

## METHODS FOR STUDYING COMPLEXATION

### MEASUREMENT OF BINDING CONSTANTS IN SOLUTION

To find out if a drug (substrate) can form a complex with any potential ligand molecule and the stability of the complex, one will need to measure the binding constants of complexes. Generally speaking, any methodology, such as surface tension measurements (Baszkin et al. 1999), which can relate the changes in one or more properties of the system which are caused by intermolecular interaction, may be utilized. Several methods that are widely used in the pharmaceutical industry, including solubility measurement, UV spectroscopic analysis, kinetic method and titration calorimetry, are described in detail here.

### Solubility Measurement

The solubility of a substance will change upon the formation of a complex with a second substance. The extent of solubility alteration directly relates to the binding affinity of the two compounds. Therefore, it is possible to evaluate equilibrium constants from solubility data (Higuchi and Connors 1965).

The heart of the solubility method is the solubility diagram which is constructed by plotting the total molar concentration of substrate ( $S_T$ ) dissolved in solution against total ligand concentration ( $L_T$ ). According to the solubility of the complex formed, phase solubility diagrams are generally classified into type A and type B diagrams as shown in Figure 8.3.

In type A diagrams, the formation of soluble complexes ( $S_mL_n$ ) results in an increase in the total amount of substrate in solution. If all complexes formed are first-order in  $L$  (i.e., if  $n = 1$  in all species), then the solubility diagram will be linear as represented by  $A_L$ . The converse is not necessarily true, but a linear diagram is often taken as evidence for  $n = 1$ . If the slope is greater than unity, then at least one complex must exist for which  $m$  is greater than 1, for it is obviously impossible for one mole of  $L$  to take more than one mole of  $S$  into solution if the complex has 1:1 stoichiometry. On the other hand, a slope of less than unity does not necessarily mean that only a 1:1 complex is formed.

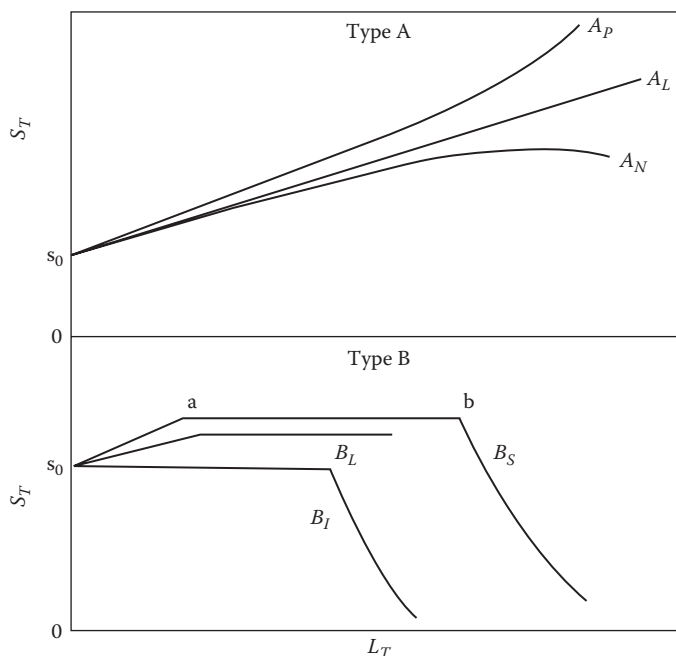


FIGURE 8.3 Phase-solubility diagrams of type A and type B systems.