



# **1000 SERIES SPECTROPHOTOMETER USER'S MANUAL**

United Products & Instruments, Inc.  
182 Ridge Road, Suite E  
Dayton, NJ 08810, USA  
TEL: 732-274-1155 FAX: 732-274-1151  
<http://www.unico1.com>

Rev.0105

# Table of Contents

<b>Introduction</b> .....	3
<b>Working Principle</b> .....	3
<b>Specifications</b> .....	4
Packing List .....	4
<b>Unpacking Instructions</b> .....	4
<b>Installation</b> .....	4
<b>1000 Spectrophotometer Operation Panel</b> .....	5
<b>Changing Cuvette Holders</b> .....	7
<b>Basic Operation</b> .....	7
Absorbance and Transmittance Measurement .....	7
Making Measurements at Different Wavelengths .....	8
<b>Analog Output</b> .....	9
<b>Lamp Changing</b> .....	9
Lamp Adjustment .....	10
<b>Absorbance Accuracy and Stray Light Checking</b> .....	10
<b>Sample Experiments</b> .....	10
Experiment 1 Brief Introduction to Beer's Law .....	10
Experiment 2 Measurement of an Absorption Spectrum .....	12
Experiment 3 Study of Beer's Law .....	13
Experiment 4 Determination of the Concentration of a Solution .....	14
Experiment 5 Determination of a Species in a Solid Sample .....	15
<b>1000 Parts List</b> .....	18
<b>Troubleshooting</b> .....	19

## Introduction

The **UNICO**<sup>®</sup> 1000 is a single beam spectrophotometer, which is designed to meet the needs of both students and instructors. Its digital display, easy operation, and wavelength range of 400 nm to 1000 nm makes this unit ideal for spectrophotometric experiments in the visible wavelength region of the electromagnetic spectrum.

## Working Principle

The spectrophotometer consists of five parts:

- 1) **Light Source** (Halogen lamp) to supply the light
- 2) **A Monochromator** to isolate the wavelength of interest and eliminate the unwanted second order radiation
- 3) **A Sample Compartment** to accommodate the sample solution
- 4) **A Detector** to receive and convert the transmitted light to an electrical signal
- 5) **A Digital Display** to show absorbency and transmittance

Figure-1 illustrates the relationship between these parts.

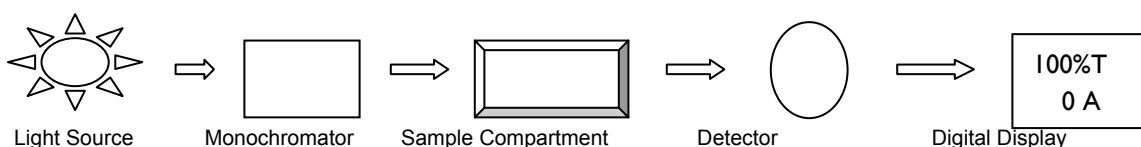


Figure-1 Block Diagram for the Spectrophotometer

In the spectrophotometer, light from the Halogen Lamp is focused on the entrance slit of the Monochromator where the collimating mirror directs the beam onto the grating. The grating disperses the light beam to produce the spectrum, a portion of which is focused on the exit slit of the Monochromator by a collimating mirror. From here the beam is passed to a Sample Compartment through one of the filters, which helps to eliminate unwanted second order radiation from the diffraction grating. Upon leaving the Sample Compartment, the beam is passed to the silicon photodiode Detector and causes the Detector to produce an electrical signal that is shown on the Digital Display window.

The **UNICO**<sup>®</sup> 1000 incorporates a special analog output terminal that can be used to interface this spectrophotometer with a chart recorder, or with any computer interface that will accept a signal which varies from 0-1.0V DC.

## Specifications

Table-1 lists the specification for **1000**.

Table-1 **UNICO®** 1000 Specifications

<b>Wavelength Range</b>	400~1000 nm
<b>Spectral Slit Width</b>	20 nm
<b>Wavelength Accuracy</b>	± 3 nm
<b>Wavelength Readability</b>	2 nm
<b>Stray Radiant Energy</b>	2%T at 400 nm
<b>Transmittance Range</b>	0%T to 100%T
<b>Absorbance Range</b>	0A to 1.99A
<b>Photometric Accuracy</b>	± 2.0%T
<b>Photometric Noise level</b>	± 1.0%T
<b>Power Requirements</b>	110 V/60 Hz or 230 V/50 Hz
<b>Dimensions</b>	308 (L) x 408 (W) x 156 (H) mm)
<b>Net Weights</b>	5.5 kg

## Unpacking Instructions

Carefully unpack the contents and check the materials against the following packing list to ensure that you have received everything in good condition:

### **Packing List**

<u>Description</u>	<u>Quantity</u>
Spectrophotometer .....	1
Power Cord .....	1
Cuvette (round) .....	Box of 12
Square Cuvette Adapter .....	1
Dust Cover.....	1
User Manual.....	1

## Installation

1. Place the instrument in a suitable location away from direct sunlight. In order to have the best performance from your instrument, keep it as far as possible from any strong magnetic or electrical fields or any electrical device that may generate high-frequency fields. Set the unit up in an area that is free of dust, corrosive gases and strong vibrations.
2. Remove any obstructions or materials that could hinder the flow of air under and around the instrument.
3. Select either **230V** or **115V** on the Voltage Selector Switch on the backside of the **1000** shown in Figure-2, to match your local main voltage supply.

4. Turn on your **UNICO® 1000** and allow it to warm up for 15 minutes before taking any readings.

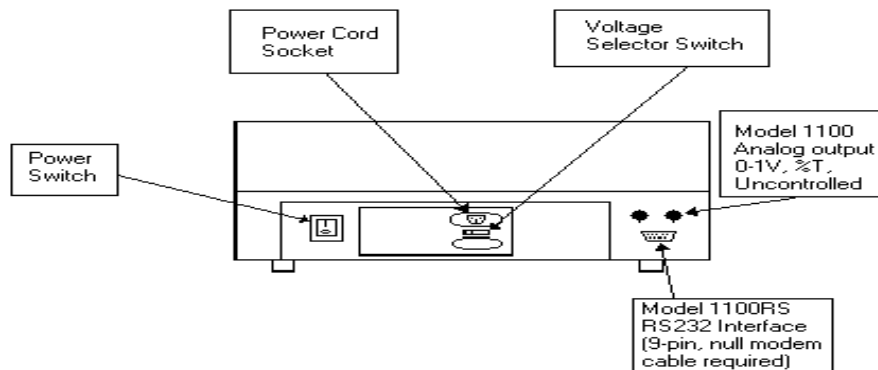


Figure-2 Backside of **UNICO® 1000**

## **1000 Spectrophotometer Operation Panel**

The key operating components of the **1000** spectrophotometer are shown in Figure-3. The power switch and analog output jack is located on the back of the instrument.

**Power Switch:** *Turns the instrument on and off.*

**MODE Button:** *Switch between **Trans** and **Abs** measurement mode.*

**0A/100%T Knob:** *Adjust Digital Display reading to 100%T or 0.000A when blank reference solution is in Sample Compartment.*

**Sample Compartment:** *Accept 10 mm test tube or 10 mm square cuvette (the square cuvette adapter is required).*

**Second Order Filter Lever:** *There are built in filters used to eliminate stray reflections and improve the precision of your measurements. To change the filter position, simply put your hand on the second order filter lever knob and pull it out or push it in until the appropriate color is shown for the wavelength range you are using. The color convention is shown in Table-2.*

**WAVELENGTH (Wavelength Control) Knob:** *Select desired wavelength in nanometers (nm).*

**Wavelength Readout Window:** *Display desired wavelength.*

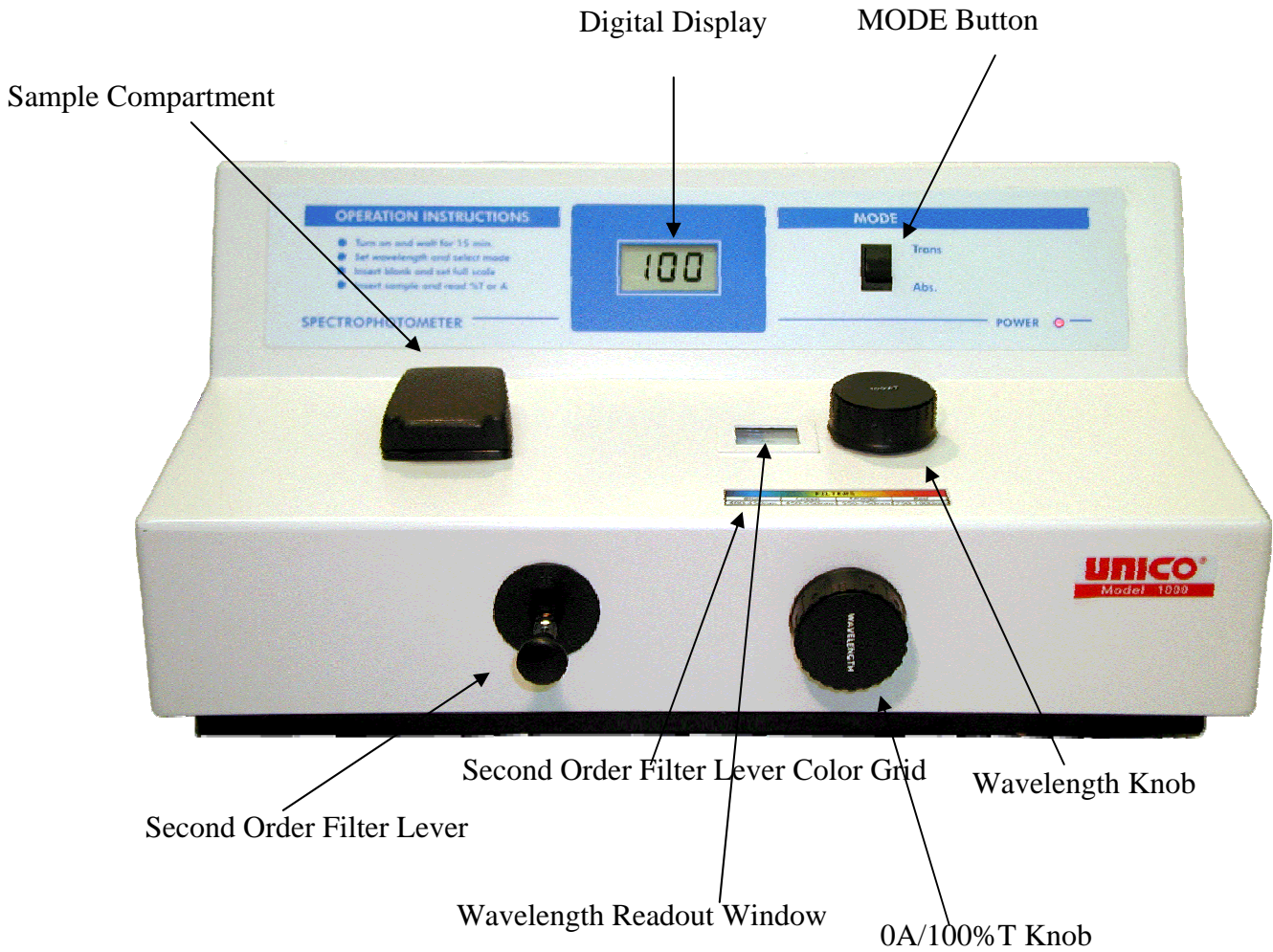


Figure-3 UNICO® 1000 Spectrophotometer

Table-2 Color Convention Chart for the Second Order Wavelength Filters

Filter Color	Wavelength Range
Blue	400~449
White	450~549
Orange	550~749
Red	750~1000

This easy to read chart can also be found on the front panel of the spectrophotometer.

## Changing Sample Holders

Your **1000** comes standard with the **S-1100-102 Sample Holder**. **UNICO®** offers three additional holders as optional accessories, please refer to Table-3 “**1000 Parts List**” in **1000 Parts List** of this manual.

Follow the steps below to change the Cuvette **Sample Holder**:

- Open the lid of the **Sample Holder**, and locate the **Sample Holder Locking Screw** as Figure-4 shown
- Use **Allen Wrench (S-1100-521)** to loosen the **Screw** counterclockwise
- Remove the **Sample Holder** you want to change, insert the one you want to install, align it properly, and fasten the **Screw**

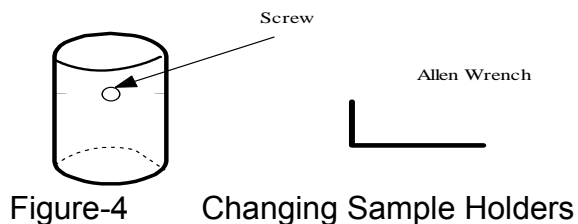


Figure-4 Changing Sample Holders

## Basic Operation

Simple **OPERATION INSTRUCTIONS** are printed on the front panel of your **UNICO® 1000**.

### **Absorbance and Transmittance Measurement**

Making a measurement of absorbance or transmittance is a two-step process. In the first step, a test tube filled with a transparent solution is placed into the sample compartment. This solution will typically be distilled water, but can be the aqueous (in water) medium that you are using for your experiment. The transmittance of this solution is made to read 100%T (0A). This process is called blanking. Blanking must be done each time a new wavelength is selected.

Note: When using square cuvettes, use the cuvette adapter provided to cradle the cuvette for insertion into the spectrophotometer.

The second step is to remove your blanking solution and replace it with the sample solution. The absorbance or transmittance can be read from the digital display.

The step-by-step procedure for making an absorption/transmittance measurement is as follows:

1. Turn on the spectrophotometer by pressing the power switch (the power switch is located on the right side of the back of the instrument.) In order to stabilize the lamp and detector; allow the instrument to warm up for at least 15 minutes.
2. Push the Mode button to choose either the Trans or Abs operating mode.
3. Turn the wavelength control knob to the desired wavelength.
4. Move the second order filter lever position to the appropriate filter setting (see chart above). For example, if your wavelength dial is set at 470 nm, pull out the second order filter lever until the white dot is showing (see Table-2).
5. Fill a test tube full with blanking solution and with a tissue, wipe off any residue and fingerprints on the outside of the test tube. If the test tube has no guide mark, some may wish to use a permanent marker to draw an approximate  $\frac{1}{4}$  inch vertical line at the lip of the test tube or cuvette (this ensures that you minimize the bearing of any differences in reflection due to small changes in the thickness in the wall of the test tube). Place a test tube into the sample compartment with the test tube guide mark aligned with the mark at the top of the compartment (this mark is located at the front of the sample compartment). Be sure that the test tube has been firmly pressed into the sample compartment and the lid of the sample compartment has been closed.
6. Adjust the display to 100%T or 0.00A by turning the 100%T/ 0A knob.
7. Remove the test tube from the sample compartment and empty the blank solution.
8. Rinse the test tube twice with small volumes of the sample solution to be measured, then fill the test tube  $\frac{2}{3}$  full with the solution and wipe it clean.
9. Place the test tube in the sample compartment, aligning the guide marks (if any).
10. Close the lid of the sample compartment.
11. Read the %T or absorbance from the digital display.
12. Remove the test tube from the sample compartment, empty it and repeat **Step 7** to **10** for any additional samples.

### **Making Measurements at Different Wavelengths**

Repeat **Step 4 to Step 12** above and remember to blank your spectrophotometer whenever a measurement is being made at different wavelengths. In addition,



when operating at a fixed wavelength for an extended period of time, check to make sure that the transmittance reads 100%T when the blanking solution is placed into the sample compartment. If not, repeat the blanking process again.

## Analog Output

Your **UNICO® 1000** Spectrophotometer is provided with two banana jacks at the rear of the spectrophotometer for interfacing with a computer or a strip chart recorder. When the Mode button is in the Trans position, the output transmittance varies from 0-1 V as the transmittance varies from 0-100%T. When the Mode button is in Abs position, the output voltage varies from 0-2 V as the absorbance varies from 0-2A. Most popular computer interfaces, in conjunction with a voltage sensor can be used to read the absorption.

## Lamp Changing

1. Remove any cuvettes from the sample compartment.
2. Unplug your spectrophotometer and turn it upside down. Locate the screw that holds the lamp housing in place (refer to the diagram shown below). **DO NOT LOOSEN THE SCREW.**
3. Remove the screw and retain. **BE CAREFUL, THE BULB MAY BE HOT.**
4. Remove the old bulb and place the leads of the new bulb into the holes in the lamp socket. Secure the bulb with the metal bracket. Use replacement bulbs with the **UNICO®** Item Number **S-1100-505**.
5. Replace the lamp-housing panel.

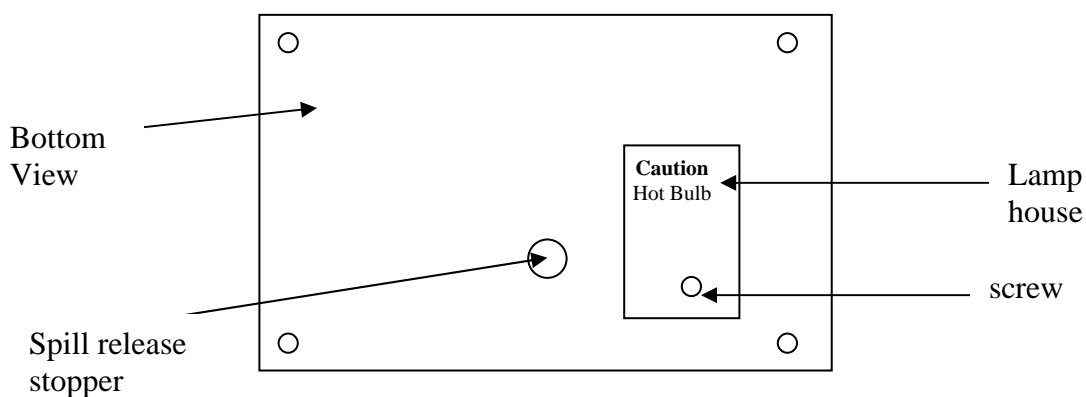


Figure-5 Bottom View of the **1000** Spectrophotometer

## Lamp Adjustment

The position of your lamp has been factory-adjusted to its optimal setting.

1. Loosen the screw on the lamp panel to reveal the light bulb within the lamp housing.
2. Make certain that the light bulb is fully inserted into its holder. The bulb socket has been permanently mounted in the optimal position by the factory. If this does not solve the problem, continue to **Step 3**.
3. The lamp socket is mounted on an "L"-shaped bracket. This bracket is held in place by 2 screws. The last option is to set your spectrophotometer to %T then slightly loosen the 2 screws such that you may move the bracket a little. Adjust this bracket so that you reach the optimum transmittance reading. Then tighten the 2 screws in the optimum position.

Tip: Don't loosen the screws too much as you adjust the bracket. This will help lessen the chance of missing the optimum positioning or movement of the bracket when securing after the optimum position has been achieved.

4. Close the lamp house door and tighten the thumbscrew.

## Absorbance Accuracy and Stray Light Checking

Specification:  $\pm 2\%$  at 1A (1100),  $\pm 1\%$  at 1A and 2A.

The absorbance accuracy and Stray Light should be checked against a set of neutral density filters accurately calibrated to the NIST standards. Contact **UNICO**<sup>®</sup> Customer Service for more information (1-800-588-9776).

## Sample EXPERIMENTS

### Experiment 1      A Brief Introduction to Beer's Law

A spectrophotometer is primarily used to identify unknown substances and to determine their concentrations. The following principles outline how this is accomplished.

Matter absorbs energy when it interacts with an energy source such as sound or light. Due to its distinctive atomic structure, each substance only absorbs energy between certain levels. Since energy is proportional to frequency,  $E=hc/\lambda$  (where  $h=6.6262 \times 10^{-34}$  J = Planck's constant,  $c=2.998 \times 10^8$  m/s = the speed of light in a vacuum (often rounded to  $3.00 \times 10^8$  m/s), and  $\lambda$  = wavelength of light). Every substance has a characteristic absorbance spectrum (which means that there is a wavelength where the atoms of a substance become highly excited).

For example, hydrogen (H<sub>2</sub>) absorbs light at the following wavelengths in the visible region: 410.7 nm, 433.8 nm, 485.7 nm, and 657.9 nm. We can verify the existence

of hydrogen ( $H_2$ ) in an unknown sample by comparing the wavelengths absorbed by the unknown sample to the wavelengths at which hydrogen absorption is known.

A spectrophotometer is required to measure absorption spectrums in order to identify an unknown substance. The spectrophotometer is used to measure the amount of light absorbed at distinct wavelength. This can be plotted and the graph can be used to identify the presence of a particular substance.

A spectrophotometer can also be used to determine the concentration of an unknown sample. When a light beam is incident to a sample, part is absorbed, and part is transmitted. The transmittance (T) is defined as the ratio of the transmitted intensity of the light beam (X) to the initial intensity of the light beam (Y), or:

$$T=X/Y.$$

The transmittance varies from 0 to 100. A transmittance of 0%T represents complete absorption by the sample, whereas 100%T represents no absorption by the sample.

The absorbance (A) is defined as:

$$A= \log (1/T).$$

When the transmittance is 100% ( $T=1$ ),  $A=\log(1)=0$  and increases as the transmittance decreases. The maximum value of the absorbance read by the **1000** is 2.0. This represents a Transmittance of:

$$T=1/10^{2.0} \text{ or about } 1\%. \text{ This is a very small transmittance and is essentially zero.}$$

It is found that absorbance is directly proportional to concentration so that:

$$A=EBC$$

Where:

A=The absorbance

C=The concentration in moles/liter, and

B=The width of the sample cell or cuvette in cm

E=A proportionality constant called the molar absorptivity (measured in liters/mole-cm)

The linear relationship between absorbance and concentration and sample cell width is called Beer's Law.

If we have a standard sample with a known absorbance and concentration and a measured absorbance, it is easy to determine an unknown concentration of the sample substance via linear interpolation (see Figure-4 below).

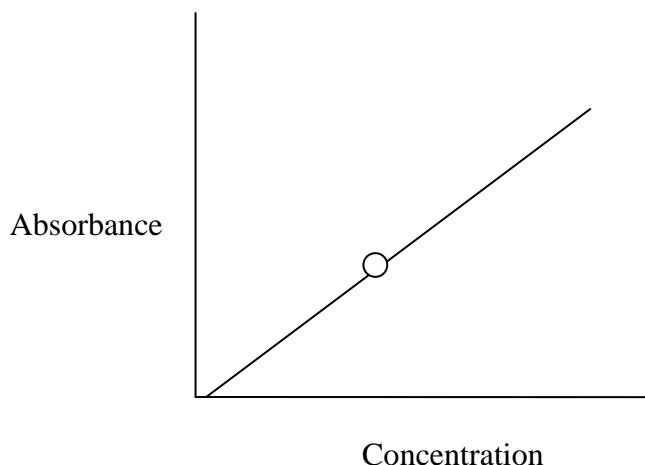


Figure-4 Absorbance vs. Concentration

### Experiment 2 Measurement of an Absorption Spectrum

The absorption spectrum of a substance helps identify it. In this experiment, you will measure the absorbencies of  $\text{Cr}^{3+}$  (chromium III ions) at a series of wavelengths in the visible light region and draw its absorption spectrum.

The materials required for this experiment are 20 ml 0.5M  $\text{Cr}^{3+}$  (aq) solution and two round cuvettes.

Procedure:

1. Place the wavelength dial at a position of 400 nm and adjust the second order filter lever position.
2. Fill one cuvette with de-ionized water and insert it into the sample compartment. Blank the instrument according to the procedure in Basic Operation. Then, remove the cuvette.
3. Fill another cuvette (or the same cuvette rinsed thoroughly with the stock solution) with the  $\text{Cr}^{3+}$  stock solution and inset it into the sample compartment. Record the absorbance of the solution at this wavelength and then remove the cuvette.

Repeat **Step 1** through **Step 3** for wavelengths from 400 to 650 nm at 20 nm intervals. Make sure that the meter/display 0%T and 100%T readings are adjusted at each new wavelength.

Calculations:

1. Study the data and identify the points where the peaks are likely to be found. Make measurements at smaller intervals (e.g. 5 nm) around these points to locate the peaks more accurately.
2. On regular graph paper, label the horizontal axis wavelength, and mark it from 400 to 650 nm in 20 nm intervals. Label the vertical axis absorbance and mark it in equal intervals from zero to a convenient round value above your highest absorbance.

3. Plot the absorbance for wavelength measured. Draw a smooth curve through the data points.

### **Experiment 3 Study of Beer's Law**

Beer's Law is the basis for the determination of the concentration of an unknown solution. It states that there is a linear relationship between the absorbance and the concentration of the absorbing substance.

In order to verify Beer's law, we can measure the absorbance for different concentrations of a substance at the same wavelength, plot them on a piece of graph paper and see if the data points lie along a straight line.

Actually, the points probably will not form an ideal straight line, due to the uncertainty in the measurements.

The materials required for this experiment are 500 ml 0.04 g/l thymol blue solution, 40 ml 1 M HCL (aqueous), a 50 ml burette, a 10 ml graduated cylinder, a 100 ml volumetric flask, five round cuvettes, and four Erlenmeyer flasks.

Procedure:

1. Fill the burette to the top calibration line (50 ml) with the thymol blue stock solution.
2. Deliver 5 ml of the thymol blue solution from the burette into the volumetric flask. Measure 10 ml HCL (aq) in a graduated cylinder and add to the flask. Dilute the flask to the mark with de-ionized water, cap the flask, and mix the diluted solution thoroughly. Transfer this first standard to an Erlenmeyer flask, calculate its concentration, and label the flask.
3. Rinse the volumetric flask with de-ionized water and repeat step 2 for 15 ml, 20 ml, and 30 ml of HCL. These are standards 2, 3, and 4.
4. Place the wavelength at 545 nm and the second order filter in the white position. Fill a cuvette with de-ionized water and insert it into the sample compartment. Blank the instrument according to the procedure in **Basic Operation**.
5. Fill another cuvette with the first standard and insert it into the sample compartment. Measure the absorbance of the first standard at this wavelength.
6. Repeat step 5 for the other three standards.

Calculations:

1. On a sheet of graph paper, label the horizontal axis concentration and mark it in equal intervals from 0 to 0.02 M. Label the vertical axis absorbance and mark it in equal intervals from 0 to a convenient round value above your highest data point.
2. Plot absorbency versus concentration for the four standard solutions.
3. Using a transparent ruler, draw a straight line from the origin such that there are equal numbers of points above and below the line. This is the Beer's law plot.

#### **Experiment 4      Determination of the Concentration of a Solution**

You will prepare a Beer's Law plot from a series of molybdenum blue standards and determine the amount of glucose in a soft drink. The glucose is able to reduce  $\text{Cu}^{2+}$  to  $\text{Cu}^+$  under the reaction conditions. The  $\text{Cu}^+$  is then used to reduce phosphomolybdic acid (PMA) to molybdenum blue, which absorbs light at a wavelength of 780 nm. The amount of molybdenum blue formed is directly proportional to the amount of glucose originally present in the solution.

The materials required for this experiment are 50 ml 2 g/l glucose stock solution, 12 ml alkaline copper titrate solution, 12 ml phosphomolybdic acid (PMA) solution, 10 ml unknown soft drink sample, two 600 ml beakers, a 50 ml burette, six Erlenmeyer flasks, a 2 ml pipette, a 10 ml pipette, six 25 ml test tubes, six round or square cuvettes, and a hot plate.

Procedure:

1. Fill the burette to the top calibration line (50 ml) with the glucose stock solution. Deliver 5 ml of the glucose solution from the burette into the volumetric flask. Dilute the flask to the mark with de-ionized water, cap the flask, and mix the diluted solution thoroughly. Transfer this first standard to an Erlenmeyer flask, calculate its concentration, and label the flask. Rinse the volumetric flask with de-ionized water and repeat step 2 for standards 2, 3, and 4 using 10 ml, 15 ml, and 20 ml of the stock solution.
4. Pour 10 ml of the soft drink into the 100 ml volumetric flask, swirl it until bubbling stops, and dilute to the mark. Transfer this solution to an Erlenmeyer flask. Rinse the volumetric flask, pipette 10 ml of the diluted soft drink solution into it, dilute and mix. Transfer this solution to another Erlenmeyer flask. Rinse the pipette and volumetric flask. Pipette 10 ml of the second dilution into the volumetric flask, dilute and mix. This final unknown solution is the 1:1000 dilution of the original soft drink.
5. Label six test tubes. Fill them with 25 ml of water using a pipette, mark the solution level, and empty them. Pipette 2 ml of water, the unknown and the four standards into the tubes, respectively. Rinse the pipette after each use.
6. Using a graduated cylinder, add 2 ml of the alkaline copper titrate solution to each test tube and stir by swirling the tubes. Rinse the graduated cylinder well.
7. Fill half of a 600 ml beaker with water and place it on a hot plate to boil. Prepare an ice-water bath in a second 600 ml beaker.
8. Immerse the six tubes into the boiling water for exactly six minutes to oxidize the glucose. Remove the tubes from the boiling water and immerse them in the ice water bath for exactly 3 minutes to allow them to cool.
9. Using a graduated cylinder, add 2 ml to the phosphomolybdic acid solution to each tube and stir by swirling them. Allow the solution two minutes to react, and then dilute them to the 25 ml mark with de-ionized water.
10. Place rubber stopper in each tube and mix by inverting the tubes several times.

11. Set the wavelength to 780 nm and put the second order filter lever in the red position. Fill a cuvette with a blank solution and insert it into the sample compartment. Blank the instrument according to the procedure in **Basic Operation**.
12. Fill another cuvette with the first standard and measure its absorbance on the spectrophotometer.

Repeat **Step 12** for the other three standards and the unknown solution.

Calculation:

1. On a piece of regular graph paper, label the horizontal axis concentration and mark it in equal intervals from 0 to the value of standard 4. Label the vertical axis absorbance and mark it in equal intervals from 0 to a convenient round value above your highest data point.
2. Plot absorbance versus concentration for the four standards. Draw the best straight line through the origin and the four points.
3. Using the working graph determine the concentration of the unknown solution.
4. Multiply the concentration of the unknown by 1000 to get the concentration of glucose in the original soft drink.

### **Experiment 5      Determination of a Species in a Solid Sample**

In order to determine the amount of a species in a solid sample, it is necessary to make an extraction solution containing that species with a proper solvent and then measure the concentration of the species in the solution using the same method described in the last experiment.

As an example, you will determine the amount of iron in a vitamin tablet. Iron II forms a colored complex with 1, 10 phenanthroline, which absorbs light energy at 508 nm. A working curve will be prepared for standard solutions of Iron II-phenanthroline complex, and the concentration of an unknown sample will be determined from the working curve data.

The materials required for this experiment are a vitamin tablet, 50 ml 0.1M HCL (aq) solution, 50 ml 0.1g/l  $\text{Fe}^{+2}$  (aq) solution, 10 ml 1% (w/v)  $\text{HONH}_3 \text{CL}$  (aq) (hydroxylamine hydrochloride solution, 10 ml 1M  $\text{NaC}_2\text{H}_3\text{O}_2$  (aq) sodium acetate) solution, 10 ml 1% (w/v) 1,10- phenanthroline (ortho) solution, a 100 ml beaker, a 50 ml burette, five Erlenmeyer flasks, a 50 ml graduated cylinder, a 10 ml pipette, six round cuvettes, a hot plate, a stirring rod, a gravity funnel, and some filter paper.

Procedure:

1. Fill the burette to the top calibration line (50 ml) with the  $\text{Fe}^{+2}$  stock solution.
2. Deliver 5 ml of the stock solution from the burette into a clean 100 ml volumetric flask. Measure 10 ml of the acetate solution and 10 ml of the phenanthroline solution into the graduated cylinder respectively and add to the flask. Allow the mixture to stand for five minutes, dilute the flask to the mark with de-ionized water, cap the flask, and mix the diluted solution thoroughly. Transfer this first standard to the Erlenmeyer flask, calculate the concentration of the standard and label the flask.
3. Rinse the volumetric flask with de-ionized water and repeat **Step 2** for 10 ml, 15 ml, and 20 ml of the stock solution from the burette. These are standards 2, 3, and 4.
4. Place the vitamin tablet into a 100 ml beaker. Measure 50 ml of the 0.1 M HCL (aq) into the graduated cylinder and add it to the beaker.
5. Place the beaker on a hot plate and heat the extraction solution to boiling. Gently boil the acid extraction solution for 15 minutes. Break apart the tablet with a clean stirring rod while boiling. Remove the beaker from the hot plate and allow the solution to cool to room temperature.
6. Transfer the solution from the beaker to the 100 ml volumetric flask. Dilute with de-ionized water and mix thoroughly. This is your diluted extraction solution.
7. Filter 20 ml of the diluted extraction mixture through filter paper in a gravity funnel into a flask or beaker.
8. Wash the volumetric flask thoroughly with de-ionized water. Pipette 10 ml of the diluted extraction solution into the flask, dilute to the mark, and mix thoroughly. This second diluted extraction solution is the unknown solution. Transfer the solution to an Erlenmeyer flask and label it.
9. Place the second order filter lever in the white position and set the wavelength to 508 nm. Fill a cuvette with de-ionized water and insert it into the sample compartment. Blank the instrument according to the procedure in **Basic Operation**.
10. Fill another cuvette with the first standard. Insert the cuvette into the sample compartment and record the absorbance.
11. Repeat step 10 for the other three standards and the unknown.

**Note:** The range of iron levels in the vitamin tablets can be quite broad. As a result, the prepared unknown solution may not yield an absorbance value that can be properly interpreted from the standard curve. Therefore, you may have to make adjustments in the concentration of iron in the unknown solution by trial and error. If the absorbance of the unknown turns out to be higher than that of standard 4, go back to **Step 8** and use smaller amount (e.g. 5 ml) of the diluted extraction solution in making the unknown. If the absorbance of the unknown is lower than that of standard 1, use a larger amount (e.g. 20 ml) instead. Keep doing the above until the desired unknown is obtained.



Calculations:

1. On a regular piece of graph paper, label the horizontal axis concentration and mark it in equal intervals from 0 to the value of standard 4. Label the vertical axis absorbance and mark it in equal intervals from zero to a convenient round value above your highest data point.
2. Plot absorbance versus concentration for the four standard solutions. Draw the best straight line through the four points and the origin of the graph.
3. Use the working graph to determine the concentration of Iron II ion in the unknown solution. Using the dilution data from the experiment, calculate the concentration of the diluted extraction solution and the mass of iron (in mg) present in the vitamin tablet.

## **1000 Parts List**

Table-3      **1000 Parts List**

<b>Item Number</b>	<b>Description</b>
S-1000	<b>UNICO</b> <sup>®</sup> Model <b>1000</b> Spectrophotometer 20 nm Bandpass
	Wavelength range: 400~1000 nm. Voltage preset at 110V
	Complete with 10 mm Test Tube Cuvettes (12 pcs.)
	10 mm Cuvette Adapter, Dust Cover, User Manual
S-1000-E	Same as Model <b>1000</b> but preset at 220 V
<b>Accessories</b>	
S-1100-101	Experiment manual includes safety in the lab, 10 experiments, instructor guide
S-1100-102	Test Tube Sample Holder for 10 mm diameter Test Tubes
S-1100-103	Square Cuvette Adapter for 10 mm Cuvettes
S-1100-113	Test Tube Sample Holder for ¾ inch Test Tubes
S-1100-114	Test Tube Sample Holder for 1 inch Test Tubes
S-1100-115	COD Vial Sample Holder
S-1100-220	Power Cord, European plug
S-2100-115	Holmium Oxide Filter 10 mm x 10 mm x 45 mm (requires <b>S-1100-103</b> )
S-2100-116	Didymium Filter 10 mm x 10 mm x 45 mm (requires <b>S-1100-103</b> )
<b>Glassware</b>	
S-90-301	Test Tube Cuvette, 10 mm diameter, 12 pcs.
S-90-302P-100	Disposable Cuvette, Polystyrene, 10 mm pathlength, 100 pcs.
S-90-302P-500	Disposable Cuvette, Polystyrene, 10 mm pathlength, 500 pcs.
S-90-304G	Square Cuvettes, Optical Glass, Set of 2
S-90-305P	Disposable semi-micro Cuvette, Polystyrene, 10 mm pathlength, 500 pcs.
<b>Misc. and Replacement Items</b>	
S-1100-505	Tungsten-Halogen Lamps, Package of 2 (6V 10W G4 type)
S-1100-508	Printer Paper, Package of 3
S-1100-511	User Manual
S-1100-512	Dust Cover
S-1100-513	Fuse, 3A, quantity 1 (size 5 x 20)
S-1100-521	Allen Wrench

## Troubleshooting

<b>PROBLEM</b>	<b>Possible Cause</b>	<b>Solution</b>
<b>Instrument Inoperative (Power indicator has no light)</b>	Power cord not connected to outlet	<i>Plug instrument in</i>
	Dead Power outlet Wrong voltage setting	<i>Change to a different outlet</i>
	Internal fuse blown or defective electronic component	<i>Call an authorized service engineer</i>
<b>Instrument can not set 100%T (0.000A)</b>	No Cuvette Adapter in the Sample Compartment	<i>Cuvette Adapter must be in Sample Compartment to open sample holder shutter</i>
	Light beam blocked: <ul style="list-style-type: none"> <li>• Holder misaligned</li> <li>• Shutter</li> </ul>	<i>Check sample holder</i>
	Lamp is old or defective	<i>Replace Lamp</i>
	Lamp is off alignment	<i>Refer to Lamp Replacement instructions in this manual</i>
	Defective electronic component	<i>Call an authorized service engineer</i>
<b>%T can not be set to 00.0%T</b>	Sample holder	<i>Remove Cuvette Adapter or test tube</i>
	Sample holder shutter	<i>May be stuck open Close shutter</i>
	Defective electronic component	<i>Call an authorized service engineer</i>
<b>Incorrect Transmittance to Absorbance correlation</b>	Bubbles or particles in solution	<i>Check sample preparation and analytical procedure</i>
	Defective electronic component	<i>Call an authorized service engineer</i>
<b>Digital Display does not change regardless of sample concentration</b>	Concentration reading “frozen”	<i>Change MODE from C to F, T or A and back to C Restart measurement procedure</i>
	Wrong wavelength setting	<i>Check sample procedure and wavelength setting</i>
	Insufficient sample volume	<i>Fill cuvette with more sample</i>
	Stray sample preparation vapors	<i>Prepare the sample away from the instrument. Use proper ventilation</i>
	Bubbles or particles in solution	<i>Check sample preparation and analytical procedure</i>
	Defective electronic component or loose wiring	<i>Call an authorized service engineer</i>

<b>PROBLEM</b>	<b>Possible Cause</b>	<b>Solution</b>
<b>Instrument drift and noise</b>	No sufficient warm up time Significant temperature change Lamp not adjusted properly	<i>Check lamp has been properly installed or has moved during transit Refer to Lamp Replacement instructions in this manual</i>
	Lamp old or defective	<i>Replace with a new lamp</i>
	Sample Holder Misaligned	<i>Refer to Lamp Replacement instructions in this manual</i>
	Unstable power supply Defective or dirty detector or defective electronic component	<i>Call an authorized service engineer</i>
<b>Incorrect readings obtained</b>	Insufficient sample volume	<i>Fill cuvette with more sample</i>
	Wrong wavelength setting Failed to blank (0A/100%T) Failed to set 0%T	<i>Check analytical procedure and wavelength setting Check wavelength accuracy according to procedure in this manual</i>
	Stray sample preparation vapors	<i>Prepare sample away from instrument. Use proper ventilation</i>
	Bubbles or particles in solution	<i>Check sample preparation and analytical procedure</i>
	Instrument out of electronic calibration	<i>Call an authorized service engineer</i>

### **UNICO Authorized Service Contact Information**

UNICO  
182 Ridge Road, Suite E,  
Dayton, NJ 08810, USA  
Tel.: 800-588-9776 or 732-274-1155  
E-mail: [info@unico1.com](mailto:info@unico1.com)