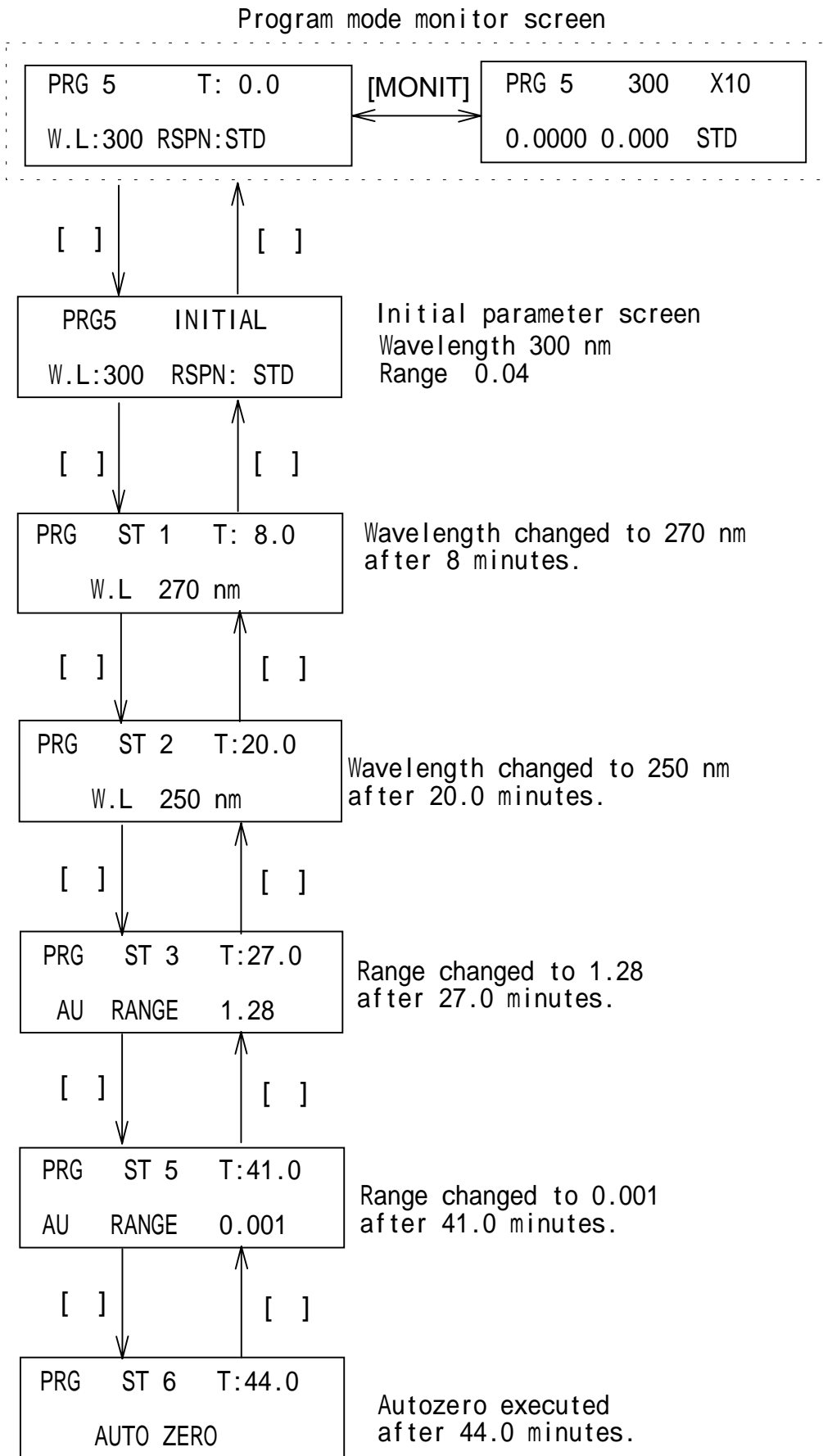


Figure 6.8 Example Time Program

6.5 Time Program Operation

6.5.1 Running a time program

Start the program from the program monitor screen.

(1) Starting the time program:

Press the [PRGM RUN] key after the baseline stabilizes. The time program will start. The [PRGM RUN] key lamp will light, showing that a time program is currently in progress.

(2) Stopping the time program:

Press the [PRGM RUN] key while a program is in progress. The [PRGM RUN] key lamp will be turned off and the time program will stop.

6.5.2 Changing parameters while a program is running

The settings for sensitivity, wavelength, response speed, and lamp off timer can be changed while a program is running. These changes can be made from the display screens designated by boxes drawn with double lines in Fig. 6.9. The pre-amplified output can also be displayed.

The settings changes are valid until the program progresses and the next group of settings are activated.

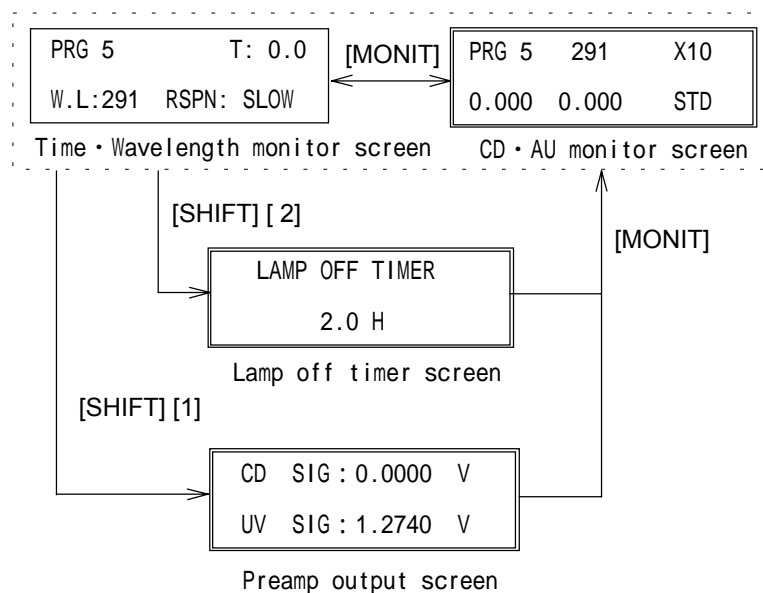


Figure 6.9 Changing Parameters While Program is Running

7. KEY OPERATIONS IN SPECTRUM MODE

This chapter outlines the operating procedures for the SPECTRUM mode.

7.1 Outline

In addition to simultaneous monitoring of CD and UV chromatograms, the CD-1595 chiral detector allows simultaneous CD and UV spectral measurements by stop-flow scanning. Please note that this function is primarily meant to find the optimum wavelength for the compound of interest to be monitored. In order for the detector to maximize the sensitivity, a Hg-Xe lamp and a monochromator with a relatively wide spectral bandwidth (20nm) are incorporated. Therefore, a spectrum obtained by a detector with this configuration has limited accuracy to evaluate it qualitatively. For such purpose, please use CD spectropolarimeter such as one from JASCO J-series products .

Spectral measurement is carried out by the single-beam method. This means that a measured spectrum includes background spectra contributed by spectrometer characteristics, mobile phase, etc. In order to obtain the true spectrum, such background spectra must be subtracted from the measured spectrum that is known as baseline correction. This is done by measuring the background first when no sample is being eluted, i.e., the baseline spectrum, then, the spectrum of the sample is measured. Finally, the detector will output the true spectrum.

7.1.1 Principles of Operation

The following operations are performed in the SPECTRUM mode:

- 1) The baseline spectra (CD and UV) are acquired then stored into memory #10.
- 2) Spectra of sample compounds are measured and stored into memories #0-9.
- 3) Baseline correction is executed by subtracting the baseline spectrum from the measured spectra. Spectra stored in the specified memory number are output as baseline-corrected ones to the recorder or integrator output.

Steps 1), 2) and 3 are described in detail in Sections 7.2, 7.3 and 7.4, respectively.

(NOTE)

The baseline spectra are automatically stored into memory #10. Measuring new baseline spectra delete the previously stored spectra.

7.1.2 Operation

SPECTRUM mode key operations are summarized below (refer to Fig. 7.1):

- 1) Change to SPECTRUM mode by pressing [SCAN].
- 2) Enter "0" and press [EDIT/ENTER] after step 1) to select the baseline measurement and baseline correction operations. Refer to Section 7.2.
- 3) Enter "1" and press [EDIT/ENTER] after step 1) to select spectrum measurement.

- 4) Enter "n" and press [EDIT/ENTER] after step 3) to set memory no "n". Refer to Section 7.3.
- 5) Press [MONIT] to return to NORMAL OPERATION or TIME PROGRAM mode.

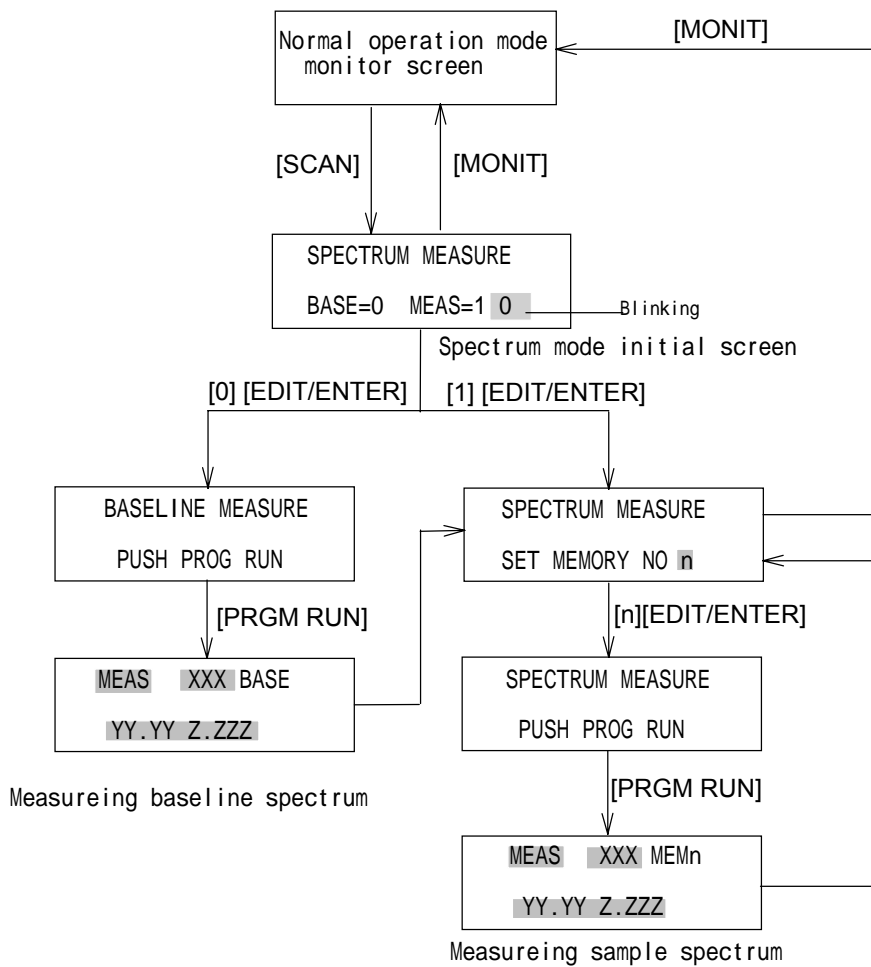


Figure 7.1 Key Operations and Corresponding Displays in SPECTRUM mode

- (NOTE 1)
Pressing [MONIT] while in any one of the SPECTRUM mode displays will restore either the NORMAL OPERATION or TIME PROGRAM MONITOR display.
- (NOTE 2)
Output of spectra obtained in a time program is carried out in SPECTRUM mode. In addition, the baseline spectrum is measured in the SPECTRUM mode even if spectrum measurement of a sample is carried out in a time program.
- (NOTE 3)
Stored spectra will NOT be output unless a baseline spectrum has been acquired and stored into memory #10.
- (NOTE 4)
When the power is turned off, the baseline spectrum (stored in memory #10) and all the measured spectra (stored in memories #0-9) will not be backed up.

7.2 Baseline Spectrum Acquisition

The measured spectrum will include the spectrum of the sample to be measured as well as the solvent and instrument backgrounds. In order to obtain a spectrum of the sample only, the backgrounds must be subtracted from the measured spectra. This subtraction process is called baseline correction.

The following outlines the steps for correcting the baseline.

- 1) Display the BASE CORRECTION display (refer to Fig. 7.1).
- 2) Press [PRGM RUN].
- 3) A wavelength scan is executed and in a few seconds, the initial SPECTRUM mode display reappears.

Note that the wavelength range is 220 – 420nm.

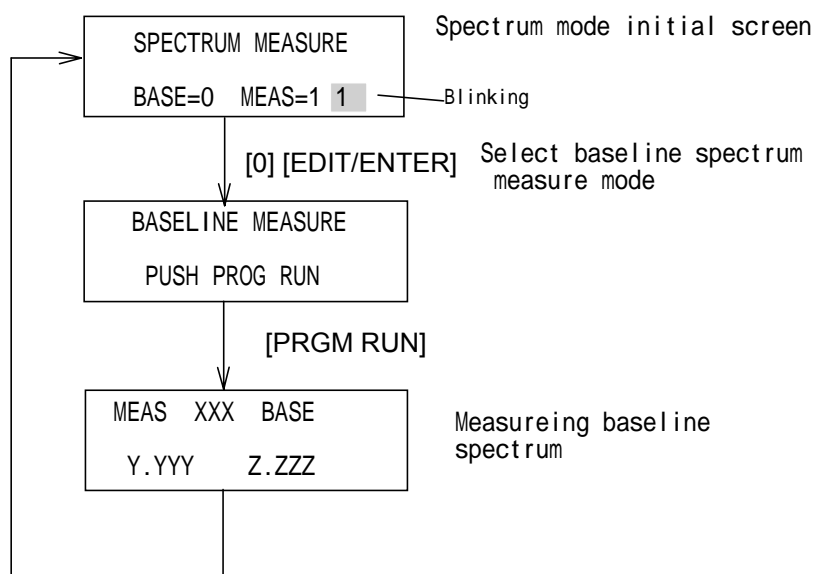


Figure 7.2 Procedure for executing baseline correction

7.3 Spectrum Measurement

This section outlines the procedure for measuring the spectrum of a sample component.

- 1) Display the SPECTRUM MEASURE display (refer to Fig. 7.1).
- 2) Enter "1" for spectrum measurement.
- 3) Assign a memory (0 - #9) into which the spectrum is to be stored.
- 4) Press [PRGM RUN].
- 5) A spectrum scan is performed and the initial SPECTRUM mode display reappears after a few seconds.

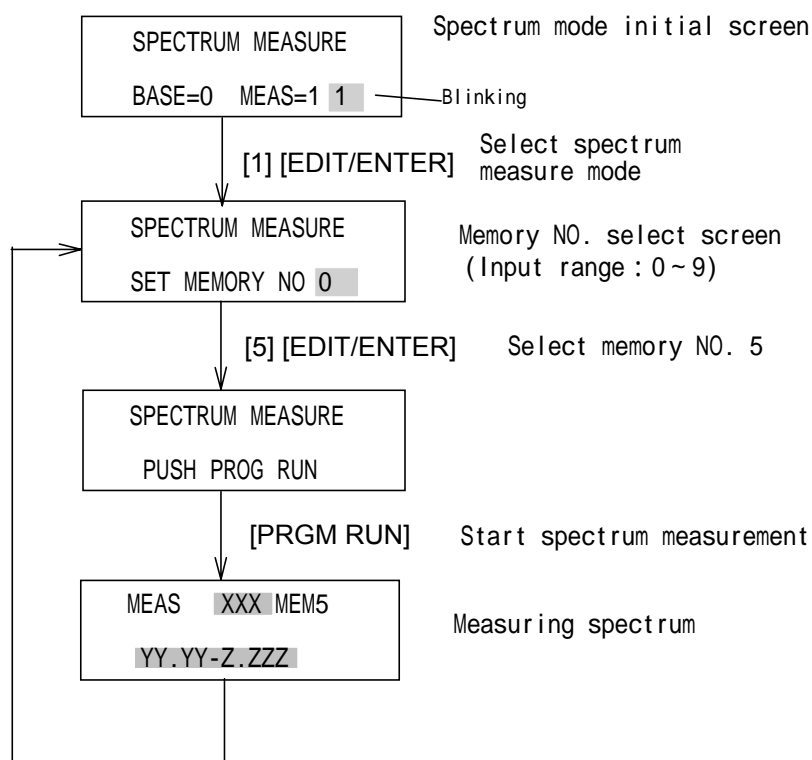


Figure 7.3 Procedure for measuring a spectrum

7.4 Spectra Output

This section outlines the procedure for outputting spectra (obtained in Sections 7.2 and 7.3) to the recorder and integrator output terminals. The recorder output level can be set as a spectrum output parameter and will include wavelength markers at 100 nm intervals. However, the integrator output does not include the wavelength marker. Note that the integrator level cannot be set as a spectrum output parameter, a separate procedure is required (refer to Section 5.7). A spectrum is output at 4 nm/sec. If the recorder chart speed is set to 100mm/min, the whole spectrum (220 –420 nm) will appear with 83mm length. These items are summarized in Table 7.2.

Table 7.2 Spectrum Output

	Integrator Output	Recorder Output
Output Level	Determined separately	Determined by this procedure
Speed	4 nm/sec	4 nm/sec
Wavelength Marker	none	every 100 nm

- 1) Push [SHIFT] [SCAN] to display the SPECTRUM OUTPUT screen. (refer to Fig. 7.4).
- 2) Push [], then "MEMORY NO. n " blinking. Select the memory location (#0-9) where the spectrum to be output is stored. In this example #5 will be set. Press [EDIT/ENTER] to set the memory location number.
- 3) Push [PRGM RUN]. A spectrum is output to the recorder or integrator. When data output is over, SPECTRUM OUTPUT screen reappears automatically.

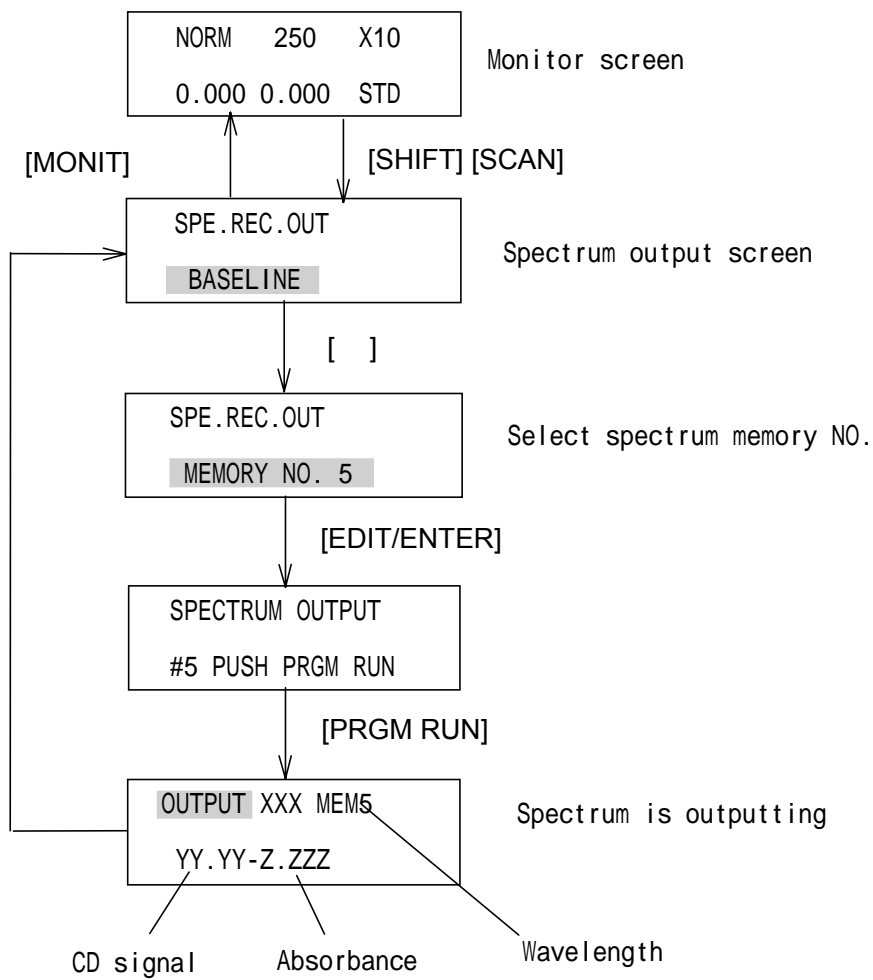


Figure7.4 Procedure for outputting a spectrum

(NOTE 1)

Baseline spectrum has been measured before outputting sample spectrum. If not so, the outputted sample spectrum will not be output.

(NOTE 2)

In case [SHIFT] [SCAN] are pressed while no sample spectrum has been measured, screen will not changed to spectrum output screen .

8. Principle

8.1 CD Measurement Principle

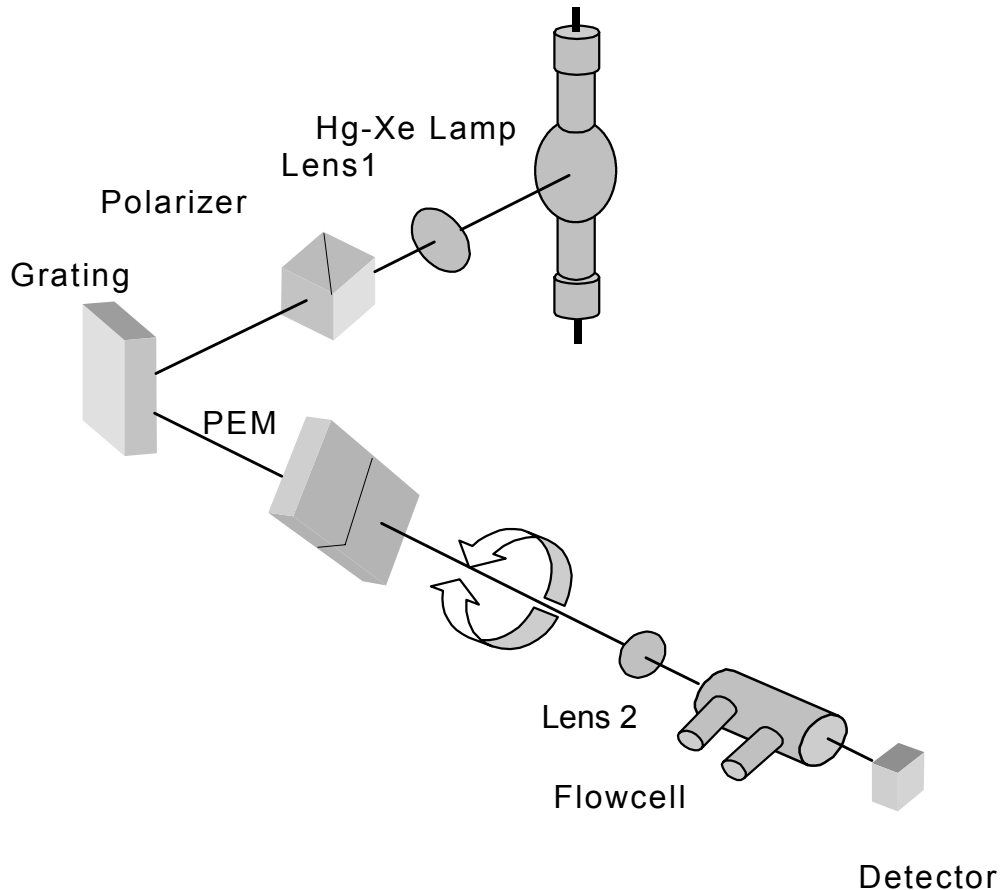


Fig.8.1 Optical diagram

The light emitted from the light source(Hg-Xe Lamp) is collimated by the Lens 1 and directed to the polarizer. The collimated light(unpolarized) is converted to linearly polarized light by the polarizer. The linearly polarized light is dispersed to each wavelength and directed to the PEM(photo-elastic modulator) by the grating. Then the light is converted to LCP(left circularly polarized light) and RCP(right circularly polarized light) alternately (with 50kHz) by the PEM and pass through the flow cell. The light which pass through the flow cell directed to the photodiode, the light signal energy is transformed to electric signal.

If there are no CD active components in the flow cell, the intensities of LCP and RCP electric signals are the same, resulting DC electric signal.

(See Fig.8.2)

If there are CD active components in the flow cell, LCP and RCP electric signals show different intensity, resulting AC electric signal.(See Fig.8.3)

The electric signal is converted to digital signal by the A/D converter, calculated to produce CD signal and UV signal by the CPU. These CD signal and UV signal are re-converted to analogue signal by the D/A converter and outputted as a output signal.

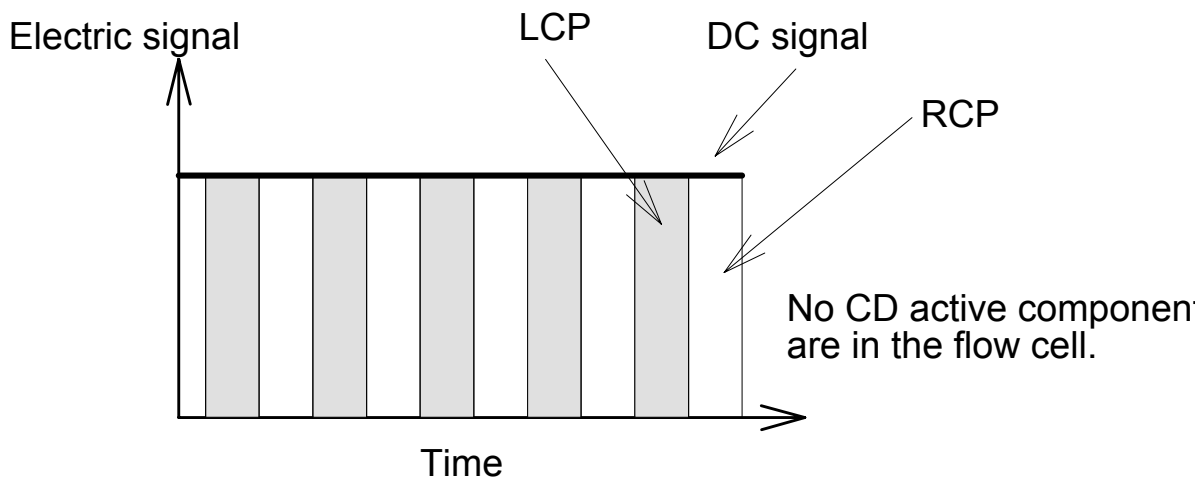


Fig.8.2 No active components in the flow cell

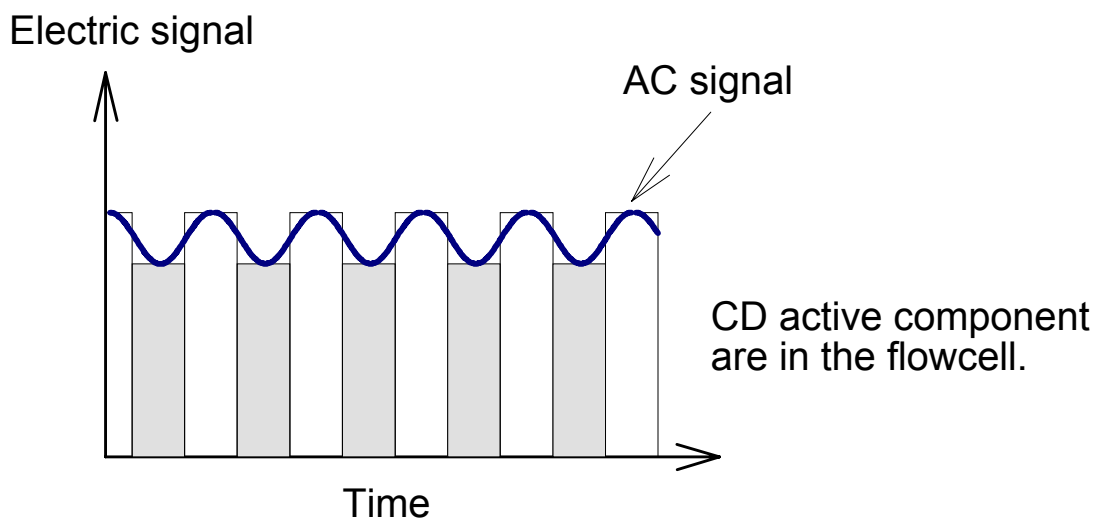


Fig.8.3 Active components in the flow cell



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