AL- MUSTAQBAL UNIVERSITY
College Of Health And Medical Techniques
Prosthetic Dental Techniques Department
Second Grade
Second Semester

Advanced chemistry

Lecture 9 (The theoretical part)

(Spectroscopic Methods of Analysis)

By:

Assist.Prof.Dr.Ahmed A. AL-Khafagi

Spectroscopic Methods of Analysis

Before the beginning of the twentieth century most quantitative chemical analyses used gravimetry or titrimetry as the analytical method. With these methods, analysts achieved highly accurate results, but were usually limited to the analysis of major and minor analytes. Other methods developed during this period extended quantitative analysis to include trace level analytes. One such method was colorimetry. One example of an early colorimetric analysis is Nesslers method for ammonia, which was first proposed in 1856. Nessler found that adding an alkaline solution of HgI2 and KI to a dilute solution of ammonia produced a yellow to reddish brown colloid with the color determined by the concentration of ammonia. A comparison of the samples color to that for a series of standards was used to determine the concentration of ammonia. Equal volumes of the sample and standards were transferred to a set of tubes with flat bottoms. The tubes were placed in a rack equipped at the bottom with a reflecting surface, allowing light to pass through the solution. The colors of the samples and standards were compared by looking down through the solutions. Until recently, a modified form of this method was listed as a standard method for the analysis of ammonia in water and wastewater. Colorimetriy, in which a sample absorbs visible light, is one example of a spectroscopic method of analysis. At the end of the nineteenth century, spectroscopy was limited to the absorption, emission, and scattering of visible, ultraviolet, and infrared electromagnetic radiation. During the twentieth century, spectroscopy has been extended to include other forms of electromagnetic radiation (photon spectroscopy), such as X-rays, microwaves, and radio waves, as well as energetic particles (particle spectroscopy), such as electrons and ions.

7.1. Definition of some terms

frequency

The number of oscillations of an electromagnetic wave per second (v).

wavelength

The distance between any two consecutive maxima or minima of a electromagnetic wave (λ).

$$\lambda = \frac{v}{\nu} = \frac{c}{\nu} \quad \text{(In vacuum)}$$

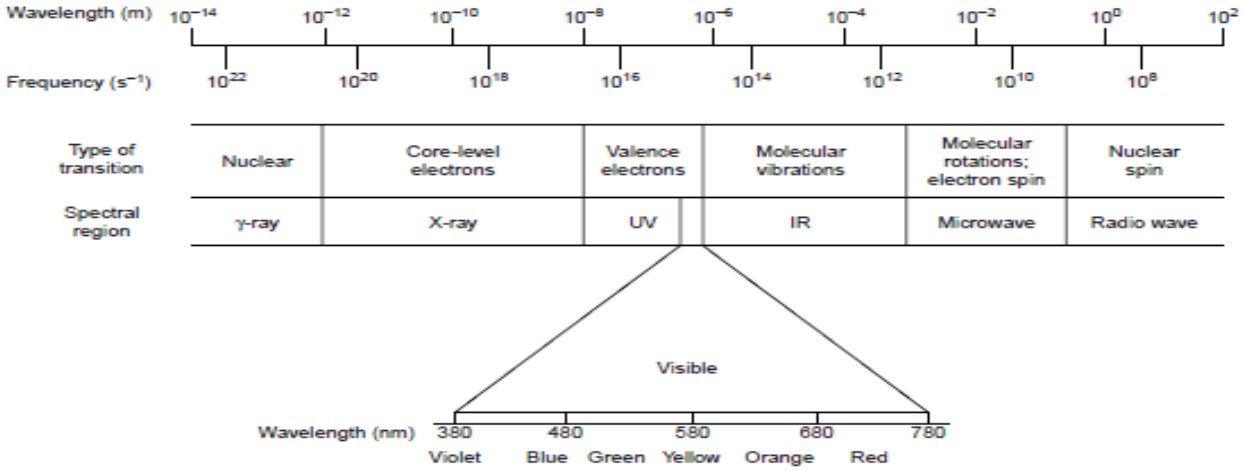
Wave number

The reciprocal of wavelength(ύ).

$$\bar{\nu} = \frac{1}{\lambda}$$

Wave numbers are frequently used to characterize infrared radiation, with the units given in reciprocal centimeter (cm–1).

Figure 16 The electromagnetic spectrum showing the colors of the visible



spectrum.

Absorbance

The attenuation of photons as they passthrough a sample (*A*).

Absorbance spectrum

A graph of a sample's absorbance of electromagnetic radiation versus wavelength (or frequency or wave number).

Emission

The release of a photon when an analyte returns to a lower-energy state from a higher-energy state.

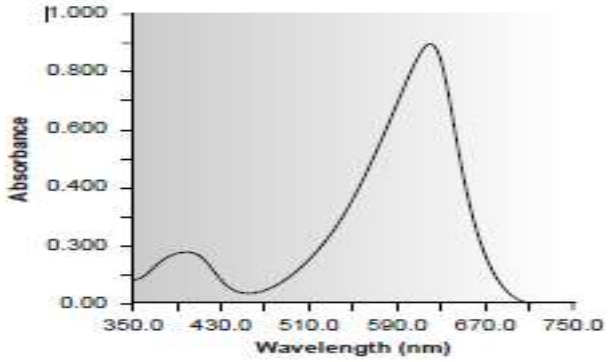


Figure 17 Ultraviolet/visible absorption spectrum forbromothymol blue.

7.2. Transmittance and Absorbance

The attenuation of electromagnetic radiation as it passes through a sample is described quantitatively by two separate, but related terms: transmittance and absorbance. **Transmittance** is defined as the ratio of the electromagnetic radiation's power exiting the sample, *P*T, to that incident on the sample from the source, *P*0, (Figure 18.a).

$$T = \frac{P_T}{P_0}$$

Multiplying the transmittance by 100 gives the percent transmittance (%*T*), which varies between 100% (no absorption) and 0% (complete absorption). All methods of detection, whether the human eye or a modern photoelectric transducer, measure the transmittance of electromagnetic radiation.

To compensate for this loss of the electromagnetic radiation's power, we use a method blank (Figure 18.b). The radiation's power exiting from the method blank is taken to be *P*0.

An alternative method for expressing the attenuation of electromagnetic radiation is absorbance, *A*, which is defined as

$$A = -\log T = -\log \frac{P_T}{P_0} = \log \frac{P_0}{P_T}$$

Absorbance is the more common unit for expressing the attenuation of radiation because, it is a linear function of the analyte's concentration.

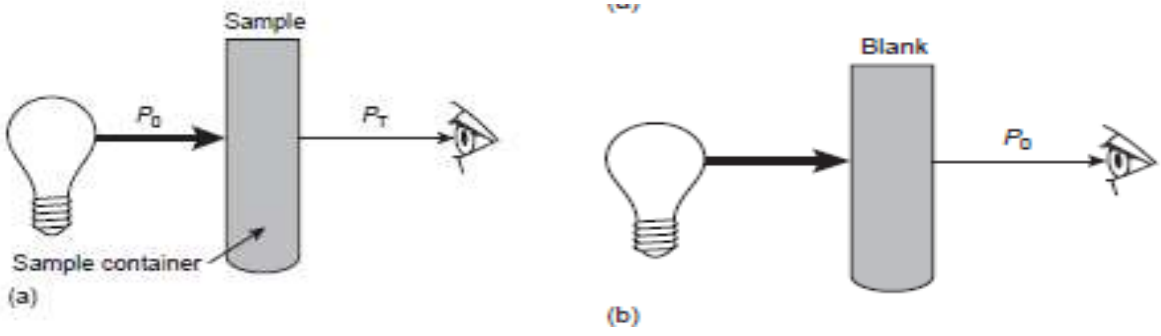


Figure 18 (a) Schematic diagram showing theattenuation of radiation passing

'A sample has a percent transmittance of 50.0%. What is its absorbance?

SOLUTION

With a percent transmittance of 50.0%, the transmittance of the sample is 0.500. Substituting into equation 10.2 gives

$$A = -\log T = -\log(0.500) = 0.301$$

through asample; P_0 is the radiant power from thesource and P_T is the radiant powertransmitted by the sample. (b) Schematicdiagram showing that P_0 is redefined as theradiant power transmitted by the blank,correcting the transmittance in (a) for anyloss of radiation due to scattering, reflectionor absorption by the cuvette, and absorption by the sample's matrix.

7.3. Absorbance and Concentration: Beers Law

When monochromatic electromagnetic radiation passes through an infinitesimally thin layer of sample, of thickness *dx,* it experiences a decrease in power of *dP* (Figure 19). The fractional decrease in power is proportional to the sample's thickness and the analyte's concentration, *C;* thus

$$\frac{-dP}{P} = \alpha C dx$$

where *P* is the power incident on the thin layer of sample, and a is a proportionality constant. Integrating the left side of above equation from *P* = *P*0 to *P* = *P*T, and the right side from *x* = 0 to *x* = *b,* where *b* is the sample's overall thickness

$$-\int_{P=P_0}^{P=P_T} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx$$

$$\ln\left(\frac{P_0}{P_T}\right) = \alpha b C$$

Converting from ln to log, and substituting above equation, gives

$$A = abC$$

where *a* is the analyte's absorptivity with units of cm–1 conc–1. When concentration is expressed using molarity, the absorptivity is replaced by the molar absorptivity, e (with units of cm–1 M–1)

$$A = \varepsilon bC$$

The absorptivity and molar absorptivity give, in effect, the probability that the analyte will absorb a photon of given energy. As a result, values for both *a* and e depend on the wavelength of electromagnetic radiation.

Beer's law The relationship between a sample'sabsorbance and the concentration of theabsorbing species (*A* = a*bC*).

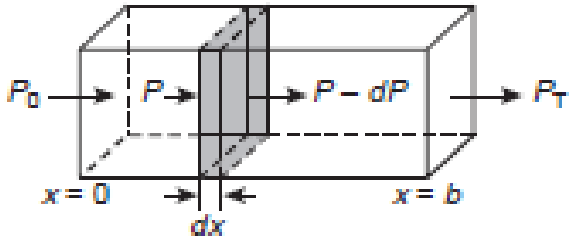


Figure 19 Factors used in deriving the Beer–Lambertlaw.

EXAMBLE / A 5.00 ´ 10–4 M solution of an analyte is placed in a sample cell that has a path length of 1.00 cm. When measured at a wavelength of 490 nm, the absorbance of the solution is found to be 0.338. What is the analyte's molar absorptivity at this wavelength?

SOLUTION

Solving equation 10.5 for ε and making appropriate substitutions gives

$$\varepsilon = \frac{A}{bC} = \frac{0.338}{(1.00\text{ cm})(5.00 \times 10^{-4}\text{ M})} = 676\text{ cm}^{-1}\text{ M}^{-1}$$

7.3.1. Beers Law and Multicomponent Samples

Beer's law can be extended to samples containing several absorbing components provided that there are no interactions between the components. Individual absorbances, A_i, are additive. For a two-component mixture of X and Y, the total absorbance, A_{tot}, is

$$A_{tot} = A_X + A_Y = \varepsilon_X bC_X + \varepsilon_Y bC_Y$$

Generalizing, the absorbance for a mixture of *n* components, *A*m, is given as

$$A_m = \sum_{i=1}^{n} A_i = \sum_{i=1}^{n} \varepsilon_i bC_i$$

7.3.2. Limitations to Beers Law

According to Beer's law, a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line with an intercept of 0 and a slope of ab or εb. In many cases, however, calibration curves are found to be nonlinear (Figure 20). Deviations from linearity are divided into three categories: fundamental, chemical, and instrumental.

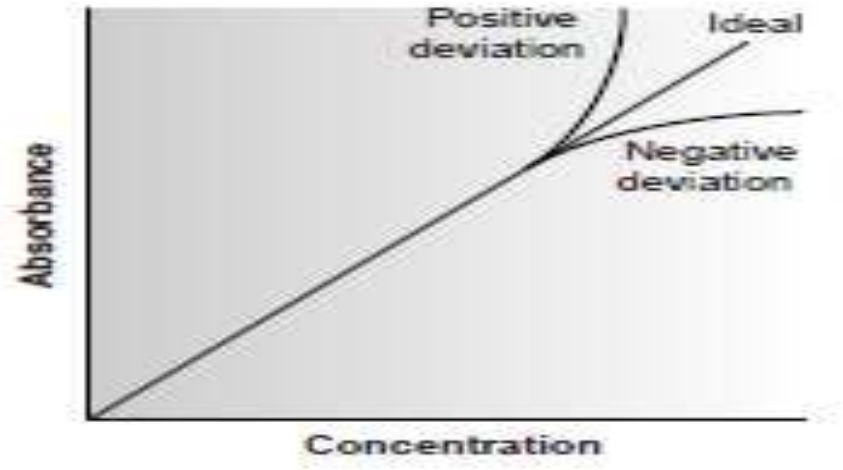


Figure 20 Calibration curves showing positive andnegative deviations from Beer's law.

7.3.2.1.Fundamental Limitations to Beers Law

Beer's law is a limiting law that is valid only for low concentrations of analyte. There are two contributions to this fundamental limitation to Beer's law. At higher concentrations the individual particles of analyte no longer behave independently of one another. The resulting interaction between particles of analyte may change the value of ε. A second contribution is that the absorptivity, *a*, and molar absorptivity, ε, depend on the sample's refractive index. Since the refractive index varies with the analyte's concentration, the values of *a* and e will change. For sufficiently low concentrations of analyte, the refractive index remains essentially constant, and the calibration curve is linear.

7.3.2.2.Chemical Limitations to Beers Law

Chemical deviations from Beer's law can occur when the absorbing species is involved in an equilibrium reaction. Consider, as an example, an analysis for the weak acid, HA. To construct a Beer's law calibration curve, several standards containing known total concentrations of HA, C_{tot}, are prepared and the absorbance of each is measured at the same wavelength. Since HA is a weak acid, it exists in equilibrium with its conjugate weak base, A–

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

If both HA and A– absorb at the selected wavelength, then Beers law is written as

$$A = \varepsilon_{HA} b C_{HA} + \varepsilon_A b C_A$$

where C_{HA} and C_A are the equilibrium concentrations of HA and A–. Since the weak acid's total concentration, C_{tot}, is

$$C_{tot} = C_{HA} + C_A$$

$$A = \varepsilon_A b C_{tot}$$

7.3.2.3.Instrumental Limitations to Beers Law

There are two principal instrumental limitations to Beer's law. The first limitation is that Beer's law is strictly valid for purely monochromatic radiation; that is, for radiation consisting of only one wavelength. However, even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Using polychromatic radiation always gives a negative deviation from Beer's law, but is minimized if the value of e is essentially constant over the wavelength range passed by the wavelength selector. For this reason, as shown in Figure 21, it is preferable to make absorbance measurements at a broad absorption peak. In addition, deviations from Beer's law are less serious if the effective bandwidth from the source is less than one tenth of the natural bandwidth of the absorbing species. When measurements must be made on a slope, linearity is improved by using a narrower effective bandwidth.

Stray radiation is the second contribution to instrumental deviations from Beer's law. Stray radiation arises from imperfections within the wavelength selector that allows extraneous light to "leak" into the instrument. Stray radiation adds an additional contribution, *P* stray, to the radiant power reaching the detector;thus

$$A = \log \frac{P_0 + P_{stray}}{P_T + P_{stray}}$$

For small concentrations of analyte, *P*stray is significantly smaller than P_0 and P_T, and the absorbance is unaffected by the stray radiation. At higher concentrations of analyte, however, *P*stray is no longer significantly smaller than *P*T and the absorbance is smaller than expected. The result is a negative deviation from Beer's law.

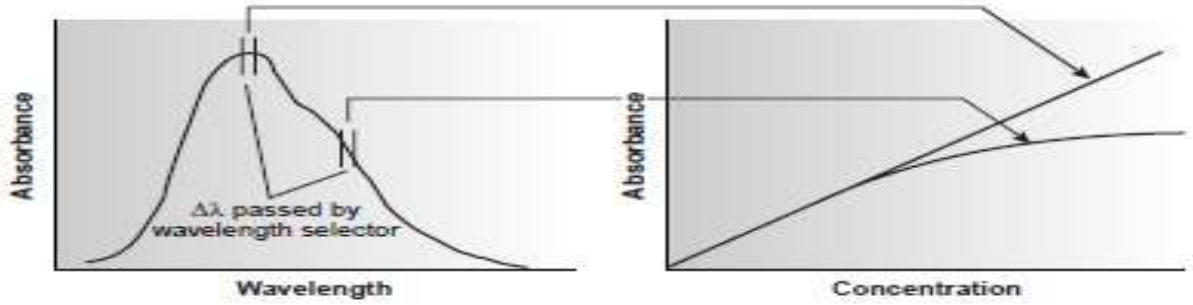


Figure 21 Effect of wavelength on the linearity of aBeer's law calibration curve.

The concentrations of Fe^{3+} and Cu^{2+} in a mixture can be determined following their reaction with hexacyanoruthenate (II), $Ru(CN)_6^{4-}$, which forms a purple-blue complex with Fe^{3+} ($\lambda_{max} = 550$ nm), and a pale green complex with Cu^{2+} ($\lambda_{max} = 396$ nm).[12] The molar absorptivities (M^{-1} cm^{-1}) for the metal complexes at the two wavelengths are summarized in the following table.

	ε_{550}	ε_{396}
Fe^{3+}	9970	84
Cu^{2+}	34	856

When a sample containing Fe^{3+} and Cu^{2+} is analyzed in a cell with a pathlength of 1.00 cm, the absorbance at 550 nm is 0.183, and the absorbance at 396 nm is 0.109. What are the molar concentrations of Fe^{3+} and Cu^{2+} in the sample?

SOLUTION

Substituting known values into equations 10.11 and 10.12 gives

$$A_{550} = 0.183 = 9970C_{Fe} + 34C_{Cu}$$

$$A_{396} = 0.109 = 84C_{Fe} + 856C_{Cu}$$

To determine the C_{Fe} and C_{Cu} we solve the first equation for C_{Cu}

$$C_{Cu} = \frac{0.183 - 9970C_{Fe}}{34}$$

and substitute the result into the second equation.

$$0.109 = 84C_{Fe} + 856\left(\frac{0.183 - 9970C_{Fe}}{34}\right) = 4.607 - (2.51 \times 10^5)C_{Fe}$$

Solving for C_{Fe} gives the concentration of Fe^{3+} as 1.80×10^{-5} M. Substituting this concentration back into the equation for the mixture's absorbance at a wavelength of 396 nm gives the concentration of Cu^{2+} as 1.26×10^{-4} M.

7.4. Buffer Systems

Buffer systems are systems in which there is a significant (and nearly equivalent) amount of a weak acid and its conjugate base—or a weak base and its conjugate acid—present in solution. This coupling provides a resistance to change in the solution's pH. When strong acid is added, it is neutralized by the conjugate base. When strong base is added, it is neutralized by the weak acid. However, too much acid or base will exceed the buffer's *capacity*, resulting in significant pH changes.

$$HA + H_2O \leftrightarrow H_3O^+ + A^-$$

$$A^- + H^+ \rightarrow HA$$

$$HA + OH^- \rightarrow H_2O + A^-$$

Consider an arbitrary weak acid, HA, and its conjugate base, A-, in equilibrium. The addition of a strong acid will cause only a slight change in pH due to neutralization. Likewise, the addition of a strong base will cause only a slight change in pH.

Buffers are useful when a solution must maintain a specific pH. For example, blood is a buffer system because the life processes in a human only function within a specific pH range of 7.35 to 7.45. When, for example, lactic acid is released by the muscles during exercise, buffers within the blood neutralize it to maintain a healthy pH.

7.4.1. Making a Buffer

Once again, let's consider an arbitrary weak acid, HA, which is present in a solution. If we introduce a salt of the acid's conjugate base, say NaA (which will provide the A- ion), we now have a buffer solution. Ideally, the buffer would contain equal amounts of the weak acid and conjugate base. Instead of adding NaA, what if a strong base were added, such as NaOH? In that case, the hydroxide ions would neutralize the weak acid and create water and A- ions. If the solution contained only A- ions, then a strong acid like HCl were added, they would neutralize and create HA.

7.4.2. Buffers and pH

To determine the pH of a buffer system, you must know the acid's **dissociation constant**. This value, *Ka* (or *Kb* for a base) determines the strength of an acid (or base). It is explored more thoroughly in the Equilibrium1 unit, but for now it suffices to say that this value is simply a measure of strength for acids and bases. The dissociation constants for acids and bases are determined experimentally.

The **Henderson-Hasselbalch equation** allows the calculation of a buffer's pH. It is:

$$\mathrm{pH} = \mathrm{pK}_a + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

For a buffer created from a base, the equation is:

$$\mathrm{pH} = \mathrm{pK}_b + \log \frac{[\mathrm{B}]}{[\mathrm{HB}^+]}$$

Using these equations requires determining the ratio of base to acid in the solution