

Introduction to Bioelectronics

Sreeja Guduri (2021102007)

LAB REPORT 2: Spectrophotometry

AIM:

1. Determine the absorption spectrum of sugars (glucose, sucrose, and fructose) by measuring the absorbance of light of different wavelengths
2. Construct a standard curve for glucose and calculate the slope for this compound. Then, using this value you will determine the concentration of an unknown dilution of glucose solution.

THEORY:

Spectrophotometry is the measurement of the interaction of light with matter. It is used for measuring the quantity of light that is absorbed or that passes through (is transmitted) by a sample solution or mixture. Using a spectrophotometer, which measures the absorptivity of a solution towards light of specific wavelengths (visible or not), allows us to determine concentration.

The basis of finding concentration using spectrophotometry is that the proportion of light that is absorbed by a solution of a particular compound is a function of the concentration of that compound. This allows for a quantitative analysis of concentration of a substance from the Beer-Lambert relationship (below). A spectrophotometer will direct light of a specific wavelength on your solution. This light is the incident light. The light that passes through the solution is the transmitted light. The absorbance (A) of the solution is the log of the ratio of these two measures:

$$A \text{ (Absorbance)} = \log_{10} (\text{Intensity of incident light} / \text{Intensity of transmitted light})$$

Experiment A:

Finding the absorption spectrum of different sucrose samples using the spectrophotometer.

MATERIALS REQUIRED:

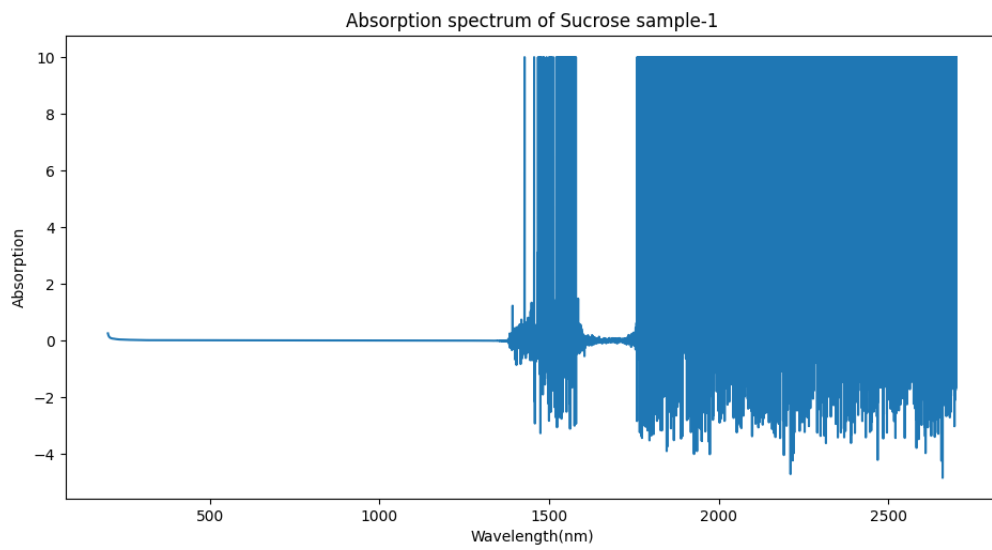
1. Spectrophotometer → The analytical instrument you will use is a spectrophotometer. As mentioned above, a spectrophotometer measures the intensity of light transmitted through a solution. It consists of two principal parts: a spectrometer and a photometer. Using a white light source and a monochromator (a prism), the spectrometer of the instrument is designed to provide discrete wavelengths of light at a known intensity. The photometer consists of a photoelectric tube sensitive to the wavelengths of light provided by the spectrometer and a galvanometer to quantitate the intensity of the light. The sample to be measured is inserted between the spectrometer and the photometer. By comparing the intensity emitted by the spectrometer to the intensity measured by the photometer, absorbance by the solution can be calculated by the machine.
2. Sucrose samples of different concentrations

PROCEDURE:

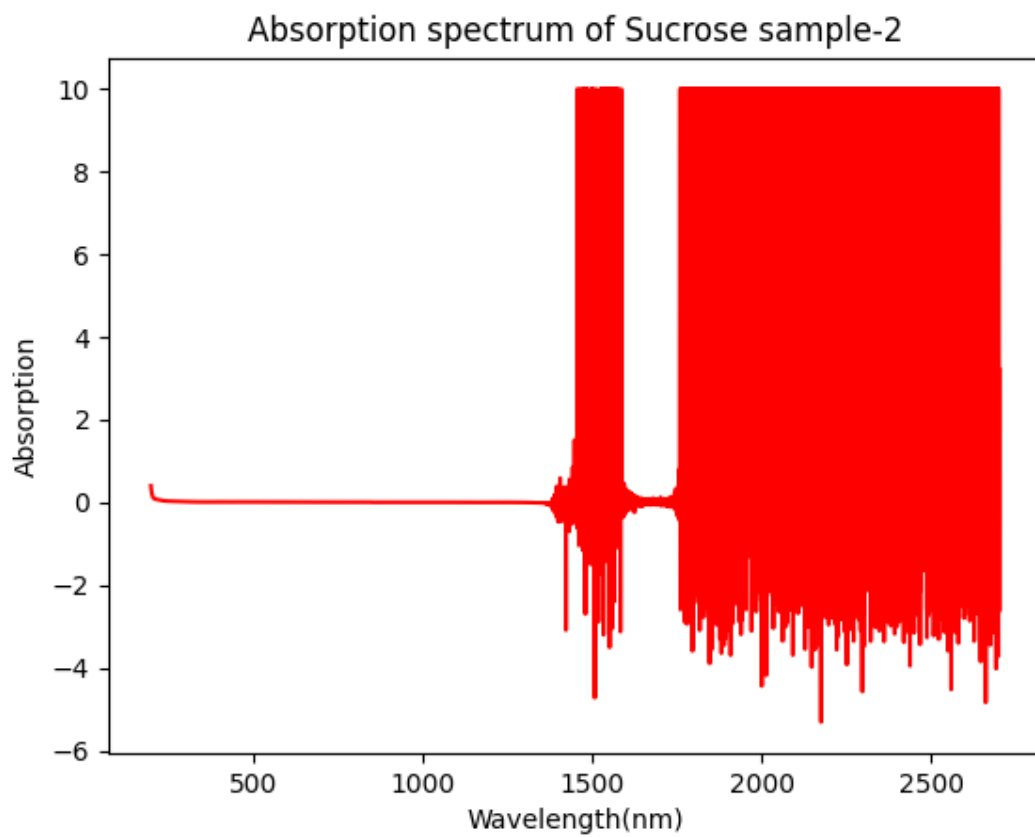
1. Place the sucrose sample in a thoroughly cleaned cuvette. Also add some distilled water into another clean cuvette.
2. The distilled water cuvette is placed as the 'reference' in the spectrophotometer and the sucrose sample is placed at the 'sample' slot.
3. We run the spectrophotometer using the reference sample as the 'zero' value. This is done so that any inconsistencies that arise due to atmospheric conditions do not effect our readings.
4. The values obtained from the spectrophotometer are saved in a CSV file.

OBSERVATIONS AND PLOTS:

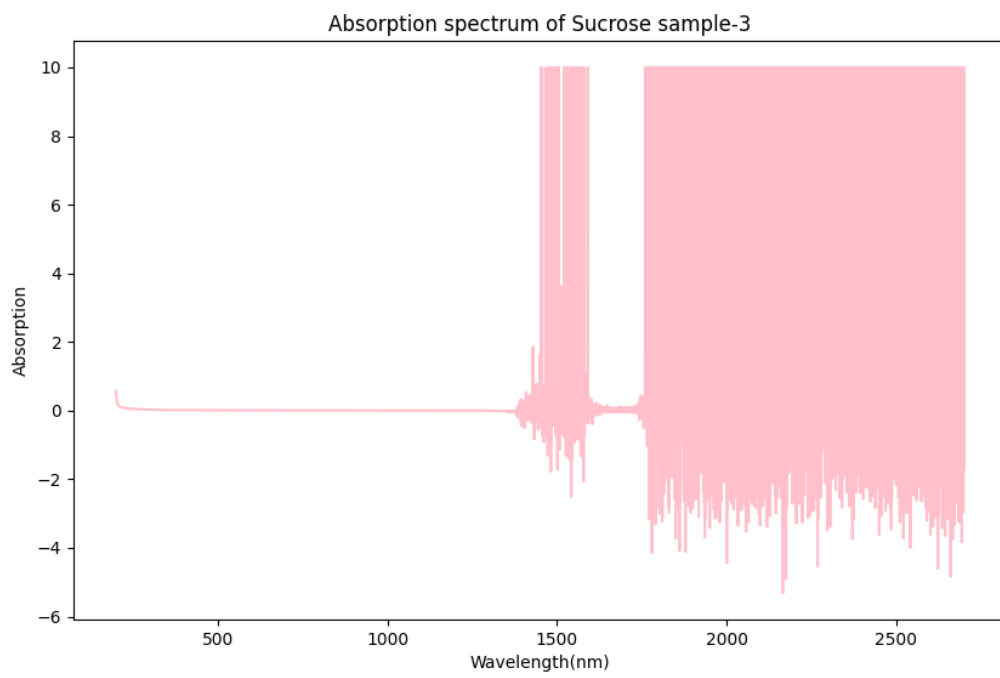
Using python codes, the values stored in the CSV files have been converted into plots to obtain the 'Absorption VS Wavelength' plots for different concentrations of sucrose samples.



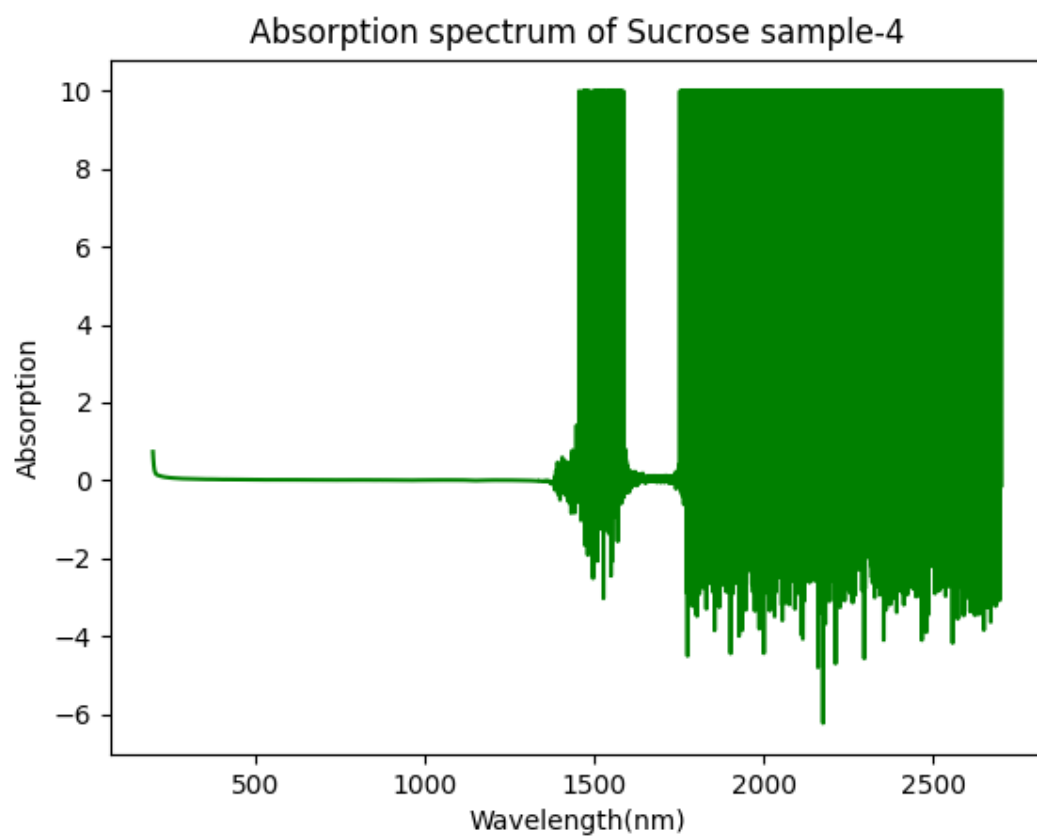
Absorption Spectrum for SUCROSE_0.01M



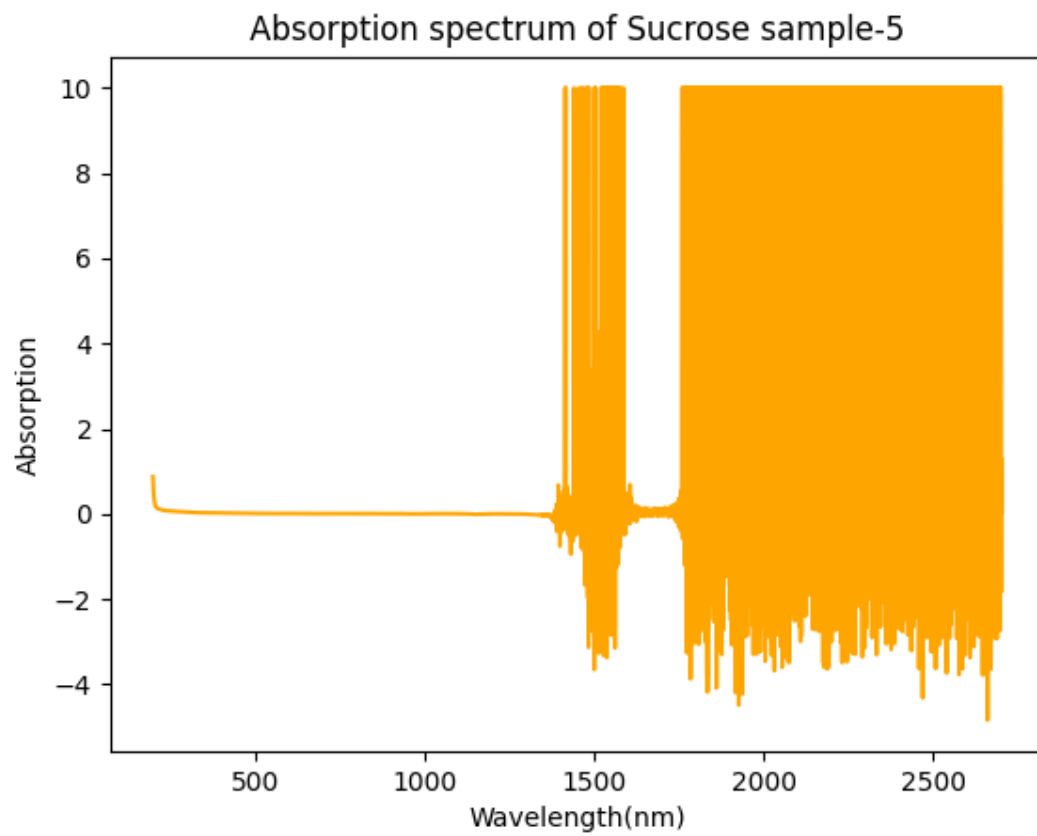
Absorption Spectrum for SUCROSE_0.02M



Absorption Spectrum for SUCROSE_0.03M

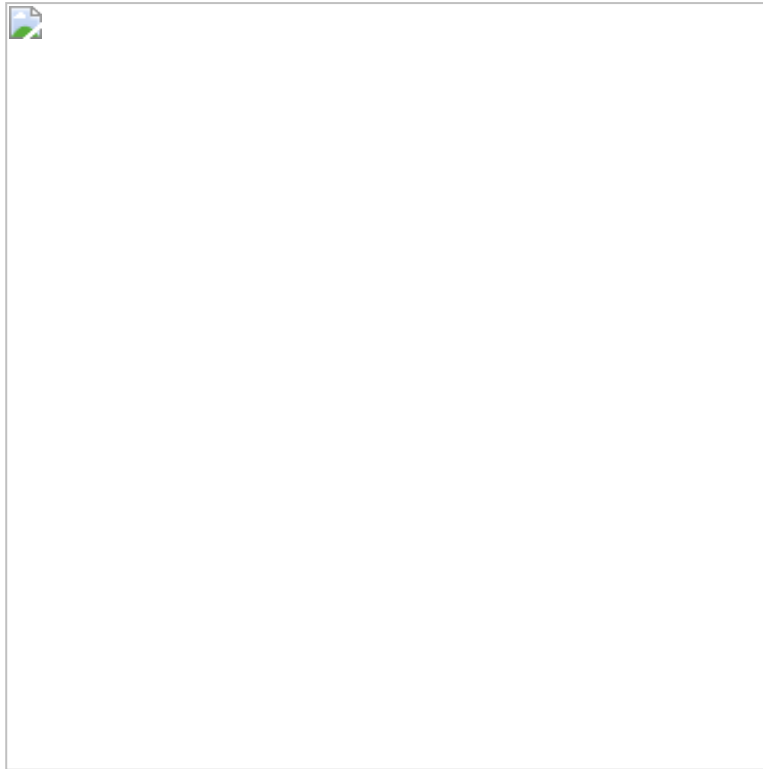


Absorption Spectrum for SUCROSE_0.04M



Absorption Spectrum for SUCROSE_0.05M

IMAGES:



Spectrophotometer

Experiment B:

We verify the Beer-Lambert's law by plotting the absorption VS concentration curve for 5 different wavelengths of light.

MATERIALS REQUIRED:

1. Spectrophotometer
2. Sucrose samples of different concentrations.

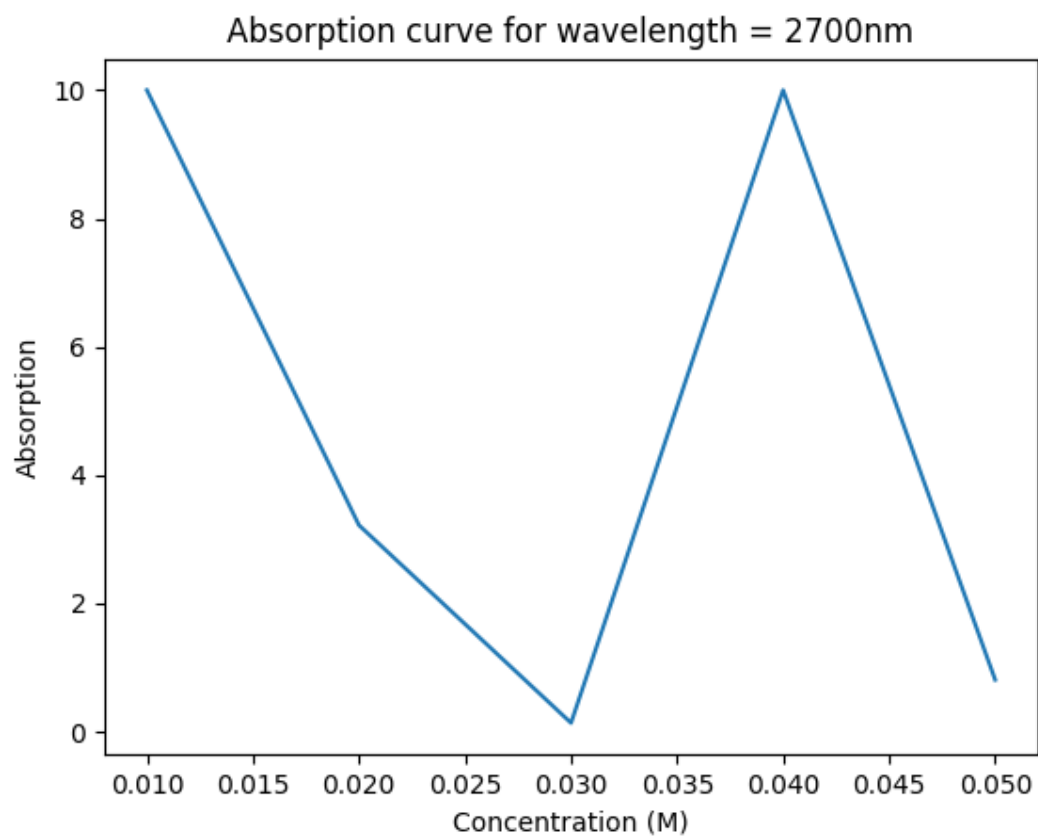
PROCEDURE:

1. Place the sucrose sample in a thoroughly cleaned cuvette. Also add some distilled water into another clean cuvette.
2. The distilled water cuvette is placed as the 'reference' in the spectrophotometer and the sucrose sample is placed at the 'sample' slot.

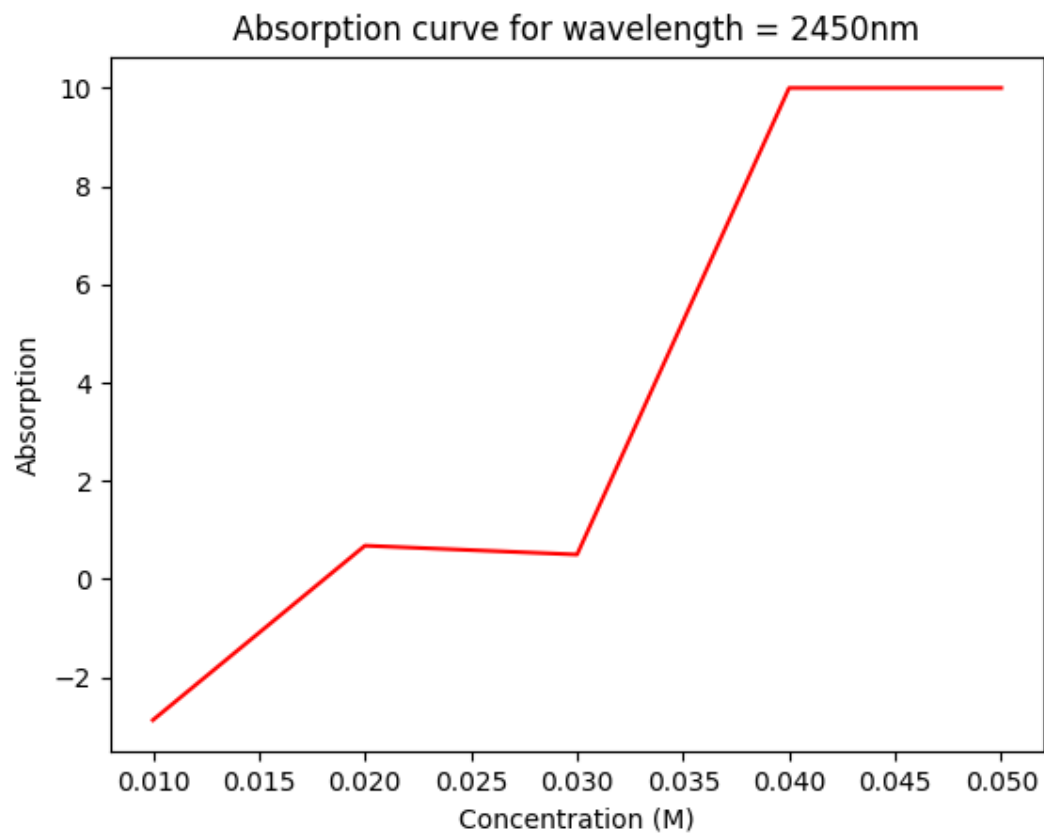
3. We run the spectrophotometer using the reference sample as the 'zero' value.
This is done so that any inconsistencies that arise due to atmospheric conditions do not effect our readings.
4. The values obtained from the spectrophotometer are saved in a CSV file.

OBSERVATIONS AND PLOTS:

Using python codes, we pick five different wavelengths, and plot the absorption VS concentration curves.



Absorption VS Concentration curve for wavelength of 2700nm



Absorption VS Concentration curve for wavelength of 2450nm