INSTRUMENTAL ANALYSIS METHODS

GENERAL INFORMATION

In order to perform quantitative and qualitative analysis of organic substances, the changing physical properties of these substances are utilized. Various methods have been developed for this purpose. With each of these methods, a changing physical property of the substance is measured. If the method is developed for the physical property of the substance, it is referred by the name of that physical property. For example, the method developed to measure the mass of the material is gravimetry, the method developed to measure the volume of the substance is called volumetry, the method developed to measure the electrical potential of the substance is called potentiometry, the method developed to measure the absorption of the substance is called absorbimetry. If these physical properties of the substance are measured by means of a tool, such methods of analysis are called Instrumental Analysis Methods. With the application of this instrumental methods, it is possible to get the accurate, precision and precisely results of the qualitative and quantitative analysis of organic compounds both in short time and in the amount of miligram (or even microgram). Among the methods of instrumental analysis, spectroscopic methods are the most common methods. With the application of spectroscopic methods, the interaction of matter with radiation such as emission, absorption, scattering, distortion and dispersion of electromagnetic radiation, and the results of this interaction are examined for analytical purposes.

A group of methods including UV-Visible Field Spectroscopy (Colorimeter, IR, X-Ray Spectroscopy, NMR, Electron-Spin Spectroscopy etc.) have been developed on the basis of absorbing electromagnetic radiation and applied for analysis.

Electromagnetic Radiation

Electromagnetic radiation is a form of energy that travels at great speed in space. But there are other energies that are emitted by waves in space, but these (every energy emitted in waves) are not radiations. The radiation spreads in space without losing anything from its energy. The rays in the visible area, the rays in the UV and IR regions, X-rays ve γ rays, microwaves and radio waves are all types of radiation that can be exemplified to electromagnetic radiation. The characteristics that differentiate them from each other is their frequencies (number of vibrations per unit time). (For example, human eye can detect the radiation between frequency values λ: 400-750 nm and the radiations in this area are visible in purple, blue, orange and red color). The electromagnetic spectrum is the range of frequencies (the spectrum) of electromagnetic radiation and their respective wavelengths and photon energies. Electromagnetic spectrum is obtained when the frequencies with different frequency values are sorted by taking these values into consideration. In Table 2.1.1, an electromagnetic spectrum is formed by ordering the different types of radiation according to their frequencies (also based on wavelength and energy levels and results in practice).In Table 2.1.1, an electromagnetic spectrum is formed by ordering frequencies (at the same time according to wavelength and energy levels and results in practice) of different types of radiation.

Table 2.1.1. Electromagnetic spectral zones

Spectral region	Wavelength	Excitation
Gamma rays	0.0001-0.01 nm	Core reactions
X rays	0.01-10 nm	Transition of internal electrons
Far UV	10 -200 nm	Ionization of atoms or molecules
Near UV	200 -380 nm	Transition of external orbital electrons

Visible	380 -780 nm	Transition of external orbital electrons
Near IR	0.78 -1.5 µ	Molecular vibration and rotation
IR	1.5 -25 µ	Molecular vibration and rotation
Far IR	25µ -1mm	Molecular rotation
Micro Waves	0.1 – 100 cm	Molecular rotation
Radio Waves	100 cm – 10-15 km	Nuclear magnetic resonance

A substance, depending on its specificity, absorbs only some of the rays of various wavelengths (from ultraviolet rays to radio waves), and does not have any relationship with others. The structure and concentration of the substance can be determined by using this feature, ie, qualitative and quantitative analysis can be done. For this purpose, the rays which have different wavelengths are sent to the substance and then rays which are absorbed by the substance (depending on its properties) can be determined by these enstruments. Only the wavelengths which are suitable for the property of the compounds are absorbed so it can be easy to determine the structure of the compound.

However, in practice it is not appropriate to make a single device that will radiate all these wavelengths and determine which of them are absorbed, so devices running between certain wavelengths have been developed. For example, the wavelengths of radio waves up to thousands of meters can be analyzed with Magnetic Resonance, the wavelength of 200-750 nm rays can be analyzed with UV (ultraviolet) Visible spectrophotometry and wavelengths of 2500-25000 nm can be analyzed with Infrared Spectrophotometer. The methods of analysis carried out using these instruments are called NMR, UV-Visible and IR spectroscopy, respectively.

Basic Properties of Electromagnetic Radiation

Multiple basic properties of radiation can be mentioned here (wavelength, period, frequency, speed, number of waves). However, we must first say that there is both the wave and photon properties of the radiation (besides the wave property of a beam, have a particle property, and each of these particles are called photons) and that the genus will be expressed by frequency or energy per photon. The intensity of the light is related to the number of photons in the light beam and is measured by the energy units. In the absorption of the light energy, depending on the variable amount of energy it will take or receive, the molecule absorbs a single-frequency (certain level) radiation. This applies to all molecules of the compound, so that absorption lines are visible in the spectrum. However, since each molecule at a certain electronic level is also at various levels of vibration and rotation, these are reflected in the spectrum as absorption bands and peaks. Other main features of radiation can be listed as follows:

Wavelength (λ): Distance between corresponding points of two consecutive waves . Wavelength is expressed in units such as meter, cm, mm, micron (μ), nanometer (nm), angstrom (Å). The relationship between them;

1 cm = 104 μ = 107 nm = 108 Å (1μ = 1000 mμ), (1 nm = 1 mμ = 10 Å)

Frequency (ν): The number of vibrations per second. At the same time, the number of period per second (1 / p) of a beam is called frequency. The unit is hertz (1 million hertz: 1 MegaHertz).

Period (P): This is the time required for two consecutive wave peaks to pass through a certain point. The unit is (sec). The period is the time it takes a wave to complete one cycle.

Speed (c): The path taken by the beam (ray) in unit time. The speed of each type of beam(ray) in vacuum is the same and indicated by (c) (c = 3.1010 cm / sec). The speed of a beam (ray) is reduced while passing through from the vacuum to any medium and is indicated by (ci). Accordingly, the beam(ray) speed in any environment is expressed by the equation $(c_i = v . \lambda)$.

Wave number ($\bar{v}$): The number of waves at a unit distance (eg. 1 cm). The unit is cm^{-1}. ($\bar{v}$) = 1 /λ

Particle Properties of Radiation, Energy and Wavelength Relations of the Particles

We have said that the beam has a particle property as well as a wave property, and these particles are called photons. Photons have a certain amount of energy and this determines the frequency of radiation. The energy of the radiation is found by taking into account the basic features given above. Accordingly, the energy increase in the case of a molecule absorbing radiation;

$E = h . v = h . c / \lambda$ (h = Planck constant: 6.63 x 10^{-27} erg.sn)

The above equation (taking into account that the masses of the photon (N) are different from each other) can be written;

$E = N. h. v = N. h . c / \lambda$

It is understood that the energy of photon (light rays) is inversely proportional to the wavelength.

During the application of each spectroscopic method, the aforementioned energies, in which the photons (light rays) have, are absorbed by the molecules for different purposes. The data obtained by determining the absorbed light rays by the appropriate tool (Spectrophotometer) and evaluating the data (Spectrum) helps us to conclude the analysis of the absorbing substance.

Radiation Absorption and Lambert-Beer Law

The most important event that occurs as a result of the interaction of compound with the beam is the absorption of the beam. If a beam containing the rays of various wavelengths is passed through a transparent medium, it is seen that some wavelengths disappear. This is called the absorption of the beam. When the rays of certain wavelengths are absorbed, the energy of the beam passes to the substance and the molecules, atoms, electrons of the matter become more energized, which is called the stimulation of atoms or molecules (Figure 2.1.1).
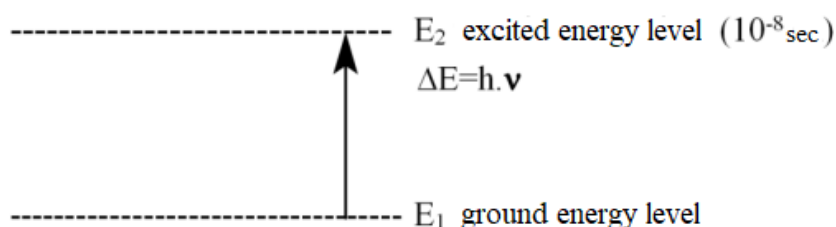
$$E_2 \text{ excited energy level } (10^{-8} \text{sec})$$
$$\Delta E = h.v$$
$$E_1 \text{ ground energy level}$$

Figure 2.1.1. Excitation of atoms or molecules

The atoms or molecules that remain in the excited state for 10-8 seconds return to the basic energy level and give back the energy they receive while in the excited state in the form of heat or light. It is called **phosphorescence** when the energy is returned slowly as light (for hours); **fluorescence** when the energy is returned in a shorter time (immediate).

When a Power of light coming to the matter in a certain direction and leaving from substance in the same direction, some part of the light is absorbed by the solution and some part of it is reflected, so its strength decreases and becomes (I). The relationship between the values (Io) and (I) referred to herein is expressed by the **Lambert-Beer Law**, and UV-Spectroscopy is utilized. According to this law, the decrease in the intensity of the beam transmitted to a transparent solution is related to the intensity of the beam transmitted, the concentration of molecules in the solution through which it passes, and the distance of the path of the beam transmitted. Therefore, if the operating conditions are kept constant (in terms of the intensity and the path of the transmitted light), the result is directly related to the concentration of the atoms and molecules in the solution, and this is utilized for quantitative analysis in UV spectroscopy.

The Lambert-Beer Law can be expressed as follows:

Log Io / I = A (Absorbance) = k. l .c

Io = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

l = length of sample cell (cm)

c = Concentration (g / l)

k = Absorption coefficient

ε = If the concentration is mol / liter, the absorption coefficient is indicated by epsilon and is called molar absorptivity. (molar extinction coefficient)

A = Absorbance (optical density, extinction), and vice versa (T) is transmittance, that is, permeability and is expressed as **T = I / Io.**

With Beer Law, it is stated that absorption is a constant independent from concentration, cell thickness and light intensity. But it does not give an idea about the effect of temperature, wavelength and solvent. In practice the effect of temperature can be neglected. The solvent must be specified in each spectrum. But for constant temperature and for a certain solvent, the absorption may not be constant. If the absorbance (A) is plotted against the concentration (c), a line through zero point is obtained according to Beer's law (figure 2.1.2). Sometimes there may be deviations from Beer law. They are generally in the form of chemical interactions with the solvent, polymerization and deviations from the device. The line obtaining from Absorbency concentration graphic can be used as a calibration curve, when is drawn for a certain compound. In such studies, it is necessary not to exceed the absorbance values of 0.2-0.9. In order to establish a linear relationship and obtain a precise result, the best results are obtained when the molar concentration of the solution is between $10-3$ and $10-5$.
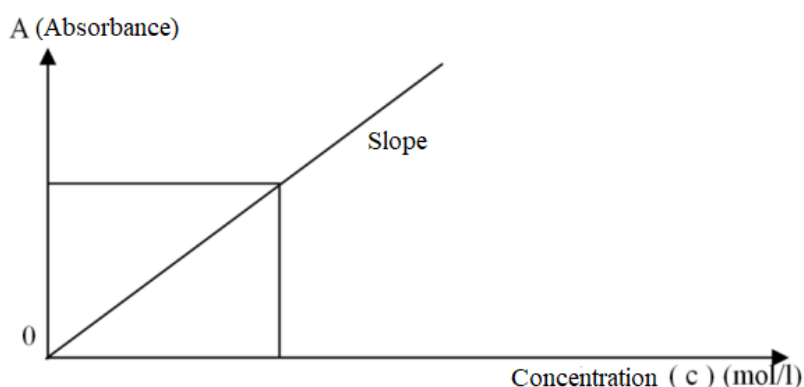
Figure 2.1.2. Calibration curve

The instrumental analysis methods performed by measuring the physical properties of the substance by using a tool allow accurate, precise and quantitative or qualitative determination of the amounts of mg of organic compounds. These spectroscopic methods will be examined under the following headings:

- Ultraviolet- Visible spectroscopy
- Infrared spectroscopy
- Nuclear magnetic resonance spectroscopy
- Mass spectroscopy

ULTRAVIOLET- VISIBLE SPECTROSCOPY

Visible and ultraviolet (UV) spectroscopy is based on the evaluation of the spectra of the radiation absorbed by the electronic transitions in the molecules (in order to provide them with the energy required). The area of the electromagnetic radiation that contains the rays of the wavelength of 200-400 nm is the UV (Ultraviolet / Ultraviolet) region. Since the rays between 10-200 nm are absorbed by the oxygen in the air, this area is called Vacuum UV (Far UV) and it is operated in this area with the help of special mechanisms and in the air-free environment (vacuum). The area used in the rays at 200-400 nm is called the ultraviolet (UV) region. However, since the rays in this area are absorbed by ordinary glass, the prism glass and lenses used in UV spectroscopy are made of quartz glass. The portion of the electromagnetic spectrum lying between about 400 and 750 nm is the visible region. Light waves with wavelengths between these limits appear colored to the human eye.

Different types of light sources are used for UV regions to obtain radiation at different wavelengths. Advanced spectrophotometers have both types of radiation source and change the frequency properly and automatically. The resulting radiation or radiation beams absorbed through the transmitted beam are sensitively detected by the device and transferred to the spectrum (as absorbance band). The graph of the relationship between the amount of absorption and wavelength or frequency of light is called the absorption spectrum. (in other words, the graph of the relationship between absorbance and transmittance is called absorption spectrum) (Figure 2.2.1).
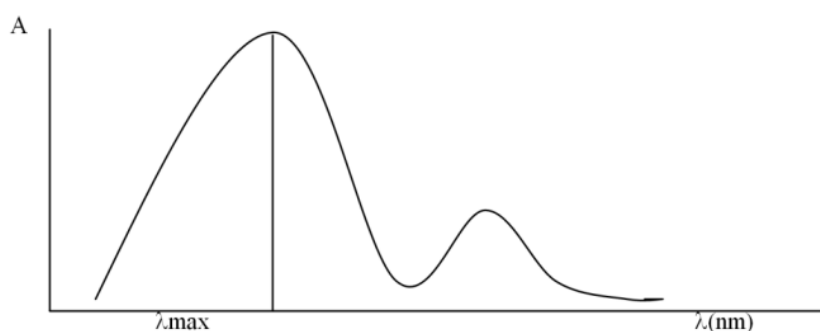

Figure 2.2.1. Absorption spectrum

Absorption of Organic Substances in UV and Visible Regions

The electrons or bond electrons of outer layer of matter absorb the beam energy or electromagnetic energy. When they absorb the light rays, they rise to higher energy levels as previously mentioned. Some of the bonds have low energies and absorb low-energy (long wavelength) rays. Some of the bonds have larger energies, which absorb higher energy (short wavelength) rays. This difference, that is, the absorption of rays of different wavelengths by different bonds gives us the opportunity to analyze. For example, a molecule that does not carry a single bond and a functional group, absorb a very short wavelength and a high energy, but since the same beams are also absorbed by other molecules in the air, it is necessary to work in a vacuum

without air. Since the electrons in the molecules bearing functional group can more readily reach their upper energy levels, the lower energy (wavelength longer) rays may be sufficient to stimulate them.

Theory of Absorption and Spectrum Explanation

In addition to some other theories on the subject, the main theory used in explaining the formation of absorption and spectra is the Molecular Orbital Theory. The theory is based on the presence of molecular bond orbitals, anti-bond orbitals and electrons responsible for absorption in the analyzed molecules.

Molecular Orbital: The electron cloud that is not localized and occupied by the bond electrons between the two atoms is called molecular orbital.

Molecular Bond Orbital: It is the orbital which is formed by the overlaping of two atom orbits and has a lower energy level than the energy levels of the orbitals of these atoms. It can occur in two ways:

The orbitale, in which the electrons make a mono-valence (single) bond, is called the sigma molecular orbital and is indicated by (σ). (single bonds are also referred to as σ bonds of sigma electrons).

The orbitale in which the bonds make the double bonds, is called the pi molecular orbital and indicated by (π). (double bonds are also referred to as π bonds of π electrons).

Antibonding Orbital: It is the orbital which is formed by the overlaping of two atom orbits and has a higher energy level than the energy levels of the orbitals of these atoms. It is indicated by ($\sigma*$) and ($\pi*$).

Absorption and Electrons Responses for Absorption: Electrons that responsible for absorption (also referred to as outer layer electrons) can be listed as follows;

- electrons connecting atoms in the molecule

- Single bond, (σ) electrons

- Double bond, (π) electrons

- the unconjugated (n) electrons on hetero atoms (such as O, S, N and X) of molecule

Absorption and Chromophore Groups:

The electrons listed above, if they encounter with the energy of the appropriate level of energy, absorb them. the absorption takes place by transferring from the initial bond-orbital energy level (σ), (π) and (n) to the anti-bond energy levels ($\sigma*$) and ($\pi*$).