

An introduction to colorimetry

Before the beginning of the twentieth century most quantitative chemical analyses used gravimetry or titrimetry as the analytical method. With these methods, analysts achieved highly accurate results, but were usually limited to the analysis of major and minor analytes. Other methods developed during this period extended quantitative analysis: include trace level analytes. One such method was colorimetry.

Colorimetry is a scientific technique that is used to determine the concentration of colored compounds in solutions by the application of the Beer–Lambert law, which **states that the concentration of a solute is proportional to the absorbance**.

Different molecules are found to absorb light with different wavelengths, ranging from IR radiation (760-1000 nm), visible light (380—780 nm), and UV radiation (100–400 nm). We say “there is an absorption of light at different wavelength by different analyte”.

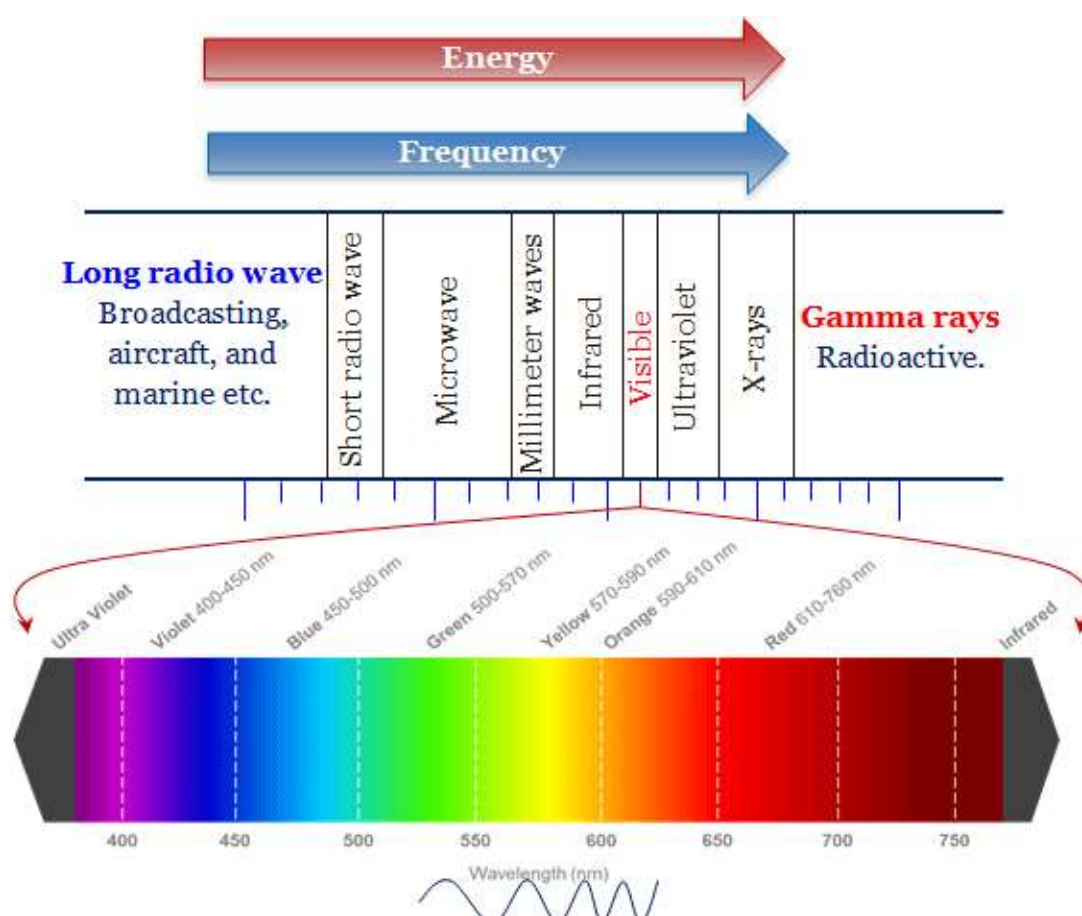


Figure 1: Electromagnetic Spectrum

The **intensity of light passing through a sample containing the analyte is altered** because of absorption. The measurement of this change, which we call **absorbance**, serves as our signal. The instrument we use is called a **spectrophotometer**. For most spectrometers and colorimeters, the useful absorbance range is from 0.1 to 1. Absorbance values greater than or equal to 1.0 are too high.



Figure 2: spectrophotometer

A **calibration curve** is a way to identify the concentration of an unknown substance. These curves use data points of known substances at varying concentrations. In most cases, a calibration curve shows that **absorbance is directly proportional to the concentration of analyte**. Thus, by matching absorbance of an unknown sample with a calibration curve, researchers can find where an unknown substance plots, and hence the concentration in the unknown sample.

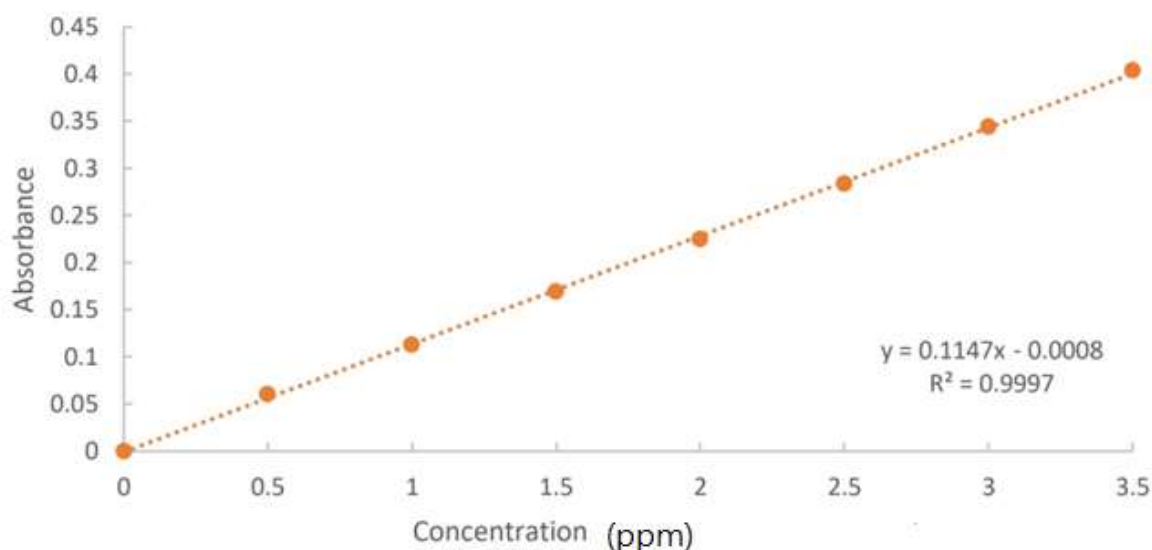


Figure 3: a typical calibration curve

Most of the chemical elements and a large number of compounds may be determined colorimetrically or spectrophotometrically, frequently at concentrations smaller than 1 mg/dm^3 (1 ppm).

Colorimetric measurement is still possible even when the analyte seems to be “colorless”. A “**coloring reagent**” can be added, which react with the analyte to form a coloured substances. The absorbance is now proportional to the concentration of the “coloured substance”, which in turn is still proportional to the concentration of analyte in the sample.

One example of an early colorimetric analysis is Nessler's method for ammonia, which was first proposed in 1856. Nessler found that adding an alkaline solution of HgI_2 and KI to a dilute solution of ammonia produced a yellow to

reddish brown colloid with the color determined the concentration of ammonia. A comparison of the sample's color to that for a series of standards was used to determine the concentration ammonia. Equal volumes of the sample and standards were transfer, to a set of tubes with flat bottoms. The tubes were placed in a rack equipped at the bottom with a reflecting surface, allowing light to pass through the solution. The colors of the samples and standards were compared by looking down through the solutions. Until recently, modified form of this method was listed as a standard method for the analysis of ammonia in water and wastewater.

Spectroscopic Analysis of nitrite content in daily-life samples - Background

Nitrate ions (NO_3^-) and nitrite ions (NO_2^-). Nitrate itself is relatively non-toxic but its metabolites, nitrite, is associated with methaemoglobinaemia (commonly known as Blue Baby Syndrome). Nitrite might also react with amines to form carcinogenic nitrosamines in the stomach. Nitrates and nitrites occur naturally in the environment. They are important plant nutrients and can be used in fertilisers. The recommended maximum limits by the World Health Organization in drinking water are 3 mg L^{-1} for nitrite ions.

Name _____

Class _____

Group _____

Title of Experiment

Spectroscopic Analysis of nitrite content in daily-life samples

Objective

- To construct a calibration curve for analysis of nitrite (NO_2^-) content in aqueous solutions
- To determine the concentration of nitrite ions spectroscopically with UV/Vis spectrophotometer

Grouping

Classes are divided into groups of 5 students. Three students of each group are responsible for construction of calibration curve, whereas the others are responsible for sample analysis.

Procedure**Part A: Construction of calibration curve****Reagents provided**

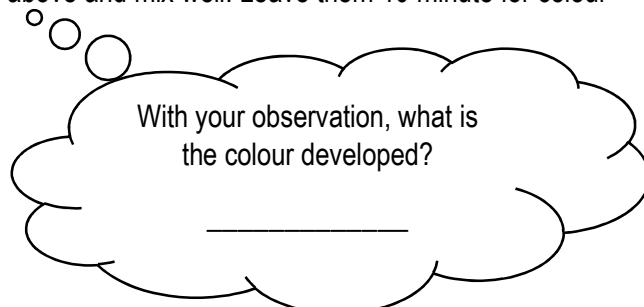
- Standard aqueous solution of sodium nitrite (NaNO_2), with concentration of 1000 ppm. This is the **stock solution** (NaNO_2)
- Colouring reagent, a mixture of H_3PO_4 , sulfonamides, and N-(1-naphthyl)-ethylenediamine-dihydrochloride

Procedures

- Pipette **1 ml** of the **stock solution** into a **100 ml** volumetric flask and add distilled water to the graduation mark. This is the **Solution I**. Concentration **Solution I** is _____ ppm
- Prepare a series of **working standard solutions** with 50 ml volumetric flasks according to the table below:

	Blank	Standard solution A	Standards solution B	Standards solution C	Standards solution D
Volume of Solution I /ml	0				
Volume of distilled water added	Add distilled water until the graduation mark of 50 ml volumetric flask				
Concentration of the standard formed / ppm	0				

- Add 2 ml of colour reagent into four working standards above and mix well. Leave them 10 minute for colour development.



4. Measure the absorbance of the solution by using UV-vis spectrophotometer at a wavelength of about 540 nm. Handle the vials with great care. Between each measurement, rinse the vials thoroughly with distilled water and dry them with lens tissue.

There are totally 5 measurements in this part. Make sure vials have been put in both cartridge in all measurements according to the table

	1 st measurement	2 nd measurement	3 rd measurement	4 th measurement	5 th measurement
Vial in reference cartridge	Blank	Blank	Blank	Blank	Blank
Vial in sample cartridge	Blank	Standard solution A	Standards solution B	Standards solution C	Standards solution D

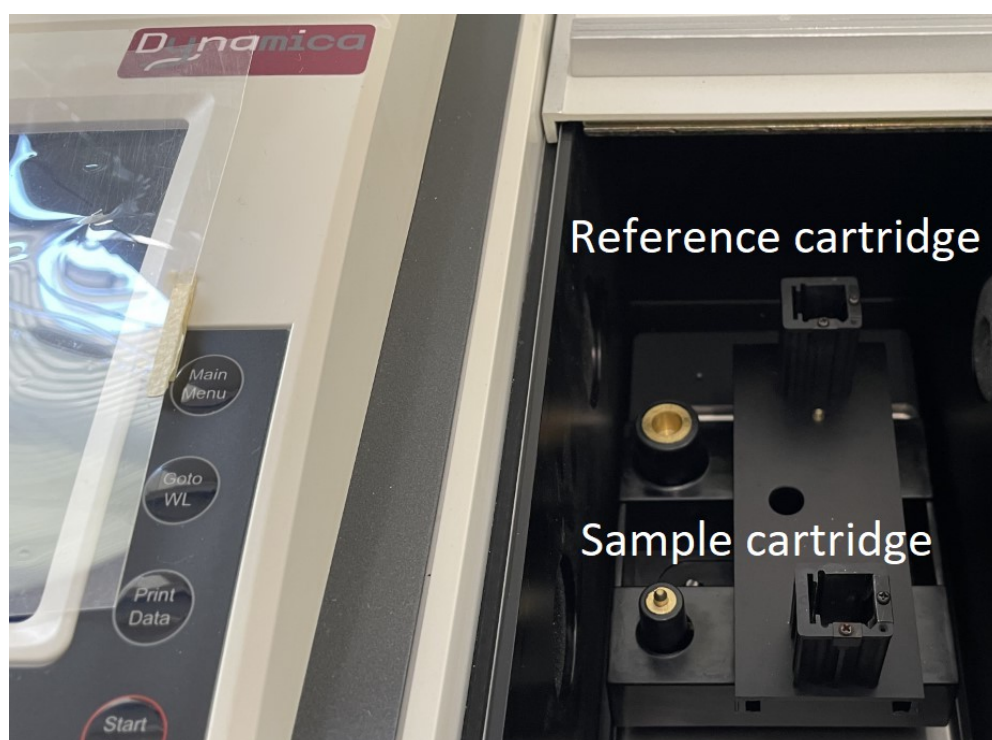


Figure 4: How vials should be put correctly

5. Construct the calibration curve by plotting the measured absorbances against the concentration, in ppm, of the standard solutions.

Part B: Measurement of absorbance with daily life samples**Reagents provided**

1. Colouring reagent, a mixture of H_3PO_4 , sulfonamides, and N-(1-naphthyl)-ethylenediamine-dihydrochloride
2. Authentic samples
 - a. 1 kg of Choi Sum immersed into 2L of water for 20 minutes
 - b. water from Sun Kei Fish Pond
 - c. tap water from chemistry laboratory

Procedures

1. Add the authentic sample into 50 ml volumetric flask until the graduation mark.
2. Add 2 ml of colour reagent into four working standards above and mix well. Leave them 10 minute for colour development.
3. Measure the absorbance of the solution by using UV-vis spectrophotometer at a wavelength of about 540 nm. Handle the vials with great care.

Results**Part A: Construction of calibration curve**

	Sample	2nd measurement	3 rd measurement	4 th measurement	5 th measurement
Vial in reference cartridge	Blank	Blank	Blank	Blank	Blank
Vial in sample cartridge	Blank	Standard solution A	Standards solution B	Standards solution C	Standards solution D
Absorbance					

The equation for the calibration curve obtained is _____
 with $R^2 =$ _____

Part B: Measurement of absorbance with daily life samples

	1 st measurement
Vial in reference cartridge	Blank
Vial in sample cartridge	Name of Sample: _____
Absorbance	

Based on the calibration curve, the concentration of nitrite ions in the sample was found to be _____

Question to be discussed

1. Why is it necessary to construct a calibration curve if we want to determine the concentration of an analyte with colorimetric experiment?

2. If the absorbance of a sample is found to be higher than 1, what should be done to the procedure of the experiment?

3. Suggest ONE assumption in this experiment.

4. Suggest some advantages of using colorimetry over tradition test-tube scale experimental analysis, such as titration.
