



UNIT-IV

COMPLEXATION AND PROTEIN BINDING:

Methods of analysis - Complexation:

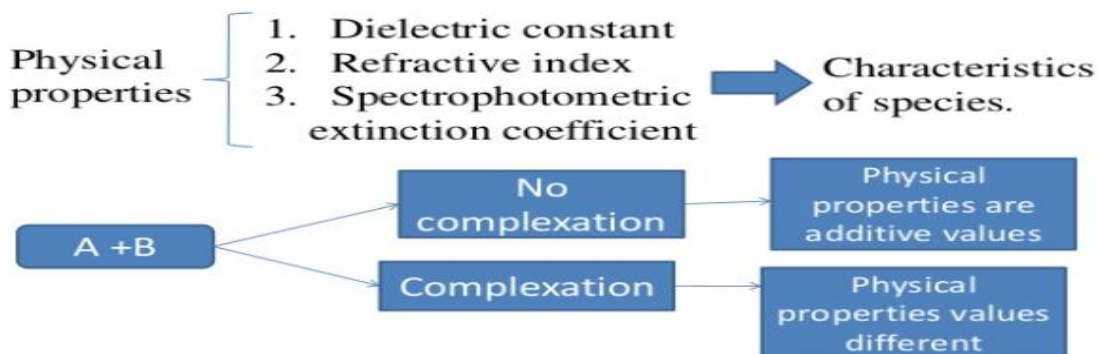
Estimation of 2 parameters

- ✓ Stoichiometric ratio of Ligand: Metal / Donar : Acceptor
- ✓ Stability Constant of complex.

Methods:

- ✓ Method of continuous variation.
- ✓ Distribution method
- ✓ Solubility method
- ✓ pH titration method.

Method of continuous variation.



Method of continuous variation:

- Principle - When there is no complexation between the species, the value of property is additive. On complexation these properties changes but additive rule do not hold good. The change in the characteristics proves that the complexation has been taken place.
- Let's take two species A and B whose individual dielectric constant in solid form and Absorbance in solution form were measured. Then two species in

both forms were mixed. The dielectric constant and absorbance were determined.

- The individual values are subtracted with mixed additive values and result was found out.
- If result is zero then no complexation and if result is not zero then there is complexation.

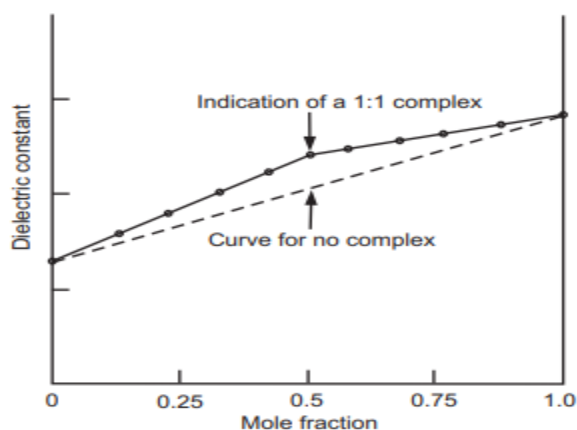


Figure: Dielectric Constant Plotted Against the Mole Fraction

pH Titration Method:

- Principle - This method is applicable for that complex that produces the changes in pH on interaction. The significant change in pH will determine that complexation has been taken place.
- Let us take 75 ml of glycine solution and it is titrated with strong alkali NaOH solution. The pH was recorded. A graph was drawn between pH and volume of NaOH added.
- In another test, complex solution of glycine and copper salt is titrated. The change in pH with increments of NaOH solution also recorded. A graph was drawn between pH and volume of NaOH added.
- The two plots are compared and it is seen that the plot of glycine with copper is well below that of the pure glycine, which indicated that complexation is obtained throughout the titration range.
- Stability constant:

$$\text{Log } \beta = 2 \times p [A]$$

$$P [A] = pK_a - \text{pH} - \log ([HA]_{\text{initial}} - [NaOH])$$

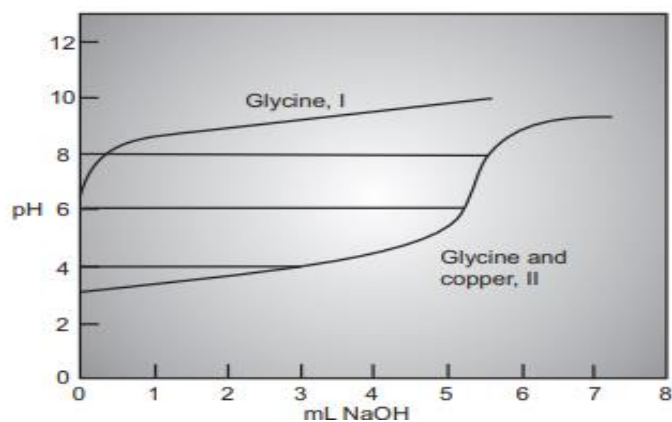
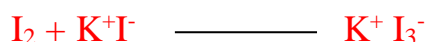


Figure: Titration of Glycine and of Glycine in the Presence of Cupric Ions

Distribution Method:

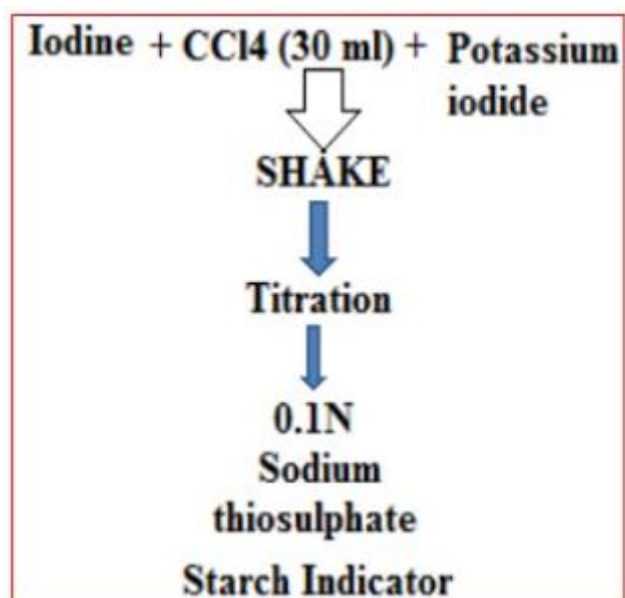
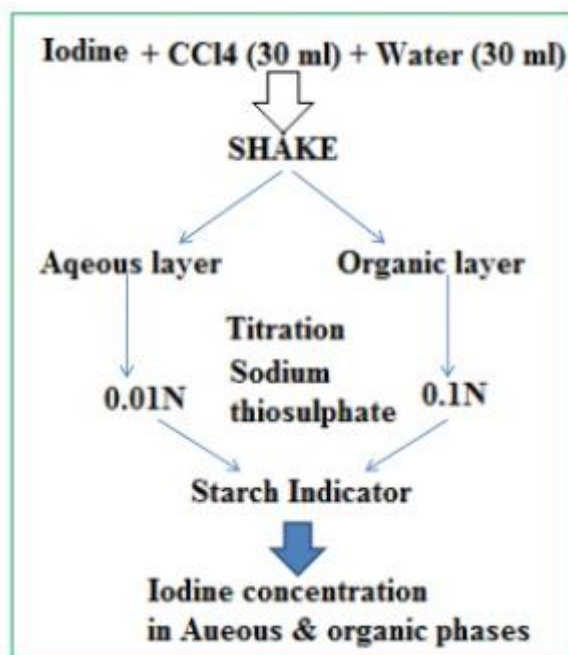
- The method of distributing a solute between two immiscible solvents can be used to determine the stability constant for certain complexes.
- The distribution behavior of a solute between two immiscible liquids is expressed by distribution or partition co-efficient.
- Principle - When a solute complexes with an added substance, the solute distribution pattern changes depending on the nature of the complex.
- The complexation of iodine by potassium iodide.



- The Equilibrium stability constant,

$$K = \frac{[K^+ I_3^-]}{[I_2] [K^+I^-]}$$

- The distribution coefficient of iodine between disulfide and water is 625.
- The K value of Iodine-Potassium iodide complex is 954.
- This change in distribution coefficient proves that the complexation has taken place.



Solubility Method:

- Principle - When the component in a mixture produce a complex, the solubility of one of the components may be increased or decreased. The change in solubility is a sign of complexation.
- The experimental data can be used to analyse complexes in terms of donor-acceptor ratio and equilibrium stability constant.
- Experiment:
 1. Caffeine (Complexing agent) taken in different concentrations

2. Add PABA, Agitate, Filter & analyze drug content.

➤ Example – PABA and Caffeine and Paracetamol – Caffeine.

$$K = \frac{[\text{PABA} - \text{Caffeine}]}{[\text{PABA}] - [\text{Caffeine}]}$$

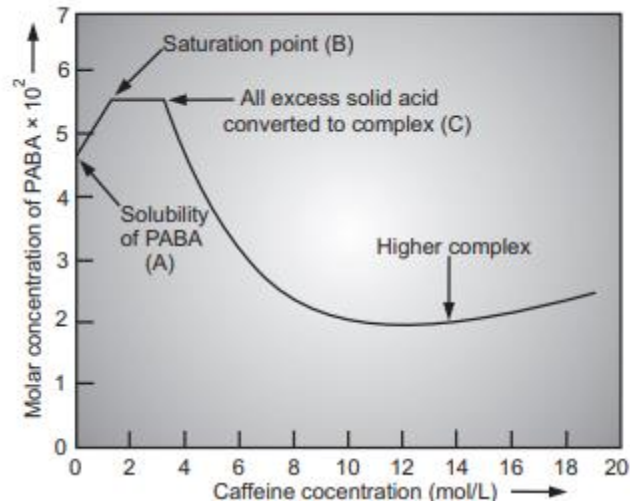
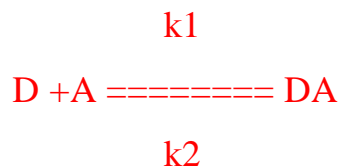


Figure: The Solubility of Para-Aminobenzoic Acid (PABA) in the Presence of Caffeine

Spectroscopy Method:

➤ The study of donor acceptor (D-A) or charge transfer complexation is generally undertaken with absorption spectroscopy in the visible and UV regions of the spectrum.



➤ Where, D and A represents electron donor and acceptor, k1 and k2 are interaction rate constants.

➤ $K = k_1 / k_2 =$ Equilibrium or stability constant for complexation.

➤ The absorbance A of the charge transfer band is measured at a definite wavelength and the constant K is obtained from the Benesi-Hildebrand equation.

➤ $A_0/A = (1/\epsilon) + (1/K\epsilon) (1/D_0)$

- Where, A_0 and D_0 are the initial concentration of acceptor and donor species in mole/litre. E is the molar absorptivity of the charge-transfer complex at its particular wavelength and K is the stability constant in litre/mole.
- A plot of A_0/A versus $1/D_0$ results in straight line with a slope of $1/K\varepsilon$ and an intercept of $1/\varepsilon$.
- The spectrometric method used to investigate the interaction of nucleic acid bases with catechol, epinephrine and isoproterenol.

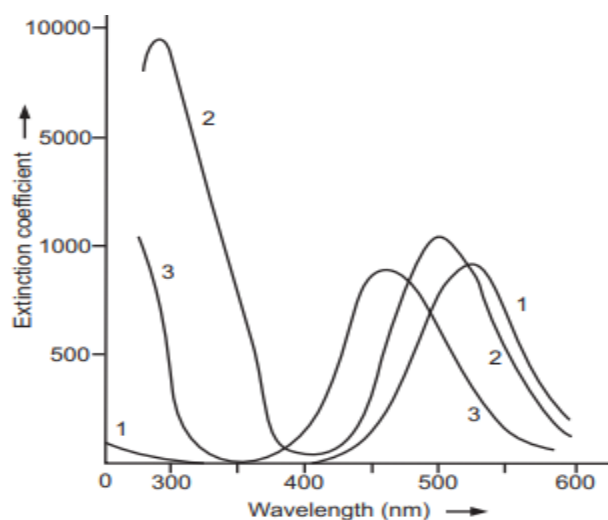


Figure: Absorption Curves of Iodine in the Non-complexing Solvent

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