PLEASE READ THIS MANUAL CAREFULLY BEFORE ATTEMPTING TO USE ULTROSPEC K

If you have any comments with regard to this manual, we will be pleased to receive them at:

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Ultrospec K

1. INTRODUCTION

1.1 GENERAL DESCRIPTION

The LKB 4053 Ultrospec K is an advanced, easy to use ultra-violet/visible spectrophotometer designed to carry out a comprehensive range of spectrophotometry measurements including enzyme kinetics for which a powerful and versatile program has been specially written. The instrument can perform absorbance, transmittance, concentration, rate kinetics and standard curve determinations at wavelengths from 200 to 900 nm.

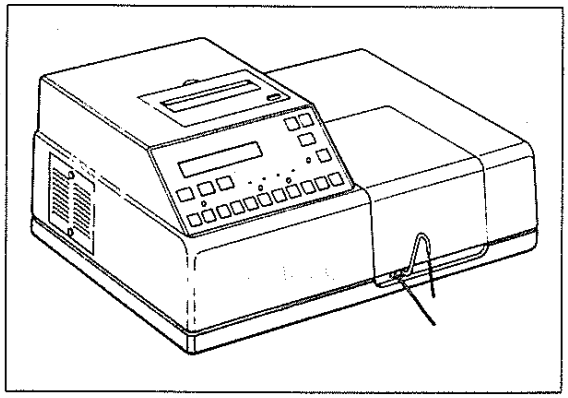


Fig.1.1 General view of Ultrospec K

Ease of operation is assured by the built-in microprocessor which performs most of the repetitive tasks traditionally carried out by the user, and the logically designed keyboard incorporating 18 tactile membrane key pads used for parameter setting.

Prompts, operator entries, instrument status and results are presented on a 20 character alphanumeric display and hard copy of selected parameters, graphical and tabular information is provided by the built-in 40 column alphanumeric printer.

A variety of different cell types, including a Peltier temperature controlled (20-50°C) flowcell, can be rapidly located in the sample chamber simply by changing cell holders. Autofill control for the rapid handling of small sample volumes is also an integral part of the instrument.
Communication for external data handling is possible via a bidirectional RS232C serial interface. Terminals with a 100 mV/1 A analogue output voltage are provided for chart recorder connection. An LKB Net connection is also provided for LKB network accessories.

Ultrospec K can be operated in three modes: the Ultrospec Mode where the instrument is used as a conventional spectrophotometer; the Kinetics Mode where the instrument is used to carry out well defined kinetics assays; and the Standard Curve Mode where the instrument produces a 'calibration curve' from a set of standards which is used to calculate sample values.

The optical system is shown schematically in Fig.1.2. The light output from the tungsten and deuterium lamps passes through the filter disc. This disc contains a series of apertures and filters, and 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. After passing through the filter, the two light sources are combined to form a single light beam which then enters the monochromator. This incorporates a diffraction grating and slits which are used to select a narrow band of wavelengths for transmission to the test sample in the cell compartment. The monochromator used is of a Czerny-Turner configuration with 1200 lines/mm holographic diffraction grating; the grating drive mechanism compensates for back-lash. Dark current compensation is facilitated by a solenoid-operated shutter also in the monochromator. From the monochromator the light passes through the cuvette to the solid state detector unit.

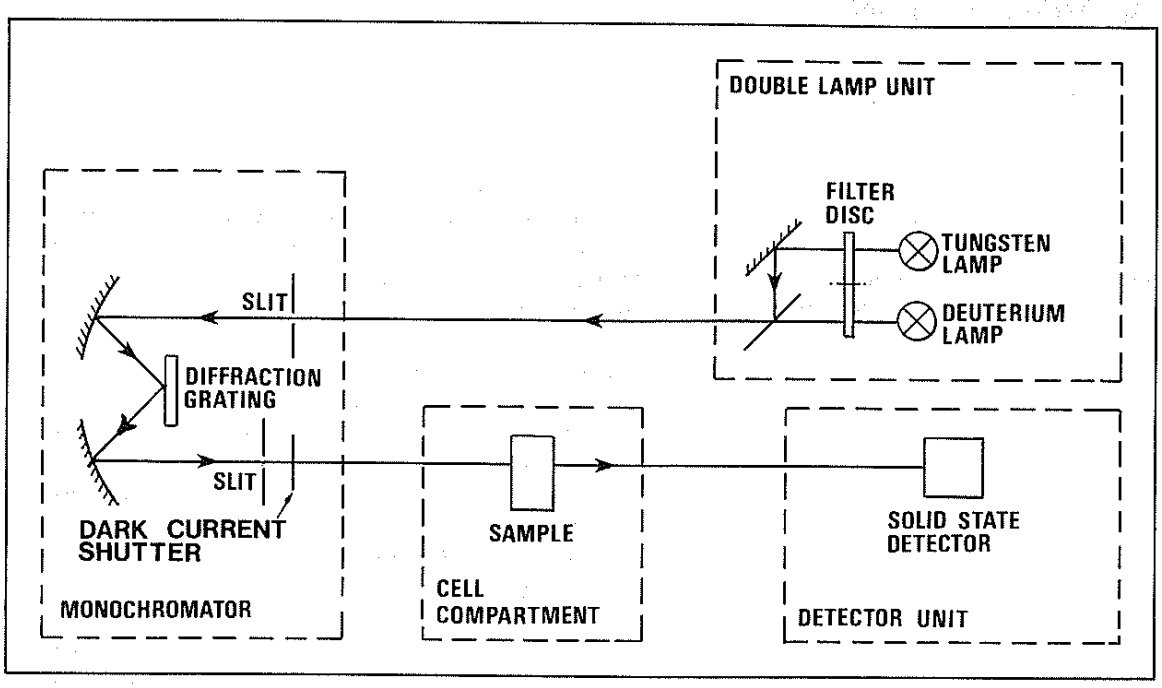


Fig.1.2 The optical system

1.2 ACCESSORIES

Numerous optional accessories are specially designed for use with Ultrospec K. A list of these are given below and full details of each accessory, including installation and operating instructions, can be found in part III of this instruction manual.

Four-position cell holder; also water heated version
Six-position cell holder; also water-heated version
Cell holder for a rectangular cell or flowcell of up to 10 mm pathlength
Cell holder for a rectangular cell or flowcell of up to 50 mm pathlength
Cell holder for a 100 mm pathlength cylindrical cell
Water heated cell holder for a single cell of up to 40 mm pathlength
Test tube holder
Finger Screw
External dot matrix printer
Replacement flowcell for Autofill K
Baseplate

In addition, a full range of cells for Ultrospec spectrophotometers is supplied by LKB. Ask your local LKB representative for further details.

1.3 SPECIFICATIONS

Monochromator	Czerny-Turner configuration with 1200 lines/mm holographic grating
Wavelength range	200-900 nm
Wavelength calibration	Automatic upon switch on
Wavelength accuracy	+1 nm
Wavelength reproducibility	$\mp$0.5 nm
Simple Scanning Ranges:	Absorbance with offset facility and %T

Scan speed:	2 nm/sec nominal: tabular peak identification with optional peak plot by means of integral printer.
Peak check	±10 nm about chosen wavelength
Light sources	Deuterium arc lamp Tungsten halogen lamp
Detector	Single solid-state silicon photocell
Bandwidth	5 nm
Stray light	<0.05%T at 220 nm <0.05%T at 340 nm Measured according to ANSI/ASTM E387-72
Stability	±0.002A/h at 0A after warm up
Noise	±0.001A near 0A at 600 nm ±0.002A near 2A at 600 nm
Photometric range	-0.301 to 3.0A 0.001 to 9999. in concentration 0.0 to 200%T
Photometric reproducibility	Within 0.5% of absorbance value
Photometric linearity	±1.0% or ±0.005A, to 3.0A, whichever is the greater
Sample compartment	Single cell Peltier-thermostatted cell holder complete with 80 uL flowcell, peristaltic pump and tubing
Data outputs	Analogue: 100 mV per 1A Digital: RS 232C & LKB Network
Manual	Operation and maintenance manual
Dimensions	Overall: 50x36x24 cm; weight 20Kg Sample compartment: 15x15x11 cm
Power requirements	100/115/125/200/220/240 V (±10%), 50/60 Hz, 150 VA

AUTOFILL K

Principle	peristaltic pump
Modes	sample recovery, sample to waste and wash
Volume range	0.25 to 9.99 mL
Volume setting	time or volume modes with calibration facility
Flowcell	10 mm pathlength: 80 uL volume UV grade silica
Temperature control	Peltier heating/cooling element
Temperature range	20 - 50°C in 1°C steps
Temperature accuracy	±0.1°C at 37°C
Temperature stability	±0.1°C at constant ambient temp.
Cross contamination	typically 0.5% for 1 mL of aqueous solution
Pump materials	element (pumphead) tubing 1.6 mm ID and 1.6 mm wall thickness Marprene (product of Watson - Marlow): transport tubing 1.0 mm ID and 0.5 mm wall thickness PTFE

We reserve the right to alter specifications without notice in accordance with our policy of continuing product development and improvement.

2. INSTALLATION

Before installing Ultrospec K as described in section 2.1 below, inspect the instrument for any signs of damage caused in transit. If any damage is apparent then inform the instrument supplier immediately and do not proceed with instrument installation.

2.1 INSTALLATION PROCEDURE

Proceed as follows to install the LKB Ultrospec K spectrophotometer:

a) Check that the instrument supplied is of the correct voltage rating to suit the mains power you intend to use and ensure that the correct mains plug has been wired to the mains input lead.

b) Check that the fuses supplied are of the correct rating. Two identical fuses need to be loaded into the back of Ultrospec K (see Fig.2.1 - the rear panel), this being done as follows:

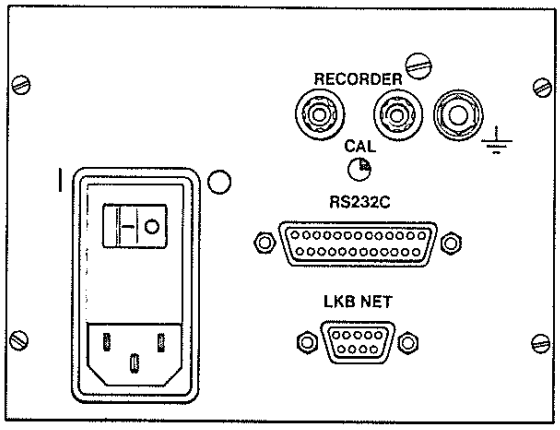


Fig.2.1 Rear panel layout

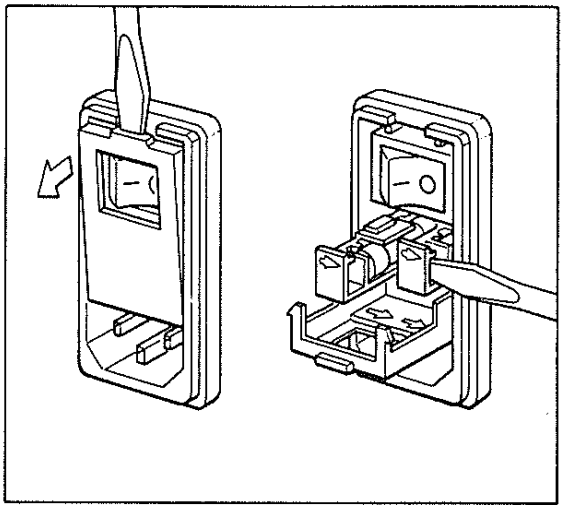


Fig.2.2 Loading the fuses

i) ensure that the instrument is switched OFF
ii) remove the mains input lead if it has already been connected
iii) insert the tip of a small screw driver behind the top edge of the black plug/switch panel (see Fig.2.2) and lever the panel (which is hinged at the bottom) outwards. This will reveal two black drawers, on the front of each of which is a white arrow pointing to the right. The correct direction of these arrows is also indicated by identical arrows present on the back of the panel which act as a guide when replacing the drawers
iv) pull out each drawer by inserting the tip of a small screw driver behind the right edge (point of arrow) of the front panel of each drawer and pulling forward
v) clip each fuse into the sprung-loaded drawers and slide the drawers back in position making sure to line up the white arrows in the same direction as those indicated on the back of the plug/switch panel
vi) lift and clip the plug/switch panel back into position

c) Connect the mains input lead to the input socket and the mains supply.

d) Switch on the instrument.

e) Check all LEDs, indicators and lamps. (See section 3. Instrument Operation).

3. INSTRUMENT OPERATION

3.1 KEYBOARD

The keyboard comprises 18 spillproof tactile membrane keys which can emit a soft click when they have been pressed correctly to carry out the appropriate operation. See Fig. 3.1.

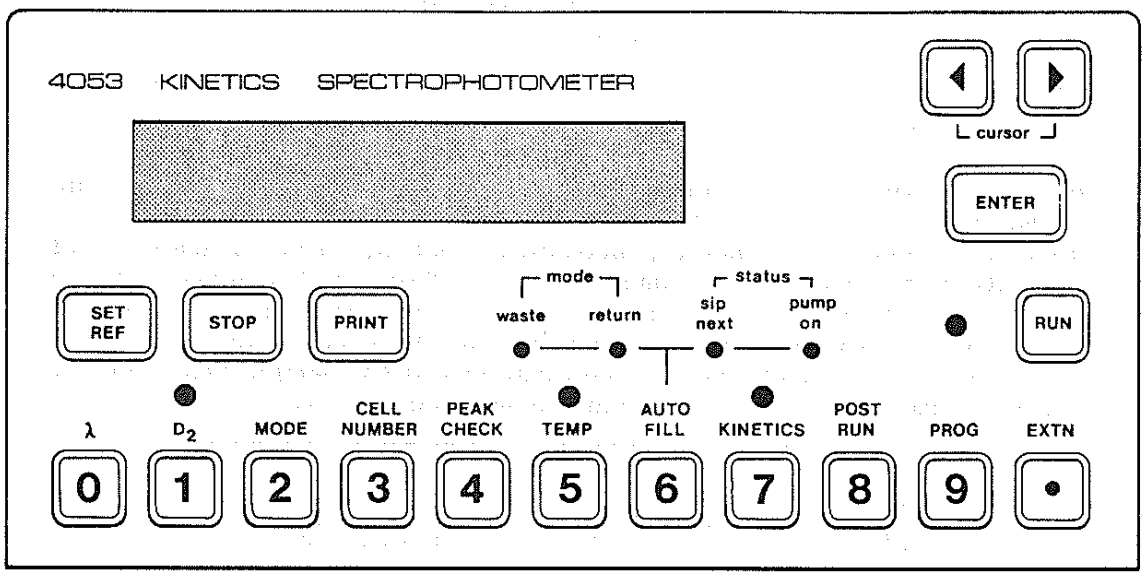


Fig.3.1 Instrument keyboard

The bottom row of 11 keys are dual function/numeric keys:

λ/0, D_2/1, mode/2, cell number/3, peak check/4, temperature/5, Autofill/6, kinetics/7, post run/8, program/9, extension/.

Pressing one of these will either select the function or number (0 to 9) indicated on the relevant key. The microprocessor recognises the current status of each key and is thus able to control operation of the instrument with minimal parameter setting from the user.

The other 7 keys are used as their identity indicates:

Set Ref: Measures the reference or sample blank and sets the display to 0.000A or 100% T.

Stop: Interrupts the procedure being carried out and returns the instrument to the 'Ultrospec Mode'.

Print: Prints out instrument status when in 'Ultrospec mode'.

Run: Prepares the instrument to begin a measurement run according to the selected program when the instrument is in the Kinetics or Standard Curve Mode.

Enter: Inputs the selected information shown on the display. This key is pressed after the user has selected the desired units, values or display option for the particular function in question. This key will emit a beep if it does not accept the input.

Cursors: The right and left arrow cursor keys are used to select either functional, units or numerical options presented on the display when parameter setting, or increments or decrements the wavelength by 1 nm when in the Ultrospec Mode. (See note overleaf).

Note: When a cursor key is repeatedly pressed, on reaching the end of the display line (which will be to the right or left of the line depending on the cursor used) it will wrap around to the opposite end of the line and continue jumping from option to option in the selected direction until the repeated pressing is stopped.

A detailed description of how each key can be used to select the required options is given in sections 3.6 onwards.

3.2 DISPLAY PANEL

Ultrospec K incorporates a green, vacuum fluorescent display which can present a maximum of 20 alphanumeric characters. It is multi-functional in that it displays a variety of menus, prompts, results, error messages and other information regarding instrument status. Two display examples are shown below. Fig.3.2 shows the display when in the Ultrospec measurement mode. Three sets of information are presented in this mode, these being the selected wavelength in nanometres, sample number and the sample measurement value in the selected units (in this case absorbance units).

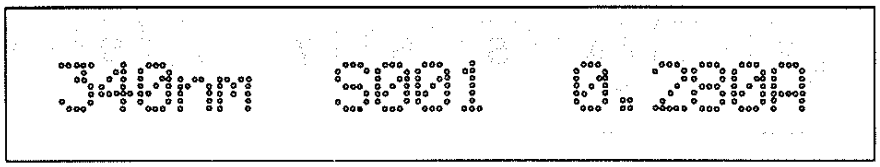


Fig.3.2 Display of Ultrospec measurement mode

Fig.3.3 shows the display after the MODE/2 key has been pressed: the current mode operational will be flashing. A menu is displayed from which the user can select the required measurement mode by means of the cursor keys. The 'ENTER' key is then pressed to enter choice and to continue.

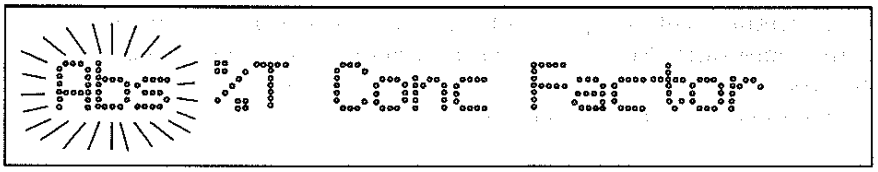


Fig.3.3 Display of measurement mode menu

A more detailed account of the display functions and their use, including parameter setting, will be found in sections 3.6 onwards.

Note i) The display examples used throughout this instruction manual are typical displays and as such the user should refer to them for guidance only and not expect the instrument to display exactly what is illustrated herein.

ii) When the user is prompted to select an option or change numerical information then the relevant option or number will flash on the display. In the case of numeric changes, once the digit has been dealt with, the next number to the right will then flash unless the end of the numerical sequence has been reached. In the case of options, if the flashing option is required then 'ENTER' must be pressed; if not then a cursor key must be used to move to the required option which will again flash before 'ENTER' is pressed.

iii) If at any time an error message is continuously presented on the display, press the 'STOP' key to escape. See section 4, Error Messages, for further details.

3.3 CELL COMPARTMENT

The cell compartment is designed to accommodate both the automatic micro-volume aspirator, Autofill K, and the numerous optional cell holders. Autofill K is supplied as standard with Ultrospec K and details of its installation and operation can be found in part II of this instruction manual. Details of the installation and operation of the various types of cell and test tube holders can be found in part III of this manual which deals with all optional accessories.

Note i) Whenever a cell holder needs to be removed from the cell compartment and replaced with either the same or different cell holder, the user must carry out the cell holder replacement routine as described in section 3.17.3 (Extn + Cell Number keys). After this procedure has been carried out, the mode of operation of Ultrospec K and the cell holder located in the cell compartment still need to be compatible with each other before sample measurement can begin. This is checked by the instrument software and if they are not then the error message 'Incompatible turret' is displayed which indicates that either the program or cell holder needs to be changed to achieve compatibility.
The cell holder replacement routine also allows the user to check which cell holder is currently in use without having to open the lid of the cell compartment.

ii) The instrument may be unable to carry out automatic calibration or cell holder identification if a cell holder is not screwed down correctly or, in the case of Autofill K, if it is not also plugged in. Correct and careful cell holder installation is therefore required.

3.4 PRINTER

Ultrospec K incorporates a thermal dot matrix printer which is capable of printing up to 40 alphanumeric characters per line. The printer is used to produce both text and graphical information. Examples of typical print-outs are given in the relevant sections of this instruction manual.

Note If the printer fails to print after Ultrospec K has been installed then first press the Extn + Mode keys and enter 'N' in response to the 'External printer Y/N' prompt. See section 3.17.2 for further details. (An external printer may have been used during instrument testing).

The printer paper is advanced forward by pressing the PF (Paper Feed) button located in the front right corner of the cover plate. One quick press advances the paper a few millimetres; the longer you press the more the paper advances.

LKB supplies the correct printer paper (part number 4073-237 for a pack of 5 rolls). A red mark printed on the paper appears when only 30 cm of paper remains and a new roll should be loaded when this mark is visible.

Printer paper is easily loaded into the printer as follows (see Fig.3.4):

i) remove the printer cover plates by first inserting a finger or suitable implement in the depression located at the back edge of one of the plates and lifting it upwards, removing it and then removing the second plate.

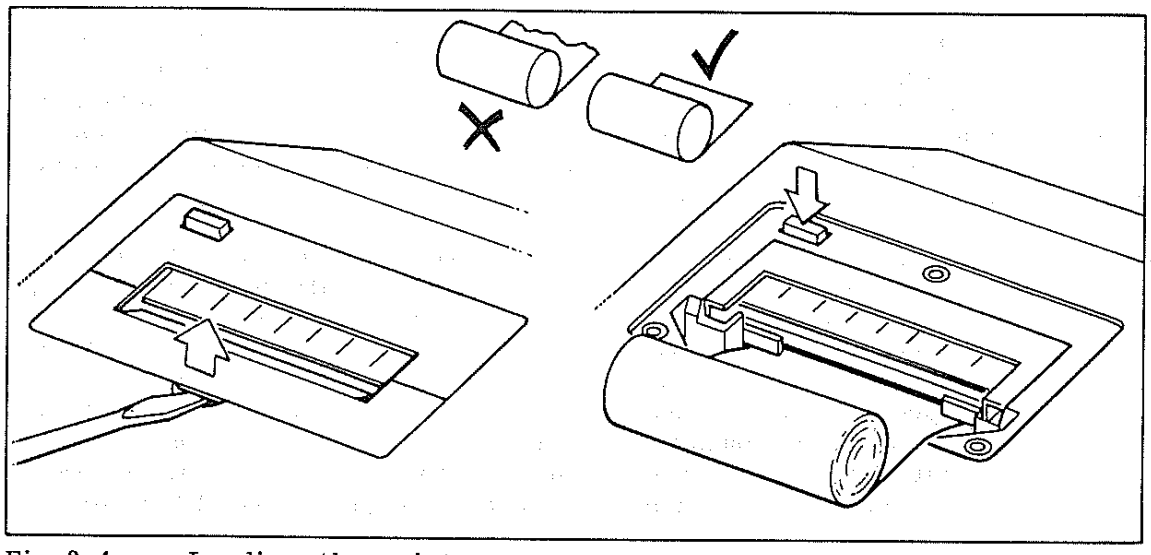


Fig.3.4 Loading the printer paper

ii) ensure that Ultrospec K is switched ON
iii) cut the leading edge of the paper so that it is parallel to the roll shaft
iv) align the paper to be straight when you insert it into the paper inlet. Do not insert paper with the wrong surface facing up
v) press the PF button to feed the paper until the leading edge passes the paper cutter
vi) locate the paper roll in its trough
vii) replace the printer cover plates ensuring that the paper exits through the slit

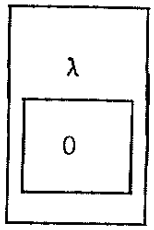
An external Epson FX 80 dot matrix printer with serial interface can be used in conjuction with Ultrospec K. For further details of this see section 4, part III (Ultrospec K Accessories) of this instruction manual.

3.5 SWITCH-ON

Each time Ultrospec K is switched on, it will go through a self identity and instrument calibration routine which is sequentially presented on the display panel. This procedure includes program and memory checking, a time and date check, turret identification and finally a seven step calibration routine (this takes about 80 seconds during which each step is displayed) after which the instrument goes to a wavelength of 340 nm and is ready for operation.

Note i) We recommend removal of the sample in the lightpath from the cell holder before the instrument undergoes a calibration routine. See 'calibration failed' error message, section 4.

3.6 THE λ/0 KEY



When this key is pressed, a prompt will be displayed asking the user to set the required wavelength:

Wavelength 340nm

Each digit can be changed by pressing the relevant numeric key (the function/numeric keys have now been automatically set to numeric status). The digits will flash from left to right in turn as the required number is entered. Alternatively, the cursor keys can be used to move to the digit which requires changing before the numeric key is pressed. When the required wavelength is displayed (say 500 nm) the user must press the 'ENTER' key to input this data and the Ultrospec K will drive to the selected wavelength after which the display will present information similar to the following:

500nm CELL1 0.134A

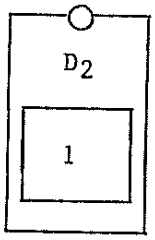
Note i) The wavelength range of Ultrospec K is between 200 and 900 nm. If a value below 200 nm is entered then the display will revert to 200 nm (the minimum wavelength allowed); if a value above 900 nm is entered then the display will revert to 900 nm (the maximum wavelength allowed).

ii) It takes approximately 30 seconds to change from a wavelength of 200 nm to 900 nm and vice versa.

iii) If a wavelength below 325 nm is selected when the D_2 lamp is OFF, then it will be automatically switched ON. It should then be left for a minimum of 5 minutes - preferably 30 minutes - to stabilise before sample measurement.

When in numeric status, pressing this key will display the number 0 in the appropriate position. See also section 3.1 for left/right cursor movement for incrementing/decrementing wavelength by 1 nm.

3.7 THE D_2/1 KEY

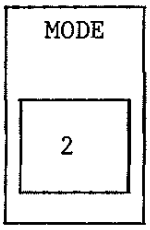


When the D_2 key is pressed, the deuterium lamp is switched ON or OFF. If switched ON, the green LED flashes while the lamp arc is striking and remains on once the lamp is on. The lamp should be allowed to stabilise for at least 5 mins before taking readings. The green LED is OFF when the D_2 lamp is OFF. When in numeric status, pressing this key will display the number 1 in the appropriate position.

Note i) A warning message on the display indicates a lamp failure.

ii) If the selected wavelength is below 325 nm when the D_2 supply is switched off, the wavelength will automatically revert to 325 nm.

3.8 THE MODE/2 KEY



When the MODE key is pressed, a prompt will be displayed asking the user to select the required mode:

```
Abs %T Conc  Factor
```

Abs = absorbance %T = % transmission
Conc = concentration

The user can either accept the currently flashing mode or select an alternative one by using a cursor key. When the desired mode has been selected, 'ENTER' must be pressed. If 'Conc' or 'Factor' has been selected, a further entry will be required as indicated on the display, e.g. :

```
Conc 0001.000
```

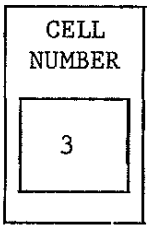
The display will show the currently selected concentration value or factor. If a factor is entered, it is multiplied by the absorbance value to produce the concentration value. If a concentration value is entered, it is used to determine the factor.

The required value is selected by entering numbers (the instrument has now been automatically set to numeric status) to replace the current number. Again the cursor keys can be used to move around the display. When the required value or factor has been selected, 'ENTER' must be pressed. The display will then show, e.g. :

```
500nm CELL2  142.8C
```

When in numeric status, pressing this key will display the number 2 in the appropriate position.

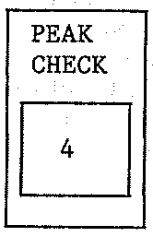
3.9 THE CELL NUMBER/3 KEY



When the CELL NUMBER key is pressed while a 4 or 6 cell holder is located in the sample chamber, the cell number displayed will increment by 1, and if this is accepted by the user 'ENTER' must be pressed to rotate the cell holder by 1 position. Alternatively, a different number within the 1-4 or 1-6 range can be entered to locate the required cell in the measurement position.
When in the Autofill Mode, this key allows presetting of sample number.

When in numeric status, pressing this key will display the number 3 in the appropriate position.

3.10 THE PEAK CHECK/4 KEY



When the PEAK CHECK key is pressed, the display will show the wavelength over which the Ultrospec K will scan to detect the highest reading. This range will be between +10 and -10 nm of the selected wavelength. At the same time the user can respond to a displayed prompt by selecting either the peak check or reference facility:

```
490 to 510 Peak Ref
```

If 'Peak' is entered then 'Peak checking..' will be displayed after which the wavelength, cell number and reading at which the highest reading was detected will be displayed:

```
503  CELL2  1.724A
```

If there is no relevant baseline (i.e. W or W + D_2) stored in the instrument memory then 'No baseline!' will be displayed.

In transmission mode, peak check will find the highest %T reading, i.e., find an absorbance trough.

The 'Ref' option is designed to allow the user a quick method of automatically setting a baseline over the short wavelength range displayed on pressing the 'Peak check' key. This 'Ref' option is called the Baseline Patch Facility.

The patch is created in the stored temporary baseline and it should be noted that this is done when either the tungsten lamp or both the tungsten and D_2 lamps are on. If the lamp status is changed then the patch will disappear. The patched baseline always overwrites whatever is stored in the temporary baseline over the 20 nm wavelength range in question.

WARNING: It is possible to use the Baseline Patch Facility even when Ultrospec K has been initialised without a permanent baseline. If this is done then it must be noted that in this case only the 20 nm patch of baseline will consist of valid readings.

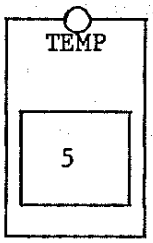
If 'Ref' has been selected, then 'Creating patch..' will be displayed until this has been completed after which the display will return to the standard wavelength/cell/reading format.

Note i) If the D_2 lamp is ON then the Peak Check is restricted to 200 - 220 nm when the selected wavelength is< 210 nm, and to 880 - 900 nm when the selected wavelength is >890 nm.

ii) If the D_2 lamp is OFF then the Peak Check is restricted to 325 - 345 nm when the selected wavelength is<335 nm, and to 880 - 900 nm when the selected wavelength is >890 nm.

When in numeric status, pressing this key will display the number 4 in the appropriate position.

3.11 THE TEMP/5 KEY



The TEMPERATURE function can only be used when the Autofill cell holder incorporating a Peltier heating system is located in the sample chamber and identified (see section 3.17.3). If this key is pressed when a different cell holder is present, then the message 'No Peltier fitted' will be displayed. If this happens then press the 'STOP' key to return to the Ultrospec mode.

When this TEMP key is pressed when the Autofill system is in use, the user is given the option of turning the temperature control on:

Temperature on Y/N

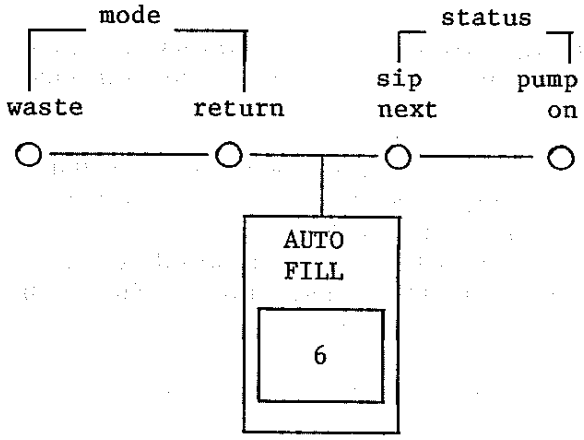
If Y is selected and the temperature was previously off, the display will change to:

Temperature 37°C

otherwise the current temperature is displayed if the temperature option was already in operation. At this stage the user may enter the displayed value or change it before pressing 'ENTER'. A temperature range of 20 -50°C in 1°C steps can be entered. If a temperature above or below this range is entered then the display will revert to 50°C or 20°C as appropriate.
If the entered temperature is higher or lower than the cell temperature, the green LED will flash until the desired temperature has been reached and stabilised after which the LED will remain lit.

When in numeric status, pressing this key will display the number 5 in the appropriate position.

3.12 THE AUTOFILL/6 KEY



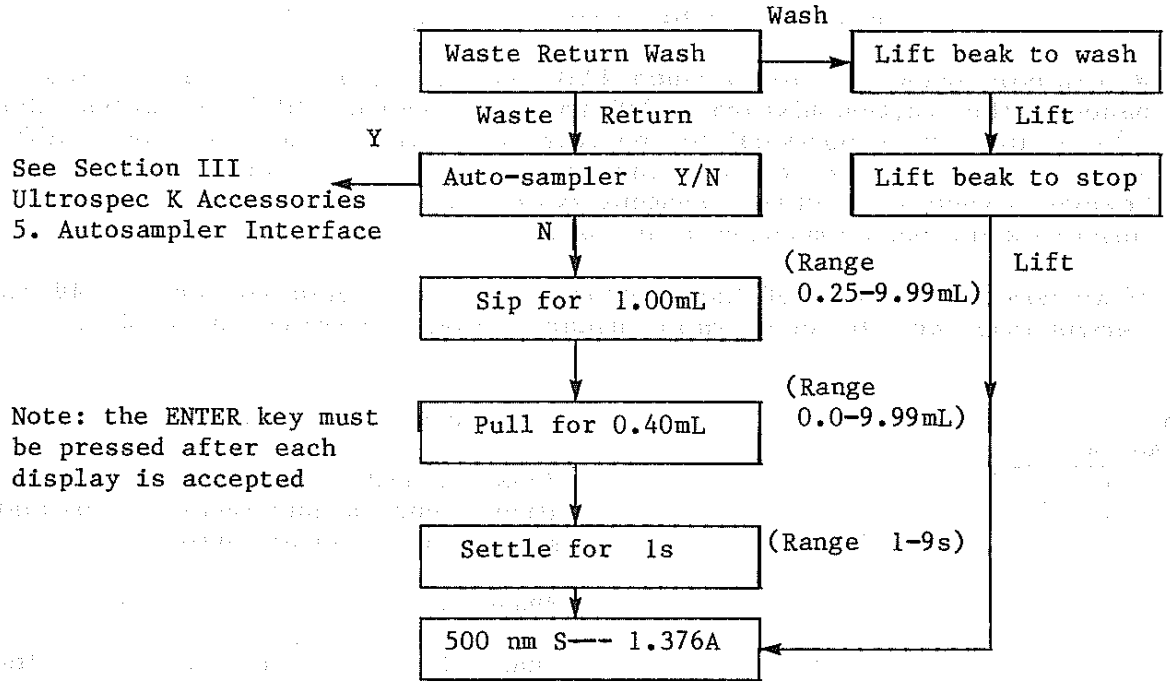
When the Autofill key is pressed but the Autofill cell holder is not located in the sample compartment (see section 3.17.3), the message 'No Autofill fitted' will be displayed. The 'STOP' key can then be pressed to escape to the standard Ultrospec Mode display.

See also section 2 (Autofill operation), part II of this instruction manual.

The following flow diagram outlines the various choices open to the user when the Autofill system is in use. Please note the following definitions:

Sip volume: the volume of sample required
Pull volume: the volume required to pull the sip volume into the optimum position in the flowcell (equivalent to the volume of air drawn in behind the sip volume to achieve this). See Fig.2.2, section 2, part II (Autofill K) of this instruction manual
Settle time: time allowed for the reading to stabilise after the sample has been pumped into the flowcell

When the AUTOFILL key is pressed with the Autofill system in operation (in volume mode), the following choice of options are possible:



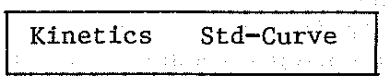
Return time = Sip time + Pull time + 2.5 secs

Note The appropriate mode and status LEDs associated with this key indicate the current Autofill status.

When in numeric status, pressing this key will display the number 6 in the appropriate position.

3.13 THE KINETICS/7 KEY

When the KINETICS key is pressed, either the Kinetics or Standard Curve mode can be selected:



3.13.1 Kinetic Measurements

The usual way of measuring the rate of an enzyme reaction is to monitor the change in concentration of one of the substrates involved in, or products produced by the reaction. Take, for example, the alanine transaminase (ALT) enzyme reaction:

$$\text{alpha-ketoglutarate} + \text{alanine} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{glutamate}$$

If we want to measure the rate of production of pyruvic acid, as this this cannot be done directly we can link the pyruvate to another enzyme reaction involving NADH and the enzyme lactate dehydrogenase (LDH) thus:

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^+$$

We can now follow the rate at which NADH is used up by measuring the absorbance of the reaction mixture at 340 nm and, because LDH is in excess, this rate is directly proportional to the rate of pyruvate produced from a different enzyme reaction to which the above reaction is coupled. This type of linkage is such a useful measurement system because about 80% of all enzyme measurements can be monitored this way.

If we now plot a curve of the absorbance of the reaction mixture at 340 nm versus time, we will get a graph similar to that illustrated in Fig.3.5.

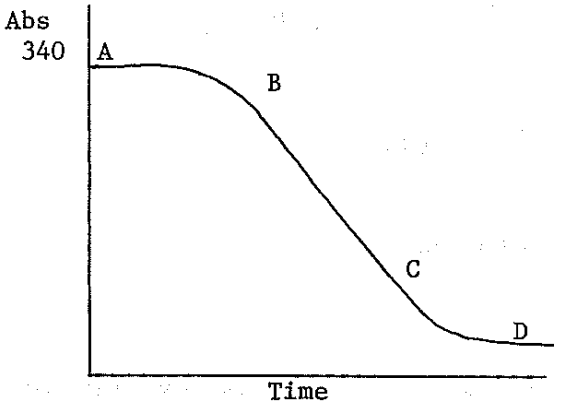


Fig.3.5 Plot of absorbance at 340 nm vs time for an alanine transaminase enzyme reaction

The curve can be split into 3 phases:

Phase 1, A-B; reactant mixing, formation of enzyme-substrate complex and attainment of linear phase.

Phase 2, B-C; the linear phase.

Phase 3, C-D; tail off, one of the reactants becomes rate limiting, the reaction approaches and reaches equilibrium thus reducing the net reaction rate to zero.

The rate of the reaction is the slope of the linear portion of the plot and therefore the change in absorbance per unit time dA/t.

To convert the change in absorbance to a change in concentration, the following formula is used:

$$dC = \frac{dA}{ExL}$$

where dC = concentration
dA = absorbance
L = path length (normally 1 cm)
E = molar absorptivity (molar extinction coefficient) of the compound being measured (for NADH, E = 6300 litres/mol/cm)

The rate of change of concentration (dC/t) can then be used to calculate enzyme activity:

$$\text{Enzyme activity} = dC/t \times \frac{Vt}{Vs}$$

where Vt = total volume of the reaction mixture
Vs = volume of sample

Units of Enzyme Activity

There are two internationally accepted units for enzyme activity:

1. International Unit of Enzyme Activity - U or IU
   Defined as that amount of enzyme activity which will convert 1 micromole of substrate per minute at 25°C

2. Katal - kat
   Defined as that amount of enzyme which will convert 1 mole of substrate per second

   $1 \text{ IU} = 1.67 \times 10^{-8} \text{ kat}$
   $1 \text{ kat} = 6 \times 10^{7} \text{ IU}$

The katal is not used in many countries although it is a recognised SI unit.
Since we have defined our calculations as the rate of change of concentration (mol/litre), the results are therefore as activity per unit volume - IU/L or kat/L.

Conversion Factor

In order to simplify the calculation of enzyme activity, variables such as molar absorptivity and sample volume can be combined to produce a conversion factor. This can be done for Ultrospec K so that the conversion factor is used to produce results in the appropriate units directly from the change in absorbance measured by the spectrophotometer.

The variables, in the correct units when working in IU/L, are given in the following equation:

$$\text{Factor} = \frac{Vt \times 10^{6}}{E \times L \times Vs}$$

where Vt = total reaction volume (mL)
Vs = sample volume (mL)
E = molar absorptivity (L/mol/cm)
L = path length (normally 1 cm)

and the factor has the units of U.s/L

Now in the alanine transaminase enzyme reaction we have taken as an example, if we have 0.2 mL of test sample in a total reaction volume of 2.20 mL, the conversion factor is calculated as follows:

$$\text{Factor} = \frac{2.20 \times 10^6}{6300 \times 1 \times 0.2}$$

$$= 1746 \text{ U.s/L}$$

The rate of change of absorbance, dA/t, is calculated by performing a linear regression analysis on numerous points from the linear portion of the Abs. vs time plot (Fig.3.5) to give a value for the slope in change in Abs. per minute. This is convenient for working in the IU unit, but to work in microkatals the conversion factor must be divided by 60.

To calculate the enzyme activity, simply multiply the rate of change in absorbance by the conversion factor;

Enzyme activity (IU/L) = dA/min x Factor

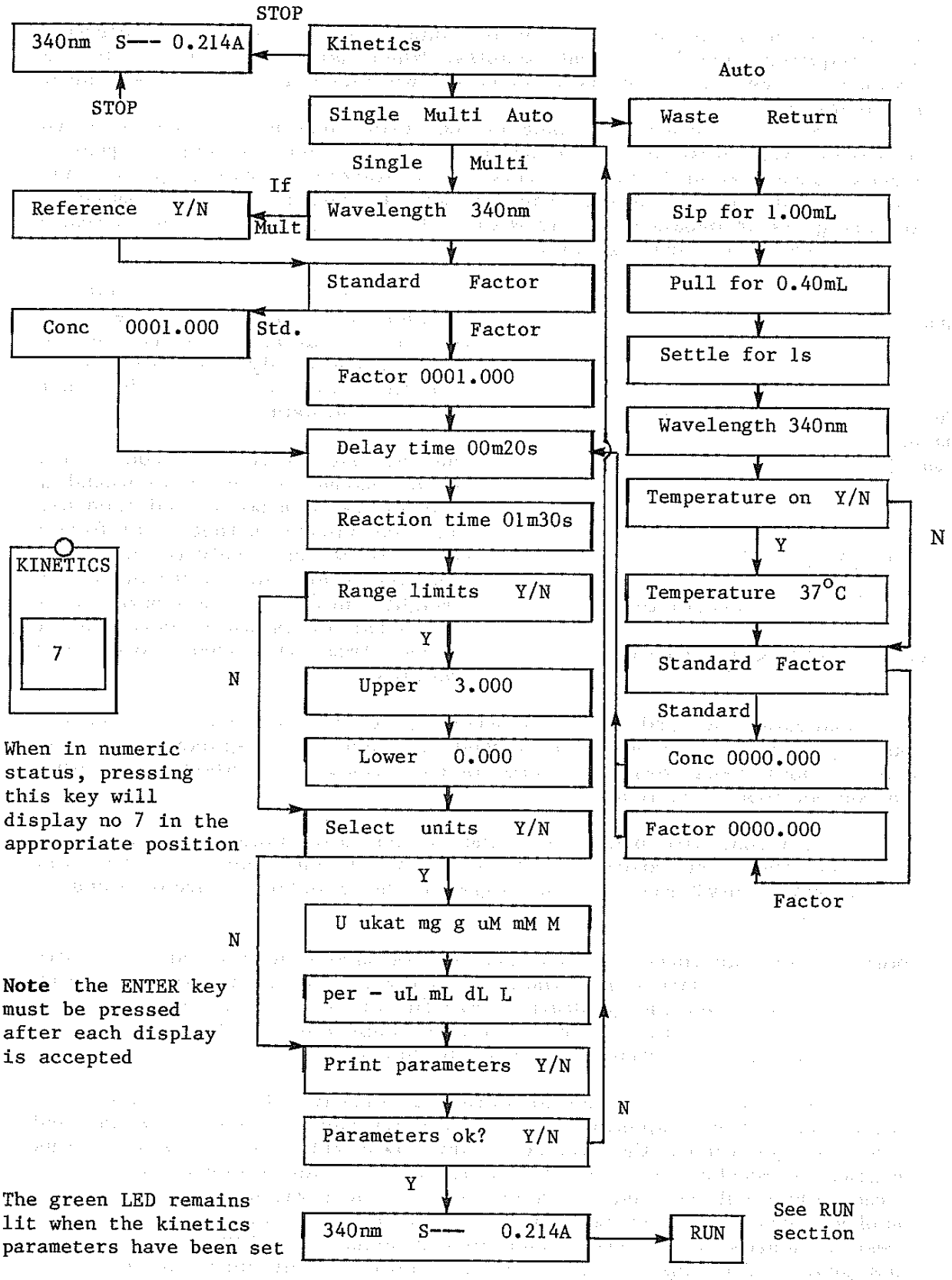
3.13.2 Kinetics Mode

Read section 3.13.5, Parameter Setting, before attempting to operate in this mode.

A reference sample can be measured when the 'Multi' mode has been selected. This sample consists of the biological sample minus the enzyme required to initiate the kinetic reaction and is used to compensate for any drift of background caused by spurious reactions within the sample during the time set for reaction rate measurements.

A standard or factor value can be entered when in any of the 'Single', 'Multi' or 'Auto' modes. The conversion factor is explained in the previous section. If a standard value is entered, then a comparison is made between this value and reaction rate of the standard sample (which must be the first sample measured) to derive a factor which is then used to calculate the value of all further samples.

If the Kinetics Mode is selected then the following display sequence will occur:


STOP
340nm S— 0.214A
Kinetics
STOP
Auto
Single Multi Auto
Waste Return
Single
Multi
If
Reference Y/N
Mult
Wavelength 340nm
Sip for 1.00mL
Standard Factor
Pull for 0.40mL
Conc 0001.000
Std.
Factor
Settle for 1s
Factor 0001.000
Wavelength 340nm
Delay time 00m20s
Temperature on Y/N
N
Reaction time 01m30s
Y
KINETICS
7
Range limits Y/N
Temperature 37°C
Y
Standard Factor
N
Upper 3.000
Standard
Lower 0.000
Conc 0000.000
When in numeric status, pressing this key will display no 7 in the appropriate position
Select units Y/N
Factor 0000.000
Y
Factor
N
U ukat mg g uM mM M
Note the ENTER key must be pressed after each display is accepted
per - uL mL dL L
Print parameters Y/N
N
Parameters ok? Y/N
Y
The green LED remains lit when the kinetics parameters have been set
340nm S— 0.214A
RUN
See RUN section

3.13.3 Standard Curve Measurements

A standard curve is required to enable one to determine the concentration of a required analyte in test samples. When using Ultrospec K in the Standard Curve Mode there is a choice of two curve fitting methods -Linear or Quadratic.
A linear curve is drawn by measuring the absorbance of a number of known increasing concentrations of the particular analyte and plotting absorbance versus concentration (Fig.3.6). This linear standard curve graph is then used to read off the correct concentration of analyte in a test sample by comparing its absorbance reading with that of the graph in order to relate it to concentration (see Fig.3.6).

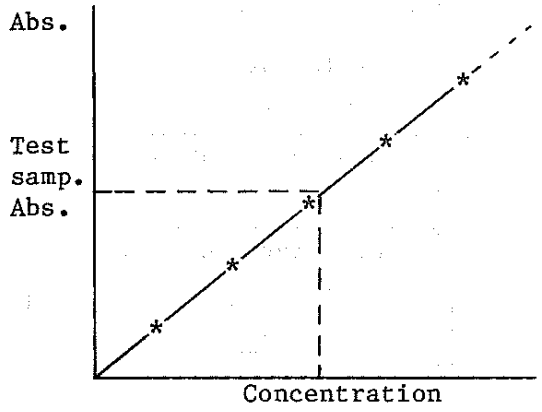


Fig.3.6 Plotting a standard curve

One of the more precise methods of determining the way absorbance varies with concentration is to analyse the data mathematically and attempt to find the equation of the line that best fits the data.

The simplest and most common method is to assume a linear relationship between the data points and then use the least squares method to perform a linear regression analysis to determine the slope and intercept of the straight line. The line drawn is is then used to estimate concentration values from measured absorbance readings.

If a quadratic standard curve is selected, it is fitted using a quadratic least squares fit. The equation is used to check for imaginary roots and also for both roots less than zero concentration. The method of selecting the correct root is as follows:

> If both roots are below the value of the first standard concentration then the first positive root is taken, if not then the first (and possibly only) root in the range of the standard concentrations is taken.

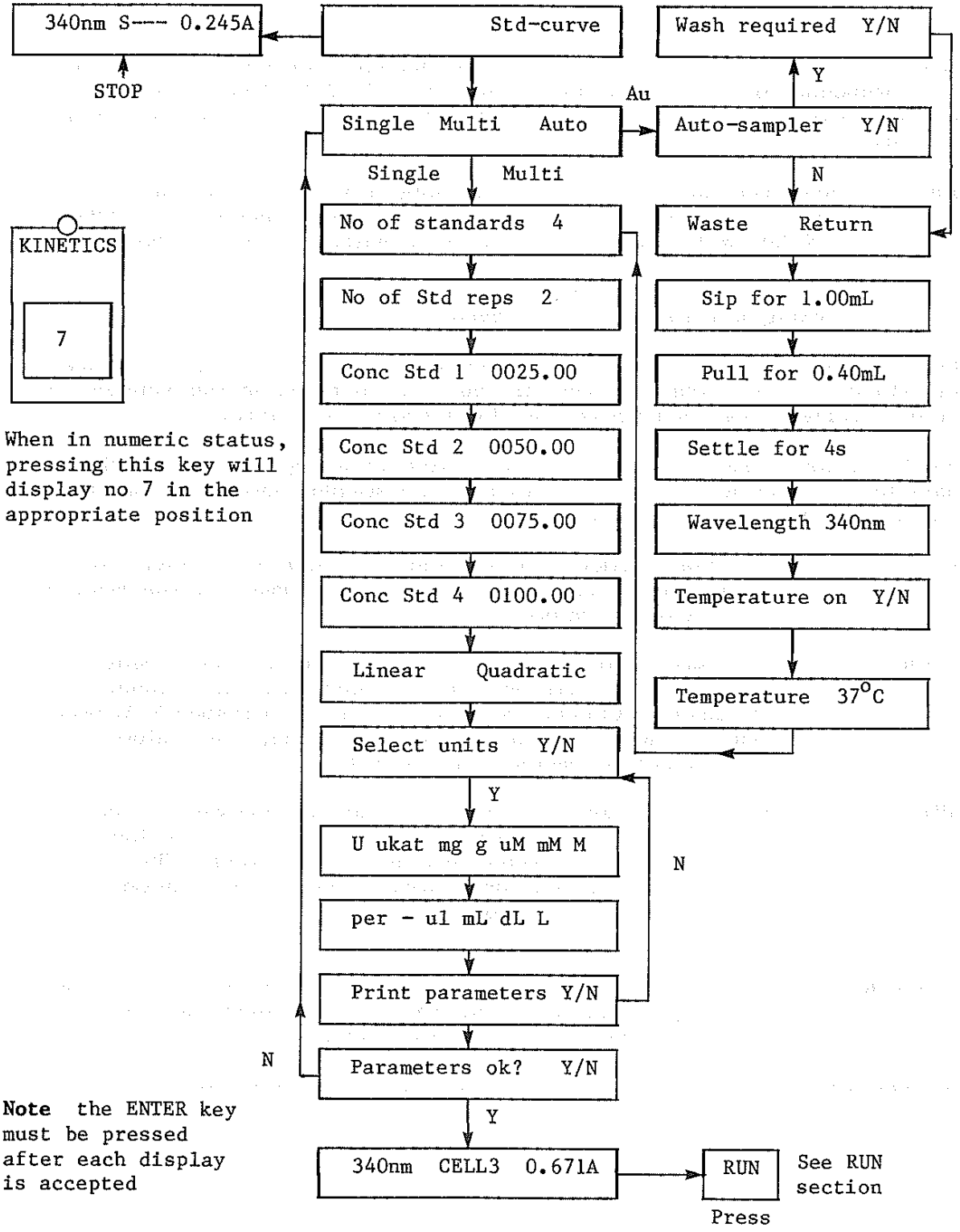
Note: If less than three standard samples are used (and entered) to construct the standard curve when the quadratic curve fit method has been selected, then the quadratic curve fit cannot be implemented and the message '3 Stds: Linear fit' will be displayed and the linear curve fit method automatically carried out instead.

If the PROGRAM key is used to store the current list of parameters (see section 3.16) after completing a standard curve run in which a new standard curve was generated, the standard curve data will also be saved. If this program is recalled to carry out subsequent standard curve assays, the stored curve will be used to calculate the concentration of unknown test samples unless it is overwritten with new data by answering 'Y' to the 'Fresh standards Y/N ' prompt which appears after the 'RUN' key is pressed. This prompt also appears if a standard curve is currently stored in the working memory after RUN is pressed when in the Standard Curve Mode.

Different standard curves can be stored under different file locations in the program catalogue. A reference sample (user determined) is measured prior to standard curve samples to set the background reading to zero.

3.13.4 Standard Curve Mode

Read section 3.13.5, Parameter Setting, before attempting to operate in this mode. If the Standard Curve Mode is selected then the following display sequence will occur:



3.13.5 Parameter Setting

Note the following comments and definitions to help parameter setting.

The default values presented on the display as you proceed with parameter setting are those which are stored in the current program. In order to change just one parameter (for example), accept each default value as presented by pressing the 'ENTER' key except for the parameter you wish to change.

None of the changed parameters become permanently stored until you enter 'Y' in response to the the prompt 'Parameters OK? Y/N'. When this is done the currently active program is overwritten with the new parameters selected.

Note i) This also has the effect of erasing any data points stored up until 'Y' was entered and thus subsequent pressing of the 'POST RUN' key will cause a 'No data' error message to be displayed.

ii) On entering 'Y', any standard curve data currently in the working memory will be destroyed.

Whenever the 'STOP' key is pressed during parameter setting, the display will return to the Ultrospec Mode. If you wish to re-enter the Kinetics or Standard Curve mode then press the KINETICS/7 key again.

The 3 options presented on the display after pressing the KINETICS key relate to the type of cell holder located in the sample chamber and whether parallel samples are to be assayed

Single — this assay option can be used with any cell holder except Autofill. One sample is measured at a time and the results are printed separately

Autofill — this assay option is the same as the single mode except that the Autofill system is used (either with or without the Autosampler optional accessory - see III Ultrospec K Accessories, section 5). When using this mode, the Autofill (or Autosampler) parameters must be set

Multi — this assay option can be used in conjunction with either the 4 or 6 sample cell holder. All the samples are monitored in parallel and the results printed out together. The MULTI mode is recommended for assays which involve kinetic reactions having long reaction times

Delay time — the period between the start of the kinetic reaction and the beginning of sample measurement during which the reaction reaches its linear (zero order) phase.

Reaction time — the period during which the sample is monitored

Range limits — the user can enter upper and lower range limits, in absorbance units, for the expected limits of absorbance of the test samples. This allows the instrument to monitor the absorbance so that samples outside this range can be flagged by 'out of range' being printed beside the relevant sample result.

Ref cell — This is a sample which lacks one essential reactant needed for a reaction to occur and is used to measure background absorbance. This is automatically subtracted from the absorbance readings of active samples prior to any calculations.

Units

IU = International Units
ukat = microkatals

g = grams
uM = micromoles
mM = millimoles
M = moles
uL = microlitre
mL = millilitre
dL = decilitre
L = litre

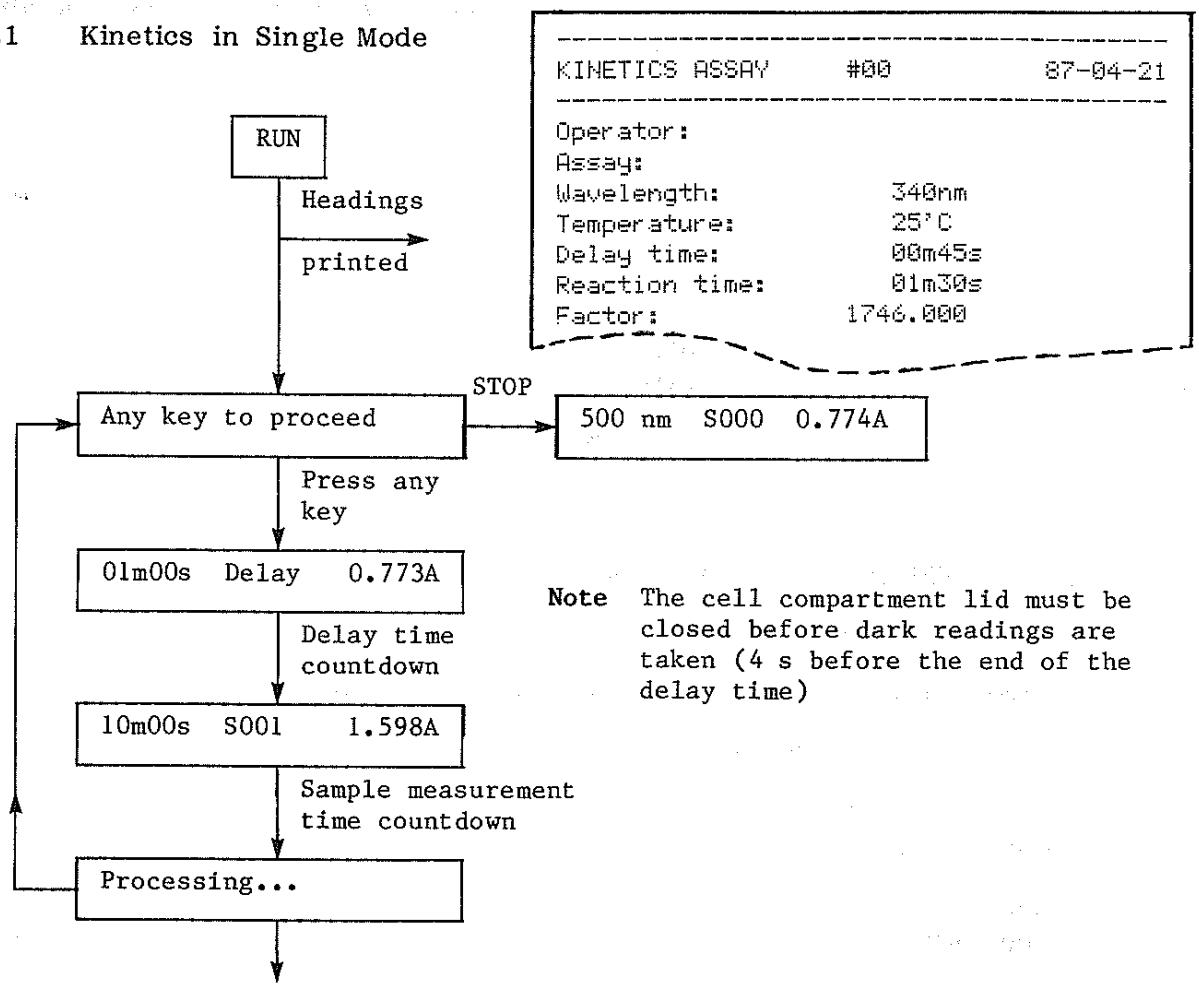
Table of Parameter Setting Ranges

Delay time	5s - 59m 59s
Reaction time	2s - 59m 59s (Multi mode min. = 24 secs.)
Factor	0-9999.999
Lower (range limits)	0 - 3.0
Upper (range limits)	0 - 3.0

3.14 THE RUN KEY

After the RUN key has been pressed, follow the instructions presented on the display to initiate sample measurement. These vary depending on the modes and parameters selected as typified by the following flow diagrams. The RUN LED lights up during spectrophotometric measurement and therefore during a kinetics run it will stay lit throughout most of the run whereas during a standard curve run it will flash on and off intermittantly.

3.14.1 Kinetics in Single Mode

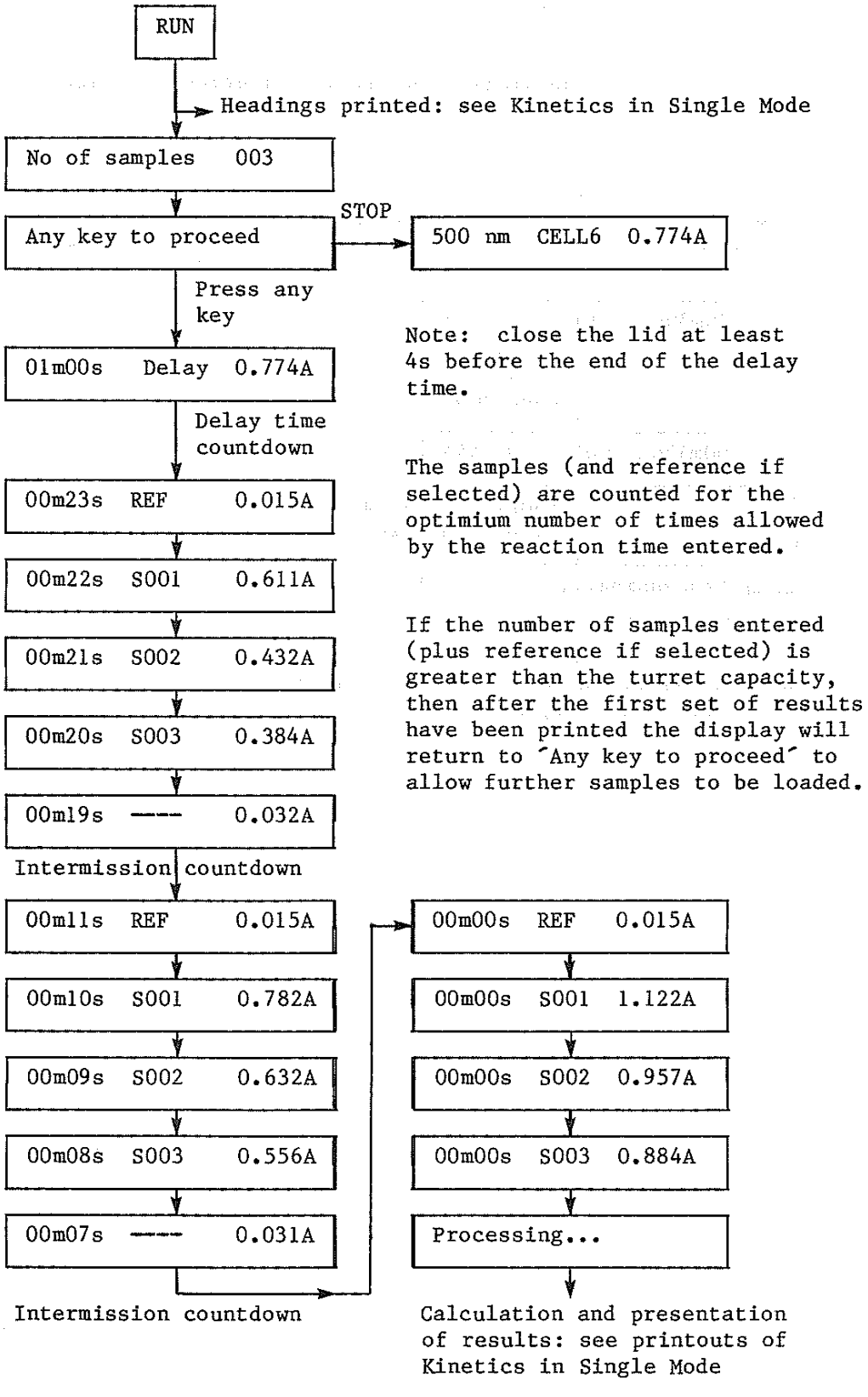


Note The cell compartment lid must be closed before dark readings are taken (4 s before the end of the delay time)

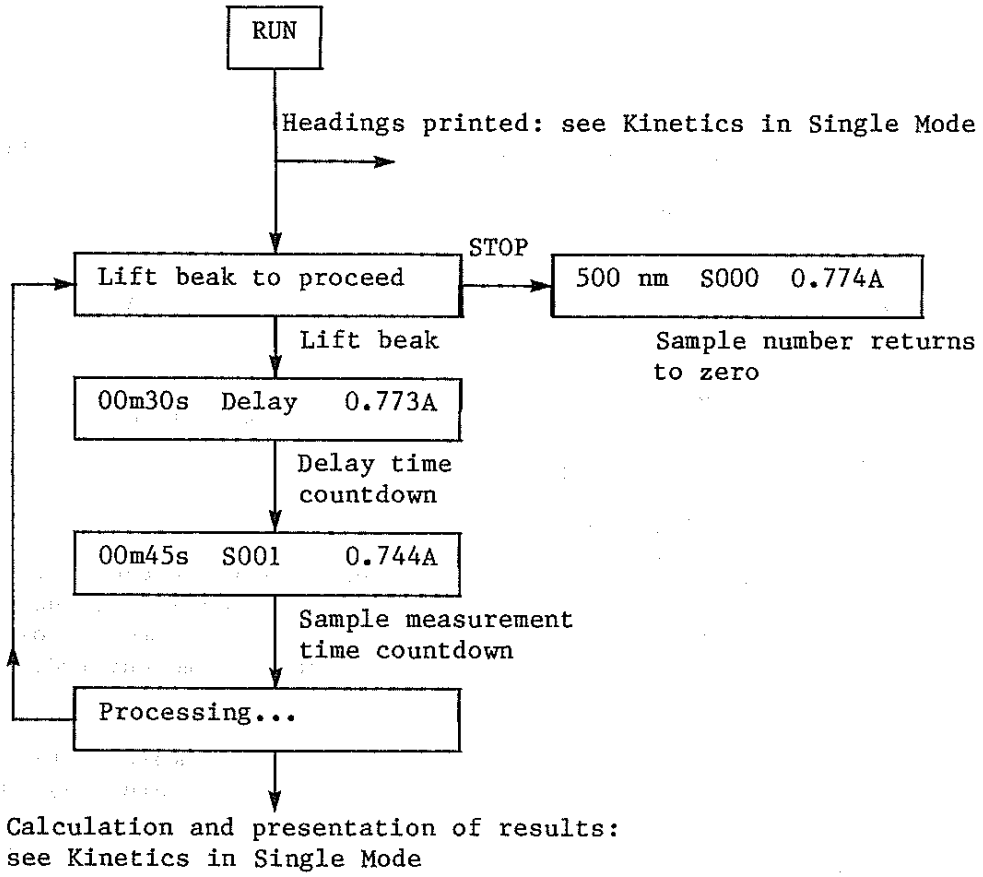
```
Sample number        S001
Init A    1.376      Final A    1.349
Slope  -  0.018      Linearity  0.999

Result      31.292 IU/L

Sample number        S002
Init A    1.338      Final A    1.315
Slope  -  0.015      Linearity  0.999

Result      26.092 IU/L
```

3.14.2 Kinetics in Multi Mode (E.g., 1 Reference plus 3 Test Samples)

Note: The start solution should be added to all samples loaded in the turret before responding to the 'Any key to proceed' prompt.



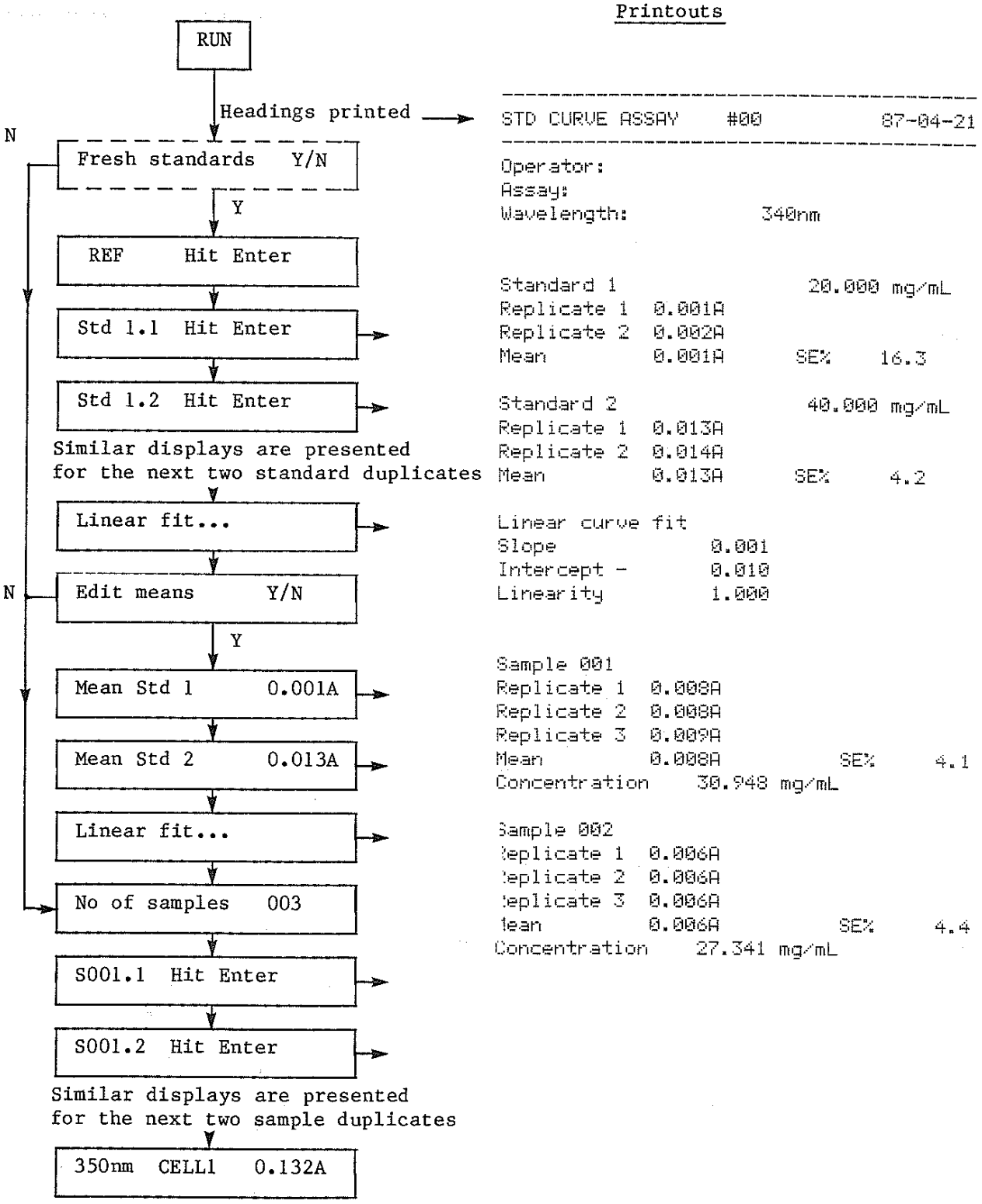
3.14.3 Kinetics in Auto Mode



3.14.4 Standard Curve in Single Mode

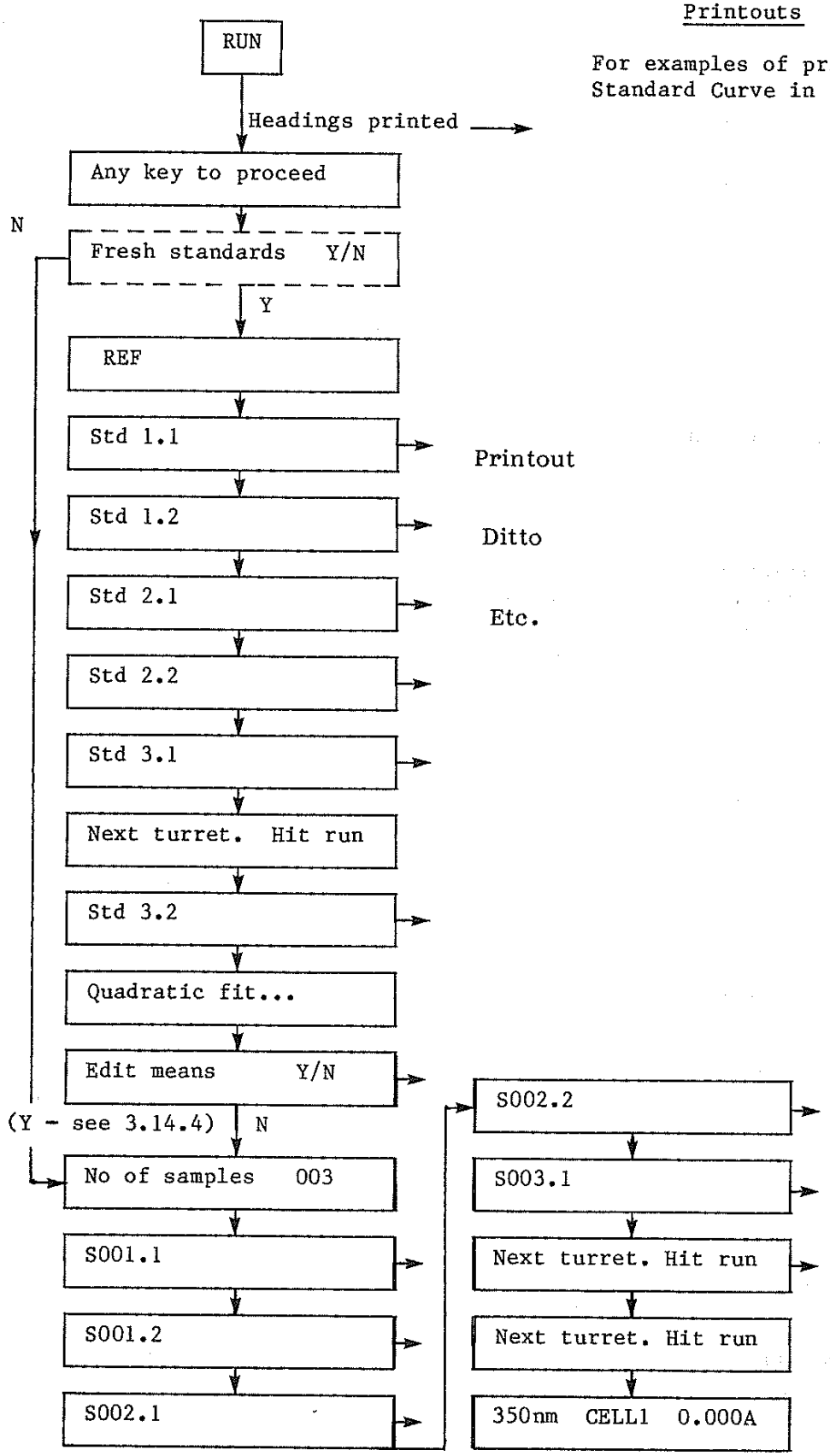
(E.g., 2 duplicate standards and 3 duplicate samples)

Note: A new Standard or Test Sample must be loaded into the measurement cell before ENTER is pressed to initiate each sample measurement.



3.14.5 Standard Curve in Multi Mode

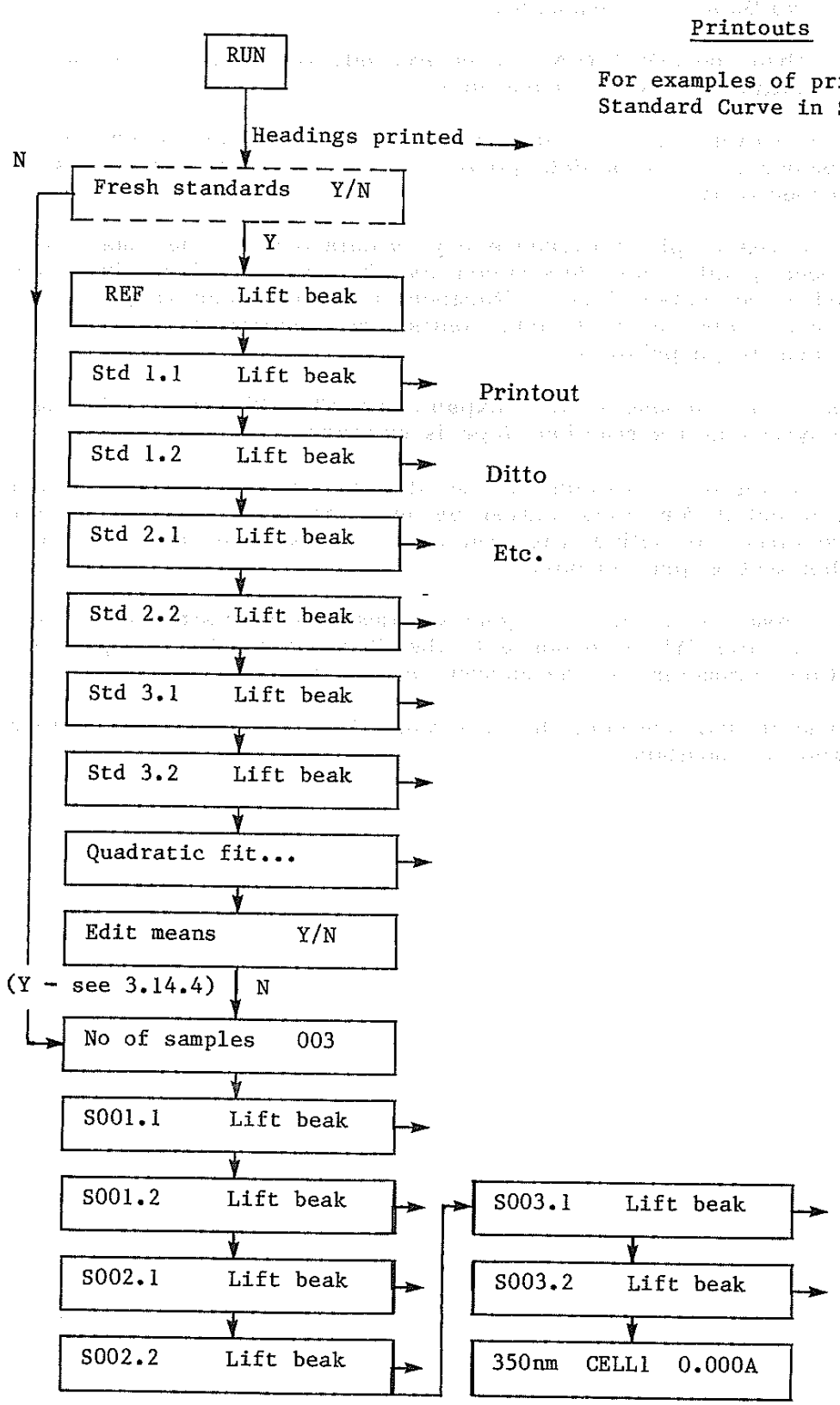
(E.g., 3 duplicate standards and 3 duplicate samples using a 6-cell turret)



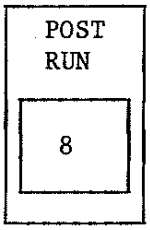
3.14.6 Standard Curve in Auto Mode

(E.g., 2 duplicate standards and n duplicate samples)

The displayed information is the same as that for the single mode except that you are asked to lift the Autofill beak where appropriate:



3.15 THE POST RUN/8 KEY



The POST RUN function allows the user to look at and recalculate certain data stored during measurement of the six most recent enzyme kinetics samples. If there is no data stored in the instrument memory on pressing this key then 'No Data!' is displayed.

When the POST RUN key is pressed, the choice of options outlined below is is possible.

When 'Data' or 'Graph' is selected for a particular sample (user enters the sample number), a list of data points or a plot of the data points is printed out respectively.

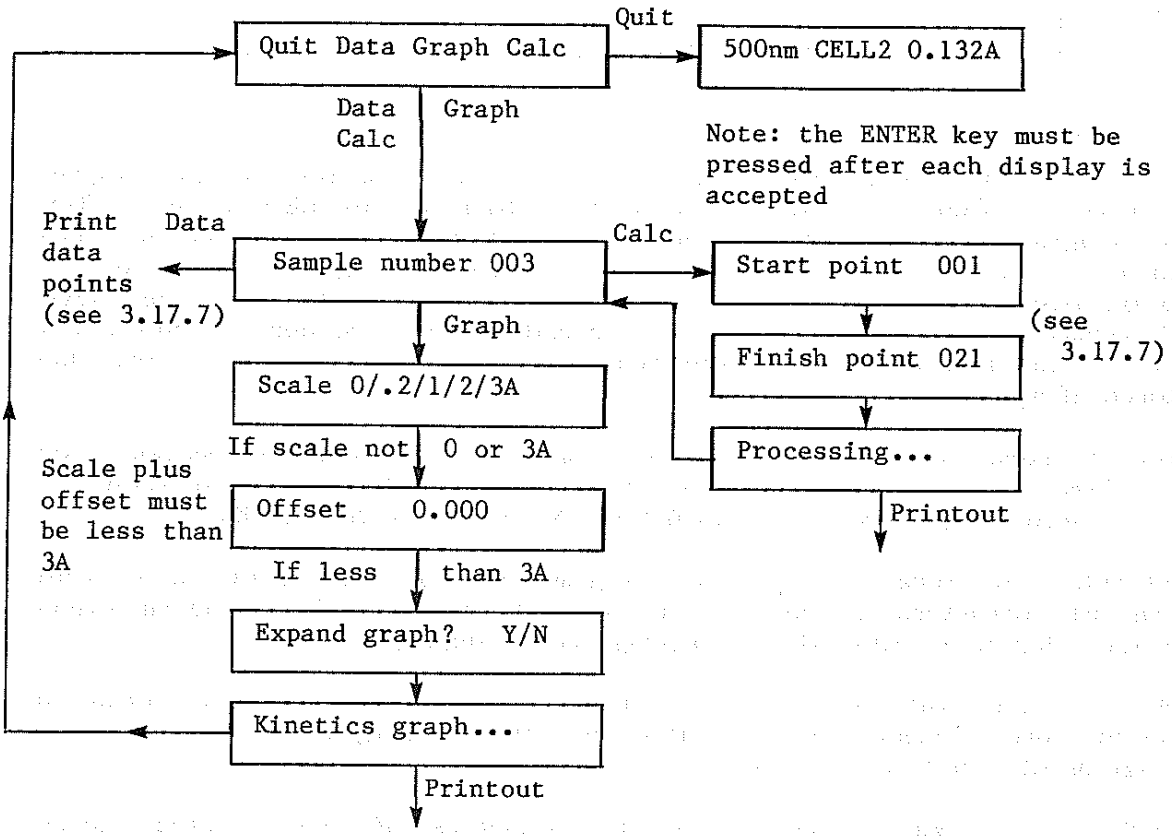
The time axis of the graph is marked every 10 data points, the time interval between each point being determined by the number of readings per sample selected - see section 3.17.7, 'Number of readings per sample'.
The time interval over which 10 data points are measured appears at the beginning of each graph printout.

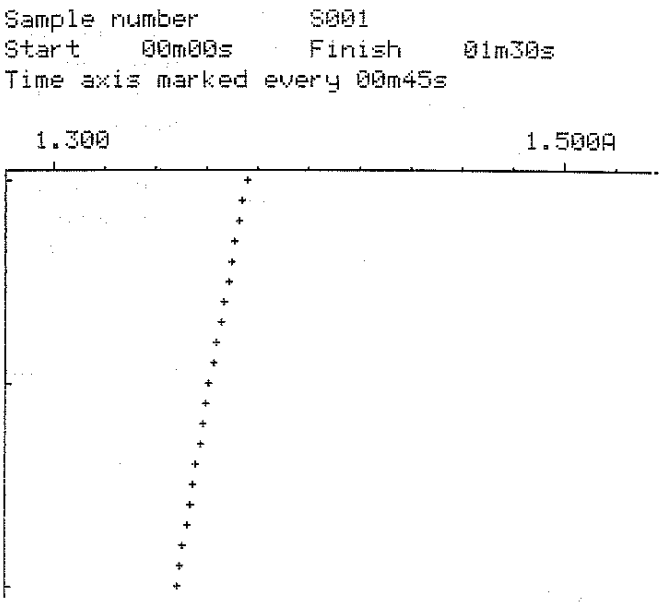
If 'Y' is entered in response to the 'Expand graph? Y/N' prompt, the time axis of the enzyme kinetics reaction slope is expanded x 8.

When 'Calc' is entered, a section of the stored enzyme kinetics reaction slope can be selected for recalculation by entering the 'Start point' and 'End point' required to define the limits of this section and the data relevant to this section printed out.

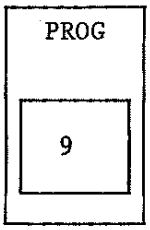
Note: Data from the previous enzyme kinetics run is destroyed as soon as you enter 'Y' in response to the 'Parameters OK?' prompt after setting parameters of the current assay run.

When in numeric status, pressing the Post Run/8 key will display the number 8 in the appropriate position.


Quit Data Graph Calc
Quit
500nm CELL2 0.132A
Data
Calc
Graph
Note: the ENTER key must be pressed after each display is accepted
Print data points (see 3.17.7)
Data
Sample number 003
Calc
Start point 001
Finish point 021
(see 3.17.7)
Graph
Scale 0/.2/1/2/3A
If scale not
0 or 3A
Processing...
Printout
Scale plus offset must be less than 3A
Offset 0.000
If less
than 3A
Expand graph? Y/N
Kinetics graph...
Printout


Sample number S001
Start 00m00s Finish 01m30s
Time axis marked every 00m45s
1.300
1.500A

3.16 THE PROG/9 KEY



The PROGRAM function allows the user to permanently store up to 20 programs (collections of parameters) and recall individual ones to carry out particular assays with little or no further need for additional parameter setting.

When the PROGRAM key is pressed, the user can choose one of 4 displayed options:

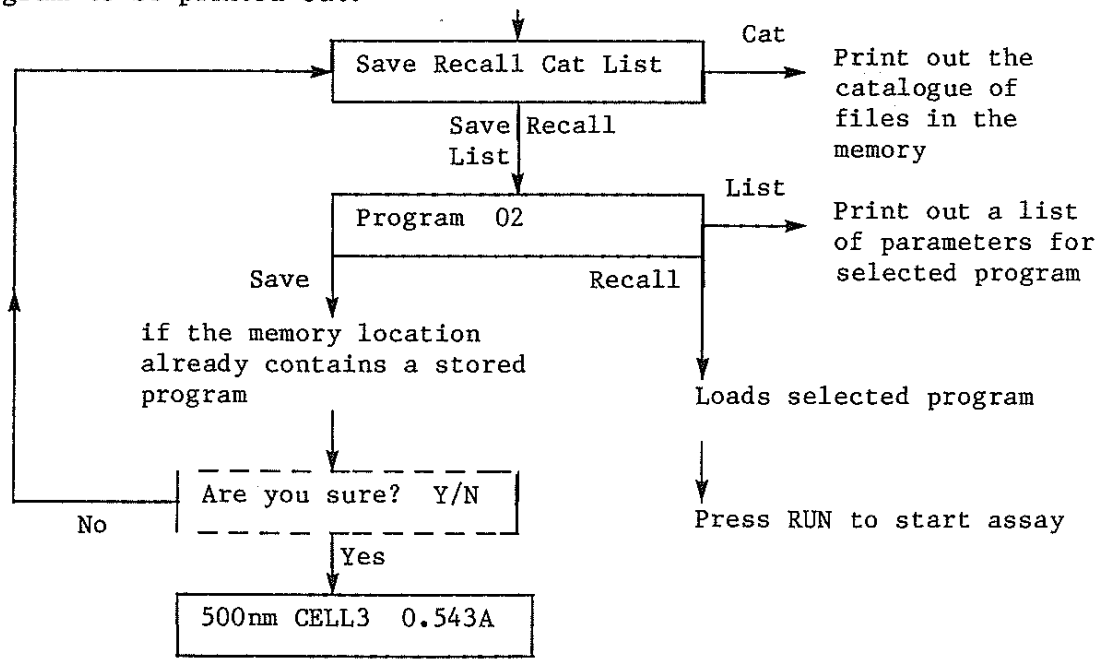
SAVE this saves (stores) the current list of parameters in the program location specified (from 01 to 20) if the KINETICS key LED is on . After the program has been stored, the instrument returns to exactly the same status it was in immediately prior to program storage. If you use this option after running a standard curve assay, you will also save the curve fit parameters so that when you next recall there is no need to run a new set of standards. Autofill calibration and operation parameters are also stored if appropriate.

Note If there is already a program stored in the selected location then 'Are you sure? Y/N' will be displayed. If YES is entered then the stored program will be OVERWRITTEN WITH THE NEW ONE.

RECALL this recalls a selected program (01-20) and resets the current program parameters to those of the recalled program. If no program exists in the selected location then 'No program' is displayed.

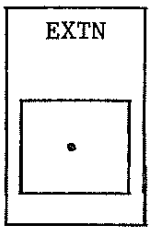
CAT this will cause a catalogue of all the file locations stored in the instrument memory to be printed out thus showing which locations have program files stored in them.

LIST this will cause a list of parameters of the selected stored program to be printed out.



When in numeric status, pressing this key will display the number 9 in the appropriate position.

3.17 THE EXTENSION KEY



This key is used in combination with several other keys to carry out many different functions. In each case the EXTENSION key is first pressed, followed by one of the other keys described below. When the EXTENSION key is pressed the display will present:

Extn..

When the second key is pressed, the options open to the user will be displayed.

When in numeric status, pressing this key will display a decimal point in the appropriate position.

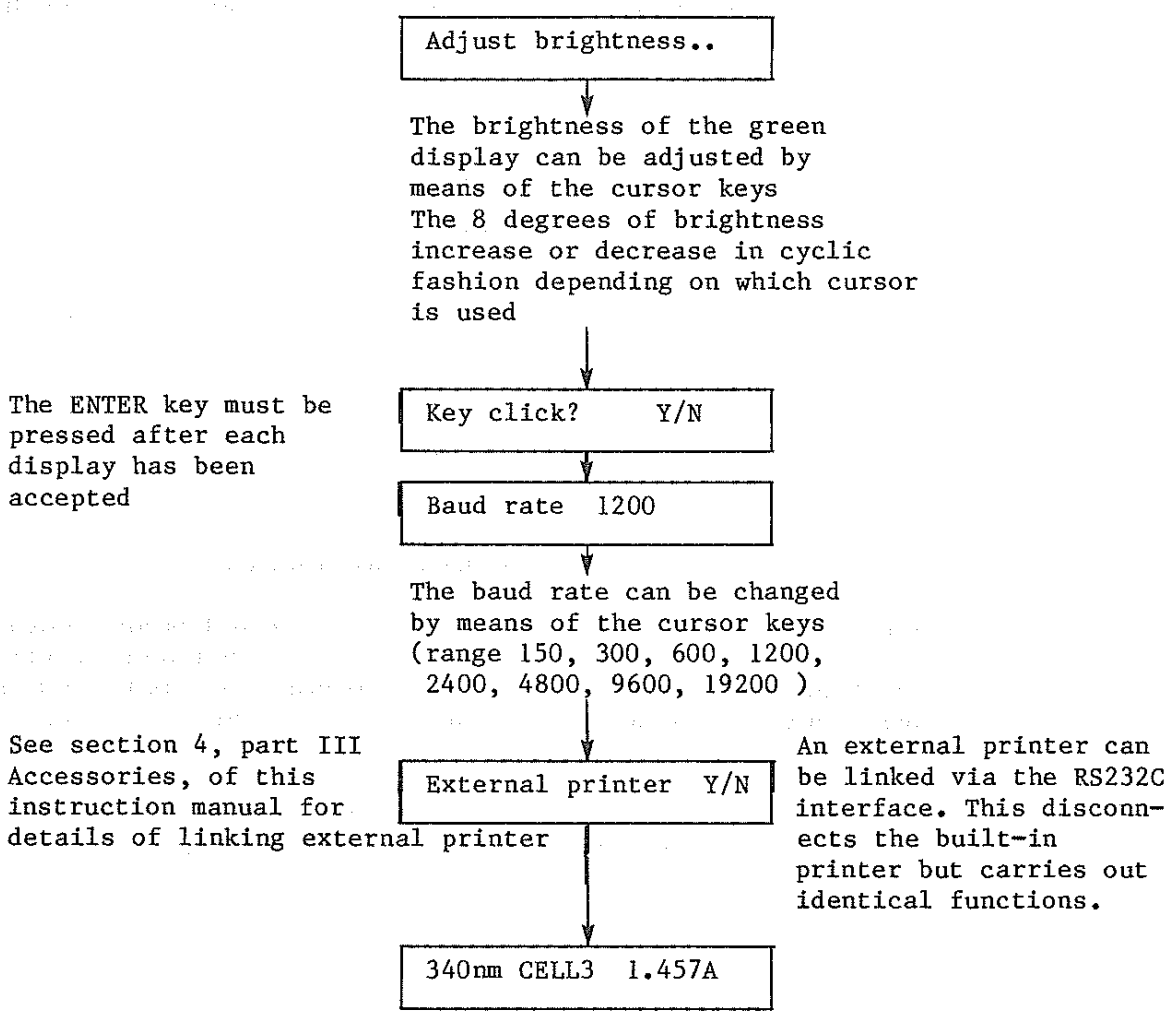
3.17.1 Extn + λ Instrument Recalibration

This combination of keys will initiate an instrument recalibration routine like that which occurs after instrument switch-on. It is included to allow the user the assurance of being able to recalibrate the instrument if he/she is unsure about its previous use, and to allow service personnel to check calibration routines.

Note We recommend the removal of the sample in the lightpath from the cell holder before the instrument undergoes a calibration routine. See 'calibration failed' error message, section 4.

3.17.2 Extn + Mode Change Instrument Functions

This combination of keys allows several instrument functions to be selected or changed as indicated by the display sequence outlined below:

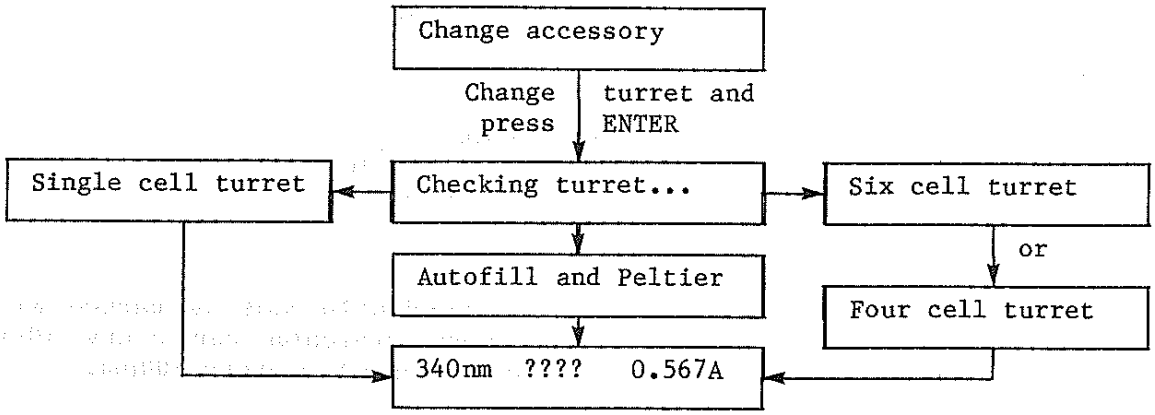


3.17.3 Extn + Cell Number Cell Holder Replacement Routine

This combination of keys is used to initiate the turret (cell holder) replacement routine. This routine also allows the user to check which turret is in use without the need to open the lid of the sample chamber.

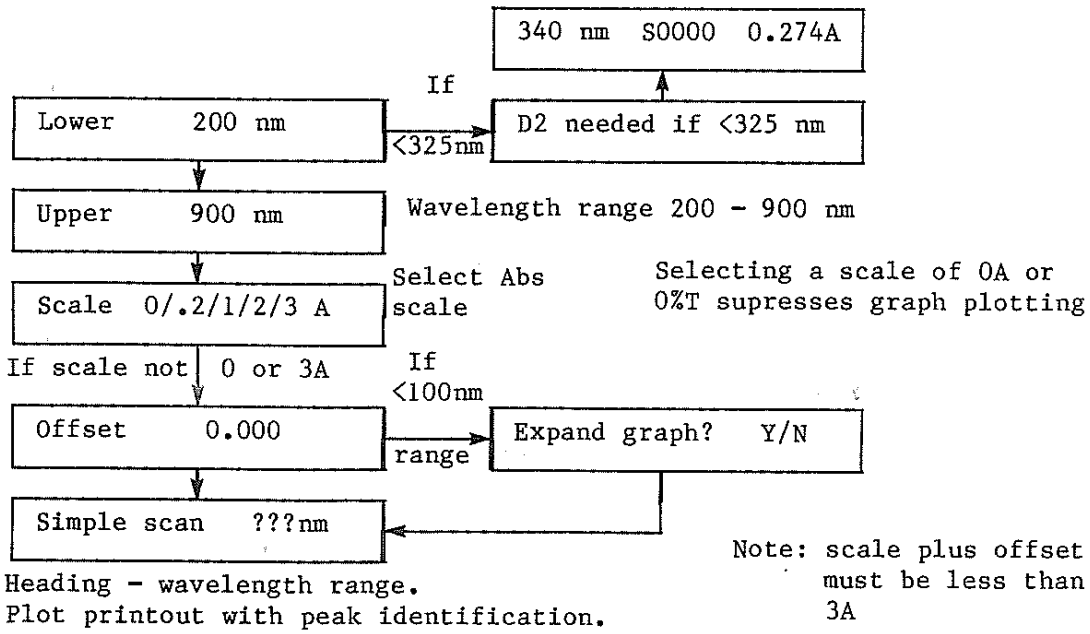
Note This routine must be initiated before a turret is removed from the sample chamber. After the turret has been replaced, this routine allows Ultrospec K to reorganise its mode of presenting cell information to the user. When Autofill K is being fitted into the sample compartment, ensure that it is securely located and plugged in during this procedure. If a water-thermostatted, multicell holder is being used then ensure that cell holder 1 is in the lightpath and that the tubing is not twisted before attempting this procedure otherwise the tubes may tangle.
If this routine is inititiated after a turret has been removed from the sample compartment, the message 'Unknown turret' may be displayed. If this happens, replace the turret, press STOP and carry out the turret replacement routine correctly.

On pressing the CELL NUMBER key, follow the cell replacement routine as outlined in the following display sequence. On changing the turret, only one of the four possible turret recognition paths shown is carried out.



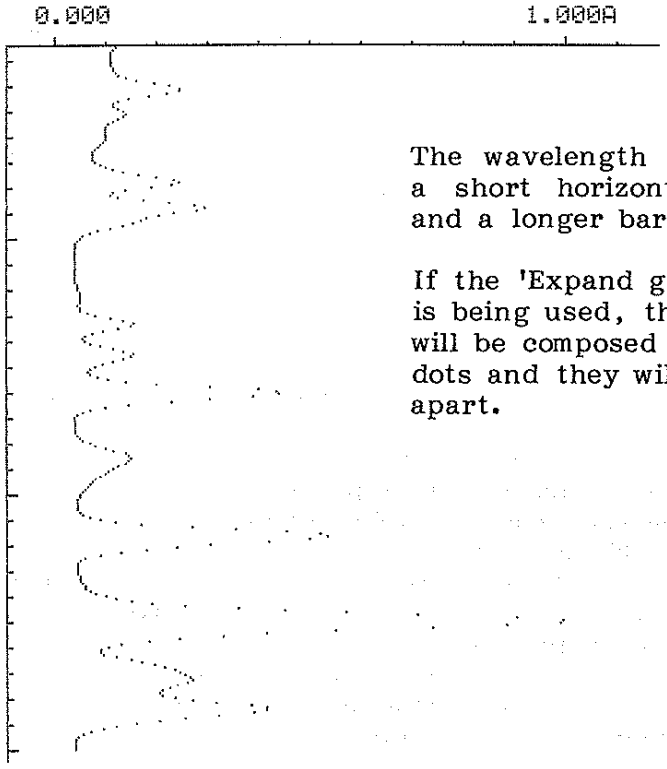
3.17.4 Extn + Peak Check Simple Wavelength Scanning

A simple wavelength scanning feature which includes a plot printout (Abs. or %T vs wavelength) is carried out by pressing these two keys. Each dot of the final plot represents 1 nm of wavelength but if the selected wavelength range is less than 100 nm then the user is given the opportunity of expanding the plot scale with the 'Expand graph' option which replaces the dots with crosses spaced a greater distance apart. After the graph is plotted, the position and magnitude of the peaks found are printed, even if they are outside the absorbance range of the scan selected.
On pressing the EXTN + PEAK CHECK key, the following options are diplayed when in the absorbance mode (a scanning printout is given overleaf):



Simple scan 225 to 500 nm

Wavelength axis marked every 10nm



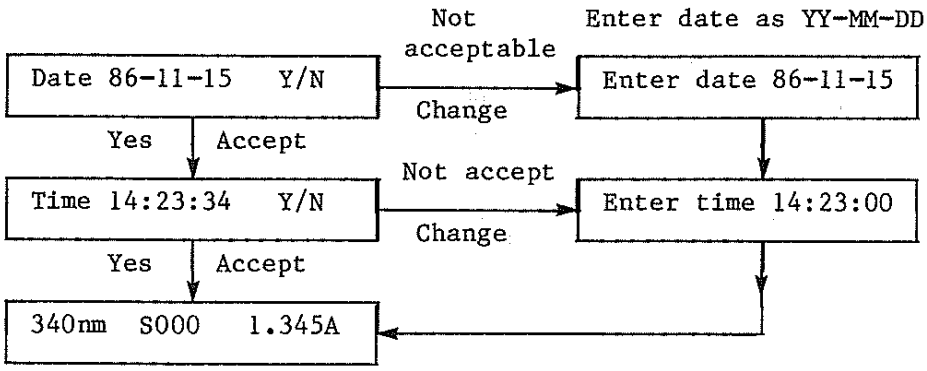
The wavelength axis is marked with a short horizontal bar every 10nm and a longer bar every 100nm.

If the 'Expand graph? Y/N' facility is being used, then the data points will be composed of crosses not dots and they will be spaced further apart.

Wavelength (nm)	Abs (A)
242	0.249
251	0.140
278	0.247
288	0.297
333	0.155
345	0.155
361	0.443
386	0.153
417	0.535
450	0.998
473	0.271
485	0.415

3.17.5 Extn + Temperature Set Date and Time

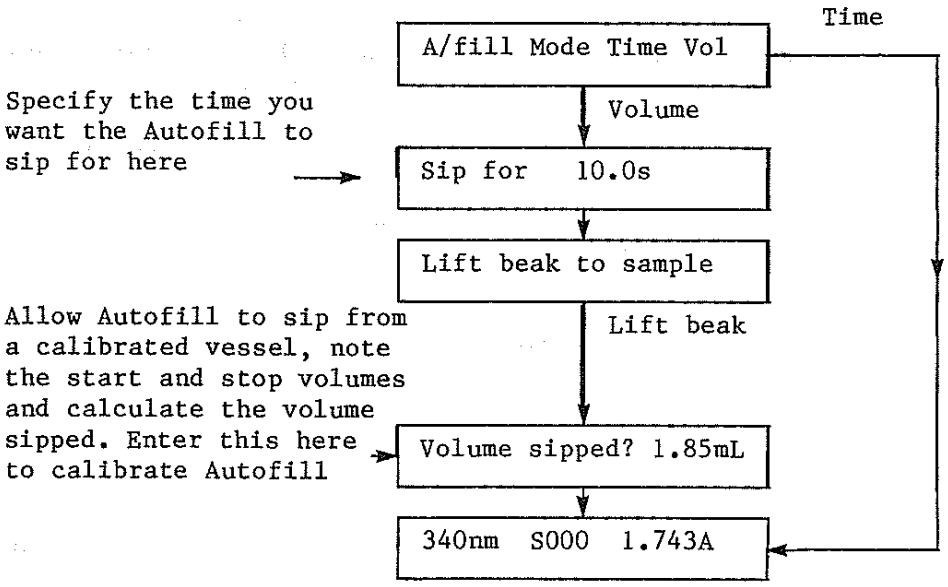
This combination of keys allows the user to set or change the date and time. On pressing the TEMP key the display presents:



The ENTER key must be pressed after each display has been accepted. When it is pressed, the time value is accepted but any seconds set are ignored.

3.17.6 Extn + Autofill Autofill K Calibration

This combination of keys allows the user to calibrate the Autofill in volume mode or to revert to time mode. When the AUTOFILL key is pressed, the following display sequence occurs:



Note i) Autofill calibration is automatically stored in the instrument memory as part of currently selected parameters and can also be stored as an integral part of a program if required.

ii) The Autofill should be calibrated when the PTFE tubing is renewed and each time a liquid differing in viscosity is used.

3.17.7 Extn + Kinetics Number of Readings per Sample

This combination of keys allows the user to set the default number of readings taken during a kinetic measurement. Any number between 2-100 can be set and this is 21 on leaving the manufacturer. On pressing the KINETICS key the following display is presented:

```
No of readings  021
```

This parameter should not require changing often and 21 readings will be found adequate for most applications in the single and auto modes (see section on KINETICS IN MULTI MODE for calculation of readings which can be taken when in this mode). However, if very short or very long reaction times are encountered, then the number of readings can be changed.

Note i) The maximum rate of readings per sample is 1 per second and so if the reaction time in seconds is less than the requested number of readings, this will result in fewer readings than requested.

ii) If a large number of readings is selected then the data processing time will increase.

3.17.8 Extn + Program Baseline Storage

This combination of keys allows the user to transfer baselines from temporary to permanent memory for either the tungsten lamp (W), the tungsten plus D_2 lamp (W+D), or both of these two alternative lamp modes.

On pressing the PROGRAM key the following options will be displayed:

```
Baseline W  W+D  Both
        ↓
Are you sure?  Y/N
        ↓
340nm  CELL3  0.654A
```

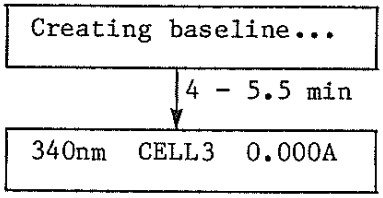
Note If any baselines are currently stored in the memory, then this function will overwrite them.

3.17.9 Extn + Set Ref — New Baseline Creation

This combination of keys allows a new temporary baseline to be created under the lamp conditions selected (either tungsten or tungsten + deuterium). Wait at least 1 hour after instrument switch-on before attempting this function.

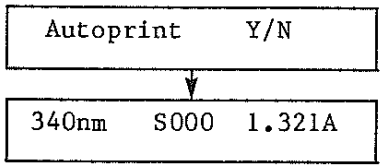
It takes an average time of 4 minutes 10 seconds to create a new baseline when using the tungsten lamp only, and 5 minutes 13 seconds when both lamps are used.

On pressing the EXTN + SET REF key, the display will present:



3.17.10 Extn + Print — Setting the Auto Print Mode

This combination of keys allows the Auto Print Mode to be selected. If 'Y' is entered in response to the 'Auto print Y/N', every time the Autofill beak is lifted or the multi-cell turret changes position the information presented on the display will be printed out along with the time.



WARNING Extn + < will request an access code which if correctly given will bring into use utility functions which are reserved for the sole use of service engineers. Inadvertant use of these utilities may cause resetting of all software and loss of stored programs and baselines.

4. ERROR MESSAGES

An error message will be displayed when either a fault condition arises or an operator error is made. All error messages require a response before the instrument can be operated further. However, if the various responses fail to allow this then contact an LKB service engineer for advice. The table below lists the error messages and the appropriate response to each one.

Error Message	Response
No baseline	Press 'ENTER' if you want to continue operating the instrument without a baseline. To create a new baseline press the Extension key followed by Set Ref (see section 3.17.9). To transfer this baseline to permanent memory use the Extn + Program keys (see section 3.17.8).
Calibration failed	This indicates that the light path is blocked. Trace and remove the reason for the blockage which might be due to a drop of water in the flowcell for instance. Also check to see if any tubing is blocking the light. Switch OFF Ultrospec K and then ON again to recalibrate the instrument. We recommend removal of the sample in the lightpath from any cell holer before undergoing a calibration routine.
No peltier fitted	This indicates that the TEMP key was pressed without the Autofill installed. If the Autofill is installed in response to this message then ensure that the Extn + Cell No. keys are used to enable the instrument to recognise the new cell holder (see section 3.17.3).
No Autofill Fitted	This indicates that the AUTOFILL key was pressed without the Autofill installed. Follow the same response as that outlined above.
Incompatible turret	This indicates that the program being used is incompatible with the cell holder installed. Change either the program or cell holder to achieve compatibility. If the latter, then the Extn + Cell No. keys must be used to identify the cell holder (3.17.3).

Unknown turret	If the 6-cell turret has been fitted into the sample compartment but not screwed down firmly with the result that it rotates loosely, this message may be displayed on operation of the instrument. Cancel the message by pressing STOP and then carry out the turret replacement routine correctly as described in section 3.17.3.
Zero slope	A zero slope has been calculated when in Standard Curve Mode. Check the values reported for the standards. Cancel the message by pressing STOP and then re-read the standards.
No data!	This indicates that there is no data stored in the instrument when the POST RUN key is pressed to recall data stored during measurement of the most recent enzyme kinetics samples. See section 3.13.5, Parameter Setting for further details.
No program	This indicates that a program does not exist in the catalogue location selected when using the PROG(RAM) key to recall a stored program.
D2 lamp failure	The deuterium lamp is no longer working. Check fuse (F6) and/or replace lamp. See section 5.2 This message is cancelled by pressing the STOP key.
D2 lamp not struck	The deuterium lamp has failed to strike at its first attempt. Allow lamp to cool for a few minutes before pressing the D_2/1 key again. This message is cancelled by pressing the STOP key.

5. MAINTENANCE

WARNING If the cover is removed when the deuterium lamp is switched on, high DC voltages and high levels of UV radiation are present. UV protection eyeshields with side windows must be worn and prolonged exposure avoided.
When the instrument is ON and the cover removed, ensure that nothing penetrates the fan guard and contacts the rotating blades.

Only the instrument lamps, fuses and air filter can be maintained by the user. Any other maintenance operation, rectification or repair must be carried out by an LKB service engineer.

5.1 LAMP OUTPUT CHECK

With a new instrument, a stored baseline should ensure that a clear light path reading at any wavelength is typically within ± 0.035 absorbance units of zero. As the lamps age however, their output reduces and the stored baseline becomes less flat; it will show greater deviations from zero (or a spread of more than 0.070 AUs around zero).

If a permanent baseline has NOT been stored during the life of the instrument, the absorbance value displayed at 325 nm for tungsten and at 200 nm for deuterium (D_2) when using a clear light path will indicate the reduction in energy caused by lamp aging. The acceptable range for the tungsten lamp is ± 0.2 AUs and for the deuterium lamp is ± 0.25 AUs.

If new permanent baselines HAVE been re-stored during lamp aging, the values obtained when measuring clear beams will not indicate the total reduction in lamp energy caused by aging.

The D_2 lamp has a life indicator fitted on its power supply connector circuit board (see Fig.5.2). This indicator has a range of 1,000 hours and moves from right to left to show the the age of the lamp. If for any reason a reduction of D_2 lamp energy is suspected, the lamp age indicator should be read before a decision is made to either replace it or realign it.

Misalignment or reduced light output from the tungsten or D_2 lamp results in an increase in absorbance. Realign or replace the lamps as appropriate.

5.2 LAMP REPLACEMENT

WARNING The deuterium and tungsten lamps become very hot during use. Ensure that they are cool before changing them. We recommend that an LKB sevice engineer replaces the deuterium lamp.

To replace either of the two lamps proceed as follows:

i) Switch OFF the instrument and disconnect the mains input.
ii) Release the instrument cover by unscrewing the two screws under the front edge of the instrument base either side of the sample compartment, and the two screws on either side of the cover at the back of the instrument. Lift the cover upwards and tilt backwards taking care to clear the connecting ribbon cable.
iii) Remove the two screws holding the lamp cover in position and lift off.

Note: If either lamp is accidently touched by fingers, the glass must be cleaned with methanol.

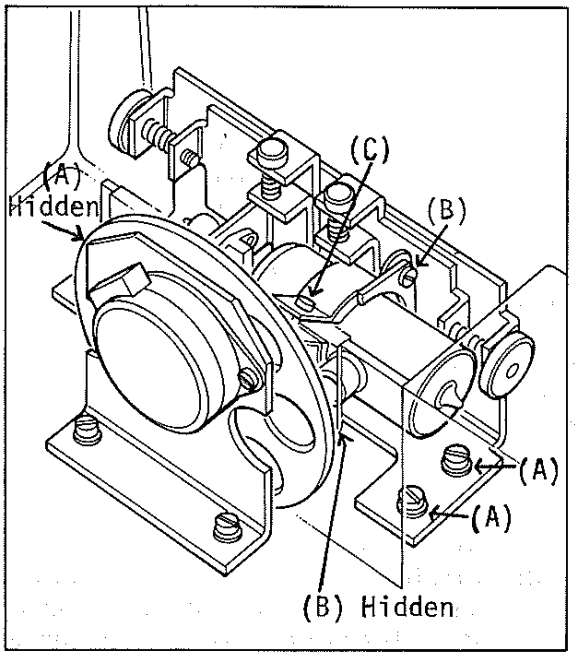


Fig.5.1 Lamp compartment

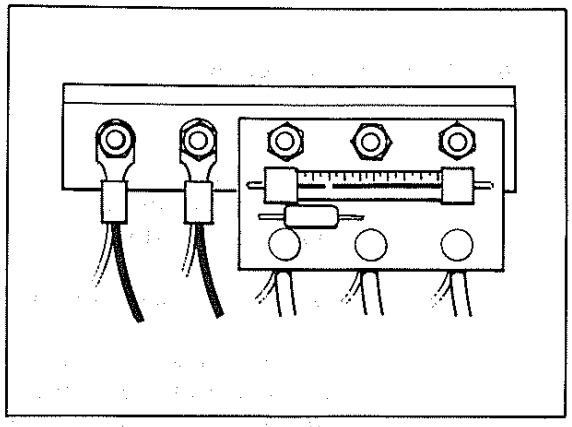


Fig.5.2 Lamp power supply connections

iv) To remove the D_2 lamp from the holder, unscrew the terminal screws on the D_2 printed circuit board (Fig.5.2), unscrew the two locating screws (A) (Fig. 5.1) and remove the lamp assembly. Unscrew the two holder screws (B) and clamp screw (C) to release the lamp.

v) To replace the D_2 lamp, place the lamp squarely in the clamp and holder assembly and fit screws (C) and (B). Refit the holder to the frame. Connect the lamp supply leads to the terminal block.

vi) To remove the tungsten lamp, slide the lamp out of the clip. Slide the replacement lamp into the clip.

vii) Focus the lamps as described in the next section, place the lamp cover in position and replace the retaining screws.
Note: Ensure the lamp supply leads do not obstruct the light path.

viii) Refit the instrument cover.

5.3 LAMP ALIGNMENT

To focus either lamp, proceed as follows with the instrument switched ON:

i) Remove the instrument cover.

ii) **Coarse adjustment:** Switch on the lamp to be focussed and slacken the clamping screw (D) or (E) as appropriate (see Fig.5.3). Place a piece of white paper over the window into the monochromator and vary the vertical and horizontal adjusting screws (F) and (G) or (H) and (J) to centre the light beam on the window (Fig.5.3). Ensure the filter wheel is in the open position on the relevant light beam.

Fine adjustment: Expose the detector lens to view by securing the photometer shutter open (located inside the cell compartment towards the right hand side) with a piece of adhesive tape. Ensure that the tape does not cover the lens or obscure the light path. Cover the cell compartment with a piece of black paper or cloth to exclude room light. Select instrument transmission mode and turn one lamp adjustment screw a little.

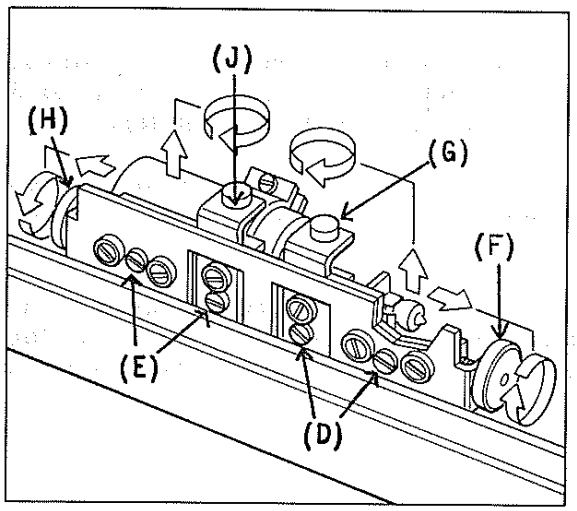


Fig.5.3 Lamp adjustment screws

Before further adjustment, allow a few seconds for the display to stabilise. This procedure should be continued until the maximum value is displayed. Repeat this procedure for the other adjusting screw. Set the wavelength to 360 nm when adjusting lamps.

Note During fine adjustment, the lamp adjusting screws should be moved by very small increments.

iii) Re-tighten the clamping screws, remove tape holding shutter and refit the instrument cover.

Note After changing and aligning lamps, a new permanent baseline should be created and stored (see section 3.17.8 & 9).

5.4 FUSE REPLACEMENT

Details of how to load fuses into Ultrospec K are given in section 2.1.

5.5 AIR FILTER CLEANING

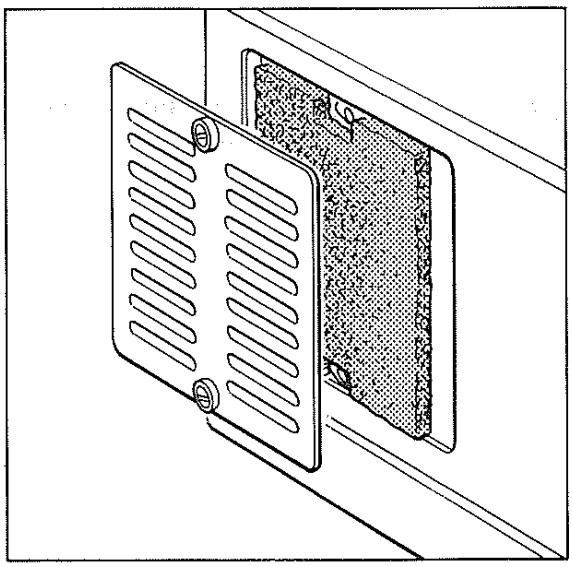


Fig.5.4 The air filter

The air filter (Fig.5.4) is situated behind the air cooling fan plate located on the left hand side of the instrument cover. It is removed by unscrewing the two plate retaining screws and lifting it out. The filter should be cleaned at regular intervals by washing it under running tap water and then drying it thoroughly at room temperature before replacing it.

Note Switch OFF Ultrospec K before removing and cleaning the air filter.

5.6 BATTERY REPLACEMENT

The non-volatile RAM memory, (which is used to store permanent baselines, assay programs and powers the instrument clock) is protected for up to 5 years by means of a long life 3V lithium battery, size DL2N (CR2N).

However, in order to make sure that this memory is fully protected towards the end of the battery life, we recommend that the battery is replaced after approximately four years.

WARNING Print out all stored program parameters before changing the battery because these are lost once the battery is removed. The printed parameters can be used to enter and re-store the programs after a new battery has been installed.

A new battery is installed as follows:

a) Switch OFF Ultrospec K and remove its cover (see 5.2). The battery will be seen on the front panel of the circuit board compartment.
b) Remove the positive lead from the top plate of the battery holder and unscrew the top plate.
c) Take out and replace the battery, positive terminal upwards.
d) Replace the top plate, lead and instrument cover.
e) Switch Ultrospec K back ON.
f) Create and store new baseline (see sections 3.17.8/9).
g) Enter new date and time (see section 3.17.5).
   Note This is essential for accurate Autofill and kinetics timings and hence for correct operation of the instrument.
h) Enter and re-store individual programs (see section 3.16) using the parameters printed out prior to battery replacement.

Note Instrument warranty becomes invalid if non-authorised personnel carry out maintenance procedures other than those detailed above.

6. RS232C INTERFACE

An RS232C interface port (see Figs.2.1 and 6.1) is fitted on the rear panel of 4053 Ultrospec K to enable an external microcomputer to control the operation of the instrument or to allow a printer to be connected for production of hard copy results. This port is initially set to a data rate of 1200 baud, but this rate can be altered by means of the Extn + Mode keys as described under section 3.17.2.

Any meaningful sequence of keys pressed on the Ultrospec K keyboard may be sent as binary ASCII code according to the reference table presented below, and will be interpreted immediately on receipt as if the corresponding key(s) had been pressed in the same order.

In addition to the listed symbols, "?" (3FH), which does not exist on the Ultrospec K keyboard, may be sent. In this case Ultrospec K will respond by transmitting via the RS232C interface an ASCII string representing the reading which may include one or more " " (2OH) as padding depending upon the mode in which the instrument is being used.

Communications Cross-Reference Table

4053 Keyboard	**ASCII**	**Hex**	**Decimal**
SET REF	'S'	53H	83
STOP (Halt)	'H'	48H	72
PRINT	'P'	50H	80
BEAK	'M'	4DH	77
(Left cursor)	'<'	3CH	60
(right cursor)	'>'	3EH	62
ENTER	(CR)	0DH	13
RUN	'R'	52H	82
	'0'	30H	48
D2	'1'	31H	49
MODE	'2'	32H	50
CELL NUMBER	'3'	33H	51
PEAK CHECK	'4'	34H	52
TEMP	'5'	35H	53
AUTOFILL	'6'	36H	54
KINETICS	'7'	37H	55
POST RUN	'8'	38H	56
PROG	'9'	39H	57
EXTN	'.'	2EH	46
does not exist	'?'	3FH	63 returns 4053 status reading in ASCII

Note There are keypress sequences not described above which may affect Ultrospec K and make it appear the the instrument has developed a fault (e.g., the keyboard will not respond). These command sequences were used during manufacture or are used when servicing. Persistent commands can be terminated by pressing the STOP key, ASCII "H" (48H). If the STOP key fails to solve the problem then switch off the instrument and then on again.

6.1 RS232C CONNECTOR

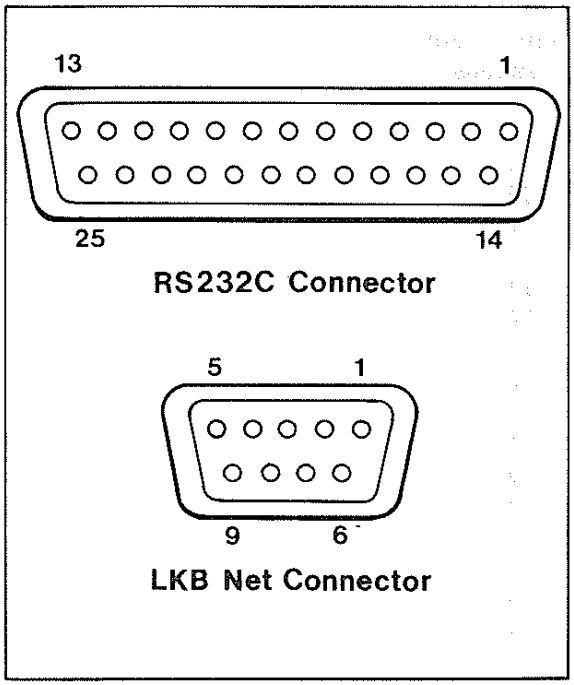
The RS232C connector (Fig.6.1) pins have the following functions:

Pin No.	Signal Title	Function
1	Shield	Cable shielding
2	Receive data	Data from microcomputer
3	Transmit data	Data to microcomputer/printer
7	Ground	Ground

(This configuration conforms to a DCE - data communication equipment)

6.2 LKB NET CONNECTOR

The LKB Net connector (Fig.6.1) is also located on the back panel of the instrument and its presence takes into account future development of LKB instruments which can be linked to Ultrospec K.



The following table lists the LKB Net connector pins and their signals:

LKB Net Pin Connections

Pin No	Signal Title
1	Status
2	Shield
3	Stop
4	Start
5	Ground
6	Disable out
7	Data
8	Disable in
9	Not connected

Fig.6.1 RS232C and LKB Net Connectors

Note Both connectors are fitted to the rear panel with jack screw posts. RS232C or NET cables should therefore be fitted with 4.40 UNC jack screws to provide good connection without the possibility of accidental disconnection. The jack screw ports are also earthed and so if a cable is used which is screened, the screen can be connected to the jack screws to provide maximum interference protection.

II
Autofill K

1. INTRODUCTION

Autofill K is an automatic microvolume sampling device which incorporates a Peltier heating/cooling element, pump system, flowcell and associated tubing. It comes as standard with Ultrospec K and is already fitted into the cell compartment when supplied to the customer. The Autofill is controlled by the electronics of Ultrospec K to which it is linked inside the cell compartment by means of a lead and connector. Parameter setting for microvolume sampling is carried out by using the spectrophotometer keyboard and the unit can be easily calibrated directly in sample volume units.
Autofill K can be used in any of the Ultrospec K measurement modes.

1.1 SPECIFICATIONS

Principle	Peristaltic pump
Modes	Sample recovery, sample to waste and wash
Volume range	0.25 to 9.99 mL
Volume setting	Time or volume modes with calibration facility
Flowcell	10 mm pathlength; 80 uL volume UV grade silica
Temperature control	Peltier heating/cooling element
Temperature range	20 to 50°C in 1°C steps
Temperature accuracy	± 0.1°C at 37°C
Temperature stability	± 0.1°C at constant ambient temp.
Cross contamination	typically 0.5% for 1 mL of aqueous solution
Pump materials	Pumphead tubing 1.6 mm ID 1.6 mm wall thickness Marprene (product of Watson-Marlow): transport tubing 1.0 mm ID 0.5 mm wall thickness PTFE

We reserve the right to alter specifications without notice in accordance with our policy of continuing product development and improvement.

1.2 FITTING AUTOFILL K TUBING

Ultrospec K (4053-011/012) is supplied with Autofill K located in the cell compartment but without its tubing fitted: the tubing, flowcell, beak assembly and waste reservoir is supplied separately as a Fluidics Kit.
Before fitting the tubing as outlined below, remove Autofill K from the Ultrospec K cell compartment to facilitate this procedure.

The 4053 Fluidics Kit contains the following items:

4001-184	Flowcell including inlet and outlet transport PTFE tubing
40 00 5143	Light shield grommet (black plastic blanking plug)
41 52 0245	A length of PTFE transport tubing
42 00 5136	Marprene pump tubing
4153 0112	Waste reservoir bottle
40 00 5152	Modified bottle top
40 00 7168	Beak assembly

Autofill K is fitted with tubing as follows (refer to Fig.1.1):

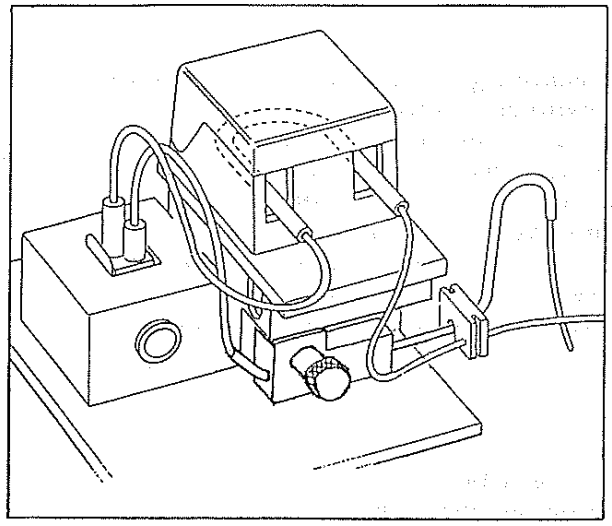


Fig.1.1 Fitting Autofill K tubing

a) Take the beak assembly and feed the piece of transport tubing with the shorter knurled finger screw through from the pivot end (see Fig. 1.1) until a few centimetres exit from the other end. Unscrew the knurled pivot screw from the pump assembly, place the beak assembly onto the pivot screw and replace the screw in the pump assembly. Tighten the pivot screw and ensure the beak assembly moves freely up and down to operate the microswitch.

b) Remove the protective tape from the top of the flowcell and screw the inlet PTFE transport tubing from the beak assembly into the top of the flowcell directly above the white arrow painted on the front of the flowcell. See Fig 1.1.

c) Screw the outlet PTFE transport tubing (by means of the longer, knurled finger screw) into the top of the flowcell.

d) Place the flowcell in the flowcell compartment. The tubing exiting from the Autofill beak should be pulled out until the tubing slack is taken up to leave it routed as indicated in Fig.1.1.

e) Cut the flowcell outlet PTFE transport tubing to a length of 15 cm and push the free end into one end of the Marprene pump tubing until a tight fit is achieved.

f) Push one end of the PTFE transport tubing supplied (41 52 0252) into the other end of the pump tubing until a tight fit is achieved.

g) Raise the transparent plastic lid of the pump head compartment, lay the flowcell end of the pump tubing in its locating slot and then feed the tubing around the backplate by rotating the pump rollers by hand in a clockwise direction to lead the tubing into the correct position as indicated in Fig.1.1.

Note Ensure that the PTFE transport tubing does not cause kinking of the pump head tubing when both types of tubing are connected and the pump tubing is fitted in the pump compartment.

h) Lay the outlet end of the pump tubing in its locating slot, fit each end of the pump tubing into the two sprung white plastic clamps by pulling back the higher inside arm of each clamp, and then close the pump compartment lid.

i) Feed the transport tubing which goes to the waste reservoir bottle (and connected to the pump tubing) through the hole in the light shield grommet and pull until the tubing slack is taken up and left under slight tension. Before Autofill K is used, the free end of the waste tubing should also be pushed through the hole in the bottle top screwed onto the waste reservoir bottle.

j) Replace Autofill K in the Ultrospec K cell compartment ensuring that when the light shield grommet is fitted onto the front lower lip of the cell compartment, it passes over the base of the Autofill beak. See following section 1.3, Fitting Autofill K, before replacing Autofill K.

Note Before operating Autofill K, ensure that you have carried out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.3 FITTING AUTOFILL K

Autofill K is refitted as follows after removing a multi-cell holder or single cell holder including the accessory baseplate:

a) Remove the small black plastic blank currently fitted into the front lower lip of the cell compartment (and save for re-use).
b) Place the Autofill in the cell compartment so that the two front and single rear knurled mounting screws locate with the corresponding three threaded holes in the floor of the cell compartment. Check that the position of the Autofill and flowcell (which should be full of bubble-free water) is optimal by moving the baseplate until a minimum absorbance reading is obtained. To avoid baseplate distortion, carefully tighten each of the mounting screws a small amount clockwise until the screws are 'finger tight' (reverse this procedure for Autofill removal).
c) Plug the Autofill lead into the socket provided in the left hand wall of the sample compartment and lock the connector.
d) Fit the slotted black plastic blank (light shield grommet) required for the Autofill over the Autofill beak and onto the front lower lid of the sample compartment. The tubing to waste should already be fitted through the hole in this blank but if it is not (as when fitting the tubing - see Fig.1.1) then pass it through the hole before fitting the blank.
e) Close the cell compartment cover.
f) Use the Extn + Cell No. keys of Ultrospec K to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

2. AUTOFILL OPERATION

Autofill K can be used in any of the Ultrospec K measurement modes:

In the basic Ultrospec measurement mode simply by pressing the AUTO-FILL/6 key after which the Autofill parameters are set, see section 3.12, part I..

In the Kinetics measurement mode by either:

a) pressing both the AUTOFILL/6 key (after which the Autofill parameters are set) and KINETICS/7 key (after which the Kinetics mode is selected, Autofill use is selected and the kinetics parameters set), see section 3.13.2, part I.
b) recalling a stored program which already incorporates kinetic mode and Autofill parameters by pressing the PROG/9 and RUN keys

In the Standard Curve measurement mode:

a) pressing both the AUTOFILL/6 key (after which the Autofill parameters are set) and KINETICS/7 key (after which the Standard Curve mode is selected, Autofill use is selected and the standard curve parameters set), see section 3.13.4, part I.
b) recalling a stored program which already incorporates standard curve mode and Autofill parameters by pressing the PROG/ 9 and RUN keys

Autofill K parameter setting is described in section 3.12, part I of this instruction manual.
Autofill calibration is described in section 3.17.6, part I of this instruction manual.

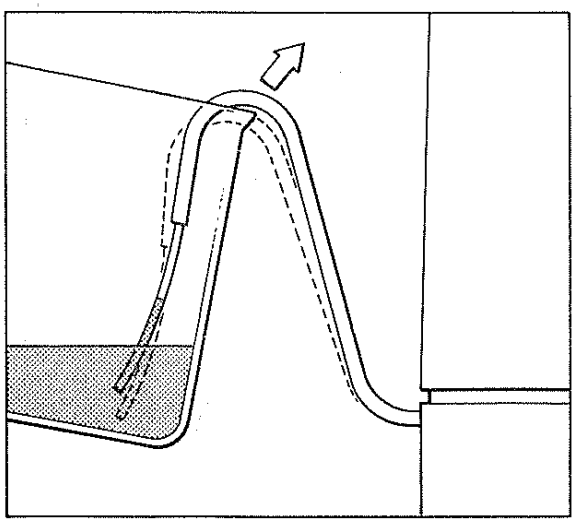


Fig.2.1 The lift beak action

Whenever an Autofill program is fully operational, the instruction 'Lift beak' will be displayed at appropriate times during the assay run. This is carried out by lifting the Autofill beak with either a test tube, beaker or other suitable vessel as shown in Fig.2.1. This momentary action will draw the required volume of test sample into the correct position in the flowcell and initiate sample measurement.

When you hear a 'beep' sound, remove the sample vessel to allow the 'pull' volume to be drawn in. You have 1 s in which to do this before the 'pull' volume is drawn in.

When the Autofill K parameters are being set, you will be asked to enter a time for the 'pull'. This refers to the pull volume which is the volume required to pull the sip volume into the optimum position in the flowcell (equivalent to the volume of air drawn in behind the sip volume to achieve this).

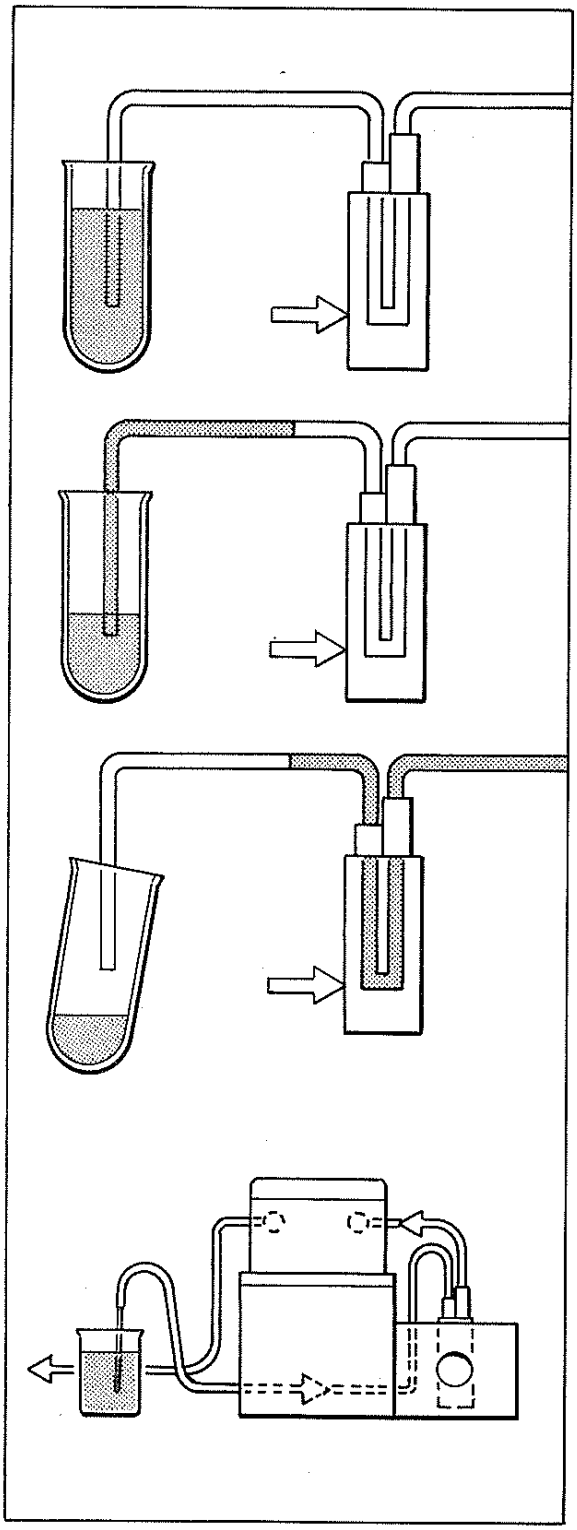


Fig.2.2 Liquid flow sequence after lifting the Autofill beak

Both the sip and pull volumes are illustrated in Fig.2.2 opposite. After the beak has been lifted by the sample vessel, the sample volume is drawn into the tubing as illustrated in the first two diagrams of the sequence shown in the figure.

When the 'beep' sound is heard the vessel should be removed to allow the pull volume to be drawn in. A programmed delay time of 1 second after the 'beep' has sounded allows you to remove the sample vessel before the pull action begins.

The third diagram in Fig.2.2 shows the optimum position of the test sample in the flowcell after the pull time has elapsed.

The fourth diagram illustrates the the flow of test sample or wash solution from the input vessel through the flowcell and out to waste.

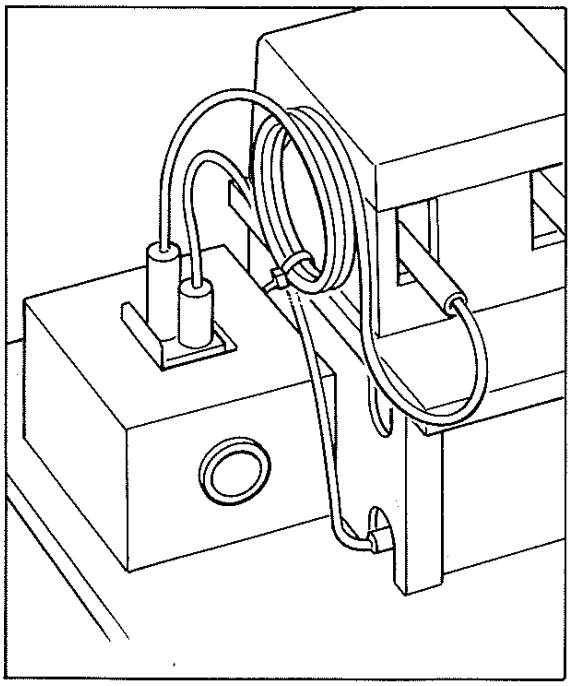


Fig.2.3 Position of 'buffer' tubing

If you want to save test samples for further analysis then we recommend the use of an extra length of cleaned 'buffer' tubing fitted as shown in Fig. 2.3. This will allow each sample to be returned after measurement to a collecting vessel with no (or minimal) contamination from the larger pump tubing with which samples will have no contact.

3. MAINTENANCE

Only the Autofill K flowcell and pump tubing are user maintained. Any other maintenance operation or rectification requires an LKB service engineer.

3.1 GENERAL MAINTENANCE

The following procedure should be carried out to maintain cleanliness of the unit, minimise sample contamination and prolong tubing life:

a) At the end of each working day, flush the system with a dilute solution of suitable non-residual surfactant detergent and leave the system filled with solution. Before commencing operation, flush the system with a sufficient volume of distilled water.
b) Release the tension of the pump tubing by removing it from the pump head compartment (see section 3.3 below) if the Autofill unit is likely to remain unused for long periods.

3.2 FLOWCELL CLEANING

The flowcell should be cleaned at regular intervals. Cleaning is necessary when small air bubbles form and remain in the flowcell upon filling.
The flowcell is cleaned as follows:

a) Prepare a suitable cleaning solution (such as methanol or a non-residual surfactant detergent).
b) Using the Autofill wash mode (see section 3.12, part I of this instruction manual), flush the flowcell and associated tubing thoroughly with the cleaning solution.
c) Flush the system through with a sufficient volume of distilled water, again using the wash mode.

If the above procedure fails to clean the flowcell sufficiently, then perform the following more vigorous cleaning procedure:

a) Remove the tubing connectors from the top of the flowcell.
b) Introduce a suitable cleaning solution (such as a dilute chromic acid solution) by means of a syringe and ideally leave overnight
   WARNING CHROMIC ACID IS CORROSIVE AND TOXIC.
   HEAT IS GENERATED IF CHROMIC ACID IS MIXED WITH WATER. HANDLE WITH CARE.
c) Remove the cleaning solution using a syringe.
d) Reconnect the tubing connectors.
e) Flush the flowcell with a sufficient volume of distilled water using the Autofill wash mode.

3.3 REPLACING PUMP TUBING

To replace the Autofill pump tubing proceed as follows:

a) Switch OFF Ultrospec K.
b) Raise the transparent plastic cover of the pump head.
c) Remove one end of the pump tubing from the sprung white plastic clamp by pulling back the higher inside arm of the clamp. Unwind the tube from the pump head compartment, rotating the pump head by hand while doing this to facilitate the operation.
d) Remove the other end of the pump tubing from its clamp as described above.

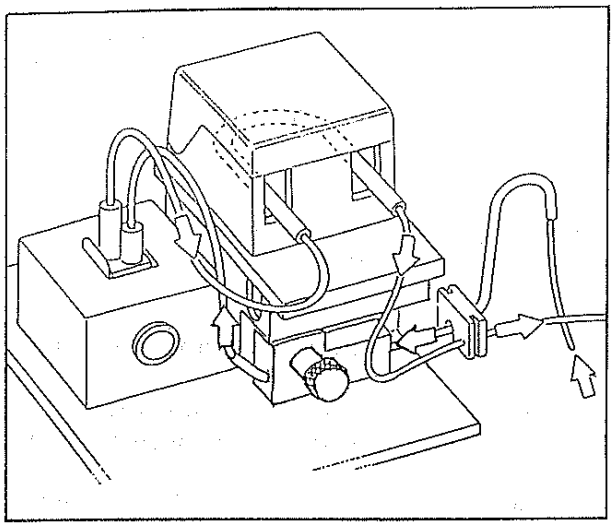


Fig.3.1 Positioning of Autofill tubing

e) Pull out the PTFE transport tubing from each end of the Marprene pump tubing.
f) Connect a new piece of pump tubing 200 mm long, as supplied by LKB, to the two pieces of PTFE tubing. Ensure that the new tubing is pushed fully onto the connectors.
g) Refit one end of the pump tubing into the locating slot of the pump head compartment and then feed the tubing around the backplate by rotating the pump rollers by hand to lead the tubing into the correct position.
h) Refit the other end of the pump tubing into the locating slot. Ensure that the PTFE transport tubing lines do not cause kinking of the Marprene pumphead tubing when the latter is reconnected, and that the tubing is under slight tension.
i) Close the plastic pump head cover.
j) Switch Ultrospec K back ON..
k) Operate the system in both the waste and return modes (see section 3.12, part I of this instruction manual) a number of times to ensure that the pump tubing is located and functioning correctly. The correct liquid flow when in the waste mode is shown in Fig.3.1 as are the correct tube connections and positioning.
l) Flush the system with a suitable non-residual surfactant detergent followed by a large volume of distilled water.

Note Instrument warranty becomes invalid if non-authorised personnel carry out maintenance procedures other than those recommended in the user maintenance sections of this instruction manual, or if they carry out repair work.

III
Ultrospec K Accessories

1. 4072 CELL AND TEST TUBE HOLDERS

1.1 4072-001 FINGER SCREW

The accessory finger screw must be used with each of the six and four-position accessory cell holders. The threaded end of the finger screw passes through the central location hole of the multi-cell holder and is used as such to locate and secure the cell holder in the cell compartment.

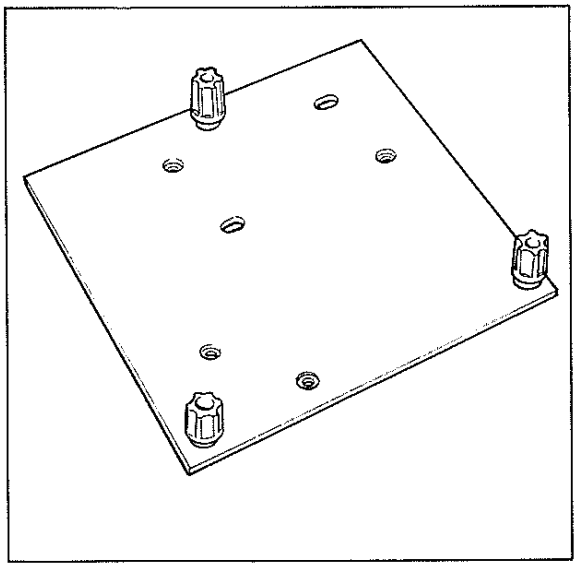
1.2 4072-100 BASEPLATE

The accessory baseplate (Fig.1.1) is secured to the bottom of the cell compartment and is used as a fixed base on which single cell and test tube holders can be located. This baseplate must be used with the following accessories:

4072-020	Cell holder for a single rectangular cell or flowcell of up to 10 mm pathlength
4072-030	Cell holder for a single rectangular cell or flowcell of up to 50 mm pathlength
4072-050	Cell holder for a 100 mm pathlength cylindrical cell
4072-061	Test tube holder for test tubes 9-16 mm OD and up to 100 mm in height
4072-070	Single water heated cell holder for cells of up to 40 mm pathlength

1.2.1 INSTALLATION

The accessory baseplate is installed as follows:



a) Raise the cell compartment cover of Ultrospec K and remove the six or four-position cell holder, or Autofill K if present.
b) Place the accessory baseplate into the cell compartment so that the three knurled mounting screws locate with the threaded holes in the cell compartment base.
c) To avoid baseplate distortion, carefully tighten each of the mounting screws a small amount clockwise until the screws are 'finger tight'.
d) To avoid baseplate distortion on removal, the reverse of procedure c) should be carried out.

Fig 1.1 Accessory baseplate

1.3 4072-015 FOUR-POSITION CELL HOLDER

The four position long pathlength cell holder (Fig.1.2) can accommodate cells of up to 50 mm pathlength. It is used in conjunction with the 4072-001 finger screw accessory.

1.3.1 INSTALLATION

The 4072-015 cell holder is installed as follows:

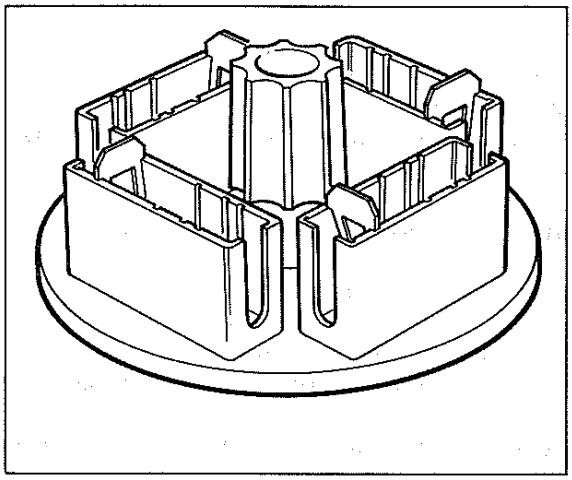


Fig.1.2 Four-position cell holder

a) Raise the cell compartment cover of Ultrospec K and remove either Autofill K or any other cell holder (and baseplate if necessary) present.
b) Place the four-position cell holder in the cell compartment so that the central knurled mounting screw locates with the central threaded hole in the cell holder pulley, and the off-centre locating peg fits into the well provided in the pulley. Tighten the screw 'finger tight'.
c) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
d) Close the cell compartment cover.

e) Use the Extn + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.4 4072-088 WATER HEATED FOUR-POSITION CELL HOLDER

The water heated four position cell holder (Fig.1.3) can accommodate cells of 10 mm pathlength only. It is used in conjunction with the 4072-001 finger screw accessory and a circulating water bath.

1.4.1 INSTALLATION

The 4072-088 cell holder is installed as follows:

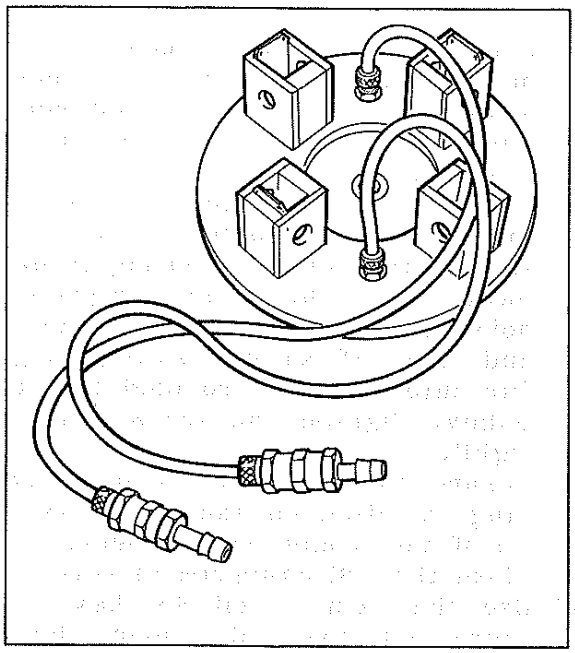


Fig.1.3 Water heated four-position cell holder

a) Raise the cell compartment cover of Ultrospec K and remove either Autofill 'K or any other cell holder (and baseplate if necessary) present.
b) Place the 4072-088 cell holder in the cell compartment so that the central knurled mounting screw locates with the central threaded hole in the cell holder pulley, and the off-centre locating peg fits into the well provided in the pulley. **Ensure that cell 1 is in the light path.** Tighten the screw 'finger tight'.
c) Locate the small plastic block and spacer supplied and lead the two water pipes (tubing) through the holes provided. (See Fig.1.6 section 1.6).
d) Remove the small black plastic blanking plug fitted on the front lower lip of the cell compartment (and save for future use).
e) Lead the two water pipes (tubing) through the hole created and through the replacement blanking plug provided. Fit the blanking plug.
f) Fit the small plastic block to the lower left corner of the cell compartment with the screw and spacer allowing sufficient slack in the pipes to allow the cell holder to rotate.
g) Connect the water pipes to a standard laboratory circulating water bath.
h) Close the cell compartment cover.
i) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

Note i) To avoid overly twisting the cell holder tubing, this accessory will rotate fully in one direction followed by a full rotation in the opposite direction.
ii) Allow test samples to reach the required temperature before performing measurements.

1.5 4072-005 SIX-POSITION CELL HOLDER

The six position long pathlength cell holder (Fig.1.4) can accommodate cells of up to 10 mm pathlength. To aid identification, position 1 cell holder is blue, positions 2 to 6 being black. This cell holder is used in conjunction with the 4072-001 finger screw accessory.

1.5.1 INSTALLATION

The 4072-005 cell holder is installed as follows:

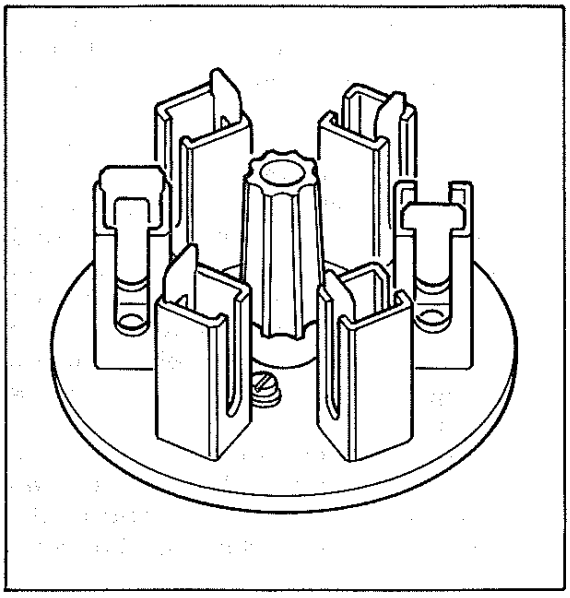


Fig.1.4 Six-position cell holder

a) Raise the cell compartment cover of Ultrospec K and remove either Autofill K or any other cell holder (and baseplate if necessary) present.
b) Place the six-position cell holder in the cell compartment so that the central knurled mounting screw locates with the central threaded hole in the cell holder pulley, and the off-centre locating peg fits into the well provided in the pulley. Tighten the screw 'finger tight'.
c) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
d) Close the cell compartment cover.
e) Use the Extn + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.6 4072-090 WATER HEATED SIX-POSITION CELL HOLDER

The water heated six position cell holder (Fig.1.5) can accommodate cells of 10 mm pathlength only. It is used in conjunction with the 4072-001 finger screw accessory and a circulating water bath.

1.6.1 INSTALLATION

The 4072-090 cell holder is installed as follows:

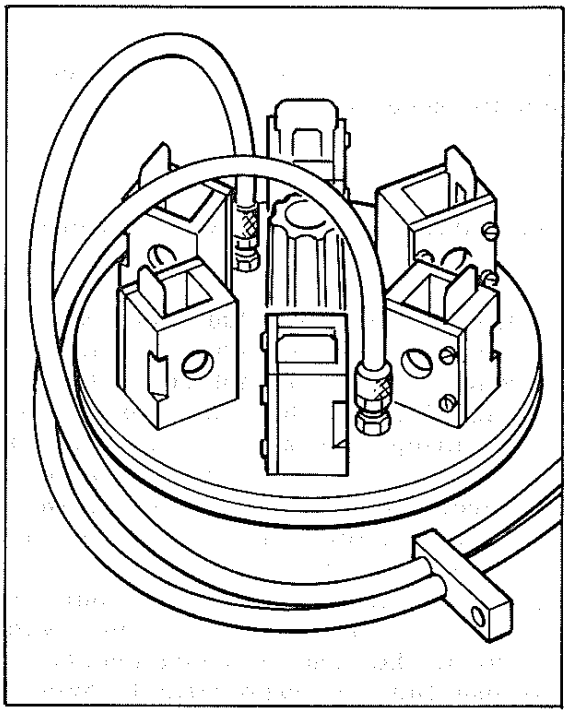


Fig.1.5 Water heated six-position cell holder

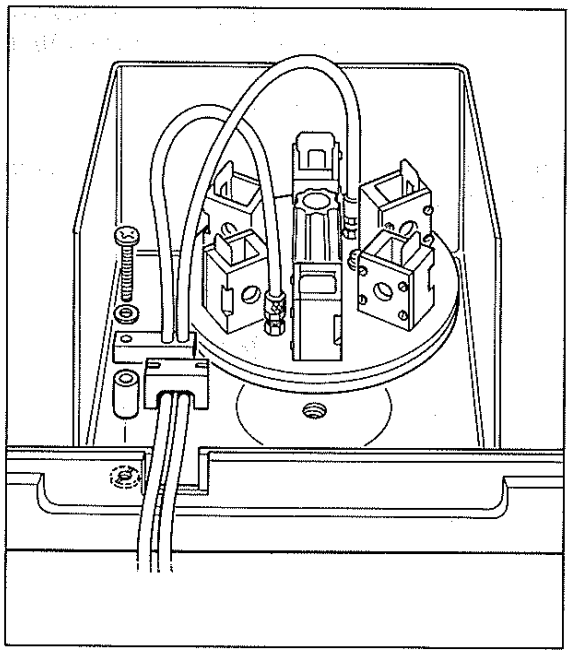


Fig.1.6 Fitting the tubing

a) Raise the cell compartment cover of Ultrospec K and remove either Autofill K or any other cell holder (and baseplate if necessary) present.
b) Place the 4072-090 cell holder in the cell compartment so that the central knurled mounting screw locates with the central threaded hole in the cell holder pulley, and the off-centre locating peg fits into the well provided in the pulley. **Ensure that cell 1 is in the light path.** Tighten the screw 'finger tight'.
c) Locate the small plastic block and spacer supplied and lead the two water pipes (tubing) through the holes provided. (See Fig.1.6).
d) Remove the small black plastic blanking plug fitted on the front lower lip of the cell compartment (and save for future use).
e) Lead the two water pipes (tubing) through the hole created and through the replacement blanking plug provided. Fit the blanking plug.
f) Fit the small plastic block to the lower left corner of the cell compartment with the screw and spacer allowing sufficient slack in the pipes to allow the cell holder to rotate.
g) Connect the water pipes to a standard laboratory circulating water bath.
h) Close the cell compartment cover.
i) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

Note i) To avoid overly twisting the water heated six-position cell holder tubing, this accessory will rotate fully in one direction followed by a full rotation in the opposite direction.

ii) Allow test samples to reach the required temperature before performing measurements.

1.7 4072-020 SINGLE CELL HOLDER

This cell holder (Fig.1.7) accommodates a single cell of 10 mm pathlength and must be used with the 4072-100 baseplate accessory.

1.7.1 INSTALLATION

The 4072-020 cell holder is installed as follows after removing Autofill K or a multi-cell holder:

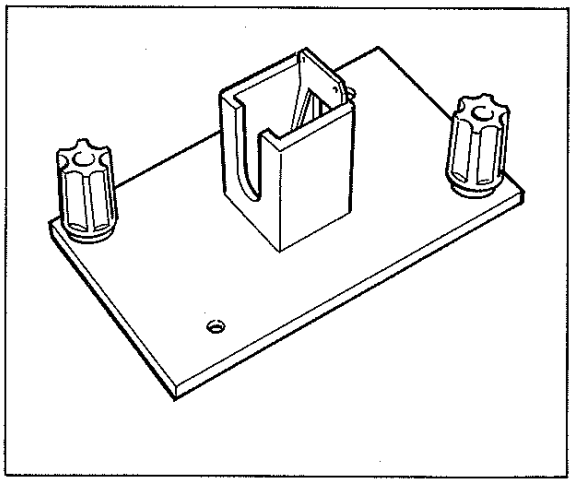


Fig.1.7 Single cell holder

a) Install the 4072-100 baseplate as described in section 1.2.1 above.
b) Place the cell holder onto the baseplate so that the two knurled mounting screws locate with the threaded holes in the baseplate.
c) Carefully tighten each screw a small amount until the screws are 'finger tight'.
d) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
e) Close the cell compartment cover.
f) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.8 4072-030 SINGLE RECTANGULAR CELL HOLDER

This cell holder (Fig.1.8) accommodates a single rectangular cell of up to 50 mm pathlength or a flowcell of up to 50 mm pathlength. The 4072-100 baseplate accessory must be used with this cell holder.

1.8.1 INSTALLATION

The 4072-030 cell holder is installed as follows after removing Autofill K or a multi-cell holder:

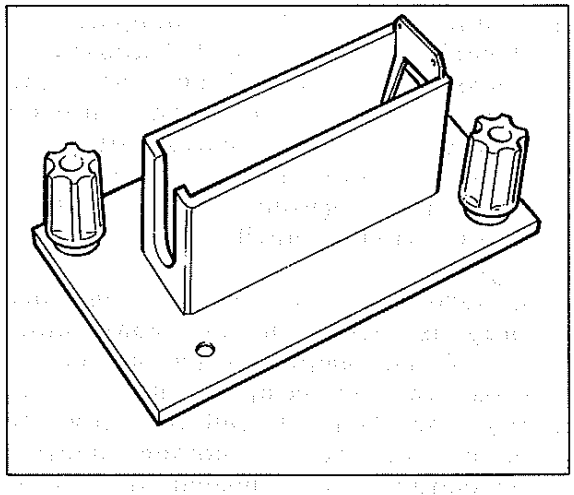


Fig.1.8 Single rectangular cell holder

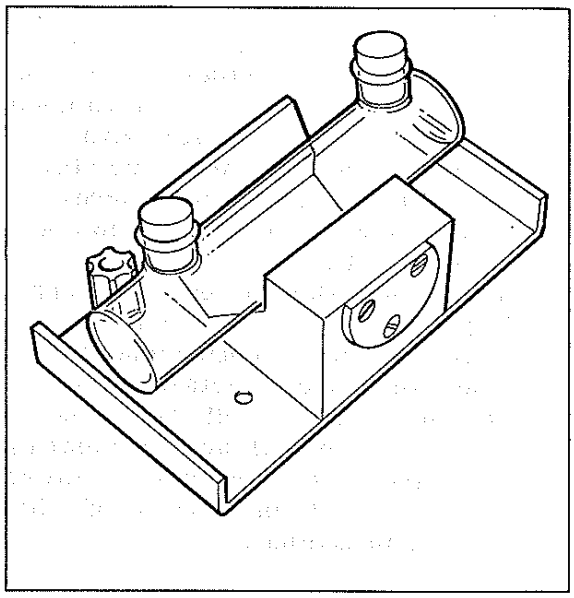
a) Install the 4072-100 baseplate as described in section 1.2.1 above.
b) Place the cell holder onto the baseplate so that the two knurled mounting screws locate with the threaded holes in the baseplate.
c) Carefully tighten each screw a small amount until the screws are 'finger tight'.
d) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
e) Close the cell compartment cover.
f) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.9 4072-050 SINGLE CYLINDRICAL CELL HOLDER

This cell holder (Fig.1.9) accommodates a single cylindrical cell of path-length 100 mm x 22 mm OD. The 4072-100 baseplate accessory must be used with this cell holder.

1.9.1 INSTALLATION

The 4072-050 cell holder is installed as follows after removing Autofill K or a multi-cell holder:



a) Install the 4072-100 baseplate as described in section 3.2 above.
b) Place the cell holder onto the baseplate so that the knurled mounting screw locates with the threaded hole in the baseplate.
c) Carefully tighten the screw a small amount until it is 'finger tight'.
d) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
e) Close the cell compartment cover.
f) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

Fig.1.9 Cylindrical cell holder

1.10 4072-070 WATER HEATED SINGLE CELL HOLDER

This water heated single cell holder (Fig.1.10) accommodates a cell of up to 40 mm pathlength. The 4072-100 baseplate accessory and a standard laboratory circulating water bath must be used with this cell holder.

1.10.1 INSTALLATION

The 4072-070 cell holder is installed as follows after removing Autofill K or a multi-cell holder:

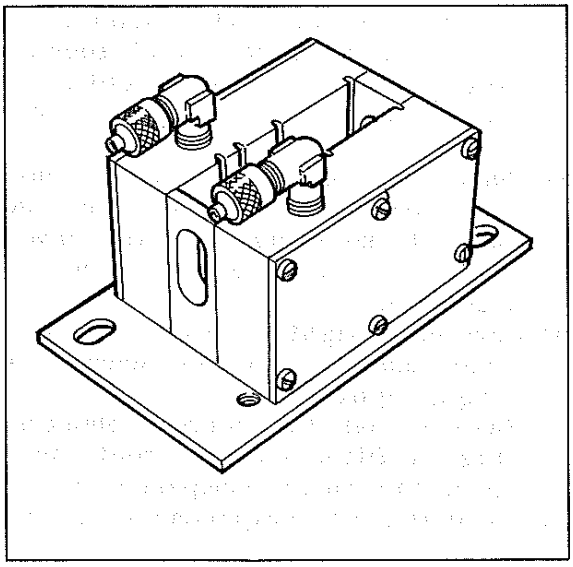


Fig.1.10 Water heated single cell holder

a) Install the 4072-100 baseplate as described in section 1.2.1 above.
b) Place the cell holder onto the baseplate so that the two knurled mounting screws locate with the threaded holes in the baseplate.
c) Carefully tighten each screw a small amount until the screws are 'finger tight'.
d) Remove the small plastic blanking plug fitted on the lower lip of the cell compartment (save for future use).
e) Lead the two water pipes (tubing) from the cell holder through the hole created and through the replacement blanking plug. Fit the blanking plug.
f) Close the cell compartment cover.
g) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

1.11 TEST TUBE HOLDER

The test tube holder (Fig.1.11) accommodates test tubes 9 to 16 mm in diameter and up to 100 mm in height. The 4072-100 baseplate accessory must be used with this test tube holder.

1.11.1 INSTALLATION

The 4072-061 test tube holder is installed as follows after removing Autofill K or a multi-cell holder:

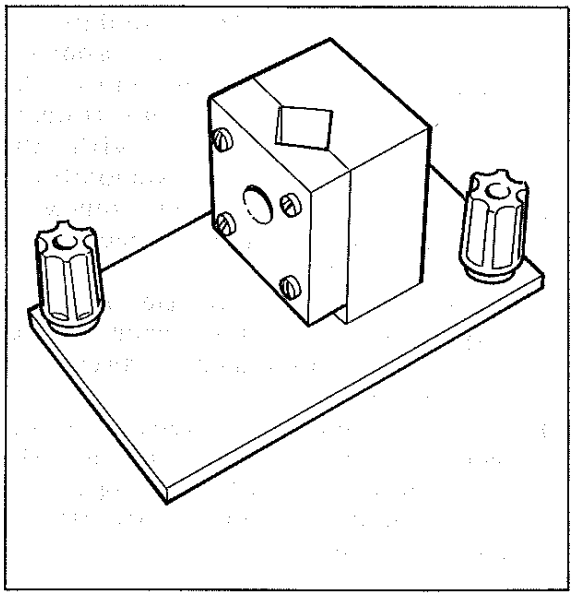


Fig.1.11 Test tube holder

a) Install the 4072-100 baseplate as described in section 1.2.1 above.
b) Adjust the test tube holder to accommodate the required test tube.
c) Place the test tube holder onto the baseplate so that the two knurled mounting screws locate with the threaded holes in the baseplate.
d) Carefully tighten each screw a small amount until the screws are 'finger tight'.
e) Ensure that the correct blanking plug is fitted on the front lower lip of the sample compartment.
f) Close the cell compartment cover.

g) Use the Extn. + Cell No. keys to carry out the cell holder identity procedure as described under section 3.17.3 in part I of this instruction manual.

2. 4001-184 REPLACEMENT AUTOFILL FLOWCELL

This replacement flowcell for Autofill K comes complete with tubing kit. Refer to part II, Autofill K, of this instruction manual for details of how to use this flowcell.

3. 4004 REPLACEMENT LAMPS

Replacement deuterium and tungsten halogen lamps are supplied by LKB. The part nos. for these are:

4004-050 Deuterium lamp
4004-060 Tungsten halogen lamp

See the following sections of part I of this instruction manual to help with lamp checking and replacement:

5.1 Lamp output check
5.2 Lamp replacement
5.3 Lamp alignment

4. 4073-231/2 EXTERNAL PRINTER

The external printer used with Ultrospec K is an Epson FX-80 series dot matrix printer. This is supplied by LKB complete with the RS232C serial interface required to link the printer to Ultrospec K.
Use of an external printer disables the built-in printer but otherwise the information printed is identical to that of the built-in printer.
If the user already has an Epson FX-80 printer incorporating a parallel interface, then LKB can supply the RS232C interface as an optional accessory to upgrade the printer for use with Ultrospec K. The part no. for this interface is 4073-236 which comprises a printed circuit board, earth wiring and detailed instructions on how to install the RS232C interface in the Epson FX-80 printer.

The part nos. for the external printer are:

4073-231 External digital printer for use at 110/120 V, 50/60 Hz
4073-232 External digital printer for use at 220/240 V, 50/60 Hz

Each printer is supplied with detailed installation and operating instructions. After the external printer has been connected, the user will have to carry out an identity routine by pressing the Extn + Mode keys as described in section 3.17.2, part I of this instrument manual.

Note If the external printer fails to respond, first check to see if 'Y' has been answered in response to the 'External printer Y/N' prompt which will appear as one proceeds with the above identity routine.

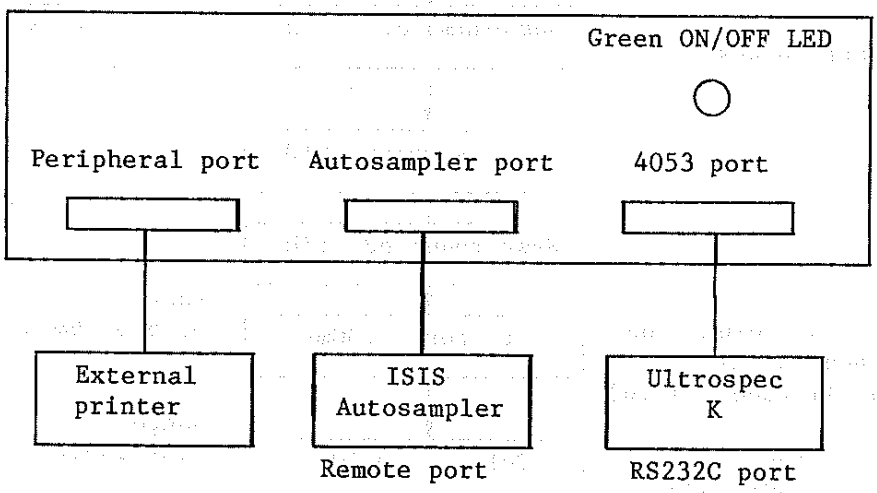
5. 4076-011/2 AUTOSAMPLER INTERFACE

An ISCO ISIS* Autosampler can be used with the LKB Ultrospec K spectrophotometer if the appropriate interface is used to facilitate this. Such an interface is manufactured by LKB Biochrom Ltd. as an accessory having the part number:

4076-011 for operation at 110 V
4076-012 for operation at 220 V

The Ultrospec K spectrophotometer, ISIS Autosampler and autosampler interface are interconnected as shown in the following diagram.
The cable linking Ultrospec K to the autosampler interface is supplied by LKB. It should be noted that as the RS232C port of Ultrospec K is now used for this link-up, the external printer port is now transferred to the autosampler interface.

LKB Autosampler Interface



The user should refer to the ISIS Autosampler Instruction Manual for instructions on how to operate the autosampler. Details of how to connect the Autofill inlet tubing to the white autosampler tubing guide is described in the instructions provided with LKB Autosampler Interface.

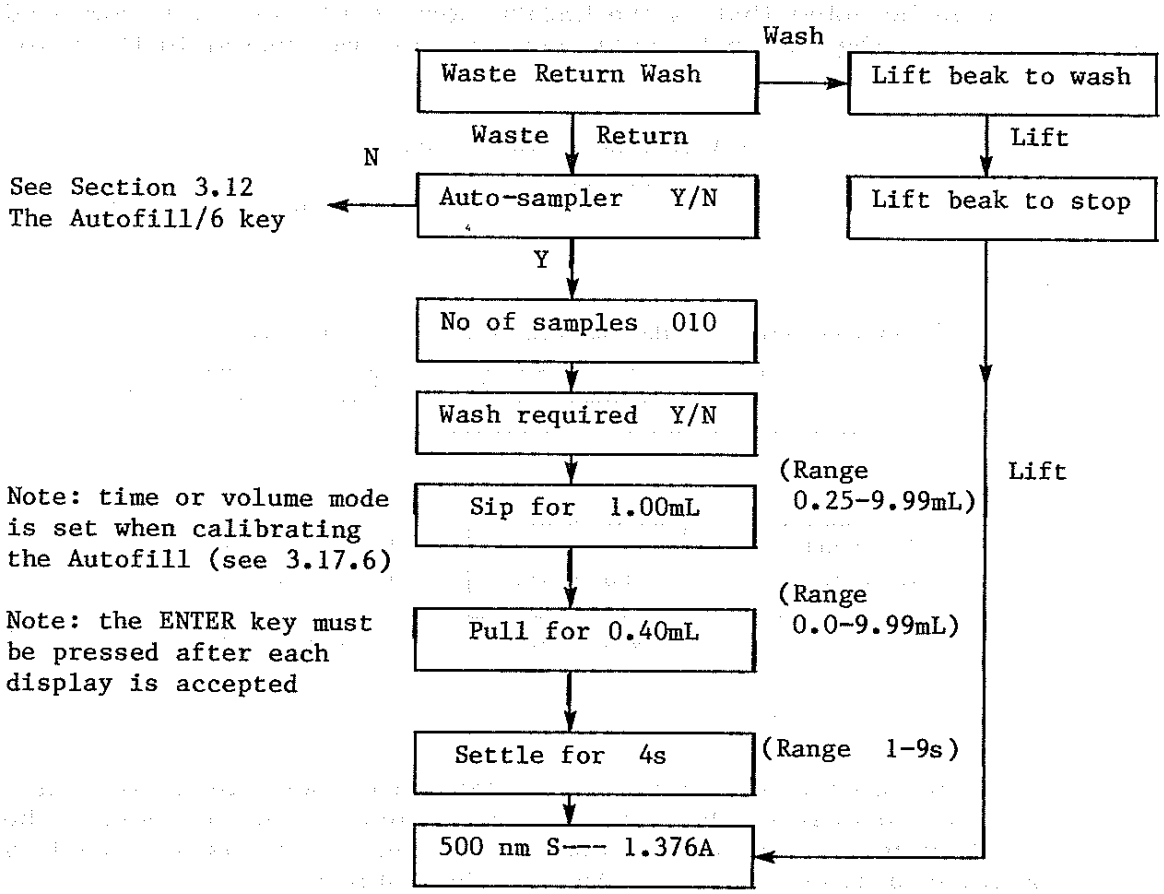
The screen display sequence presented by Ultrospec K when used in conjunction with Autofill K and the ISIS Autosampler is given overleaf.

* ISIS is a trademark of Isco, Inc., USA.

5.1 Parameter Setting for the ISIS Autosampler

Parameter setting for the ISIS Autosampler is similar to that described in section 3.2, The Autofill/6 Key. When this key is pressed the following display sequence will be presented to which the user must respond in order to achieve the desired parameter setting.

Note: As the ISIS Autosampler is used in conjunction with the Autofill K sample introduction system, it is important to ensure that Autofill K is located in the cell compartment prior to operating the ISIS Autosampler.



Return time = Sip time + Pull time + 1s