

# COLORIMETER

## Description 0358BT



**Figure 1.** The Colorimeter

## Introduction

The Colorimeter is designed to determine the concentration of a solution by analyzing its color intensity. Monochromatic light from a LED light source passes through a cuvette containing a solution sample, as shown in figure 2. Some of the incoming light is absorbed by the solution. As a result, light of a lower intensity strikes a photodiode.

The Colorimeter consists of LEDs for red (635 nm), for green (565 nm) and for blue (470 nm) light.

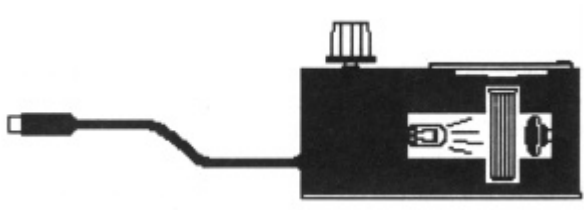


Figure 2. Colorimeter

The Colorimeter is delivered with a BT plug and can be connected to the following interfaces:

- UIA/UIB boards through the Measuring Console (via 0520 adapter)
- CoachLab
- CoachLab II
- Texas Instruments CBL data logger.

There is an adapter (art. 0520) to connect sensors with BT-plugs to 4-mm inputs.

## Transmittance and absorbance

The amount of light that penetrates a solution is known as transmittance.

Transmittance  $T$  can be expressed as the ration of the intensity of the transmitted light  $I_t$  and the initial intensity of the light beam  $I_0$  as expressed by the formula:

$$T = I_t / I_0$$

The transmittance of the sample is determined by the cell or cuvette width ( $d$ ) and the concentration of the solution ( $c$ ). The relation between  $T$ ,  $d$ ,  $c$  is:

$$T = e^{-a * d * c} \quad (a \text{ is an constant that depends on the solution and used the light wavelength})$$

From the two previous equations, the expression for absorbance is obtained:

$$A = -\log(T) = \log(1/T) = \log(e) * a * d * c = e * d * c$$

*(e is the molar absorptivity of the solution)*

For a given solution contained in a cuvette with a constant cell width, the Absorbance is proportional to the concentration:

$$A = k * c \quad (\text{this is Beer's law})$$

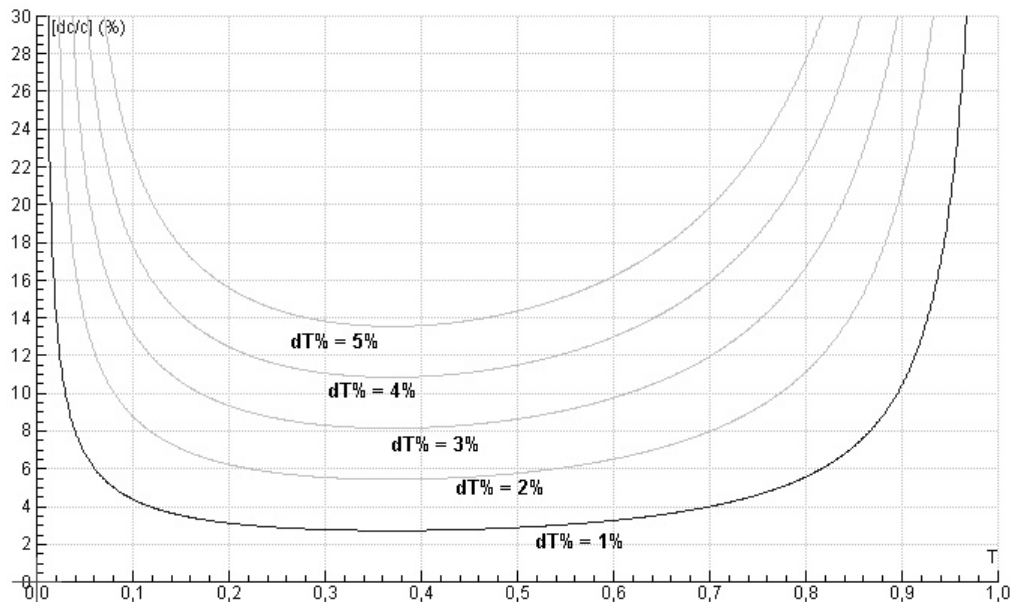
In many chemical and biological experiments this law is assumed for making calibration line for determining concentration.

### Absorbance and Transmittance ranges for the colorimeter

For best results our laboratory testing of the colorimeter indicates that absorbance or transmittance values should fall with these ranges:

|                  |               |
|------------------|---------------|
| Transmittance(T) | 0.28 - 0.90   |
| Absorbance (A)   | 0.050 - 0.550 |

Next to the limitations of the transmittance range of the colorimeter there is a measurement error in the concentration (absorbance) measurement, which strongly depends on the error in the transmittance ( $dT\%$  in figure 3). To get sufficiently reliable results calibration solutions should be used which lie in the flat part of the curve  $dT\% = 1\%$  (i.e. 1% accuracy of the transmission).



**Figure 3.** The measurement error in percent in the concentration (or absorbance) as function of the transmission for different accuracy's ( $dT\%$ ) of the transmittance range.

## **Determining of the wavelength**

You can select three LED light colors: the red (635 nm), the green (565 nm) or the blue (470 nm). There are several ways you can decide which of three wavelengths to use:

### *Method 1.*

Look at the color of the solution. Remember that the color of solution is the color of light which passes through it. You probably want to use a different color of light that will be absorbed, rather than transmitted.

For example: with a blue  $\text{CuSO}_4$  solution, use the red LED (635 nm).

### *Method 2.*

Place a cuvette containing the solution in the colorimeter. And check to see which of three wavelengths yields the highest absorbance (low transmittance).

### *Method 3.*

Directions for most colorimetry experiments express a recommended wavelength. Use the closest of the three wavelengths on the colorimeter. Even if the LED wavelength is somewhat different, a Beer's law curve can usually be obtained at almost any wavelength in the vicinity of the recommended wavelength.

## **Calibration of the colorimeter**

### *Method 1. A voltage-concentration calibration*

The general method for doing a 2-point calibration with the Colorimeter is similar to that used for most other colorimeters or spectrophotometers.

A 0% calibration is done with no light passing through cuvette. The wavelength knob on the colorimeter is turned to '0% T'. The lid of cuvette slot must be closed. In this position computer can read data from the Colorimeter. But the light source is turned off. Since the light is off it makes no difference if a cuvette is in the cuvette slot.

A 100% calibration is done with the wavelength knob turned to select one of the three LED wavelengths. This turns on the red, green, or blue LED. A blank is placed in the cuvette slot and the lid of the colorimeter is closed. The blank is a cuvette containing the solvent used on the solution being studied, usually distilled water.

The blank acts as a control by taking into account the small amount of light absorbed by the solvent and by the walls of the cuvette.

To get more points calibration the number of known sample solutions can be used.

### *Method 2. Using the Absorbance calibration of the Colorimeter*

For the voltage-absorbance calibrations (for the three wavelengths) which are placed in the Coach 5 library, the Colorimeter works like an Absorbance-meter (water as a solvent). The calibrated signal is proportional to the concentration. The proportion constant that has to be used to calculate the concentration is not taken into account because it depends on the substance that is used in the experiment.

The proportion constant between absorbance and concentration can be found by making a calibration line on paper or with help of Coach 5.

Sometimes a concentration-absorbance calibration is not necessary, for example in the investigating of a (pseudo) first-order reaction. In this type of mechanism the rate of reaction ( $v$ ) is:

$$v = dc/dt = k \cdot c$$

According to the Beer's law a concentration and absorbance are proportional:

$$A = \epsilon \cdot c \quad \text{so} \quad c = (1/\epsilon) \cdot A$$

Combining the two equations the following expression is obtained:

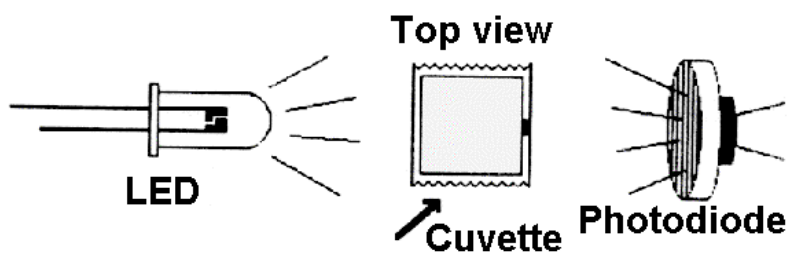
$$v = d[(1/\epsilon) \cdot A]/dt = k \cdot (1/\epsilon) \cdot A \quad \text{so} \quad dA/dt = k \cdot A$$

By the measurement of the absorbance the rate of reaction ( $k$ ) can be determined.

### **Using cuvettes**

The Colorimeter is designed to use polystyrene 1cm x 1cm cuvettes (standard disposable cuvettes). Two opposite sides of the cuvette are ribbed and are not intended to transmit light from the LED. The two smooth surfaces are intended to transmit lights. It is important to position the cuvette correctly in the colorimeter. We recommended this be done as shown in Figure 4, with the ribbed edges facing away from and toward you, and the smooth edges facing left to right. The light travels from left to right from the LED through the cuvette to the photodiode.

It should be also noted that there is often a small variation in the amount of light absorbed by the cuvette if it is rotated 180° between trials. To avoid this, you should use a water -proof marker to make a reference mark on the right side of the top of the cuvette as shown in Figure 4. Remind students to align this reference mark with the white mark on the top right side of the colorimeter each time they insert a cuvette.



**Figure 4.** Placing a cuvette in the colorimeter

Plastic cuvettes vary slightly in the amount of light they absorb. You may ignore these differences. For most experiments this variation will not have a noticeable effect on experimental results.

For best results, variation in light absorbed by individual cuvettes can be controlled either by using the same cuvette for all trials of a particular experiment or by *matching* a set of cuvettes. The easiest and the most reliable is the first method. The cuvette has to be cleaned and dry after each trial or rinsed several times with the solution that will be added to it.

As an alternative you may choose to match cuvettes. Matched cuvettes are a set of cuvettes that all absorb light (when empty) at approximately the same level. To match a set of cuvettes use clean, dry cuvettes, put a reference mark on one of the clear sides of the cuvettes so it is always oriented the same way in the cuvette slot. Place each cuvette in the batch of the Colorimeter and record transmittance values for each. When you are finished, group cuvettes according to the similar %T values. Each of these groups represents a set of matched cuvettes.

The total volume of a cuvette is 4.1 ml. Fill the cuvette for every measurement with 2.2 to 3.5 ml.

As a reminder to students mark the level with a water-proof marker on one of the ribbed sides of each cuvette.

## **Suggestions for experiments**

### ***Beer's law***

#### ***Crystal violet***

Dilute solutions of crystal violet yield a good Beer's law curve using the green LED (565 nm). A stock solution  $8 \times 10^{-5}$  M crystal violet is prepared by adding 65.3 mg of solid crystal violet to enough water to yields 2 liters of solution.

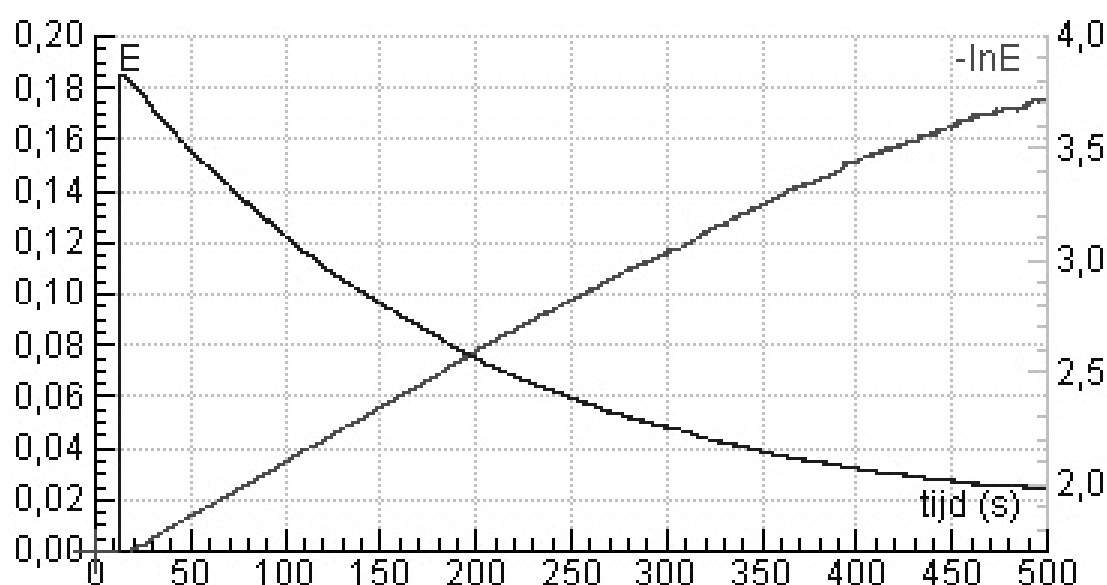
### *Copper sulfate*

Standard solutions that are 0.1 M, 0.2 M, 0.3 M and 0.4 M  $\text{CuSO}_4$  will yield a good Beer's law curve at 635 nm (red LED). Or prepare a stock solution by adding 10 g of  $\text{NH}_4\text{NO}_3$  to 10 ml 0.1 M  $\text{CuSO}_4$  and 90 ml 0,2 M  $\text{NH}_3$  (forms  $\text{Cu}(\text{NH}_3)_4^{2+}$  complex ion) and dilute to obtain standard solutions.

### ***Determination of the rate law for the reaction of crystal violet***

Add 10 ml of  $8 \times 10^{-5}$  M crystal violet solution to 5.0 ml of 0.10 M NaOH and fill with water to 100 ml.

The de-coloring of the solution takes from 15 to 20 minutes.



**Figure 5.** Measurement of the transmission during the reaction from crystal violet to sodium hydroxide.

10 ml (0.1mM) of crystal violet solution is mixed with 10 ml sodium hydroxide (0. M) in a glass and the measurement is started. Circa 3 ml of new solution is added to cuvette and the cuvette is placed in the colorimeter.

During the measurement values of  $\ln(E)$  are calculated.

The name of the Colorimeter in the sensor library of Coach 5 program is

- **Colorimeter 470 nm (0358&bt) (CMA)**
- **Colorimeter 565 nm (0358&bt) (CMA)**
- **Colorimeter 635 nm (0358&bt) (CMA).**

### Technical data

|                                                                           |                                                                                                             |
|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| For the best results the absorbance and transmittance should fall within: | Percent transmittance: 28% - 90%<br>Absorbance - 0.050 - 0.550                                              |
| Wavelengths ranges                                                        | Blue (470 nm or 4700 Å)<br>Green (565 nm or 5650 Å)<br>Red (635 nm or 6350 Å)                               |
| Supplied with                                                             | 15 cuvettes                                                                                                 |
| Connection                                                                |  BT (British Telecom) plug |

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