تفريغ لاب تحليل آلي

اسم الموضوع: Exp 3 : Ultraviolet- visible Spectroscopy (Qualitative Analysis)

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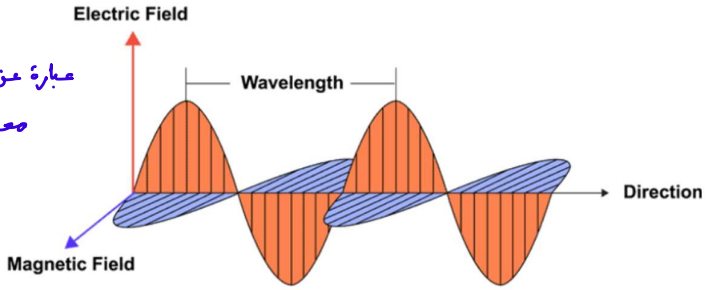
اللّٰهُمَّ سهل لي مطلبي و يسر لي
مقصدي و ارزقني بتسخير منك لهدفي
و اجعل خطواتي مباركة، اللّٰهُمَّ إن كان
ما أدعي به مستحيلاً فأنت القادر
سبحانك لا يعجزك شيء في الأرض
ولا في السماء و إن كان شراً فاجعله
خير و ارزقني به ياالله"

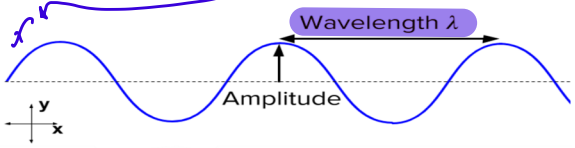
EXPERIMENT 3: Ultraviolet-Visible Spectroscopy – Qualitative Analysis

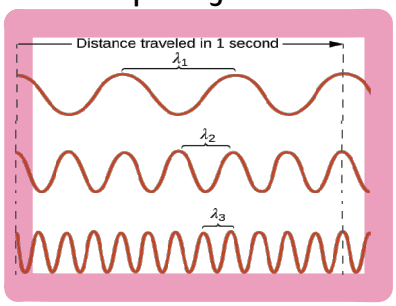
INTRODUCTION

Electromagnetic radiation is a form of energy, made up of electric and magnetic fields that move at right angles to each other. It exhibits wave-like behavior as it travels through space.



The electromagnetic radiation is classified according to the frequency of its wave (f) or according to the wavelength (λ).



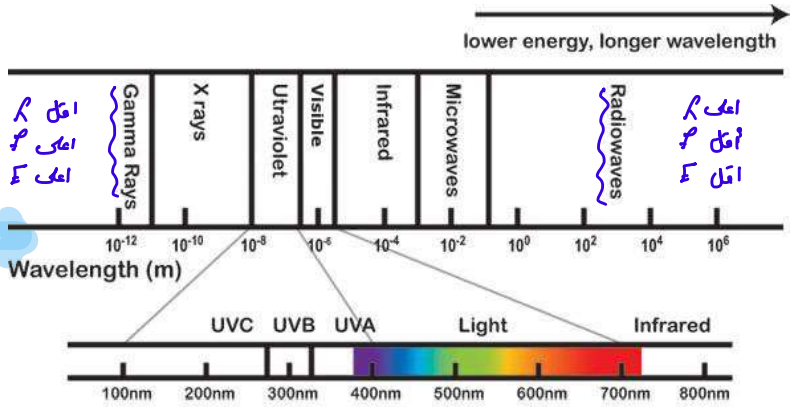


The frequencies and wavelengths of electromagnetic waves are related by the equation:

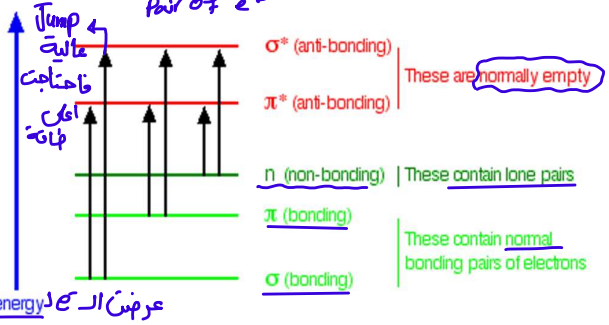
$$\lambda = \frac{v}{f}$$

Where λ (lambda) is the wavelength in (**m**), f is the frequency in (**Hz**) or (s^{-1}), and v is the velocity of the wave in (**m/s**).

The electromagnetic spectrum, in order of increasing frequency and decreasing wavelength, consists of radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays.



- **Spectroscopy** is the study of the interaction between matter and radiated energy.
- When light passes through the compound, energy from the light is used to promote an electron from a bonding or non-bonding orbital into one of the empty anti-bonding orbitals.
- An electron is excited from a full orbital into an empty anti-bonding orbital. Each jump takes energy from the light, and a big jump obviously needs more energy than a small one.



- Each wavelength of light has a particular energy associated with it. Electrons in atoms and molecules can change energy levels by emitting or absorbing a photon (light) whose energy must be exactly equal to the energy difference between the two levels. The larger the energy jump, the lower the wavelength of the light absorbed.
- Some jumps are more important than others for absorption spectrometry including jumps from pi bonding orbitals to pi anti-bonding orbitals, jumps from non-bonding orbitals to pi anti-bonding orbitals, and from non-bonding orbitals to sigma anti-bonding orbitals.

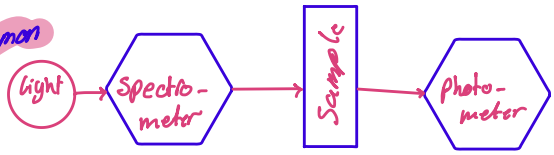
Hence, in order to absorb light in the region of Ultraviolet-visible spectroscopy (UV= 200-400 nm, visible= 400-800 nm), the molecule must contain either pi bonds or atoms with non-bonding orbitals. Remember that a non-bonding orbital is a lone pair on, say, oxygen, nitrogen, or a halogen and groups in a molecule which absorb light are known as ***chromophores***.

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

Spectrophotometer consists of two instruments, namely a **spectrometer** for producing light of any selected wavelength, and a **photometer** for measuring the

The important jumps are:

- ✓ from pi bonding orbitals to pi anti-bonding orbitals; $\pi \rightarrow \pi^*$ most common
- ✓ from non-bonding orbitals to pi anti-bonding orbitals; $n \rightarrow \pi^*$
- ✓ from non-bonding orbitals to sigma anti-bonding orbitals. $\pi \rightarrow \sigma^*$

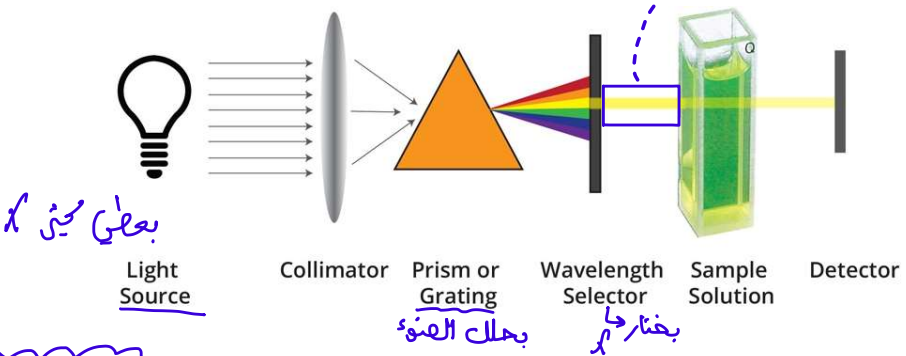


intensity of light. The instruments are arranged so that liquid (sample or blank) in a cuvette can be placed between the **spectrometer** beam and the **photometer**. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device. The signal changes as the amount of light absorbed by the liquid changes.

Spectrophotometers are divided into:

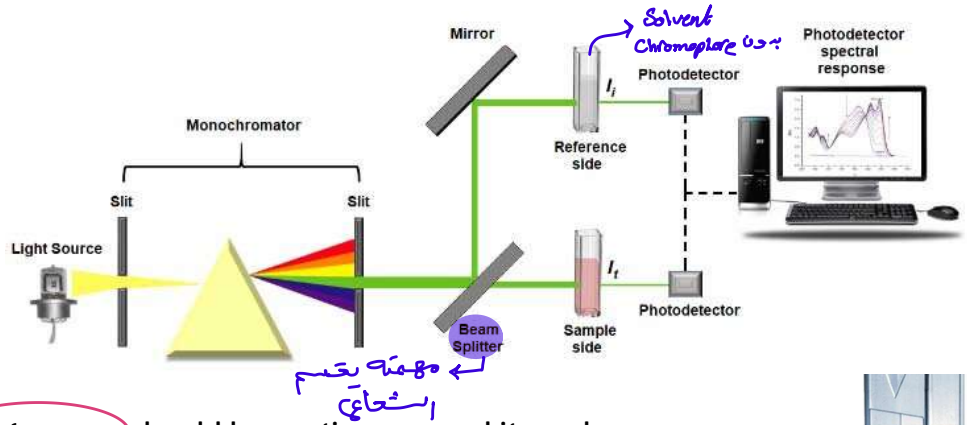
❖ **Single Beam Spectrophotometers**

Here a single beam of light is passed through a single sample container and the resulting light is detected by a detector. Single Beam Spectrophotometers are of the simplest in design hence have lower capital and maintenance prices than other spectrophotometer types.



❖ **Double Beam Spectrophotometers** (*will be used in this experiment*)

Here the light leaving the monochromator is split, using a beam splitter, into a sample beam and a reference beam. After each beam of light is passed through its respective sample/reference (blank) container, each beam is then detected by its own detector. The sample and reference are simultaneously measured/scanned, saving time, and providing for optimum accuracy. These devices ensure that any fluctuations in the light emitted from the lamp are applied equally to both the sample and the reference beams.



The **light source** should be continuous, and it can be:

- Deuterium lamp for uv radiation,
- Tungsten/halogen lamp for visible radiation, or
- Xenon lamp for uv and visible radiation.

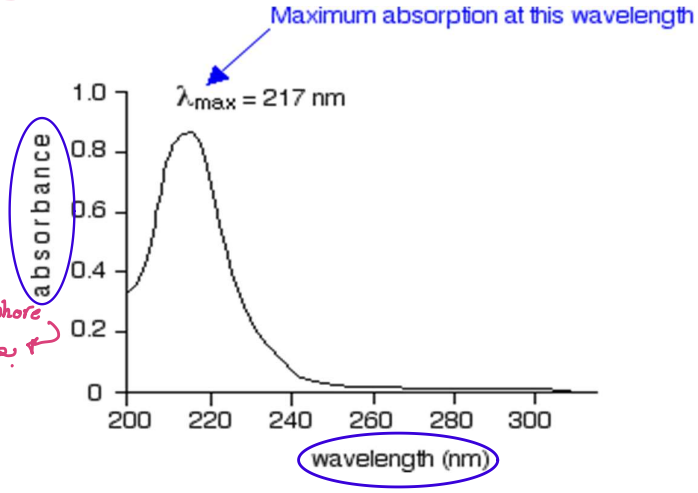
The **sample cell (cuvette)** should be transparent, and it can be:

- Quartz for UV and visible regions → إذا كانت العينة شفافة
- Glass and plastic only for the visible region → بتردا استخدمها لما تكون العينة ملونة

UV-Vis absorption spectrum

It is a plot relating the amount of light absorbed (absorbance) by a molecule at UV-Vis range of wavelengths and each compound has a characteristic UV-Vis spectrum. Sample scans over the UV-Visible wavelength range (200-400 and 400-800) nm respectively can be done using spectrophotometers,

The **wavelength of maximum absorbance** (λ_{max}) is a characteristic value, which is useful for the identification of a particular substance and is usually not affected by the concentration used.



طيب في هنوين جايين من

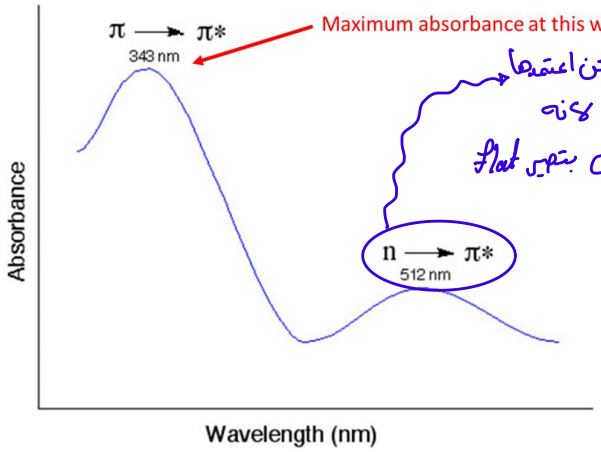
① Reference Cuvette : Solvent
without chromophore ⇒ بتعطي الجهاز قراءة الـ Solvent بس

② Sample Cuvette : Solvent with Chromophore
Analyte و Solvent بتعطي قراءة الـ

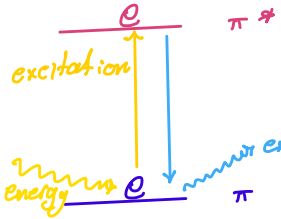
قراءة الجهاز النهائية ⇒ A Sample - A Reference

Many organic compounds give more than one maximum peak when their UV-Vis spectra are analyzed. Each peak corresponds to an electron transition from a ground state to an excited state, and more than one different transition (with different energy, and therefore, different wavelength) are allowed.

عشان يقدر الـ e ينتقل من مدار لمدار أخر لازم يكون الطاقة اللي اعطيتها اكبر من فرق الجهد بين المدارين



اي الـ Peak ممكن اعتمدها لكن مش دائمًا ، لأنه على التركيز القليل بتعمي Flat

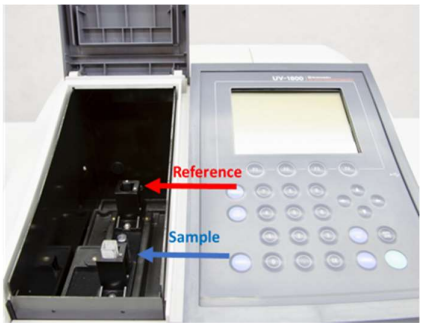


energy → voltmeter → Reading of Absorbance ⇓ على الجهاز

فكل ما يزيد λ بتقل الطاقة و بتقل القراءة

PRACTICAL PART

GLASSWARE	CHEMICALS
Volumetric flask 100 ml	Potassium permanganate
Volumetric flask 250 ml	Distilled water
Volumetric flask 25 ml	
Volumetric pipette10 ml	
Beakers	
Plastic cuvette	
INSTRUMENT	



PROCEDURE

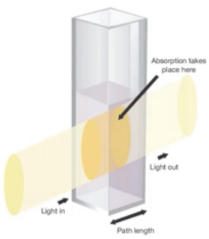
1. Prepare a stock solution of Potassium permanganate (1mg/ml).
2. Take 10ml of the above solution and dilute to 100ml with distilled water to prepare solution 1
3. Now take 10ml of the above solution (solution 1) and dilute to 25 ml with distilled water to prepare solution 2.
4. With the help of your instructor:
    a. Switch on the spectrophotometer and allow it to stabilize for 15 minutes.
    b. Set the absorbance at zero by using distilled water as the blank in each cuvette.
    c. Select baseline option by using distilled water as the blank in each cuvette.
    d. Carry out a full scan over the range of (400 – 700) nm using solution 2.
    e. Select peak pick option to display a table of peak and valley.
    f. Fill in the report sheet for the results and graph plotting.

Reminder: you should bring with you at least 1 pair of gloves, permeant marker and clean tissue paper.

Note to take in consideration

➢Where to put your sample?

- In UV spectroscopy the liquid sample is held in cuvette.
- Cuvetteis sealed at one end, and made of a clear, transparent material such as plastic, glass, or fused quartz..

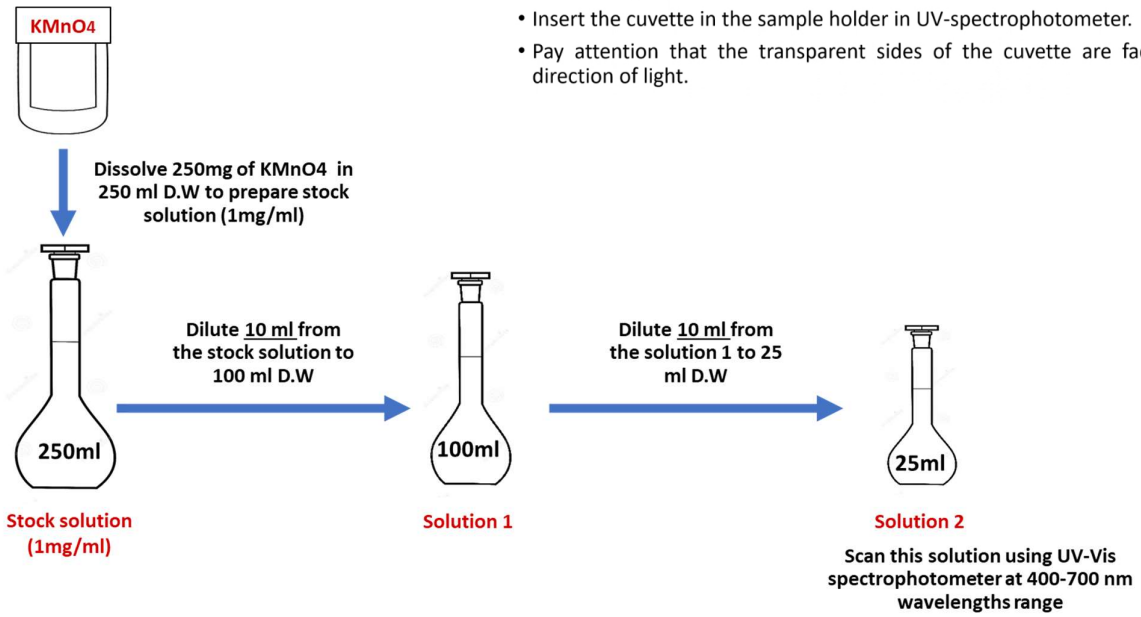


Note to take in consideration

➢In order to insert your sample in the UV-spectrophotometer for measurement do the followings:

- Clean the cuvette
- Place your liquid sample in the clean cuvette "Don't overfill the cuvette".
- Clean the transparent sides of your cuvette with a damp paper towel to remove finger prints.
- Insert the cuvette in the sample holder in UV-spectrophotometer.
- Pay attention that the transparent sides of the cuvette are facing the direction of light.

PROCEDURE DIAGRAM



Note to take in consideration

- The Double beam UV-Vis spectrophotometer have two cuvette holders one for the reference and one for sample .
- Red arrow shows cuvette holder for reference and blue arrow shows cuvette holder for sample.