

Spectrophotometric Analysis

Spectrophotometric techniques are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer. Spectrophotometry takes advantage of the dual nature of light. Namely, light has:

1. a particle nature which gives rise to the photoelectric effect
2. a wave nature which gives rise to the visible spectrum of light

A spectrophotometer measures the intensity of a light beam after it is directed through and emerges from a solution. An example with copper sulfate (CuSO_4) is shown on Figure 1.

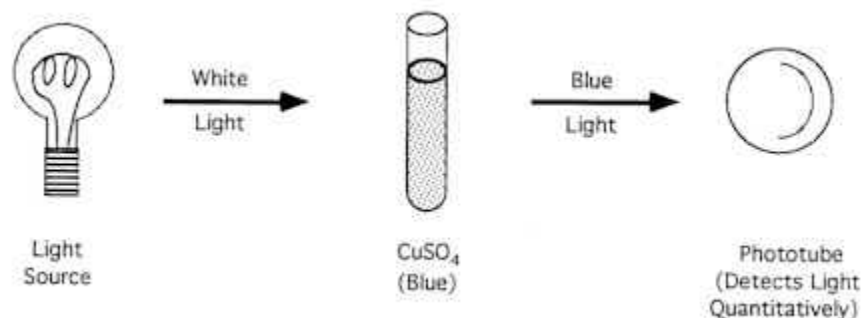


Figure 1.

The red part of the spectrum has been almost complete absorbed by CuSO_4 and blue light has been transmitted. CuSO_4 absorbs little blue light and therefore appears blue.

In spectrophotometry, greater sensitivity can be gained by directing red light through the solution because CuSO_4 absorbs strongest at the red end of the visible spectrum. For this, red wavelengths have to be isolated.

In a spectrophotometer, a light source gives off white light which strikes a prism, separating the light into its component wavelengths (See Figure 2.). Thus, lightwaves can be separated by frequency.

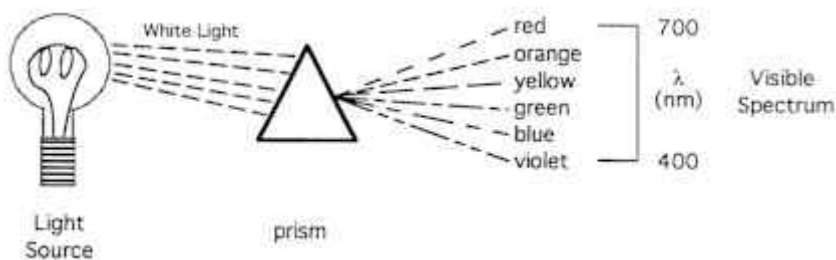


Figure 2.

The spectrophotometer can measure the amount of light (of certain frequency) transmitted or adsorbed by the solution. This light that has not been absorbed by the solution in the cuvette, will strike the phototube. The photons of light that strike the

phototube will be converted into electrical energy. This current that is produced is very small and must be amplified before it can be efficiently detected. The signal is proportional to the amount of light which originally struck the phototube and is thus an accurate measurement of the amount of light which has passed through (been transmitted by) the sample.

Different compounds having dissimilar atomic and molecular interactions have characteristic absorption phenomena and absorption spectra. Concentration of every component may be found from the spectrophotometer measurements and calibration curve made using the samples of known concentration.

Laboratory procedure

You will need:

- 4 calibration samples of water contaminated with Toluene and MTBE. Concentrations are 1 ppm, 10 ppm, 25 ppm and 50 ppm
 - Your test samples from other experiments
 - Safety equipment (gloves, glasses, etc.)
 - Cuvette
 - Notebook
 - 100-250 mL Wastewater beaker
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- Make sure that you read and understand the lab procedure.
 - Follow the safety procedure for the work in the lab.
 - Turn spectrophotometer on. Let it warm up for about 15 minutes
 - In the meantime, prepare your calibration samples
 - Rinse the quartz cuvette with deionized water several times. Careful with the cuvettes. They are very expensive!
 - Place 1 ml of sample into cuvette using a pipette. Use new pipette tip for every sample.
 - Make sure you don't touch the clear sides of the cuvette. Wipe the cuvette with very fine paper to remove any water droplets or dust. Avoid scratching the cuvette.
 - Place cuvette into spectrometer.
 - Take a reading.
 - Remove cuvette and discard liquid into beaker with wastewater.
 - Rinse the cuvette with deionized water 3 times before placing another sample.
 - Repeat the procedure.
 - Compare signal against calibration curve to obtain your concentrations

Calibration curve:

- Prepare 4 vials with 25 ml DI (de-ionized) water using a graduated cylinder.
- The stock solution is water saturated with toluene or MTBE. For toluene, the water is at a concentration of 546 mg/L; for MTBE, the water is at a concentration of 54,000 mg/L.
- Add toluene and MTBE solutions to obtain the following concentrations: 1, 10, 25 and 50 mg/L. (1 ppm = 1 mg/L)
- The required volume can be calculated according to the equation:

$$C_1 * V_1 = C_2 * V_2$$

(Where C = concentration, V =volume.)

For example to get a 10 mg/L toluene solution;

$$546 \text{ mg/L} * V_1 = 10 \text{ mg/L} * 25 \text{ mL}$$

Solving for V1;

$$V_1 = 0.458 \text{ mL} = 458 \text{ }\mu\text{L of toluene saturated water is needed.}$$

The final volume should be 25 ml, so you have to get this amount of water (i.e. 458 μ l) out from the vial with pipette, and then add this amount of toluene water back into the vial.

- Cap & shake well to make sure the calibration sample is a homogeneous solution.

References

<http://faculty.uca.edu/~march/bio1/scimethod/>