Chapter 33
Complex Formation

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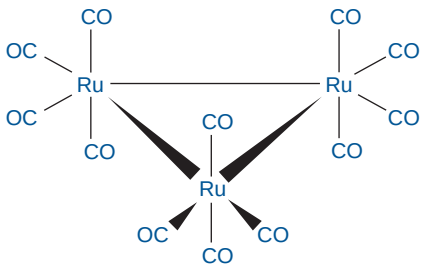
In chemistry and chemical processes the word *complex* usually refers to molecules or molecular assemblies formed by combination of *substrates*, *S*, and *ligands*, *L*. Most often complex (S_mL_n) formation is a reversible process:

$$m.S + n.L \rightleftarrows S_mL_n$$

where *m* substrate molecules, associate with n ligand molecules to form a complex of m:n stoichiometry. In this context, *complex formation*, *complexation*, *binding*, *association,* and *chelation* are often synonymous. The substrate and ligand are kept together by relatively strong coordinate covalent bonds or by weak non-covalent forces such as hydrogen bonding, van der Waals forces, electrostatic interactions, dipole forces, charge transfer, release of conformational strain, or hydrophobic interactions. The complex formation changes the physicochemical properties of its constituents, both of the substrate and the ligand, including their aqueous solubility, molar absorptivity, NMR chemical shifts, adsorption to solid surfaces, partitioning behavior, conductivity, chemical reactivity and/or pKa values. By studying such properties, for example of the substrate as a function of the ligand concentration, the complex can be identified and quantitatively described. Furthermore, the methods of chemical kinetics and thermodynamics can be applied to describe the formation and dissociation of a complex. Although most frequently, substrate and ligand molecules are associated by weak chemical forces, there are complexes where bonds are quite strong and formation of some metal complexes are virtually irreversible. Complexes are usually broadly classified into two groups based on the type of S-L bonding involved, namely *coordination complexes* and *molecular complexes*.

Coordination complexes consist of ionic substrates, most frequently transition metal ions, with bases or, in other words, products of Lewis acid-base reactions where the metal ion (an acid) accepts a pair of electrons from the ligand (the base) to form a coordinate covalent bond. Examples of such complexes are $[Ag(NH_3)_2]^+$, $[Co(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{4-}$. Other common types of coordination complexes are organometallic complexes that are complexes formed between organic groups and metal atoms such as vitamin B12 (a porphyrin containing a cobalt atom), and cluster complexes where the central metal ion consists of a three-dimensional cell of several directly bonded metal ions such as triruthenium dodecacarbonyl ($Ru_3(CO)_{12}$):



A **coordination complex** is called a *chelate* if the same substrate (metal ion) binds with two or more sites on a ligand.

Molecular complexes consist of non-covalently bound substrates and ligands. These include complexes of relatively small substrates and ligands such as drug-cyclodextrin complexes, complexes between small substrates and a large ligand such as drug-protein complexes (e.g., plasma protein binding) and complexes between large substrates and a small ligand such as some protein-polyalcohol complexes. Molecular complexes also include molecular dimers, ion-pairs, intramolecular interactions (such as base-base interactions in the DNA helix) and clathrate compounds (cage compounds) where a cage-like lattice of one type of molecules (e.g., hydroquinone molecule) entraps a second type of molecules (e.g., methanol molecule). Pharmaceutical co-crystals can also be considered a type of molecular complex with components subjected to hydrogen bonding and other forces in the crystal lattice. Examples of these types of molecular complexes include isoniazid and 4-aminosalicylic acid where the carboxylic acid function can be shown to interact with the pyridine nitrogen.

The following is a more detailed description of these complexes with several examples of pharmaceutical interest.

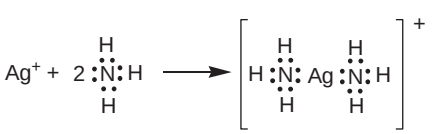
COMPLEX BONDING

Chemical bonding and bonding forces within and between molecules is covered in Chapter 26. The following relates to forces that participate in complex formation.[1,2]

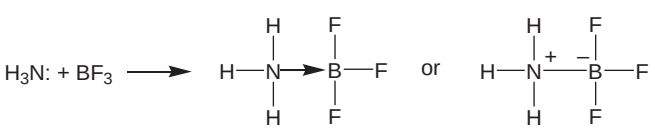
A coordinate covalent bond is a covalent bond where both electrons come from the same atom unlike a simple covalent bond which is formed between two atoms with each atom supplying one electron. For example, when silver ions (Ag^+) interact with ammonia (NH_3) to form the silver-ammonia coordinate complex ($[Ag(NH_3)_2]^+$) both of the electrons forming the covalent bond come from ammonia. However, each of the three H-N covalent bonds in ammonia is formed by one electron from a hydrogen atom and one electron from the ammonia atom.

$$Ag^+ + 2(:NH_3) \rightarrow [Ag(NH_3)_2]^+$$

or



Coordinate covalent bonds are sometimes designated by arrows indicating that both electrons are coming from, for example, the nitrogen or by plus and minus signs indicating that the nitrogen end of the bond has become positive because the electron pair has moved away from the nitrogen towards the boron which has thus become negative:



Several different theories have been developed in order to describe coordinate covalent bonding such as *molecular orbital theory* (also called *ligand field theory*) that applies quantum mechanical description of coordinate bonds, *electrostatic theory* where the substrate ions are treated as spherical charges

Table 33-1. Some examples of the Hard-Soft Classification of Lewis Acids and Bases

	Acids	Bases
Hard	H^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Zn^{2+}, $AlCl_3$, CO_2	H_2O, OH^-, F^-, Cl^-, PO_4^{3-}, SO_4^{2-}, ClO_4^-, NO_3^-, NH_3, CH_3COO^-
Soft	Cu^+, Ag^+, Au^+, Hg^{2+}, Hg_2^{2+}, Pd^{2+}, Pt^{2+}; I_2, Br_2	I^-, SCN^-, CN^-, CO, C_6H_6

and the ligands as dipoles, and *valence bond theory*, a quantum mechanical theory wherein the ligand donates a pair of electrons to a vacant orbital associated with the ion.

Another concept that is used to describe coordination complexes is the concept of *hard and soft acids and bases* (HSAB). A *hard acid* is defined as a small Lewis acid (i.e. electron-pair acceptor) atom with high positive charge density and low polarizability whereas a *soft acid* is large and polarizable. A *hard base* has high electronegativity and low polarizability whereas a *soft base* is polarizable (Table 33-1). Polarizability is a measure of the ease with which the electron cloud can be deformed under the influence of a charge field. In general, hard acids bind preferably with hard bases, and soft acids bind preferably with soft bases.

Non-covalent intermolecular forces (i.e., between molecules) participating in a complex formation are, in general, relatively weak forces compared to intramolecular forces (i.e., within a molecule) such as covalent bonds. The following types of non-covalent forces are known to participate in complex formations:

Electrostatic interactions are the consequence of classical attraction and repulsion effects between charges such as charge-charge, charge-dipole, and dipole-dipole interactions.

Dispersion force is a quantum mechanical effect where synchronization of the electronic motion between two molecules results in momentary dipole moments and consequently attraction between the molecules. Dispersion force is also called the *van der Waals force*. However, some authors use the term van der Waals force for all non-covalent forces.

Hydrogen bonding is an interaction involving formation of hydrogen bond (*H*-bond) between a proton donor (*HA*) and a proton acceptor (*B*):

$$A - H + B \rightleftarrows A - H \cdots B$$

The *A-H* bond has a covalent character but the $H \cdots B$ bond is predominantly electrostatic. The strength of the hydrogen bond is controlled in part by the acid strength of *HA* and the base strength of *B*, but it is also affected by the solvent.

Charge-transfer interaction is a consequence of electron transfer from an electron donor molecule to an electron acceptor molecule. Frequently, charge-transfer complexes involve electron transfer between metal atoms and ligands. However, charge-transfer interactions are also known in other inorganic as well as organic compounds. A well-known charge-transfer complex is the dark blue or purple iodine/starch complex.

Hydrophobic interaction results from the tendency of liquid water to exclude non-polar molecules. The water molecules form a "cage" around two or more non-polar molecules keeping them together in a kind of a complex or loosely associated molecules. Hydrophobic interactions are commonly observed during inclusion complex formations where a non-polar moiety of larger molecule enters a somewhat lipophilic cavity.

Release of conformational strain is known to participate in formation of cyclodextrin inclusion complexes where the cyclodextrin molecule frequently undergoes significant conformation changes upon complex formation. However, it has been argued that relief of conformational strain is not a driving force for cyclodextrin complex formation.[2]

Release of high-energy water molecules from, for example, a cyclodextrin cavity does participate in inclusion complex formation. In aqueous solutions the somewhat lipophilic cyclodextrin cavity is occupied by high-energy water molecules (i.e., water not capable of fulfilling all of its hydrogen-bonding requirements) that are expelled during inclusion complex formation. However, based on some thermodynamic observations it has been argued that the exclusion of water molecules from the cavity is not a driving force of cyclodextrin complex formation.[2] This is based on the fact that although the cavity-bound water molecules are of higher energy (i.e., are enthalpy rich) they have more conformational freedom (i.e., form fewer hydrogen bonds). Consequently, although expulsion of water molecules from the cavity is accompanied by a negative enthalpy change, the free energy change of the overall process is not necessary negative.

METAL-ION COORDINATION COMPLEXES

Metal-ion coordination complexes, sometimes simply called metal complexes, consist of a central metal ion bonded to one or more ligands that are electron-pair donors such as a nitrogenous base (e.g., ammonia), an ion (e.g., chloride ion), or an aromatic compound (e.g., ferrocene). The number of bonds between the metal ion and the ligand or ligands is called the *coordination number* of the complex. Metal ions can have more than one coordination number. The maximum number is defined by the size, charge and electronic structure of the metal ion. Coordination numbers are normally between 2 and 9 with the most common coordination numbers being 4 and 6. For example, the anticancer drug cisplatin has a coordination number of 4 (Fig. 33-1).

The *trans* stereoisomer of cisplatin is transplatin (Fig. 33-1). Both of these geometrical isomers have square planar structure, that is, the metal substrate and the four ligand groups all lie in the same plane. Most metal-ion coordination complexes of coordination number 4 have a square planar structure but some are tetrahedral where the central metal ion is in the center of a tetrahedron with each of the four ligands located in the four corners. Most complexes with coordination number 6 are octahedral, that is, the bonds lie along the x, y, and z axes of a coordinate system with the metal ion at the origin. Example of such a complex are the *cis* and *trans* isomers of dichlorotetraamminecobalt(III) chloride (Figure 33-2).

Metal ions that are found within cells form coordination complexes with small molecules such as porphyrins that are themselves bound to proteins. The function of such metalloproteins can be the transport of oxygen or nitric oxide, or can be enzymatic in nature. For example, the heme unit (Figure 33-3) is a coordination complex of iron and a porphyrin that is responsible for binding oxygen in hemoglobin and myoglobin.

A chelate (from Greek word "chelè" meaning "claw") is formed when a ligand uses more than one donor atom to bind a single metal atom. Such ligands are called chelating agents, chelants, or chelators. Chelates tend to be more stable than comparable complexes containing only one binding site and are used in drug formulations to, for example, bind metal ions that catalyze drug oxidation, thus increasing the shelf-life of the drug product. Examples of such chelating agents include citric acid, tartaric acid

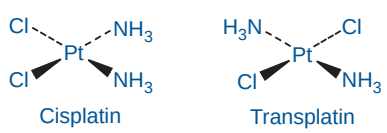


Figure 33-1. Cisplatin and transplatin.

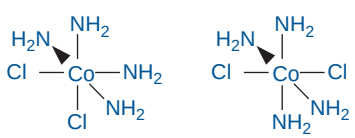


Figure 33-2. Equivalent ways to draw an octahedral complex.

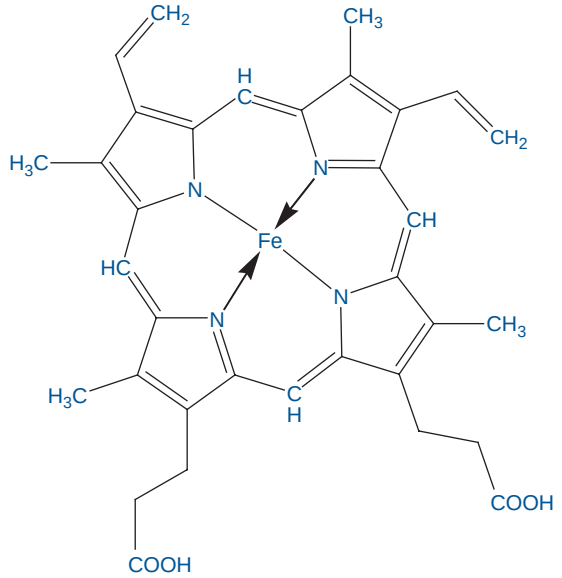


Figure 33-3. Heme, a coordination complex of iron and a porphyrin. Two of the bonds are coordination covalent bonds indicated by arrows.

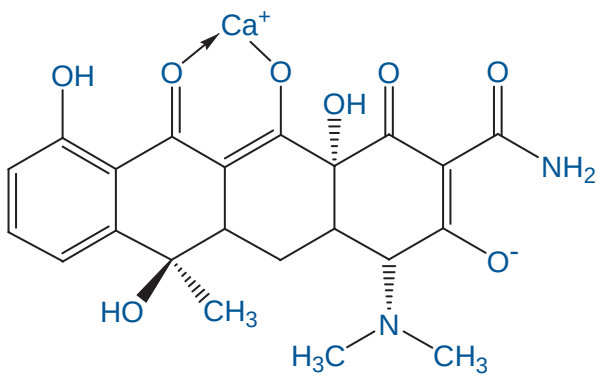


Figure 33-4. Calcium (Ca^{2+}) tetracycline complex (chelate).

and EDTA (ethylenediamine tetraacetic acid). Some drugs can also be chelating agents and bind ions. For instance tetracycline forms hydrophilic chelates with ions such as calcium (Ca^{2+}), iron (Fe^{3+}, Fe^{2+}), aluminium (Al^{3+}), and magnesium (Mg^{2+}), i.e. complexes that possess poor oral bioavailability (Fig. 33-4).[3] Milk and milk products, mineral supplements and antacids containing polyvalent cations ingested simultaneously with tetracycline antibiotics can reduce their oral bioavailability by as much as 90%.[4] Nalidixic acid, ciprofloxacin and other quinolones do also form chelates with polyvalent ions that can reduce their oral bioavailability. The intensity of the effect depends both on the nature of the drug and the cation, as well as on the doses used. The drugs that bind metal ions should be taken either two to three hours after or before ingestion of cation containing products such as dairy products, antacids and mineral supplements. These types of drug-metal ion interactions are covered in Chapter 70, Drug Interactions.

β-Lactam antibiotics form chelates with metal ions such as Cu^{2+} (Fig. 33-5). The β-lactam ring is much more susceptible to specific base hydrolysis (i.e., toward OH^- attack) when complexed than when uncomplexed. Thus, formation of such chelates can significantly decrease the shelf-life of β-lactam antibiotics.

MOLECULAR COMPLEXES

Molecular complexes can be classified according to 1) the bonding or interaction between substrate and ligand (e.g., electrostatic interaction, charge-transfer, hydrogen bonding and hydrophobic interaction), 2) type of substrate and ligand forming the complex (e.g., small molecule—small molecule, small molecule—macromolecule, enzyme—substrate, drug—receptor and antigen—antibody) or 3) type of structure formed (e.g., self-assembled aggregate, micelle, clathrate, and inclusion complex). Molecular complexes consist of one or more substrates and ligands that are, in general, held together by relatively weak, noncovalent forces. In aqueous solutions, free molecules are most often in dynamic equilibrium with molecules bound within the complex but tightly bond complexes are not unknown. Much of the pioneering work on molecular complexes was published by Takeru Higuchi and coworkers.[5]

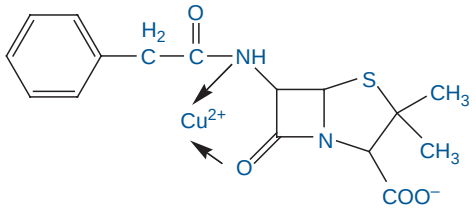


Figure 33-5. Copper (Cu^{2+}) benzylpenicillin complex (chelate).

Drug-excipient interactions are quite common in pharmaceutical formulations and frequently such interactions are the result of formation of molecular complexes. Formation of such complexes will affect the physicochemical and biological properties of the drug bound to the excipient such as its aqueous solubility, release from the drug formulation and bioavailability.

Non-ionic water-soluble polymers, such as polyvinyl pyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC), are commonly used to enhance viscosity and to form hydrogels. In aqueous solutions, PVP forms coils and is able to bind drug molecules via non-covalent binding, and PVP is known to form complexes with various drugs.[6] PVP binds iodine in the presence of iodide ions (povidone-iodine) and as such is used as antibacterial agent for treatment and prevention of skin or wound infections (Fig. 33-6). Both PVP and HPMC can enhance aqueous solubility of drugs through the formation of water-soluble complexes and have been used as solubility and dissolution enhancers.[7,8]

Ionic water-soluble polymers, such carboxymethylcellulose (CMC), are able to bind drugs. For example, in aqueous solutions CMC forms complexes with some basic drugs such as atenolol, diphenhydramine, lidocaine and propranolol.[9] Gum Arabic and alginates, both of which are anionic polysaccharides, are known to interact with drugs affecting their aqueous solubility and chemical stability. Chitosan (a linear polysaccharide composed of randomly distributed glucosamine) is positively charged at acidic pH and forms nano-sized complexes with negatively charged DNA and RNA. Chitosan has been used in gene delivery.[10] Alginates form drug complexes and have also been used to modify drug delivery.[11]

Drug-drug interactions in the form of complexes are also well known. Thus, salicylates form complexes with benzocaine, and the anticancer drugs paclitaxel, doxorubicin, and etoposide have been shown to form dimers and trimers in aqueous solutions as well as etoposide–paclitaxel complexes.[12] Cocaine and morphine form a binary complex in aqueous solutions that

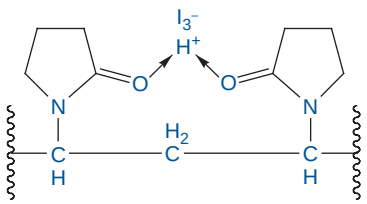


Figure 33-6. The iodine—PVP complex.

can affect their pharmacokinetics and biological activity.[13] β-Lactam antibiotics form complexes with aminoglycosides,[14] heparin forms complexes with drugs such as polymyxin B and streptomycin, and caffeine and nicotinamide form water-soluble complexes with a number of drugs and vitamins.[5,15,16]

Drug-food interactions can also be due to drug complexation.[17] Dietary fibers reduce oral drug absorption by drug complexation.[18] These material can include psyllium, guar gums, and other carbohydrates such as cyclodextrins. Cholestyramine and related bile acid sequestrates interact with bile acids to reduce their ability to be reabsorbed and thereby accelerating cholesterol elimination. These are strong ion exchange resins which interact with substrates through electrostatic forces to form the desired complexes.

INCLUSION COMPLEXES

Inclusion complexes are molecular complexes characterized by entrapment of the substrate (guest) in a cage (host) consisting of one or more ligand molecules. Cyclodextrin complexes are examples of guest-host complexes where a single ligand molecule entraps one or more guest molecules. Some other relatively large molecules, such as, water-soluble dextrins, crown ethers and calixarenes, are also capable of forming this type of monomolecular inclusion complex.[19,20] In contrast several small molecules, like hydroquinone, bile acids, thiourea, and urea assemble to form channels (tunnels), clathrates (cages), molecular sandwiches or other types of hydrophilic nanostructures around lipophilic molecules. For example, hydroquinone molecules form a cage like structure around small molecules like methanol through hydrogen bonding of the hydroquinone molecules whereas urea can form channels around straight-chain hydrocarbons and 13-*cis*-retinoic acid.[21,22]

COMPLEX STABILITY

STOICHIOMETRY AND BINDING CONSTANTS

The two most important characteristics of complexes are their stoichiometry and the numerical values of their stability constants. If m substrate molecules (S) associate with n ligand molecules (L) to form a complex (S_mL_n) the following overall equilibrium is attained:

$$m \cdot S + n \cdot L \overset{K_{m:n}}{\rightleftarrows} S_mL_n \tag{1}$$

where $K_{m:n}$ is the stability constant (also known as binding constant, formation constant or association constant) of the substrate-ligand complex. The stability constant can be written as:

$$K_{m:n} = \frac{[S_mL_n]}{[S]^m \cdot [L]^n} \tag{2}$$

where the brackets denote molar concentrations. In general, higher order complexes are formed in a stepwise fashion where a 1:1 complex is formed in the first step and 1:2 (or 2:1) complex in the next step and so on:

$$S + L \rightleftarrows SL$$
$$SL + L \rightleftarrows SL_2$$

Consequently the stability constants can be written as:

$$K_{1:1} = \frac{[SL]}{[S]\cdot[L]} \tag{3}$$

$$K_{1:2} = \frac{[SL_2]}{[SL]\cdot[L]} \tag{4}$$

The **reciprocal values** (e.g., $1/K_{1:1}$) are known as dissociation constants or instability constants. The physicochemical properties of free substrate molecules are somewhat different from those of the compound bound to the ligand molecules. Likewise, the physicochemical properties of free ligand molecules are different from those of the ligand in the complex. In general, any methodology that can be used to observe these changes in additive physicochemical properties may be utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants. These include changes in solubility, changes in chemical reactivity, changes in UV/VIS absorbance, changes in fluorescence, NMR chemical shifts, changes in substrate retention (e.g., in liquid chromatography), changes in pKa values, potentiometric measurements and effects on substrate permeability through artificial membranes. Furthermore, because complexation will influence the physicochemical properties of the complexation media, methods that monitor these media changes can also be applied to study the complexation. These include measurements of conductivity changes of an aqueous medium, determinations of freezing point depression, viscosity measurements and solution calorimetric titrations. The stoichiometry of the substrate/ligand complexes and the numerical values of their stability constants should be independent of the methodology applied and sometimes that is the case. In some cases, method-dependent differences in the stability constants can be discerned. These inconsistencies can be explained by coexistence of several different types of substrate-ligand complexes in the complexation media and concentration dependent changes in their relative contribution to the overall complexation effect.

The van't Hoff equation is used to explain the effects of temperature on equilibrium constants such as stability constants of complexes:

$$InK = Intercept - \frac{\Delta H^0}{R} \cdot \frac{1}{T} \tag{5}$$

where ΔH^0 is the standard enthalpy change, R is the gas constant and T is the temperature in Kelvin. A plot of T^{-1} *versus* lnK will give a straight line with slope equal to $-(\Delta H/R)$. The standard free energy change (ΔG) for equilibrium processes is calculated from Eq. 6:

$$\Delta G^0 = -R \cdot T \cdot InK \tag{6}$$

with the standard free entropy change (ΔS^0) derived from the following relationship:

$$\Delta G^0 = \Delta H^0 - T \cdot \Delta S^0 \tag{7}$$

DETERMINATION OF BINDING CONSTANTS

Various analytical methods can be applied to obtain the stoichiometry and numerical values of a given substrate-ligand complex. The following are descriptions of just a few common methods that are applied during drug formulation studies. However, it must be emphasized that most of these methods were developed under ideal conditions (e.g., in dilute aqueous solutions) or conditions that rarely can be found in pharmaceutical formulations. Thus, the determined values should be characterized as observed and not exact values (i.e., observed stoichiometry, observed stability constant etc.).

The solubility method

The most common application of complexes in pharmaceutical formulations is their usage as solubilizers of poorly soluble drugs. In such cases, the stoichiometry of the substrate-ligand complexes and the numerical values of their stability constants are frequently obtained from phase-solubility diagrams, i.e., plots of substrate solubility vs. ligand concentration. The phase-solubility technique was developed by Higuchi and Connors and it is based on research related to how complexes of different ligands such as caffeine, polyvinylpyrrolidone, and some aromatic acids affects the aqueous solubility of substrates.[5,23,24] Experimentally, an excess of a poorly water-soluble substrate is introduced into several vials to which a constant volume of an aqueous vehicle containing successively higher concentrations of ligand are added. The vials are shaken or otherwise agitated at constant temperature until equilibrium is established. The suspensions are then filtered and the total concentration of dissolved substrate ($[S]_T$) determined based on an appropriate

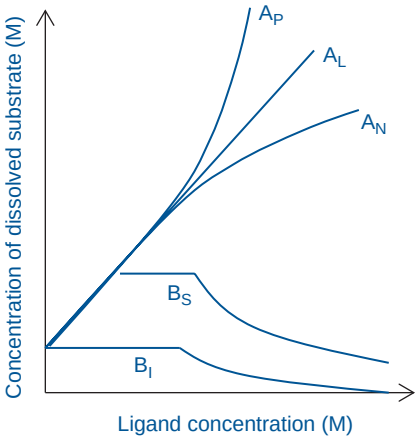


Figure 33-7. Schematic presentation of A- and B-type phase-solubility diagrams with applicable subtypes (A_P, A_L, A_N, B_S, and B_i).

analytical technique. The phase-solubility profile is then constructed by assessing the effect of the ligand concentration on the apparent solubility of the substrate. The phase-solubility method does not give insight as to how the complexes are formed or as to the forces involved. To study these types of effects, only methodologies that monitor physicochemical properties of the included substrate molecule or the ligand molecule can be applied, such as changes in the UV-visible or NMR spectra. Also, due to the coexistence of several different types of substrate—ligand complexes, the stability constants obtained from the phase-solubility profiles are observed constants. Based on the shape of the generated phase-solubility relationships, several types of behaviors can be identified.[25] Phase-solubility diagrams fall into two major types: A and B (Fig. 33-7).

A-TYPE PROFILES

In A systems, the apparent solubility of the substrate increase as a function of ligand concentration. Three subtypes have been defined: A_L profiles indicate a linear increase in solubility as a function of ligand concentration, A_P systems indicate an isotherm wherein the curve deviates in a positive direction from linearity (i.e., the ligand solubilization is proportionally more effective at higher concentrations) and A_N relationships indicate a negative deviation from linearity (i.e., the ligand solubilization is proportionally less effective at higher concentrations). Taken as a whole, these isotherms indicate that water-soluble complexes are being formed with solubilities higher than that of the uncomplexed substrate. A_L-type relationships are first order with respect to ligand and first or higher order with respect to the substrate, that is, SL, S_2L, S_3L, and so on. If the slope of the A_L isotherm is greater than unity, higher order complexes are assumed to be involved in the solubilization. Although a slope of less than one does not exclude the occurrence of higher order complexes, a one-to-one complex is often assumed in the absence of other information. A_P systems suggest the formation of higher order complexes with respect to the ligand at higher ligand concentrations, for example, SL_2, SL_3, and so on. The stoichiometry of the formed complexes has historically been implied by the extent of curvature of the phase-solubility profile. Thus, an isotherm best fit to a quadratic function suggest the formation of a one-to-two (SL_2) complex, one best fit to a cubic function suggests a one-to-three complex (SL_3), and so forth. A_N profiles have several explanations including bulk changes imparted to the solvent by the ligand at various concentrations, that is, the ligand is acting as a chaotrope or kosmotrope or is altering the bulk properties of the media by changing its viscosity, surface tension, or conductivity and/or self-association of the ligand at high concentrations.

Equilibrium constants can be derived from A-type phase-solubility profiles in a number of ways. As discussed previously, the equilibrium constant for a complexation of interest is given by Equation 1. The intrinsic substrate solubility is given as S_0 and a formed complex is represented by SL:

$$[S] = S_0 \tag{8}$$

$$[S]_T = S_0 + m \cdot [S_m L_n] \tag{9}$$

$$[L]_T = [L] + n \cdot [S_m L_n] \tag{10}$$

where $[S]$ is the concentration of free substrate (i.e., dissolved substrate that is not bound to ligand), $[S]_T$ is the total concentration of dissolved substrate (i.e., both free and bound to a water-soluble complex), $[L]_T$ is the total concentration of ligand in solution and [L] is the concentration of free ligand (i.e., dissolved ligand molecules that are not forming complex with the substrate). The values for $[S_mL_n]$, $[S]$ and $[L]$ can be derived as:

$$[S_m L_n] = \frac{[S]_T - S_0}{m} \tag{11}$$

$$[L] = [L]_T - n \cdot [S_m L_n] \tag{12}$$

where S_0 is the solubility of the substrate in the absence of the L. For equilibria that are first order with respect to the ligand (n = 1), the following equation can be obtained:

$$[S]_T = \frac{m \cdot K \cdot (S_0)^m \cdot [L]_T}{1 + K \cdot (S_0)^m} + S_0 \tag{13}$$

A plot of $[S]_T$ versus $[L]_T$ for the formation of S_mL complex should, therefore, give a straight line with the y-intercept representing S_0 and the slope defined as:

$$Slope = \frac{m \cdot K \cdot (S_0)^m}{1 + K \cdot (S_0)^m} \tag{14}$$

Therefore, if m is known, the K can be calculated, meaning that for one-to-one complexation, that is, m = 1, the following equation will apply:

$$K_{1:1} = \frac{Slope}{S_0 \cdot (1 - Slope)} \tag{15}$$

It should be noted that in the circumstance where a series of complexes of the form $SL + S_2L + \ldots + S_mL$ are present, an A_L-type profile would still be observed and simple phase-solubility analysis would not be able to distinguish between the simple 1:1 (one-to-one) and higher order complexes. If the slope of the isotherm is greater than unity, higher order complexes are indicated and equation 13 can be used with substitution of various trial values of m to suggest the stoichiometry and magnitude for the equilibrium constant.

Frequently, the y-intercept is not equal to S_0 and this can cause considerable error in the value of K. A more accurate method for determination of the solubilizing effect of ligands is to determine their complexation efficiency (CE), i.e., the concentration ratio between ligand in a complex and free ligand. CE is calculated from the slope of the phase-solubility diagrams, it is independent of both S_0 and the intercept, and it is more reliable when the influences of different pharmaceutical excipients on the solubilization are being investigated. For 1:1 SL complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$CE = \frac{[SL]}{[L]} = S_0 \cdot K_{1:1} = \frac{Slope}{(1 - Slope)} \tag{16}$$

And the substrate:ligand molar ratio can be calculated from the CE:

$$S : L\ molar\ ratio = 1 : \frac{(CE + 1)}{CE} \tag{17}$$

For A_P-type profiles, the equilibrium constants can also be calculated. Equations 3 and 4 apply for a system in which one

substrate molecules forms a complex with two ligand molecules and the following mass balance equations apply:

$$[S]_T = S_0 + [SL] + [SL_2] \quad (18)$$

$$[L]_T = [L] + [SL] + 2\cdot[SL_2] \quad (19)$$

Equations 3, 4, 18, and 19 can be combined and converted into the following quadratic relationship:

$$[S]_T = S_0 + K_{1:1}\cdot S_0\cdot[L] + K_{1:1}\cdot K_{1:2}\cdot S_0\cdot[L]^2 \quad (20)$$

indicating that a plot of $[S]_T$ versus $[L]_T$ (assuming that $[L] \approx [L]_T$) fitted to the quadratic relationship will allow for the estimation of $K_{1:1}$ and $K_{1:2}$. Note that at low L concentrations, $[L]_T$ is sometimes used as an estimate for $[L]$, assuming low CE, meaning that a plot of the ligand concentration versus substrate solubilized can be used to estimate the K values. A linear form of this equation can also be derived:

$$\frac{[S]_T - S_0}{[L]} = K_{1:1}\cdot S_0 + K_{1:1}\cdot K_{1:2}\cdot S_0\cdot[L] \quad (21)$$

Theoretically, higher order complexation can be further examined with higher order curve fitting meaning that the same formalism can be used when $K_{1:3}$, $K_{1:4}$,..., $K_{1:m}$ values are present (the cubic equation is given as an example):

$$[S]_T = S_0 + K_{1:1}\cdot S_0\cdot[L] + K_{1:1}\cdot K_{1:2}\cdot S_0\cdot[L]^2 + K_{1:1}\cdot K_{1:2}\cdot K_{1:3}\cdot S_0[L]^3 \quad (22)$$

It should be emphasized that frequently the K-values obtained are apparent values that do not describe the actual process on a molecular level. Coexistence of several different types of ligand complexes and the nonideality of substrate saturated solutions complicate exact determination of the K-values from phase-solubility diagrams. In addition substrate molecules are known to self-associate in aqueous solution and to interact with other pharmaceutical excipients and because these equilibria will reduce the availability of the substrate for complex formation, this may lead to errors in the K-value determinations. Although correlation is often found between phase-solubility diagrams and the stoichiometry of substrate-ligand complexes determined by other means such as NMR, some discrepancies can be found in the literature.[26]

The origin of A_N-type phase-solubility profiles is uncertain. One possibility is ligand enforced structural changes of the aqueous complexation medium. Another possibility is self-association of ligand molecules at higher concentrations.

B-type profiles indicate formation of complexes with limited water solubility. Two subclasses have been described including B_s and B_I systems (Fig. 33-7). B_s-type isotherms can be interpreted in the following manner. As the ligand concentration increases, a soluble complex is formed which increases the total solubility of the substrate. At a particular point in this solubilization process, the maximum solubility of the substrate is achieved which is the sum of S_0 plus any substrate solubilized in the form of the ligand complex (SL). Additional ligand generates additional complex which precipitates but so long as solid substrate remains, dissolution and complexation can occur to maintain the value of $[S]_T$. During this plateau phase, the following equilibrium is assumed to occur:

$$S_S \rightleftarrows S_{Aq} + L \overset{K}{\rightleftarrows} (SL)_{Aq} \rightleftarrows (SL)_S \quad (23)$$

where S_S is the solid substrate, S_{Aq} is the dissolved substrate, K is the stability constant of the complex, $(SL)_{Aq}$ is the dissolved complex, and $(SL)_S$ is the solid complex. At some point, all of the solid substrate will have been consumed in the above described process and further addition of the ligand results in the formation of additional insoluble inclusion complex which precipitates and further depletes the total substrate concentration, $[S]_T$. Finally, the solubility observed in the systems is associated with the solubility of the precipitated complex. If the same complex which forms in the ascending portion the phase-solubility profile precipitates in the plateau phase, the increase in the substrate concentration from S_0 to the plateau should be equal to the intrinsic solubility of the complex.

The kinetic method

The second most common application of complexes in pharmacy is to influence drug kinetics, usually to decrease drug degradation in pharmaceutical formulations. Complexes are always under kinetic equilibrium in aqueous solutions, i.e., the molecules forming a complex are in equilibrium with free molecules in the solution, where the rate of formation and dissociation of the complex is diffusion controlled. In kinetic studies the stability constant ($K_{m:n}$ in Equation 1) can be determined from the stabilizing or destabilizing effects of a ligand on a given substrate. If a ligand has for example stabilizing effect on a substrate molecule in aqueous solution, then the rate of disappearance of the substrate will decrease when the substrate-to-ligand concentration ratio is decreased (i.e., at increasing ligand concentration). In the following, we assume that the substrate degradation is first order and that a 1:1 SL complex is being formed. The first-order rate constant for degradation of the free substrate (k_f) is determined in the aqueous complexation media when no ligand is present. The first-order rate constant for the degradation of the substrate within the ligand complex (k_c) and $K_{1:1}$ can then be determined from the degradation profile (Fig. 33-8) and the observed first-order rate constant (k_{obs}) for the rate of disappearance of the substrate:

$$k_{obs} = k_f\cdot f_f + k_c\cdot f_c \quad (24)$$

where f_f is the fraction of free substrate and f_c is the fraction of substrate in complex. If we assume that only a 1:1 substrate-ligand complex (SL) is being formed and that the total substrate concentration ($[S]_T$) is the sum of the concentration of free substrate ($[S]$) and the concentration of the complex ($[SL]$) the following equations are obtained:

$$[S]_T = [S] + [SL] \quad (25)$$

$$[L]_T = [L] + [SL] \quad (26)$$

$$K_{1:1} = \frac{[SL]}{[S]\cdot[L]} \quad (27)$$

$$f_f = \frac{[S]}{[S]+[SL]} = \frac{1}{1+K_{1:1}\cdot[L]} \quad (28)$$

$$f_c = 1 - f_f = \frac{K_{1:1}\cdot[L]}{1+K_{1:1}\cdot[L]} \quad (29)$$

$$k_{obs} = \frac{k_f + k_c\cdot K_{1:1}\cdot[L]}{1+K_{1:1}\cdot[L]} \quad (30)$$

$$-\frac{d[S]_T}{dt} = k_{obs}\cdot[S]_T = \left(\frac{k_f + k_c\cdot K_{1:1}\cdot[L]}{1+K_{1:1}\cdot[L]}\right)\cdot[S]_T \quad (31)$$

If the total ligand concentration is much greater than the total substrate concentration ($[L]_T \geq 10\cdot[S]_T$) then it can be assumed that $[L] \approx [L]_T$:

$$k_{obs} = \left(\frac{k_f + k_c\cdot K_{1:1}\cdot[L]}{1+K_{1:1}\cdot[L]}\right) \quad (32)$$

Equation 32 can then be rearranged into several different formats including that of the Lineweaver-Burk plot where $(k_f - k_{obs})^{-1}$ *versus* $([L]_T)^{-1}$ will give a straight line in which k_c can be obtained from the intercept and $K_{1:1}$ from the slope:

$$\frac{1}{k_f - k_{obs}} = \frac{1}{K_{1:1}\cdot(k_f - k_c)}\cdot\frac{1}{[L]_T} + \frac{1}{k_f - k_c} \quad (33)$$

Alternatively, k_c and $K_{1:1}$ can be obtained by simple non-linear fitting of k_{obs} according to equation 32.

UV/vis spectroscopic method

It is also possible to determine the value of $K_{1:1}$ by observing spectrophotometric or spectroscopic changes of the substrate

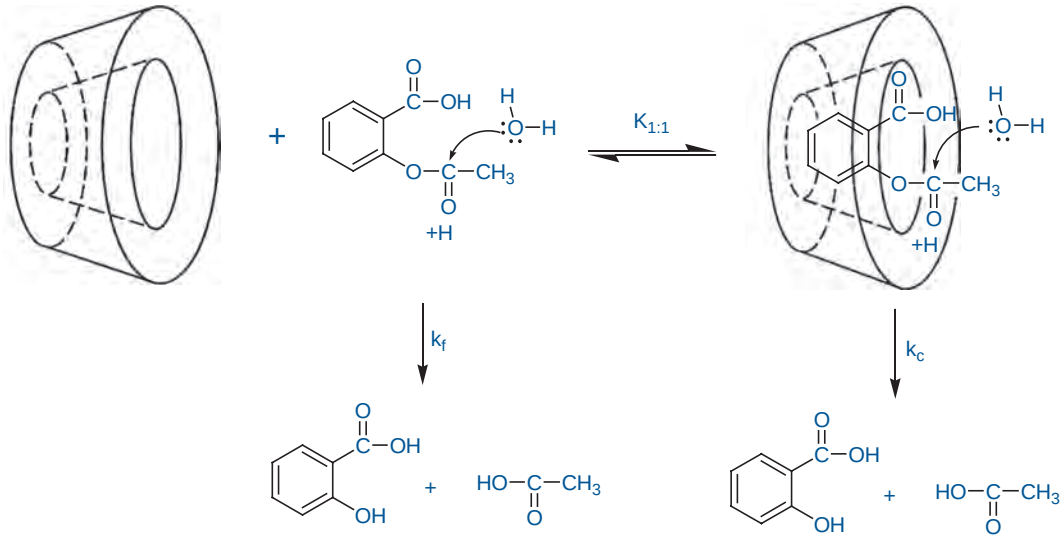


Figure 33-8. Under acidic conditions unionized aspirin forms 1:1 complex with β-cyclodextrin and its derivatives. When cyclodextrin is added to the aqueous aspirin solution the lipophilic aromatic ring will be taken into the cyclodextrin cavity where the ester linkage is more sterically hindered against nucleophilic attack by water molecules than outside it (i.e., $k_f > k_c$).

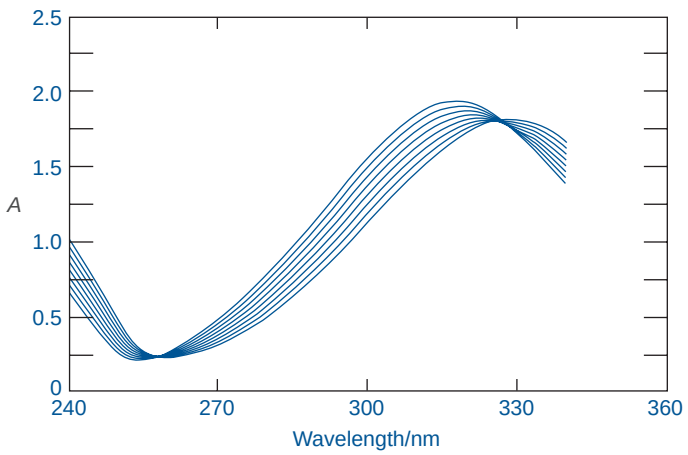


Figure 33-9. Ultraviolet absorption spectrum of *p*-nitrophenol in the presence of varying concentrations of α-cyclodextrin. The *p*-nitrophenol concentration is $1.99 \cdot 10^{-4}$ M, and the cyclodextrin concentration ranges from zero (topmost spectrum) to 0.01 M.

upon binding. Figure 33-9 shows how the ultraviolet spectrum of *p*-nitrophenol changes upon complexation with α-cyclodextrin. The spectrometric changes which occur are similar to those encountered when a substrate is dissolved in solvents of decreased polarity (e.g., water → dioxane). In the case of cyclodextrin guest-host complexes, the chromophore of the guest (substrate) is transferred from a polar aqueous environment to a less polar environment within the cyclodextrin cavity (ligand). The presence of well-defined isosbestic points is consistent with the assumption of 1:1 stoichiometry.

A wavelength showing significant changes in absorption is selected and the changes in absorption titrated assuming that Beer's law is obeyed:

$$A_0 = \varepsilon_s \cdot b \cdot [S]_T \tag{34}$$

where A_0 is the absorbance of a substrate solution when no ligand is present, ε_S is the molar absorptivity of the substrate, b is the path length and $[S]_T$ is the total substrate concentration. In the presence of a ligand the absorbance is:

$$A_L = \varepsilon_s \cdot b \cdot [S] + \varepsilon_L \cdot b \cdot [L] + \varepsilon_{11} \cdot b \cdot [SL] \tag{35}$$

where ε_L is the absorptivity of the ligand and ε_{11} is the absorptivity of the complex. Combining equation 35 with the mass balances $[S]_T = [S] + [SL]$ and $[L]_T = [L] + [SL]$ (i.e., Equations 25 and 26) gives:

$$A_L = \varepsilon_s \cdot b \cdot [S]_T + \varepsilon_L \cdot b \cdot [L]_T + \Delta\varepsilon_{11} \cdot b \cdot [SL] \tag{36}$$

where $\Delta\varepsilon_{11} = \varepsilon_{11} - \varepsilon S - \varepsilon L$. If the solution absorbance is measured against reference solution containing same concentration of ligand ($[L]_T$) but no substrate ($[S]_T = 0$) the measured absorbance will be:

$$A = \varepsilon_S \cdot b \cdot [S]_T + \Delta\varepsilon_{11} \cdot b \cdot [SL] \tag{37}$$

Combining Equations 3 and 37 gives the Benesi-Hildebrand equation:

$$\frac{b}{\Delta A} = \frac{1}{K_{1:1} \cdot [S]_T \cdot \Delta\varepsilon_{11}} \cdot \frac{1}{[L]_T} + \frac{1}{[S]_T \cdot \Delta\varepsilon_{11}} \tag{38}$$

where ΔA is the difference in absorbance in the presence and absence of ligand. Most frequently the $[S]_T$ is kept constant whereas $[L]_T$ is varied. Then a plot of $b/\Delta A$ *versus* $1/[L]_T$ should give a straight line for a 1:1 complex with the ratio intercept/slope yielding $K_{1:1}$.

NMR spectrometry

Changes is the NMR spectra of the substrate and/or the ligand (i.e., chemical shifts, coupling constants, nuclear Overhauser effects, and spin-spin and spin-lattice relation times) can be used to probe the solution geometry of complexes as well as to give kinetic information on their association and dissociation. If the changes in chemical shift of, for example, the substrate molecule is titrated then a modified Benesi-Hildebrand Equation 38 can be used:

$$\frac{1}{\Delta\delta} = \frac{1}{K_{1:1} \cdot \Delta\delta_{max}} \cdot \frac{1}{[L]_T} + \frac{1}{[S]_T \cdot \Delta\delta_{max}}$$

where $\Delta\delta$ is the change in chemical shift at particular ligand concentration and $\Delta\delta_{max}$ is the limiting change in chemical shift at infinite ligand concentration (i.e., when all the substrate molecules in the solutions are bound to the ligand). A plot of $1/\Delta\delta$ *versus* $1/[L]_T$ should give a straight line for a 1:1 complex with the ratio intercept/slope yielding $K_{1:1}$.

CYCLODEXTRINS

Cyclodextrins are cyclic oligosaccharides consisting of (α-1,4)-linked D-glucopyranose units, with a hydrophilic outer surface and a lipophilic central cavity. The natural α-, β-, and γ-cyclodextrins consist of 6, 7, and 8 glucopyranose units, re-

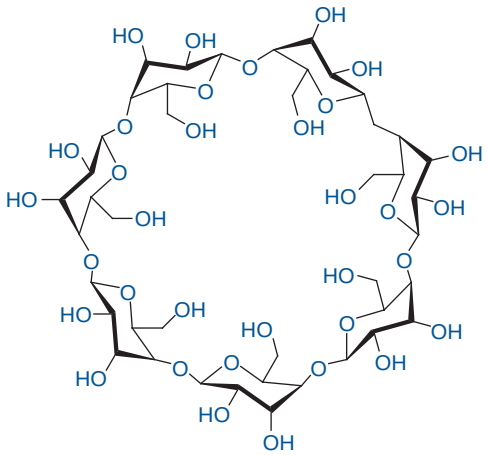


Figure 33-10. The structure of β-cyclodextrin.

spectively (Fig. 33-10 and Table 33-2).[25,27,28] The diameters of the central cavities are about 5, 6, and 7 Å for α-, β-, and γ-cyclodextrin, respectively. Larger cyclodextrins containing more than eight glucopyranose units do exist but are of limited pharmaceutical interest. Although the natural cyclodextrins and their complexes are hydrophilic, their aqueous solubility can be rather limited. This is thought to be due to relative strong binding of the cyclodextrin molecules in the crystal state. Random substitution of the hydroxy groups can result in dramatic improvements in their aqueous solubility. Cyclodextrin derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin and sulfobutylether β-cyclodextrin (Table 33-2). The molar degree of substitution (MS) is defined as the average number of substituents that have reacted with one glucopyranose repeat unit. In an aqueous environment, cyclodextrins form inclusion complexes with many lipophilic molecules through a process in which water molecules located inside the central cavity are replaced by either a whole molecule, or more frequently by some lipophilic structure of the molecule (Fig. 33-8). Cyclodextrin complexation of a drug molecule changes the physicochemical properties of the drug, such as its aqueous solubility, chemical stability and ability to permeate biological membranes. Because the cyclodextrin molecule is hydrophilic on the outside the complex formation usually increases the water-solubility of lipophilic water-insoluble drugs. Once included in the cyclodextrin cavity, the drug molecules may be dissociated from the cyclodextrin molecules through complex dilution or competitive binding by some other suitable molecule (e.g., lipids) or, if the complex is located in close approximation to a lipophilic biological membrane (e.g., mucosa), the drug may be transferred to the matrix for which it has the highest affinity. Importantly, because no covalent bonds are formed or broken during the guest-host complex formation, the complexes are in dynamic equilibrium with free drug and cyclodextrin molecules. In aqueous solutions the rates for formation and dissociation of drug-cyclodextrin complexes are very close to the diffusion-controlled limits and the complexes are continuously being formed and dissociated.[29] For cyclodextrin complexes the value of $K_{1:1}$ (Equations 1 and 15) is frequently between 10^1 and 10^3 M^{-1} and $K_{1:1}$ greater than 5×10^3 M^{-1} is rarely observed. The effects of cyclodextrins on drug solubility, bioavailability, chemical stability and delivery through biological membranes have been investigated by a number of research groups.[28,30]

Cyclodextrins enhance drug delivery through biological membranes by increasing drug permeation through the unstirred water layer that is located adjacent to the membrane surface or, in other words, by increasing the availability of dissolved drug molecules juxtaposed to the membrane surface. Cyclodextrins only enhance drug permeation when a water layer is present at the membrane exterior. Such water layers can consist of mucus or an aqueous vehicle such as o/w creams or hydrogels. Cyclodextrins do not enhance drug permeation from vehicles that do not form an unstirred water layer, such as lipophilic ointments and w/o creams. The effect also depends on the physicochemical properties of the drug. Better enhancement is obtained for lipophilic drugs that are poorly soluble in water that form water-soluble complexes with cyclodextrins with stability constants ($K_{1:1}$) that are between about 50 and 5000 M^{-1}.[30]

In aqueous solutions free drug molecules are in equilibrium with drug molecules bound in a complex and, thus, the release of drug molecules from cyclodextrin-containing vehicle will depend on the drug/cyclodextrin molar ratio and the $K_{m:n}$ value (Eq. 1). Furthermore, pharmaceutical excipients will affect the $K_{m:n}$ value. Thus it is of utmost importance to optimize the drug vehicle with regard to the amount of cyclodextrin. Too much or too little cyclodextrin will result in less than optimal drug bioavailability.

Recently it has been discovered that cyclodextrin molecules and their complexes self-associate in aqueous solutions to form nanoparticles.[31] The general observation is that the aggregate formation increases with increasing cyclodextrin concentration. The anomalously low solubility of β-cyclodextrin is explained by the intensity of nanoparticle formation (i.e., aggregation). The same explanation is valid for the observed peculiarity of aqueous γ-cyclodextrin solutions, which are known to become spontaneously turbid at concentrations of about 1% (w/v) or above. The fraction of molecules participating in nanoparticle formation is often very low. For example, the mass contribution of the nanoparticles in aqueous 12 mM α-cyclodextrin solution does not exceed 0.8%, that of β-cyclodextrin is only 0.0011% in 10 mM in β-cyclodextrin solution, and that of γ-cyclodextrin is only 0.02% in 12 mM γ-cyclodextrin solution.[32-34] However, formation of cyclodextrin complexes enhances the cyclodextrin aggregation.[35] In some cases the mass contribution of the nanoparticles in aqueous complexation media can be well above 50%. Another interesting feature of cyclodextrin nanoparticles is their shape, such as disks, rods and fibers. Thus, cyclodextrins

Table 33-2. The natural cyclodextrins and some of their derivatives.

Cyclodextrin	Synonyms	MS	MW* (Da)	Solubility (mg/ml)
α-Cyclodextrin	alfadex		972.8	130
β-Cyclodextrin	betadex		1135	18.4
2-Hydroxypropyl-β-cyclodextrin	hydroxypropyl betadex	0.65	1400	> 600
Sulfobutylether β-cyclodextrin sodium salt		0.9	2163	> 500
Methylated β-cyclodextrin		1.8	1312	> 600
		0.57	1191	200
γ-Cyclodextrin	gammadex		1297	249
2-Hydroxypropyl-γ-cyclodextrin	hydroxypropylgammadex	0.6	1576	> 600

*The molecular weights (MW) of the cyclodextrin derivatives will depend on their molar degree of substitution (MS), i.e., the number of substituents per glucopyranose repeat unit.

and cyclodextrin complexes form supramolecular complexes at elevated cyclodextrin concentrations.

Due to their favorable toxicological profile cyclodextrins are frequently preferred to organic solvents during *in vitro/in vivo* evaluation of new chemical entities.

Worldwide there are between 30 and 40 different cyclodextrin-containing drug products on the market and few examples are shown in Table 33-3. In most cases cyclodextrins are used as solubilizers, either to enhance dissolution and oral bioavailability of poorly-soluble drugs in solid dosage forms or to replace organic solvents in parenteral dosage forms. Cyclodextrins are also used to increase both chemical and physical stability of drugs, including both peptide and protein drugs, to reduce drug-drug and drug-excipient interactions and to convert liquids to solid powders. Cyclodextrins can also have some adverse effects. For example, excess cyclodextrin can hamper drug absorption from the gastro-intestinal tract and permeation of drugs through skin and other biological membranes. In fact, cyclodextrins have been used to prevent permeation of topically applied sunscreen agents into skin and to reduce absorption of fat from the gastrointestinal tract.

COMPLEXES IN PHARMACY

APPLICATION TO DRUG DELIVERY

Complexation may affect physicochemical and biopharmaceutical properties of drugs, such as aqueous solubility, chemical stability, dissolution rate, partition coefficient, permeability, rate of absorption, bioavailability, biological activity, volatility and physical state (e.g., converting liquid drug into solid drug complex). Pharmaceutical formulators sometimes apply complexation agents in their formulation design. For example, cyclodextrins and their derivatives are commonly applied functional pharmaceutical excipients with products containing these materials associated with numerous therapeutic categories and administration routes (Table 33-3). These materials are present in formulations intended for parenteral (intravenous, intramuscular, intracavernosal), oral, sublingual, buccal, nasal, ophthalmic, and dermal use. Parenteral use of cyclodextrins include drug products intended to deliver prostaglandins with examples including prostaglandin E1 formulated with α-cyclodextrin (αCD) (alprostadil alfadex). This formulation is used in the treatment of various vascular complications including Buerger's disease. More recently, the material has been shown to be of benefit in male erectile dysfunction when administered intracavernosally. Studies have found that it is useful in patients who do not respond to oral sildenafil (Viagra®, Pfizer) treatment. Although β-cyclodextrin (βCD) is contraindicated parenterally, its derivatives can be safely administered using the oral route. 2-Hydroxypropyl-β-cyclextrin (HPβCD) is available in several intravenous products including in the Sporanox® IV solution (itraconazole) product. Itraconazole is a triazole-type drug which exerts its effect by inhibiting fungal P450 and inhibiting the biosynthesis of ergosterol, an essential component of the fungal membrane. The compound is noteworthy in that it was the first approved orally bioavailable agent with significant clinical activity against both candidiasis and *Aspergillus spp*., the two most common human fungal pathogens. Intravenous use of itraconazole is indicated for empiric therapy of idiopathic fever as well as for blastomycosis (pulmonary and extra-pulmonary), histomycosis (pulmonary and disseminated, non-meningeal) and aspergillosis (pulmonary and non-pulmonary). Cyclodextrins were enabling in this

Table 33-3. Examples of cyclodextrin-containing drug products.

Drug/Cyclodextrin	Therapeutic usage	Formulation	Trade Name
α-Cyclodextrin (αCD)			
Alprostadil	To treat erectile dysfunction	IV solution	Caverject Dual
β-Cyclodextrin (βCD)			
Cetirzine	Antihistamine drug	Chewing tablets	Cetrizin
Dexamethasone	Anti-inflammatory steroid	Ointment, tablets	Glymesason
Ethinylestradiol and drospirenone	Birth control	Tablets	Yaz
Iodine	Throat disinfection	Solution	Mena-Gargle
Nicotine	Nicotine replacement product	Sublingual tablets	Nicorette
Nimesulide	Non-steroidal anti-inflammatory drug	Tablets	Nimedex
Omeprazole	To treat gastroesophageal reflux	Tablets	Omebeta
Piroxicam	Non-steroidal anti-inflammatory drug	Tablets, suppository	Brexin
Tiaprofenic acid	Non-steroidal anti-inflammatory drug	Tablets	Surgamyl
2-Hydroxypropyl-β-cyclodextrin (HPβCD)			
Indomethacin	Non-steroidal anti-inflammatory drug	Eye drop solution	Indocid
Itraconazole	Antifungal agent	Oral and i.v. solutions	Sporanox
Mitomycin	Anticancer agent	IV infusion	MitoExtra
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)			
Aripiprazole	Antipsychotic drug	IM solution	Abilify
Maropitant	Anti-emetic drug (motion sickness in dogs)	Parenteral solution	Cerenia
Voriconazole	Antifungal agent	IV solution	Vfend
Ziprasidone mesylate	Antipsychotic drug	IM solution	Geodon
Randomly methylated β-cyclodextrin (RMβCD)			
Cloramphenicol	Antibacterial agent	Eye drop solution	Clorocil
γ-Cyclodextrin (γCD)			
Tc-99 Teoboroxime	Diagnostic aid, cardiac imaging	IV solution	CardioTec
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)			
Diclofenac sodium salt	Non-steroidal anti-inflammatory drug	Eye drop solution	Voltaren Ophtha
Tc-99 Teoboroxime	Diagnostic aid, cardiac imaging	IV solution	CardioTec

product due to their solubilizing effect on itraconazole which has an estimated aqueous solubility at neutral pH of about 1 ng/mL. The formulation contains 40% w/v HPβCD and increases the solubility of itraconazole to 10 mg/mL (or approximately 100,000-fold). Based on i.v. doses of itraconazole between 200 and 400 mg and the formulation containing 10 mg/mL itraconazole in a 40% HPβCD solution, the i.v. dose of HPβCD is between 8 and 16 g/day. Another widely used β-cyclodextrin derivative is the sulfobutylether β-cyclodextrin sodium salt (SBEβCD). This anionic excipient is found in a number of parenteral products including the intravenous formulation for voriconazole (Vfend, Pfizer) as well as intramuscular preparations for ziprasidone (Geodon, Zeldox, Pfizer) and aripiprazole (Abilify, BMS). In the case of voriconazole, the cyclodextrin solubilizes the antifungal such that its solubility increases from 0.2 mg/mL at pH 3 in the absence of cyclodextrin to 10 mg/mL using 15% w/v SBEβCD. Based on standard injection doses of the API, 3.5 to 7 g of the cyclodextrin are administered. 2-Hydroxypropyl-γ-cyclodextrin (HPγCD) is also available in an approved intravenous product.

Oral use of cyclodextrins includes products containing αCD, βCD, and HPβCD. An oral formulation has been developed and marketed for itraconazole. This dosage form provides for increased and more consistent oral bioavailability of itraconazole relative to other solid formulation in various subpopulations with a fraction absorbed of 85% and oral bioavailability of 55%. Based on oral doses of 200 mg itraconazole, the dose of HPβCD is 8 g/day. This formulation also allowed the effective treatment of esophageal candidiasis. Solid oral dosage forms containing βCD include tablets containing ethinylestradiol and drospirenone (Yaz®, Bayer), piroxicam (Brexin®, Chiesi) and prostaglandin E2 (Prostarmon E®, Ono). Cyclodextrins are also used to enhance drug absorption from suppositories as well as to increase the bioavailability of drug in eye drops and nasal sprays.[28,30]

Complexation does not always improve the physicochemical and biopharmaceutical properties of drugs and, as previously mentioned, some *incompatibilities* (i.e., drug-drug and drug-excipient interactions) as well as drug-food interactions are the results of unwanted drug complexation.

Other complexing agents commonly used in drug formulations are, for example, EDTA and citric acid for complexation of metal ions. Metal ions catalyze various drug degradation pathways such as oxidation. However, the complex bound metal ions are inactive. Polyalcohols, such as sorbitol, are added as stabilizers to lyophilized protein drugs. The polyalcohols form complexes with proteins and prevent their self-aggregation.

COMPLEXES IN PHARMACEUTICAL ANALYSIS

Complexation is an essential aspect of many types of bioanalysis. Determination of metal ions is frequently based on metal ion coordination complexes. *Complexometric* (chelatometric) titration is often based on EDTA complexation of metal ions. Spectrophotometric (i.e., colorimetric) determinations of metal ions are sometimes based on complex formations. Recently, complexes have been applied in *chiral chromatography*. Cyclodextrins have found numerous applications in this regard in part due to their ability to interact with compounds by complexation as well as their molecular recognition abilities. These properties have allowed cyclodextrin to aid in chiral analysis in gas and high performance liquid chromatography as well as capillary electrophoresis and NMR assessments. Chromatographically, cyclodextrins can be added to the mobile phase or bound to the stationary phase with both approaches being widely exploited. Cyclodextrins are chiral sugars and can be useful adjuncts in circular dichroism measurements due to their ability to induce chirality in non-chiral substrates. Furthermore, cyclodextrins can increase fluorescence quantum yield by shielding the excited singlet state in the central cavity thus protecting it from quenching and related effects. *Phosphorescence* can likewise be allowed in solution by providing pseudo-ordered stated within the cyclodextrin cavity in which the triplet state can relax.

DRUG-PROTEIN BINDING

After a drug has been absorbed into plasma, or after it has been injected into the blood circulation, the drug molecules are carried by the blood circulation to the target tissue within the body where they bind to receptors. The drug molecules are also carried to the eliminating organs such as the liver and kidneys. Blood plasma and the various body tissues contain proteins that are able to form complexes with drugs. Formation of such complexes is reversible and is normally referred to as drug-protein binding. Irreversible drug-protein binding is usually a consequence of covalent binding of drug molecules to proteins, not complexation, and may account for certain types of adverse effects such as allergy, carcinogenicity, teratogenicity or tissue toxicity. Drug-protein binding does affect the ability of drug molecules to permeate biological membranes and their ability to interact with enzymes and receptors. Only unbound drug molecules permeate membrane barriers and interact with drug receptors or undergo metabolism and glomerular filtration, and, thus, drug-protein binding will affect the drug pharmacokinetics (see Chapter 55).

Plasma protein binding is the result of complex formation between drug molecules and plasma proteins, such as human serum albumin (HSA), lipoprotein, glycoprotein, and α-, β-, and γ-globulin, of which HSA is the most important and most studied. HSA consists of 585 amino acid residues having calculated molecular weight of 66.47 kDa. Normal plasma concentration of HSA is between 35 and 55 mg/ml but it varies with age, exercise, stress and disease. One of the functions of HSA is to act as a transporter for poorly soluble drugs. However, HSA is very non-specific complexing agent that besides drugs binds water, various cations, fatty acids and thyroid hormones. Also plasma α_1-acid-glycoprotein (α-AGP) has been shown to bind a variety of drugs. Other plasma proteins play a smaller role in drug-protein binding.[36,37] In general, weak bonds such as hydrophobic bonds, van der Waals dispersion forces, hydrogen bonds, and ionic interactions are involved in the protein binding of drugs. Each protein molecule may possess several binding sites (i.e. bind several drug molecules) and, thus Equation 1 is not precisely applicable but it can be used to explain the basis of binding of drug (D) to a protein (P) to form a drug-protein complex (PD):

$$P + D \underset{}{\overset{K}{\rightleftharpoons}} PD \tag{40}$$

If one drug molecule is bound to one protein molecule then (see Equations 28 and 29 for comparison):

$$r = \frac{\text{concentration of bound drug}}{\text{total concentration of protein}} = \frac{[PD]}{[PD]+[P]} = \frac{[PD]}{[P]_T} = \frac{K \cdot [D]}{1 + K \cdot [D]} \tag{41}$$

where K is the binding constant or the equilibrium constant of the complex formation and $[D]$ is the concentration of free drug. However, because each protein has n number of independent binding sites we get:

$$r = \frac{n \cdot K \cdot [D]}{1 + K \cdot [D]} \tag{42}$$

Then the large protein molecule may contain more than one type of binding sites:

$$r = \frac{n_1 \cdot K_1 \cdot [D]}{1 + K_1 \cdot [D]} + \frac{n_2 \cdot K_2 \cdot [D]}{1 + K_2 \cdot [D]} + \ldots \tag{43}$$

where n_1 is the number of binding sites of type 1 with binding constant K_1, n_2 is the number of binding sites of type 2 with binding constant K_2, and so on. If only one type of binding site exists then Equation 42 can be converted to:

$$\frac{1}{r} = \frac{1}{n \cdot K} \cdot \frac{1}{[D]} + \frac{1}{n} \tag{44}$$

Plotting *1/r* against *1/[D]* will give a straight line where the value of *K* is obtained from the slope and the number of binding sites (*n*) from the intercept. The concentration of free drug can be determined by dialysis. The fraction of bound drug (β) is expresses as:

$$\beta = \frac{\text{concentration of bound drug}}{\text{total concentration of drug}} = \frac{[PD]}{[PD]+[D]} = \frac{n \cdot K \cdot [P]_T}{1 + K \cdot [D] + n \cdot K \cdot [P]_T} \quad (45)$$

The plasma protein binding is non-linear and, thus, the fraction of drug bound is dependent on the concentration of both drug and protein. However, at very low free drug concentrations (*[D]*) β becomes essentially independent of drug concentration but β increases with increasing protein concentration ($[P]_T$). Drugs with high *K* value may saturate the protein resulting in a decrease in β with increasing drug concentration. Drugs with relatively high β values are susceptible to drug-drug interactions due to competitive drug protein binding.

Enzymes are proteins that catalyze reactions and like all proteins, enzymes are long, linear chains of amino acids that fold to produce a three-dimensional structure. Enzymes (*E*) form an intermediate complex (*ES*) with the substrate which is then converted to product upon release of the enzyme:

$$E + S \underset{}{\overset{K}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \quad (46)$$

K is the binding constant or the equilibrium constant of the complex formation and k_2 is the rate constant for the catalyzed reaction. Enzymes can be highly specific catalyzing only certain class of chemical reactions or even only one particular reaction. Enzyme catalysis is covered in Chapter 32.

Drug receptors (*R*) are most often, but not always, proteins that interact with the drug molecules (*D*) to form a drug-receptor complex (*DR*) (see Chapter 53):

$$D + R \underset{}{\overset{K}{\rightleftharpoons}} DR \quad (47)$$

The complex formation changes the receptor that consequently leads to a biological activity and pharmacological response and/or adverse toxic effects. The forces involved in drug-receptor binding are the same as responsible for drug-protein binding such as ionic interactions, hydrogen bonds van der Waals dispersion forces and hydrophobic bonds. Formation of irreversible covalent bonds between drug and receptor leads to long-lasting effects.

COMPLEXES IN THERAPEUTICS

Complexes are very common in biological systems. As mentioned above drugs interact with proteins, including enzymes and receptors, through complex formations. For example, biological activity of some antimicrobial and antineoplastic agents is based on complex formation with DNA base-pairs. Molecular complexes in biological systems include DNA base-pairing and folding of proteins. Charge-transfer interactions play an important role in some membrane-transport processes. Metal ion coordination complexes are important parts of many biologically active compounds.[38] Examples of such compounds are hemoglobin (iron), cytochrome (iron), carboxypeptidase A (zinc), carbonic anhydrase (zinc), superoxide dismutase (zinc and copper), vitamin B_{12} (cobalt), chlorophyll (magnesium), and urease (nickel).

ACKNOWLEDGMENTS

Kenneth A. Connors, PhD is acknowledged for his contribution to previous editions of this work.

REFERENCES

1. Müller-Dethlefs K, Hobza P. Noncovalent interactions: a challenge for experiment and theory. *Chem Rev* 2000; 100:143–167.
2. Liu L, Guo Q-X. The driving forces in the inclusion complexation of cyclodextrins. *J Incl Phenom Macroc Chem* 2002; 42:1–14.
3. Palm GJ *et al.* Specific binding of divalent metal ions to tetracycline and to the Tet repressor/tetracycline complex. *J Biol Inorg Chem* 2008; 13:1097–1110.
4. Leyden JJ. Absorption of minocycline hydrochloride and tetracycline hydrochloride. Effect of food, milk, and iron. *J Am Acad Dermatol* 1985; 12:308–312.
5. Riley CM *et al.* eds. *Takeru Higuchi, a Memorial Tribute. Volume 3 - Equilibria and Thermodynamics.* Lawrence: Allen Press, 1991.
6. Plaizier-Vercammen JA, de Nève RE. Interaction of povidone with aromatic compounds II: Evaluation of ionic strength, buffer concentration, temperature, and pH by factorial analysis. *J Pharm Sci* 1981; 70:1252–1256.
7. Rácz I. *Drug Formulations*. Budapest: John Wiley and Sons, 1989.
8. Loftsson T *et al.* The effect of water-soluble polymers on aqueous solubility of drugs. *Int J Pharm* 1996; 127:293–296.
9. Ramírez Rigo MV *et al.* A linear free energy relationship treatment of the affinity between carboxymethylcellulose and basic drugs. *Mol Pharm* 2004; 1:383–386.
10. Mao S *et al.* Chitosan-based formulations for delivery of DNA and siRNA. *Adv Drug Deliv Rev* 2010; 62:12–27.
11. Cafaggi S *et al.* Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin-alginate complex. *J Control Rel* 2007; 121:110–123.
12. Lorenz SA *et al.* Using solution phase hydrogen/deuterium (H/D) exchange to determine the origin of non-covalent complexes observed by electrospray ionization mass spectrometry: in solution or in vacuo? *J Am Soc Mass Spectrom* 2001; 12:795–804.
13. Garrido JMPJ *et al.* Spectroscopic and electrochemical studies of cocaine-opioid interactions. *Anal Bioanal Chem* 2007; 388:1799–1808.
14. Greenslade MD *et al.* Interactions between β-lactam and aminoglycoside antibiotics. *Int J Pharm* 1985; 26:133–144.
15. Lim L-Y, Go M-L. Caffeine and nicotinamide enhances the aqueous solubility of the antimalarial agent halofantrine. *Eur J Pharm Sci* 2000; 10:17–28.
16. Evstigneev MP *et al.* Effect of a mixture of caffeine and nicotinamide on the solubility of vitamin (B2) in aqueous solution. *Eur J Pharm Sci* 2006; 28:59–66.
17. Singh BN. Effects of food on clinical pharmacokinetics. *Clin Pharmacokinet* 1999; 37:213–255.
18. Dongowsk G *et al.* Interactions between food components and drugs. Part 4: Influence of pectins and bile salts on propranolol absorption. *Int J Pharm* 1996; 144:233–239.
19. Kralj M *et al.* Biomedical potentials of crown ethers: prospective antitumor agents. *Chem Med Chem* 2008; 3:1478–1492.
20. Rodik RV *et al.* Calixarenes in bio-medical researches. *Curr Med Chem* 2009; 16:1630–1655.
21. Brown ME, Hollingsworth MD. Stress-induced domain reorientation in urea inclusion compounds. *Nature* 1995; 376:323–327.
22. Thakral S, Madan AK. Urea co-inclusion compounds of 13 cis-retinoic acid for simultaneous improvement of dissolution profile, photostability and safe handling characteristics. *J Pharm Pharmacol* 2008; 60:823–832.
23. Higuchi T, Connors KA. Phase-solubility techniques. *Adv Anal Chem Instrum* 1965; 4:117–212.
24. Repta AJ. Alteration of apparent solubility through complexation. In: Yalkowski SH, ed. *Techniques of solubilization of drugs.* New York: Marcel Dekker, 1985: 135–157.
25. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Deliv Rev* 2007; 59:645–666.
26. Loftsson T *et al.* Self-association of cyclodextrins and cyclodextrin complexes. *J Pharm Sci* 2004; 93:1091–1099.
27. Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. *Int J Pharm* 2007; 329:1–11.
28. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. *J Pharm Pharmacol* 2010; 62:1607–1621.
29. Stella VJ *et al.* Mechanisms of drug release from cyclodextrin complexes. *Adv Drug Deliv Rev* 1999; 36:3–16.

30. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes. *J Pharm Pharmacol* 2011; 63:1119–1135.
31. Messner M *et al.* Self-assembled cyclodextrin aggregates and nanoparticles. *Int J Pharm* 2010; 387:199–208.
32. Bonini M *et al.* Self-assembly of beta-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering. *Langmuir* 2006; 22:1478–1484.
33. Wu A *et al.* Micrometer-sized rodlike structure formed by the secondary assembly of cyclodextrin nanotube. *J Coll Interf Sci* 2006; 302:87–94.
34. He W *et al.* Cyclodextrin-based aggregates and characterization by microscopy. *Micron* 2008; 39:495–516.
35. Messner M *et al.* Self-assembly of cyclodextrins: the effect of the guest molecule. *Int J Pharm* 2011; 408:235–247.
36. Bertucci C, Domenici E. Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. *Curr Med Chem* 2002; 9:1463–1481.
37. Vuignier K *et al.* Drug-protein binding: a critical review of analytical tools. *Anal Bioanal Chem* 2010; 398:53–66.
38. Frezza M *et al.* Novel metals and metal complexes as platforms for cancer therapy. *Curr Pharm Des* 2010; 16:1813–1825.