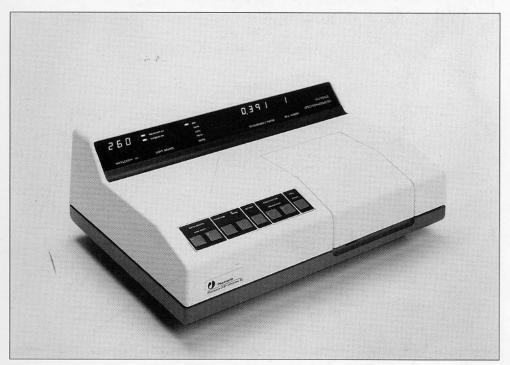
PHARMACIA LKB BIOTECHNOLOGY

VCSC BIOL Teaching Thimann 230

Ultrospec® III UV/Visible Spectrophotometer



Instruction Manual



1. INTRODUCTION

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1. INTRODUCTION

1.1 GENERAL DESCRIPTION

The Ultrospec III is designed as a simple to use instrument capable of producing rapid measurement of light absorption or transmission in both ultra-violet and visible spectra. Ease of operation is achieved by using a microprocessor to perform most of the repetitive tasks usually requiring prolonged operator action.

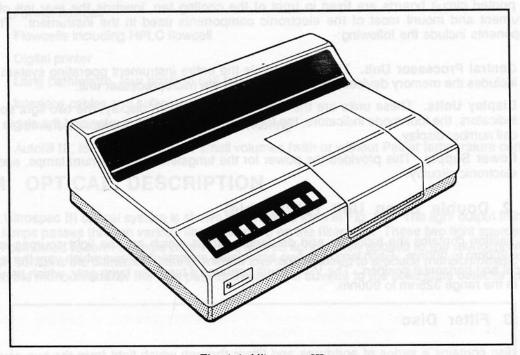


Fig. 1.1 Ultrospec III

Ultrospec III is capable of performing absorbance, concentration and transmittance measurements on up to five samples plus one reference at wavelengths from 200nm to 900nm (UV/Visible) or 325nm to 900nm (Visible). All operator inputs are made using eight keys which comprise the keypad. A display panel mounted at the top of the unit provides indication of instrument status and digital displays of the selected wavelength, cell number and measurement. Samples are loaded into cells which are placed in the six position cell holder under a cover at the right of the instrument. This cell holder is controlled by the microprocessor under operator guidance.

For those customers who wish to perform tablet dissolution monitoring a conversion kit is available that converts Ultrospec III for use as a tablet dissolution monitor.

1.2 COMPONENT DESCRIPTION

The instrument is built on a rigid chassis which mounts all the major components. This chassis is enclosed in a moulded cover which protects the major components from the ingress of dirt.

Functionally, the instrument comprises ten major components: five printed circuit boards, double lamp unit (UV/Visible only - single lamp fitted to Visible unit), filter/lamp selection disc, monochromator, sample compartment and detector.

1.2.1 Printed Circuit Boards

The printed circuit boards are fixed in front of the cooling fan, towards the rear left of the instrument and mount most of the electronic components used in the instrument. These components include the following:-

- a) Central Processor Unit. This unit controls the entire instrument operating system and includes the memory devices, interface units and the microprocessor unit.
- b) Display Units. These units are the three digit wavelength display, the two light source indicators, the four mode indicators, the four digit measurement display and the single digit cell number display.
- c) Power Supply. This provides the power for the tungsten and deuterium lamps, and the electronic circuitry.

1.2.2 Double Lamp Unit (UV/Visible)

This section contains the tungsten and deuterium lamps which are the light sources in the range 200nm to 900nm. Each lamp has two associated alignment screws which vary the lamps vertical and horizontal position. The Visible unit contains a tungsten lamp only, which provides light in the range 325nm to 900nm.

1.2.3 Filter Disc

This disc contains a series of apertures and filters through which light from the two sources passes. A motor rotates the disc so that light from a selected source can pass through a given aperture. The motor then stops and holds the disc in this position.

1.2.4 Monochromator

The monochromator receives the beam of light from the filter disc then, using a diffraction grating and slit, selects light of a small band of wavelengths for transmission to the sample. The monochromator used is of the Czerny-Turner configuration, with a 1200 lines/mm holographic diffraction grating, this being driven by a mechanism designed to ensure zero back-lash. Dark current compensation is facilitated by a solenoid operated shutter.

1.2.5 Sample Compartment

The sample compartment contains either the standard six position cell holder or any of the accessory cell holders (see section 4) into which the sample cuvettes are loaded. Monochromatic light enters and exits the sample compartment through focussing lenses at either side of the sample compartment. For TDS applications provision is made to accommodate the 8 position cell holder.

1.2.6 Detector

The detector unit is contained in the compartment to the right of the sample compartment. This detector is a solid state device which gives a high degree of accuracy and repeatability.

1.3 ACCESSORIES

A range of cells and accessories is available for the Ultrospec III as described below:-

Standard cells

Test tube holders

Long pathlength cells

Water heated cell holders

Micro and semi-micro cells

Funnel flowcell

Flowcells including HPLC flowcell

Digital printer

Long pathlength, four position cell holder

Interface cables and software packages for PCs

Conversion kit for tablet dissolution monitoring

Autofill III, for rapid handling of small volumes (with or without Peltier temperature control)

1.4 OPTICAL DESCRIPTION

The Ultrospec III optical system is shown in simplified form in Fig. 1.2. The light output from the two lamps passes through various filters mounted on the filter disc. These two light sources are then combined to form a single beam. The filtered light is then applied to the monochromator which contains the diffraction grating, mirrors and slits required to produce monochromatic light. From the monochromator the light passes through the cuvette to the solid state detector unit.

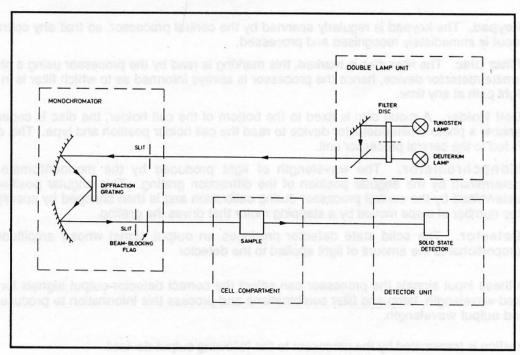


Fig. 1.2 Optical system

1.6 SPECIFICATION

The instrument specification is described below:-

Light sources Tungsten halogen (325nm to 900nm)

Deuterium (200nm to 325nm) - UV/Visible only

Monochromator Czerny Turner configuration with holographic

diffraction grating (1200 lines/mm)

Wavelength range 200nm to 900nm (UV/Visible)

325nm to 900nm (Visible)

Wavelength calibration Automatic upon switch on

Wavelength accuracy ±1nm Wavelength reproducibility ±0.5nm

Detector type Single solid state silicon photodiode

Photometric range -0.3 to 3.0A

0 to 9999 concentration with adjustable decimal

point 0.1 to 200%T

Peak check ±10nm about chosen wavelength

Absorbance ratio Automatic absorbance ratio calculation for two

values of entered wavelength

Absorbance difference calculation for

two values of entered wavelength

Simple kinetics and timed measurements Repetitive automatic measurement at specified

time intervals reporting absorbance or

concentration as absolute or change with time.

Wavelength scanning Automatic wavelength drive and analog signal

output to an accessory chart recorder simultaneously driven at 10 nm/sec.

Bandwidth 5nm

Stray light <0.05% at 220nm measured according to

ANSI/ASTM

<0.05% at 340nm E387-72

Stability ±0.002A/hr near 0A after warm up

Noise at 600nm ±0.001A near 0A

±0.002A near 2A

Photometric linearity ±1.0% or ±0.005A, to 3.0A whichever is the

greater

Photometric reproducibility Within 0.5 of absorbance value

Sample compartment Six position cell holder for 10mm cells with

automatic cell change as standard (UV/Visible

and Visible).

Data outputs Analogue: 100mV per 1A

Digital: RS232C & Pharmacia LKB Network

Manuals Instruction manual

Dimensions overall (w x d x h) $50 \times 36 \times 19$ cm:

Sample compartment: 15 x 14 x 8cm

Weight 15k

Power requirements 100/115/125/200/220/240V (±10%),

50/60HZ, 110VA

Specifications are measured after the Ultrospec III has warmed up and at constant ambient temperature, and are typical of a production instrument.

3. OPERATION

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3. OPERATION

3.1 INTRODUCTION

The three operator areas of the Ultrospec III are the keypad, display panel and cell holder.

3.1.1 Keypad

The keypad comprises eight individual keys, in five groups, which together offer the user the extensive flexibility of the 10 functions, (referred to as Fn0-Fn9) without detracting from the simple, routine operating procedures, Fig. 3.1 shows the keypad layout.

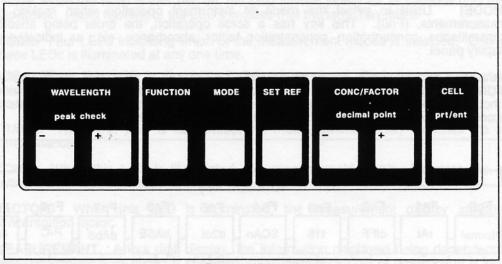


Fig. 3.1 Keypad

The keypad offers operator interaction with the instrument on several levels:

routine abs, %T, and concentration measurements (Fn0) enhanced measurement operations (Fn1-Fn4) enhanced instrument conditions, (Fn5-Fn9)

The individual keypad operations are outlined below:-

a) [-WAVELENGTH] [+WAVELENGTH]. These keys select the wavelength of light used for the measurement. Press and hold either [+WAVELENGTH] or [-WAVELENGTH] to increment or decrement the wavelength to the desired value as shown on the display. An automatic ramp function increases the rate of change of wavelength with the length of time the key is pressed until a predetermined maximum rate is reached.

[peak check]. Pressing both keys simultaneously initiates the peak check routine, (see section 3.3.4).

enhanced operations [-WAVELENGTH] [+WAVELENGTH]. When pressed in the context of enhanced measurement operation, (Fn1-Fn4) these keys retain their wavelength selection capability.

b) [FUNCTION]. This key is used to select the required instrument function, from Fn0 to Fn9. At switch on the instrument defaults to Fn0 which automatically prepares Ultrospec III for the simple routine measurement operations of absorbance, %T, concentration, or peak check. The use of [FUNCTION] to present the enhanced operations and conditions requires operator interaction with the keypad where the functions Fn0 - Fn9 are scrolled through as shown in Figure 3.2.

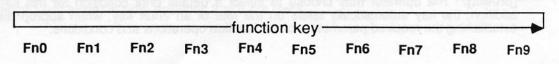


Fig 3.2 Function Selection

Selection of Fn1-Fn4 presents the enhanced measurement operations, as listed:

Fn1 absorbance ratio

Fn2 absorbance difference

Fn3 simple kinetics

Fn4 wavelength scanning

Selection of Fn5-Fn9 presents access to the enhanced instrument conditions, as listed:

Fn5 pre-set deuterium control

Fn6 utilities

Fn7 kinetics result presentation

Fn8 cell change and printer modes

FN9 baud rate settings

When the required function has been selected it is only on pressing [MODE] that the full extent of the choice within that function is revealed in readiness to be entered.

[MODE] Used to select the mode of instrument operation when making routine measurements, (Fn0). This key has a scroll operation, the order being absorbance, transmittance, concentration, concentration factor, absorbance, etc., as indicated on the display panel.

enhanced operations & conditions [MODE]. The key is also used in conjunction with [FUNCTION] to scroll through the various options available to the operator within the functions Fn1-Fn9. The wide scope of available operations and conditions are readily accessed by scrolling with [MODE] as illustrated by the following diagram, Fig. 3.3.

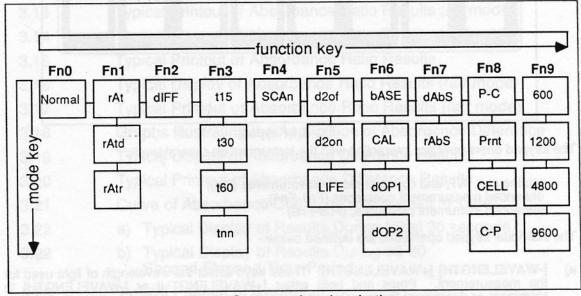


Fig 3.3 Structure of mode selections

- c) [SET REF]. When pressed, this key sets the absorbance readout to 0.000A, or transmittance readout to 100.0%T.
- d) [-CONC/FACTOR] [+CONC/FACTOR]. Used when preparing the Ultrospec III for a concentration measurement to vary the concentration readout to the known value. Pressing [+CONC/FACTOR] or [-CONC/FACTOR] individually increments or decrements the displayed value.

[decimal point]. Pressing both keys simultaneously, shifts the displayed decimal point sequentially, the series being 0000., 000.0, 00.00, 0.000, 0000., etc.

e) [CELL]. Controls the cell holder to select one of the four or six sample positions, eight positions for TDS applications. This key is pressed until the desired cell number is displayed. If the key is held pressed for more than one complete revolution of the cell holder, then the cell holder identification routine is initiated, (see section 3.1.3).

[prt/ent]. The operator may choose to select a default print condition for this key. In addition, the key automatically takes on the role of an enter key, when appropriate, for establishing the required parameters of the function operations and conditions.

3.1.2 Display Panel

The display panel comprises three digital display units and six indicator LEDs.

Fig. 3.4 shows the layout of the display panel, the display functions of which are as follows:-

- a) WAVELENGTH. A three digit display indicating the selected wavelength in nanometres. A flashing display indicates that the selected wavelength requires the deuterium lamp and that this has not been switched on. The display is also used to indicate the selected function (when [FUNCTION] is pressed), several function messages and instrument error messages as listed in section 5.
- b) LIGHT SOURCE. Two LEDs indicating which of the light sources is switched ON.
 - **TUNGSTEN ON.** When illuminated indicates that the tungsten lamp is switched ON. **DEUTERIUM ON.** When illuminated indicates that the deuterium lamp is switched ON. Initially, when this lamp is switched on, the LED flashes slowly for 15 seconds until the lamp has struck. Rapid flashing of the LED indicates that the lamp has failed.
- c) MODE. Four LEDs indicating which of the measurement modes is selected. Only one of these LEDs is illuminated at any one time.
 - ABS. When this LED is illuminated, the measurement display indicates the sample absorbance. When this LED flashes it indicates that either the absorbance ratio mode or the absorbance difference mode has been selected, (see sections 3.4.1 & 3.4.2).

TRANS. When this LED is illuminated, the measurement display indicates the sample transmittance.

CONC. When this LED is illuminated, the measurement display indicates the sample concentration in concentration units.

FACTOR. When this LED is illuminated, the measurement display indicates the concentration factor.

- d) **MEASUREMENT.** A four digit display, the information displayed being dependent on the selected function and mode. If this display flashes then an out of range value is indicated. Other error messages are also displayed on this display, (see section 5).
- e) CELL NUMBER. A single digit display indicating the cell number currently in the light path.

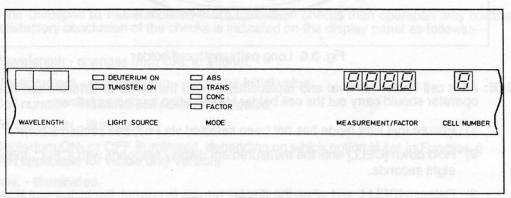


Fig. 3.4 Display panel

3.1.3 Cell Holder

The standard cell holder supplied with the UV/Visible and Visible instrument has six positions as shown in Fig. 3.5. This cell holder is located under the light proof cover at the right of the instrument. It can be removed for cleaning or replacement by unscrewing the thumb screw at the cell holder centre. The sample cells or cuvettes are a push fit in the holders, the spring clip in each location firmly clamping the cuvettes in position. When handling cells, only hold the cell using the ground faces. A cell containing solvent only is usually placed in position 1 to act as a reference, the samples for analysis being loaded into positions 2 to 6. To aid identification, position 1 cell holder is blue, positions 2 to 6 are black.

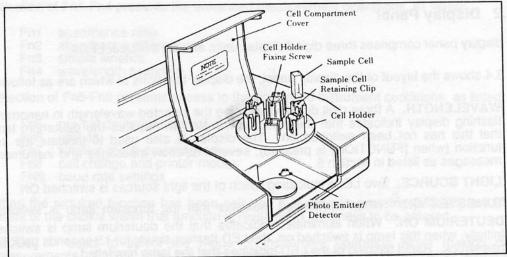


Fig. 3.5 Cell compartment layout with six cell holder

The optional four position, long pathlength cell holder is shown in Fig. 3.6. This cell holder also uses spring clips to retain the cells, these clips being positioned according to the pathlength of the cell in use.

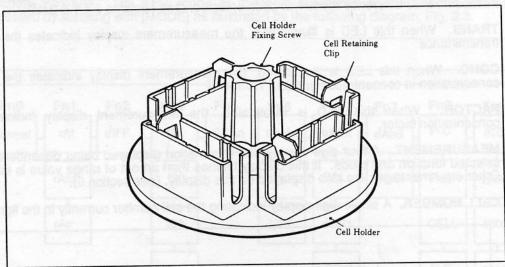


Fig. 3.6 Long pathlength cell holder

NOTE: After cell holder removal and replacement, (with the same or different cell holder) the operator should carry out the cell holder identification routine as follows:

- 1) Ensure that Prnt mode has not been selected via Fn8, see section 3.5.4).
- Hold down [CELL] until the measurement display responds with 'CELL', this will take eight seconds.
- 3) Release [CELL], and when the display returns to normal, the instrument is ready for use.

Following the installation of a single cell holder, when the operator presses [CELL] the display panel should respond with an incremental count of 0-9.

3.2 GETTING STARTED

3.2.1 Initial Switch-ON

Before switching on the Ultrospec III ensure that:-

- The cell holder is empty and in place.
- 2) The cell compartment cover is closed.
- The main supply voltage selector is set to the appropriate voltage.

To switch ON the Ultrospec III, set the main ON/OFF rocker switch located at the rear of the instrument to ON. When switched ON the Ultrospec III automatically performs a cell holder identification routine prior to the five step wavelength calibration sequence. This takes approximately two minutes, during which the cell compartment cover must remain closed. While these calibration checks are being performed the digital displays on the display panel are as follows:-

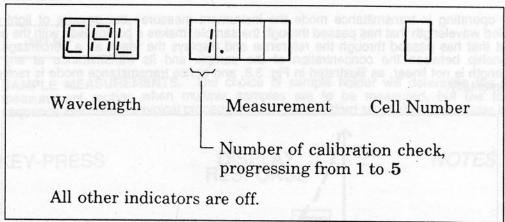


Fig. 3.7 Calibration display

If the calibration checks fail, the entire display begins to flash and the instrument will continue to attempt calibration.

When the Ultrospec III has completed these calibration checks then operation may commence. The satisfactory conclusion of the checks is indicated on the display panel as follows:-

- a) Wavelength changes from CAL to 360nm.
- b) Measurement changes from - - to the initial value.
- c) Cell number indicates cell number 1 selected.
- d) Tungsten ON illuminated.
- e) Deuterium ON- or OFF illuminated, depending on which option is set in Function 6 (not applicable for visible only version)
- f) Abs. illuminated.
- g) All other indicators are OFF.

INFORMATION, HELP, ERROR MESSAGES

- If the instrument fails to calibrate then:
 - a) check the sample compartment for clear beam.
 - b) check light emmission from source.
 - c) a fault may exist, see section 5 error codes.

The Ultrospec III is now ready for operation. Prior to any measurements certain preparatory operations should be carried out. These are referred to as initial checks in the following section, 3.2.2.

3.2.2 Initial Checks.

- 1) Load the reference cell into the cell holder position 1 (blue).
- If a measurement wavelength of less than 325nm is required, select the deuterium ON mode of Fn5. To achieve optimum stability, allow the lamp to warm up for 30 minutes prior to any measurements.
- If a serial printer is to be used the necessary instrument conditions (Fn8 & Fn9) should be selected now.

3.3 ROUTINE MEASUREMENT OPERATIONS (FN0)

At switch on Ultrospec III automatically defaults to Fn0, with routine measurement readily accessible to the user. In order to access the following routine measurement operations, (Fn0), when Ultrospec III has been used for alternative functions ensure that Fn0 is selected and entered prior to proceeding.

3.3.1 Transmittance Measurement

When operating in transmittance mode the instrument measures the amount of light of the specified wavelength that has passed through the sample, makes a comparison with the amount of light that has passed through the reference and displays the result as a percentage. The relationship between the concentration of the sample and its transmittance at any given wavelength is not linear, as illustrated in Fig. 3.8, and hence transmittance mode is rarely used experimentally.

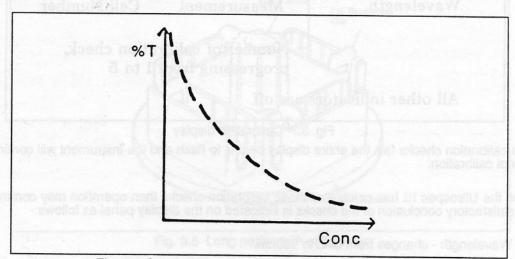


Fig. 3.8 Graph of % transmission against concentration

a) INITIAL CHECKS. Carry out the checks as identified in 3.2.2 for :-

reference cell,

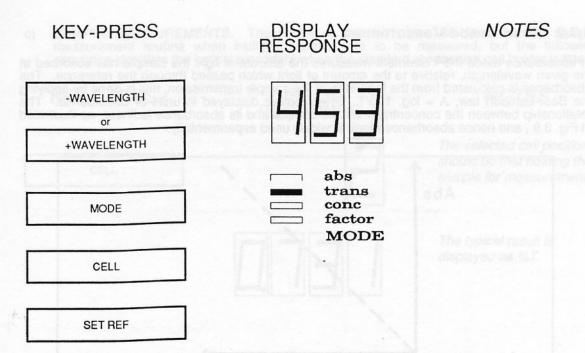
deuterium lamp,

use of printer.

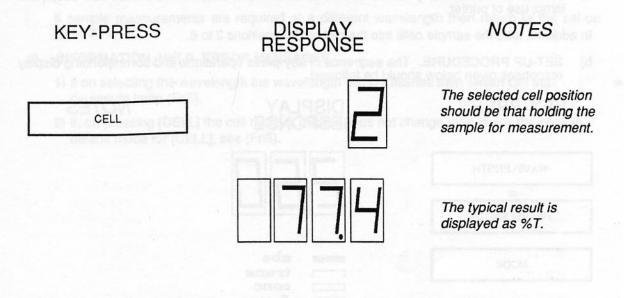
In addition:-

load the sample cells into the cell holder positions 2 to 6.

SET-UP PROCEDURE. The sequence of key-press operations and corresponding display responses shown opposite should be followed.



c) SAMPLE MEASUREMENTS. The choice of sample holder will determine the sample measurement routine when multiple samples are to be measured, but the following sequence identifies the typical procedure when the standard six position cell holder is fitted.



To measure the transmittance of further samples at the same wavelength then repeat above measurement steps.

If sample measurements are required at a different wavelength then return to the set-up procedure.

- d) INFORMATION, HELP, ERROR MESSAGES.
 - If on selecting the wavelength the wavelength display flashes then switch ON the deuterium lamp (Fn5).
 - 2) If, on pressing [CELL] the cell number display does not change then check the chosen default mode for [CELL], see (Fn8).

3.3.2 Absorbance Measurement

In absorbance mode the instrument measures the amount of light the sample has absorbed at the given wavelength, relative to the amount of light which passed through the reference. The absorbance is calculated from the logarithm of the sample transmission, this is done by applying the Beer-Lambert law, A = log. 100/T. The result is displayed in units of absorbance. The relationship between the concentration of the sample and its absorbance is linear, as illustrated in Fig. 3.9, and hence absorbance mode is widely used experimentally.

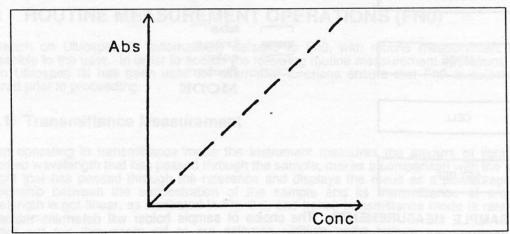
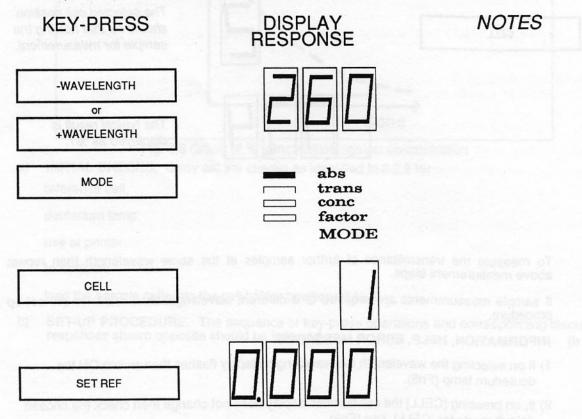


Fig 3.9 Graph of absorbance against concentration

 a) INITIAL CHECKS. Carry out the checks as identified in 3.2.2 for: reference cell; deuterium lamp; use of printer.

In addition, load the sample cells into the cell holder positions 2 to 6.

 SET-UP PROCEDURE. The sequence of key-press operations and corresponding display responses given below should be followed. 

c) SAMPLE MEASUREMENTS. The choice of sample holder will determine the sample measurement routine when multiple samples are to be measured, but the following sequence identifies the typical procedure when the standard six position cell holder is fitted.

| KEY-PRESS | DISPLAY RESPONSE | NOTES |
|--------------|---------------------|---|
| CELL | | The selected cell position should be that holding the sample for measurement. |
| | | The typical result is displayed as %T. |
| CELL SIGN OF | | |

To measure the absorbance of further samples at the same wavelength then repeat above measurement steps.

If sample measurements are required at a different wavelength then return to the set-up procedure.

- d) INFORMATION, HELP, ERROR MESSAGES.
 - 1) If on selecting the wavelength the wavelength display flashes then switch ON the deuterium lamp (Fn5).
 - 2) If, on pressing [CELL] the cell number display does not change then check the chosen default mode for [CELL], see (Fn8).

3.5 ENHANCED INSTRUMENT CONDITIONS (FN5-FN9)

Ultrospec III offers the operator access to a number of instrument conditions via function selections.

Having selected the instrument conditions to suit the operator's needs, the selections become default parameters which are stored in non-volatile memory and hence are automatically recalled the next time the instrument is switched on. In order to change the default setting the operator simply selects the relevant function and enters an alternative mode of choice.

3.5.1 Deuterium Lamp Control (Fn5).

The deuterium lamp is the source of the ultra-violet energy and hence is only found in the uvvisible instrument version, enabling the operating wavelength range to extend from 900nm to 200nm rather than from 900nm to only 325nm as in the visible instrument. The energy output of the lamp will deteriorate with age, hence it is advisable not to switch the lamp on if the sample measurements do not require wavelengths below 325nm, but if the instrument is frequently operated with the deuterium lamp on it is advisable to intermittently check the deuterium lamp usage. An aged lamp will eventually lead to a deterioration in the performance of the instrument, see section 8 maintenance.

Ultrospec III offers the operator access to these instrument conditions via the selection of function 5.

The function 5 modes may be selected as follows:-

MODE d2on switches on the deuterium lamp

MODE d2oF switches off the deuterium lamp

MODE LIFE displays the deuterium lamp usage

The operator should select the appropriate mode by following the necessary key-press routines as outlined in the following explanations:-

a) MODE d2on. When an operating wavelength of 325nm or less is required

| KEY-PRESS | DISPLAY RESPONSE | NOTES |
|-----------|-------------------------------|-------|
| FUNCTION | F _n 5 | |
| | | |
| MODE | | |
| | | |
| ENTER | housed by any broadly broadly | |

NOTE: In certain circumstances normal instrument operation may be suspended on pressing [ENTER] as instructed above, which will be indicated by a display of 60 second count-down. This situation will occur if the deuterium lamp was switched off less than 60 seconds prior to selecting the d2on mode. The operator may either:

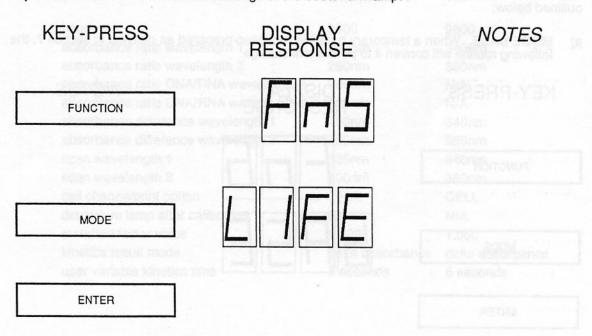
a) wait for the count-down to elapse when the lamp will be switched on and control returned to the operator,

01

- b) halt the process by pressing [FUNCTION], whereupon the control of the instrument will be returned to the operator but the deuterium lamp will remain switched off.
- b) MODE d2oF. When the operating wavelengths are in the range 325nm-900nm.

| KEY-PRESS | DISPLAY RESPONSE | NOTES |
|-----------|---------------------|-------|
| FUNCTION | Fn5 | |
| MODE | d2oF | |
| ENTER | | |

c) MODE LIFE. To establish the usage of the deuterium lamp.



NOTE: The instrument displays the number of hours for which the deuterium lamp has been switched on, for about 5 seconds, in the measurement display. This feature is purely for customer information and should not be regarded as evidence in the case of a warranty claim on a deuterium lamp which fails inside 750 hours. The instrument contains a hardware indicator internally which is used in the event of any dispute.

d) INFORMATION, HELP, ERROR MESSAGES.

- 1) The operator is prevented from switching the deuterium lamp off and on again within any 60 second period as difficulty may be experienced; as the lamp ages so it becomes increasingly difficult to strike when it is hot, the 60 second interval suffices as a cooldown period in most circumstances. However, as the lamp becomes significantly aged this 60 second period may not allow the lamp to cool-down enough to strike in which case the operator should assume the responsibility of waiting longer before switching the deuterium lamp back on, and should also check the lamp usage via function 5, mode LIFE.
- 2) When the wavelength display flashes on the selection of a wavelength of 325nm or less, this is an indication that the deuterium lamp is not switched on as required.
- 3) At instrument switch on the deuterium lamp may or may not be automatically switched on, this can be selected by the operator via the utilities options of function 6, with dOP1 or dOP2, see section 3.5.2 utilities.

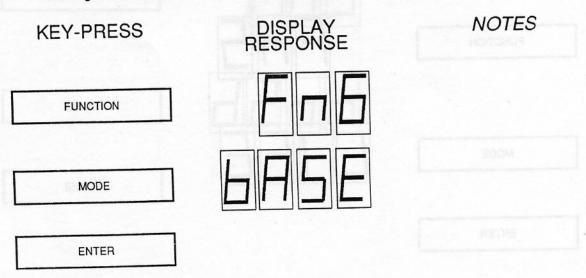
3.5.2 Utilities (Fn6).

Various instrument conditions for which constant access is not required are referred to as utilities. These are selected as the various modes of function 6 as follows:-

| MODE BASE | converts a temporary baseline to permanent storage. | |
|-----------|---|--|
| MODE CAL | recalibrate and reset Ultrospec III default parameters. | |
| MODE dOP1 | deuterium lamp automatically on at instrument switch on. | |
| MODE dOP2 | deuterium lamp automatically off at instrument switch on. | |

The user should select the appropriate mode by following the necessary key-press routines as outlined below:

a) MODE bASE. When a temporary baseline has been prepared as detailed in section 7, the following routine will convert it to permanent storage.



NOTE: Ultrospec III has now stored the temporary baseline as a permanent baseline