# Activity 2: Spectrophotometry

## Part 1: Preparing a standard solution of helianthine by dilution

Dilute the stock standard solution of helianthine provided (0,120 g.L$^{-1}$) to make a working standard solution (0,0120 g.L$^{-1}$) using a volumetric flask 100 mL.

## Part 2: Visible absorption spectrum of helianthine

Use your standard solution (part 1) to make the spectrum of helianthine. For that:

1. Set the program « Spectre » on the laptop connected to the spectrophotometer (so that) to record absorbance every 1 nm between 345 and 800 nm then print the spectrum.

2. Read off the wavelenght at which the absorbance of helianthine is maximum ($\lambda_{max}$) on the graph.

## Part 3: Dosage of an unknown solution of helianthine

1. Preparing the calibration range

Use your standard solution (part 1) to make the calibration range. For that

    - Prepare 6 test tubes numbered from 0 to 5 containing:

        • Standard solution: 0 – 2 – 4 – 6 – 8 – 10 mL

        • The amount of distilled water needed for a final volume of 10 mL

    - Homogenize.

2. Preparing samples

    - Prepare 2 test tubes labelled $S_1$ and $S_2$ containing:

        • 5 mL of unknown solution

        • The amount of distilled water needed for a final volume of 10 mL

    - Homogenize.

3. Measuring absorbances

    - Set the spectrophotometer wavelenght to $\lambda_{max}$.

    - Read absorbances against the blank: set the reference of the spectrophotometer with the tube 0.

    - Read absorbances of every tube (calibration and samples) at the same time.

## *Results*

    1. Make a table showing: the composition, the concentration and the absorbance of every tube.

      Give the calculation of the concentration of the tube 2 as example.

    2. Complete the table with the absorbance values after reading.

    3. Plot the calibration curve: A = f($\rho_{helianthine}$) on graph paper.

    4. Use the calibration curve to determine the concentration of the unknown solution.

# DILUT1ON

**1. What' a solution? How to prepare a solution?**

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Solution = ......................... + .........................

When you're working in a biochemistry lab, it's essential to know how to calculate and prepare a dilution.

**2. How to define a dilution?**

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**3. How to make a solution by dilution?**

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**4. Define "stock solution" and "diluted (or working) solution"**

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**5. Define "amount of solute"**

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**6. What is "the law of conservation of mass" (explain)**

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**7.** You need to prepare 100 ml of a 0.5 mol.L$^{-1}$ solution from a 2.0 mol.L$^{-1}$ stock solution.
   How to prepare this diluted solution?

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**8. How to express the dilution? (There are two ways).**

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**9. How to express the dilution ?**

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<u>Application:</u>  Activity 2 : Spectrophotometric part 1
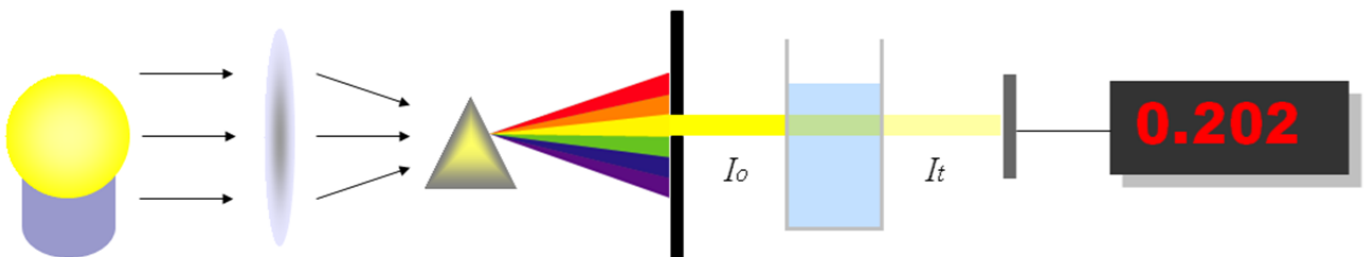
# spectrophotometer

## 1. Presentation of spectrophotometer



## 2. How does a spectrophotometer work? Watch the video.

a. The spectrophotometer is made of:

b. What's the diffraction grating used for?

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c. Why is the grating rotated?

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d. What does the light beam pass through?

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e. What's the transmittance?

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f. What's the absorbance?

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g. What does the detector measure?

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h. About cuvette. How do you hold it? How do you fill it? How do you transport it?

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# spectrophotometric Assay

**1. What is a relationship between Absorbance and concentration of solution?**

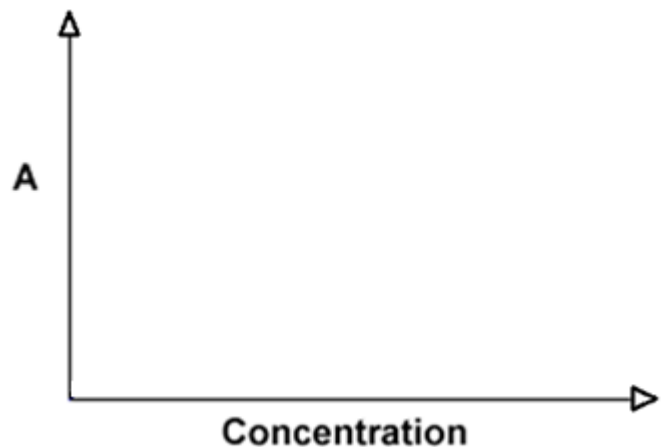..........................................................................................................................................................
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**2. What is the significance and units of A, e, b and c?**

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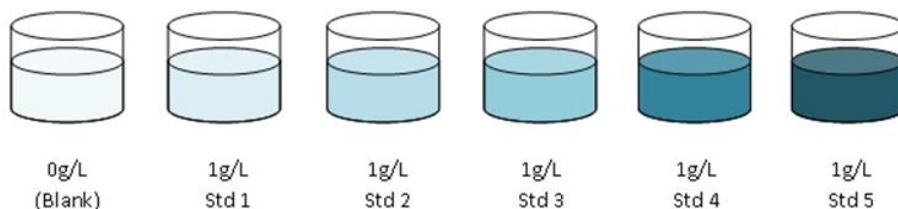
**3. If we plot absorbance against concentration: Can you describe us the graph? Is it always true?**
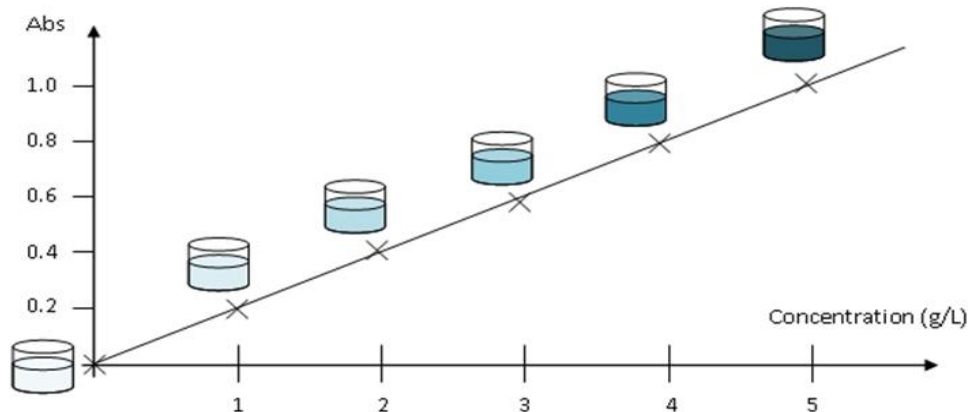
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A

Concentration

**4. How to prepare a standard curve?**

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| 0g/L (Blank) | 1g/L Std 1 | 1g/L Std 2 | 1g/L Std 3 | 1g/L Std 4 | 1g/L Std 5 |

2. ....................................................................................................................
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5. How to find the concentration of the unknown solution?

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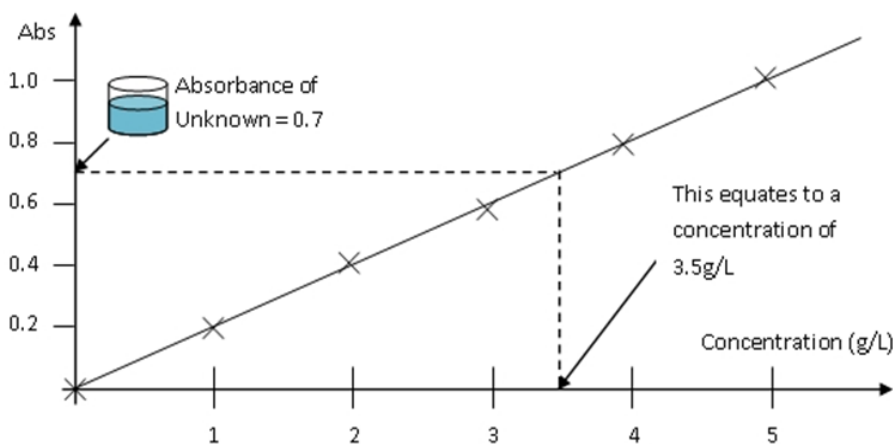


## Table of spectrophotometric results

| n° of tube | 0 | 1 | 2 | 3 | 4 | 5 | $S_1$ | $S_2$ |
|---|---|---|---|---|---|---|---|---|
| **Working standard solution of helianthine** (0,0120 g.L$^{-1}$) (mL) | 0 | 2 | 4 | 6 | 8 | 10 | 0 | 0 |
| **Unknown solution of helianthine** (mL) | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 |
| Distilled water qs* 10 mL (mL) | 10 | 8 | 6 | 4 | 2 | 0 | 5 | 5 |
| **Concentration of Helianthine** (g.L$^{-1}$) | 0 | | | | | | $C_{S1}=$ | $C_{S2}=$ |
| Absorbance ($\lambda$ = 465nm) | | | | | | | | |

*qs or QS : « quantum satis » meaning the amount which is needed.