To determine the concentration of Co^{2+} ion in water sample by spectrophotometer

Spectrophotometry. Many substances dissolve to give coloured solutions. The higher the concentration of solute, the more light is absorbed and the less light is transmitted through the sample. Spectrophotometry is a simple technique used to measure absorbance of solutions.

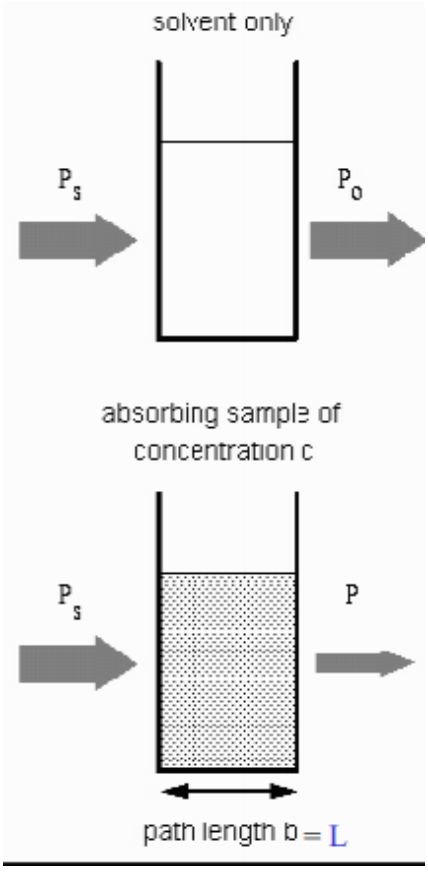
About: When the solution contains a *specific molecule* that absorbs light at a *specific wavelength.*

The intensity of light hitting the detector after passing through a "blank" solution is measured – this is a solution that is identical to the sample but doesn't contain the solute**. This measurement is called "I_0".**

Then the intensity of light hitting the detector after passing through the sample is measured**. This measurement is "I".**

What is a blank in spectrophotometry?

A blank is a sample containing everything except for the significance analyte. The blank is a sample of the solvent itself.



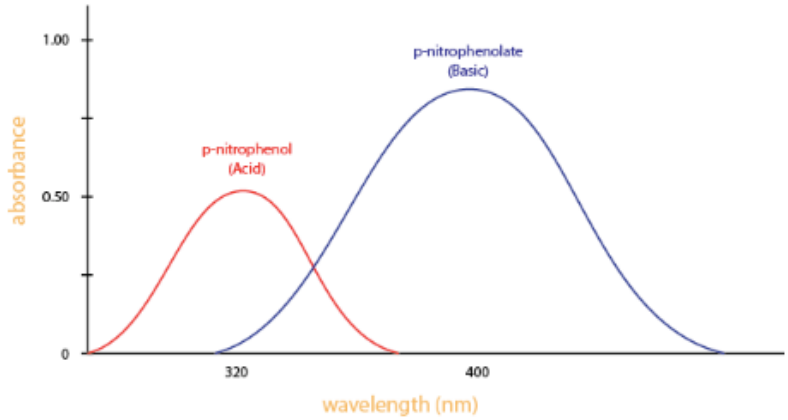


Figure 3: Absorbance of two different compounds

Isobestic point:

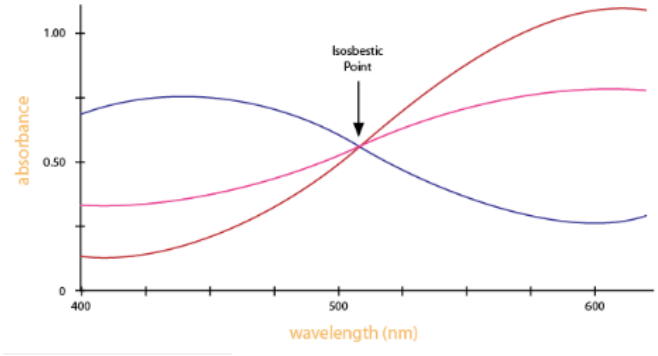


Figure 4: An example of isosbestic point

What are transmittance and absorbance?

Consider monochromatic light transmitted through a solution; with an incident intensity of I_0 and a transmitted intensity of I.

The transmittance, T, of the solution is defined as the ratio of the transmitted intensity, I, over the incident intensity, I_0:

$$T = \frac{I}{I_0}$$

and takes values between 0 and 1. However, it is more commonly expressed as a percentage transmittance:

$$T(\%) = 100\frac{I}{I_0}$$

Experimental measurements are usually made in terms of **transmittance** (T), which is defined as:

$T = I / I_o$

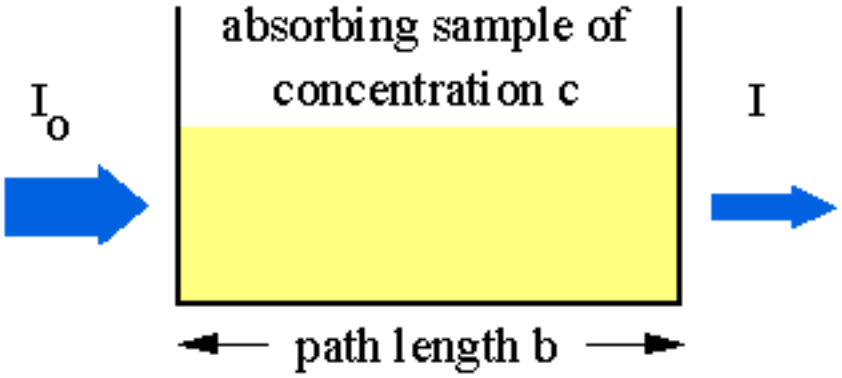
where I is the **light intensity** after it passes through the sample and I_o is the **initial light intensity**. The relation between A and T is: or The absorbance, *A*, of the solution is related to the transmittance through the following relations:

$$A = \log_{10} \frac{I_0}{I}$$
$$A = -\log_{10} T$$

or

$A = -\log T = -\log (I / I_o)$.

Absorption of light by a sample



The absorbance has a logarithmic relationship to the transmittance; with an absorbance of 0 corresponding to a transmittance of 100% and an absorbance of 1 corresponding to 10% transmittance.

The Absorbance of a Solution

For each wavelength of light passing through the spectrometer, the intensity of the light passing through the reference cell is measured. This is usually referred to as I_o - that's I for Intensity.

The intensity of the light passing through the sample cell is also measured for that wavelength - given the symbol, I. If I is less than I_o, then the sample has absorbed

some of the light (neglecting reflection of light off the cuvette surface). A simple bit of math is then done in the computer to convert this into something called the absorbance of the sample - given the symbol, A. The absorbance of a transition depends on two external **assumptions.**

1. The absorbance is directly proportional to the concentration (c) of the solution of the sample used in the experiment. (**Beer's Law)**
2. The absorbance is directly proportional to the length of the light path (l), which is equal to the width of the cuvette. (**Lambert's law**)

Assumption one relates the absorbance to concentration and can be expressed as

$$A \propto c$$

The absorbance (A) is defined via the incident intensity I_o and transmitted intensity I by

$$A = \log_{10}\left(\frac{I_o}{I}\right)$$

Assumption two can be expressed as

$$A \propto l$$

Combining Equations 1 and 3:

$$A \propto cl$$

This proportionality can be converted into an equality by including a proportionality constant (ϵ).

$$A = \epsilon cl$$

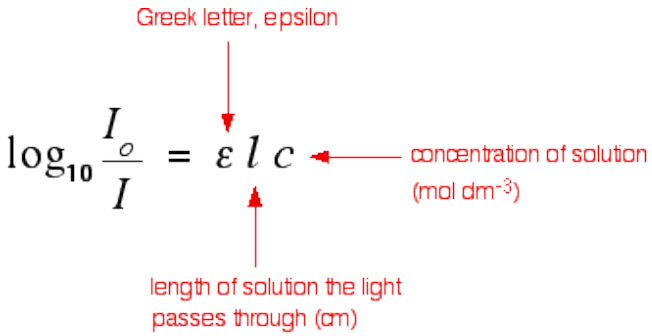
This formula is the common form of the *Beer-Lambert Law*, although it can be also written in terms of intensities:

$$A = \log_{10}\left(\frac{I_o}{I}\right) = \epsilon lc$$

The constant ϵ is called **molar absorptivity** or **molar extinction coefficient** and is a measure of the probability of the electronic transition. On most of the diagrams you will come across, the absorbance ranges from 0 to 1, but it can go higher than that. An absorbance of 0 at some wavelength means that no light of that particular wavelength has been absorbed. The intensities of the sample and reference beam are both the same, so the ratio I_o/I is 1 and the $\log_{10}$of 1 is zero.

The Beer-Lambert Law

You will find that various different symbols are given for some of the terms in the equation - particularly for the concentration and the solution length.



The Greek letter epsilon in these equations is called the **molar absorptivity** - or sometimes the molar absorption coefficient. The larger the molar absorptivity, the more probable the electronic transition. In uv spectroscopy, the concentration of the sample solution is measured in mol L^{-1} and the length of the light path in cm. Thus, given that absorbance is unitless, the units of molar absorptivity are L mol^{-1} cm^{-1}.

Limitations of the Beer-Lambert law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- deviations in absorptivity coefficients at ***high concentrations (>0.01M)*** due to electrostatic interactions between molecules in close proximity
- **scattering of light due to particulates** in the sample
- **fluoresecence or phosphorescence** of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

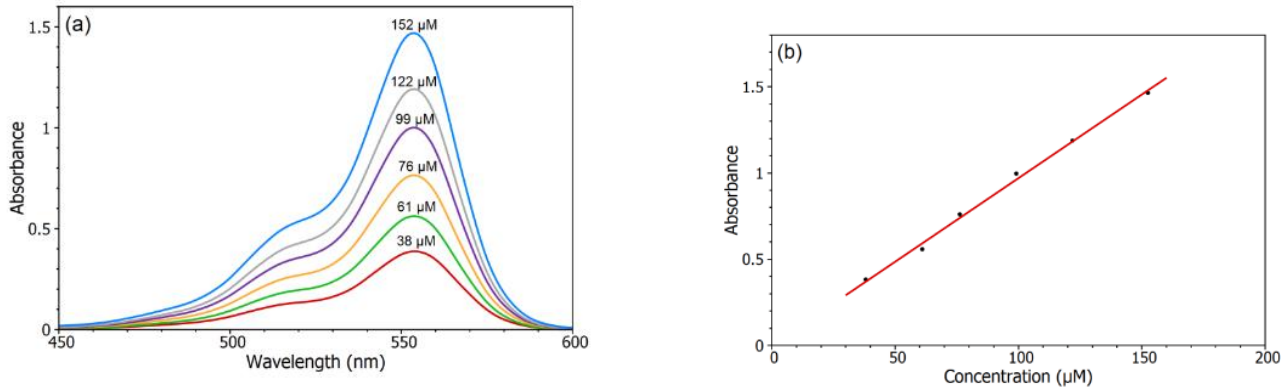


Figure 3 (a): Absorption spectra of Rhodamine B solutions with different concentrations in water (b) Calibration curve of Rhodamine B in the water at measured at λ_{max}.

AIM: To determine the concentration of Co^{2+} ion in water sample by spectrophotometer.

INSTRUMENT: Spectrophotometer, Filter, Cobalt Nitrate

PROCEDURE:

(1) Preparation of 0.2M standard cobalt nitrate solution: Prepare a stock solution of 0.2M cobalt nitrate (5.82 g in 100 mL distilled water). Dilute this stock solution quantitatively as to get desired concentration of 0.04M, 0.08M, 0.12M, 0.16M solution in 100 mL SMF.

(2) Strictly follow the instructions given on the instrument.

(3) Measure the one of the above Co^{2+} solution against water as a 'Blank' or 'Reference' using each of the filters in turn given with the instrument. The filter giving the maximum absorbance (510 nm) will be most suitable for measuring the absorbance (A) (Known as λ_{max}).

(4) Determine the absorbance (A) (from %Transmittance) of a test solution against water as a blank. Carry out replicate measurements so as to get the corresponding readings.

(5) Repeat the experiment for unknown Co^{2+} concentration.

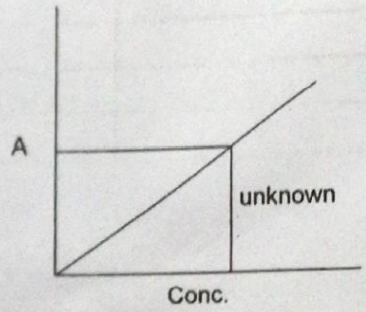
(6) Plot a graph absorbance (A) against concentration of Co^{2+} ion. A linear curve must be obtained if the Beer- Lambert's law is valid. Find the concentration of the unknown Co^{2+} solution from this curve from its absorbance.

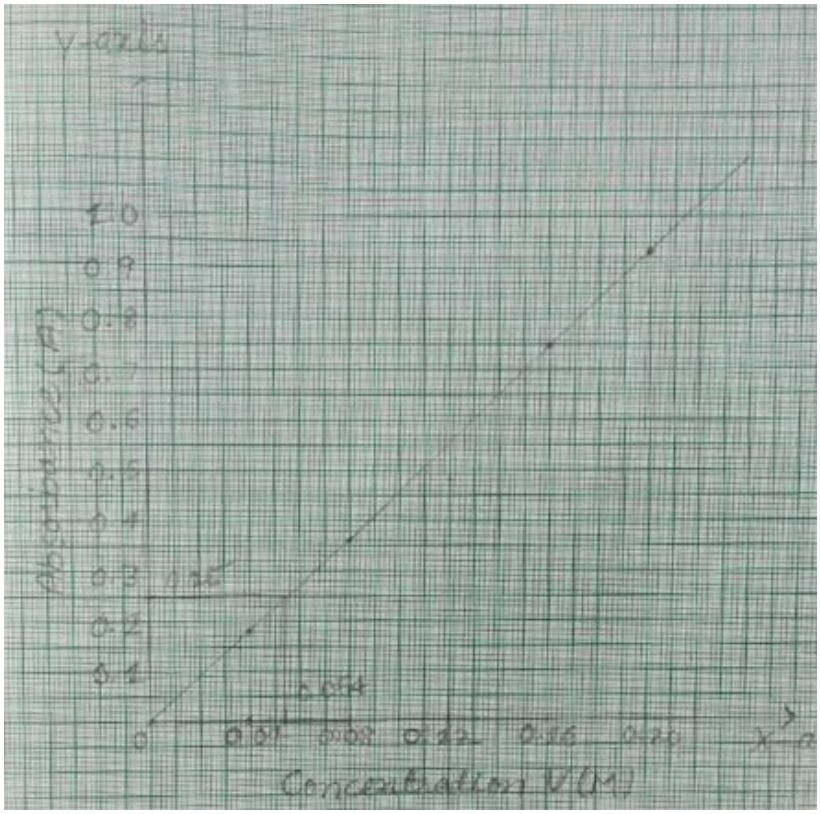
(7) Tabulate the observations as follows :

OBSERVATION TABLE:

No.	Concentration 'V' (M)	% Transmittance (T%)	Absorbance (A) (From Table)
1	0.04	70 %	0.18
2	0.08	46 %	0.36
3	0.12	36 %	0.48
4	0.16	22 %	0.75
5	0.20	14 %	0.93
6	Unknown	58 %	0.25

GRAPH:





Result:

1. The straight-line natures group of concentration *vs* absorbance at λ_{max} 510 nm which confirms the Beer-Lambert's Law.

2. From the graph the concentration of unknown Co^{2+}solution = ……. ??? M