

The stability constant is evaluated from the linearized plot according to:

$$K_{11} = \frac{y\text{-intercept}}{\text{slope}} \quad (8.17)$$

Note that the reference solution contains the same total ligand concentration  $L_T$  as the sample solution. Even if the ligand does not absorb at the analytical wavelength, it is recommended to include it in the reference solution so that both the sample and reference have the same refractive index.

Both binding isotherm Equation 8.15 and double reciprocal plots Equation 8.16 are expressed as a function of free ligand concentration, which is not known. By means of the mass balance on ligand,  $L$  can be related to the known total ligand concentration  $L_T$ .

$$L_T = [L] = \frac{S_T K_{11} [L]}{1 + K_{11} [L]} \quad (8.18)$$

For multiple equilibria, consider a system containing only two complex states, namely, 1:1 ( $SL$ ) and 1:2 ( $SL_2$ ). Development following the lines of the treatment for the 1:1 system results in the following:

$$\frac{\Delta A}{b} = \frac{S_T (\beta_{11} \Delta \varepsilon_{11} [L] + \beta_{12} \Delta \varepsilon_{12} [L]^2)}{1 + \beta_{11} [L] + \beta_{12} [L]^2} \quad (8.19)$$

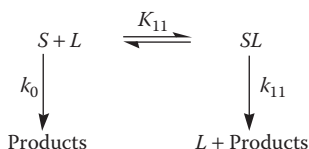
$$L_T = [L] = \frac{S_T (\beta_{11} [L] + 2\beta_{12} [L]^2)}{1 + \beta_{11} [L] + \beta_{12} [L]^2} \quad (8.20)$$

where  $\beta_{11} = K_{11}$  and  $\beta_{12} = K_{11} K_{12}$ . By using nonlinear regression analysis, the parameters can be evaluated.

For multiple equilibria, spectroscopy is not in general a very useful tool. This is because the absorbance is not a direct measure of extent of binding, but rather is proportional to it. As a consequence, each stoichiometric state in the system adds two unknown parameters, a binding constant and a molar absorptivity, to the isotherm, so the number of parameters is twice the number of complex states.

### Kinetic Method

The stabilization of  $S$  as the result of inclusion complex formation occurs non-covalently and may be the result of either a microsolvant effect, or of a conformational effect (Szejtli 1982). The kinetic method utilizes this reduction in rate of the reaction of  $S$  when  $L$  is present to obtain information about the nature of the complex. The basic assumption is that the decreased reactivity is the sole result of complexation, the complexed  $S$  being less reactive than free  $S$ . The kinetic scheme can be represented as below:



Kinetic scheme for a system in which a substrate degrades at a different rate inside the complex than the substrate alone, where  $k_0$  is the rate constant for decomposition of uncomplexed  $S$ ,  $k_{11}$  is the rate constant for the complex  $SL$  and  $K_{11}$  is the stability constant for complex formation. Assuming the degradation is under pseudo-first-order conditions, the theoretical rate equation is:

$$v = k_0 [S] + k_{11} [SL] \quad (8.21)$$