Ultraviolet-Visible Spectroscopic Analysis

Many aromatic organic molecules show changes in their UV-visible absorption spectrum on inclusion by CDs (Cramer et al. 1967; Connors 1987) (Nagabhushanam et al. 2013). Generally, the spectral changes observed are similar to the effects caused by changes in solvent. These changes must be due to a perturbation of the electronic energy levels of the guest, caused either by direct interaction with the CD, by the exclusion of solvating water molecules, or by a combination of these two effects.

The stability constant of a complex can be determined from the analysis of a series of absorption spectra (Connors 1987). Consider the system in which a single 1:1 complex (*SL*) is formed, with the complex and free substrate (*S*) having significantly different absorption spectra. A wavelength is selected at which the molar absorptivity of substrate (ε_s) and complex (ε_{11}) are different. Then at a particular total concentration of substrate (S_T), in the absence of ligand, the solution absorbance is given by Equation 8.9,

$$A_0 = \varepsilon_s b S_T \tag{8.9}$$

where b is the cell path length. In the presence of ligand at total concentration L_T, assuming that Beer's law is followed by all species, the absorbance of a solution containing the same total substrate concentration can be expressed as Equation 8.10,

$$A_L = \varepsilon_s b[S] + \varepsilon_L b[L] + \varepsilon_{11} b[SL] \tag{8.10}$$

which, combined with the mass balances on *S* and *L*, gives:

$$A_L = \varepsilon_s b S_T + \varepsilon_L b L_T + \varepsilon_{11} b[SL] \tag{8.11}$$

where $\Delta\varepsilon_{11} = \varepsilon_{11} - \varepsilon_s - \varepsilon_L$, in which ε_L is the molar absorptivity of the ligand. By measuring the solution absorbance against a reference containing ligand at the same total concentration L_T, the measured absorbance becomes

$$A_L = \varepsilon_s b S_T + \varepsilon_{11} b[SL] \tag{8.12}$$

Combining Equation 8.12 with the stability constant definition, gives:

$$\Delta A = A - A_0 = K_{11} \Delta\varepsilon_{11} b[S][L] \tag{8.13}$$

When combined with the mass balance equation for *S*, Equation 8.14 is obtained which, when substituted back into Equation 8.13, gives Equation 8.15.

$$[S] = \frac{S_T}{1 + K_{11}[L]} \tag{8.14}$$

$$\frac{\Delta A}{b} = \frac{S_T K_{11} \Delta\varepsilon_{11}[L]}{1 + K_{11}[L]} \tag{8.15}$$

Equation 8.15 is the binding isotherm, which shows the hyperbolic dependence of absorbance on free ligand concentration.

By simple algebra, a double-reciprocal plot form, often called the Bensi-Hildebrand equation, can be derived.

$$\frac{b}{\Delta A} = \frac{1}{S_T K_{11} \Delta\varepsilon_{11}[L]} + \frac{1}{S_T \Delta\varepsilon_{11}} \tag{8.16}$$