

Methods of Analysis

A determination of the *stoichiometric ratio* of ligand to metal or donor to acceptor (n) and a quantitative expression of the *stability constant* for complex formation are important in the study and application of coordination compounds. A limited number of the more important methods for obtaining these quantities is presented here.

Method of Continuous Variation

Job suggested the use of an additive property such as the spectrophotometric extinction coefficient (dielectric constant or the square of the refractive index may also be used) for the measurement of complexation. If the property for two species is sufficiently different and if no interaction occurs when the components are mixed, then the value of the property is the weighted mean of the values of the separate species in the mixture. This means that if the additive property, say dielectric constant, is plotted against the mole fraction from 0 to 1 for one of the components of a mixture where no complexation occurs, a linear relationship is observed, as shown by the dashed line in Figure 10-7. If solutions of two species A and B of equal molar concentration (and hence of a fixed total concentration of the species) are mixed and if a complex forms between the two species, the value of the additive property will pass through a maximum (or minimum), as shown by the upper curve in Figure 10-7. For a constant total concentration of A and B , the complex is at its greatest concentration at a point where the species A and B are combined in the ratio in which they occur in the complex. The line therefore shows a break or a change in slope at the mole fraction corresponding to the complex. The change in slope occurs at a mole fraction of 0.5 in Figure 10-7, indicating a complex of the 1:1 type.

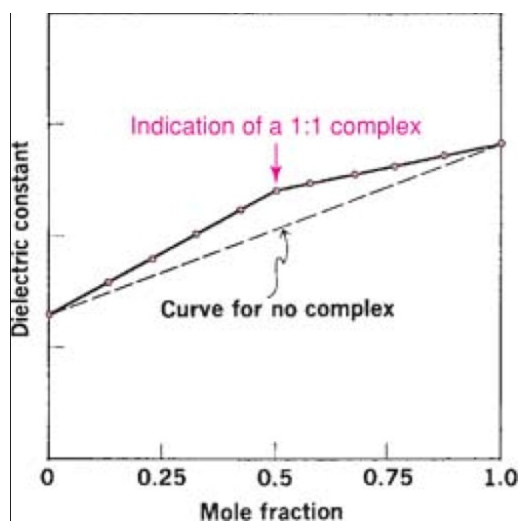


Fig. 10-7. A plot of an additive property against mole fraction of one of the species in which complexation between the species has occurred. The dashed line is that expected if no complex had formed.

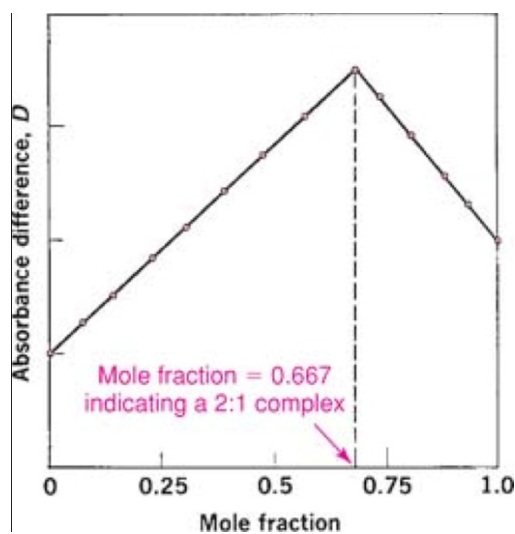


Fig. 10-8. A plot of absorbance difference against mole fraction showing the result of complexation.

When spectrophotometric absorbance is used as the physical property, the observed values obtained at various mole fractions when complexation occurs are usually subtracted from the corresponding values that would have been expected had no complex resulted. This difference, D , is then plotted against mole fraction, as shown in Figure 10-8. The molar ratio of the complex is readily obtained from such a curve. By means of a calculation involving the concentration and the property being measured, the stability constant of the formation

If the magnitude of the measured property, such as absorbance, is proportional only to the concentration of the complex MA_n , the molar ratio of ligand A to metal M and the stability constant can be readily determined. The equation for complexation can be written as



and the stability constant as

$$K = \frac{[MA_n]}{[M][A]^n} \quad (10-4)$$

or, in logarithmic form,

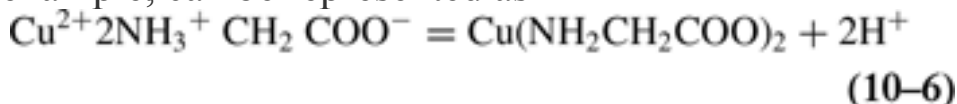
$$\log [MA_n] = \log K + \log [M] + n \log [A] \quad (10-5)$$

where $[MA_n]$ is the concentration of the complex, $[M]$ is the concentration of the uncomplexed metal, $[A]$ is the concentration of the uncomplexed ligand, n is the number of moles of ligand combined with 1 mole of metal ion, and K is the equilibrium or *stability* constant for the complex. The concentration of a metal ion is held constant while the concentration of ligand is varied, and the corresponding concentration, $[MA_n]$, of complex formed is obtained from the spectrophotometric analysis.⁴¹ Now, according to equation(10-5), if $\log [MA_n]$ is plotted against $\log [A]$, the slope of the line yields the stoichiometric ratio or the number n of ligand molecules coordinated to the metal ion, and the intercept on the vertical axis allows one to obtain the stability constant, K , because $[M]$ is a known quantity.

Job restricted his method to the formation of a single complex; however, was modified it so as to treat the formation of higher complexes in solution. and used spectrophotometric techniques to investigate 1:2 metal–ligand complexes of copper and barbiturates. A greenish-yellow complex is formed by mixing a blue solution of copper (II) with thiobarbiturates (colorless). By using the Job method, an apparent stability constant as well as the composition of the 1:2 complex was obtained.

pH Titration Method

This is one of the most reliable methods and can be used whenever the complexation is attended by a change in pH. The chelation of the cupric ion by glycine, for example, can be represented as

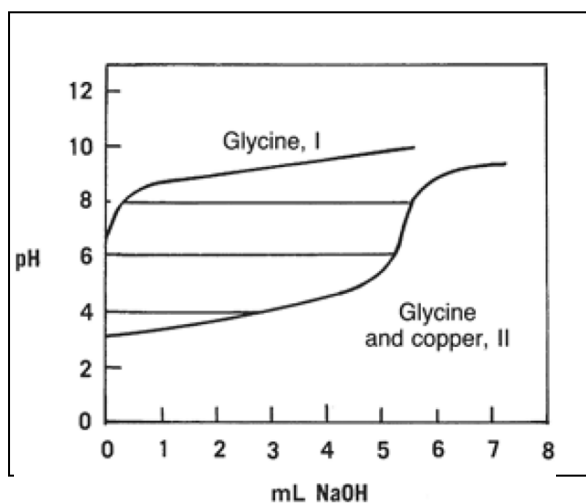


Because two protons are formed in the reaction of equation (10-6), the addition of glycine to a solution containing cupric ions should result in a decrease in pH. Titration curves can be obtained by adding a strong base to a solution of glycine and to another solution containing glycine and a copper salt and plotting the pH against the equivalents of base added. The results of such a potentiometric titration are shown in Figure 10-9. The curve for the metal–glycine mixture is well below that for the glycine alone, and the decrease in pH shows that complexation is occurring throughout most of the neutralization range. Similar results are obtained with other zwitterions and weak acids (or bases), such as *N,N'*-diacetylenediamine diacetic acid, which has been studied for its

complexing action with copper and calcium ions.

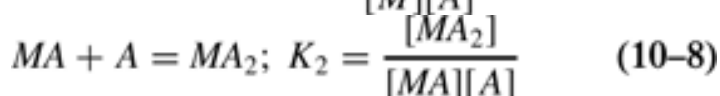
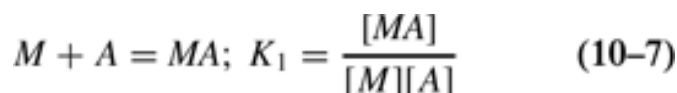
Fig.

ions.



10-9. Titration of glycine and of glycine in the presence of cupric. The difference in pH for a given quantity of base added indicates the occurrence of a complex.

The results can be treated quantitatively in the following manner to obtain stability constants for the complex. The two successive or stepwise equilibria between the copper ion or metal, *M*, and glycine or the ligand, *A*, can be written in general as



and the overall reaction, (10-7) and (10-8), is

$$M + 2A \rightleftharpoons MA_2; \beta = K_1 K_2 = \frac{[MA_2]}{[M][A]^2} \quad (10-9)$$

- K_1 and K_2 the *formation constants*, and the equilibrium constant
- β , for the overall reaction is known as the *stability constant*.
- $\bar{n}$ is the number of ligand molecules bound to a metal ion.

The *average* number of ligand groups bound per metal ion .

$$\bar{n} = \frac{\text{Total concentration of ligand bound}}{\text{Total concentration of metal ion}} \quad (10-10)$$

or

$$\bar{n} = \frac{[MA] + 2[MA_2]}{[M] + [MA] + [MA_2]} \quad (10-11)$$

Although n has a definite value for each species of complex (1 or 2 in this case), it may have any value between 0 and the largest number of ligand molecules bound, 2 in this case. When $\bar{n} = 1$, equation (10-11) becomes

$$[MA] + 2[MA_2] = [M] + [MA] + [MA_2] \quad (10-12)$$

Employing the results in equations (10-9) and (10-12), we obtain the following relation:

$$\beta = K_1 K_2 = \frac{1}{[A]^2} \text{ or } \log \beta = -2 \log [A]$$

and finally

$$p[A] = \frac{1}{2} \log \beta \text{ at } \bar{n} = 1 \quad (10-13)$$

where $p[A]$ is written for $-\log [A]$.

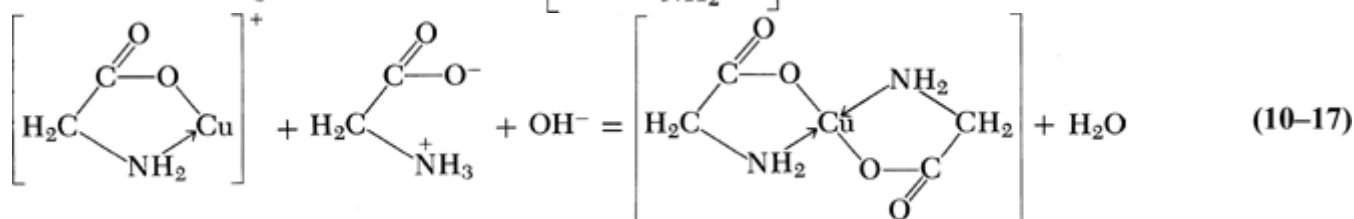
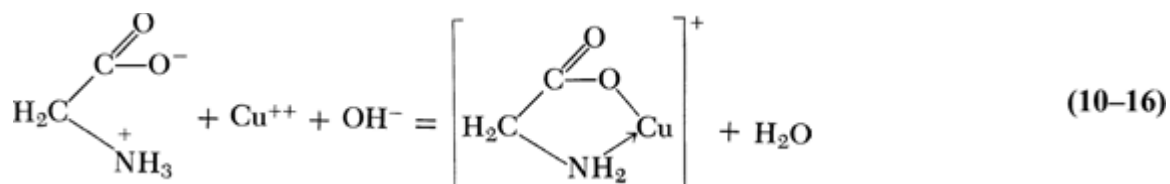
$$p[A] = \log K_1 \text{ at } \bar{n} = \frac{1}{2} \quad (10-14)$$

$$p[A] = \log K_2 \text{ at } \bar{n} = \frac{3}{2} \quad (10-15)$$

It should now be possible to obtain the individual complex formation constants, K_1 and K_2 , and the overall stability constant, β , if one knows two values: $[\bar{n}]$ and $p[A]$.

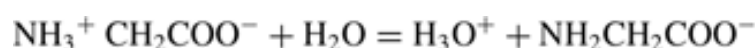
Equation (10-10) shows that the concentration of bound ligand must be determined before $\bar{n}$ can be evaluated.

The horizontal distances represented by the lines in Figure 10-9 between the titration curve for glycine alone (curve I) and for glycine in the presence of Cu^{2+} (curve II) give the amount of alkali used up in the following reactions:



This quantity of alkali is exactly equal to the concentration of ligand bound at any pH, and, according to equation (10-10), when divided by the total concentration of metal ion, gives the value $\tilde{n}$.

The concentration of free glycine $[A]$ as the “base” $\text{NH}_2\text{CH}_2\text{COO}^-$ at any pH is obtained from the acid dissociation expression for glycine:



$$K_a = \frac{[\text{H}_3\text{O}^+][\text{NH}_2\text{CH}_2\text{COO}^-]}{[\text{NH}_3^+\text{CH}_2\text{COO}^-]} \quad (10-18)$$

or

$$[\text{NH}_2\text{CH}_2\text{COO}^-], = [A] = \frac{K_a[HA]}{[\text{H}_3\text{O}^+]} \quad (10-19)$$

The concentration $[\text{NH}_3^+ \text{CH}_2\text{COO}^-]$, or $[HA]$, of the acid species at any pH is taken as the difference between the initial concentration, $[HA]_{\text{init}}$, of glycine and the concentration, $[\text{NaOH}]$, of alkali added. Then

$$[A] = K_a \frac{[HA]_{\text{init}} - [\text{NaOH}]}{[\text{H}_3\text{O}^+]} \quad (10-20)$$

or

$$-\log, [A] = p[A] = pK_a - \text{pH} - \log, ([HA]_{\text{init}} - [\text{NaOH}]) \quad (10-21)$$

where $[A]$ is the concentration of the ligand glycine.

The values of $\tilde{n}$ is the number of ligand molecules bound to a metal ion.

The *average* number of ligand groups bound per metal ion .

and $p[A]$ at various pH values are then plotted as shown in Figure 10-10.

The curve that is obtained is known as a *formation curve*. It is seen to reach a limit at $\tilde{n} = 2$, indicating that the maximum number of glycine molecules that can combine with one atom of copper is 2.

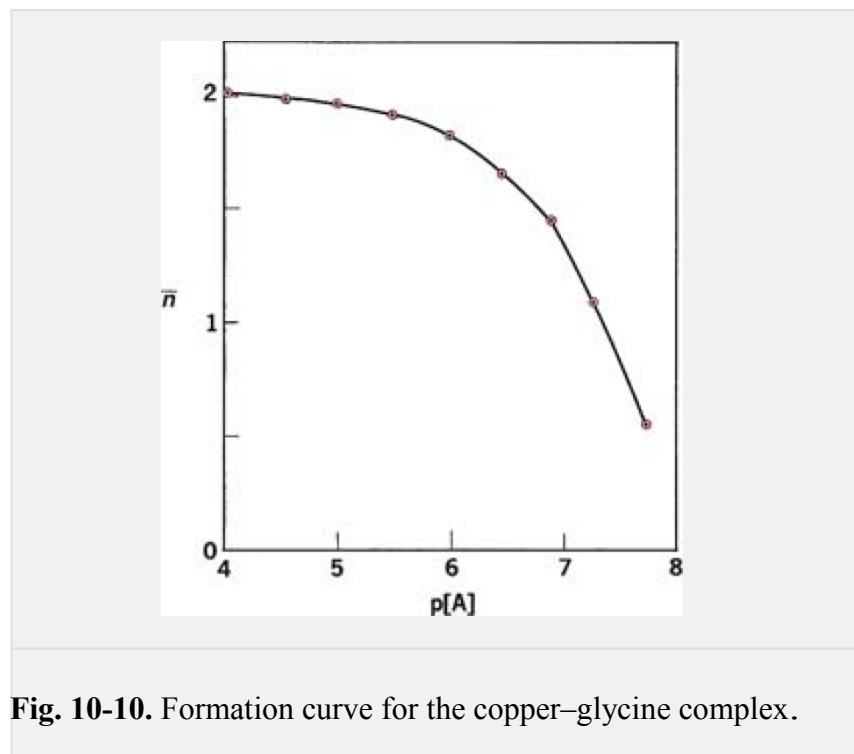


Fig. 10-10. Formation curve for the copper–glycine complex.

Distribution Method

The method of distributing a solute between two immiscible solvents can be used to determine the stability constant for certain complexes. The complexation of iodine by potassium iodide may be used as an example to illustrate the method. The equilibrium reaction in its simplest form is



Additional steps also occur in polyiodide formation; for example, $2\text{I}^- + 2\text{I}_2$ [right harpoon over left harpoon] I_6^{2-} may occur at higher concentrations, but it need not be considered here.

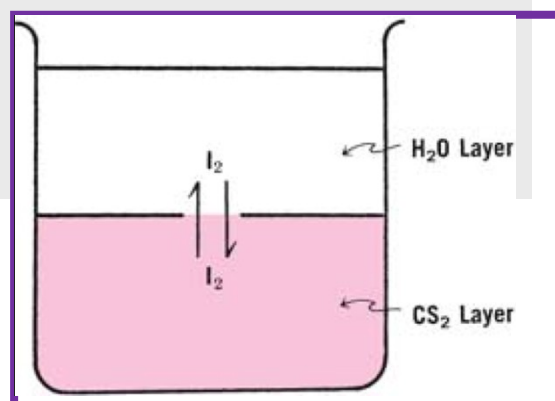
Example

Free and Total Iodine

When iodine is distributed between water (w) at 25°C and carbon disulfide as the organic phase (o), as depicted in Figure 10-11, the distribution constant $K(o/w) = C_o/C_w$ is found to be 625. When it is distributed between a 0.1250 M solution of potassium iodide and carbon disulfide, the concentration of iodine in the organic solvent is found to be 0.1896 mole/liter. When the aqueous KI solution is analyzed, the concentration of iodine is found to be 0.02832 mole/liter.

In summary, the results are as follows:

- Total concentration of I_2 in the aqueous



- layer (free + complexed iodine): 0.02832, mole/l
- Total concentration of KI in the aqueous
- layer (free + complexed KI): 0.1250 mole/l
- Concentration of I₂ in the CS₂ layer (free): 0.1896 mole/l
- Distribution coefficient, $K(o/w) = [I_2]_o/[I_2]_w = 625$

The species common to both phases is the free or uncomplexed iodine; the distribution law expresses only the concentration of *free* iodine, whereas a chemical analysis yields the *total* concentration of iodine in the aqueous phase. The concentration of free iodine in the aqueous phase is obtained as follows:

$$[I_2]_w = \frac{[I_2]_o}{K(o/w)} = \frac{0.1896}{625} = 3.034 \times 10^{-4} \text{ mole/liter}$$

To obtain the concentration of iodine in the complex and hence the concentration of the complex, [I₃⁻], one subtracts the free iodine from the total iodine of the aqueous phase:

$$\begin{aligned} [I_2]_{\text{complexed}} &= [I_2]_{w, \text{total}} - [I_2]_{w, \text{free}} \\ &= 0.02832 - 0.000303 \\ &= 0.02802 \text{ mole/liter} \end{aligned}$$

According to equation (10-22), I₂ and KI combine in equimolar concentrations to form the complex. Therefore,

$$[KI]_{\text{complexed}} = [I_2]_{\text{complexed}} = 0.02802 \text{ mole/liter}$$

KI is insoluble in carbon disulfide and remains entirely in the aqueous phase.

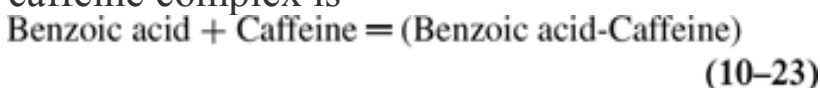
The concentration of *free* KI is thus

$$\begin{aligned} [KI]_{\text{free}} &= [KI]_{\text{total}} - [KI]_{\text{complexed}} \\ &= 0.1250 - 0.02802 \\ &= 0.09698 \text{ mole/liter} \end{aligned}$$

and finally

$$\begin{aligned} K &= \frac{[\text{Complex}]}{[I_2]_{\text{free}} [KI]_{\text{free}}} \\ &= \frac{0.02802}{0.000303 \times 0.09698} = 954 \end{aligned}$$

the reaction between caffeine and benzoic acid to form the benzoic acid-caffeine complex is



and the stability constant for the reactions at 0°C is

$$K = \frac{[\text{Benzoic acid-Caffeine}]}{[\text{Benzoic acid}][\text{Caffeine}]} = 37.5 \quad (10-24)$$

The results varied somewhat, the value 37.5 being an average stability constant. caffeine exists in aqueous solution primarily as a monomer, dimer, and tetramer, which would account in part for the variation in K as observed

Solubility Method

According to the solubility method, excess quantities of the drug are placed in well-stoppered containers, together with a solution of the complexing agent in various concentrations, and the bottles are agitated in a constant-temperature bath until equilibrium is attained. Aliquot portions of the supernatant liquid are removed and analyzed.

used the solubility method to investigate the complexation of *p-aminobenzoic acid (PABA) by caffeine*. The results are plotted in Figure 10-12.

- **The point A** at which the line crosses the vertical axis is the solubility of the drug in water. With the addition of caffeine, the solubility of PABA rises linearly owing to complexation.
- **At point B** the solution is saturated with respect to the complex and to the drug itself. The complex continues to form and to precipitate from the saturated system as more caffeine is added.
- **At point C** all the excess solid PABA has passed into solution and has been converted to the complex. Although the solid drug is exhausted and the solution is no longer saturated, some of the PABA remains uncomplexed in solution, and it combines further with caffeine to form higher complexes such as (PABA-2 caffeine) as shown by the curve at the right of the diagram.

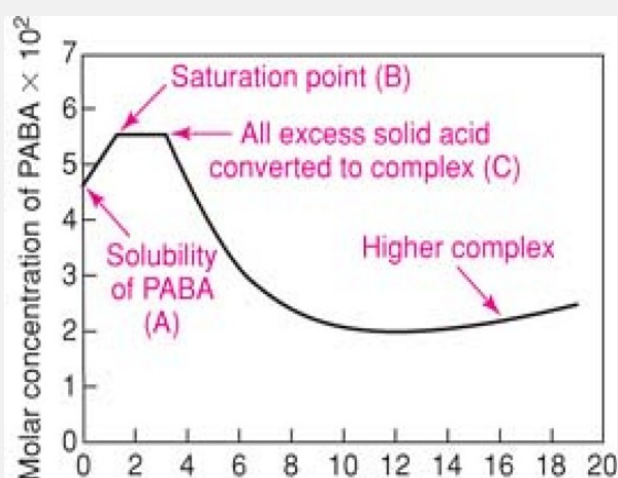


Fig. 10-12. The solubility of *para*-aminobenzoic acid (PABA) in the presence of caffeine.

Spectroscopy and Charge Transfer Complexation

Absorption spectroscopy in the visible and ultraviolet regions of the spectrum is commonly used to investigate electron donor–acceptor or charge transfer complexation.

- ✓ When iodine is analyzed in a noncomplexing solvent such as CCl_4 , a curve is obtained with a single peak at about 520 nm. The solution is **violet**.
- ✓ A solution of iodine in benzene exhibits a maximum shift to 475 nm, and a new peak of considerably higher intensity for the charge-shifted band appears at 300 nm. The solution is **red to brown**.
- ✓ A solution of iodine in diethyl ether shows a still greater shift to lower wavelength and the appearance of a new maximum. The solution is **red to brown**. Their curves are shown in Figure 10-13. In benzene and ether, iodine is the electron acceptor and the organic solvent is the donor.

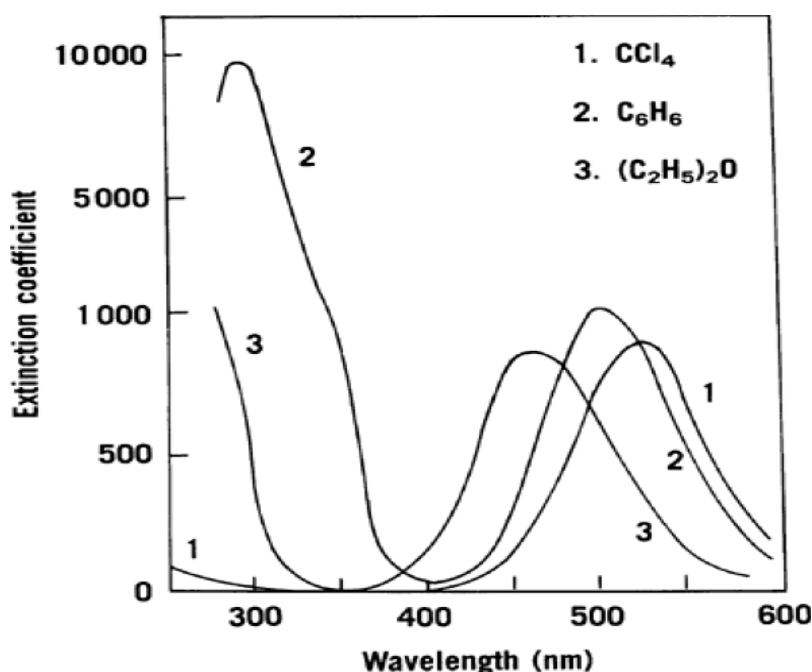


Fig. 10-13. Absorption curve of iodine in the noncomplexing solvent (1) carbontetrachloride and the complexing solvents (2) benzene and (3) diethyl ether.

The complexation constant, K , can be obtained by use of visible and ultraviolet spectroscopy. The association between the donor D and acceptor A is represented as



where $K = k_1/k_{-1}$ is the equilibrium constant for complexation (stability constant) and k_1 and k_{-1} are the interaction rate constants. When two molecules associate according to this scheme and the absorbance A of the charge transfer band is measured at a definite wavelength, K is readily obtained from the Benesi–Hildebrand equation:

$$\frac{A_0}{A} = \frac{1}{\epsilon} + \frac{1}{K\epsilon} \frac{1}{D_0} \quad (10-28)$$

A_0 and D_0 are initial concentrations of the acceptor and donor species, respectively, in mole/liter, ϵ is the molar absorptivity of the charge transfer complex at its particular wavelength, and K , the stability constant, is given in liter/mole or M^{-1} . A plot of A_0/A versus $1/D_0$ results in a straight line with a slope of $1/(K\epsilon)$ and an intercept of $1/\epsilon$, as observed in Figure 10-14.

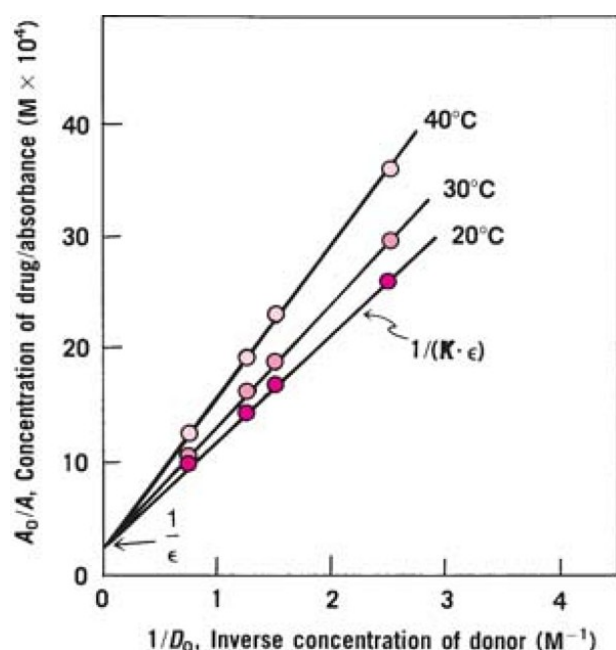


Fig. 10-14. A Benesi–Hildebrand plot for obtaining the stability constant, K , from equation(10-28) for charge transfer complexation.

With the assumption of 1:1 complexes, the equilibrium constant, K , for charge transfer interaction was obtained from **Benesi–Hildebrand** plots at three or four temperatures, and ΔH° was obtained at these same temperatures from the slope of the line as plotted in Figure 10-15.

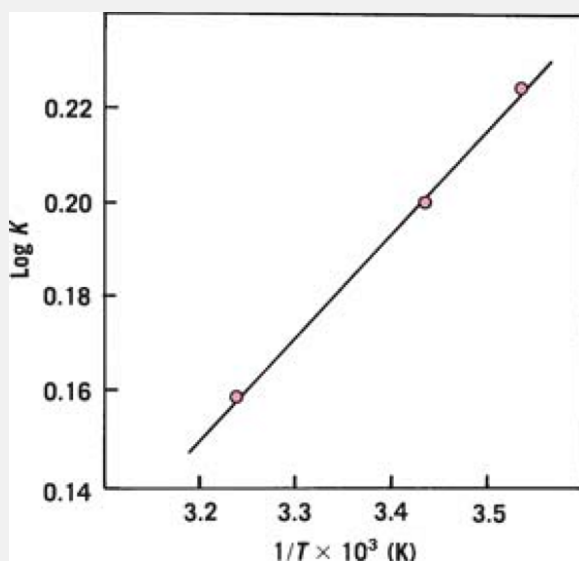


Fig. 10-15. Adenine–catechol stability constant for charge transfer complexation measured at various temperatures at a wavelength of 340 nm.

Other Methods

A number of other methods are available for studying the complexation of metal and organic molecular complexes. They include NMR and infrared spectroscopy, polarography, circular dichroism, kinetics, x-ray diffraction, and electron diffraction.

Protein Binding

A complete analysis of protein binding, including the multiple equilibria that are involved, would go beyond our immediate needs. Therefore, only an abbreviated treatment is given here.

Binding Equilibria

the interaction between a group or free receptor P in a protein and a drug molecule D as



The equilibrium constant, disregarding the difference between activities and concentrations, is

$$K = \frac{[PD]}{[P][D_f]} \quad (10-30)$$

or

$$K[P][D_f] = [PD] \quad (10-31)$$

where K is the association constant, $[P]$ is the concentration of the protein in terms of free binding sites, $[D_f]$ is the concentration, usually given in moles, of

free drug, sometimes called the ligand, and $[PD]$ is the concentration of the protein–drug complex. K varies with temperature and would be better represented as $K(T)$; $[PD]$, the symbol for bound drug, is sometimes written as $[D_b]$, and $[D]$, the free drug, as $[D_f]$.

If the total protein concentration is designated as $[P_t]$, we can write

$$[P_t] = [P] + [PD]$$

or

$$[P] = [P_t] - [PD] \quad (10-32)$$

Substituting the expression for $[P]$ from equation (10-32) into (10-31) gives

$$[PD] = K[D_f]([P_t] - [PD]) \quad (10-33)$$

$$[PD] = K[D_f][PD] = K[D_f][P_t] \quad (10-34)$$

$$\frac{[PD]}{P_t} = \frac{K[D_f]}{1 + K[D_f]} \quad (10-35)$$

Let r be the number of moles of drug bound, $[PD]$, per mole of total protein, $[P_t]$; then $r = [PD]/[P_t]$, or

$$r = \frac{K[D_f]}{1 + K[D_f]} \quad (10-36)$$

The ratio r can also be expressed in other units, such as milligrams of drug bound, x , per gram of protein, m . Equation (10-36) is one form of the Langmuir adsorption isotherm. Although it is quite useful for expressing protein-binding data, it must not be concluded that obedience to this formula necessarily requires that protein binding be an adsorption phenomenon.

Expression (10-36) can be converted to a linear form, convenient for plotting, by inverting it:

$$\frac{1}{r} = \frac{1}{K[D_f]} + 1 \quad (10-37)$$

If ν independent binding sites are available, the expression for r , equation (10-36), is simply ν times that for a single site, or

$$r = \nu \frac{K[D_f]}{1 + K[D_f]} \quad (10-38)$$

and equation (10-37) becomes

$$\frac{1}{r} = \frac{1}{\nu K} \frac{1}{[D_f]} + \frac{1}{\nu} \quad (10-39)$$

Equation (10-39) produces what is called a **Klotz reciprocal plot**.

An alternative manner of writing equation (10-38) is to rearrange it first to

$$r + rK[D_f] = \nu K[D_f] \quad (10-40)$$

Data $\frac{r}{[D_f]} = vK - rK$ (10-41)

presented according to equation (10-41) are known as a **Scatchard plot**. The binding of bishydroxycoumarin to human serum albumin is shown as a Scatchard plot in Figure 10-17.

- Graphical treatment of data using equation (10-39) heavily weights those experimental points obtained at low concentrations of free drug, D , and may therefore lead to misinterpretations regarding the protein-binding behaviour at high concentrations of free drug. Equation (10-41) does not have this disadvantage and is the method of choice for plotting data. Curvature in these plots usually indicates the existence of more than one type of binding site.
- Equations (10-39) and (10-41) cannot be used for the analysis of data if the nature and the amount of protein in the experimental system are unknown. For these situations, **Sandberg et al.** recommended the use of a slightly modified form of equation (10-41):

where $\frac{[D_b]}{[D_f]} = -K[D_b] + vK[P_t]$ (10-42) $[D_b]$ is the concentration of bound drug. Equation (10-42) is plotted as the ratio $[D_b]/[D_f]$ versus $[D_b]$, and in this way K is determined from the slope and $vK[P_t]$ is determined from the intercept.

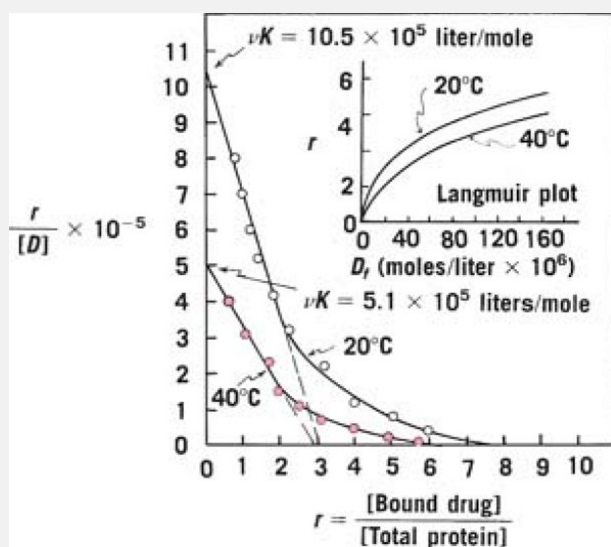


Fig. 10-17. A Scatchard plot showing the binding of bishydroxycoumarin to human serum albumin at 20°C and 40°C plotted according to equation (10-41). Extrapolation of the two lines to the horizontal axis, assuming a single class of sites with no electrostatic interaction, gives an approximate value of 3 for v .

The binding constant, K indicating extremely weak affinity of the drug for the sites, but this class may have a large number of sites and so be considered unstatutable.