# Colorimeter Sensor

***(Product No. 3275)***

Transmittance range: 0 - 110%T
Resolution: 0.1%

Absorbance range: 0.0500 - 1.0500 Abs.

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## Contents



## Introduction

The *Smart Q* Colorimeter measures the amount of light penetrating a solution. It can be used for investigations which result in a change in colour or opacity e.g. Beer's law and rate of reaction experiments. It is supplied calibrated and the stored calibration is automatically loaded into the **EASY**SENSE unit when the Colorimeter is connected.

The Colorimeter is supplied with

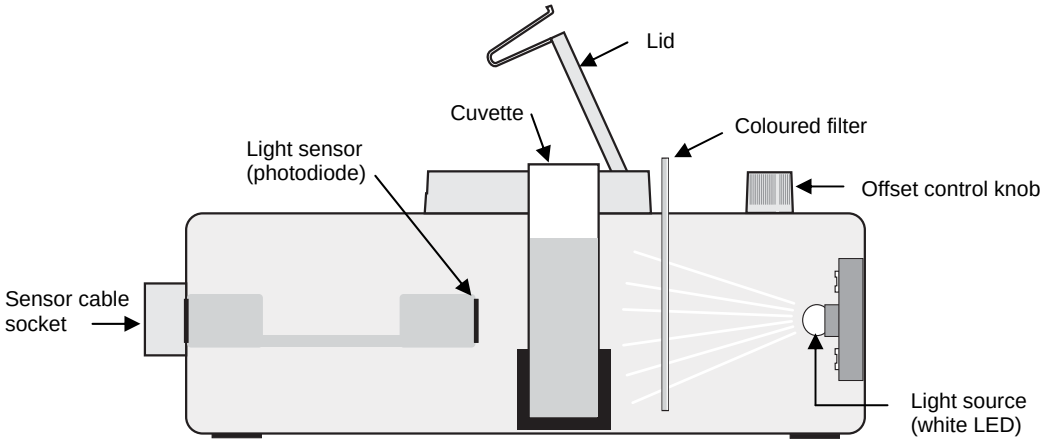
- Four coloured filters of different wavelengths.

| Red - 630 nm | Orange - 600 nm | Green - 560 nm | Blue - 470 nm |
|---|---|---|---|

- Five cuvettes - made of optical polystyrene, with a capacity of 4.0 ml.

The *Smart Q* Colorimeter can be used with two ranges:

1. Transmittance / Transmission (**%T**) - the amount of light being received referenced to light sent.
2. Absorbance (**Abs**) - the amount of light absorbed by the solution.




The Colorimeter uses a white LED as a light source, which has the advantage of not heating the solution being studied. The white light from the LED passes through a cuvette that contains the sample solution. Some of the light will be absorbed by the solution and as a result the light received by the photodiode will be of lower intensity.

An offset control knob allows the user to set a maximum value for the investigation. Turning the knob clockwise will increase the light from the LED.

- For the **Transmission range**: (%T) - Use the **weakest** solution to set a transmission value of **100%** or slightly below.

- For the **Absorbance range: (Abs)** - Use the **strongest** (darkest) solution in the range to give an Absorbance of **0.70 Abs**. Alternatively use the **weakest** (lightest) solution to give an absorbance of **0.05 Abs**.

The Colorimeter has a snap-shut lid to prevent stray light entering the unit from the environment. If a cuvette is in the Colorimeter and the lid is opened, a small spring in the base of the cuvette holder will push the cuvette above the moulding. This gives the user easy access to the cuvette and improves handling.

***Note:*** *Take care when opening the lid that the spring tension is not released too quickly. If an uncapped full cuvette springs up too quickly, some spillage may take place.*

To open, push back the catch and allow the lid to open slowly.

To close, push down the lid until the catch snaps into place.

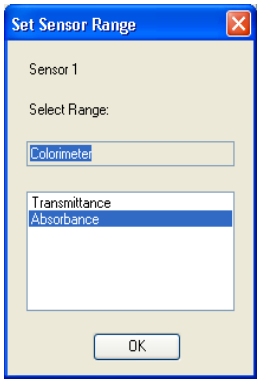
## Connecting

- Push one end of the sensor cable (supplied with the **EASY**SENSE unit) into the shaped socket on the Colorimeter with the locating arrow on the cable facing upwards.
- Connect the other end of the sensor cable to an input socket on the **EASY**SENSE unit.
- The **EASY**SENSE unit will detect that the Colorimeter is connected and display light values using the currently selected range i.e. either Transmission (**%T**) or Absorbance (**Abs**). If the range is not suitable for your investigation, set to the correct range.

Power is provided to the Colorimeter from the **EASY**SENSE unit. The use of an LED as the light source means there is no warm up time or drift.

## To set the range

To alter the currently selected range:




- Connect the Colorimeter to the **EASY**SENSE unit.
- Start the **EASY**SENSE program and select one of the logging modes from the Home page e.g. EasyLog. Select **Sensor Config** from the **Settings** menu.
- Select the Colorimeter from the list (it will be listed using its current range) and click on the **Change Range** button.
- The current range will be highlighted. Select the required range and click on OK.
- Close Sensor Config. Click on New and then Finish for the change in range to be detected by the logging mode.

The range setting will be retained until changed by the user. With some **EASY**SENSE units it is possible to change the range from the unit. Please refer to the **EASY**SENSE unit's user manual.

## Coloured filters

| Red - 630 nm | Orange - 600 nm | Green - 560 nm | Blue - 470 nm |
|---|---|---|---|

Coloured filters can be used to select wavelength. Select a filter that produces light which will be absorbed by the solution rather than transmitted through it. For example, a solution of copper sulphate is blue because it transmits blue light, so using an orange or red filter will provide a light source that is absorbed by the solution i.e. the filter selected should not be the same colour as the solution.

To test if the 'right filter' is being used, place a cuvette with the solution in the Colorimeter and check with the coloured filters provided to see which yields the largest value for Absorbance.

The coloured filters will reduce the amount of light reaching the photodiode. The LED can be made brighter by turning the offset knob on the top of the Colorimeter.

## Using Cuvettes

The optical qualities of the container holding the test solutions should be identical throughout the experiment. The optical polystyrene cuvettes supplied with the Colorimeter are manufactured to be within 1% absorbance to each other. For class experiments they can be considered as identical. The design of the cuvette and holder is such that the position and distance between light source, photodiode and experiment remains constant.

The cuvette has two clear faces and two ribbed faces. The clear faces are the optical surfaces and the ribbed faces are used for handling the cuvette. There can be slight difference in transmission if the cuvette is rotated by 180 degrees

***Note:*** *A small mark on one of the clear faces of the cuvette will assist in orientation of the cuvette in the Colorimeter*

The cuvettes supplied hold 4.1 $cm^3$ of liquid. Small caps are provided with the cuvettes. These are useful when solutions are mixed by shaking the cuvette or to prevent evaporation during the course of an experiment.

**Note:** *With some experiments (for example rate of reaction) the time taken in fitting the cap can result in loss of early data.*

If cuvettes are to be moved in and out of the Colorimeter limit the maximum volume to 3.5 $cm^3$ or use a cap. If the cuvette is being filled whilst in the Colorimeter, a volume of up to 4.0 $cm^3$ can be used.

Small scratches on the surface of the cuvette can affect results. The optical face of the cuvettes will in time become fogged leading to a loss of transmission. This will not be a problem if the same cuvette is used throughout an experiment.

If replacement cuvettes are required they should be 10 mm x 10 mm with a 4 ml capacity e.g. Scientific & Chemical Supplies, Part No. **CUV 010 020**.

## Practical information

- Do not let liquids enter the body of the Colorimeter.
- Do not use organic compounds from the aromatic, halogenated, aliphatic, ketone, aldehyde or ester groups in the polystyrene cuvettes.

  ***Note:*** *The plastics mix used for the cuvettes can differ from manufacturer to manufacturer.*

## The relationship between Transmittance (T) and Absorbance (A)

Transmission = **T,** Absorbance = **A**

The amount of light that penetrates a solution is known as transmittance. It is a ratio of the intensity of the light transmitted (**It**) to the intensity of the original light beam (**Io**).

$$T = \frac{It}{Io}$$

The transmittance of a solution varies to Log(base10) with three factors,
1. The Molar absorptivity of the solution **E**
2. The cell or cuvette width **b**
3. The Molar concentration **C**

This is expressed as $\text{Log}\left(\frac{I}{T}\right) = EbC$

Absorbance would at first appear to have an inverse relationship to Transmittance. In reality the light transmitted by a solution as the concentration increases does not show any obvious linearity. The true relationship is inverse and logarithmic (base10).

$A = \text{Log}\left(\frac{I}{T}\right)$ or $A = EbC$

By using cuvettes of identical optical properties **E** and **b** will remain constant and the equation known as Beer's law is produced.

$$A = kC$$

**C** is the molar concentration of the solution, **k** is a constant, and so absorbance can be used to measure the concentration of a solution.

The linear relationship between Absorbance and concentration does not hold across the whole of the Transmittance range. Absorbance values below 0.045**A** (**T** = 90%) and above 0.700**A** (**T** = 20%) are considered to be unreliable. Experiments using the absorbance range should be designed to fit within these values.

| Transmission value (%T) | Appearance of solution | Absorbance value (Abs) | Interpretation |
|---|---|---|---|
| 20 | Light | 0.7 | Little light is being absorbed; most light is passing through the solution. |
| 90 | Dark | 0.045 | Most light is being absorbed; little light is passing through the solution. |

## Investigations

**Examples of investigations that use the 'Transmission' range**
*Rate of reaction experiments e.g. sodium thiosulphate and acid*
*Growth of yeast in a sugar solution*
*Digestion of starch by amylase*
*Use of oxygen in respiration*
*Growth curve of chlorella*

**Examples of investigations that would use the Absorbance range**
*Beer's Law*
*Rate reaction of crystal violet*
*Colorimetric determination of manganese in a steel paper clip*
*Estimation of chlorine in water*
*Determination of glucose concentration*

## Beer's Law

Beer's law can be used to determine the concentration of an unknown solution.
There are several solutions that can be easily made in the laboratory to demonstrate Beer's law.

Examples:

### *Crystal violet*

Use a green filter with a dilute solution of crystal violet. E.g. make up a stock solution of 8.0 x $10^{-5}$ mol $dm^{-3}$ crystal violet, by adding 65.3 mg of crystal violet to 2 litres of water. Dilutions can then be made up to cover a range of Absorbance.

***Note:*** *Crystal violet is an intense stain and care needs to be taken when using it. To decolourise cuvettes and glassware rinse with dilute acid. For more intense stains leave the acid in contact for longer or increase the strength of the acid.*

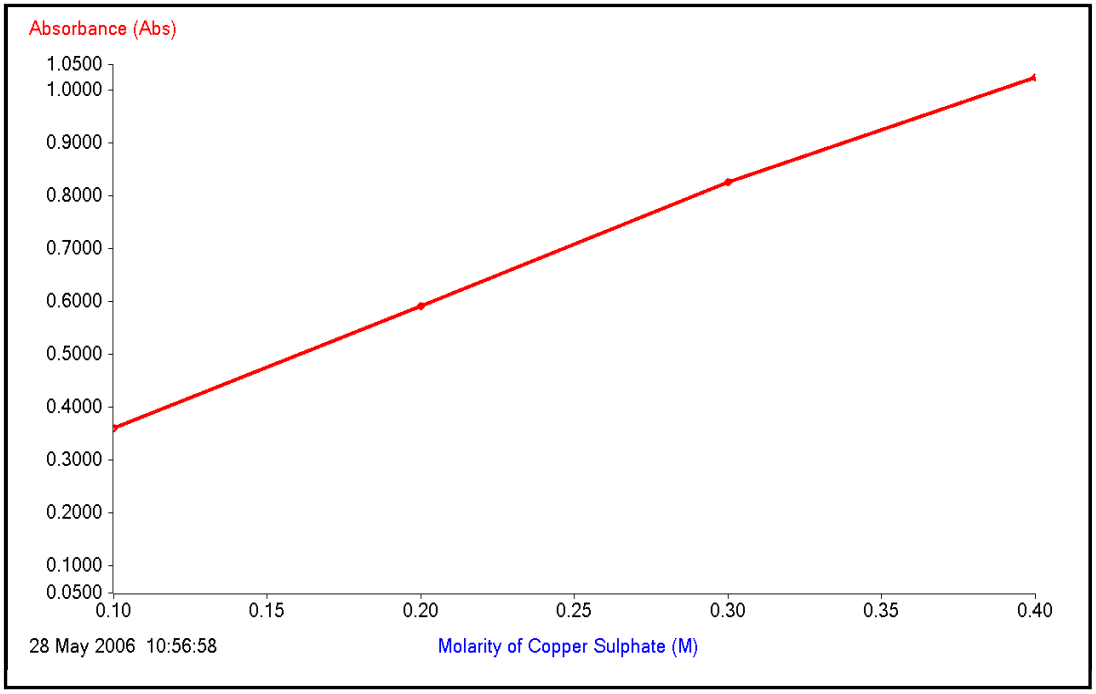
### *Food Colouring*

Food colours diluted with water can be used to demonstrate Beer's law. A litre of water can be coloured by the addition of 5 - 6 drops of the colouring. This represents a 100% concentration; further dilutions can then be made to create 80, 60, 40, and 20% solutions.

- For blue food colouring use an orange or red filter.
- For red food colouring use a green filter.
- For green food colouring use a red or orange filter.
- For yellow/orange food colouring use a blue filter

### *Copper Sulphate*

Use 0.1, 0.2, 0.3 and 0.4 mol $dm^{-3}$ solutions with the red filter.




For all the solutions the method is the same.

1. Prepare the solutions and cuvettes to be tested, i.e. 80% solution, 60%, 40%, and 20%.
2. Attach the Colorimeter to the **EASY**SENSE unit.
3. Open the **EASY**SENSE program and select **Snapshot** from the Home page. The Y-axis should show **Absorbance** (Abs), if not change the range.
4. Select **Pre-log Function** from the **Tools** menu.

5. Select a **Preset** function, with **General** from the first drop-down list and then **Asks for Value** from the second list. Next. Type ‘Concentration’ as the name and enter the units to be used e.g. %. Finish.
6. From the **Options** icon select **X-Axis** and select **Channel.** OK. If necessary, click below the X-axis so that ‘Concentration’ is displayed.
7. Select **Test** mode from the **Tools** menu and place the strongest coloured solution into the Colorimeter. Close the lid and insert the appropriate colour filter (into the slot at the front of the Colorimeter).
8. Adjust the offset knob anticlockwise to give an absorbance value close to 0.7 Abs. Remove the cuvette.
9. Click on the **Start** icon to begin. Place the weakest coloured solution in the Colorimeter and click in the graph area to record the Absorbance value of the sample. Type the concentration into the ‘enter value box’. OK.
10. Repeat using the other test solutions (work in order of weakest to strongest colour).
11. Click on the **Stop** icon to finish recording.

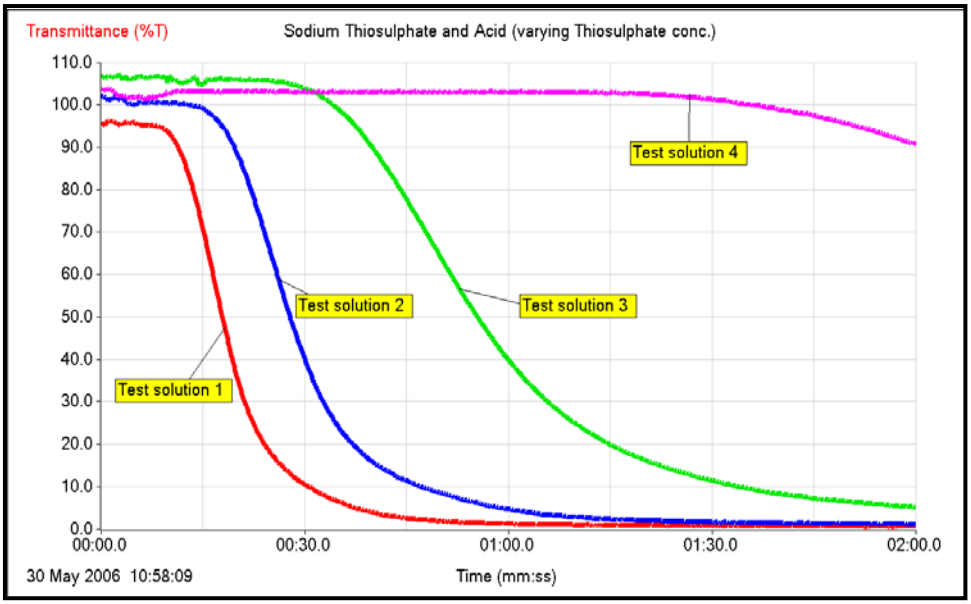
***Note:*** *If the Absorbance values of the weaker solutions give the same value repeat the experiment, using the weakest of these solutions to set the absorbance to 0.05.*

## Rate of reaction kinetics using sodium thiosulphate and hydrochloric acid

Sodium thiosulphate will decompose to produce colloidal sulphur in the presence of an acid catalyst. The effect of concentration on a reaction can be studied by keeping the concentration of the acid constant but changing the concentration of the thiosulphate.

1. Attach the Colorimeter to the **EASY**SENSE unit.
2. Open the **EASY**SENSE program and select **EasyLog** from the Home page. The Y-axis should show **Transmission (%)**, if not change the range.
3. Fill a cuvette with water, place in the Colorimeter and insert the blue filter. Select **Test** from the **Tools** menu and adjust the offset knob to give a transmission reading of 100%. Remove the cuvette.
4. Place 0.5 $cm^3$ of 1mol $dm^{-3}$ hydrochloric acid into a cuvette. Place the cuvette in the Colorimeter.
5. Use a pipette or syringe to add 3.5 $cm^3$ of 40 g/litre thiosulphate to the cuvette (the addition will mix the two solutions). Close the lid and click on the **Start** icon to begin.
6. The length of time taken for the solution to become opaque will increase with each test solution so continue recording for at least another minute after the transmission level has dropped to near zero and then click on the **Stop** icon to finish. Wash and dry the cuvette.
7. Select **Overlay**.
8. Repeat the experiment from step 4 using the test solutions shown in the table below.

| Test solution | 40 g/L Thiosulphate ($cm^3$) | Water ($cm^3$) | 1mol $dm^{-3}$ HCl ($cm^3$) | Total Volume ($cm^3$) |
|---|---|---|---|---|
| 1 | **3.5** | **0** | 0.5 | 4.0 |
| 2 | **2.5** | **1** | 0.5 | 4.0 |
| 3 | **1.5** | **2** | 0.5 | 4.0 |
| 4 | **0.5** | **3** | 0.5 | 4.0 |




The reaction produces colloidal sulphur, which will make the solution opaque and blocks the light transmission. The results will show a fall in transmission with time.

## Using protease enzyme to study the effect of enzyme concentration on an enzyme catalysed reaction

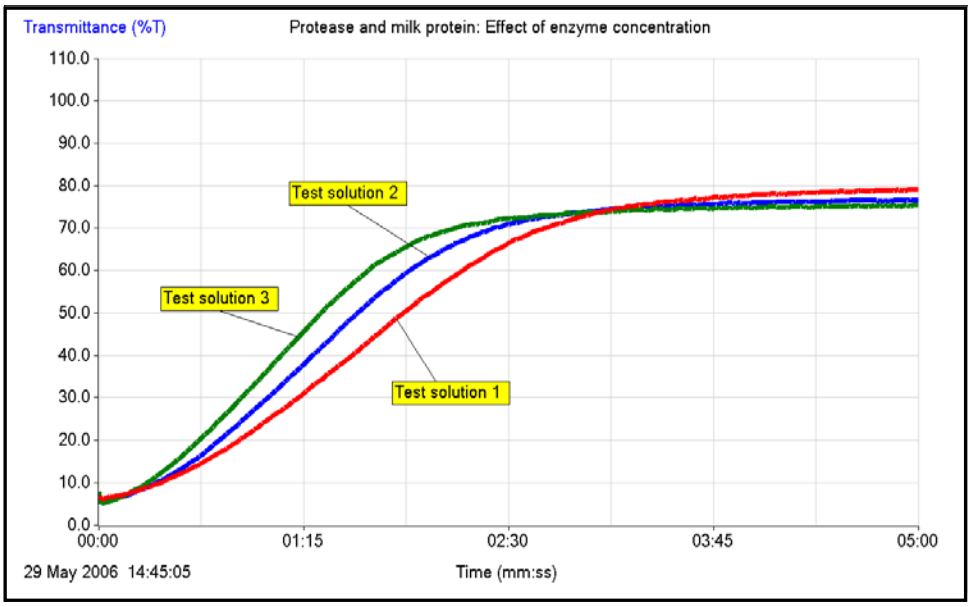
Enzymes work by digesting a substrate and producing smaller fragments (the product). Milk protein can be digested with a protease enzyme and the original opaque white colour of the milk powder solution is replaced with a faint straw-coloured solution of amino acid fragments.

This experiment was conducted at room temperature using:

- Neutrase (protease enzyme) supplied by NCBE Reading, made up to a 0.05% v/v solution.
- Supermarket skimmed milk powder made to a 1% w/v solution.

1. Prepare a standard reference cuvette by placing 2 $cm^3$ of enzyme e.g. 0.05% v/v neutrase, into a cuvette and adding 3 $cm^3$ of a 1% w/v solution of milk powder. Leave this for 5 - 10 minutes until it becomes clear.
2. Attach the Colorimeter to the **EASY**SENSE unit.
3. Open the **EASY**SENSE program and select **EasyLog** from the Home page. The Y-axis should show **Transmission (%)**, if not change the range
4. Place the reference cuvette into the Colorimeter and insert a blue filter. Select **Test Mode** from the **Tools** menu and adjust the offset knob to give a transmission reading of 100%.
5. Place 1$cm^3$ of enzyme and 1$cm^3$ of water into a cuvette. Place the cuvette in the Colorimeter.
6. Use a pipette or syringe to add 2 $cm^3$ of milk powder solution to the cuvette, close the lid and click on the **Start** icon to begin.
7. When the readings level out, click on the **Stop** icon to finish. Wash and dry the cuvette.
8. Select **Overlay**.
9. Repeat the experiment from step 5 using the test solutions shown in the table below.

| Test solution | Volume of enzyme ($cm^3$) concentration | Volume of milk solution, 1% w/v ($cm^3$) | Volume of water ($cm^3$) | Total Volume ($cm^3$) |
|---|---|---|---|---|
| 1 | **1.0** | 2 | 1.0 | 4.0 |
| 2 | **1.5** | 2 | 0.5 | 4.0 |
| 3 | **2.0** | 2 | 0 | 4.0 |




The experiment will give results showing light transmission increasing with time. **Gradient** can be used to discover the rise in reaction rate with increase in the enzyme concentration.

The colour of the enzyme solution may result in the end point of each reaction being different. This could form the start point for a discussion about unexpected results and errors.

*The enzyme for this experiment was supplied by the National Centre for Biotechnology Education: www.ncbe.reading.ac.uk.*

## Warranty

All Data Harvest Sensors are warranted to be free from defects in materials and workmanship for a period of 12 months from the date of purchase provided they have been used in accordance with any instructions, under normal laboratory conditions. This warranty does not apply if the Sensor has been damaged by accident or misuse.

In the event of a fault developing within the 12 month period, the Sensor must be returned to Data Harvest for repair or replacement at no expense to the user other than postal charges.

**Note:** Data Harvest products are designed for **educational** use and are not intended for use in industrial, medical or commercial applications.

WEEE (**W**aste **E**lectrical and **E**lectronic **E**quipment) Legislation

Data Harvest Group Ltd is fully compliant with WEEE legislation and is pleased to provide a disposal service for any of our products when their life expires. Simply return them to us clearly identified as ‘life expired’ and we will dispose of them for you.