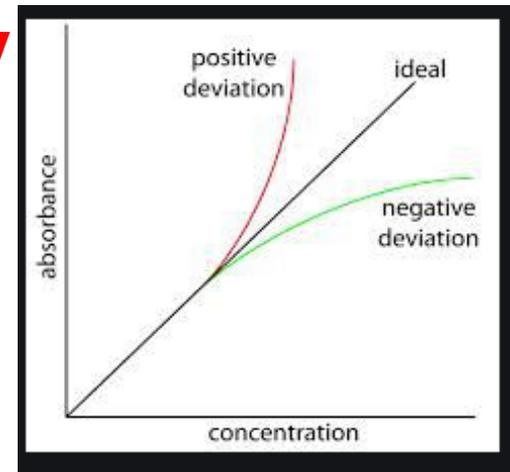# Chapter 13

UV-Visible Molecular Absorption Spectrophotometry

Apparent deviations from beer's law

- **Beer's law, a calibration plot of A vs. c from measurements on a series of standards should be linear with an intercept of zero.**

- **However, calibration curves are sometimes found to be nonlinear or have a nonzero intercept.**

- **These effects are rarely due to Beer's law being invalid, but rather are a consequence of measurement conditions in which the assumptions used to derive Beer's law are not valid.**

- **The terms positive deviation and negative deviation from linearity are used to describe nonlinear calibration curves that bend toward or away from the concentration axis, respectively.**

- **Hence in the case of a negative deviation, the calibration slope decreases with increasing analyte concentration.**

- **What specific instrumental and chemical effects that cause such apparent deviations from Beer's law?**

Assumptions for Beer's law derivation

TABLE 3-1

Assumptions of the absorption law

1. The incident radiation is monochromatic.
2. The absorbers (molecules, atoms, ions, etc.) act independently of each other.
3. The incident radiation consists of parallel rays, perpendicular to the surface of the absorbing medium.
4. The pathlength traversed is uniform over the cross section of the beam. (All rays traverse an equal distance of the absorbing medium.)
5. The absorbing medium is homogeneous and does not scatter the radiation.
6. The incident flux is not large enough to cause saturation effects. (Lasers can cause such effects, as discussed in Chapters 11 and 15.)

Nonzero Intercept

- **A nonzero intercept is usually due to an improper blank measurement or adjustment or to nonequivalent measurement conditions for the blank and standard solutions.**

- **The blank measurement should account for all the attenuation of the incident beam by the sample cell filled with standard solution except for that due to the analyte.**

- **This implies that the reference transmission factor T_r (attenuation by a sample cell filled with blank solution at a given wavelength) should be identical for the standard and blank measurements.**

- **Differences can occur because of an improper blank**

- **[i.e., the composition of the blank solution is different from the matrix of the standard solutions such that the absorption by nonanalyte species (or the refractive index which affects the reflection loss is different].**

- If different and nonequivalent sample cells are used for blank and standard measurements, differences in cell wall absorption and scattering or reflection properties can cause a nonzero intercept.

- With double-beam instruments, differences in cell properties are adjusted by zeroing the absorbance with blank in both cells.

- In single-beam instruments, either one sample cell should be used for all solutions or a measurement should be made with the blank solution in each cell. Correction factors can be developed to account for sample cells with different transmission characteristics.

- Drifts in the lamp radiance, PMT or signal processor gain, or detector responsivity can also cause apparent nonzero intercepts.

- For example, if the lamp radiance is constantly decreasing in a single-beam instrument and the blank measurement is always made before the standard measurement, the transmittance calculated will always be too low; this yields a positive intercept corresponding to the absorbance change due to drift between the blank and sample measurement.

- Random error can also result in a nonzero intercept.

- When calibration data are fit by least-squares methods, the intercept is normally not zero.

- Errors in preparation of standards can cause similar effects.

- Statistical methods can be used to evaluate the uncertainty in the intercept and determine if it differs significantly from zero.

Nonlinearity due to Chemical Equilibria

- If several species absorb at a given wavelength, the additive form of Beer's law can be used:

$$A = \sum_{i=1}^{n} A_i = b \sum_{i=1}^{n} \varepsilon_i c_i$$

- Assume that the blank compensates for the absorbance due to concomitants, but consider that the analyte can exist in several chemical forms in solution, which may be in equilibrium.
- If only one of the chemical forms absorbs at the monitored wavelength, we can rewrite Beer's law as

$$A = \varepsilon b f c_a$$ **Conc of absorbing form**

- f is the fraction of C_a that exists in the absorbing form.
- A linear calibration curve is observed only if f is independent of the analytical concentration (C_a).
- A negative or positive deviation in the calibration curve is observed if f decreases or increases with increasing C_a, respectively.

- The situation becomes more complex if two or more chemical forms of the analyte absorb at the analysis wavelength.

- Consider an equilibrium between a monomer (M) and a dimer (D) in solution (2M $\rightleftharpoons$ D) with an equilibrium constant K $(K = C_D/C^2_M)$.

- The total absorbance due to both species from the additive equation is given by

$$A = b(\varepsilon_D c_D + \varepsilon_M c_M)$$

- The analytical concentration (C_a) is found from

$$c_a = c_M + 2c_D = c_M + 2Kc^2_M \qquad (13\text{-}41)$$

- **If equation 13-41 is solved for C_M and C_D, and the results are substituted into equation 13-40, we obtain**

$$A = \frac{b}{2}\left[\varepsilon_D c_a + (2\varepsilon_M - \varepsilon_D)\frac{(8Kc_a + 1)^{1/2} - 1}{4K}\right]$$

$$(13\text{-}42)$$

- **If $\varepsilon_D = 2\,\varepsilon_M$, the second term vanishes and a linear calibration curve is obtained.**
- **In other cases, the contribution of the second term in equation 13-42 to the total absorbance decreases with increasing analytical concentration and causes a positive or negative deviation depending on the sign of the quantity $(2\,\varepsilon_M - \varepsilon_D)$**
-

$$A = \frac{b}{2} \left[\varepsilon_D c_a + (2\varepsilon_M - \varepsilon_D) \frac{(8Kc_a + 1)^{1/2} - 1}{4K} \right]$$

$$(13\text{-}42)$$

- If $\varepsilon_D > 2\varepsilon_M$, a positive deviation is observed
- By contrast, a negative deviation occurs if $\varepsilon_D < 2\varepsilon_M$ because the increase in dimer absorbance does not compensate for the loss in monomer absorbance.
- Negative and positive deviations due to dimer formation are observed with methylene blue when the absorbance is monitored at 664 and 600 nm, respectively.

- **Many types of equilibria involving the analyte are possible.**

- **For example, if a metal is determined by the absorbance of a metal complex, the total absorbance can be due to a number of complexes involving different numbers of ligands associated with the metal or polynuclear complexes.**

- **Each complex can have a different spectrum and thus different molar absorptivities at a given wavelength.**

- **The relative fraction of each metal complex can vary with the total metal concentration.**

- **Sometimes it is possible to choose the initial ligand concentration so that only one metal complex forms in a significant amount.**

- **Alternatively, with a sufficient excess of ligand, the fraction of each complex formed can be independent of the initial metal concentration.**

- **Many equilibria involving analyte species are pH dependent.**
- **A classic example is Cr(VI) in solution**

$$Cr_2O_7^{2-} + H_2O \rightleftharpoons 2H^+ + 2CrO_4^{2-}$$
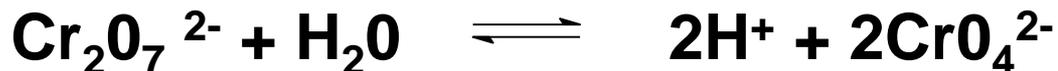
- **The relative fraction of yellow chromate to orange dichromate varies with the Cr(VI) concentration and the pH; positive or negative deviations can be observed depending on the monitored wavelength**
- **For such pH-dependent equilibria, the pH can be adjusted or buffered to force the equilibrium predominantly in one direction.**
- **For ex-ample, at high pH ($\geq$ 12), Cr(VI) exists primarily as yellow chromate, and a linear calibration curve is observed at an analysis wavelength of 370 nm.**
- **At very low pHs, nonlinearity is observed at most wavelengths because of equilibria between several absorbing species (e.g., $HCr_2O_7^-$, H_2CrO_4, chromium sulfate complexes).**

- **Equilibria involving metal complexes or the acid and base forms of a species are also pH dependent.**
- **Absorption spectra of phenol red at various pH values is shown below**
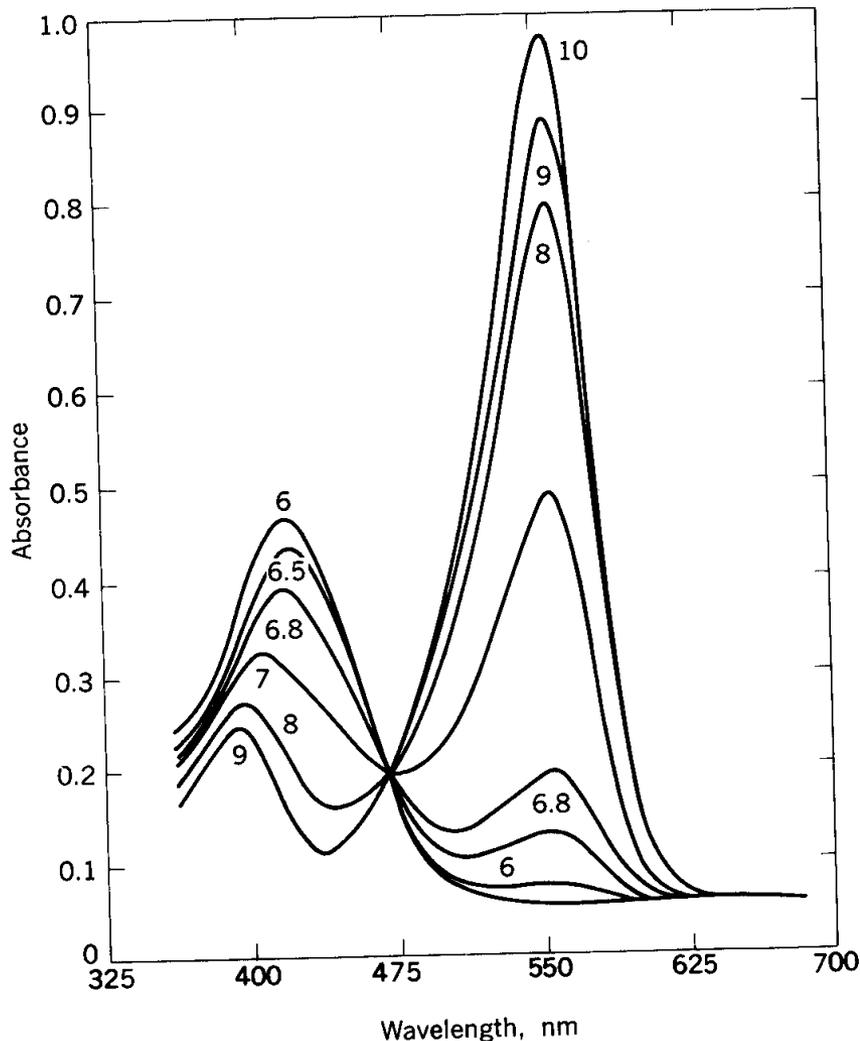
- **The absorbance at a given wavelength is given by $A = b(\varepsilon_u c_u + \varepsilon_p c_p)$ where the subscripts p and a refer to protonated and unprotonated forms of phenol red.**
- **At most wavelengths, A is seen to depend on pH because c_u and c_P vary with pH.**
- **At the isobestic point ($\lambda \sim 495$ nm), $\varepsilon_u = \varepsilon_u$ and A is proportional to the total indicator concentration, but A is independent of the pH and the fraction in each form.**

- **Absorption spectra of the indicator phenol red are shown as a function of pH.**

- **The plot of absorbance vs. total indicator concentration can be made linear by adjusting the pH of all solutions to a value that results in the indicator being totally unprotonated or protonated or that maintains a constant ratio for the concentration of the two forms for any total indicator concentration.**

منحنی کالیبراسیون خطی فنل رد
چگونه حاصل می شود؟

- The Figure shows that the absorbance is independent of pH at about 495 nm.
- This wavelength is called the isobestic or isoabsorptive point.
- An isobestic point is observed when two absorbing species in equilibrium have the same molar absorptivity at one wavelength.
- In fact, the observation of an isobestic point is a criterion to prove the existence of two interconvertible absorbing forms of a species with overlapping spectra.
- The isobestic point is an analytically useful wavelength because a linear calibration curve is obtained at this wavelength without controlling such solution conditions as pH.

EXAMPLE

- **The molar absorptivities of the weak acid HIn (Ka = 1.42 X 10^{-5}) and its conjugate base In⁻ at 430 and 570 nm were determined by measurements of strongly acidic and strongly basic solutions of the indicator (where essentially all of the indicator was in the HIn and In⁻ forms respectively). The results were**

	ϵ_{430}	ϵ_{570}
HIn	6.30×10^2	7.12×10^3
In⁻	2.06×10^4	9.61×10^2

Derive absorbance data for unbuffered solutions having total indicator concentrations ranging from 2 x 10^{-5} to 16 x 10^{-5} M.

- **Let us calculate the molar concentrations of [HIn] and [In⁻] of the two species in a solution in which the total concentration Of indicator is 2.00 X 10⁻⁵ M.**

Here,
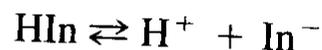
$$HIn \rightleftarrows H^+ + In^-$$

and

$$K_a = 1.42 \times 10^{-5} = \frac{[H^+][In^-]}{[HIn]}$$

From the equation for the dissociation process, we may write

$$[H^+] = [In^-]$$

Furthermore, the sum of the concentrations of the two indicator species must equal the total molar concentration of the indicator. Thus,

$$[In^-] + [HIn] = 2.00 \times 10^{-5}$$

Substitution of these relationships into the expression for K_a gives

$$\frac{[In^-]^2}{2.00 \times 10^{-5} - [In^-]} = 1.42 \times 10^{-5}$$

Rearrangement yields the quadratic expression

$$[In^-]^2 + 1.42 \times 10^{-5} [In^-] - 2.84 \times 10^{-10} = 0$$

The positive solution to this equation is

$$[In^-] = 1.12 \times 10^{-5}$$

$$[HIn] = 2.00 \times 10^{-5} - 1.12 \times 10^{-5}$$
$$= 0.88 \times 10^{-5}$$

We are now able to calculate the absorbance at the two wavelengths. Thus, substituting into Equation 7–10 gives

$$A = \epsilon_{In^-} b[In^-] + \epsilon_{HIn} b[HIn]$$

$$A_{430} = 2.06 \times 10^4 \times 1.00 \times 1.12 \times 10^{-5}$$
$$+ 6.30 \times 10^2 \times 1.00 \times 0.88 \times 10^{-5}$$
$$= 0.236$$

Similarly at 570 nm,

$$A_{570} = 9.61 \times 10^2 \times 1.00 \times 1.12 \times 10^{-5} +$$
$$7.12 \times 10^3 \times 1.00 \times 0.88 \times 10^{-5} = 0.073$$

Additional data, obtained in the same way, are shown in Table 7–2.

TABLE 7-2
Concentration and Absorbance Data Derived by the Technique Shown in Example 7-1

M_{HIn}	[HIn]	[In$^-$]	A_{430}	A_{570}
2.00×10^{-5}	0.88×10^{-5}	1.12×10^{-5}	0.236	0.073
4.00×10^{-5}	2.22×10^{-5}	1.78×10^{-5}	0.381	0.175
8.00×10^{-5}	5.27×10^{-5}	2.73×10^{-5}	0.596	0.401
12.00×10^{-5}	8.52×10^{-5}	3.48×10^{-5}	0.771	0.640
16.00×10^{-5}	11.9×10^{-5}	4.11×10^{-5}	0.922	0.887
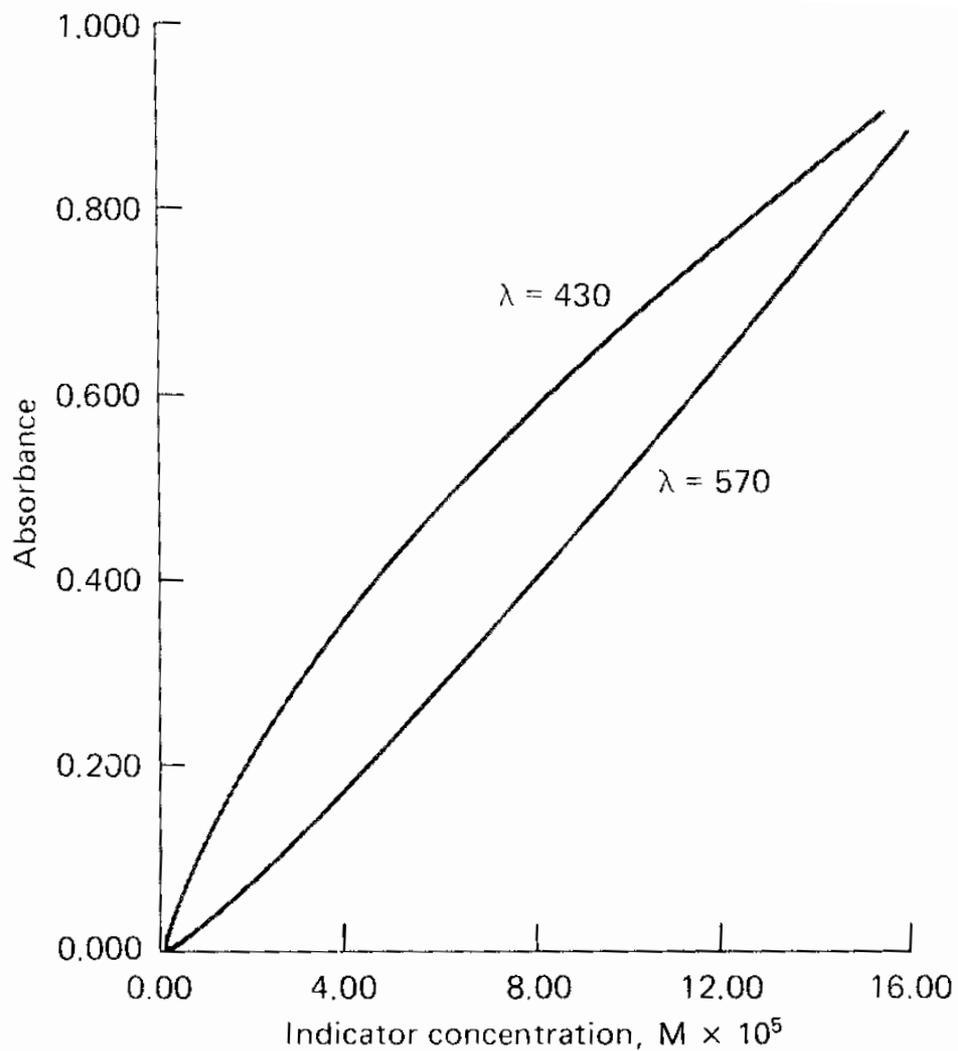
FIGURE 7–5 Chemical deviations from Beer's law for unbuffered solutions of the indicator HIn. For data, see Table 7–2.

Nonlinearity due to Other Chemical Effects

وابستگی ضریب جذب مولی به غلظت

- **Nonlinearity can also occur if the analyte molar absorptivity is dependent on the analyte concentration.**

- **Such effects are usually minor and occur at relatively high concentrations (> 10^{-2} M).**

- **Differences in solute-solvent interactions, solute-solute interactions, or hydrogen bonding at high concentrations can change the chemical or electrostatic environment and hence the absorptivity of the analyte.**

- **Changes in the solution refractive index (η) with analyte concentration can also cause nonlinearity by altering the position, size, or solid angle of the image transmitted to the detector, the reflectance loss at the cell wall-solution interface of the analyte molar absorptivity.** $\dfrac{\varepsilon\eta}{(\eta^2+2)^2}$

- **In the last case, ε in Beer's law is replaced by**

$$\frac{\varepsilon\eta}{(\eta^2+2)^2}$$

Nonlinearity due to Polychromatic Radiation

- Beer's law is valid only for monochromatic radiation
- Consider a simple example in which the sample cell is illuminated with two monochromatic lines of λ_1 and λ_2
- If we assume that Beer's law is followed for each wavelength; Transmittance, $T = E_s/E_r$ (Es and Er are readout signals). Then for a polychromatic radiation:

$$T = \frac{E_1 10^{-\varepsilon_1 bc} + E_2 10^{-\varepsilon_2 bc}}{E_1 + E_2} \qquad (13\text{-}43)$$

- where E_1 and E_2 are the reference readout signals observed at wavelengths λ_1 and λ_2, respectively, when measured separately.
- Let us assume that λ_1 is the desired wavelength and that the radiation at λ_2 represents the undesired contribution.
- If we let $r = E_2/E_1$, equation 13-43 becomes

$$T = \frac{10^{-\varepsilon_1 bc}}{1 + r} \times [1 + r \times 10^{(\varepsilon_1 - \varepsilon_2)bc}] \qquad (13\text{-}44)$$

- **Note that *r is* the weighting factor for the undesired contribution and accounts for differences in the source radiance, wavelength selector optical efficiency, reference transmission factor, or detector responsivity at the two wavelengths. From equation 13-44, the absorbance is given by**

$$A = \varepsilon_1 bc + \log(1 + r) - \log[1 + r \times 10^{(\varepsilon_1 - \varepsilon_2)bc}]$$

$$(13\text{-}45)$$

- **If this equation is differentiated with respect to *bc,* we find that the effective absorptivity *(ε)* is given by**

$$\varepsilon = \varepsilon_1 - \frac{r(\varepsilon_1 - \varepsilon_2)10^{(\varepsilon_1 - \varepsilon_2)bc}}{1 + r \times 10^{(\varepsilon_1 - \varepsilon_2)bc}} \qquad (13\text{-}46)$$

- **Clearly, *A is* not proportional to c and ε is not independent of c except when *r* equals 0 or∞ (monochromatic radiation) or $\varepsilon_1 = \varepsilon_2$**

- **Two limiting cases may be considered:**

1. bc ⟶ 0, Thus 13-46 equation becomes

$$\varepsilon = \frac{\varepsilon_1 + r\varepsilon_2}{1 + r}$$

- **Linearity is observed with an effective molar absorptivity that is a weighted average of ε_1 and ε_2.**
- **For large absorbance *(bc ---> ∞)* with $\varepsilon_1 \gg \varepsilon_2$ we find that $\varepsilon = \varepsilon_2$.**
- **Hence the least absorbed wavelength controls the calibration slope because effectively all the radiation at λ_1 is absorbed, and further increases can only attenuate the radiation at λ_2 .**

Apparent instrumental deviations with polychromatic radiation

- **Adherence to Beer's law is observed only with truly monochromatic radiation**

- **Unfortunately, the use of radiation that is restricted to a single wavelength is seldom practical, because devices that isolate portions of the output from a continuous source produce a more or less symmetric band of wavelengths around the desired one**

The following derivation shows the effect of polychromatic radiation on Beer's law.

Consider a beam consisting of just two wavelengths λ' and λ''. Assuming that Beer's law applies strictly for each of these individually, we may write for radiation λ'

$$A' = \log \frac{P_0'}{P'} = \epsilon'bc$$

or

$$P_0'/P' = 10^{\epsilon'bc}$$

and

$$P' = P_0'10^{-\epsilon'bc}$$

Similarly, for λ''

$$P'' = P_0''10^{-\epsilon''bc}$$

When an absorbance measurement is made with radiation composed of both wavelengths, the power of the beam emerging from the solution is given by $(P' + P'')$ and that of the beam from the solvent by $(P_0' + P_0'')$. Therefore, the measured absorbance A_M is

$$A_M = \log \frac{(P_0' + P_0'')}{(P' + P'')}$$

Substituting for P' and P'' yields

$$A_M = \log \frac{(P_0' + P_0'')}{(P_0'10^{-\epsilon'bc} + P_0''10^{-\epsilon''bc})}$$

or

$$A_M = \log(P_0' + P_0'') - \log(P_0'10^{-\epsilon'bc} + P_0''10^{-\epsilon''bc})$$

Now, when $\epsilon' = \epsilon''$, this equation simplifies to

$$A_M = \epsilon'bc$$

- **Relationship between A_m and concentration is no longer linear when the molar absorptivities differ**
- **Moreover, greater departures from linearity can be expected with increasing differences between ε' and ε''.**
- **This derivation can be expanded to include additional wavelengths; the effect remains the same.**
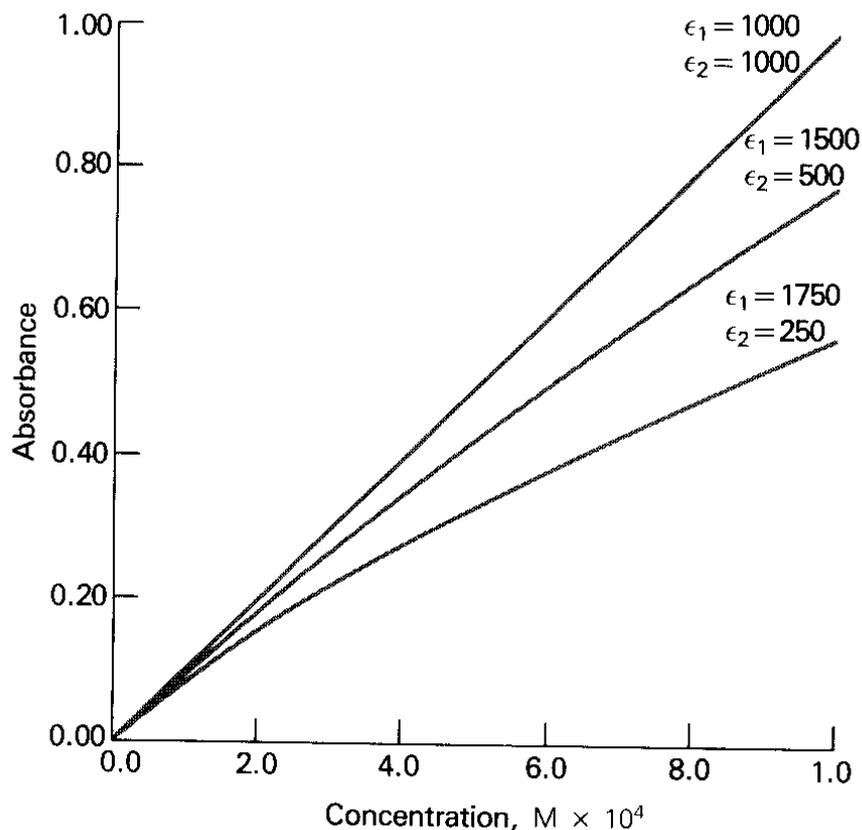
- **Deviations from Beer's law with polychromatic light.**

- **Here, two wavelengths or radiation λ_1, and λ_2 have been assumed for which the absorber has the indicated molar absorptivities.**

- **It is an experimental fact that deviations from Beer's law resulting from the use of a polychromatic beam are not appreciable, provided the radiation used does not encompass a spectral region in which the absorber exhibits large changes in absorption as a function of wave-length. This observation is illustrated in the Figure.**
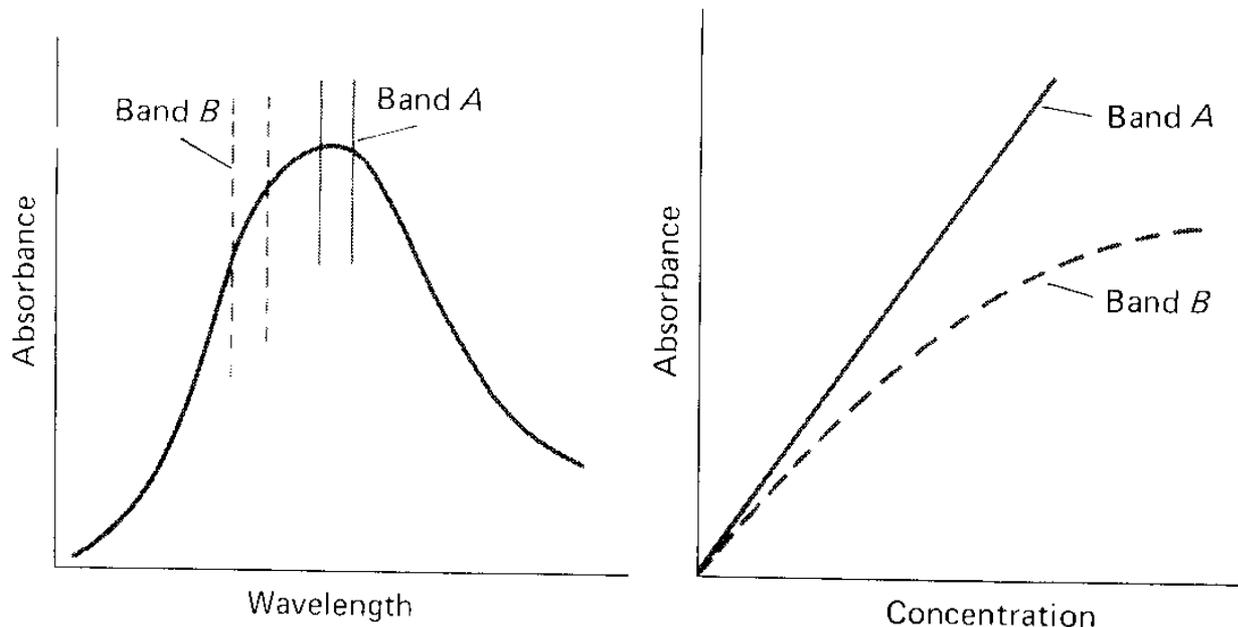

- **The effect of polychromatic radiation upon the Beer's law relationship.**
- **Band A shows little deviation, because ε does not change greatly throughout the band.**
- **Band B shows marked deviations because ε undergoes significant changes in this region.**

- It is also found experimentally that for absorbance measurements at the maximum of narrow peaks, departures from Beer's law are not significant if the effective bandwidth of the monochromator or filter $\Delta\lambda_{eff}$ is less than 1/10 of the half width of the absorption peak at half height.

- Half-widths of many absorption bands are in the range 50 to 100 nm.

- Thus a spectral bandpass of 5 nm or less is usually adequate.

- However, there are many exceptions to this rule. For example, the absorption bands of rare earth ions are quite narrow.

- Significant nonlinearity due to polychromatic radiation is observable with a spectral bandpass greater than 1 nm, as illustrated in the Figure
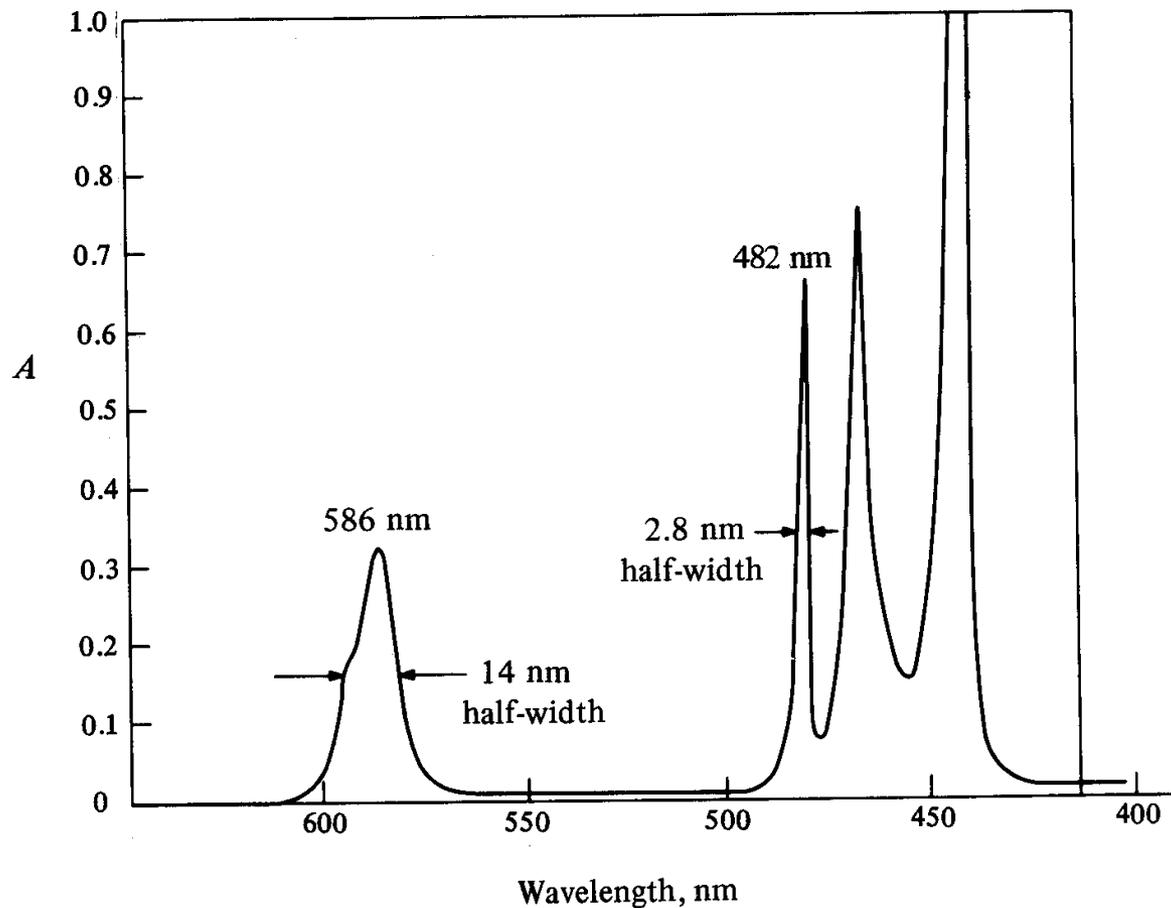
(a)

The absorption spectrum of 0.25 M Pr $^{3+}$ 1M HCl is shown to possess several narrow absorption bands.

(b)
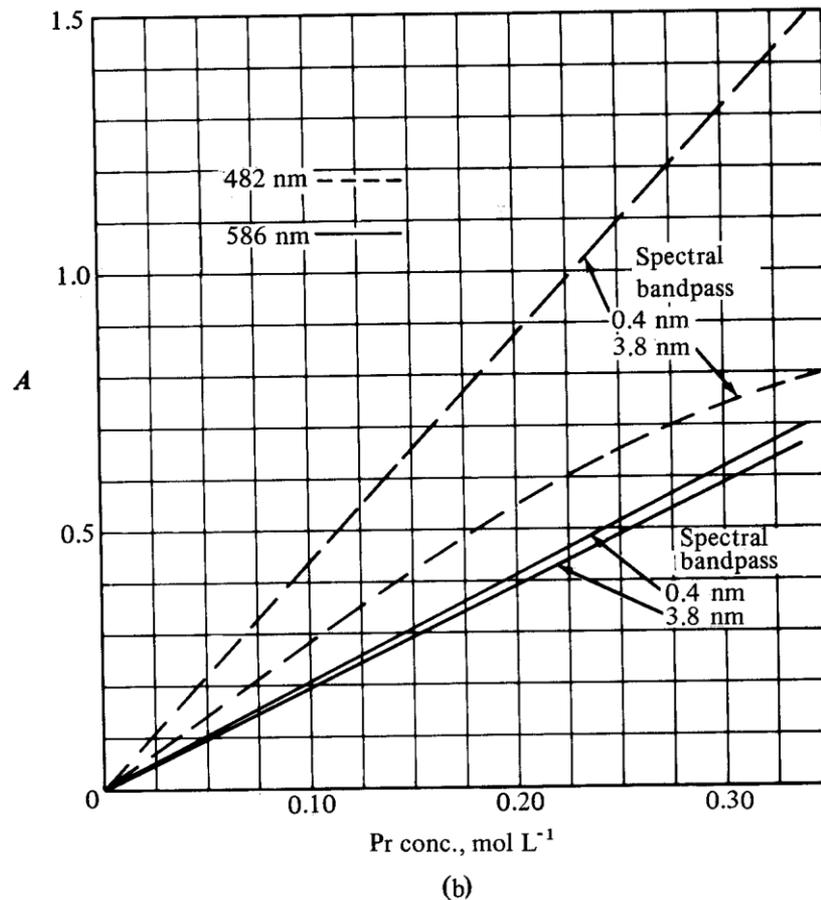
- **The calibration curves are for measurements at 482 and 586 nm with spectral bandpasses of 0.4 and 3.8 nm.**
- **For the narrow band at 483 nm, there is a significant decrease in the initial slope and negative deviation at higher concentrations with a 3.8 nm spectral bandpass** $, s/\Delta\lambda = 1.36 \geq 0.1$

- In general, absorption bands in the UV region are narrower than those in the visible region.

- In the gas phase, absorption bands exhibit vibronic structure as discussed earlier, such that a spectral bandpass less than 1 nm is necessary to record faithfully the absorption spectrum and to make quantitative measurements.

Nonlinearity due to Stray Radiation

- **Stray radiation or stray light occurs when the wavelength selector passes wavelengths outside its nominal range.**

- **For a monochromator, the amount of stray radiation is normally expressed as the stray radiation fraction (f) or percent (% f), defined as**

Stray radiant power

$$f = \frac{\Phi_{SR}}{\Phi_0}$$

Radiant power output Within $\lambda_0 \pm s$

- **The stray radiation readout fraction (r) or the relative contribution of stray radiation to the ideal reference signal can be defined as**

$$r = \frac{E_{SR}}{E_r}$$

- **In a spectrophotometer, room light leaks into the sample compartment or detector housing can also contribute to the total stray radiation signal.**
- **The presence of stray radiation usually causes the measured transmittance to be larger than it should be because the majority of the stray radiation is at wavelengths less strongly absorbed by the analyte than radiation within the bandpass.**
- **As the analyte concentration increases, this effect progressively worsens and causes a negative deviation in the calibration curve because the stray radiant power transmitted becomes a larger fraction of the total transmitted radiation power.**

- **For simplicity we will first assume that the stray radiation is not absorbed by the analyte.**
- **Thus the read-out signal due to the stray radiation component is identical for the reference and analyte solutions.**
- **In this case, the measured transmittance (T') is given by**
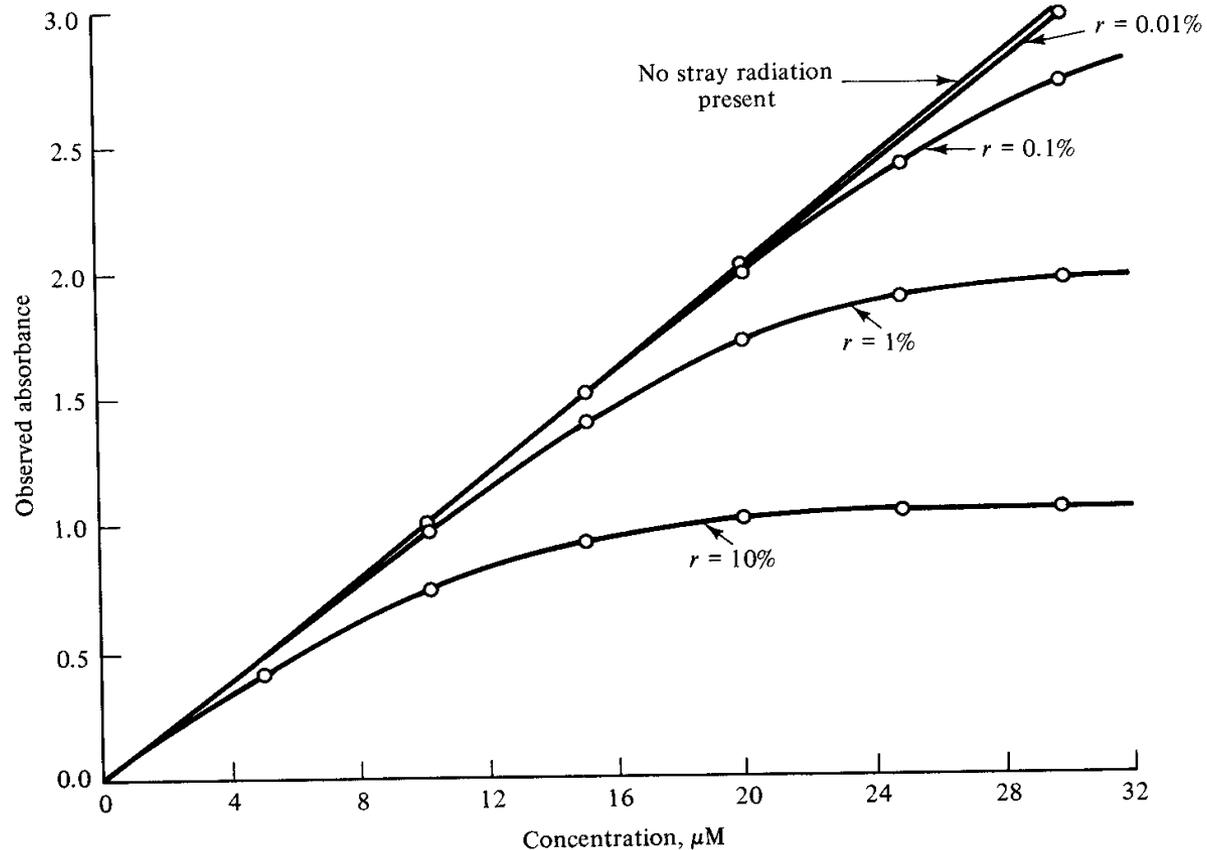
$$T' = \frac{E_s + E_{SR}}{E_r + E_{SR}}$$

- **Divide by E_r and use the definition for r**

$$T' = \frac{T + r}{1 + r} \approx T + r$$

- **where T is the ideal transmittance when r = 0.**
- **The measured absorbance (A') is given**

$$A' = -\log T' = -\log(T + r) + \log(1 + r)$$
$$\approx -\log(T + r)$$

Effect of stray radiation

- Calibration plots derived from equation for A' given above are shown for different values of the stray radiation readout fraction *(r)* where $\varepsilon b = 10^5$ *L* mol^{-1}.
- *As r* increases in value, the degree of negative deviation increases and the maximum limiting value of A' decreases.

Other instrumental causes of nonlinearity

- **Variability in pathlength**
- **Multiple reflections**
- **Circular dichroism**
- **Fluorescence**
- **Miscellaneous: Noniead performance of the detector, signal processing or readout device**

Methodology and performance

- **Sample treatment**
- **Choice of solution conditions**
- **Choice of wavelength and spectral bandpass**
- **Choice of instrument**
- **Performance characteristics: Precision, detection limit, linearity, accuracy,**

Applications

Qualitative Analysis

- **The spectral position of an absorption band is indicative of the presence or absence of certain structural features or functional groups in a molecule**

- **Also, compilations of UV-visible absorption spectra of many compounds are available to compare to the absorption spectrum of a pure unknown.**

- **Usually, a match between the reference and unknown spectra is not sufficient proof of the identity of the compound because the positions and intensities of the few absorption bands are not greatly affected by minor differences in structure, particularly for large molecules.**

- **Thus spectrophotometry is not considered a major tool for qualitative analysis; such techniques as NMR, IR, and mass spectrometry are more often employed for positive identification.**

Fundamental Applications

- **Spectrophotometry is a major tool for studying chemical equilibria and kinetics.**

- **Often it is possible to choose appropriate wavelengths to monitor the absorbance(s)of one or more reactants, products, or intermediates in the presence of other species.**

- **The concentrations are then determined by applying Beer's law and known molar absorptivities.**

- **For equilibrium studies, known concentrations of reactants are mixed, and the absorption spectrum of the reaction mixture is obtained after equilibrium is reached.**

- **The final concentrations of reactants and products are determined from the measured absorbances at selected wavelengths and stoichiometric relationships; these are then used to calculate equilibrium constants.**

- **Spectrophotometry is also used to determine the stoichiometry of reactions and, in particular, of metal complexes.**

- **The dependence of the solution absorbance on the ratio of the metal ion (M) and ligand (L) analytical concentrations is used to determine the molar ratio *l/m***

- **For simplicity, assume that only one complex is formed and that only the complex absorbs at the monitored wavelength.**

Molar ratio method

- The absorbances of a series of solutions with different ligand-to-metal ion concentration ratios are measured
- The metal ion analytical concentration (c_m) is kept constant while the ligand analytical concentration (C_L) is varied.
- As shown in the Figure, the value of c_L/c_M at the break point in the plot of A vs. c_L/c_M equals l/m.
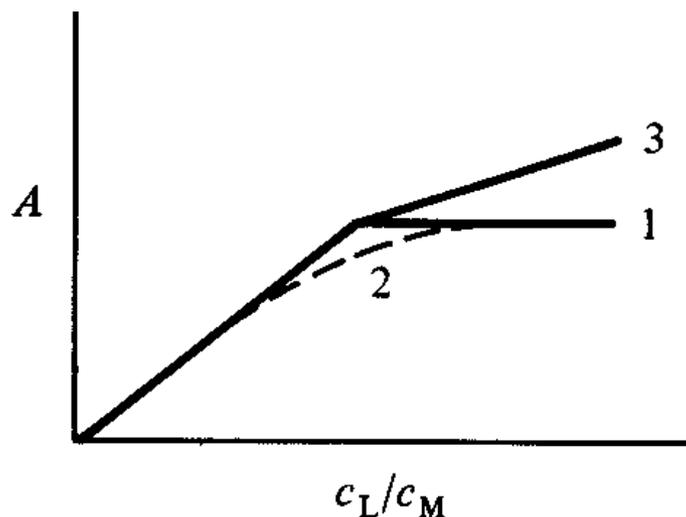
Continuous variations or Job's method

- The absorbance of a series of solutions is measured in which the mole fraction X of the ligand is varied from 1 to 0 as shown in the Figure
- The total number of moles of L and M is held constant.
- The mole fraction yielding the maximum absorbance X_m is related to the stoichiometric ratio in the complex by

  $l/m = X_m(1 - X_m)$.
- In both the molar ratio and continuous variation methods, negligible dissociation of the complex is often assumed; extrapolation techniques can be used if the dissociation is not excessive as shown in the figure.
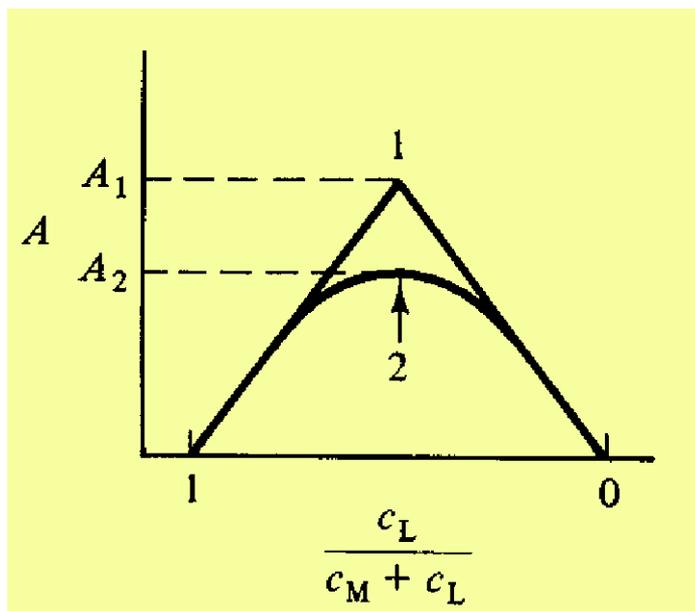
Mollard method

- It involves the measurement of the absorbance of only two solutions.
- One measurement is made with metal ion analytical concentration c_m and a large excess of ligand to yield absorbance A_M.
- The second absorption measurement is made on a solution with an excess of metal ion compared to the ligand analytical concentration c,, to yield absorbance A_1,.
- The amount of excess reactant in both cases is adjusted so that dissociation of the complex is negligible and the concentration of the metal complex is determined by the concentration of limiting species.
- Under these conditions,

$$l/m = (c_L A_M)/(c_M A_L)$$

Slope ratio method

- **It is a variation of the Mollard method**
- **A series of solutions with excess L and different c_m and with excess M and different c_L are prepared.**
- **The ratio of the slopes of plots of A_M vs. c_m and A_L VS- c_L is equal to l/m.**
- **Curvature of the plots indicates that dissociation of the complex is significant.**

Example

- consider the continuous variations method applied to a simple 1:1 metal complex (l = m = 1).

- If A_1 and A_2 are the extrapolated and measured absorbances at the maximum, respectively, the equilibrium concentrations (denoted by brackets) are given by

$$[ML] = c_M \frac{A_2}{A_1}$$

$$[M] = [L] = c_M \left(1 - \frac{A_2}{A_1}\right)$$

where c_M is the initial (analytical) metal ion concentration. Thus the formation constant, $K = [ML]/[M][L]$, is calculated from

$$K = \frac{A_2/A_1}{c_M(1 - A_2/A_1)^2}$$

- **In kinetics studies, spectrophotometry is used to monitor the disappearance of reactants or the formation of products or intermediates.**
- **If Beer's law is valid for the absorbing species, the rate can be calculated from the rate of change in absorbance**
  _[dc/dt = (dA/dt)/εb]._
- **Thus absorbance monitoring provides a tool to deter-mine rate laws and rate constants and to elucidate the mechanisms of reactions.**
- **Some other fundamental applications include the determination of molecular parameters and the molecular weight.**
- **The shape, width, and wavelength of the absorption bands of a pure compound can be used to elucidate the energy-level structure of the molecule,**
- **If a compound is derivatized with a reagent possessing a chromophore of known molar absorptivity that is not affected by the coupling, the molecular weight (MW) can be calculated from the measured absorbance and the formula**

$$MW = \frac{\varepsilon c b}{A} \qquad (13\text{-}56)$$

where c is concentration of the species in g L^{-1}.

Conventional Quantitative Determinations

- The analyte is usually determined by measuring the absorbance of an absorbing product after equilibrium has been reached for the reaction of the analyte with selective reagents.

- Many analytical reactions have been proposed for a host of species, and often numerous derivatization reactions are available for a given analyte.

- Only a small percentage of all developed procedures are accepted and used routinely.

- In certain applications, the absorbance of the analyte is measured directly provided that it is the predominant absorbing species at the analysis wavelength or if it is separated from potential absorbing species before detection.

- For example, nitrate in natural water is determined by measuring the absorbance at 220 nm.

- This technique is normally used only as a screening procedure because dissolved organic matter can also absorb at the same wavelength.

- Often a second absorbance measurement is made at 270 nm, where nitrate does not absorb and is used to correct for interferences from organic species.

Determination of Inorganic Species

- **Molecular absorption spectrophotometry is widely used to determine metals, cationic species, anionic species, and complex ions.**

- **Although atomic spectrometric techniques are most commonly used to determine metals because of the selectivity and detectability provided, molecular absorption spectrophotometry is still used in selected applications.**

- **Molecular spectrophotometric instrumentation is usually less expensive and can be made more portable for field studies or highly automated for high sample throughput.**

- **Spectrophotometric techniques can also be used to determine specific oxidation states of metal ions.**

Determination of Organic Species

- The analytical reactions used for the determination of many organic species can be organized around the specific functional group in the molecule that reacts with the reagent(s).

- The Table lists some common reagents used for different functional groups and typical molar absorptivities for the absorbing products that are meas-ured.

- The specificity and efficiency of the analytical reaction and the molar absorptivity depend on the type and placement of other functional groups in the molecule.

- Because many of these reactions are selective for only a type of functional group, they are used when the analyte is the primary species in the sample with the reactive moiety.

Multicomponent Determinations

- **The additivity of absorbances from several species is the basis for determining several analytes in one sample.**
- **For two species x and y, the absorbance of the sample is measured at two wavelengths and**

$$A_1 = b(\varepsilon_{x1}c_x + \varepsilon_{y1}c_y) \qquad (13\text{-}62)$$

$$A_2 = b(\varepsilon_{x2}c_x + \varepsilon_{y2}c_y) \qquad (13\text{-}63)$$

where the subscripts 1 and 2 indicate that quantity measured at λ_1 and λ_2, respectively. These two equations can be solved for c_x and c_y in terms of measured or known quantities:

$$c_x = \frac{\varepsilon_{y2}A_1 - \varepsilon_{y1}A_2}{b(\varepsilon_{y2}\varepsilon_{x1} - \varepsilon_{y1}\varepsilon_{x2})} = \alpha_1 A_1 - \alpha_2 A_2 \qquad (13\text{-}64)$$

$$c_y = \frac{\varepsilon_{x1}A_2 - \varepsilon_{y1}A_2}{b(\varepsilon_{y2}\varepsilon_{x1} - \varepsilon_{y1}\varepsilon_{x2})} = \beta_1 A_1 - \beta_2 A_2 \qquad (13\text{-}65)$$
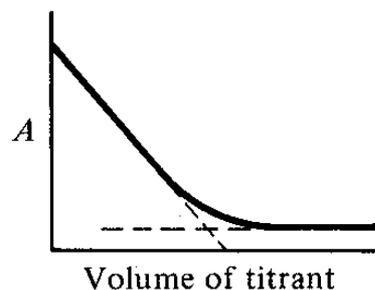
Spectrophotometric Titrations

- A titration is the process by which the quantity of an analyte (A_n) is determined by adding a standard solution of a titrant (T) with which the analyte reacts in a known and stoichiometric manner.

- A detector (manual or automatic) is required to signal when the amount of titrant added is chemically equivalent to the amount of analyte present.

- This point of chemical equivalence is called the equivalence point, while that actually measured is denoted the endpoint.

- If a good endpoint detector and reaction are used, the accuracy of a titration depends predominantly on the accuracy with which the titrant concentration is known.

- The latter is usually determined by titration against a primary standard substance.

- In spectrophotometric titrations, the spectrophotometer serves as the detector, monitoring the sample transmittance or absorbance at a suitable wavelength during the addition of increments of the titrant. The spectrophotometer sample cell is the titration vessel.

- For a titration reaction of the form

    An + T ⇆ P

- where P is the product, the absorption due one or more of the species involved is monitored.

- Instrumental titration methods can also be based on _fluorescence_ or _chemiluminescence_ detection

- The plot of the absorbance versus titrant volume is called a spectrophotometric titration curve and many shapes are possible, depending on the absorbing species

- Normally, the absorbance values plotted are corrected for dilution by the titrant by multiplying the measured absorbance values by $(V_T + V_{An})/V_{An}$ where V_{An} and V_T are the volumes of the sample containing the analyte and the titrant, respectively.

-

- Ideally, the endpoint is indicated by a sharp change in absorbance at the intersection of the two straight-line regions of differing slopes on either side of the equivalence point region.

- If the titration or indicator reaction is not quantitative near the equivalence point, a rounded intersection results.

- The linear segments bfore and after the endpoint can be extrapolated to locate the endpoint if conditions are adjusted so that Beer's law applies.
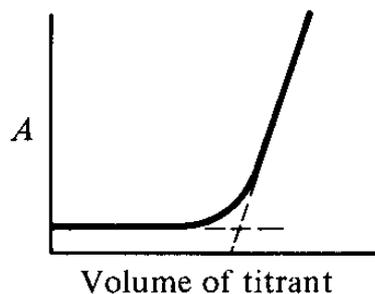
(a)

(c)
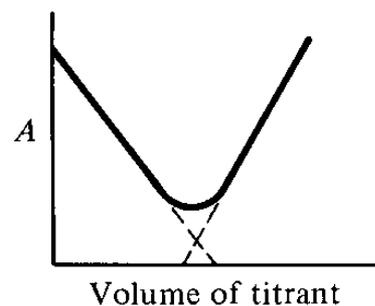
(e)

Shapes of spectrophotometric titration curves. The curve in

(a) is typical of a titration where only the titrant (T) absorbs [titration of As(III) with bromine] or where an indicator reacts with excess T.
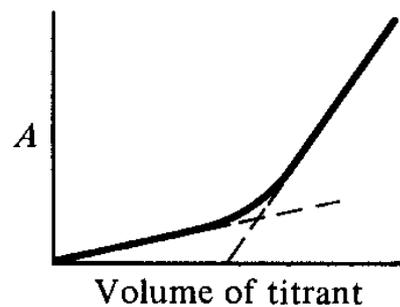
(c) When an absorbing analyte is converted to a nonabsorbing product with a nonabsorbing titrant,

(e) an absorbing analyte is converted to a non-absorbing product by an absorbing titrant.
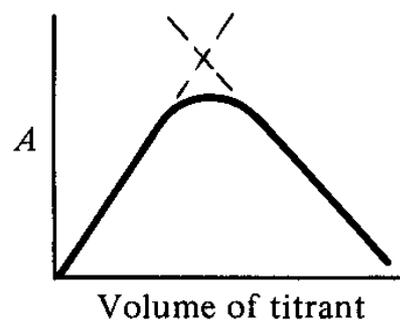
(b)

(d)

(f)

Shapes of spectrophotometric titration curves. (b) is characteristic of a titration reaction in which the only the product absorbs (e.g., titration of Cu(II) with EDTA).
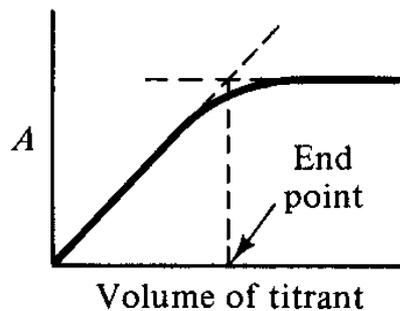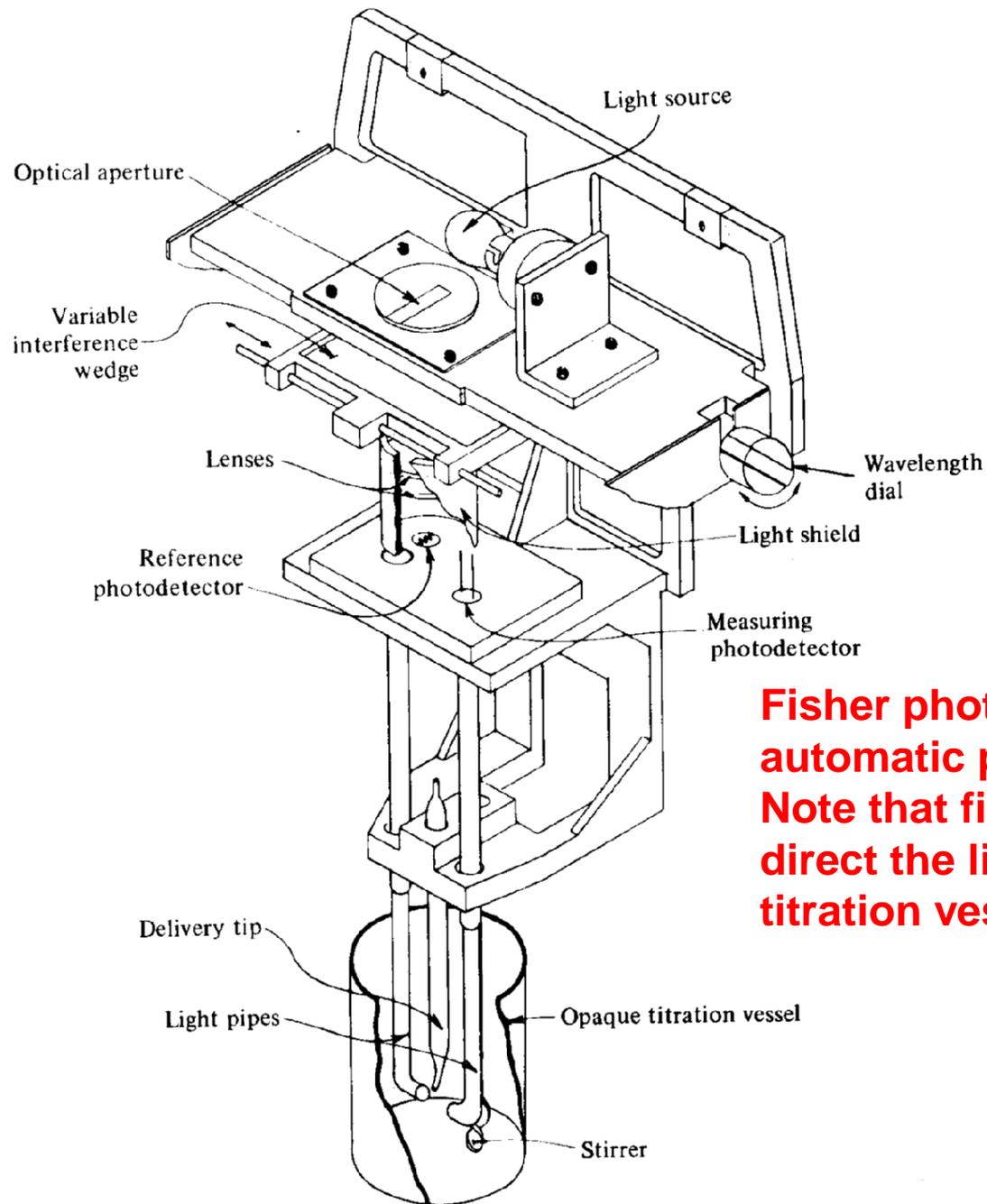
In curves (d) to (f), two species in-volved in the titration reaction absorb. In figure (e) an absorbing analyte is converted to a non-absorbing product by an absorbing titrant. The curve in (d) could result if both the titrant and product absorb but the analyte does not absorb or if two different metal complexes with different molar absorptivities are formed at the monitored wavelength during the titration of a nonabsorbing metal with a nonabsorbing ligand. The latter sit-uation could also produce a titration curve of the shape shown in (f). In all the cases above, the rounding near the endpoint is due to the titration reaction not going to completion. The dashed ex-trapolated portions indicate the behavior ob-served if the titration reaction goes to completion.

Advantages of spectrophotometric titrations

- The determination of the endpoint can be more precise because the spectrophotometer can better detect smaller changes in color shade or absorbance than the eye.

- The titration could be carried out in turbid or colored solution

- Titration reactions with unfavorable equilibrium constants (i.e., where the color change at the endpoint in not sharp) can be employed because measurements taken away from the endpoint can be used to extrapolate to the true endpoint.

- These advantages allow titrations to be extended to lower analyte concentrations ($\leq 10^{-4}$ M).

- In contrast to conventional spectrophotometric measurements, it is not necessary to measure the absolute absorbance or to calibrate the absorbance scale with standards.
- The presence of other species which absorb or scatter does not cause interference.
- Concomitants that react with the titrant do interfere.
- Analytical reactions can be used in cases where neither the analyte nor its reaction product absorb if a suitable absorbing indicator is available to react with the excess titrant.
- A sufficient excess of indicator must be added to provide a reasonable linear region beyond the equivalence point
- .Commercial spectrophotometric titration systems are available which include a spectrophotometer or photometer, a titration vessel, an automated buret, and often specialized data handling and plotting capabilities.
- A diagram of a commercial titration accessory is shown in the Figure

Light source

Optical aperture

Variable interference wedge

Lenses

Wavelength dial

Light shield

Reference photodetector

Measuring photodetector

Delivery tip

Light pipes

Opaque titration vessel

Stirrer

Fisher photometric accessory for automatic photometric titrations. Note that fiber optics are used to direct the light beam through the titration vessel.