Chapter IV : physical analysis methode :

1. UV-visible spectrometry
2. IR
3. RX

## Introduction :

**Physico-chemical analysis** is an area of analytical chemistry based on the relationship between the measurable **physical properties** of a substance and its **qualitative and quantitative composition**. Because these physical properties are measured using specific tools or machines, these techniques are commonly referred to as **instrumental methods**.

**Optical analytical methods** or **spectroscopy** represent a wide range of physico-chemical methods and are based on the interaction of electromagnetic radiation with the analyte. As a result of this interaction, atoms, molecules, ions or radicals are excited, which is associated with absorption and spontaneous emission of radiation.

### 3. Fields Of Application Of Spectroscopy

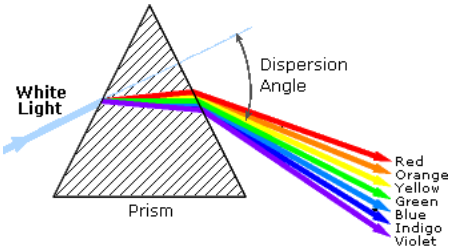
Spectroscopy makes it possible to explain a large number of phenomena that constantly surround us: the color of our clothes, the color of the sky ... In laboratories, it allows :

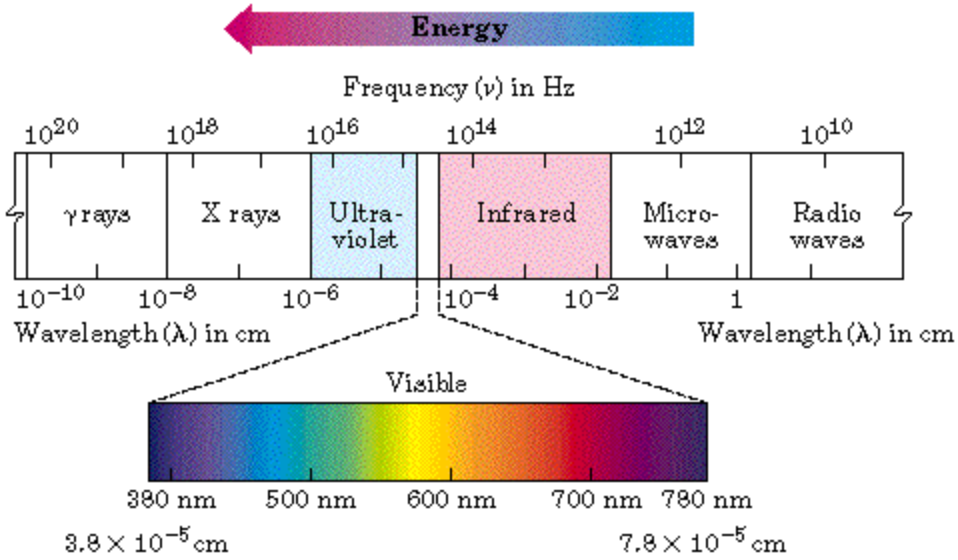
- the identification of molecules
-the determination of the structures
-the study of reaction kinetics
-the determination of the reaction mechanisms
-the dosages

Medical analyzes (MRI, scintigraphy, mammography ...) It also knows important applications in astrochemistry.

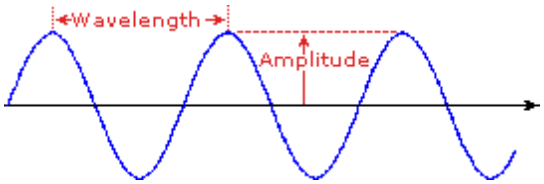
### 4. Electromagnetic radiation

The electromagnetic radiation, extending from the radio waves to cosmic rays. All these apparently different forms of electromagnetic radiations travel at the same velocity but characteristically differ from each other in terms of frequencies and wavelength



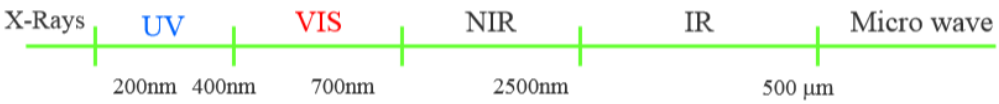




we are interested in interactions of the electric field with the matter Although a wave is frequently characterized in terms of its wavelength, often the terms such as wavenumber (ν), frequency (ν), cycles per second (cps) or hertz (Hz) are also used.

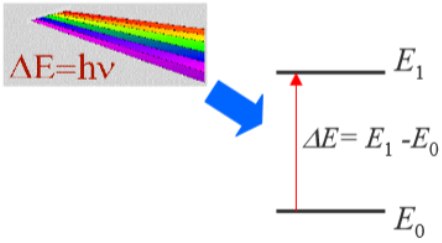



***Christian Huygens 1692:***
**Developed a wave theory of light**

## I. Type of transition :




| Type of Radiation | Type of Transition |
|---|---|
| X-Rays | Inner Electron |
| UV/VIS | Outer Electron |
| IR | Molecular vibration |
| Micro Wave | Molecular rotation |




- outer electron :

(1) σ ,π and n electrons

(2) *d* and *f* electrons

(3) charge transfer electrons

**Energy level of a molecule**

**Energy of a molecule :**

A molecule has its own energy E which comprises :

**For the movement of atoms :**

**Rotational energy (Er):** associated with the rotational movements about an axis passing through the center of inertia.

**A vibration energy (Ev):** associated with the movements of the atoms around their equilibrium position without there being any overall movement of the molecule.

**For the electrons :**

**An electronic energy (Ee):** associated with electronic transitions, the transitions are generally between a binding orbital (or single doublet) and an anti-binding orbital (or vacant non-binding).

The clean energy of the molecule can be expressed in the form: E= Er+Ev+Ee

**Arrangement of energy levels :**

The energy difference between two levels e (ΔEe ), between two levels of vibrational (ΔEv ) or between two levels of rotational mixing (ΔEr) is not of the same order of magnitude : $\Delta Er \ll \Delta Ev \ll \Delta Ee$

It can be considered that to each electronic energy level E_e correspond several vibrational energy levels V, and that to each vibrational energy level correspond several rotational energy levels Er.

## II. Ultraviolet and Visible Spectroscopy

This absorption spectroscopy uses electromagnetic radiations between 190 nm to 800 nm and is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions. Since the absorption of ultraviolet or visible radiation by a molecule leads transition among electronic energy levels of the molecule, it is also often called as electronic spectroscopy.

each substance absorbs light in a different way, a unique and specific relationship exists between the substance and its UV/VIS spectrum. The spectrum can then be used to identify or quantify a substance.

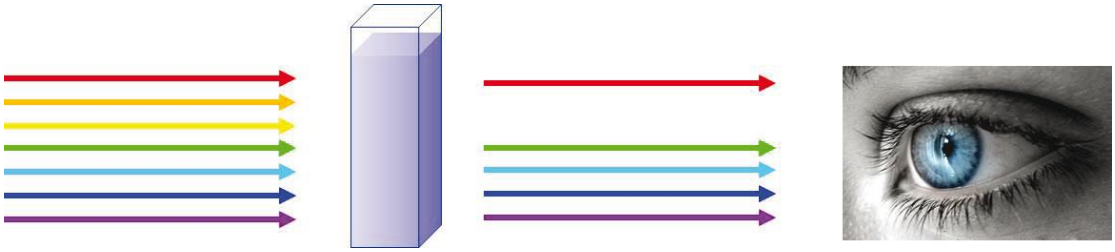
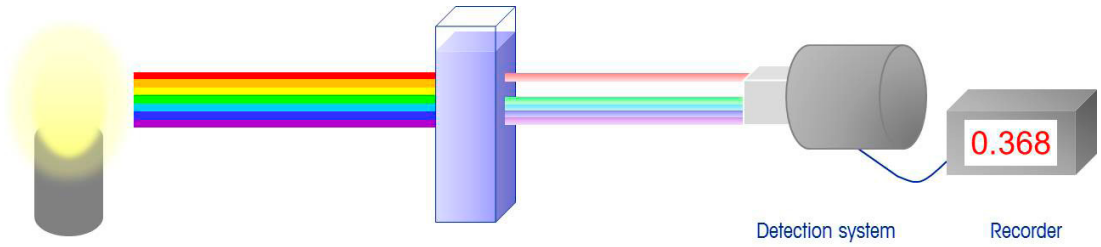


Figure : Light passing through a sample solution is partially absorbed by the components

UV/VIS spectroscopy is usually applied to organic molecules, inorganic ions or complexes in solutions, although solid materials such as films or glass can be analyzed as well. The obtained UV/VIS spectra are very useful for quantitative measurements of a specific compound. In fact, the concentration of an analyte in solution can be determined by measuring the absorbance at a specific wavelength. From the absorbance value of the sample, its concentration can be calculated,

### II.1. Measurement principle

A UV/VIS spectrophotometer measures the intensity of light passing through a sample solution in a cuvette, and compares it to the intensity of the light before it passes through the sample. The main components of a UV/VIS spectrophotometer are a light source, a sample holder, a dispersive device to separate the different wavelengths of the light (e.g. a monochromator), and a suitable detector.




Light source Cuvette with sample solution

## II.2. Transmittance and absorbance The detector in a UV/VIS spectrophotometer :

measures the intensity of light after passing through the sample solution. This fraction of light collected by the detector is called the transmitted intensity, *I*. The intensity of the transmitted light is attenuated by the sample solution due to, for instance, absorption of light at specific wavelengths. Therefore, its value is lower than the original intensity *I0* at the light source.

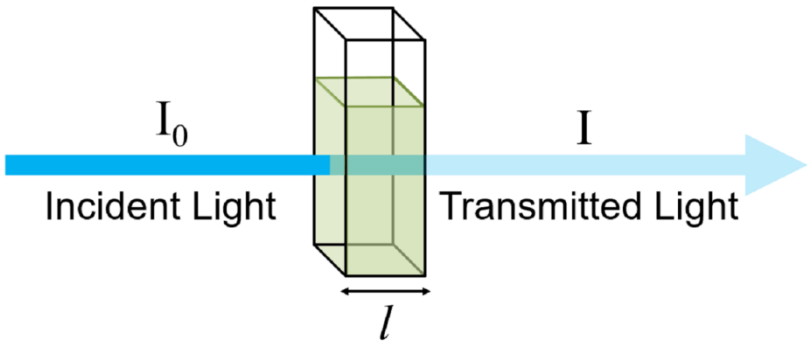



Figure 1: Light attenuation by absorption of sample molecules in solution

a) **absorption B** (Io - I/Io) - indicates how much of the incoming radiation was absorbed

b) **transparency T** (I/Io) - transmittance, which in turn indicates how much radiation has passed through the solution.

From that follows: B = 1 - T resp. B = 100-T (%).

A negative decimal logarithm of permeability is called **absorbance A**, logarithm of the ratio of the intensity of light entering (Io) to the intensity of light leaving the color layer (I)

$$\mathbf{A = - \log T} = (1/T) = \mathbf{\log} (I_0/I)$$

The relationship between radiation absorption, solution layer thickness and solution concentration is expressed by the **Lambert-Beer law**, which states that absorbance (A) is proportional to layer thickness (d) and solution concentration in $mol.l^{-1}$ (c)

$$\mathbf{A = \varepsilon . d . c = \log} (I_0/I)$$

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV spectroscopy.

The Lambert-Beer law allows for the determination of the sample concentration from the measured absorbance value. If the extinction coefficient $\varepsilon$ and the path length $d$ are known, then concentration $c$ can be calculated from absorbance $A$ as given below:

$$\mathbf{C = A/ \varepsilon . d}$$

## II.3. Nature of Electronic Transitions :

The total energy of a molecule is the sum of its electronic, its vibrational energy and its rotational energy. Energy absorbed in the UV region produces changes in the electronic energy of the molecule. As a molecule absorbs energy, an electron is promoted from an occupied molecular

**1. excitation :** $M + h\nu \longrightarrow M^*$

The lifetime of the excited species: $10^{-8}$ - $10^{-9}$ s

**2. relaxation** (conversion of the excitation energy to heat) $M^* \longrightarrow M$ + heat

The absorption of ultraviolet or visible radiation generally results from excitation of bonding electrons.

A compound appears coloured if it selectively absorbs light in the visible region. The main function of absorbed energy is to raise the molecule from ground energy state ( $E_0$ ) to higher excited energy state ( $E_1$ ). The difference is given by:

$$\mathbf{\Delta E = E_1 - E_0 = h\nu = hc/\lambda}$$

ΔE depends upon how tightly the electrons are bound in the bonds and accordingly, absorption will occur in UV or visible range, for example; If the electrons of a molecule are tightly bound as in compounds containing sigma bonds (e.g. saturated compounds) no light of region will be absorbed. The light of UV region will only be absorbed and hence compound appears colourless.

If the electrons of molecule are loosely bound as in unsaturated compound. Such absorption may occur in visible region and substance will appear as coloured.

Generally, there are three types of electronic transitions – namely:

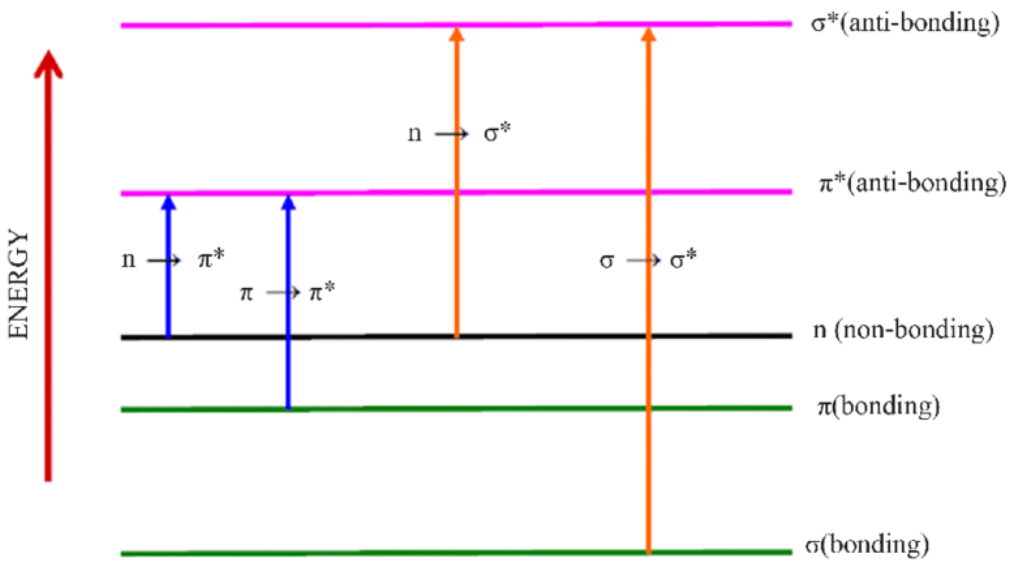
**1. Transition involving σ ,π and n electrons** (in most organic molecules)

2. **Transition involving d and f electrons** (most transition metal ions, lanthanide and actinide series absorb UV or visible light due to d and f electrons transition respectively)

3. **Transition involving charge transfer electrons**

(involving the transfer of an electron between two orbitals one of which is associated predominantly with the ligand and the other with the metal).

σ → σ*, n → σ*, n → π*, and π → π*



Alkanes can only undergo σ □ σ* transitions. These are high-energy transitions and involve very short wavelength ultraviolet light (< 150 nm). These transitions usually fall out-side the generally available measurable range of UV-visible spectrophotometers (200-1000 nm). The σ→ σ* transitions of methane and ethane are at 122 and 135 nm, respectively. In alkenes amongst the available σ →σ* and π→ π* transitions, the π →π* transitions are of lowest energy and absorb radiations between 170-190 nm.

In saturated aliphatic ketones the lowest energy transition involves the transfer of one electron of the nonbonding electrons of oxygen to the relatively low-lying π* anti-bonding orbital. This n → π* transition is of lowest energy (~280 nm) but is of low intensity as it is symmetry forbidden. Two other available transitions are n →π* and π → π*. The most intense band for these compounds is always due to π →π* transition.

In conjugated dienes the π→ π* orbitals of the two alkene groups combine to form new orbitals – two bonding orbitals named as $\pi_1$ and $\pi_2$ and two antibonding orbitals named as $\pi_3$* and $\pi_4$*. It is apparent that a new π → π* transition of low energy is available as a result of conjugation. Conjugated dienes as a result absorb at relatively longer wavelength than do isolated alkenes

**Chromophores :** The group of atoms containing electrons responsible for the absorption is called chromophore. Most of the simple un-conjugated chromophores give rise to high energy transitions of little use. Some of these transitions have been listed in tables 2 and 3.

| Chromofor, příklad sloučeniny | Přechod | $\lambda_{max}$ (nm) |
|---|---|---|
| $H_2O$ | $\sigma \rightarrow \sigma^*$ | 183 |
| C-C a C-H, $CH_4$ | $\sigma \rightarrow \sigma^*$ | cca 170, 173 |
| C-X, $CH_3OH$, $CH_3NH_2$, $CH_3I$ | $n \rightarrow \sigma^*$ | 180-260, 187, 215, 258 |
| C=C, $H_2C=CH_2$ | $\pi \rightarrow \pi^*$ | 160-190, 162 |
| $H_2C=CH-CH=CH_2$ | $\pi \rightarrow \pi^*$ | 217 |
| C=O, H−CH=O | $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ | 270, 170-200, 270, 185 |
| $H_2C=CH-CH=O$ | $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ | 328, 208 |
| C=N | $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$ | 190, 300 |
| N=N | $n \rightarrow \pi^*$ | 340 |
| C=S | $n \rightarrow \pi^*$ | 500 |
| $NO_2$ | $n \rightarrow \pi^*$ | 420-450 |
| N=O | $n \rightarrow \pi^*$ | 630-700 |

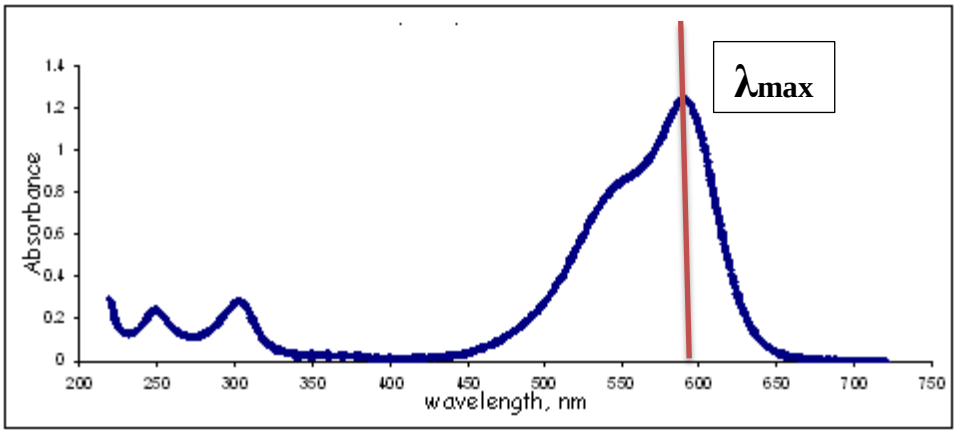
**TABLE 14-1** Absorption Characteristics of Some Common Chromophores

| Chromophore | Example | Solvent | $\lambda_{max}$, nm | $\varepsilon_{max}$ | Transition Type |
|---|---|---|---|---|---|
| Alkene | $C_6H_{13}CH=CH_2$ | *n*-Heptane | 177 | 13,000 | $\pi \rightarrow \pi^*$ |
| Alkyne | $C_5H_{11}C\equiv C-CH_3$ | *n*-Heptane | 178 | 10,000 | $\pi \rightarrow \pi^*$ |
| | | | 196 | 2000 | — |
| | | | 225 | 160 | — |
| Carbonyl | $CH_3CCH_3$ (O) | *n*-Hexane | 186 | 1000 | $n \rightarrow \sigma^*$ |
| | | | 280 | 16 | $n \rightarrow \pi^*$ |
| | $CH_3CH$ (O) | *n*-Hexane | 180 | large | $n \rightarrow \sigma^*$ |
| | | | 293 | 12 | $n \rightarrow \pi^*$ |
| Carboxyl | $CH_3COOH$ | Ethanol | 204 | 41 | $n \rightarrow \pi^*$ |
| Amido | $CH_3CNH_2$ (O) | Water | 214 | 60 | $n \rightarrow \pi^*$ |
| Azo | $CH_3N=NCH_3$ | Ethanol | 339 | 5 | $n \rightarrow \pi^*$ |
| Nitro | $CH_3NO_2$ | Isooctane | 280 | 22 | $n \rightarrow \pi^*$ |
| Nitroso | $C_4H_9NO$ | Ethyl ether | 300 | 100 | — |
| | | | 665 | 20 | $n \rightarrow \pi^*$ |
| Nitrate | $C_2H_5ONO_2$ | Dioxane | 270 | 12 | $n \rightarrow \pi^*$ |



## PRESENTATION OF SPECTRA

The ultraviolet–visible spectrum is generally recorded as a plot of absorbance versus wavelength. It is customary to then replot the data with either e or log e plotted on the ordinate and wavelength plotted on the abscissa. Figure 3, most are described by indications of the wavelength maximal and absorptivities of the principal absorption peaks.




λmax - wavelength where maximum absorbance occurs

## Characteristics Features of UV-Vis Absorption Spectrum

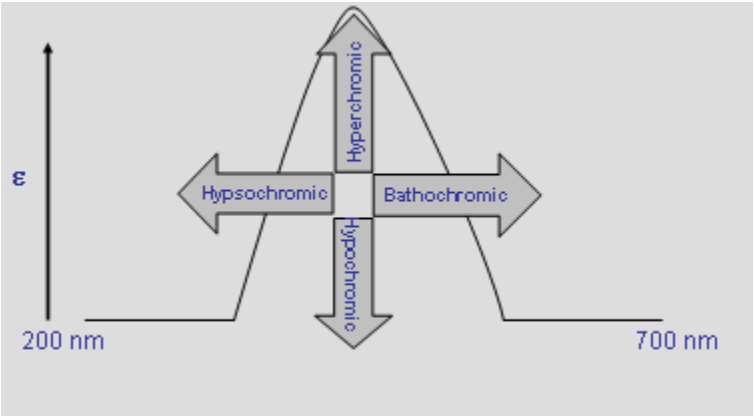
- ✓ is usually recorded as plot of absorption versus λ or ε versus λ at which maximum absorption is observed.
- ✓ An absorption band is characterized by its shape, that is, by its width and intensity.
- ✓ The intensity distribution is related to the probability of the transition to a given vibrational sublevel.
- ✓ The position of the peak is characterized by λmax
- ✓ The intensity of the peak is characterized by ε max
- ➢ In general, substituents may have any of four effects on a chromophore:

1.Bathochromic shift (red shift) – a shift to longer **λ ;** lower energy

2.Hypsochromic shift (blue shift) – shift to shorter **λ** higher energy

3.Hyperchromic effect – an increase in intensity **(ε )**

4.Hypochromic effect – a decrease in intensity(**ε**)




The Magnitude of Molar Absorptivities :

- ✓ Empirically, molar absorptivities (ε values) that range from 0 up to a maximum on the order of $10^5$ l $mol^{-1}$ $cm^{-1}$ are observed in UV-visible molecular absorption spectrometry.
- ✓ Commonly ε 104–105l $mol^{-1}$ $cm^{-1}$ for an allowed transition and is on the order of 10–100 for a forbidden transition.
- ✓ The magnitude of the absorptivity is an indication of the probability of the electronic transition.
- ✓ High values of ε give rise to strong absorption of light at the specified wavelength; low values of ε result in weak absorption of light.

**Factors affecting the position intensity of the absorption band**

- ✓ The positions and intensities of the absorption bands are sensitive to:
  **1. substituents close to the chromophore,**
  **2. conjugation with other chromophores, and**
  **3. Solvent effects**.

**1.Effect of Auxochrome *(*substituents close to the chromophore)**

In general, auxochromic substitution of chromophores causes bathochromic shifts and increases in intensity for π ⟶ π* transitions, and hypsochromic or blue shifts (to shorter wavelengths) for n ⟶ π* transitions.

***2.* Conjugation Effects**

Increase in the amount of conjugated double bonds always significantly shift absorption maxima towards longer wavelength (bathochromic) and usually towards stronger intensity relative to an isolated chromophore.

**Red shift of λmax with increasing conjugation**

$CH_2$=CHC$H_2$C$H_2$CH=$CH_2$ λmax = 185 nm

$CH_2$=CHCH=$CH_2$ λ max = 217 nm

$CH_2$=CH-CH=CH-CH=$CH_2$ (trans) λmax = 263 nm

•Red shift of λmax with **number of rings**

• Benzene λmax = 204 nm

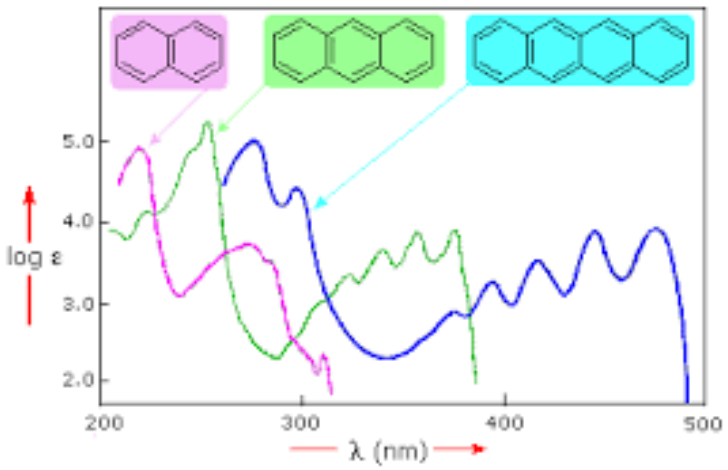
•Naphthalene λmax = 286 nm

**1,3-butadiene, CH2=CHCH=CH2**: a strong absorption band that is displaced to a longer wavelength by 20 nm compared with the corresponding peak for an unconjugated diene

on the amount of light and its wavelength absorbed by the sample, valuable information can be obtained, such as the purity of the sample. Moreover, the amount of absorbed light is related to the amount of sample, and thus, quantitative analysis is possible by optical spectroscopy.

Increasing the number of aromatic rings increases the absorption maximum

| | Benzene < Naphthalene < Anthracene < naphthacene < pentacene | | | | |
|---|---|---|---|---|---|
| **Abs. Max Abs.** | 262nm | 275 nm | 375 nm | 475 nm | 580 nm |
| **Max Log ε (Extinction)** | 3.84 | 3.75 | 3.90 | 4.05 | 4.20 |




As the **degree of conjugation increases** (i.e the number of electrons involved in the delocalized π-orbitals) the **absorption energy decreases** (> **λ**, the energy between the ground and excited state decreases) the **absorption becomes more intense** (> **ε**, increased probability of absorption)

**Example: Substituent effect on benezene absorption maximum**

| Substituent of benzene | $\lambda_{max}$ | **ε** | log **ε** | Substituent of benzene | $\lambda_{max}$ | **ε** | log **ε** |
|---|---|---|---|---|---|---|---|
| none | 204<br>256 | 7900<br>200 | 3.9<br>2.3 | $-NH_2$ | 230<br>281 | 8000<br>160 | 3.9<br>3.2 |
| $-CH_3$ | 208 | 8000 | 3.9 | -OH | 211 | 6300 | 3.8 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 261 | 300 | 2.5 | | 270 | 1500 | 3.2 |
| -Cl | 216<br>265 | 8000<br>1500 | 3.9<br>2.4 | -$NO_2$ | 251<br>280<br>330 | 9000<br>1000<br>130 | 4.0<br>3.0<br>2.1 |
| -$OCH_3$ | 220<br>272 | 8000<br>1500 | 3.9<br>3.2 | -$CH=CH_2$ | 244<br>282 | 12000<br>1600 | 4.1<br>3.2 |

**3. Solvent Effects**

Solvent polarity changes λmax since polarity changes with the movement of electron from one orbital to another.

- ✓ Polar solvents cause a blue shift (lower λ) due to salvation of the nonbonding electrons lowering the n orbitals But they cause a red shift for stabilizing the π* orbitals than do the π. On the other hand, the reverse is true if the solvent is non polar.
- ✓ Solvent can also induces significant changes in the intensity of peaks.

  Hyperchromic – Increase in absorption peak

  Hypochromic – Decrease in absorption peak

***Example***: 1.For acetone, *n to* π* transition occurs at 279 nm in n-hexane, 270 nm in ethanol and at 265 nm in water

Absorption characterstics of 2 – methylpyridine

| **Solvent** | **λmax** | **εmax** |
|---|---|---|
| Hexane | 260 | 2000 |
| Chloroform | 263 | 4500 |
| Ethanol | 260 | 4000 |
| Water | 260 | 4000 |
| Ethanol/HCl (1:1) | 262 | 5200 |

Applications:

1. Detection of Impurities

 It is one of the best methods for determination of impurities in organic molecules.

 Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.

 By also measuring the absorbance at specific wavelength, the impurities can be detected.

2. Structure elucidation of organic compounds

 It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of hetero atoms.

3. UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation.

4. UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation thus used in qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

5. This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.

6. Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

7. Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.

8. Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.

9. UV spectrophotometer may be used as a detector for HPLC.

1. الكشف عن الشوائب

· وهي واحدة من أفضل الطرق لتحديد الشوائب في الجزيئات العضوية.

* يمكن ملاحظة قمم إضافية بسبب الشوائب في العينة ويمكن مقارنتها مع المواد الخام القياسية.

* من خلال قياس الامتصاص عند طول موجي محدد ، يمكن اكتشاف الشوائب.

2. توضيح هيكل المركبات العضوية

* من المفيد في توضيح بنية الجزيئات العضوية ، كما هو الحال في الكشف عن وجود أو عدم وجود عدم التشبع ، وجود ذرات غير متجانسة.

3. يمكن استخدام التحليل الطيفي لامتصاص الأشعة فوق البنفسجية لتحديد كمية المركبات التي تمتص الأشعة فوق البنفسجية.

4. يمكن أن يميز التحليل الطيفي لامتصاص الأشعة فوق البنفسجية تلك الأنواع من المركبات التي تمتص الأشعة فوق البنفسجية المستخدمة في التحديد النوعي للمركبات. يتم تحديد الهوية من خلال مقارنة طيف الامتصاص مع أطياف المركبات المعروفة.

5. تستخدم هذه التقنية للكشف عن وجود أو عدم وجود مجموعة وظيفية في المركب. يعتبر غياب النطاق عند طول موجي معين دليلا على غياب مجموعة معينة.

6. يمكن أيضا دراسة حركية التفاعل باستخدام التحليل الطيفي للأشعة فوق البنفسجية. يتم تمرير الأشعة فوق البنفسجية عبر خلية التفاعل ويمكن ملاحظة تغيرات الامتصاص.

7. العديد من الأدوية إما في شكل مواد خام أو في شكل صياغة. يمكن فحصها عن طريق عمل محلول مناسب للدواء في مذيب وقياس الامتصاص عند طول موجي محدد.

8. يمكن قياس الأوزان الجزيئية للمركبات طيفيا ضوئيا عن طريق تحضير المشتقات المناسبة لهذه المركبات.

9. الأشعة فوق البنفسجية الطيفي يمكن استخدامها ككاشف ل هبلك.