CHROMA M260uv Colorimeter

Operator Manual

260 91 002 Issue 1 24th April 2013 ECN 788
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Contents

Intended Use

This CHROMA Model 260uv Colorimeter is intended to be used by professional chemists/biochemists. Programming the unit is easier with detailed understanding of the mechanisms of both the test kits to be used in conjunction with the instrument and the underlying principles of colorimetry.

Please note that the drop–in Gelatin filters, although sealed, are subject to deterioration when exposed to excessive heat and humidity. Storage in a cool area and in a plastic bag with silica gel moisture absorbent pack is recommended.

The unit incorporates a “Warm-up” time period which can be over-ridden. If this is done then care needs to be taken over the validity of the results.

Chapter One Introduction

1.1 Contacting Sherwood Scientific Ltd

1.1.1 If your Model 260uv Colorimeter was supplied by a third party

i.e. a Distributor in UK or Overseas, the first contact should be that Distributor for all issues of supply and operation. Please note here the details of the Company where you purchased this unit:

Company……

Tel Number……

Contact Name…….

Email……..

1.1.2 If the Instrument was obtained from Sherwood Scientific Ltd

Contact for all issues of supply and operation should be sent with model and serial number to:

info@sherwood-scientific.com
Tel +44 (0)1223 243444
Fax +44 (0)1223 243300

Introduction (continued)

1.2 Using this Manual

Reading the Operator Manual will allow the user to operate the Colorimeter. The user will be able to enter new methods, edit existing methods and run methods.

The performance to be expected is listed in Chapter 4 of this Manual.

Your CHROMA Programmable Colorimeter incorporates an Interactive Interface, designed to be used in conjunction with third party test kits. The Programming Instructions are shown in Chapter 7 of this manual and the Methods of Operation for programmed tests are in Chapter 8.

1.3 Safety

Your Model 260uv Colorimeter operates at low voltage (12 Volts DC), powered from a universal self-adjusting power supply.
CARE should be taken to use the correct adapter for the user's local supply.

1.4 Reagents

The CHROMA range of Colorimeters is designed to be used with commercially available test kits. The programming options should accommodate the vast majority of applications. Please contact your kit supplier for the details of the sample preparation, measurement procedure, standard concentrations, factors and calculations. These can then be programmed into the Colorimeter.

1.5 Sample Preparation

Samples should be prepared according to the protocols of the reagent kit manufacturer. The total volume of reagent and sample required for the Colorimeter to operate satisfactorily is dependent on the profile of the cuvette used. These are listed in Chapter 4 (see 4.1.1) of this manual.

1.6 Warranty

The Sherwood Scientific warranty statement is included at Appendix A.

Chapter Two Installation

2.1 Location

The Colorimeter should be placed on a stable bench out of direct sunlight and with sufficient space to accommodate a separate heating block if necessary.

2.2 Services Required

The Model 260uv Colorimeter is supplied with a universal power supply.

The Colorimeter can also be used with a car battery or any 12V battery giving 1.25A for field use when fitted with the appropriate battery lead (471 88 200). Visit www.sherwood-scientific.com for a complete list of accessories, consumables and spares.

2.3 Unpacking

The Model 260uv Colorimeter is packed in a custom designed box, which will protect the instrument for both air-freight and sea-freight transport. The box contains:

Description	Part No.
M260uv Colorimeter	260 00 109
Cuvettes, UV, Plastic, 400µl, pack 100	001 26 120
Bulb Assembly	260 44 001
PSU, Universal, Multi Plu, 12Vdc	001 53 313
Cuvette Holder	252 11 002
Heated Micro Cuvette Holder	267 86 001
Interference Filter 340nm	252 27 021
Gelatin Filters, set of 8	252 26 001
Operator Manual	260 91 002
Lead, Instrument to Printer/PC	926 09 052

2.4 Assembly

Remove the Colorimeter from the packaging.
Insert the Cuvette Holder or Heated Micro Cuvette Holder as required

2.5 Connecting Peripheral Devices

2.5.1 Printer

Connect the printer lead to the RS232 output at the rear of the Colorimeter.
Ensure that the printer is set up to the following condition:

9600 baud
8 bit
Parity None
Stop bit 1

Connecting Peripheral Devices (continued)

2.5.2 Heated Micro Cuvette Holder (267 86 001)

The heated micro cuvette holder (if required for the test being undertaken) should be inserted with the power lead taken through the drain hole in the instrument before inserting the holder into the instrument. This device will, when connected to a power source, automatically settle to 37º C with the indicator light showing green.

NB** the heated micro cuvette holder is not connected electronically to the CHROMA Colorimeter and its set point cannot be altered from 37ºC.* ***It will only accommodate the micro cuvette 001 26 120 or cuvettes with the same profile.

2.6 Instrument Set-up

The CHROMA Model 260uv incorporates full programmability.

However it is possible to operate the "Simple" protocol which enables the Absorbance and % Transmission of a sample to be measured, without programming, after Auto-zeroing the instrument.
In addition, simple Factor and Concentration experiments may also be carried out, without need to create Methods or include Sample Naming, if such an approach is better suited for your requirements.

The Model 260uv is supplied with one 340nm interference filter and a set of eight gelatine filters; (430, 470, 490, 520, 540, 580, 600, 710nm).

A full list of available Gelatin and Interference Filters is given in Appendix B.

Chapter Three Scientific Background

3.1 Historical

We can trace the heritage of the Sherwood CHROMA range of colorimeters back to the very first Model 2 Colorimeter developed for a Dr Rose at Hammersmith Hospital in London by Evans Electro-Selenium Ltd. (EEL) in the 1940s. Prior to the Model 2 the concentrations of analytes by reference to their colour was accomplished by the optical matching by eye; the Tintometer approach. The Model 2 was superseded by the original Model 252 Colorimeter and then in the 1980s by the CHROMA Models 254 and 257. More recently Sherwood included programmability with the introduction of the Model 260 and now we also have the Model 260uv with near UV capability.

3.2 Principles of Colorimetry

The two most basic laws which describe the foundations of a quantitative analysis by photometry are attributed to Lambert and Beer. ***Lambert's Law*** states that the intensity of transmitted light decreases logarithmically as the path length increases arithmetically. ***Beer's Law*** states that increasing the concentration of analyte had the same effect as a proportional increase in the path length. Both laws use a constant related to the amount of light absorbed per analyte molecule, i.e. specific absorption. (This property has been given many names in the literature, such as molar absorptivity and extinction coefficient. These names are simply trying to introduce the concept that different analyte molecules are able to absorb a different amount of light per molecule, which relates to the energy states of their electrons).
The combined laws are now simply referred to as ***Beer's Law*** and are expressed as follows: -

$$\log_{10} \frac{I}{I_0} = abC$$

where **a** is an absorptivity constant, **b** is related to path length and **C** is analyte concentration

or $-A = abC$

Since in most analytical work, the path-length is kept constant using a 1 cm cell, the calibration graph generated for a particular analyte is linear with a measurement given in absorbance units over a wide range.
Once the linearity of a calibration curve has been established, an analyst has the convenient option of entering a ***factor***, ***f*** on the 260 Colorimeter and reading out in Concentration units directly, shown as follows: -

$$C = fA$$

where the ***factor***, ***f***, combines the previous path length and absorptivity factors, ***a*** and ***b.***

3.3 Time Based Chemistry Test Kits

For a detailed description of time based and kinetic enzyme measurements see Chapter Six, Seven and Eight.

For an overview of time based methods with calculations see Appendix D.

Chapter Four Specifications

4.1 Sample Requirements

When used with test kits, the manufacturer of the kit will specify the individual sample parameters depending on the particular assay.

4.1.1 Total Volume Required:

Standard cuvettes:	1.4ml	Part No: 471 88 300
Micro cuvette	400µl	Part No: 001 26 120

(NOTE Only micro-cuvettes part number 001 26 120 can be used with the Heated Cuvette Holder (267 86 001) The Micro cuvettes should not be used with the standard cuvette holder (252 11 002).

4.2 Measurement Range

When used with test kits, the manufacturer of the kit will specify the individual measurement ranges depending on the particular assay.

4.2.1 Operating Range

0 to 2.999	Abs
100.0 to 0	%T
0 to 9999	Conc

4.2.2 Resolution

0.001	Abs
0.1	%T
0.001	Conc

4.2.3 Warm Up Time

15 minutes. (Instrument monitors this).

4.2.4 Wavelength Range

325 to 750nm.

4.2.5 Alternative Filters

Gelatin filters available in a range of wavelengths from 410nm to 710nm
Interference Filters available in a range of wavelengths 340 to 725nm-Half Band Width 10 nm
Hi Spec filter at 430 nm Half Band Width 2 nm for EBC beer colour method

4.2.6 Heated Micro Cuvette Holder (267 86 001)

Controls to 37° C ±0.1°C
Only for use with micro-cuvette (001 26 120)

Chapter Five Product Description

5.1 Controls and Indicators

5.1.1 Typical Front Panel Layout

5.1.2 The LCD Display:
Has three lines with a maximum of 16 characters per line. When results are displayed the digits are shown over two rows in double size.

The buttons were designed to be universally understood.

5.1.3 Buttons

The Select buttons
These allow various choices to be displayed in the Menu sequence.

The Enter button
Allows the user to select the choice displayed in the Menu. It also accepts a reading or result and operates the Print function.

The Back Space button
Allows the user to go back one or more steps in the protocol.

Pressing these two buttons simultaneously will take the user straight back to the start-up screen.

Traditional Zero/Blank Button.

Product Description (continued)

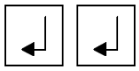
5.1.4 Modifying Numbers etc.

Where there are numbers to change i.e. concentration factor values or letters to change, i.e. to label tests and to define sample numbers or names, then the Enter button changes the decade or letter position and the select buttons are used to change the value of the number or letter.

For example to enter a concentration factor of 100 the user would do the following when prompted:

Press  to leave the 0 in the first unit column

Press  to get 1 in the second unit column then press  to accept

then press  to leave 0 in the 3d and 4th unit column.

5.2 Sample Heating and Incubation Times

Our protocols only cover the functions of the Colorimeter. They do not control the use of a heating block or water bath. We indicate when a particular incubation or measurement should start and an alarm sounds before the end of the incubation/measurement. This allows the user to transfer the cuvette back to the Colorimeter for the measurement. Provision is made to enter the temperature of a particular test in the programmed methods, but this is for completeness of method recording and reporting purposes only.

Note: For any test requiring samples to be incubated at a specified temperature (especially time based i.e. kinetic and end-point tests) it is critical that the specified temperature is actually attained by reagents and samples alike. Failure to ensure that is the case will give rise to results which are incorrect. As an example, low results are often caused by poor temperature transfer to the sample; often exacerbated by the use of micro cuvettes in an in-appropriate heater block or a heater block which is controlling at the “set” temperature but is actually lower than stated. The user is advised to establish the correct settings for their sample preparation “system” before embarking on the analysis of critical samples. A difference of just one degree Celsius from the required setting can yield results as much as 10% in error.

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Product Description (continued)

5.3 Operating Modes

5.3.1 Warm Up

There is a 15 minute warm up period built into the start-up routine. This can be over-ridden by pressing the Enter button 

5.3.2 Main Menu

5.3.2.1 Set-up

While the Model 260uv Colorimeter is warmed, you can enter **Setup** to:

- set time and date format
- set the actual time and date
- select (Uart) the RS232 settings for printer
- set the LCD screen contrast.

(for a full list of setup options see the Model 260uv Menu structure on the next page).

5.3.2.2 Simple

To use the Colorimeter for a simple Absorbance measurement, (or % Transmission), **Simple** mode is used. This only requires the instrument with appropriate filter selected to be zeroed against a blank solution (See 6.3) prior to measurement of samples. Straightforward Factor and Concentration experiments may also be performed in **Simple** Mode; they just require the entering of a factor or measurement of a 'known' standard in addition to blanking before measurement of samples.

NB In the "Simple" mode there is no sample labelling, operator identifier or storage of the methodology used.

5.3.2.3 Last

Takes the user straight back to the last used method.

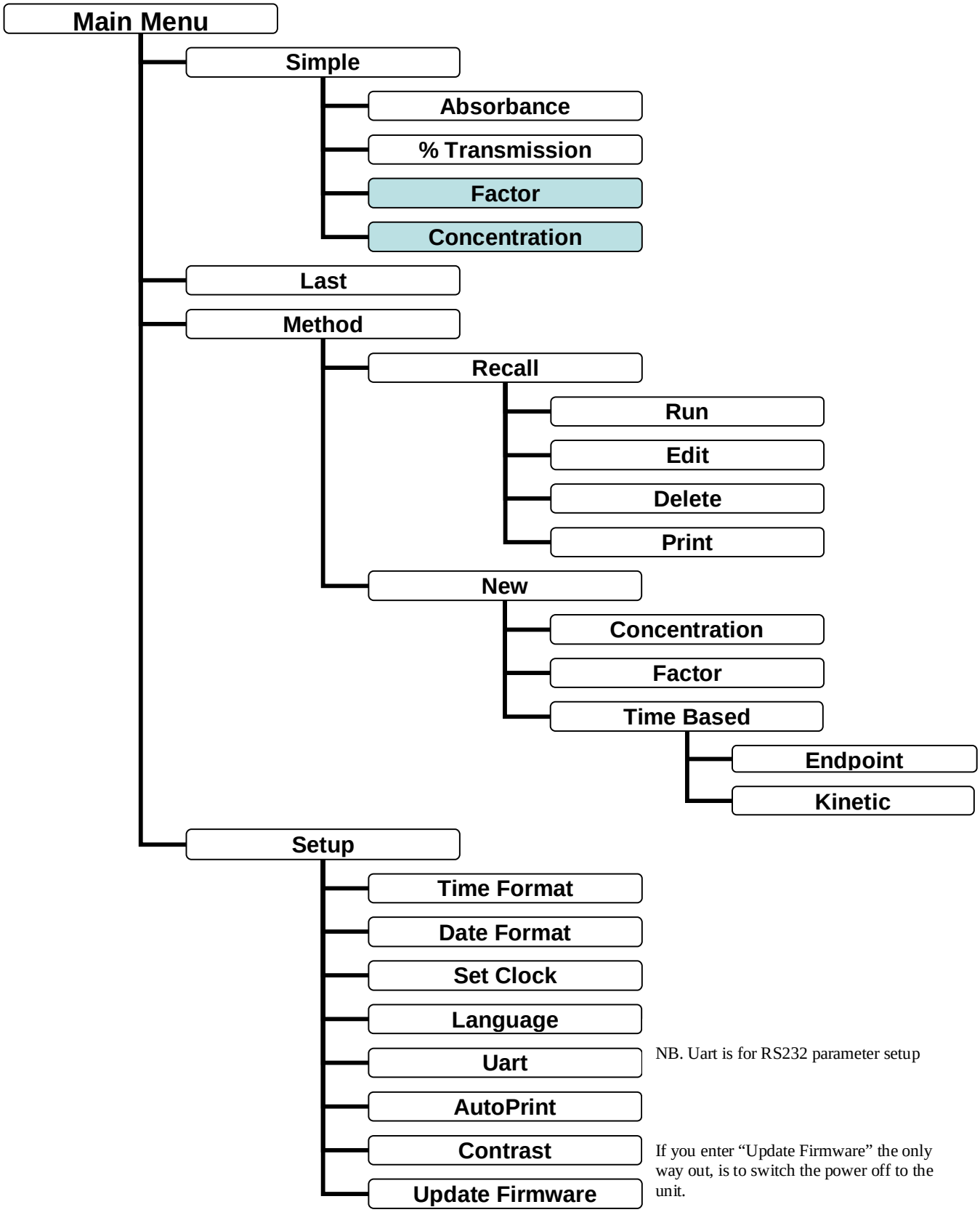
5.3.2.4 Method

Entering the **Method** screen gives two options.

1. **Recall**
   Allows an existing Method to be selected, which can then be:-
   Run, Edited, Deleted or Printed.
2. **New**
   Allows a New Method to be entered and stored.
   See Chapter 7 for detailed method entry procedures.

The Model 260uv Colorimeter Menu Structure is displayed on the next page.

5.4 Menu Structure



Chapter Six Operation

6.1 Start Up

Switch on the Colorimeter and, if needed, a heater block or water bath for sample and standard preparation. Allow the heater block, or water bath to get to the control temperature.

NB The nominal temperature setting point of a Heating Block should be checked with an accurate thermometer in a liquid sample in a cuvette (of the same profile you intend to use with your samples) in the block to ensure that the desired temperature is achieved.

The 260uv Colorimeter is fitted with a thermal switch to protect it from damage due to overheating should the cooling fan fail. When the temperature rises above the trigger point the thermal switch disconnects the power such that the lamp is extinguished. Once the instrument has cooled down sufficiently power will be restored to the lamp.

6.2 Zeroing and Calibration

Once the correct temperature has been reached and the Colorimeter has ended its warm up phase and shows "Ready" then the correct filter should be inserted. When the appropriate filter has been put in the filter slot, the user should press the Enter key to move on to the next step in the procedure/method.

It is important that the cuvette holder and filter are in their correct positions and that nothing is in the cuvette holder at this point.
The detector will saturate and the unit will "freeze" if return is pressed before the filter is in place. If that happens, switch the power off and then on again to recover normal operation.

The filter required and the actual procedures for zeroing and calibration are prompted by the menu system for each analytical procedure required.

Operation (continued)

6.3 Simple Method

The simple method allows measurement of Absorbance or % Transmission of a sample. Here there is no calibration procedure. The instrument is zeroed against a blank solution before a reading is taken of the sample. Put the blank cuvette in the cuvette holder and press the zero button when prompted.

It is important that the appropriate blank is used for the analysis; using an empty cuvette as a 'Blank' solution may lead to errors.

Then put the sample cuvette in the cuvette holder and the absorbance or % Transmission reading will be displayed. To print the result, if a printer is connected, press the enter button. When all the sample readings have been taken, press the back space button.

Many applications for the Colorimeter involve measurement of the concentration of an analyte either by:-

1. Using a ***factor*** supplied by the test kit manufacturer.
2. Comparison with a calibrator (or 'standard') of known ***concentration***.

In the Model 260uv Colorimeter's 'Simple' Menu, these two facilities are respectively denoted as:-

1. Factor.
2. Concentration.

Within the Simple Menu the Model 260uv allows Factor or Concentration experiments to be performed very simply if the user does not require to save and store a method protocol or to enter sample identifiers (name or numbers). The instrument, with appropriate filter selected, is zeroed against a blank solution after which the user is prompted to enter a factor (or a standard of known concentration and that concentration value) before the instrument is then ready to take a reading and calculate a result for an unknown sample or samples.

For detailed operational flow charts of the 'Simple' Factor or Concentration procedures, please refer to Appendix E.

Operation (continued)

6.4 Concentration Methods

As stated, the majority of applications for the Colorimeter will be the measurement of the concentration of an analyte either by:-

1. Using a ***factor*** supplied by the test kit manufacturer.
2. Comparison with a calibrator (or "standard") of known ***concentration.***

In the 260 series Colorimeters, these two methods are respectively denoted as:-

1. Factor Method
2. Concentration Method

The 'Method' section within the Model 260's Menu allows for methods to be constructed, saved, recalled and operated for assistance with GLP and SOP procedures. In addition, these 'Methods' allow the operator to enter sample identifiers (names or numbers) for subsequent sample/result correlation.

Detailed illustration of the "Setup" and "Running" of these method types is given in Chapter 7 and 8 respectively of the Manual.

6.5 Time Based Measurements

Some measurements require time to develop an absorbing reactant, which then allows the presence of the analyte of interest to be determined and quantified. These have two categories:-

6.5.1 End-Point Methods

Used when the colour needs time to develop but is then stable for, usually, at least an hour.

6.5.2 Kinetic Measurements

This Procedure is used, for example, when the analyte is an enzyme which catalyses a particular reaction. Higher concentrations of the analyte result in faster rates of reaction. The rate of change of adsorption is calculated having measured the adsorption at two different times in the constant rate period of the reaction (pre-determined by the test kit manufacturer).

Both time based measurement types can be made using a known factor or in comparison to a calibrator (or "Standard"), both supplied by the test kit manufacturer.

Operation (continued)

Consequently, in the Model 260uv Colorimeter, four method types can be entered, edited and/or run to accommodate the methodologies outlined above. They are respectively denoted as:-

1. End Point Method with Factor
2. End Point Method with Calibrator
3. Kinetic Method with Factor
4. Kinetic Method with Calibrator

In many cases the sample itself may contain interfering coloured substances. The protocols provided in the Model 260uv Colorimeter require a sample blank to be measured and then the sample. The blank result is subtracted from the absorbance of the reacted sample (this is done automatically by the Colorimeter firmware).

NOTE: When a test is measuring an increase in absorbance (e.g. the increase in concentration of an absorbing species with time) the result is given in the units set by the user. When a test is measuring a decrease in absorbance (e.g. the drop in concentration of an absorbing species with time) the result is given in the units set by the user, but with a minus sign. The minus sign is generated because the absorbance dropped over the measurement period, the minus sign must be removed to arrive at the correct concentration result.

Detailed Illustration of the "Setup" and "Running" of these method types is given in Section 7 and 8 respectively of the Manual.

6.5.3 Incubation and Measurement Times

Many kinetic measurements procedures require an initial incubation time before a second reagent is added. This time is sometimes referred to as the "delay" time. Sherwood differentiates between the incubation and the "Measurement" time during which the reaction takes place and the change in absorbance is measured.

6.6 Automated Measurements

A significant feature of your 260 series Colorimeter is its programmability. Once the correct methodology is selected and e.g. the correct calibration concentration value and wavelength programmed, each measurement operation is prompted by the instrument. Once named and saved the method can be recalled from a list of methods by name.

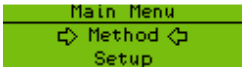
6.7 Sample Numbering

The Model 260uv Colorimeter allows both numerical and alpha-numerical sample numbering to be used so that a result can be printed against the original sample number. This is important where Good Laboratory Practice is employed.
It is possible to accept a sequential number as the default option.

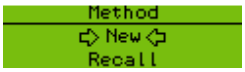
Chapter Seven Method Set-up

The following flowcharts give detailed illustration of entering a new Method of a type accommodated by the Model 260uv Colorimeter firmware (outlined in Sections 6.4 and 6.5).

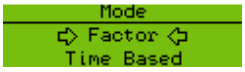
7.1 Factor Method – Set-up Procedure



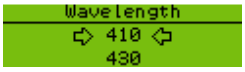
Select Method from Main Menu using >> then press ↵



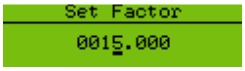
Select New from Method Menu using >> then press ↵



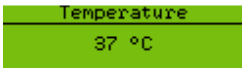
Select Factor from Mode Menu using >> then press ↵



Select wavelength from list* using >> then press ↵

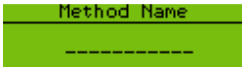


Set Factor value. Use >> and ↵ for each number column

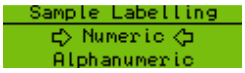


Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).

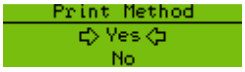
Set concentration units for the test kit using >> then ↵



Enter a method name using >> and then ↵ for each letter



Select sample labelling format using >> then press ↵

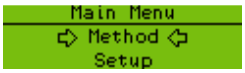


You can print the method and check it before you save it

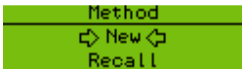
You can edit, save or cancel your new method

*See section 7.7 Filter Selection.

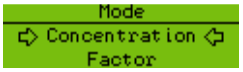
7.2 Concentration Method – Set-up Procedure



Select Method from Main Menu using >> then press ↵

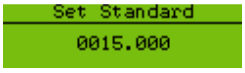


Select New from Method Menu using >> then press ↵



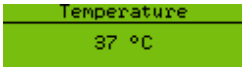
Select Concentration from Mode Menu using >> then press ↵

Select wavelength from list* using >> then press ↵

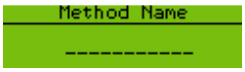


Set Standard concentration value as outlined in the test kit. Use >> then press ↵ for each number column

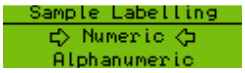
Set concentration units using >> then ↵



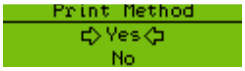
Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).



Enter a method name using >> and then ↵ for each letter



Select sample labelling format

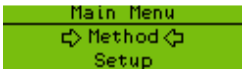


You can print the method and check it before you save it

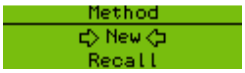
You can choose to edit, save or cancel your new method

*See section 7.7 Filter Selection.

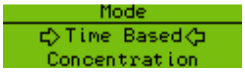
7.3 End Point Method with Factor – Set-up Procedure



Select Method from Main Menu using >> then press ↵



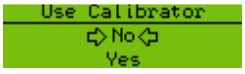
Select New from Method Menu using >> then press ↵



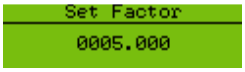
Select Time Based from Mode Menu using >> then press ↵

Select Endpoint from Mode Menu using >> then press ↵

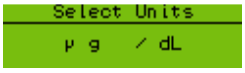
Select wavelength from list*using >> then press ↵



Select No on Use Calibrator screen using >> then press ↵

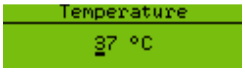


Set Factor value using >> then ↵ for each number column

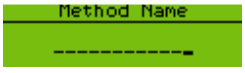


Set concentration units using >> then ↵

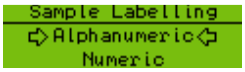
Set Incubation time using >> then ↵ for each number column



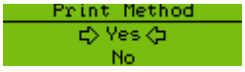
Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).



Enter a method name using >> and then ↵ for each letter



Select sample labelling format

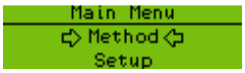


You can print the method and check it before you save it

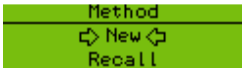
You can choose to edit, save or cancel your new method

*See section 7.7 Filter Selection.

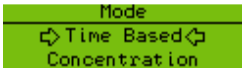
7.4 End Point Method with Calibrator – Set-up Procedure



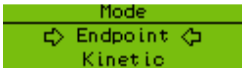
Select Method from Main Menu using >> then press ↵



Select New from Method Menu using >> then press ↵

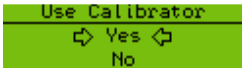


Select Time Based from Mode Menu using >> then press ↵

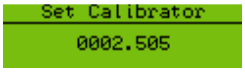


Select Endpoint from Mode Menu using >> then press ↵

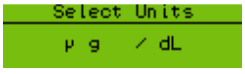
Select wavelength from list* using >> then press ↵



Select Yes on Use Calibrator Screen using >> then press ↵

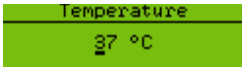


Set Calibrator value. Use >> then ↵ for each number column

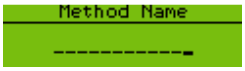


Select required units using >> then ↵ for each unit's column

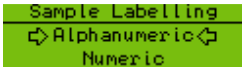
Set Incubation time. Use >> then ↵ for each number column



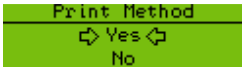
Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).



Enter a method name using >> and then ↵ for each letter



Select sample labelling format

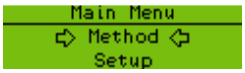


You can print the method and check it before you save it

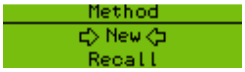
You can choose to edit, save or cancel your new method

*See section 7.7 Filter Selection.

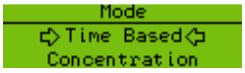
7.5 Kinetic Method with Factor Method – Set-up Procedure



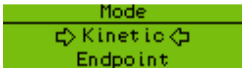
Select Method from Main Menu using >> then ↵



Select New from Method Menu using >> then ↵

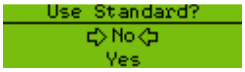


Select Time Based from Mode Menu using >> then ↵

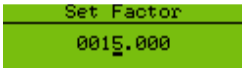


Select Kinetic from Mode Menu using >> then ↵

Select wavelength from list* using >> then ↵



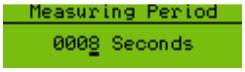
Select No on Use Standard? Screen using >> then ↵



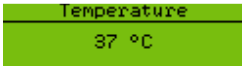
Set Factor value. Use >> then ↵ for each number column

Select required units using>> then ↵ for each unit's column

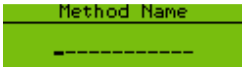
Set Incubation time. Use >> then ↵ for each number column



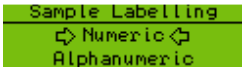
Set measuring period. Use >> then ↵ for each number column



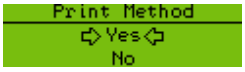
Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).



Enter a method name using >> and then ↵ for each letter



Select sample labelling format

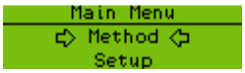


You can print the method and check it before you save it

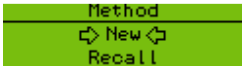
You can choose to edit, save or cancel your new method

*See section 7.7 Filter Selection.

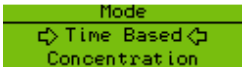
7.6 Kinetic Method with Calibrator – Set-up Procedure



Select Method from Main Menu using >> then ↵

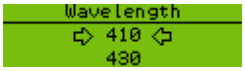


Select New from Method Menu using >> then ↵

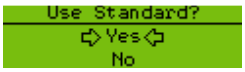


Select Time Based from Mode Menu using >> then ↵

Select Kinetic from Mode Menu using >> then ↵



Select wavelength from list* using >> then ↵

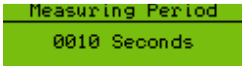


Select Yes on Use Standard? Screen using >> then ↵

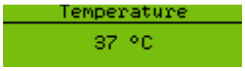
Set Standard value using >> then ↵ for each number column

Select required units using>> then ↵ for each unit's column

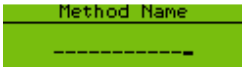
Set Incubation time. Use >> then ↵ for each number column



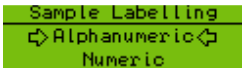
Set measuring period. Use >> then ↵ for each number column



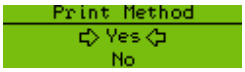
Note sample temperature if required using >> then ↵
(This facility only allows a record of the test temperature used to be printed for traceability. The Colorimeter does not control sample temperature).



Enter a method name using >> then ↵ for each letter



Select sample labelling format



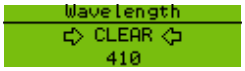
You can print the method and check it before you save it

You can choose to edit, save or cancel your new method

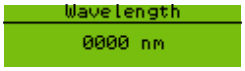
*See section 7.7 Filter Selection.

7.7 Filter Selection– Set-up Procedure

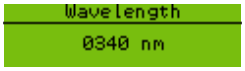
*Selecting “Clear” from the wavelength options enables a record to be made of the use of other wavelength filters (within the operating range of the instrument), the following screens appear



Select wavelength (Clear) from list* using >> then ↵



Set filter value using >> then ↵ for each number column



then ↵

The method screens continue as shown in the illustrations.

Chapter Eight Method Run Details

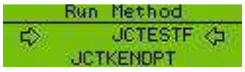
The following flowcharts give detailed illustration of running a Method of a type accommodated by the Model 260uv Colorimeter firmware (outlined in Sections 6.4 and 6.5).

8.1 Factor Method - Run Procedure

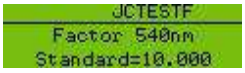
Select Method from main menu using >> then ↵

Select Recall from Method Menu using >> then ↵

Select Run from Recall Menu using >> then ↵



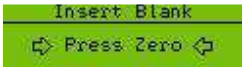
Select required Method using >> then ↵



Method details will move up and down in display.
Check details are correct and press ↵.

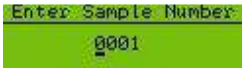
Insert the filter as prompted then ↵

The cuvette holder must be empty when you press ↵



Insert the blank in the cuvette holder and then press **0**

The instrument will be set to Zero. Press ↵

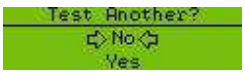


Enter sample number using >> then ↵ for each number option

Put the sample in the cuvette holder when prompted. Press ↵

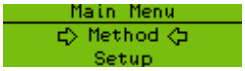
The sample OD will be shown on the display. Press ↵

The concentration of the sample will be displayed. Press↵

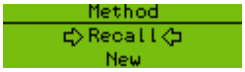


Choose Yes or No to run another sample then press ↵

8.2 Concentration Method - Run Procedure

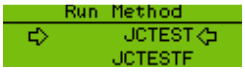


Select Method from Main Menu using >> then ↵

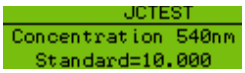


Select Recall from Method Menu using >> then ↵

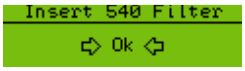
Select run from Recall Menu using >> then ↵



Select required Method using >> then ↵

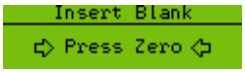


Method details will move up and down in display.
Check details are correct and press ↵.



Insert the filter as prompted then ↵

The cuvette holder must be empty when you press ↵

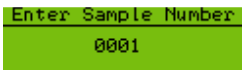


Insert the blank in the cuvette holder and then press **0**

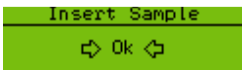
The instrument will be set to zero. Press ↵

Put the standard into the cuvette holder and press ↵

The OD of the standard will be displayed. Press ↵



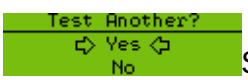
Enter sample number using >> then ↵ for each number option



Put the sample in the cuvette holder and press ↵

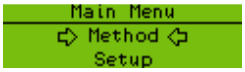
The sample OD will be displayed. Press ↵

The sample concentration will be display. Press ↵

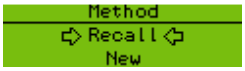


Select yes to run another sample then press ↵

8.3 End Point Method with Factor - Run Procedure

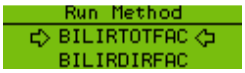


Select Method from Main Menu using >> then ↵

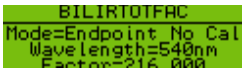


Select Recall from Method Menu using >> then ↵

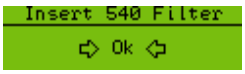
Select Run from Recall Menu using >> then ↵



Select the named method you want to run using >> then ↵

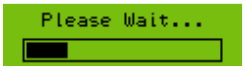


Method details will move up and down in display.
Check details are correct and press ↵.

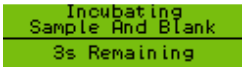


Insert the filter as prompted then ↵

The cuvette holder must be empty when you press ↵

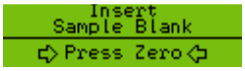


Put your sample and sample blanks in your pre-heated bath or heater block and press ↵ to start the Colorimeter timer



Just before the incubation time ends a beep will alert the user

Enter sample number using >> then ↵ for each number option.



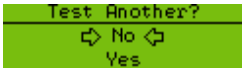
Put the blank in the cuvette holder and press **0**

The absorbance will be set to 0.000. Press ↵

Put the sample in the cuvette holder and press ↵

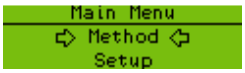
The sample absorbance will be shown and stored. Press ↵

The sample concentration will be shown. Press ↵

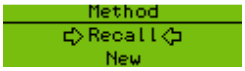


Test Another. Select Yes or NO using >> and press ↵

8.4 End Point Method with Calibrator - Run Procedure

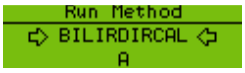


Select Method from Main Menu using >> then ↵

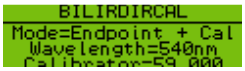


Select Recall from Method Menu using >> then ↵

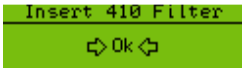
Select Run from Recall menu using >> then ↵



Select the named method you want to run using >> then ↵

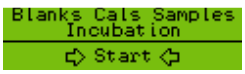


Method details will move up and down in display.
Check details are correct and press ↵.

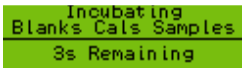


Insert the filter as prompted then ↵

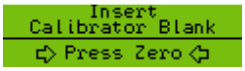
The cuvette holder must be empty when you press ↵



Put the calibrator blank in a pre-heated water bath or heater block and press ↵ to start the Colorimeter timer

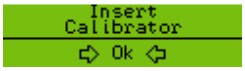


Just before the incubation time ends a beep will alert the user



Put the blank in the cuvette holder and press the **0**

The absorbance will be set to 0.000. Press ↵

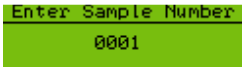


Put the calibrator in the cuvette holder and press ↵

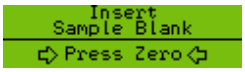
The calibrator absorbance will be shown. Press ↵

continued on next page

End Point Method with Calibrator - Run Procedure (continued)

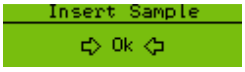


Enter the sample number using >> then ↵ for each position



When prompted, put the sample blank and press the **0**

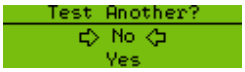
The absorbance will be set to 0.000. Press enter↵



Put the sample in the cuvette holder and press ↵

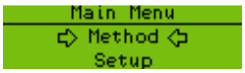
The sample absorbance will be displayed and stored. Press ↵

The sample concentration will be shown. Press ↵

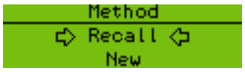


Test Another. Select Yes or NO and press ↵

8.5 Kinetic Method with Factor – Run Procedure

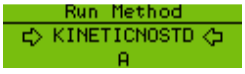


Select Method from Main menu using >> then ↵

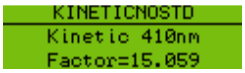


Select Recall from Method Menu using >> then ↵

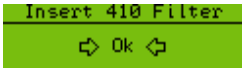
Select Run from Recall menu using >> then ↵



Select required Method using >> then ↵

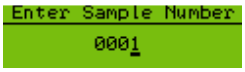


Method details will move up and down in display. Check the details are correct and press ↵.



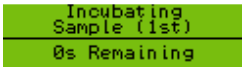
Insert the filter as prompted then ↵

The cuvette holder must be empty when you press ↵

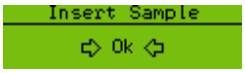


Enter sample number using the >> then ↵ for each number

Put the Sample in the preheated water bath or heater block and press ↵ to start the Colorimeter timer



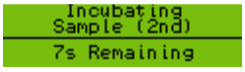
Just before the incubation time ends a beep will alert the user



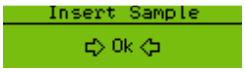
Insert the sample in the cell holder and press ↵

The optical density of the sample will be displayed. Press ↵

Return the sample to the incubator and press ↵ on the Colorimeter to start the timer again



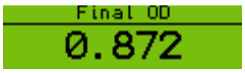
Just before the incubation time ends a beep will alert the user



Put the sample in the cuvette holder again and press ↵

Continued on next page

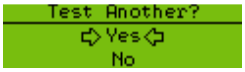
Kinetic Method with Factor – Run Procedure **(continued)**



The new optical density of the sample will be displayed. Press ↵

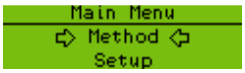
The change in Sample OD per minute will be displayed. Press ↵

The concentration of the sample will be displayed. Press ↵

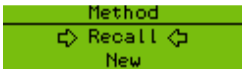


Select Yes or No and press ↵

8.6 Kinetic Method with Calibrator– Run Procedure

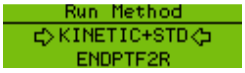


Select Method from Main Menu using >> then ↵

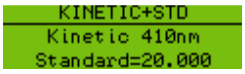


Select Recall from Method Menu using >> then ↵

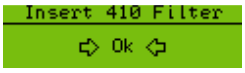
Select Run from Recall Menu using >> then ↵



Select required method using >> then ↵



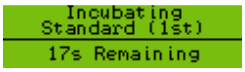
Method details will move up and down in display.
Check the details are correct and press ↵.



Insert the filter as prompted then ↵.

The cuvette holder must be empty when you press ↵

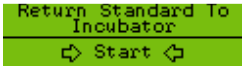
Put the Standard in the pre-heated water bath or heater block and press ↵ to start the Colorimeter timer



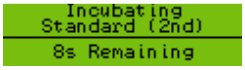
Just before the incubation time ends a beep will alert the user

Insert the standard in the cuvette holder and press ↵

The optical density of the Standard will be displayed. Press ↵



Return the standard to the incubator and press ↵ on the Colorimeter to start the timer again

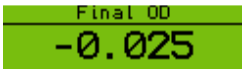


Just before the incubation time ends a beep will alert the user

Insert the standard in the cuvette holder again and press ↵

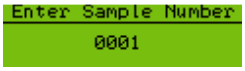
Continued on next page

Kinetic Method with Calibrator– Run Procedure (continued)



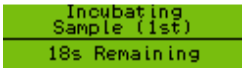
The new optical density of the Standard is shown. Press ↵

The change in Optical density of the Standard is shown. Press ↵

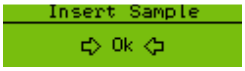


Enter the sample number using >> then ↵ for each number

Put the sample in the pre-heated water bath or heater block and press ↵ to start the Colorimeter timer



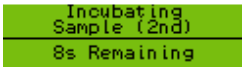
Just before the incubation time ends a beep will alert the user



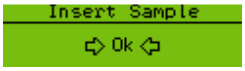
Put the sample in the cuvette holder and press ↵

The optical density of the Sample will be shown. Press ↵

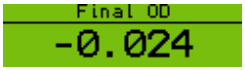
Return the sample to the incubator and press ↵ on the Colorimeter to start the timer again



Just before the incubation time ends a beep will alert the user



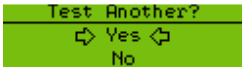
Insert the sample in the cuvette holder again and press ↵



The new optical density of the Sample will be shown. Press ↵

Change in Optical density will be shown. Press ↵

The sample concentration will be displayed. Press ↵



Choose Yes or No in answer to test another using >> then ↵

Appendix A Warranty

Sherwood Scientific Limited
Product Warranty Statement

Warranty Term: 12 Months

Sherwood Scientific Ltd (Sherwood) warrants, subject to the conditions itemised within this document, through either Sherwood personnel or personnel of its authorised distributors, to repair or replace free of all charges, including labour, any part of this product which fails within the warranty time specified above, appertaining to this particular product. Such failure must have occurred because of a defect in material or workmanship and not have occurred as a result of operation of the product other than in accordance with procedures described in the instructions furnished with this product. Conditions and specific exceptions that apply to the above statement are as follows:

1. End-user warranty time commences on the date of the delivery of product to end-user premises.

2. *Free of all charges*' statement applies only in areas recognised by Sherwood as being serviced either directly by its own personnel, or indirectly through personnel of an authorised distributor. Products purchased outside these areas requiring service during the warranty period will incur charges relative to the travel/transit costs involved. However, products purchased in such areas will be serviced during the warranty period free of all charges providing they are returned, carriage paid, to either Sherwood or by pre-arrangement to an authorised Sherwood distributor.

3. All maintenance (other than operator maintenance as described in the instructions), repairs or modifications have been made by Sherwood or Sherwood authorised personnel.

4. This product has where applicable been operated using Sherwood specified supplies and reagents.

5. Sherwood reserves the right to make any changes in the design or construction of future products of this type at any time, without incurring any obligation to make any changes whatsoever to this particular product.

6. Reagents, supplies, consumables, accessories and user maintenance items are not included in this warranty.

Product Warranty Statement (continued)

7. Repairs or replacement of any part failing due to abnormal conditions including the following, are excluded from this warranty:
    a. Flood, lightning, earthquake, tornado, hurricane, or any other natural or man-made disaster.
    b. Fire, bombing, armed conflict, malicious mischief or sprinkler damage.
    c. Physical abuse, misuse, sabotage or electrical surge.
    d. Damage incurred in moving the product to another location.

8. User agrees to permit Sherwood personnel or personnel of its authorised distributor to make changes in the product which do not affect results obtained, but do improve product reliability.

Representations and warranties purporting to be on behalf of Sherwood made by any person, including distributors and representatives of Sherwood, which are inconsistent or in conflict with the terms of this warranty (including but not limited to the limitations of the liability of Sherwood as set forth above), shall not be binding upon Sherwood unless reduced to writing and approved by an officer of Sherwood Scientific Ltd.

Except for the obligations specifically set forth in this warranty statement, in no event shall Sherwood be liable for any direct, indirect, special, incidental, or consequential damages, whether based on contract, tort or any other legal theory and whether advised of the possibility of such damages.

Neither Sherwood nor any of its third party suppliers makes any other warranty of any kind, whether expressed or implied, with respect to Sherwood Products.

Sherwood Scientific Ltd.,
1 The Paddocks,
Cherry Hinton Road,
Cambridge,
CB1 8DH,
England

Appendix B Gelatin and Interference Filters

252 15 001	FILTER, GELATIN, 430NM
252 16 001	FILTER, GELATIN, 470NM
252 17 001	FILTER, GELATIN, 490NM
252 18 001	FILTER, GELATIN, 520NM
252 19 001	FILTER, GELATIN, 540NM
252 20 001	FILTER, GELATIN, 580NM
252 21 001	FILTER, GELATIN, 600NM
252 22 001	FILTER, GELATIN, 710NM
252 26 001	FILTERS, GELATIN, SET OF 8
252 31 001	FILTER, GELATIN, 410NM
252 27 021	FILTER, INTERFERENCE, 340NM
471 79 700	FILTER, INTERFERENCE, 430NM
471 79 800	FILTER, HIGH SPEC, 430NM
471 88 900	FILTER, INTERFERENCE, 405NM
471 89 000	FILTER, INTERFERENCE, 510NM
471 89 100	FILTER, INTERFERENCE, 546NM
471 89 150	FILTER, INTERFERENCE, 600NM
471 89 155	FILTER, INTERFERENCE, 636NM
471 89 200	FILTER, INTERFERENCE, 660NM
471 89 300	FILTER, INTERFERENCE, 680NM
471 89 400	FILTER, INTERFERENCE, 725NM

Appendix D Time Based Methods for Clinical Analysis

NOTE: It may be necessary to validate the use of this instrument, together with the chosen test kit, for clinical diagnostic purposes in accordance with the relevant local regulatory authority guidelines prior to use for analysis of patient samples and generation of clinical results.

The laboratory analysis of enzymes in blood plasma is useful in the diagnosis and monitoring of various diseases especially where there is damage to tissues and organs of the body e.g. the heart, the liver and bone. Much of the raised enzyme activity, compared to the normal reference range, is due to enzymes within the cells of the tissues or organs being released into the bloodstream as a result of damage or rupture of the cell walls.
The more clinically important enzyme test requests made to laboratories are:-

- The enzymes AST (aspartate aminotransferase) and ALT(analine aminotransferase) known as the aminotransferases are often requested together :-
    - Increased levels of AST in blood plasma may be found in cases of acute hepatitis, necrosis of the liver, in severe crush injuries and after heart attacks.
    - Increased levels of ALT are found in the same liver diseases as is AST.
- High plasma levels of the enzyme Gamma GT are found in alcohol-associated liver disease e.g. cirrhosis, in hepatitis and may be moderately raised after certain drug therapy.
- Increased levels of LDH are found after acute damage to the liver or the main muscles, in cases of tumours known as lymphoma and in certain types of anaemia. A form of this enzyme termed HBDH is raised in plasma after heart attacks (myocardial infarction).
- Increased plasma levels of ALP are found in various diseases of the liver and bone.
- Raised levels of creatine kinase (CK) are found after heart attack and in trauma to muscle.

The role of these enzymes in the cells of the body is to act as a catalyst where they convert a substrate to another molecule and by-product. The enzyme actually accelerates the reaction. By careful analysis of the enzyme reaction on the substrate, where parameters such as temperature and pH are optimised, the activity of the enzyme in plasma can be easily determined.

Appendix D (continued)

There are two types of method in which enzymes or metabolic substrates can be measured in clinical chemistry:

1. End Point method
2. Reaction Rate method

Each method follows similar principles.

- Enzymes are simply used as reagents in excess to turn the blood substrate under investigation into a product that can be measured e.g. glucose by end point method.
- The enzyme of clinical significance can be measured by observing the rate at which the reaction proceeds and hence the rate that the substrate or products increase of decrease over a period of time e.g. LDH at 340 nm by the rate of reaction.

1. End Point Reaction

If the assay is intended to measure the concentration of the substrate then excess enzyme is present and the reaction goes to completion e.g. glucose by the enzyme *glucose oxidase.*

i) The substrate (Sample) mixture is placed in the cuvette. This is brought to temperature (externally in a heating device).
ii) The cuvette is then placed in the Colorimeter, the enzyme is added and the instrument is immediately zeroed.
iii) The cuvette is placed in the heating device for a period which is usually between 10-30 minutes (this is called the incubation period).
iv) At the end of the measurement time the final absorbance (OD) is measured.

A standard sample of known concentration of substrate and a blank sample containing no substrate are both treated in exactly the same way.

Calculation

After subtracting the blank absorbance reading from both the Standard and the Sample, the ratio of the Sample Absorbance and the Standard absorbance is multiplied by the standard concentration to obtain the concentration of the unknown. (OD refers to absorbance in the calculation below).

$$\frac{\text{(OD sample-OD blank)}}{\text{(OD standard –OD blank)}} \times \text{concentration of Standard} = \text{Concentration of Sample}$$

Appendix D (continued)

2. Reaction Rate

This method of analysis is used where an enzyme itself is to be measured and the substrate is added in excess to the reaction cuvette.

The calculation measures the rate of change of either the initial substrate or a particular product of the reaction. This is then either compared with the rate of change of an enzyme concentration of a fixed standard or simply multiplied by a given factor.

Due to complex reactions taking place a delay time or incubation as well as the measuring time may be used.

A. ***For immediate Reaction Rate*** (i.e. no incubation time)

i) The substrate is brought to the appropriate temperature externally.
ii) Place cuvette in light path and sample (or standard) is added.
iii) Initial absorbance is recorded and Start initiated.
iv) Sample removed and placed into heater for Measure Time.
v) 20 seconds before end of Measure Time buzzer sounds to remind user to prepare to replace cuvette into Colorimeter.
vi) At exact end of Measure Time cuvette is in light path and final absorbance noted.

Calculation

Δ OD sample x factor = sample concentration

or (Δ OD sample) x Standard concentration = Concentration of Sample
Δ OD Standard

B. ***For Delayed Reaction Rate (i.e. with Incubation time)***

Substrate is brought to temperature externally

i) Sample (or standard) added.
ii) Incubation Time started.
iii) 20 seconds before end of Incubation time buzzer sounds reminder.
iv) Cuvette placed in Colorimeter and at end of incubation Time absorbance value is noted and Start of Measurement time is pressed.
v) Sample removed and placed into heater for Measure Time.
vii) 20 seconds before end of Measure Time buzzer sounds to remind user to prepare to replace cuvette into Colorimeter.
viii) At exact end of Measure Time cuvette is in light path and final absorbance noted.

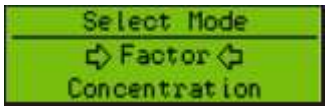
Calculation

Δ OD sample X factor = sample concentration

or (Δ OD sample) x concentration of standard = concentration of sample
(Δ OD Standard)

Appendix E "Simple" Factor Procedure

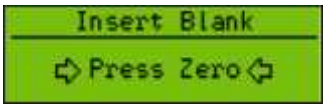
Select Simple from Main Menu using >> then ↵.



Select Factor from Main Menu using >> then ↵.

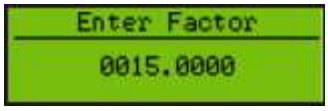
Insert the filter as prompted, then ↵.

The cuvette holder must be empty when you press ↵

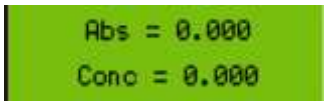


Insert the blank in the cuvette holder and then press **0**
The instrument will be set to Zero and immediately prompt the user to enter the Factor given for the test kit in use.

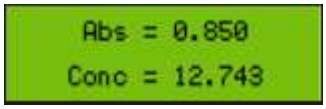
Enter the test kit factor using >> then ↵ for each number option.



(In this example we have entered a factor of 15).



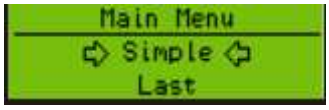
Once the factor has been entered the display changes to present the Absorbance measured and the calculated Concentration.



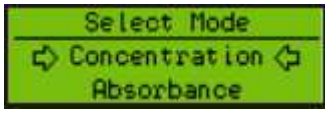
Insert a sample in the cuvette holder and the display shows Absorbance, measured and calculated Concentration. Remove the sample cuvette, replace it with another and the Absorbance and calculated Concentration will be displayed immediately. It is recommend you wait at least 10 seconds for the sample to settle before taking a reading. Once you have finished measuring all your samples press ← to return to the "Select Mode" Screen.

Appendix E (continued)

"Simple" Concentration Procedure



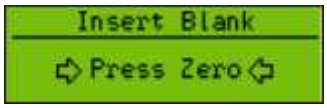
Select Simple from Main Menu using >> then ↵.



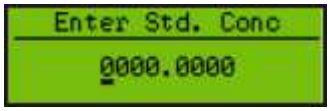
Select Concentration from Main Menu using >> then ↵.

Insert the filter as prompted then ↵.

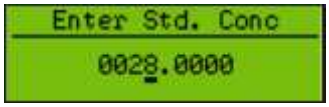
The cuvette holder must be empty when you press ↵.



Insert the blank in the cuvette holder and then press **0.** The instrument will be set to Zero and immediately prompt the user to enter the concentration of the standard to be measured.

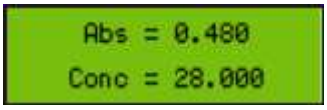


Enter the standard concentration using >> then ↵ for each number option.

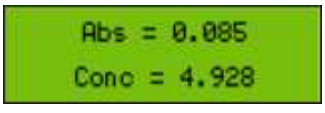


(In this example we have entered a concentration of 28).

Insert the standard in the cuvette holder, wait 10 seconds and then press ↵.



The instrument will display Absorbance measured and the Concentration of the standard.



Insert a sample in the cuvette holder and the display shows Absorbance, measured and calculated Concentration. Remove the sample cuvette, replace it with another and the Absorbance and calculated Concentration will be displayed immediately. It is recommend you wait at least 10 seconds for the sample to settle before taking a reading. Once you have finished measuring all your samples press ← to return to the "Select Mode" Screen.