
COLORIMETER BT29i

USER'S GUIDE



CENTRE FOR MICROCOMPUTER APPLICATIONS

<http://www.cma-science.nl>

Short description

The CMA Colorimeter BT29i measures the amount of light transmitted through a sample solution and it can be used to determine the concentration of a solution. It has four LED light sources emitting light of different wavelengths, violet 430 nm, blue 470 nm, green 565 nm and red 635 nm. The wavelength is selected by pressing the front panel arrow keys. The Colorimeter is delivered with 10 plastic cuvettes with caps.

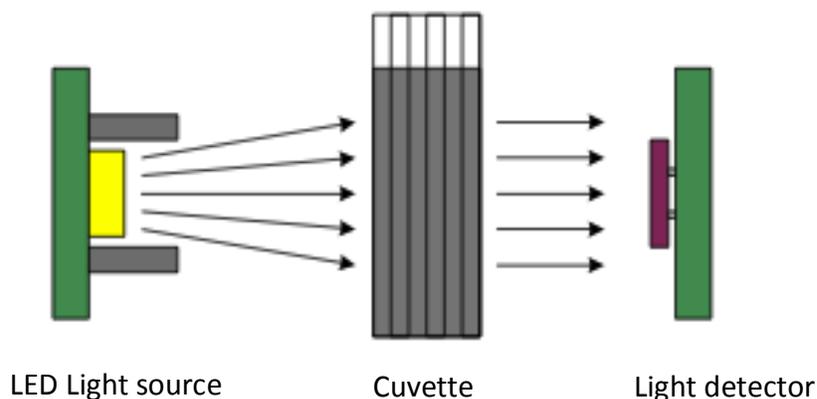
The Colorimeter can be directly connected to the analog BT inputs of the CMA interfaces. The sensor cable BT - IEEE1394 needed to connect the sensor to an interface is not supplied with the sensor and has to be purchased separately (CMA Article BTsc_1).

Sensor recognition

The Colorimeter BT29i has a memory chip (EEPROM) with information about the sensor: its name, measured quantity, unit and calibration. Through a simple protocol this information is read by the CMA interfaces and the sensor is automatically recognized when it is connected to these interfaces. If your Colorimeter is not automatically detected by an interface you have to manually set up your sensor by selecting it from the Coach Sensor Library.

How the Colorimeter works

Light from the LED passes through a cuvette that contains a sample solution. A light-sensitive photocell at the other end of the light path detects the amount of transmitted light.



The proportion of the light that passes through the solution is known as **Transmittance T** and is expressed as the ratio of the intensity of the transmitted light I_t and the initial intensity of the light beam I_0 : $T = I_t / I_0$

The reciprocal of transmittance of the sample varies logarithmically with three factors: the molar absorptivity of the solution ϵ , the cell or cuvette width b , and the molar concentration C :

$$\log\left(\frac{1}{T}\right) = \epsilon * b * C$$

Many experiments designed to use a Colorimeter require a measurement of **Absorbance A**, which is defined as:

$$A = \log\left(\frac{1}{T}\right) \text{ or } A = \epsilon * b * C$$

For a given solution contained in a cuvette with a constant cell width, one can assume ϵ and b to be constant. This leads to the equation:

$$A = k * C, \text{ where } k \text{ is a proportionality constant.}$$

This equation shows absorbance to be related directly to concentration (Beer's law) and so absorbance can be used to measure the concentration of a solution.

Transmittance can be also expressed as **Percent Transmittance** or %T. Since $T = \%T / 100$, the formula describing the relationship between absorbance and transmittance can be rewritten as: $A = \log(100 / \%T)$ or $A = 2 - \log(\%T)$

The linear relationship between Absorbance and Concentration does not hold across the whole Transmittance range. For best results our testing of the Colorimeter indicates that transmittance or absorbance values should be within these ranges:

- Transmittance: 10% - 90%
- Absorbance: 0.05 – 1.0.

Experiments using the absorbance range should be designed to fit within these values. If you have a solution that transmits very low level of light, consider diluting the solution so that it falls within this range.

Calibration

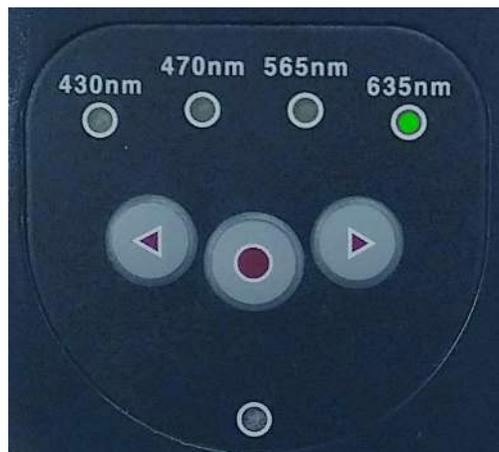
The CMA Colorimeter BT29i is supplied calibrated. The output of the Colorimeter is linear with respect to the percent transmittance. The supplied calibration function is made for Absorbance $A = 0.544 - 0.434 \cdot \ln V_{\text{out}}(V)$ ¹.

The Coach software allows selecting the calibration supplied by the sensor memory (EEPROM) or the calibration stored in the Coach 6 Sensor Library.

Before collecting data the zero-point calibration for the selected wavelength should be performed.

To perform such calibration:

- Connect the Colorimeter to an interface.
- Let the system stabilize at the desired wavelength for 5 minutes². One of the four green wavelength indicator lights is turned on when the Colorimeter is powered.
- Press the  or  button on the Colorimeter to select the correct wavelength setting for your experiment (430 nm, 470 nm, 565 nm, or 635 nm).
- Open the Colorimeter lid.
- Fill a cuvette with distilled water. This will be your blank measurement (100 % transmittance or 0 absorbance).
- Insert the sample cuvette into the cuvette holder. It is important to line up one of the smooth sides of the cuvette at the top of the cuvette slot. Notice that there can be slight difference in transmission if the cuvette is rotated by 180 degrees.
- Close the Colorimeter lid.
- Next, press the  button to begin the calibration process. Release the button when the red LED begins to flash. The calibration is finished when the LED stops flashing and the measured absorbance will be 0.000 or 0.001. The unit is now ready to collect data.



Collecting data

After the zero-point calibration is finished you can start your measurement:

- Fill a cuvette with 2.2 to 3.5 mL of the solution you want to measure (the volume of the cuvette is 4 mL). Close it with the cap. Notice that with some experiments (for example rate of reaction) the time taken in fitting the cap can result in loss of early data.

¹ Calculated as following $A = 2 - \log(\%T) = 2 - \log(28.571 \cdot V_{\text{out}}) = 0.544 - \log(V_{\text{out}}) = 0.544 - 0.434 \cdot \ln(V_{\text{out}})$

² For best results, the system should be stabilized for 5 minutes prior to calibration or data collection.

- Replace the blank cuvette in the Colorimeter with the cuvette containing the solution. Handle the cuvette by the cap and avoid touching it with your fingers.
- Close the Colorimeter cap and start your data collection.

Wavelength selection

The wavelength is selected by pressing ◀ or ▶ buttons on the front panel. There are several ways one can decide which of the four light wavelengths to use.

Method 1

Look at the color of the solution. Remember that the color of a solution is the color of light that 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 copper (II) sulfate (CuSO_4) solution, use the red LED (635 nm).

Method 2

Another easy method is to place a cuvette containing the solution in question in the Colorimeter and check to see which of the wavelengths yields the highest absorbance.

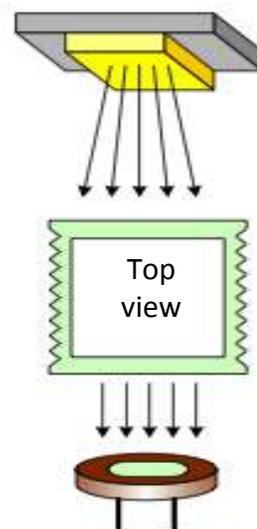
Method 3

Directions for most colorimetry experiments express a recommended wavelength. Use the wavelength closest in value to the recommended wavelength. 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.

Using cuvettes with the Colorimeter

The Colorimeter is designed to use standard disposable polystyrene cuvettes. Macro cuvettes have a 10 mm path length and are 45 mm high. The total volume of a cuvette is 4 mL. The cuvette has two smooth and two ribbed faces. The smooth faces are the optical surfaces intended to transmit the light from the LED. It is important to position the cuvette correctly in the Colorimeter, with a smooth side facing the top and the bottom of the cuvette slot, and with the ribbed edges facing left and right.

Individual plastic cuvettes vary slightly in the amount of light they absorb. For most experiments, this variation will not have a noticeable effect on experimental results and you may choose to ignore these differences. 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.



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 Colorimeter and record absorbance values for each. When you are finished group cuvettes according to the similar absorbance values.

Suggested experiments

The Colorimeter can be used in experiments such as:

- Application of Beer's law e.g. Crystal violet or Copper Sulphate.
- Measuring of unknown concentrations.
- Reaction kinetics – measuring reaction rate, reaction order or reaction equilibrium.
- Quantitative determination of biological molecules e.g. sugars, protein, vitamins.
- Quantitative determination of inorganic ions e.g. nitrate, phosphate.
- Population growth of microorganisms.

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.

Technical Specifications

<i>Sensor kind</i>	Analog, generates an output voltage between 0 - 5 V
<i>Useful Measurement range</i>	Percent Transmittance: 90% .. 10% Absorbance: 0.05 .. 1.0
<i>Wavelengths ranges</i>	Violet, 430 nm or 4300 Å Blue, 470 nm or 4700 Å Green, 565 nm or 5650 Å Red, 635 nm or 6350 Å
<i>Resolution using 12 bit AD converter</i>	0.025 %T
<i>Supply voltage</i>	5 VDC ±25 mV
<i>Supply current (typical)</i>	40 mA
<i>Calibration functions</i>	$A = 0.544 - 0.434 * \ln V_{out}(V)$ (stored in the sensor memory) $\%T = 28.571 * V_{out}(V)$
<i>Connection</i>	IEEE1394 connector for BT-IEEE1394 sensor cable. Sensor cable not delivered with the sensor.

Warranty:

The Colorimeter BT29i is warranted to be free from defects in materials and workmanship for a period of 12 months from the date of purchase provided that it has been used under normal laboratory conditions. This warranty does not apply if the sensor has been damaged by accident or misuse.

Note: *This product is to be used for educational purposes only. It is not appropriate for industrial, medical, research, or commercial applications.*

Rev. 20/11/2014