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Theme

Protocol for UV visible spectrophotometric calibration check

Submitted and defended by:

LAIDANI Rafika

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Examiners Committee

Pr. KAIL Fatiha	President	Oran 1 university
Dr. GUERNAZI Daoud	Examiner	Oran 1 university
Pr. ILES Nadia	Supervisor	Oran 1 university

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Abstract

This project outlines the protocol for calibration check of a UV-Visible spectrophotometer Shimadzu UV-3600 in Laboratory of Thin Films and Materials for Electronics. The calibration process involved preparing a series of coloured solutions with known absorbance spectra to serve as calibration standards. The spectrophotometer's performance was evaluated by measuring the absorbance of these solutions across the UV-Visible spectrum. The obtained results demonstrated the instrument's accuracy and linearity, confirming its reliability for precise spectrophotometric analysis. This protocol ensures consistent performance of the spectrophotometer, essential for high-quality quantitative measurements in various applications.

General Introduction

Since Isac Newton discovers the light dispersion through a prism, the era of spectroscopy started. Centuries later, efficient spectroscopic devices are developed and occupied a dominant place in our daily life. The principle of detection of these devices is based on interaction of matter with radiation. A Spectrophotometer is an equipment based on the analysis of transmitted light after passing through a sample (solid or liquid) to identifies chemical compounds and their concentration and other physical characteristics as absorbance, transmittance, thickness, ...Therefore, this equipment find its application in many fields. In Biology, spectrophotometers allow quantification of biomolecules like DNA, RNA, proteins, enzyme assays, cell viability assays. While in medical science, spectrophotometers are used for analysis of blood components, urine analysis, detection of diseases and pathogens, drugs screening. This device is also an important tool in pharmacology and food industry, analysis of drug formulations, determination of drug concentration in biological samples, quality control and analysis of food and beverages, determination of nutritional composition, detection of contaminants. In materials science, spectrophotometers are used for characterization of materials such as polymers, metals and ceramics, measurement of optical properties, analysis of coatings.

Regardless of the specific application, calibration of the spectrophotometer is essential for ensuring accurate and reliable measurement results. Calibration involves adjusting the instrument's settings to match known standards and correcting for any deviations or errors in the measurement process. By calibrating the spectrophotometer, scientists can minimize sources of uncertainty and obtain precise and reproducible data, enhancing the validity and reliability of their research findings. In this master work, we proposed to check the calibration of the UV-Visible spectrophotometer Shimadzu UV-3600 used for thin films and solutions characterization in Laboratory of Thin films and Materials for Electronics. For this purpose,

many dyes solutions are analysed to cover all the visible spectrum. Their absorbance spectra will be examined to check device calibration. This manuscript is organized into two chapters. In the first one, the basics about spectrophotometry are provided. The second chapter is dedicated to the description of the experimental protocol used for calibration verification of the spectrophotometer Shimadzu UV-3600 and analysing the obtained absorbance results.

Chapitre 1

Basics on spectroscopy

1.1 What is spectroscopy:

Spectroscopy is the study of the absorption and emission of light and other radiation by matter. It involves the splitting of light (or more precisely, electromagnetic radiation) into its constituent wavelengths (a spectrum), which is done in much the same way as a prism splits light into a rainbow of colours. Figure 1 illustrates the full electromagnetic Spectrum. [1]

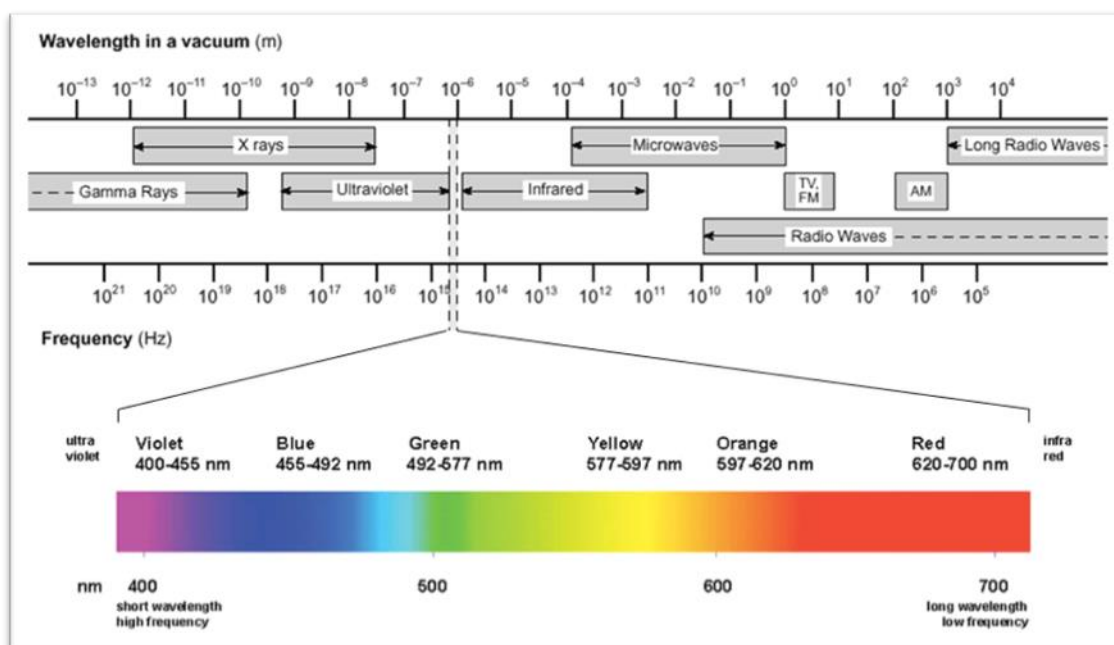


Figure 1: The electromagnetic spectrum, with the visible spectrum highlighted

1.2 Application of spectroscopy:

Today, modern spectroscopy contributes to a fundamental understanding of the interaction of electromagnetic energy with matter in the following important fields: [2]

1. Astronomy
2. Analytical organic chemistry
- food packaging
- toxicology

- | | | |
|----|-------------------------------|-------------------------------|
| 3. | Remote sensing | acoustics and speech analysis |
| 4. | Agriculture and crop analysis | national defence |
| 5. | Terrestrial geography | telecommunication |
| 6. | Forensic science | |

1.3 Classification of spectrum:

All spectra show basically the same thing: how brightness varies with wavelength. Scientists often classify spectra based on the key light-matter interactions they represent and how they are used. We take examples of light penetration a gas cloud [3]

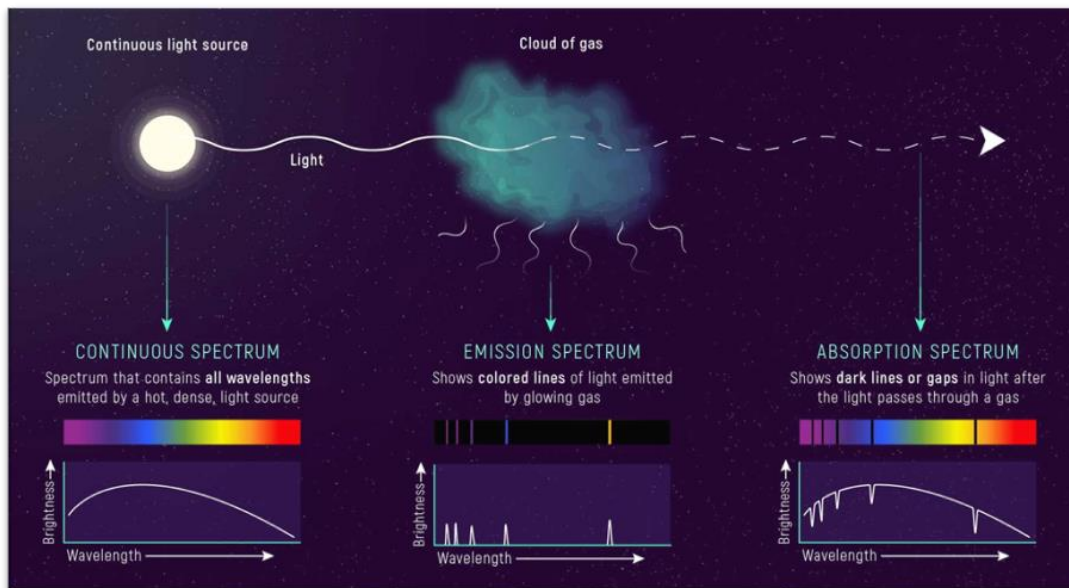


Figure 2: Types of spectrums

1.3.1 Continuous Spectrum:

A continuous spectrum contains all wavelengths of light in a certain range. Hot, dense light sources like stars, for example, emit a nearly continuous spectrum of light, which travels out in all directions and interacts with other materials in space. The broad range of colours that a star emits depends on its temperature.

1.3.2 Absorption Spectrum:

When starlight passes through a cloud of gas, some of the light is absorbed and some is transmitted through the gas. The wavelengths of light that are absorbed depends on what elements and compounds it is made of. An absorption spectrum has dark lines or gaps in the spectrum corresponding to wavelengths that are absorbed by the gas.

1.3.3 Emission Spectrum:

Starlight can also heat up a cloud of gas, exciting the atoms and molecules within the gas, and causing it to emit light. The spectrum of light that a cloud of gas emits depends on its temperature, density, and composition. An emission spectrum consists of a series of coloured lines that correspond to wavelengths emitted by the glowing gas.[3]

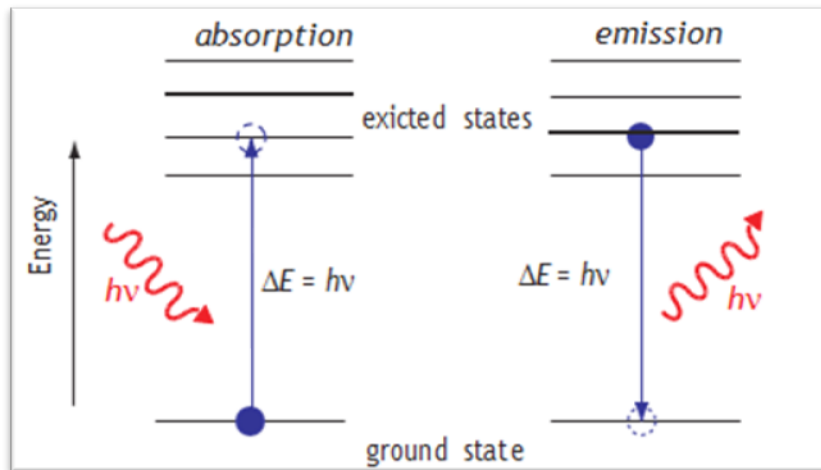


Figure 3: Energy diagram of the absorption and emission of a photon by an atom or a molecule

“Figure 3” Simplified energy diagram showing the absorption and emission of a photon by an atom or a molecule. When a photon of energy $h\nu$ strikes the atom or molecule, absorption may occur if the difference in energy, ΔE , between the ground state and the excited state is equal to the photon’s energy. An atom or molecule in an excited state may emit a photon and return to the ground state. The photon’s energy, $h\nu$, equals the difference in energy, ΔE , between the two states.

1.4 Wavelength and frequency:

Electromagnetic radiation can be considered as a combination of alternating electric and magnetic fields that travel through space. Because radiation acts as a wave, it can be classified in terms of either wavelength or frequency, which are related by the following equation:

$$\nu = \frac{c}{\lambda} \quad (\text{I.1})$$

where ν is frequency (in seconds), c is the speed of light ($3 \times 10^8 \text{ ms}^{-1}$), and λ is the wavelength (in meters).

In UV-Vis spectroscopy, wavelength is usually expressed in nanometres ($1 \text{ nm} = 10^{-9} \text{ m}$). It follows from the equations that radiation with shorter wavelength has higher energy, and, for UV-Vis spectroscopy, the low (short) [4]

1.5 Light sources:

The ideal light source would yield a constant intensity over all wavelengths with low noise and long-term stability of the output. Unfortunately, such a source does not exist. Two different light sources have historically been used in UV-visible spectrophotometers:

- The deuterium arc lamp was used to provide a good intensity continuum in the UV region and useful intensity in the visible region
 - The tungsten-halogen lamp yielded good intensity over the entire visible range and part of the UV spectrum
- More recently, a single Xenon flash lamp has been used more widely. The use of a Xenon flash lamp as a single source has significant advantages over the use of the two conventional lamps. Table 1 show the common light sources used and what useful for . [4]

Table 1 Common Sources of Electromagnetic Radiation		
Source	Wavelength Region	Useful for...
H ₂ and D ₂ lamp	continuum source from 160–380 nm	molecular absorption
tungsten lamp	continuum source from 320–2400 nm	molecular absorption
Xe arc lamp	continuum source from 200–1000 nm	molecular fluorescence
nernst glower	continuum source from 0.4–20 μ m	molecular absorption
globalar	continuum source from 1–40 μ m	molecular absorption
nichrome wire	continuum source from 0.75–20 μ m	molecular absorption
hollow cathode lamp	line source in UV/Visible	atomic absorption
Hg vapor lamp	line source in UV/Visible	molecular fluorescence
laser	line source in UV/Visible/IR	atomic and molecular absorption, fluorescence, and scattering

Table 1: Common light sources and their use for characterization

1.6 Devices used for spectroscopy:

The three main instruments are used in spectroscopy: a prism, a diffraction grating, and an interferometer.

In physical size, they may vary in length from centimetre to meters (prism from 5cm to 30cm, diffraction grating from 10cm to 1m and interferometer 5cm to 30m), depending on the precision required or the intended application. Each one has a particular use, which may include simply observing the spectrum, photographing the spectrum, or focusing on a particular detail of the spectrum.

1.6.1 Prism spectrometer:

Typically, prisms used in spectrometers to analyse the visible light region are made of glass. Rock salt, fluorite, and quartz are used in the optical components for viewing the ultraviolet regions of the spectrum. In the near-infrared region, lenses and prisms in the spectrometer are made generally of rock salt, fluorite, lithium fluoride, and potassium bromide. Prisms are not effective in the far-infrared or far-ultraviolet ends of the spectrum. The prism is shown in Figure 5.

1.6.2 Diffraction grating:

The diffraction grating, a device more versatile than a prism, is made usually of a mirror or transparent plate of glass on which are etched up from 300 and 6000 lines/mm. For a diffraction grating as the principal wavelength dispersing element, all parts of the spectrum from the far-infrared to the far-ultraviolet can be displayed and analysed. The great number of lines in a diffraction grating make the dispersion of light more accurate than that obtained with a prism. The diffraction grating is shown in Figure 4.

1.6.3 Interferometer spectrometer:

A spectrometer that uses an interferometer in the place of a prism or diffraction grating makes use of the principles of interference of light. An interferometer spectrometer provides the ultimate in wavelength resolution and precision of measurement of the spectrum provided. [2]

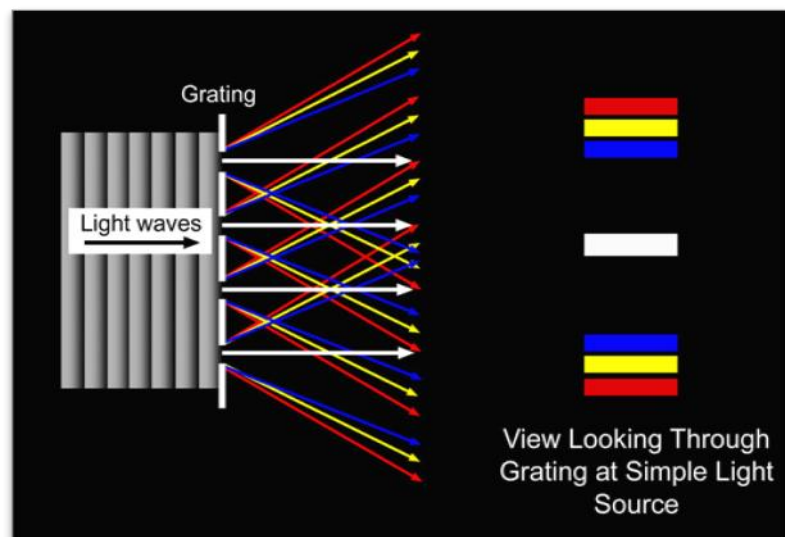


Figure 4: Diffraction grating

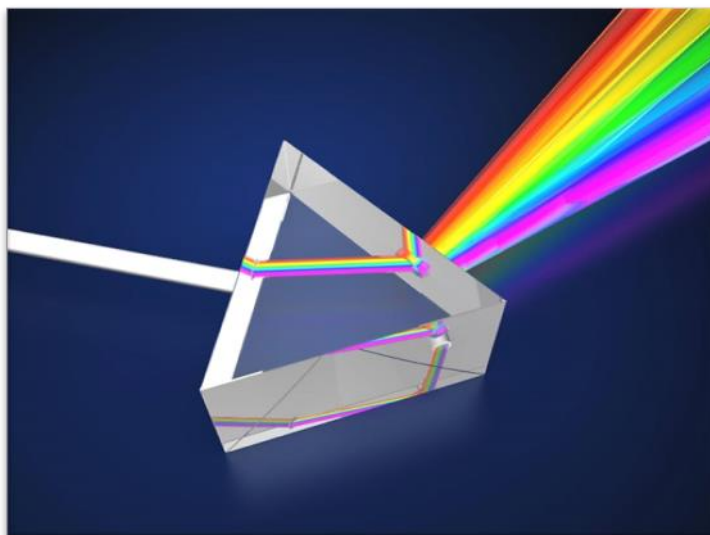


Figure 5: Prism in spectrometers

1.7 Types of spectroscopies:

1.7.1 Atomic Spectroscopy:

Atomic spectroscopy was the first application of spectroscopy developed, and it can be split into atomic absorption, emission and fluorescence spectroscopy. Atoms of different elements have distinct spectra so atomic spectroscopy can quantify and identify a sample's composition. The main types of atomic spectroscopy include atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and atomic fluorescence spectroscopy (AFS).[5]

1.7.2 Infrared Spectroscopy:

Infrared (IR) analyses compounds using the infrared spectrum, which can be split into near IR, mid-IR and far IR. Near IR has the greatest energy and can penetrate a sample much deeper than mid or far IR, but due to this, it is also the least sensitive. Infrared spectroscopy is not as sensitive as UV/Vis spectroscopy due to the energies involved in the vibration of atoms being smaller than the energies of the transitions.[5]

1.7.3 Raman Spectroscopy:

Raman spectroscopy is similar to IR, it is a vibrational spectroscopy technique, but it uses inelastic scattering. The spectrum of Raman spectroscopy shows a scattered Rayleigh line and the Stoke and anti-Stoke lines, which is different from the irregular absorbance lines of IR.[5]

1.7.4 Nuclear Magnetic Resonance:

Nuclear magnetic resonance (NMR) uses resonance spectroscopy and nuclear spin states for spectroscopic analysis. All nuclei have a nuclear spin, and the spin behaviour of the nucleus of every atom depends on its intramolecular environment and the external applied field.[5]

1.7.5 Ultraviolet and Visible Spectroscopy:

Ultraviolet (UV) and visible (Vis) spectroscopy analyses compounds using the electromagnetic radiation spectrum from 120 nm to 700 nm. Many atoms are able to emit or absorb visible light, and it is this absorption or reflectance that gives the apparent colour of the chemicals to characterise.[5]

1.8 Spectrophotometers:

1.8.1 Difference between spectrometer and spectrophotometer:

By spectrometer, we can measure the wavelength of absorbed light and reflected light while by using a spectrophotometer, we can measure the relative intensity of light absorbed and reflected. [6]

1.8.2 Application of spectrophotometers:

Chemistry: determination of concentration of substances in solutions, identification of chemical compounds, analysis of reaction kinetics.

Biology and biochemistry: quantification of biomolecules like DNA, RNA, proteins, enzyme assays, cell viability assays.

Pharmaceuticals: analysis of drug formulations, determination of drug concentration in biological samples, quality control.

Environmental science: monitoring pollutants in air, water and soil, analysis of environmental contaminants, studying environmental impacts.

Food and beverage industry: quality control and analysis of food and beverages, determination of nutritional composition, detection of contaminants.

Medical diagnostics: analysis of blood components, urine analysis, detection of diseases and pathogens, drugs screening.

Material science: characterization of materials such as polymers, metals and ceramics, measurement of optical properties, analysis of coatings and films.

Forensics: identification of trace evidence, analysis of forensics samples like bloodstains and fibres, detection of illegal substances. [7]

1.8.3 Principle of spectrophotometer:

The UV-visible spectrophotometer consists of a light source, sample holders, a monochromator, and a detector.

Light source: Hydrogen or deuterium lamps are used as UV light source, whereas for visible source tungsten filament lamp is mostly used.

Sample holders: In UV and Visible ranges, cuvettes made from quartz or ordinary glass are used as sample holders. Generally, in the UV region quartz or silica cell are used, whereas in the visible region glass cell are used and these cuvettes have a standard path length of usually 1 cm.

Monochromators: A monochromator converts polychromatic radiation into monochromatic radiations by which the wavelengths of these radiations translate into very narrow bands.

Detectors: Photovoltaic cells, phototubes and photomultiplier are commonly used detectors in the UV and visible range [8]

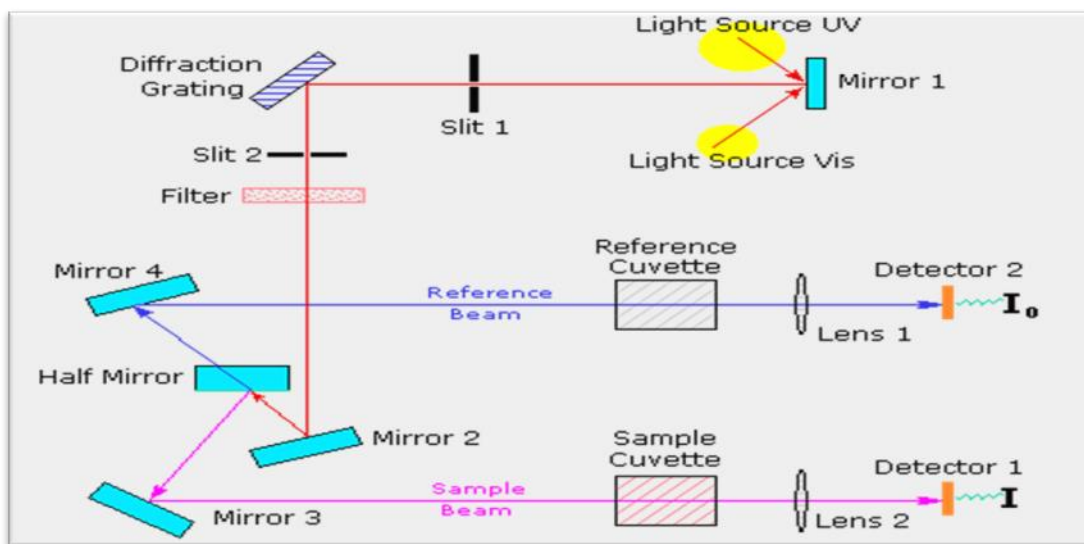


Figure 6: Principle of working of spectrophotometer

1.9 Transmittance:

Transmittance refers the amount of light energy that a material absorbs or disperses. It is expressed as the ratio between transmitted intensity I (after passing through a sample) and the incident intensity I_0 (source light before passing through the sample). Most of time transmittance T is expressed as:

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

Also, transmittance can be related to the absorbed quantity of light by the sample (Absorbance A) by:

$$A = -\log T$$

1.10 Absorbance and concentration: ‘beer-’s lambert law ‘:

When monochromatic electromagnetic radiation passes through an infinitesimal thin layer of sample of thickness dX , it experiences a decrease in its power of dP (figure 8). The fractional decrease in power is proportional to the sample’s thickness and the analyte’s concentration, C ; thus

$$-\frac{dP}{P} = \alpha C dX \quad (I.2)$$

where P is the incident power on the thin layer of sample, and α is a proportionality constant. Integrating the left side of equation 2 over the entire sample thickness b

$$-\int_{P_0}^{P_T} \frac{dP}{P} = \alpha X \int_0^b dX \quad (I.3)$$

$$\ln \frac{P_0}{P_T} = \alpha b C$$

$$A = \alpha b C$$

Converting $\ln$ to $\log$, and substituting in equation 3, gives:

$$A = \epsilon b C \quad (I.4)$$

where α is the analyte’s absorptivity with units of $cm^{-1} conc^{-1}$. If we express the concentration using molarity, then we replace α with the molar absorptivity, ϵ , which has units $cm^{-1} M^{-1}$ of or $L Mol^{-1} cm^{-1}$

The absorptivity and molar absorptivity are proportional to the probability that the analyte absorbs a photon of a given energy. As a result, value for both α and ϵ depend on the wavelength of the absorbed photon [9]

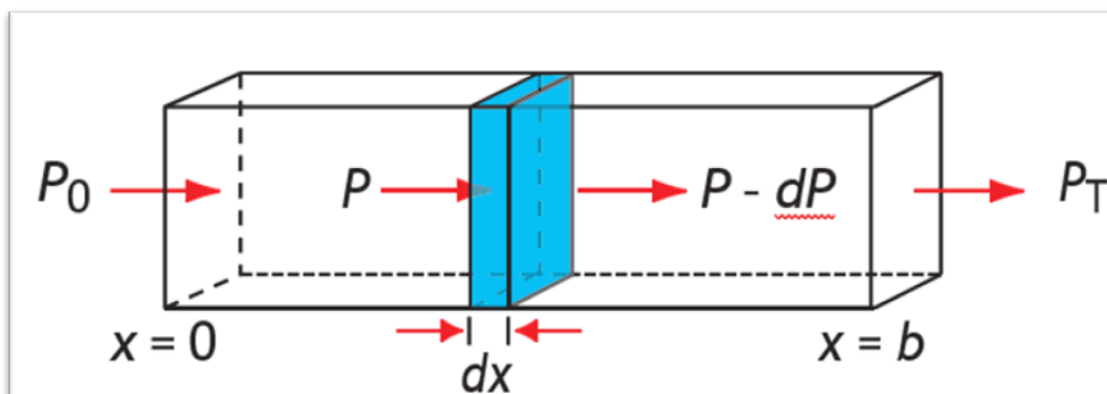


Figure 7: Path of radiation through the sample thickness to deriving the beer -lambert law.

1.10.1 Limitations to beer-Lambert's law:

Beer-Lambert's law suggests that a calibration curve is a straight line with a y intercept of zero and a slope of αb or ϵb . In many cases a calibration curve deviates from this ideal behaviour (Figure 8). Deviations from linearity are divided into three categories: fundamental, chemical and instrumental [9]

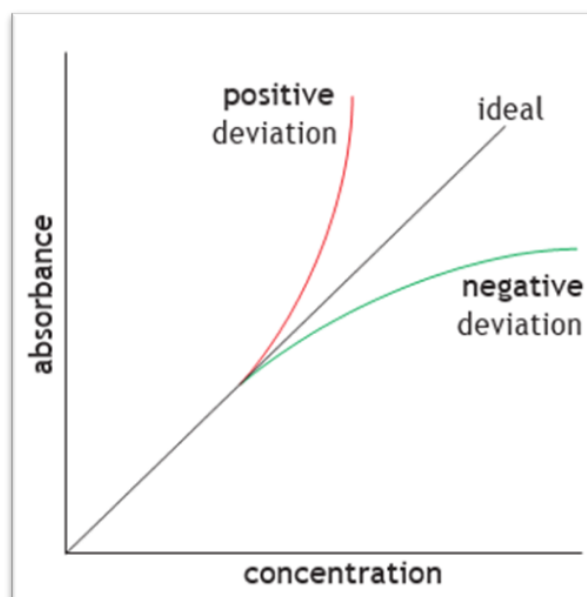


Figure 8: Calibration curves of the Beer-Lambert's law showing positive and negative deviation from the ideal beer's law calibration curve which is straight line [9].

1.11 Colorimetry and spectrophotometry:

- Colorimetry is a technique for determining the concentration of a coloured solution. It calculates the colour intensity and correlates it with the sample concentration. In colorimetry, the colour of the sample is compared to the colour of a known-colour standard. A colorimeter is a piece of equipment that may be used to test coloured samples and calculate absorptions.
- Colorimetry utilizes fixed wavelengths that are only observable in the visible spectrum, but spectrophotometry can use wavelengths from a larger range. Furthermore, a colorimeter quantifies colour by measuring the three major colour components of light (red, green, and blue), but a spectrophotometer detects the precise colour in human-visible light wavelengths. As a result, there is a distinction between colorimetry and spectrophotometry [10].

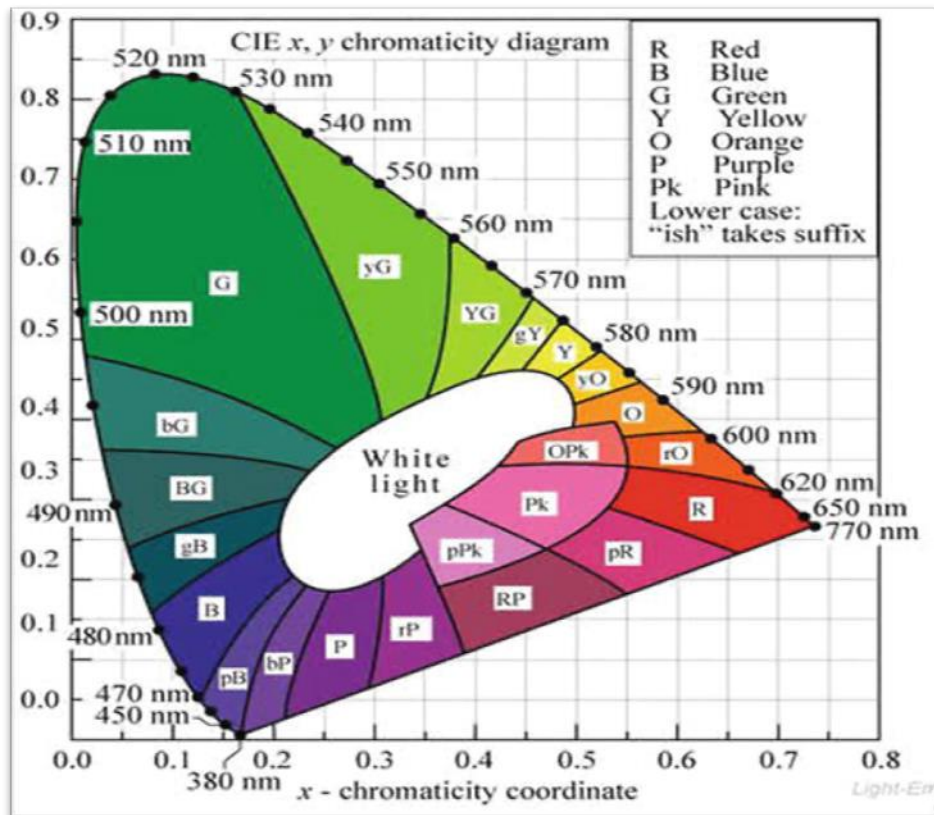


Figure 9: Chromaticity diagram CEI [11]

Figure 9 shows the CIE (Commission Internationale de l'Éclairage or International Commission on Illumination) is an international authority of light, illumination, colour, and

colour spaces. The CIE develops standards and recommendations for the measurement and description of light and colour. The CIE system measures colour using a mathematical model called the CIE XYZ colour space, this model defines colours based on three primary components: X, Y, and Z. These components represent the amount of red, green, and blue light needed to create a specific colour. By combining these values, the CIE system can accurately represent and measure colours. It's a standardized way to describe colours in a precise and consistent manner [11].

1.11.1An example of colorimetry “Application of colorimetry in food industries “:

Absorbance spectra of food dyes in the red-violet colour range. Figure 10 shows a schematic diagram of a colour wheel illustrating the emission wavelengths (inner circle) and absorption wavelengths (outer circle) of visible light (in nanometres; nm).

The inner circle represents the emission colour of the indicator dye and the outer circle represents the colour of the quencher required to absorb the emission. For example, ANG (Natrium green-2) has a peak emission wavelength of 540 nm (green), therefore it can be expected that red-violet-coloured dyes will quench the emission (B) Absorbance spectra of Brilliant Black BN, Carmine, Ponceau 4R, Allura Red and Amaranth, Calcium 4 Assay and BD Quencher. Grey shaded area represents the FLIPR TETRA detection range (FDR). (C) International Numbering System for Food Additives (INS) E number, common name(s), colour and peak absorption (λ max) of food dyes tested [12].

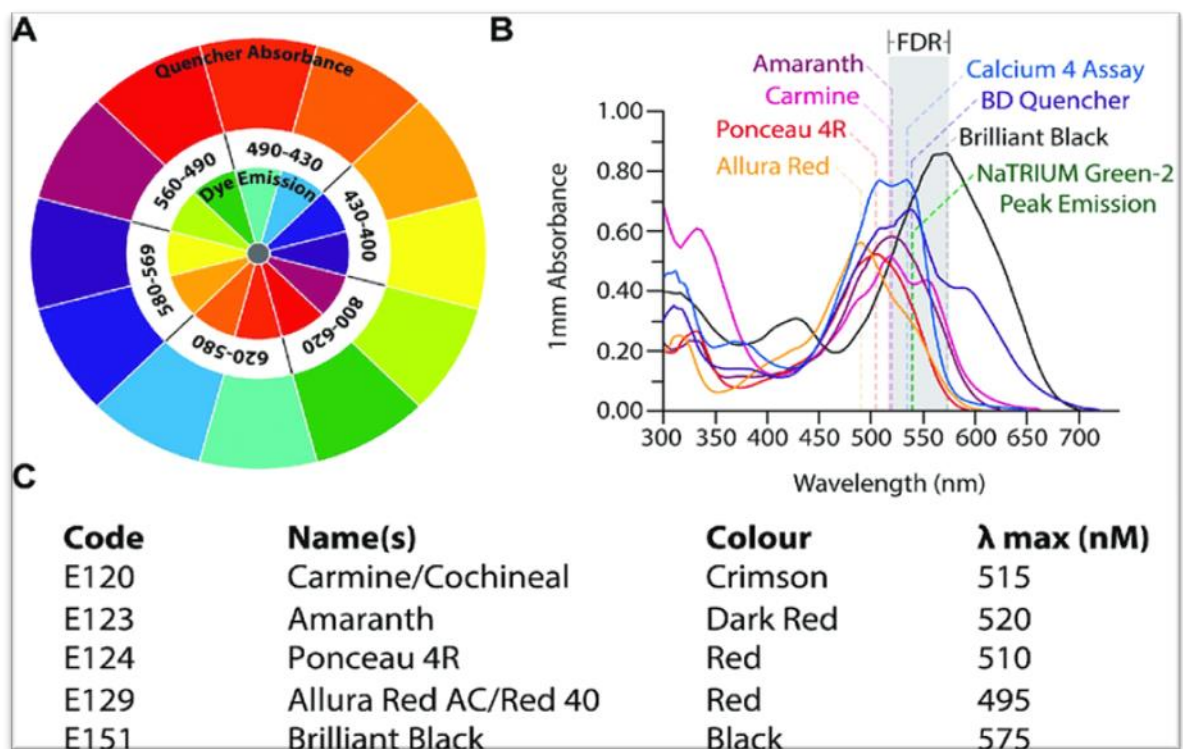


Figure 10: Absorbance spectra of food dyes and a schematic diagram of emission/absorbance wavelengths ref [12]

Chapitre 2

Measurement and calibration using spectrophotometer Shimadzu UV-3600

2.1 Introduction:

UV-visible spectrophotometer analysis plays a crucial role in a wide range of applications across various scientific disciplines, including chemistry, biology, environmental science, and material science. These instruments are commonly used to measure the absorption or transmission of light by substances in the UV and visible regions of the electromagnetic spectrum. In chemistry, UV-visible spectrophotometry is used for quantitative analysis of compounds, determining concentrations of analytes in solutions or studying reaction kinetics. Biologists employ spectrophotometers to characterize biomolecules such as proteins and nucleic acids, aiding in research on diseases and drug development. Environmental scientists rely on these instruments to monitor pollutants in air, water, and soil, contributing to environmental protection efforts. Material scientists use UV-visible spectrophotometry to study the optical properties of materials, guiding the development of new materials for various applications.

Regardless of the specific application, calibration of the spectrophotometer is essential for ensuring accurate and reliable measurement results. Calibration involves adjusting the instrument's settings to match known standards and correcting for any deviations or errors in the measurement process.

By calibrating the spectrophotometer, scientists can minimize sources of uncertainty and obtain precise and reproducible data, enhancing the validity and reliability of their research findings.

In this chapter, we will focus on checking the calibration of the UV-Visible spectrophotometer Shimadzu UV-3600 used for thin films and solutions characterization in Laboratory of Thin films and Materials for Electronics. For this purpose, many dyes solutions are analysed to cover all the visible spectrum.

2.2 Shimadzu UV- 3600 UV-Vis-Nir Spectrophotometer:

The UV-3600 UV-VIS-NIR Spectrophotometer combines research-grade UV-Vis or UV-Vis-NIR optical performance with the ease and familiarity of PC operation. It is designed for the measurement of liquid samples, the UV-3600 features are: three detectors and a high-performance double monochromator to ensure high sensitivity, reduced noise, and ultra-low stray light [13].



Figure 11: Spectrophotometer Shimadzu UV-3600

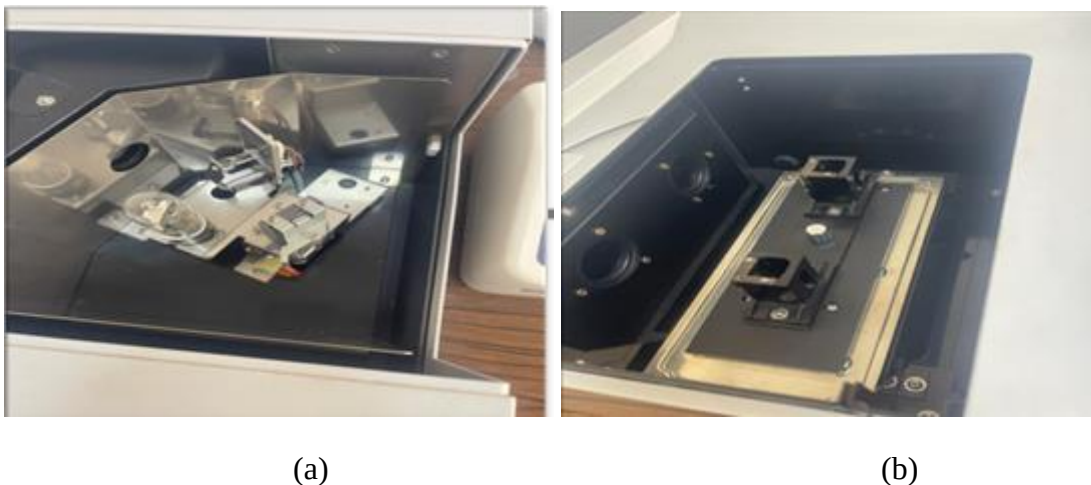


Figure 12: Component of Shimadzu UV-3600, (a) Light sources: Deuterium and Tungsten and (b) sample holder.

2.2.1 Features of Shimadzu UV-3600 spectrophotometer:

High Sensitivity - 3 Detector System:

This spectrophotometer is equipped with 3 detectors: a PMT detector (photomultiplier tube) for the ultraviolet and visible regions, and InGaAs and PbS detectors for near infrared region. The InGaAs detector bridges the gap between the PMT - PbS switching wavelength where sensitivity is typically low to ensure high sensitivity over the entire measurement wavelength range. Accuracy of absorbance noise is lesser than 0.00003 at 1500 nm [18].

High Resolution - Wide Measurement Range & Ultra-low Stray Light:

With its high-performance double monochromator, ultra-low stray light (0.00005% or less at 340 nm) is achieved at high resolution (highest resolution 0.1 nm). With a measurement wavelength range of 185 - 3300 nm, measurement is possible over a wide range including the ultraviolet, visible and near infrared regions. This allows spectroscopic analysis in a wide variety of fields [13].

Until now, conventional spectrophotometers have used a **PMT** (photomultiplier tube) for the **ultraviolet** and **visible** region and a **PbS** detector for the **near-infrared** region (see Figure 13). Neither detector, however, is very sensitive near the detector-switchover region. This

prevents high sensitivity measurement in this range. The UV-3600 makes it possible to take high-sensitivity measurements in the switchover range by using an **InGaAs** detector [8]

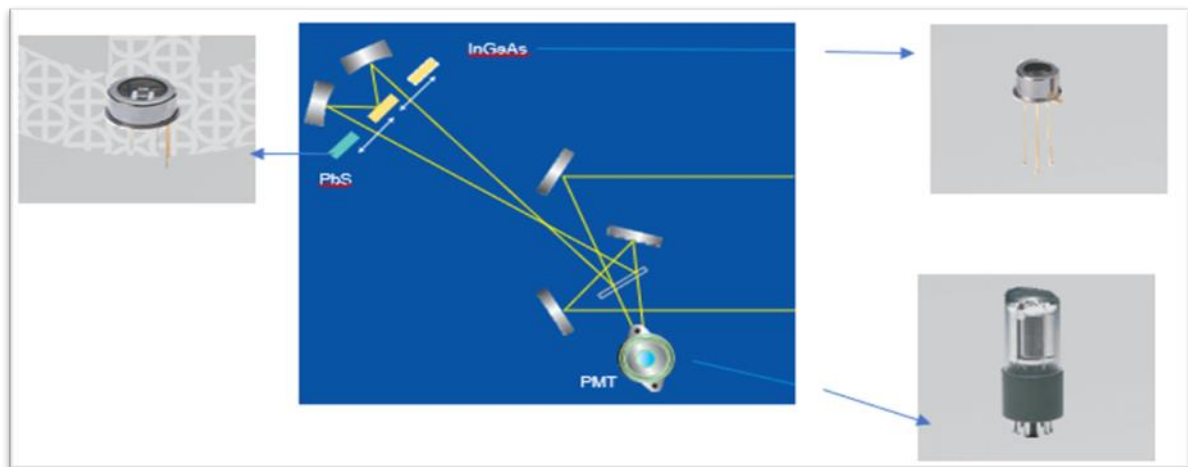


Figure 13: Optical system around detectors of spectrophotometer Shimadzu UV-3600

2.2.2 Specifications:

Shimadzu is a well-known manufacturer of UV-Visible spectrophotometers, offering various models with specific features and specifications designed to meet diverse analytical needs. The typical specifications for Shimadzu UV-Visible spectrophotometers, such as the UV-3600 are summarized on table 2.

Wavelength range	185nm - 3300nm
Spectral Bandwidth	8 steps in ultraviolet/visible region 0.1, 0.2, 0.5, 1, 2, 3, 5, 8 nm 10 steps in near-infrared region 0.2, 0.5, 1, 2, 3, 5, 8, 12, 20, 32 nm
Resolution	0.1 nm
Sampling Pitch	0.01 - 5 nm
Wavelength accuracy	UV/VIS region: ± 0.2 nm NIR region: ± 0.8 nm
Wavelength repeatability	UV/VIS region: less than ± 0.08 nm NIR region: less than ± 0.32 nm
Stray light	< 0.00008% (220 nm, NaI) < 0.00005% (340 nm, NaNO ₂) < 0.0005% (1420 nm, H ₂ O) < 0.005% (2365 nm, CHCl ₃)
Photometric range	-6 to 6 Abs
Noise	0.00005 Abs or less (500 nm), 0.00008 Abs or less (900 nm), 0.00003 Abs or less (1500 nm) Slit width 2nm, RMS value at 1 sec. response
Photometric System	Double beam
Dimensions	1020(W) x 660(D) x 275(H) mm

Table 2: Specifications of Shimadzu UV-3600 [14]

Wavelength Range: 185 nm to 3300 nm. This range allows for the analysis of a wide variety of samples, including those absorbing in the UV and visible regions.

Spectral Bandwidth: 8 steps in ultraviolet /visible region 0.1,0.2,0.5,1,2,3,5,8 nm and 10 steps are near-infrared region 0.2,0.5,1,2,3,5,8, 12,10,20,32 nm. The bandwidth defines the resolution of the spectrophotometer and can be selected based on the needs of the analysis.

Resolution: 0.1 nm, depending on the model and the selectable bandwidth. Higher resolution is necessary for distinguishing closely spaced spectral lines

Sampling Pitch: 0.01- 5 nm, sampling pitch refers to the interval at which data points are collected during a scan.

Wavelength Accuracy: ± 0.2 nm for UV/Visible region and ± 0.8 nm for NIR region. Wavelength accuracy ensures that the spectrophotometer measures the correct wavelength

Wavelength Repeatability: less than ± 0.8 nm for UV/Visible region and less than ± 0.32 nm for NIR region. This specification indicates the instrument's ability to reproduce the same wavelength in repeated measurements

Stray Light: Stray light can interfere with accurate absorbance measurements, so lower values indicate better performance.

Photometric Range: -6 to 6 Abs (Absorbance). The photometric range indicates the range of absorbance values the spectrophotometer can accurately measure

Noise: Less than 0.00005 Abs (500 nm) (RMS). Lower noise levels indicate higher sensitivity and stability of measurements.

Photometric System: Double beam optics. Double beam systems compare the sample and reference beams to improve accuracy and stability.

Dimensions: Around 1020 mm (W) $\times$ 660 mm (D) $\times$ 275 mm (H). These dimensions provide an idea of the instrument's physical size, which is important for lab space planning.

2.3 The calibration standards for spectrophotometers ASTM E925-09 (2022):

The ASTM E925-09 standard provides guidelines for the calibration of spectrophotometers used in measuring the absorbance of light. Key conclusions from this standard include:

Purpose of Calibration: The primary objective is to ensure that spectrophotometers produce accurate and consistent absorbance readings, which is critical for reliable quantitative analysis in various fields, including chemistry, biology, and materials science.

Reference Materials: The standard specifies the use of certain reference materials and solutions with known absorbance values for calibration. These reference materials help verify the accuracy and linearity of the spectrophotometer's response.

Calibration Procedures: It details specific procedures for calibrating the instrument. This includes steps for preparing calibration standards, conducting measurements, and comparing results against known values.

Wavelength Accuracy: Ensuring wavelength accuracy is crucial, as spectrophotometric measurements are highly sensitive to wavelength. The standard outlines methods to check and adjust the wavelength setting of the spectrophotometer.

Baseline Stability and Noise: The standard emphasizes the importance of baseline stability and minimizing noise. Calibration involves checking the instrument's baseline drift and noise levels to ensure they are within acceptable limits.

Performance Verification: Regular performance verification is recommended. This involves periodic checks and recalibration to maintain the spectrophotometer's accuracy over time.

Documentation and Traceability: Proper documentation of calibration procedures and results is essential for traceability and quality control. This includes maintaining records of calibration dates, standards used, and any adjustments made to the instrument.[15]

2.4 General steps of spectrophotometer calibration:

Knowing the general standards for spectrophotometers is important to ensure the device confidence. Where calibrations cannot be strictly made in SI units, the laboratory shall provide confidence in measurements by establishing traceability to appropriate measurement standards such as:

- The use of certified materials (CRMs) provided by a competent supplier to give a reliable physical or chemical characterisation of a material.
- The use of specified methods and/or consensus standards that are clearly described and agreed by all parties concerned” [UV/Vis Spectrophotometer Calibration Procedures, Published by: International Accreditation New Zealand 2005] [16]

The following measurements for spectrophotometer calibration are considered to be critical (see Figure 14):

- Wavelength calibration
- Absorbance (photometric) calibration.
- Liquid sample holder absorption.
- Stray light control.

The following are calibration parameters of UV visible spectrophotometer and their stringent limits are adopted from pharmacopeia for acceptance criteria. For instrument operation, follow the Standard Operating Procedure (SOP) and operating laboratory guidelines [17].

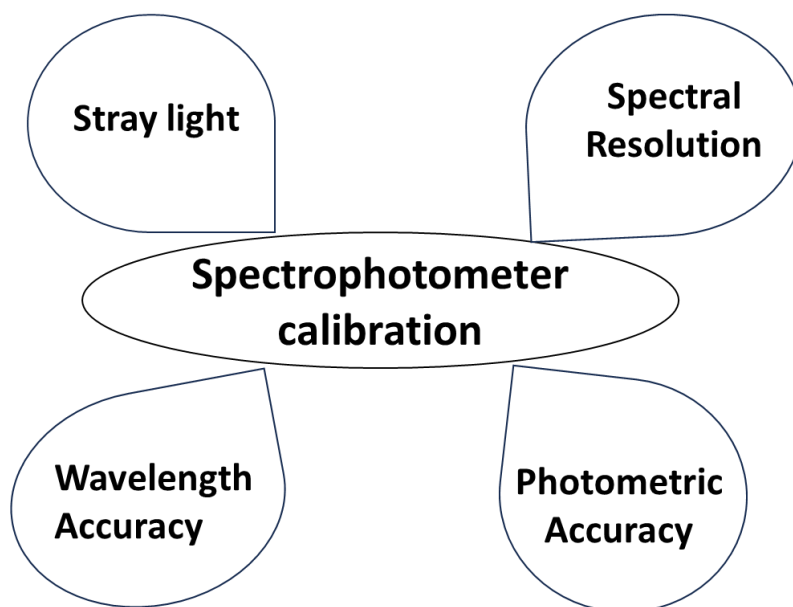


Figure 14: Main spectrophotometer calibration steps

2.4.1 Wavelength Accuracy:

There are several standards suitable for wavelength calibration. Checking of the wavelength accuracy is described as follows:

“Verify the wavelength scale using the absorption maxima of holmium perchlorate solution R. The permitted tolerance is ± 1 nm for the ultraviolet range and ± 3 nm for the visible range”.

European Pharmacopeia specifies four wavelengths for holmium perchlorate solution filters: 241.15 nm, 287.15 nm, 361.5 nm and 536.3 nm. The spectrum contains some additional peaks

which can also be used for checking the wavelength in principle.

Holmium oxide dissolved in perchloric acid is highly suitable for checking the wavelength accuracy of spectrophotometers both in the UV and the visible range. It shows a spectrum with a multitude of highly defined peaks. The heights and positions of these peaks are dependent on and will vary with the chosen slit width of the measuring instrument (see the Figure 14).

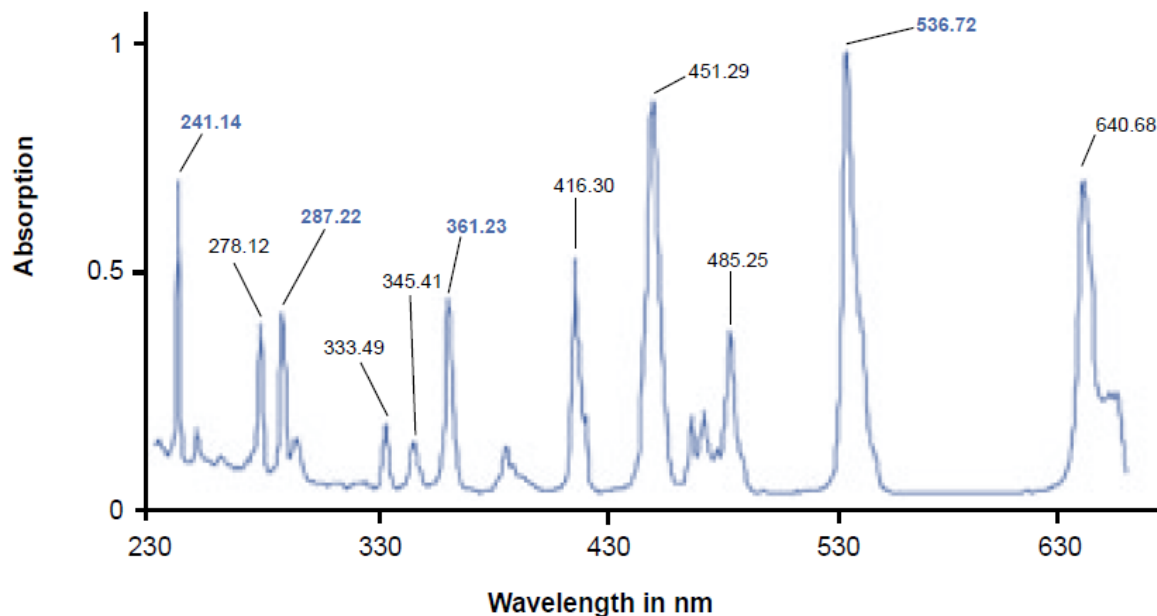


Figure 14: Typical absorption spectrum of holmium oxide dissolved in perchloric acid, recorded with a slit width of 1 nm from [Calibration standards catalogue]

2.4.2 Photometric Calibration (control of absorbance)

Checking of the photometric accuracy (absorbance) is described as follows:

“Check the absorbance using suitable filters or a solution of potassium dichromate R at the wavelength indicated in the Figure 15 which gives for each wavelength the exact values and the permitted limits of the specific absorbance. The tolerance for the absorbance is ± 0.01 . For the control of absorbance, use solutions of potassium dichromate R in sulphuric acid.” Potassium dichromate gives a spectral scan containing characteristic peak maxima at 257 nm and 350 nm and minima at 235 nm and 313 nm. The corresponding absorbance values of the filter are determined and are specified in the certificate (see Figure 15)

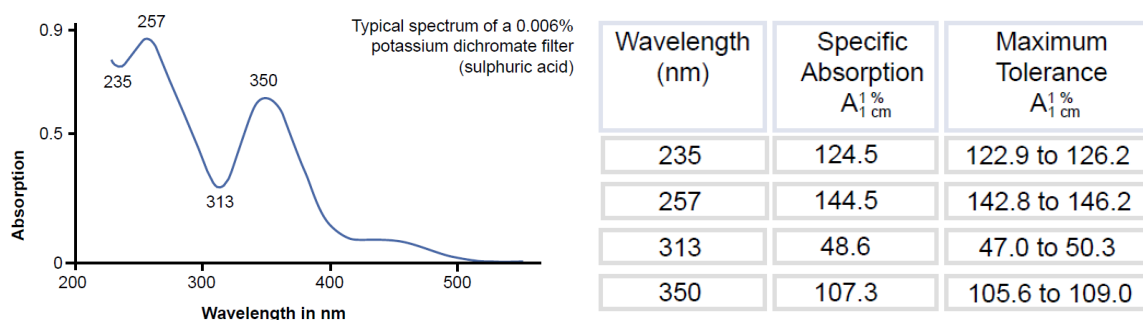


Figure 15: Spectrum of a 0.006% potassium dichromate solution filter and the corresponding wavelength, specific absorbance in %

2.4.3 Stray Light Correction

Stray light (false light) is light being detected but does not belong to the bandwidth of the chosen measuring wavelength. The effect of stray light is caused by light scattering, diffraction or malfunction of the instrument. Stray light causes a decrease in the measurable absorbance range and reduces the linear relationship between concentration and absorbance of the instrument. To measure stray light, filters are needed which would ideally absorb all light of the wavelength at which the measurement is to be performed and transmit higher wavelengths (so called cut-off filters). Stray light may be detected at a given wavelength with suitable filters or solutions: for example, the absorbance of a 12 g/l solution of potassium chloride R in a 1 cm cell increases steeply between 220 nm and 200 nm and is greater than 2.0 at 198 nm when compared with water as compensation liquid. Suitable certified reference materials may also be used.

2.4.4 Spectral resolution

Spectral resolution is a measure of the ability of an instrument to differentiate between two adjacent wavelengths. Two peaks usually are considered resolved if the minimum of the absorption between the two peaks is lower than 80% of the peak maxima. Resolution of a

modern spectrophotometer is closely related to its slit width. The smaller the slit width and associated instrumental spectral bandwidth, the higher is the spectral resolution.

For wavelength and absorbance measurements the dispersive power of the monochromator has to be considered, too. At the same time a small spectral bandwidth leads to a reduction of energy throughput and so the signal-to-noise ratio will fall.

When measuring in a high-resolution spectrophotometer, it is recommended that the spectral bandwidth should not exceed 10% of the natural bandwidth of the peak that is to be analysed. Regularly checks of the resolution power of an instrument assure that, for example adjacent peaks will be fully resolved and not blended into a combined peak with the associated wavelength. Additionally, absorbance errors will be avoided.

To check the resolution power of a spectrophotometer, a filter solution of Toluene in hexane is used as a standard. For this solution, the ratio of the peak maximum at λ_{max} (269 nm) to the absorbance of the minimum at λ_{min} (266 nm) is calculated (see Figure 16). This gives a measure of the spectral bandwidth of the instrument.

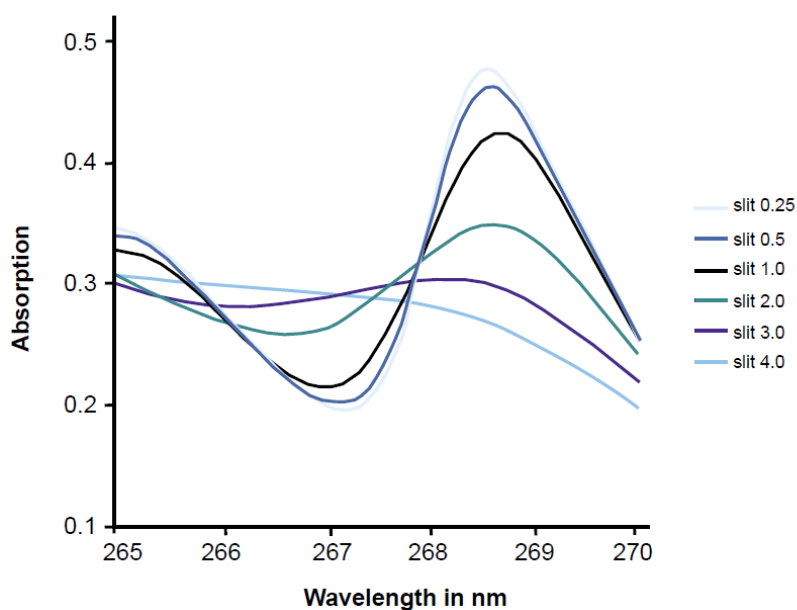


Figure 16: Typical spectra of Toluene at different slits widths

2.4.5 Baseline Correction

Baseline corrections in spectroscopy are necessary to remove background noise and ensure accurate interpretation of spectral data. This baseline represents the signal level when no light is absorbed by the sample. Ideally, this baseline should be a straight line at zero absorbance. However, in reality, various factors such as instrument noise, stray light, and sample scattering can cause the baseline to deviate from zero. This deviation is known as baseline drift or baseline noise. If not corrected, it can distort the spectral data and lead to inaccurate results.

Calibration of the spectrophotometer is required for accurate measurements of the baseline or starting absorbance. When assessing samples, this adjustment adjusts for any changes in the instrument's response.[18]

2.4.6 Regular Maintenance Checks

Calibration is a continuous process that includes maintenance inspections on a regular basis. This entails comparing the instrument's performance to known standards and ensuring that any discrepancies are identified and remedied as soon as possible.[18]

2.5 Coloured solution for calibration:

Calibrating a UV-visible spectrophotometer to ensure accurate and reliable measurements often involves using specific solutions and filters that are known for their absorbance characteristics at predetermined wavelengths. As examples of solutions and filters used for calibration:

Using references liquid solutions for spectrophotometer is an efficient way to ensure and check its calibration. For example, Adeeyinwo et al. mentioned a normal calibration method which was adopted and Ringbom-Ayre's plot was used to confirm the precision using a known sample such as Potassium permanganate (KMnO_4). The absorbance was measured for each solution at 480, 526 and 580nm using the spectrophotometer. The Spectrum of KMnO_4 was done to determine the wavelength of maximum absorption at the different concentration

by plotting the data for absorbance against wavelength [19]. Other reference solutions can be used for checking the spectrophotometric calibration, most of them are enumerated in the next sections.

2.5.1 Holmium Oxide Solution:

Colour: yellow

Use: Holmium oxide in perchloric acid solution is standard for calibrating the wavelength accuracy of UV- visible spectrophotometers. It has several well- defined peaks throughout the UV and visible spectrum (241 nm ,287 nm, 361 nm ,536 nm) [20]



Figure 17: Holmium oxide Powder

2.4.2 Potassium Dichromate Solution:

Colour: orange

Use: this solution is good for validating the absorbance accuracy. Potassium dichromate solutions are used in the visible range at specific concentrations to check absorbance at 235 nm, 257nm, 313nm and 350 nm

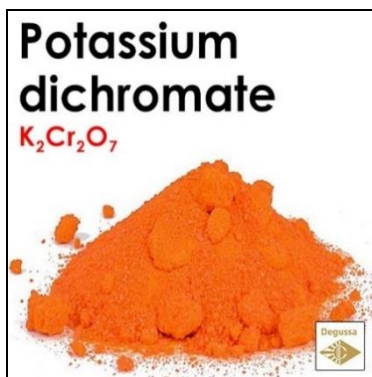


Figure 18: Potassium dichromate Powder

2.5.3 Nicotinic Acid Solution:

Colour: colourless

Use: used for UV range calibration, nicotinic acid has a peak at 261 nm although colorless, it is important for the UV calibration.



Figure 19: Nicotinic Acid

2.5.4 Cobalt chloride solution:

Colour: pink to deep blue

Use: Cobalt chloride can be used in a solution with cobalt (II) chloride hexahydrate in water. It provides a check for the visible range with absorbance maxima at about 510 nm to 515 nm when mixed with hydrochloric acid ($[Co(H_2O)_6]^{2+}$ complex)



Figure 20: Cobalt chloride solution

2.4.5 Permanganate of Potash (KMnO_4):

Colour: pink to purple

Use: KMnO_4 solution exhibits distinct absorption peaks at specific wavelengths in the UV-visible range. These peaks can be used as reference points for wavelength calibration.



Figure 21: Permanganate of potash KMnO_4

2.6 Filters:

Solid glass filters are also used for spectrophotometric calibration in visible UV part of spectrum. Some examples are given hereafter:

2.6.3 Didymium Glass Filters:

Colour: varies

Use: while not a solution, didymium glass filters are used for checking the wavelength accuracy. They exhibit sharp absorption bands suitable for calibration in the visible range (around 329 nm and 472 nm) [20]

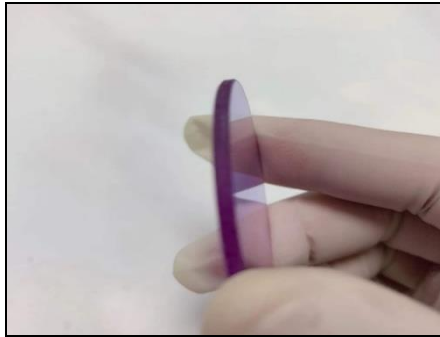


Figure 22: Purple light didymium glass

2.6.4 Neutral Density Filters:

Colour: gray (transparent)

Use: these filters are used for checking the photometric accuracy across a range of wavelengths. They provide a uniform attenuation of the light beam across the visible spectrum.



Figure 23: Neutral Density glass

2.7 Calibration check experiment on Shimadzu UV-3600:

During my master training, I had the opportunity to spend time in the Laboratory of Thin Films and Materials for Electronics of Oran 1 university. There, I was introduced to the basics to use different lab instruments, some of them were dedicated to solution preparations as precision scales, dyes in powder and others were dedicated to optical characterization as Shimadzu UV-3600 spectrophotometer. The main objective of my training was to check if this spectrophotometer is well calibrated to reproduce reliable and accurate absorbance spectra. For that purpose, an experimental protocol detailed in the next section was elaborated to characterize the spectral response of different coloured dyes with already known absorbance spectra in the visible part.

2.7.1 Coloured solutions used for experiment:

1.Blue methylene:

Blue methylene is a synthetic dye with a deep blue colour, it is used as a coloured solution for spectrophotometer calibration:

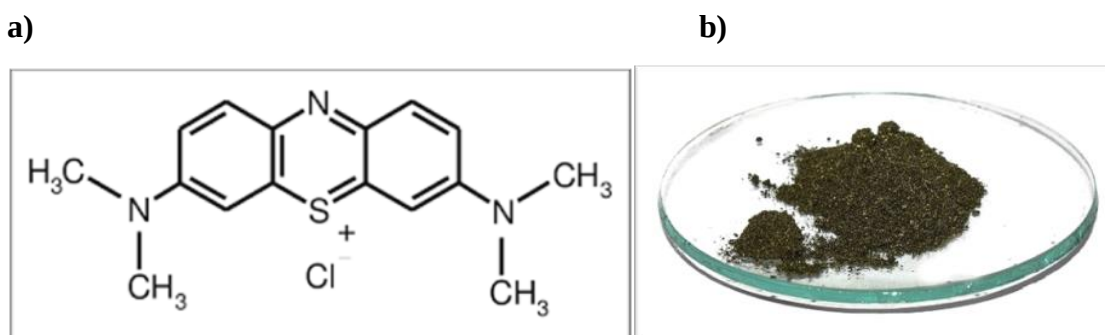


Figure 24 : Methylene blue (a). Chemical formula of methylene blue (b). Powder of methylene blue.

Chemical Structure: Methylene blue has the chemical formula $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$ and belongs to the thiazine class of dyes. Its molecular structure consists of a tricyclic aromatic ring system with a nitrogen atom as a bridge between two benzene rings. Shown above on Figure 21

Preparation: Prepare a stock solution of blue methylene by accurately weighing a known amount of the powder and dissolving it in a suitable solvent. Common solvents include distilled water or alcohol. Ensure thorough mixing to achieve a homogeneous solution.

Dilution Series: Create a series of dilutions from the stock solution to cover a range of concentrations. Dilutions can be made by adding aliquots of the stock solution to volumetric flasks and diluting to the mark with solvent. Label each flask with the corresponding concentration.

Measurement: Measure the absorbance of each blue methylene solution at specific wavelengths using the spectrophotometer. Choose wavelengths where blue methylene absorbs light effectively, typically in the visible range, such as around **665 nm** as shown on Figure 25

Calibration Curve: Plot a calibration curve using the absorbance values obtained at each concentration. The curve should demonstrate a linear relationship between concentration and absorbance, allowing for the determination of unknown concentrations based on absorbance measurements.

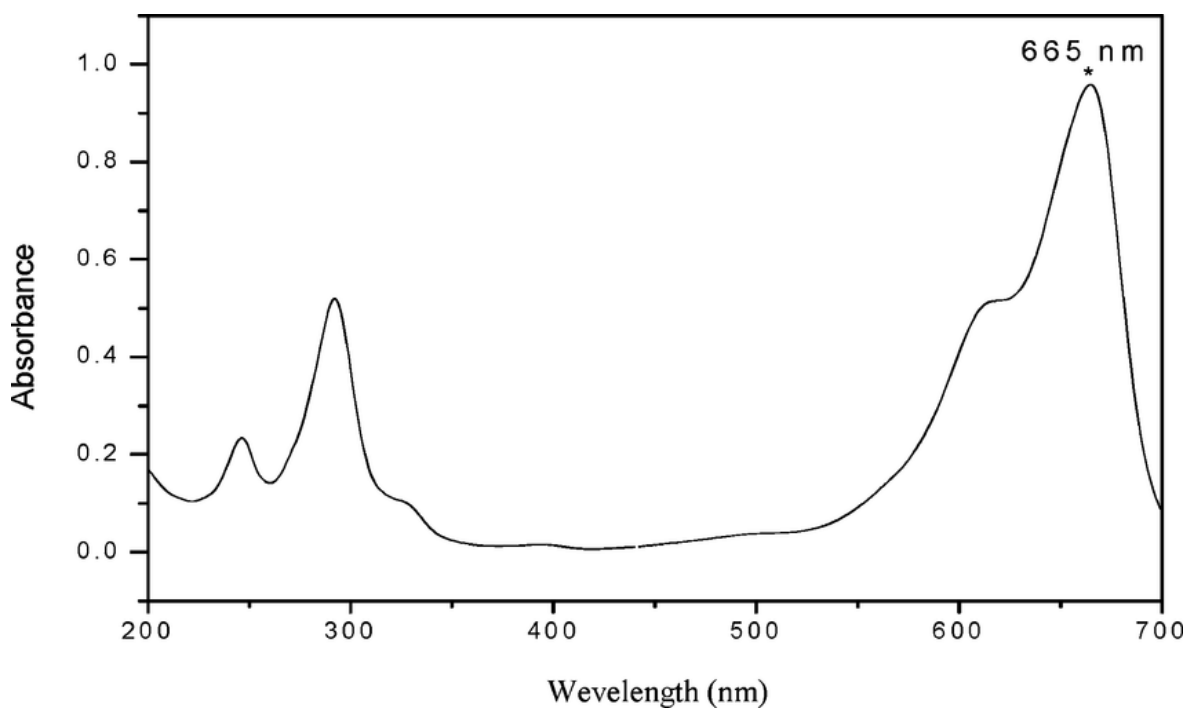


Figure 25: Absorbance spectrum of methylene blue [21]

This spectrum was taken with a UV visible spectrophotometer using a 1cm quartz cuvette filled with 10 μ M solution of methylene blue in water

2. Tartrazine:

Tartrazine, also known as E102 or Yellow 5; is a synthetic yellow dye that can be used as a colourful solution to calibrate a spectrophotometer. It has a bright yellow colour and is often used as a reference standard for wavelength calibration.

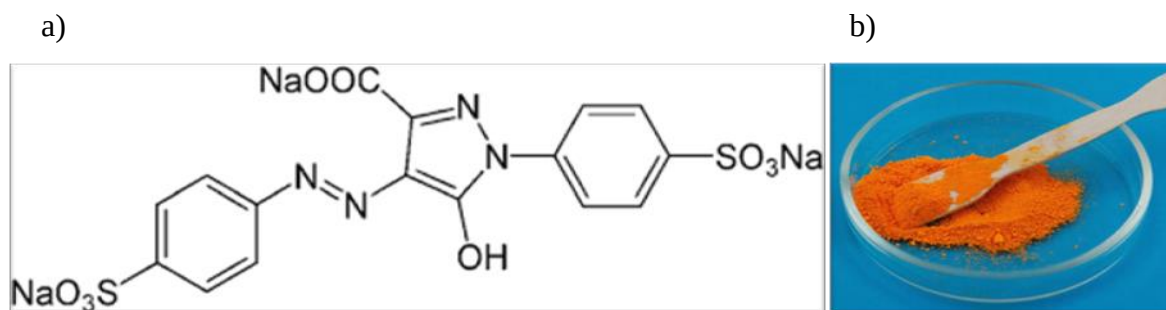


Figure 26: Tartrazine (a). Chemical formula of tartrazine (b) powder of tartrazine

Chemical Composition: Tartrazine is a synthetic organic compound classified as an azo dye. Its chemical name is sodium 5-(4-sulfonatophenylazo)-4,5-dihydro-1H-pyrazole-3-carboxylate. The molecular formula is $\text{C}_{16}\text{H}_9\text{N}_4\text{Na}_3\text{O}_9\text{S}_2$ shown above on Figure23.

Preparation: Prepare a stock solution of tartrazine by accurately weighing a known amount of the compound and dissolving it in a suitable solvent. The solvent choice depends on the solubility of tartrazine and compatibility with the spectrophotometer's operating range. Common solvents include water or alcohol.

Dilution Series: Create a series of dilutions from the stock solution to cover a range of concentrations. Dilutions can be made by adding aliquots of the stock solution to volumetric flasks and diluting to the mark with solvent. Label each flask with the corresponding concentration.

Measurement: Measure the absorbance of each solution at specific wavelengths using the spectrophotometer. Choose wavelengths where tartrazine absorbs light effectively, typically in the visible range, such as around **427 nm** as shown on Figure 27

Calibration Curve: Plot a calibration curve using the absorbance values obtained at each concentration. The curve should demonstrate a linear relationship between concentration and absorbance, allowing for the determination of unknown concentrations based on absorbance measurements.

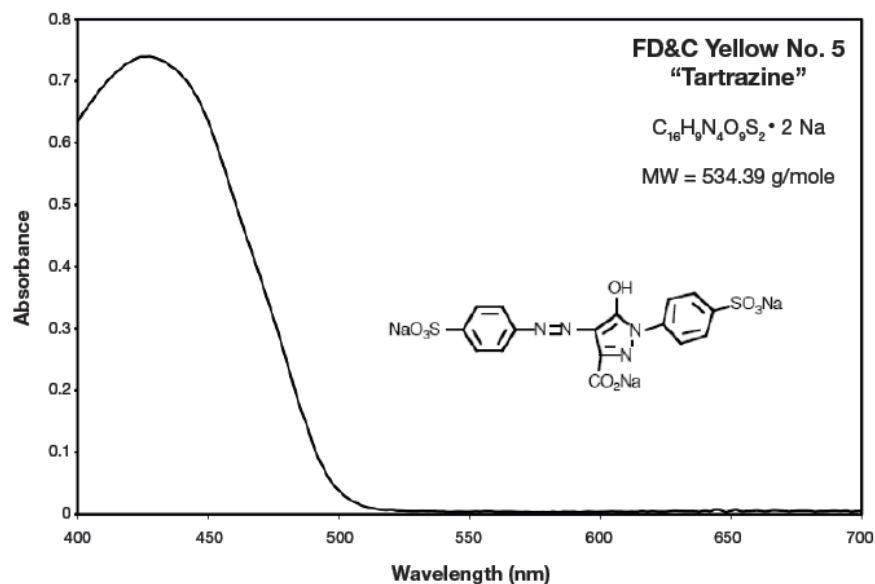


Figure 27: Absorbance spectrum of tartrazine[22]

3. Red dye:

Additif E122-carmoisine, Azorubine, food red 3, brilliant carmoisin O, azorubine S and acid red 14 are the names of the red colouring food (sin133) comes in the form of red to brown powder or granules [16]:

Chemical formula: $C_{20}H_{12}N_2Na_2O_7S_2$

Molar mass: 502.44

Melting point: >300 °C (572 °F)

Solubility in water: Soluble (120g/L)

Red dyes can belong to different chemical classes, including azo dyes, anthraquinone dyes, and others. For example:

E129 = Red 40 (Allura Red AC)

E120 = acid carminic (cochenille)

E124 = ponceau 4R (Acid Red 18)

E163 = anthocyanes

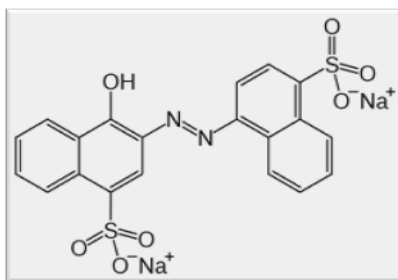


Figure 28: Chemical formula of red dye E122

The measurement of absorbance of each red dye solution at specific wavelengths using the spectrophotometer. Choosing wavelengths where red dye absorbs light effectively, typically in the visible range, such as around **600-620 nm** as shown on Figure 29

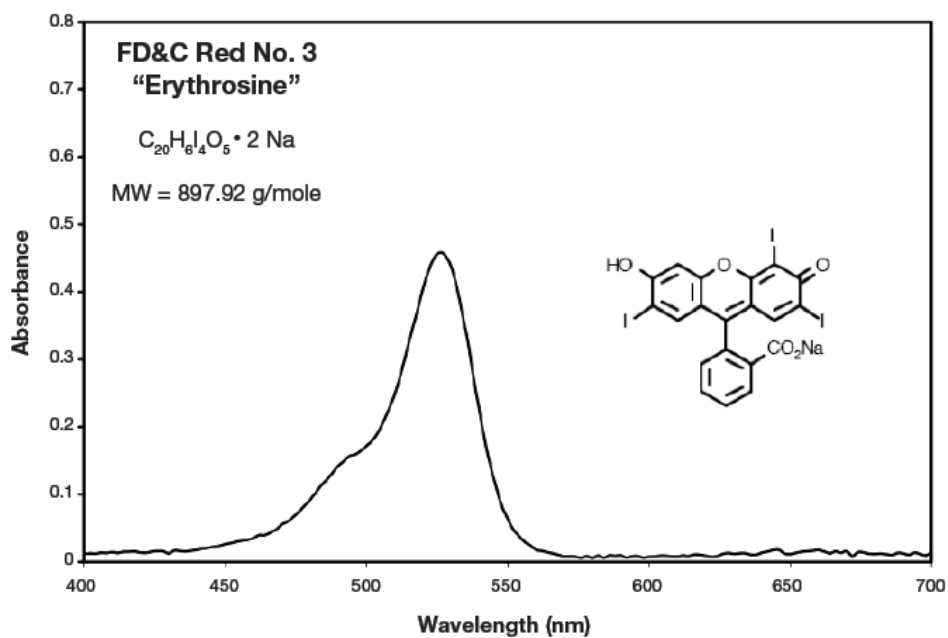


Figure 29: Absorbance spectrum of red dye[22]

4. Blue dye:

Indigotine, 5,5'-indigodisulfonic acid sodium salt, Brilliant Indigo, 4 G, C.I. Acid Blue 74, C.I. 73015, CI Food Blue 1, FD&C Blue 2, Sicovit Indigotin 85, E132 and indigotin disulfate sodium are the names of the blue colouring food (SIN132) [17]:

Chemical formula: $C_{16}H_8N_2Na_2O_8S_2$

Molar mass: 466.36 g/mol

Appearance: purple solid

Melting point: >300 °C (572 °F)

Solubility in water: 10 g/L (25 °C (77 °F))

Blue dyes also belong to various chemical classes, including azo dyes, triarylmethane dyes, and anthraquinone dyes. For examples:

E133 = Brilliant Blue FCF (Blue 1)

E132 = Carmin d'indigo (blue 2)

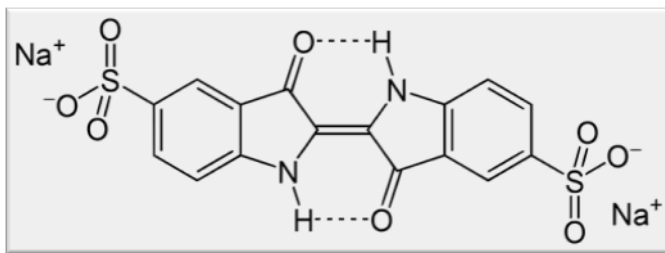


Figure 30: Chemical formula of blue dye (sin132)

The measurement of absorbance of each blue dye solution at specific wavelengths using the spectrophotometer. Choosing wavelengths where blue dye absorbs light effectively, typically in the visible range, such as around **600-620 nm** as shown on Figure 31.

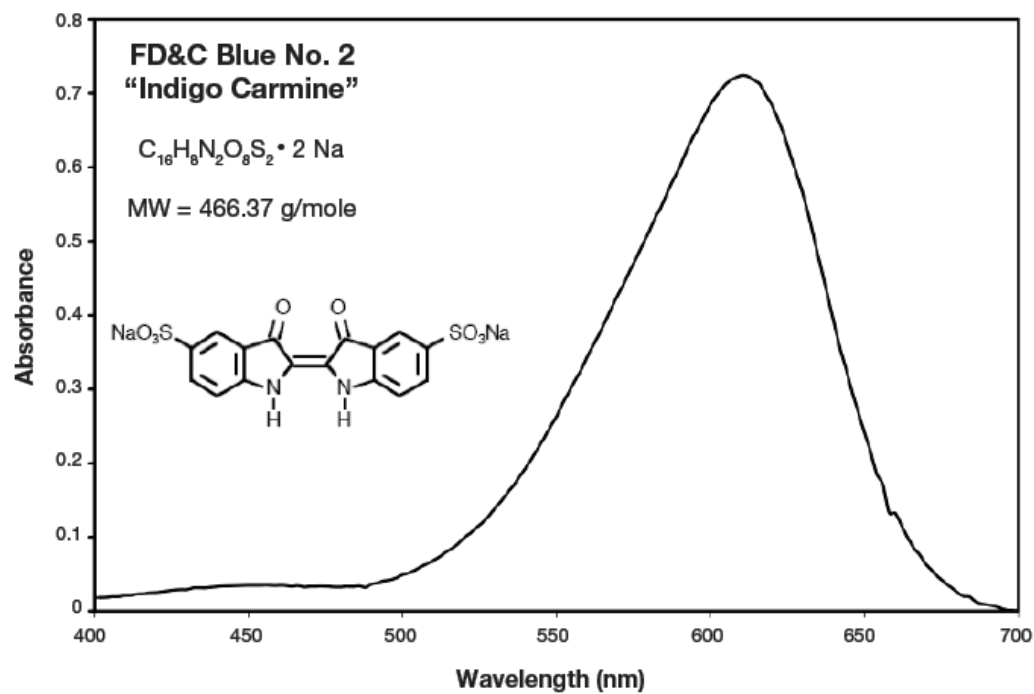


Figure 31: Absorbance spectrum of blue dye[22]

5. Others food dyes:

Dyes food is hugely used in food industry, they are classified and labelled with specific codes as shown in Table 3

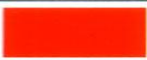
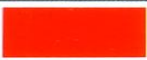
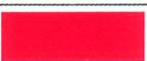
PRODUCT LIST OF LAKE COLOURS				
PRODUCT	COLOUR	COLOUR INDEX NO.	E. E. C. NO.	OTHER NAME
Lake Tartrazine		19140	E 102	F.D.&C. Yellow 5 Food Yellow 4
Lake Allura Red		16035	E 129	F.D.&C. Red 40 Food Red 17
Lake Brilliant Blue FCF		42090	E 133	F.D.&C. Blue 1 Food Blue 2
Lake Ponceau 4R		16255	E 124	Food Red 7
Lake Erythrosine		45430	E 127	F.D.&C. Red 3 Food Red 14
Lake Indigo Carmine		73015	E 132	F.D.&C. Blue 2 Food Blue 1
Lake Amaranth		16185	E 123	F.D.&C. Red 2 Food Red 9
Lake Quinoline Yellow		47005	E 104	D.&C. Yellow 10 Food Yellow 13
Lake Carmoisine		14720	E 122	Food Red 3 Azorubine
Lake Sunset Yellow FCF		15985	E 110	F.D.&C. Yellow 6 Food Yellow 3
Blended Lake		All Blended Lake Colours Available		

Table 3: Classification and codes of food dyes ref [22]

2.7.2 Molar Absorption Coefficients of Commonly used FoodDyes:

In Table 4 are given wavelength and absorption coefficient for commonly used food dyes and their symbols, Data were taken from ref [23]

Dye	E-No.	$\lambda_{\max}$ [nm]	ϵ at $\lambda_{\max}$ [$\text{l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$]
Allura Red	E129	502	22261
Amaranth	E123	522	21869
Apocarotenal	E160e	464 ^a	316746 ^a
Azorubine	E122	516	20097
β -Carotene	E160a	464	896
Betanin	E162	531	171
Brilliant Blue	E133	630	100661
Brown HT	E155	463	18367
Capsanthin	E160c	496	1542
Carmin	E120	518	3785
Chlorophyll	E140	424	20010
Curcumin	E100	430	50765
Erythrosine	E127	527	79099
Green S	E142	635	86840
Indigo Carmine	E132	610	19445
Lutein	E160b	452	546
Patent Blue V	E131	638	101460
Ponceau 4R	E124	507	19892
Quinoline Yellow	E104	413	30604
Riboflavin	E101	445	9811
Sunset Yellow	E110	482	20165
Tartrazine	E102	428	22974

Table 4: Studied food colourings with recorded wavelength of maximum of absorbance and the calculated absorption coefficients ref [24]

2.7.3 Preparation protocol of coloured solutions:

In our study, 4 coloured solutions were used for spectrophotometric analysis: Methylene blue solution, the Tartrazine solution, and red and blue food colouring.

To prepare Methylene blue and Tartrazine solutions for the calibration check of Shimadzu UV-3600 spectrophotometer, the followed steps were:

1-Materials needed:

- Methylene blue powder (high purity)
- Tartrazine (yellow5) powder
- Solvent (typically distilled water or a suitable buffer solution)
- Analytical balance
- Volumetric flasks
- Graduated cylinder or pipettes
- Stirring rod or magnetic stirrer (optional)

2- Steps of preparation:

1. Start by weighing the appropriate amount of {methylene blue / tartrazine (yellow5)} powder using an analytical balance



Figure 32: Analytical balance for weighing powder

2. Dissolve the weighed powder in a suitable solvent, such as distilled water or a specific solvent recommended for UV-vis calibration (we dissolve in distilled water)
3. Mix the solution thoroughly until the powder is completely dissolved to finally get stock solutions of the required concentrations: 50mg/l for tartrazine and 50mg/l for blue methylene
4. Dilute the solutions to different concentrations: 40mg/l and 30mg/l for tartrazine and 40mg/l, 30mg/l, 20mg/l, 10mg/l and 5mg/l for blue methylene to obtain a gradation of yellow and blue colours.

5. Store them in a dry place not exposed to the sun (because they lose their chemical properties and their required concentration) in closed flasks. It is best to use them for two days to a week (their lifetime is short).
6. Transfer the solution to a clean and dry cuvette or sample cell

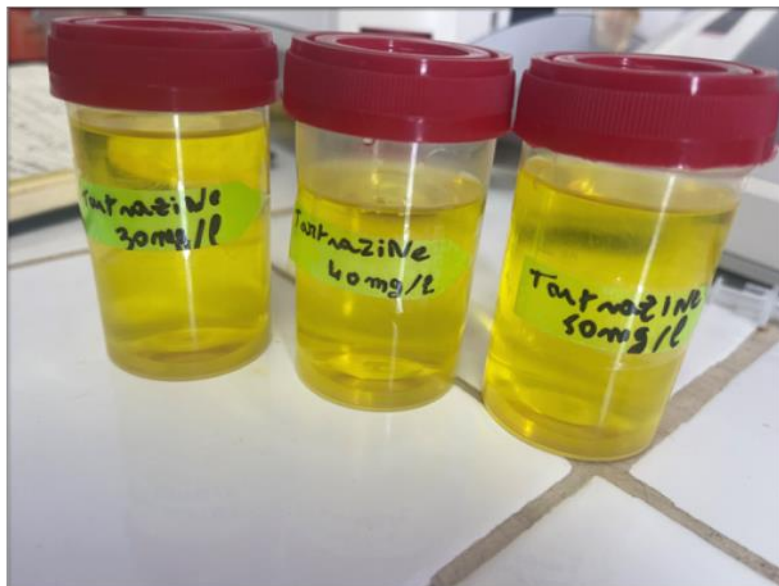


Figure 33: Tartrazine solution with different concentration



Figure 34: Methylene blue with different concentration

For red and blue dyes, we diluted the stock solution with distilled water (we prepare two concentration 10mg/l and 5mg/l for the two colourings). All the studied concentrations are summarized on Table 5

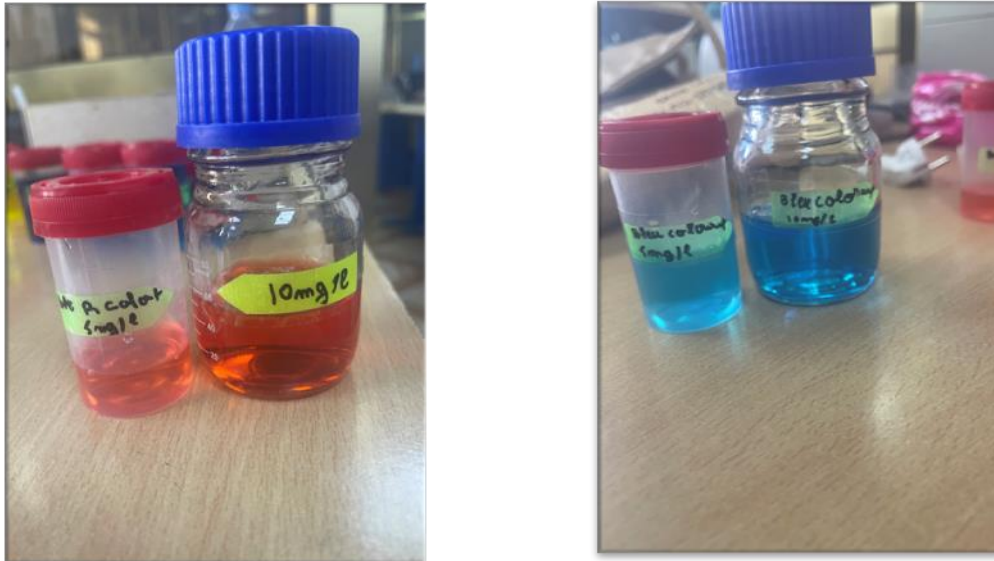


Figure 35: Red and blue dyes solutions

Dilution and mixing of solutions:

We made several dilutions with different concentration for each solution based on the principal chemical equation of dilution

$$C_0V_0 = C_1V_1 \quad (II.1)$$

Where:

C_0 : concentration of the stock solution.

V_0 : primary volume.

C_1 : concentration of diluted solution.

V_1 : volume of the final diluted solution.

Table 5: Dosage of the coloured solutions (experimented)

Tartrazine	Blue methylene	Red dye SIN122	Blue dye SIN132
C0 = 50mg/l	C0 = 50mg/l	C0 = 10mg/l	C0 = 10mg/l
C1= 40 mg/l	C1= 40 mg/l	C1 = 5mg/l	C1 = 5mg/l
C2 =30mg/l	C2 =30mg/l	/	/
/	C3 = 20mg/l	/	/
/	C4 = 10mg/l	/	/
/	C5 = 5mg/l	/	/

Also, we prepared mixed the solutions from two different dyes to get gradation of colours with known concentration and volume:

Tartrazine (5ml/5mg) + blue dye (5ml/40mg) give pistachio green

Tartrazine (5ml/40mg) + red dye (5ml/5mg) give light orange

Tartrazine (5ml/40mg) + blue methylene (4ml/30mg) give slightly dark green

Blue methylene (4ml/20mg) + red dye (5ml/5mg) give slightly dark purple

Blue methylene (4ml/5mg) + red dye (5ml/5mg) give purple less dark

Blue dye (4ml/5mg) + red dye (5ml/5mg) give purple



Figure 36: Gradation of colour of solutions obtained

2.8 Measurement protocol on Shimadzu UV-3600:

In this part, we present the calibration steps of Shimadzu uv-3600 and measurement of the dye solutions absorbance:

1. Turn on the Shimadzu UV-3600
2. Set the **baseline** before starting any operation

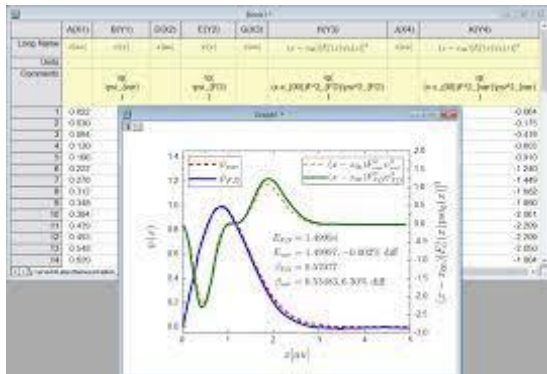
The baseline in a spectrophotometer, such as the Shimadzu UV-3600, refers to the reference level of absorbance or transmittance observed when measuring a blank solution. This baseline serves as a point of comparison for subsequent measurements of sample solutions.

In the Shimadzu UV-3600 spectrophotometer, the baseline is typically established by measuring the absorbance or transmittance of a blank solvent or reference solution within the desired wavelength range. The instrument's software allows users to set the baseline by selecting appropriate parameters and adjusting the baseline correction settings if necessary.

3. Start baseline setting before filling the reference sample with distilled water and wait

4. when the baseline adjustment is completed, we can open the small door of the spectrophotometer and fill the bowl with the coloured solution that we have prepared and place it correctly in its place and close the door and press the enter button
5. wait till finishes the analysis on all the wavelengths
6. we observe that we obtained peaks in certain wavelengths (corresponding to the absorbed colour)
7. when the operation is complete, press the ok button and saves the spectra obtained in a personal file as a data .txt
8. after getting my results as data.txt, draw them in **Originpro**:

OriginPro is a powerful software package widely used for data analysis and visualization in scientific and engineering research. It offers a comprehensive suite of tools for plotting, analyzing, and interpreting data from various sources, including experimental measurements, simulations, and statistical analyses.



2.9 Results and discussion:

To present the obtained spectra of Shimadzu uv-3600 spectrophotometer, we analyse typically the absorption peaks. These peaks indicate the wavelengths at which the sample absorbs light the most. By analysing these peaks, we can identify the compounds present in the sample and determine their concentrations. The shape of the spectrum can also provide information about the chemical structure of the compounds. It's important to compare the

obtained spectrum with reference spectra to make accurate identifications. The chromatic CEI is translated into a table (see Table 6) of wavelength domains with their corresponding observed and absorbed colour which it helps us to know the colours absorbed in each wavelength:

Wavelength Absorbed (nm)	Color Observed	Color Absorbed
400-435	Yellow- Green	Violet
435-480	Yellow	Blue
480-490	Orange	Green-Blue
490-500	Red	Blue-Green
500-560	Purple	Green
560-580	Violet	Yellow- Green
580-595	Blue	Yellow
595-605	Green-Blue	Orange
605-700	Blue-Green	Red

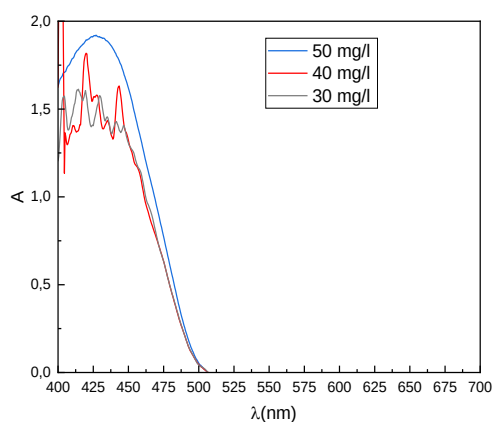
Table 6: The chromatic wavelength domains and the corresponding observed and absorbed colour [24]

On Figures 37-46 (all $A(\lambda)$ curves) are plotted our measured absorbance as function of the wavelength for all the prepared solutions.

For Tartrazine: the obtained absorbance spectra provide a net peak at 425 nm (in violet domain) for the highest concentration of 50 mg/l, this result confirms the yellow colour of tartrazine according to Table 6 Our result is in good agreement with other work [21] as shown in Figure 37. For the two other prepared solutions with concentrations of 40 mg/l and 30mg/l, we found a noise in the blue region. Therefore, another protocol with $C > 50\text{mg/l}$ for Tartrazine to get suitable results with the spectrophotometer.

For Methylene Blue: The absorbance spectrum shows a unique peak for the smallest concentration of 5 mg/l. Whereas, a satellite peak appears when the concentration increase. The maximum of absorbance for the main peak is $\lambda = 663$ nm, so a blue-green solution absorbs in red. For the satellite peak, the maximum is located around 611 nm. This spectrum is in accordance with the literature of ref [26] as shown in Figure 38.

a)



b)

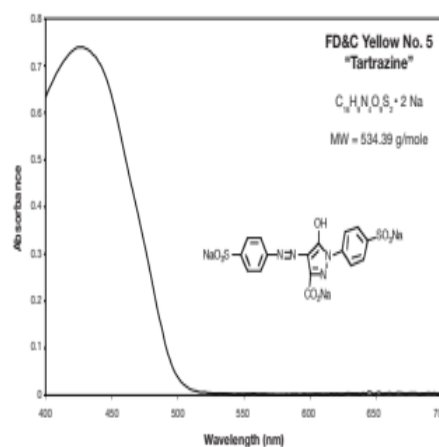
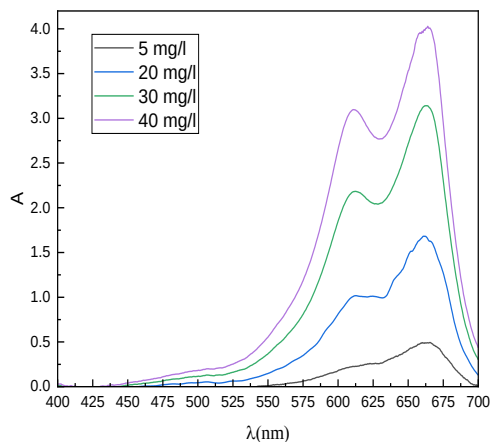


Figure 37: (a) Shimadzu uv-3600 absorbance spectrum of tartrazine. (b) literature spectrum of tartrazine ref [22] .

a)



b)

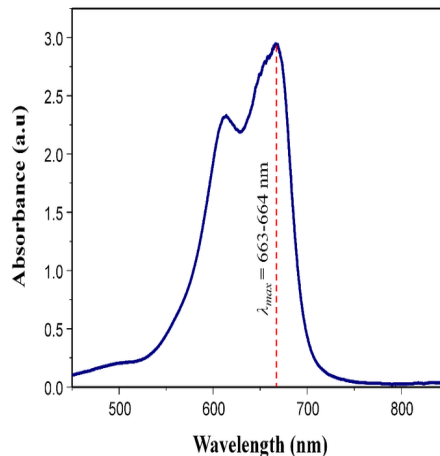


Figure 38: (a) Shimadzu uv-3600 absorbance spectrum of methylene blue. (b) literature spectrum of methylene blue ref [26].

For blue dye: Figure (a) shows the absorption of blue dye solution as a function of wavelength whereas at the concentration of 10 mg/l the absorption was 1.38 and at 5mg/l it was 0.72 in the same region at about 625 nm

-605-700 nm is the region of red colour, so a blue-green solution absorbs into red. This spectrum is in accordance with the literature of ref [21] as shown in Figure 39.

For red dye:

The absorbance spectrum shows a peak for the smallest concentration of 5 mg/l. Whereas, the biggest peak refers to the concentration of 10mg/l. The maximum of absorbance for the main peak is $\lambda = 500$ nm, so a red solution absorbs in blue-green. This spectrum is in accordance with the literature of ref [21] as shown in Figure 40.

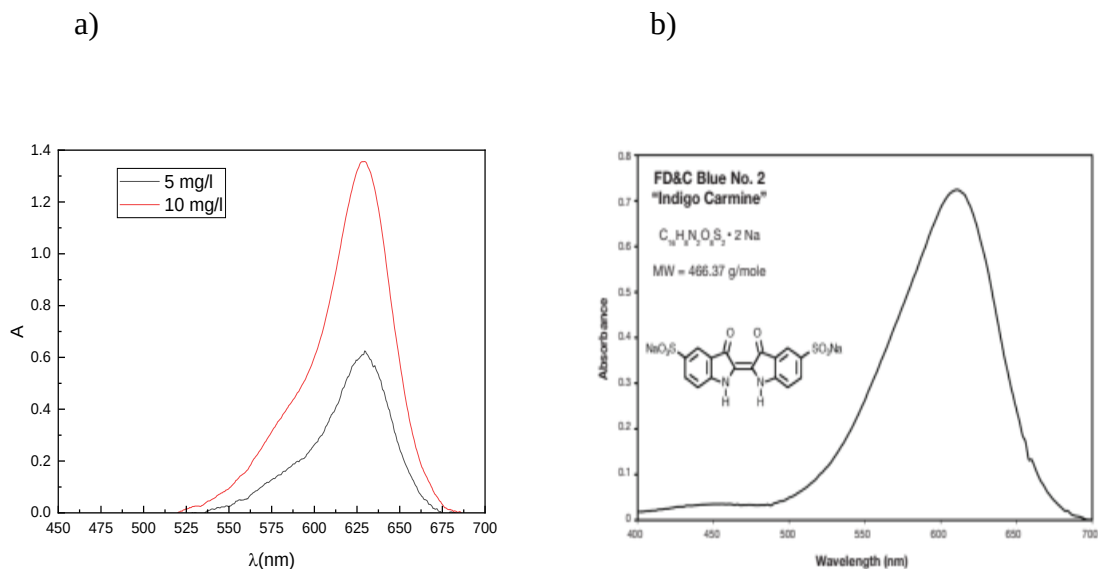


Figure39: Shimadzu uv-3600 absorbance spectrum of blue dye. (b) literature spectrum of blue dye ref [22].

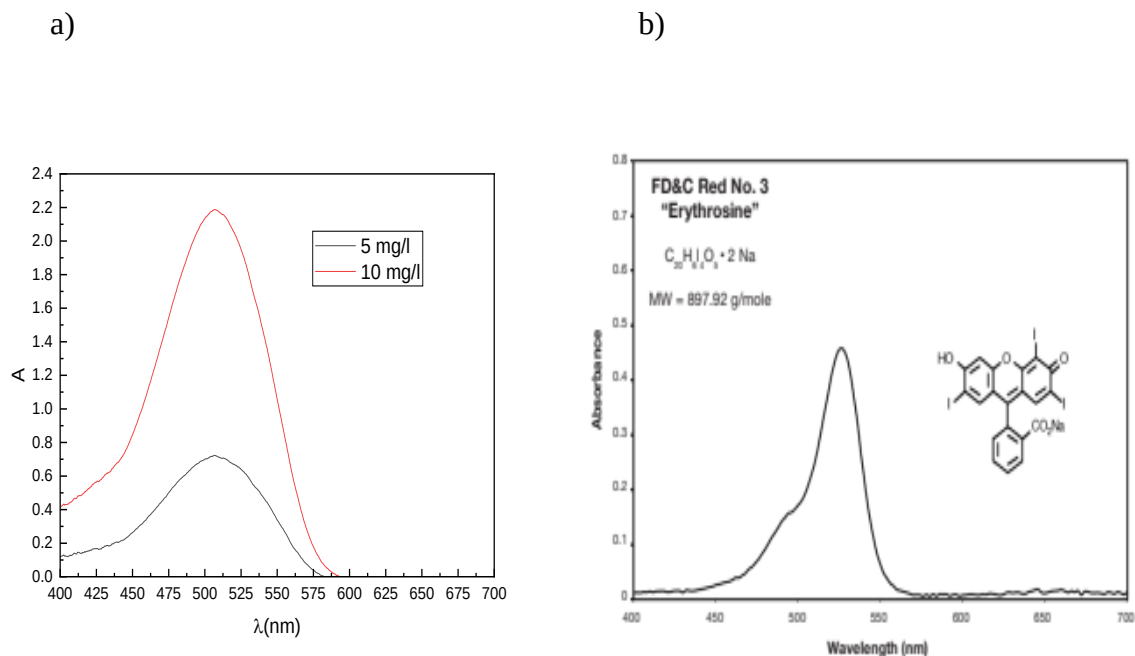


Figure 40: (a) Shimadzu uv-3600 absorbance spectrum of red dye. (b) literature spectrum of red dye [21].

Mixtures of solutions:

We mixed the solutions with known concentration and volume for getting new colourful solutions with gradation of colours:

Methylene Blue + Tartrazine:

Here, we mixed volume of 5ml of tartrazine with concentration of 40mg/l with 4ml of methylene blue with a concentration of 30 mg/l as a result we obtain a new solution with slightly dark green colour

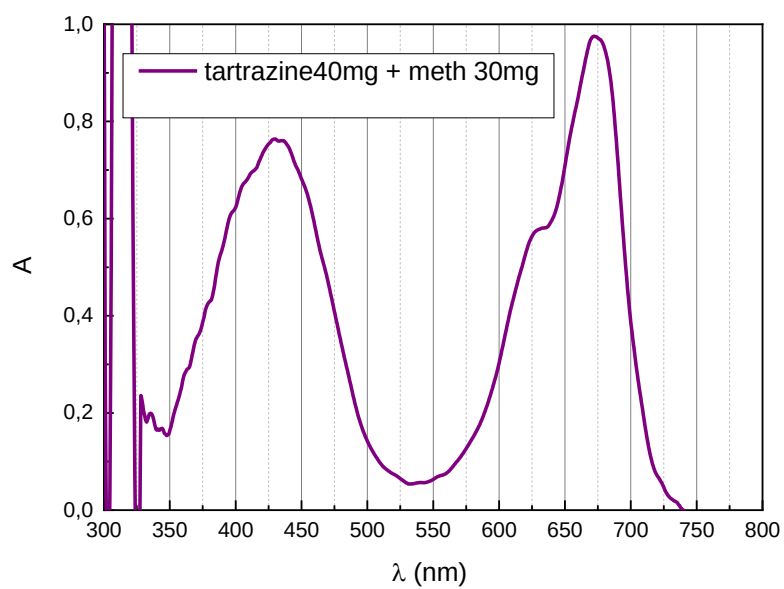


Figure 41: Absorbance spectrum of tartrazine 40mg/l + meth 30mg/l

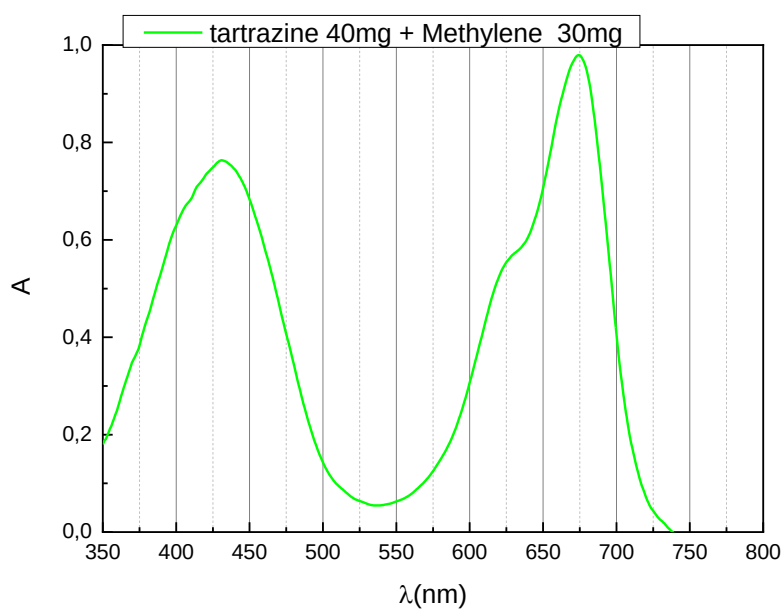


Figure 42: Absorbance spectrum of tartrazine 40mg/l + blue dye 30 mg/l

-The appearance of two peaks in two different regions, explained by the absorption of two colours which the solution contains

-These two peaks are due to the mixing of two solutions, the peak at 430 nm correspond to tartrazine solution and the one at 675 nm correspond to methylene blue this confirms that the solution was well absorbed in the ultraviolet region

Methylene Blue + Red dye:

we mixed volume of 4ml of methylene blue with concentration of 20mg/l with 5ml of red dye solution with a concentration of 5mg/l as a result we obtain a new solution with slightly dark purple

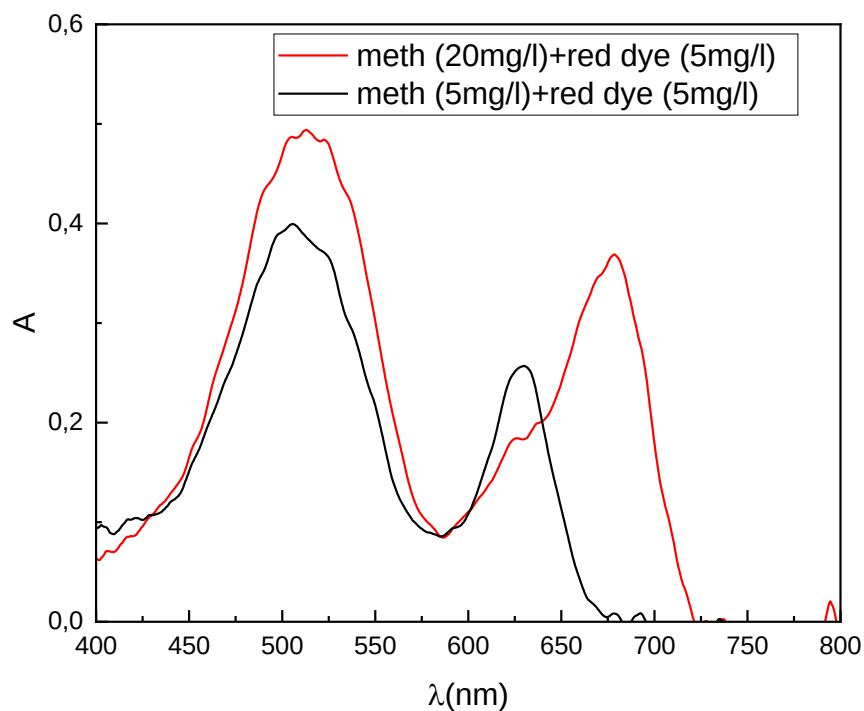


Figure 43: Absorbance Spectrum of the mixture of Methylene Blue + Red Dye

-the appearance of two peaks is due to the two colours in the mixed solution, the first peak is at 545nm and it correspond to the red dye solution and the other one is at 675nm correspond to the methylene blue solution

-we obtained the same peaks at the same regions however the absorption is decreased because the concentration of the solution decreased

Tartrazine + Blue dye:

we mixed volume of 5ml of tartrazine with concentration of 30mg/l with 5ml of blue dye with a concentration of 40 mg/l as a result we obtain a solution with pistachio green colour

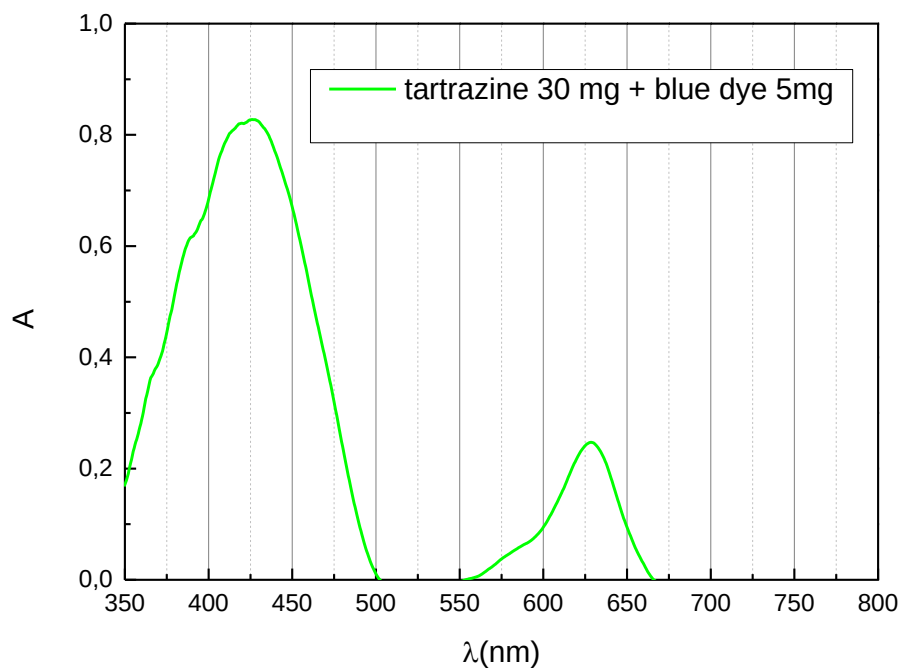


Figure 44: Absorbance spectrum of tartrazine 30mg/l + blue dye 5 mg/l

-we obtain also two peaks at two different region one at 450 nm it corresponds to yellow colour of tartrazine solution and the other one at 650 nm is due to blue colour of the blue dye solution

Tartrazine + Red dye:

we mixed volume of 5ml of tartrazine with concentration of 40 mg/l with 5ml of red dye with a concentration of 5mg/l as a result we obtain a solution with light orange colour

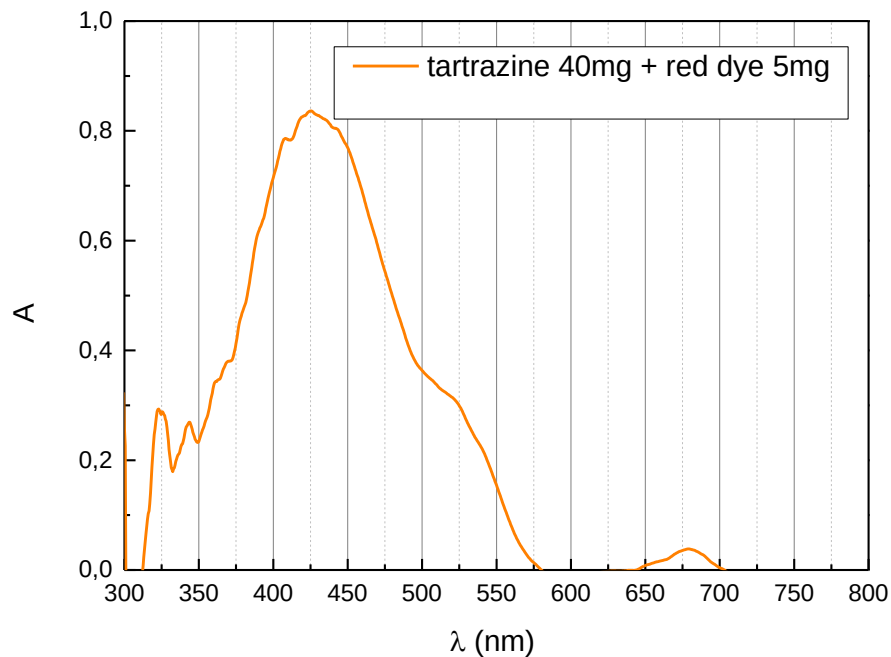


Figure 45: Absorbance spectrum of tartrazine 40mg/l + red dye 5 mg/l

-We observe the appearance of two peaks the big one at 450 nm it corresponds to tartrazine solution which the absorbance it about 0.85 because we have high concentration (40mg/l) and the small one corresponds to red dye

-We note that the difference between the two peaks is clear, and this is due to the huge difference between the two concentrations, where the second peak almost disappears

Blue + Red dye:

We mixed the two-food colouring of concentration of 5mg/l and we obtained a solution with violet colour;

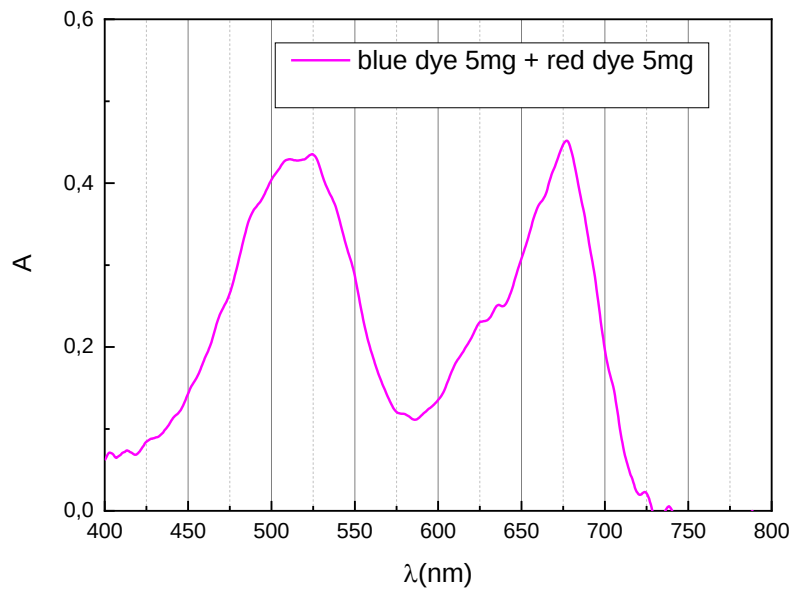


Figure 46: Absorbance spectrum of red dye 5 mg/l + blue dye 5 mg/l

-At 545 nm the appearance of the peak responsible for the blue colour, and at 675 nm for the red dye

-The absorbance was in maximum 0.5 due to the low concentration of the two solutions

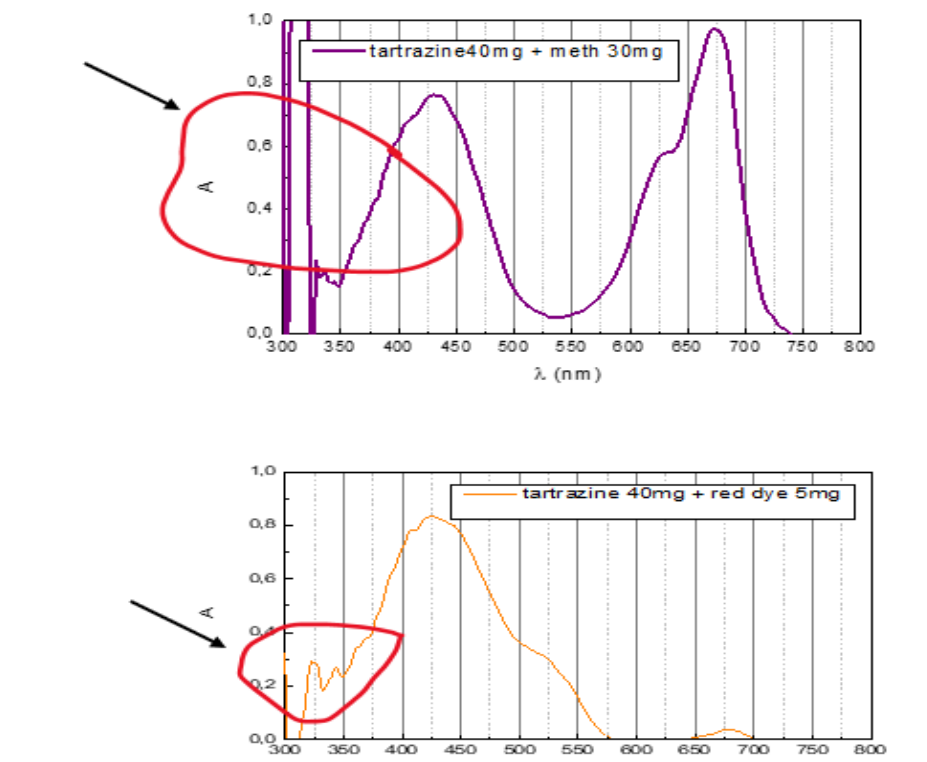


Figure 47: Noise in spectra of absorbance

These were the results obtained from spectrophotometer, but we also notice the appearance of perturbations on the peaks which it called by the noise, this is caused by:

Electrical Interference: Electrical interference from nearby electronic devices or poor grounding can introduce noise into the spectrophotometer readings.

Optical Interference: Dust, scratches, or improper alignment of optical components can lead to noise in the spectra by distorting the light passing through the sample.

Sample Impurities: Impurities in the sample being analysed can cause irregularities in the spectra, leading to noise.

Instrument Calibration: Improper calibration of the spectrophotometer or aging of the instrument components can introduce noise into the measurements. (baseline)

Environmental Factors: Changes in temperature, humidity, or air vibrations can affect the stability of the spectrophotometer, resulting in noise in the spectra.

To reduce noise in spectrophotometer spectra, we have to click on the option “smooth”

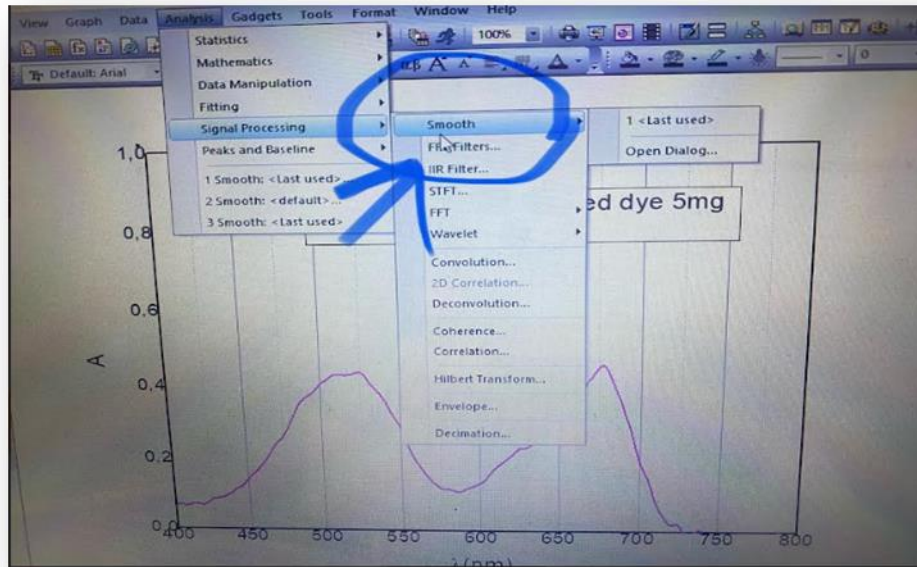


Figure 48: The option smooth for reducing noise

Calibration curves:

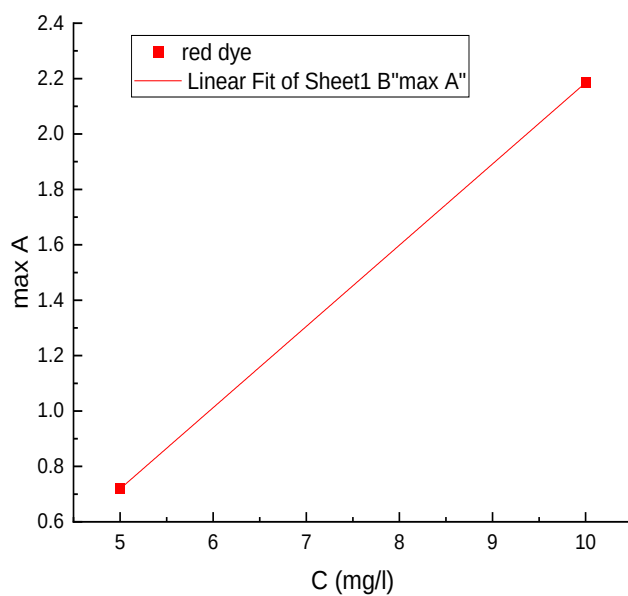


Figure 49: calibration curve of red dye SIN 122

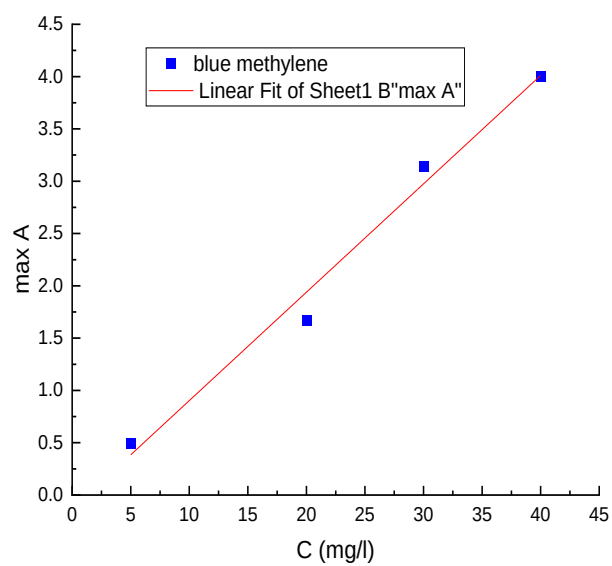


Figure 50: calibration curve of methylene blue

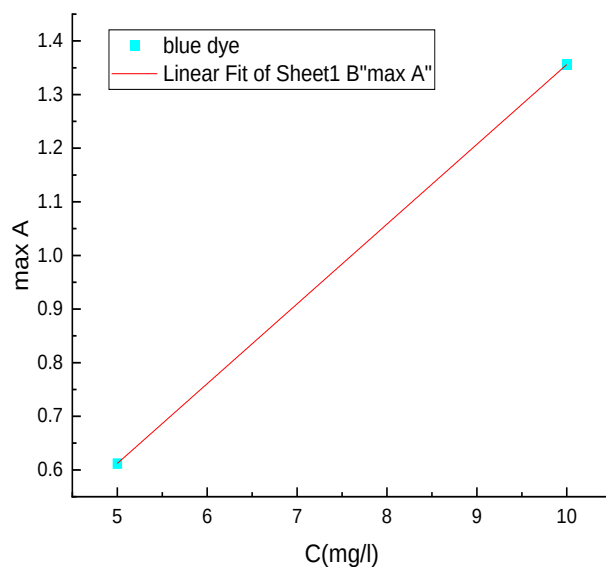


Figure 51: calibration curve of blue dye SIN 132

According to the beer lambert curves (Figures 49-51) of each solution we can summarize beers lambert law in the following table:

Table 7: A table that verifies the beer lambert law for the four solutions

Colourful solution	ϵ Measured	ϵ literature	Divergence
Blue methylene	$98504 \text{ L mol}^{-1} \text{ cm}^{-1}$	$98500 \text{ L mol}^{-1} \text{ cm}^{-1}$	4
Blue dye SIN 132	$19400 \text{ L mol}^{-1} \text{ cm}^{-1}$	$19445 \text{ L mol}^{-1} \text{ cm}^{-1}$	45
Red dye SIN 122	$20000 \text{ L mol}^{-1} \text{ cm}^{-1}$	$20097 \text{ L mol}^{-1} \text{ cm}^{-1}$	79

The Table 7 presents data for three colourful solutions: blue methylene, blue dye, and red dye, along with their calculated absorption coefficients based on Beer's Lambert law, and compares these values to the absorption coefficients reported in literature. The absorption coefficients, which quantify how much light a solution absorbs at a particular wavelength, are

derived from the Beer's Lambert curves obtained during the experiments. The calculated absorption coefficients for blue methylene, blue dye, and red dye closely match the literature values, indicating a high degree of accuracy in the experimental measurements. The minimal divergence between the experimental and literature values suggests that the experimental setup and methods used were precise and reliable. This close agreement reinforces the reliability of the absorption coefficients calculated for these solutions, demonstrating that the spectrophotometric analysis was conducted accurately and the results are dependable for further applications or studies.

Conclusion

In conclusion, the protocol for UV-visible spectrophotometer calibration involved preparing colourful solutions of blue methylene, blue dye, and red dye, and conducting measurements using the Shimadzu UV-3600 spectrophotometer in the Thin Films and Materials for Electronics Laboratory at Oran 1 University. The obtained spectra and calculated absorption coefficients were compared with literature references to assess the accuracy and reliability of the spectrophotometer. The results showed minimal divergence between the experimental and literature values, indicating that the Shimadzu UV-3600 is well-calibrated and capable of producing reliable and accurate absorbance spectra. Therefore, we have successfully addressed the problematic of verifying the results of our Shimadzu UV-3600 spectrophotometer.

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