CHAPTER II

LITERATURE REVIEW

1. Cyclodextrins

Structure and Physicochemical Properties.

Cyclodextrins (cycloamyloses, Schardinger dextrins, CDs) are water soluble, nonreducing, macrocyclic polymers, formed by the degradation and cyclization of starch by an enzyme produced by *Bacillus macerans*. Three natural CDs are readily available: α -, β -, and γ - CDs formed by six, seven, and eight D-glucose units, respectively (Figure 1). CDs with fewer than six glucose residues are too strained to exist, whereas those with more than eight residues are difficult to isolate (Loftsson and Brewster, 1996; Connors, 1997). Of these large-ring CDs, only δ - CD (containing nine glucose units) has been well characterized (Miyazawa, 1995).

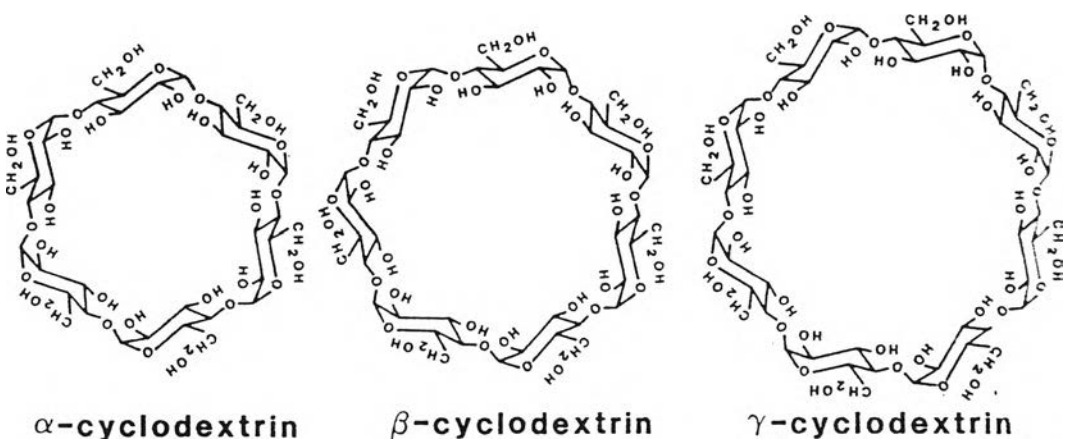


Figure 1. Structures of α - , β -, and γ - CDs.

The glucose units are joined together through α-1,4 glycosidic linkages, causing the formation of torus or cone-shaped molecules on which the narrower side bearing the primary 6-hydroxyl groups and the other the secondary 2- and 3- hydroxyl groups. The interior of the cavity is lined with (from the secondary hydroxyl rim inwards) a row of CH groups (the C-3 carbons), then a row of glycosidic oxygens, and then a row of C-5 CH groups (Figure 2). The non bonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity producing a high electron density and lending it some Lewis base character (Frank, 1975; Duchêne, Vaution, and Glomot, 1986; Connors, 1997).

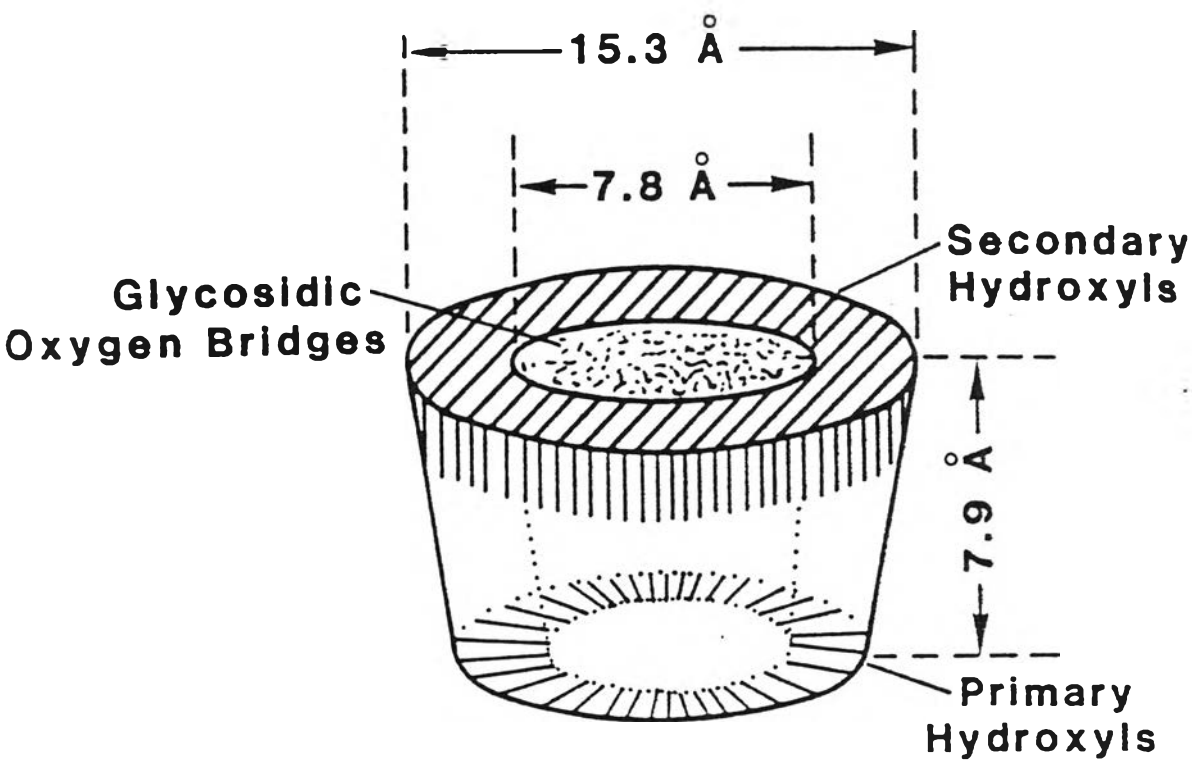


Figure 2. Functional structural scheme of β -CD.

This special conformation of CDs results in relative hydrophobicity in the cavity compared with water while the external faces are hydrophilic. These molecules have therefore been studied as ' host ' for ' guest ' molecules capable of entering, in whole or in part, the cavity and forming noncovalent host - guest inclusion complexes (Li and Purdy, 1992; Connors, 1997). Some of the important physical properties and characteristics of natural CDs are listed in Table 1.

Table 1. Physical properties and characteristics of natural CDs (Li and Purdy, 1992).

Characteristics	α -CD	β -CD	γ - CD
number of glucose units	6	7	8
molecular weight	972	1135	1297
solubility in water (g/100mL at 25°C)	14.5	1.85	23.2
cavity diameter (Å)	4.7 - 5.3	6.0 - 6.5	7.5 - 8.3
height of torus (Å)	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
pK_a values	12.33	12.20	12.08

The CDs crystallize from water as hydrates of variable compositions. Their cavities are filled with water molecules. Some are included into the CD cavity, others are integral parts of the crystal structure (crystal water). Due to the crystalline condition, there are different crystal forms of each CD. α - CD is usually encountered as the hexahydrate, α - CD. 6 H_2O, which can exist in crystal forms I and II, but the third form, α - CD. 7.57 H_2O, has been crystallized from aqueous $BaCl_2$. β - CD exists as the undecahydrate, β - CD. 11 H_2O, and as the dodecahydrate, β - CD. 12 H_2O, but these integral ratios are idealizations; the actual composition depends on the relative humidity (Steiner and Koellner, 1994). γ - CD has been crystallized as γ - CD. 13.3 H_2O but it can crystallize with 7 to 18 molecules of water (Szejtli, 1988; Connors, 1997).

From the pharmaceutical standpoint, the internal diameter (Figure 3) of α -CD (≈ 5 Å) is generally too small to include most of the active molecules. β - and γ - CDs are more appropriate (diameter ≈ 6 and 8 Å, respectively). γ - CD should obviously be the most appropriate one due to its largest cavity. However, γ - CD is not in fact intensively produced and remains impossible to use on an industrial scale (Duchêne and Wouessidjewe,1990).

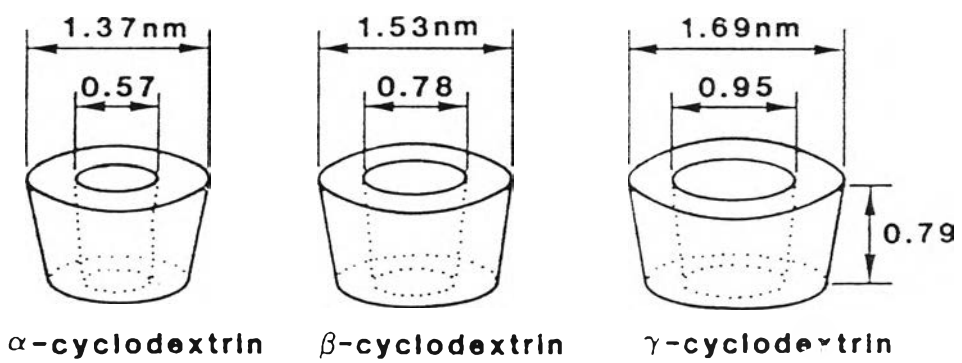


Figure 3. Molecular dimensions of CDs.

Cost is one of the most important factors in considering pharmaceutical use of CDs. Due to their high molecular weights, in the range of 1000 - 2000 Dalton, the production of dosage forms on an industrial scale would consume very large amount of CDs to account for a certain molar ratio of drug : CDs. Therefore CDs chosen must be reasonably inexpensive to be economically feasible. β - CD itself is quite inexpensive, and the cost of α - and γ - CDs are declining. Any modifications of CD structures must entail relatively inexpensive reagents and purification procedures. However, well-

characterized, pure, single-component materials are rare. For the moment, only β - CD can be used industrially (Rajewski and Stella, 1996).

In a CD molecule, the C-2 hydroxyl group of one glucose unit can form a hydrogen bond with the C-3 hydroxyl group of the adjacent glucose unit. Molecules of β - CD are capable of forming these intramolecular hydrogen bondings completely, which gives a hydrogen bond belt, that detracts from hydrogen bond formation with surrounding water molecules; this results in the least aqueous solubility (Table 1) of the series. On the other hand, in the α - CD molecule, the hydrogen bond belt is incomplete because one glucopyranose unit is in a distorted position. Consequently, instead of the six possible hydrogen bondings, only four can be established fully. The γ - CD is a non coplanar, more flexible structure, therefore, it possesses the most water solubility of the three CDs (Szejtli, 1988; Loftsson and Brewster, 1996). In addition, the work of Frank, Gray, and Weaver (1976) showing the nephrotoxicity of the unmodified CDs limited further studies of the parent CDs to nonparenteral routes. Based on the water solubility and the safety concern with CDs, numerous chemical modifications of CDs have been developed.

Modified Cyclodextrins

In order to improve drug carrier properties of the natural CDs, various functional groups have been incorporated into the CD molecules (Table 2). These chemically modified CDs can be classified into three types: hydrophilic, hydrophobic, and ionizable derivatives (Uekama and Hirayama, 1996). The hydrophilic derivatives such as methylated CDs, hydroxyalkylated CDs, and branched CDs merit special study because they exhibit very high water solubilities; these derivatives may be applied to solubilize lipophilic drugs (Sharma, Balasubramanian, and Stranbinger, 1995). In contrast, hydrophobic CDs have the ability to decrease the solubility of guest molecules so they may be used as sustained release drug carriers of water soluble drugs

Table 2. Derivatives of β- cyclodextrin (From Uekama and Hirayama, 1996).

Derivatives	Characteristics	Possible uses (dosage forms)
Hydrophilic derivatives		
Methylated β-CD	soluble in cold water and	oral, dermal,
DM-β-CD	in organic solvent,	mucosal[a]
TM-β-CD	surface active, hemolytic	
Hydroxyalkylated β-CD		
2-HE-β-CD	amorphous mixture with	oral, dermal,
2-HP-β-CD	different degrees of	mucosal[a],
3-HP-β-CD	substitution, highly water-	parenteral
2,3-DHP-β-CD	soluble (>50%), low toxicity	(intravenous)
Branched β-CD		
G_1-β-CD	highly water-soluble (>50%),	oral, mucosal[a],
G_2-β-CD	low toxicity	parenteral (intravenous)
Hydrophobic derivatives		
Alkylated β-CD		
DE-β-CD	water-insoluble, soluble in	oral, parenteral (subcutaneous)
TE-β-CD	organic solvents, surface-active	(slow-release)
Acylated β-CD		
TAcyl-β-CD	water-insoluble, soluble in	oral, dermal
	organic solvents	(slow-release)
Ionizable derivatives		
Anionic β-CD		
CME-β-CD	pK_a = 3 to 4, soluble	oral, dermal, mucosal[a]
	at pH > 4	(delayed-release)
β-CD sulphate	pK_a > 1, water-soluble	oral, mucosal[a],
β-CD phosphate		parenteral (intravenous)
Al-β-CD sulphate	water-insoluble	(slow-release)

[a] mucosal: nasal, sublingual, ophthalmic, pulmonary, rectal, vaginal, etc.

(Hirayama et al., 1988; Uekama et al., 1994). Whilst the ionizable CDs can modify the release rate of drugs depending on the pH of solution (Horikawa, Hirayama, and Uekama, 1995).

2-Hydroxypropyl- beta- cyclodextrin (2HP-β-CD) (Figure 4) is mostly utilized in the series of hydroxyalkylated β - CD derivatives because of its high water solubility. The main reason for its high solubility is that chemical manipulation frequently transforms the crystalline β - CD into amorphous mixtures of isomeric derivatives (Loftsson and Brewster, 1996).

2HP-β-CD was prepared by condensation of β - CD with propylene oxide in a sodium hydroxide solution (Pitha and Pitha, 1985; Pitha et al., 1986). In this reaction, hydroxyls of glucose residues, which are located on the outside of the torus, are etherified with 2-hydroxypropyl. The products are mixtures of many isomeric compounds. The extent of CD modification is given by the degree of substitution which is the number of substituents per molecule (Pitha et al., 1988).

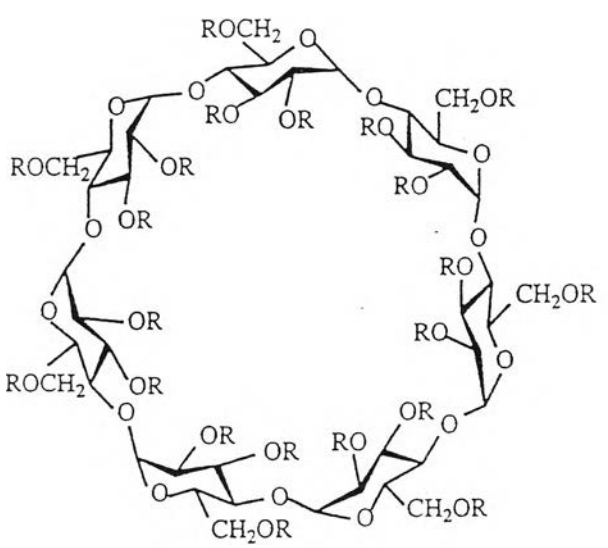


Figure 4. Structure of 2HP-β-CD.

The water solubility of 2HP-β-CD is more than 50 g/100mL at 25°C. Furthermore, these amorphous compounds are also less hygroscopic than the mother crystalline β - CD. The low hygroscopicity of 2HP-β-CD may be of advantage in pharmaceutical applications since the moisture sorption often initiates hydrolytic degradation of drugs in a solid state (Yoshida et al., 1988).

Stability Profiles of Cyclodextrins

Acid and base hydrolyses: The α - 1,4 glycosidic bonds of CDs are fairly stable in an alkaline solution, while they are hydrolytically cleaved by strong acids to give a series of linear maltosaccharides. The ring-opening rate of CDs increases with increasing cavity size, and is accelerated when the ring is distorted (Irie and Uekama, 1997). The apparent rate constants for acid hydrolysis in 1 N HCl solution at 60°C of the parent CDs are 6.7, 7.7, and 13.9 min^{-1} for α - , β -, and γ - CDs, respectively (Miyazawa et al., 1995). It should be noted that the ring-opening rate of β - CD is decelerated by the addition of guest molecules, the decrease being marked for guests with a close fit to the β - CD cavity. This reduction of rate may be attributed to the inhibition of access of catalytic oxonium ions to the glycosidic oxygen atoms from the inside of the cavity because the CD cavity is occupied by the guest (Hirayama et al., 1993).

Enzymatic degradation: The glycosidic bond cleaving rate of CDs by certain starch-degradation enzymes is much slower than that of linear sugars. The natural CDs are hydrolyzed by α - amylase that cleaves the endo type α - 1,4 glycosidic bond, but they are not hydolyzed by glucoamylase that cleaves the α - 1,4 glycosidic bond from terminal nonreducing glucose and pullulanase that cleaves the α - 1,6 glycosidic bond. Generally, the introduction of substituents on the hydroxyl groups slows down the enzymatic hydrolysis of CDs by lowering the affinity of CDs to enzymes or reducing the intrinsic reactivity of enzymes. Furthermore, CDs may be degraded by bacterial enzymes in human colon (Irie and Uekama, 1997).

Metabolism of Cyclodextrins

It is generally recognized that the gastrointestinal absorption of CDs in an intact form is limited due to their bulky and hydrophilic nature. Only a trace amount of intact β - CD is absorbed from the gastrointestinal tract of rats. The fate of the parent CDs in the gastrointestinal tract differs based on the rate of hydrolysis and enzymatic degradation. The α - and β - CDs are practically resistant to saliva amylase, stomach acid, and pancreatic amylase but they are extensively hydrolyzed in the colon. On the other hand, γ - CD is slowly digested even in the upper intestine. The primary metabolites, presumably acyclic maltodextrins, are further metabolized, absorbed, and excreted finally as CO_2 and H_2O. The chemical modification of CDs converts them into xenobiotics which may be more resistant to the intestinal hydrolases than the parent CDs. When the modified CDs are administered orally, their absorptions are low and most of them are excreted intact in the feces (Irie and Uekama, 1997).

CDs disappear rapidly from the systemic circulation and are excreted mainly through the kidney after an intravenous administration. α - and β - CDs are excreted almost completely in an intact form into the urine, whereas γ - CD is considerably degraded. The steady-state volumes of distribution (Vd_{ss}) for β - CD and 2HP-β-CD in rats, rabbits, dogs, and human beings correspond well with the extracellular fluid volumes of each species, suggesting that no deep compartment or storage in pools are involved. The total plasma clearance of 2HP-β-CD is similar to that of inulin, a polysaccharide known to be rapidly distributed in extracellular fluid, and then excreted at a rate of glomerular filtration (Irie and Uekama, 1997).

Safety Profiles of Cyclodextrins

Safety is a primary concern when new excipients intended for use in pharmaceutical formulations are considered. So the toxicological issues together with

the biological fates of CDs must be thoroughly investigated before practical use can be considered (Irie and Uekama, 1997).

It is well known that CDs are able to induce shape changes of membrane invagination of human erythrocytes and they may induce the lysis of cell at their high concentration. Miyazawa et al. (1995) reported the rank order of hemolytic activity of the parent CDs as follows: β - CD > α - CD > γ - CD. These differences are resulted by the difference in solubilization rates of membrane component by each CD. For example, α - CD's cavity, the smallest cavity size, is tightly fitted with the acyl chain of phospholipid, while the larger cavity sizes of β - and γ - CDs are looser. Furthermore, the side chain of cholesterol is preferably included in the β - CD cavity. γ - CD seems to be the least lipid selective CDs of the three natural CDs (Irie and Uekama, 1997).

In the series of β - CD derivatives, the concentrations inducing hemolysis start at 0.07, 0.3, 0.5, and 2% w/v for DM-β-CD, β - CD, 2HP-β-CD, and HE-β-CD, respectively. The hemolytic activity of CDs are correlated with their inclusion abilities toward membrane lipids rather than their intrinsic solubilities or surface activities (Yoshida et al., 1988).

All oral toxicity studies of CDs proof that they are practically nontoxic because none or only a small amount of CDs is absorbed through the gastrointestinal tract. No mortality is observed in the animals receiving the highest possible oral doses of the parent CDs. Duchêne (1988) reported that the LD_{50} values for β - CD is more than 5, 12.5, and 18.8 for dogs, mice, and rats, respectively. Based on a 52-week toxicity studies of β - CD utilizing dietary administration, no significant toxic effect is observed if the daily dose is less than 600 mg/kg in rats and less than 1800 mg/kg in dogs (Bellringer et al., 1995).

For 2HP-β-CD, the oral administrations of 16-24 g daily dose for 14 days to volunteers increases incidences of soft stools and diarrhea. 2HP-β-CD has no mutagenic effect, adverse effects on fertility, peri- and postnatal development, embryotoxic and teratogenic effects (Irie and Uekama, 1997). In a contrary, various untoward effects observed after a chronic large oral dose of 2HP-β-CD has been administered are not only caused by an increase in excretion of some vital lipophiles but also caused by solubilization and an increase in absorption of lipophilic toxicants and carcinogens present in the gastrointestinal tract (Horsky and Pitha, 1996).

Safety data of natural CDs for parenteral use show that they are toxic to the kidney which is the main organ for the removal of CDs from systemic circulation and for concentrating CDs in the proximal convoluted tubule after glomerular filtration. The nephrotoxicity of α -, and β - CDs is manifested as a series of alterations in the vacuolar organelles of the proximal tubule. Unlike such osmotic agents, α -, and β - CDs cause irreversible cellular changes and finally are toxic to the cells. The intravenous LD_{50} values in rats of α - and β - CDs are 1.00 g/kg and 0.788 g/kg, respectively (Frank et al., 1976).

Although the methylated CD, DM-β-CD, is higher water soluble than the mother β - CD (26 times more soluble than β - CD in water at 25°C on a molar basis), its systemic toxicity is higher than the mother β - CD (the intravenous LD_{50} values in rats for DM-β-CD is 220 mg/kg). The systemic toxicity of DM-β-CD may be due to its higher surface activity as well as greater ability to interact with endogenous lipids which characteristically limits its parenteral uses (Irie and Uekama, 1997).

2HP-β-CD has received the greatest attention regarding to its parenteral safety in animals and human beings (Carpenter et al., 1987; Pitha et al., 1988; Yoshida et al., 1988, Brewster et al., 1989; Brewster, Estes, and Bodor, 1990; Carpenter, Gerloczy, and Pitha, 1995). A brief summary of safety profiles of parenterally administered 2HP-β-CD

in animals and human beings is presented in Table 3. It can be concluded that 2HP-β-CD is safe for parenteral uses and its effects on the kidney are reversible (Irie and Uekama, 1997). Additionally, 2HP-β-CD shows slightly irritation reaction when it is intramuscularly injected as a single dose (100 mg/mL) into M. vastus lateralis of rabbits (Yoshida et al., 1988; Yoshida et al., 1989; Shiotani et al., 1995).

Cyclodextrin Inclusion Complexes

Drug-CD inclusion complexes are formed by substitution of included water by an appropriate guest molecule. As it was reviewed previously that the CD's cavity provides a lipophilic microenvironment into which drug molecules with proper sizes may enter and be included. During the drug-CD complex formation, no covalent bonds are involved, and the complexes are readily dissociated in an aqueous solution. For this reason, there is an equilibrium between free drug molecules and drug molecules bound within the CD cavity, which can be illustrated in equation 1, where D is the guest molecule, CD is the cyclodextrin, and D-CD is the inclusion complex. So, the measurement of the stability or equilibrium constant (K_c) or the dissociation constants (K_d) of the drug-CD complexes are important because they are indexes of changes in physicochemical properties of a compound upon inclusion, as illustrated in equations 2 and 3 (Li and Purdy, 1992; Loftsson and Brewster, 1996; Szejtli, 1998).

$$D + CD \leftrightarrow D\text{-}CD \qquad (1)$$

$$K_c = [D\text{-}CD] / [D][CD] \qquad (2)$$

$$K_d = 1/K_c = [D][CD] / [D\text{-}CD] \qquad (3)$$

Table 3. An overview of safety profiles of parenterally administered 2HP-β-CD in animals and human beings (from Irie and Uekama, 1997).

Species	Route of Administration	Dose	Remarks
Mouse	Intraperitoneal (acute)	10 g/kg	No mortality
	Intracerebral (acute)	1 μL of 40% w/v	No necrosis at the injection site
Rat	Intravenous (acute)	2 g/kg	No toxicity
	Intravenous (subacute)	5 g/kg daily for 7 days	No toxicity
	Intravenous (subchronic)	50, 100, or 400 mg/kg daily for 90 days	No toxicity at 50 mg/kg, minimal effects at higher doses
	Intravenous	50-400 mg/kg from day 6 to day 18 of pregnancy	No teratogenicity and embryotoxicity
	Intramuscular	5-40% w/v solution	No or minimal toxicity
Rabbit	Intravenous	50-400 mg/kg from day 6 to day 18 of pregnancy	No teratogenicity and embryotoxicity
Dog	Intravenous (subchronic)	50, 100, or 400 mg/kg daily for 90 days	No toxicity at 50 and 100 mg/kg, minimal effects at 400 mg/kg
	Intravenous (subchronic)	700-1000 mg/kg daily for 90 days	Reversible vacuolation of renal-tubular cells
Monkey	Intravenous (subacute)	200 mg/kg every second day for 91 days	No toxicity
	Intravenous	10 g/kg	No mortality
Human	Intravenous (acute)	infusion of 5%w/v solution at a rate of 470 mg/kg/day, total doses of 30 g over 4 days	No adverse effects
	Intravenous (acute)	infusion at a rate of 100 mg/min, total doses of 0.5-3 g	No adverse effects

The principle for determining the constants is titrating changes in the physicochemical properties of the guest molecule with the CD and the concentration dependencies are then analyzed. Although it is possible to use either guest or host changes to calculated the equilibrium constant, guest properties are frequently more comfortably assessed. These properties can be titrated by several methods, for example, aqueous solubility, chemical reactivity, molar absorptivity and other optical properties, phase solubility measurements, nuclear magnetic resonance (NMR) chemical shifts, pH-metric methods, calorimetric titration, freezing point depression, etc.

In addition, the thermodynamic parameters, i.e., the standard free energy change (ΔG), the standard enthalpy change (ΔH), and the standard entropy change (ΔS), can be analyzed from the temperature dependence of the stability constant of the complex. Almost all of the complex formation is associated with a relatively large negative ΔH and a ΔS that can be either positive or negative (Loftsson and Brewster, 1996).

It is important to realize that there is no simple construct to describe the driving force for complex formation. Although the release of enthalpy-rich water molecules from the CD cavity is probably an important driving force for drug : CD complex formation, other forces may also be involved in the complex formation and are listed here (Connors, 1997):

- relief of conformational strain energy possessed by the uncomplexed CD.
- hydrophobic interaction.
- electrostatic interaction (mainly dipole-dipole).
- hydrogen bonding (which is largely of electrostatic origin).
- induction forces (primarily dipole-induced dipole).
- London dispersion force.

Furthermore, the geometric capability and the polarity of guest molecules, the medium, and temperature are also important factors for determining the stability of the inclusion complex. Geometric rather than the chemical factors is decisive in determining the kind of guest molecules which can penetrate into the cavity (Li and Purdy, 1992).

Preparation Methods of Drug-Cyclodextrin Inclusion Complexes

Various methods have been described for preparing the inclusion complexes. However, each applied complexation method may obtain differences in the complexation effectiveness. Types of drug and CD used and the molar ratio of drug-CD for complex formation are also important factors affecting the complexation effectiveness. Most of useful methods are reviewed by Duchêne (1988) as follows.

Co-precipitation (liquid medium): For a water soluble drug, the drug molecule is added to a saturated aqueous solution of CD. The solution is then agitated for several hours or even days, until spontaneous precipitation of the inclusion is achieved. In some cases, the precipitation does not occur spontaneously; it is necessary to cool the medium at ambient temperature or even lower. A drying process (freeze-drying or spray-drying) is also applicable to separate the complex; the complex obtained from this method is amorphous with a better solubility than the crystallized product. However, this preparation method is not suitable for highly water insoluble drug.

Co-grinding (solid phase): This method is applicable to a drug molecule that is susceptible to hydrolysis. If the grinding was carried on for a sufficiently long period, the yield could be 100 %. Moreover, the microfine powder, which can be dissolved faster than large crystals, is obtained by this method. These products should be kept in a dry place where moisture can be excluded to prevent a possibly separate recrystallization of the drug molecule and CD. This is a very interesting method for industrial purposes.

Kneading: In the case of a poorly water soluble drug, the drug molecule is added to a slurry of CD. The mixture is then thoroughly kneaded to obtain a paste and then dried. This method, which is easy for industrial application, is far from being recommended for obtaining a pure inclusion.

Investigations of Drug-Cyclodextrin Inclusion Complexes

Several methods are applicable for detection of the interactions of drugs and CD molecules. Some useful techniques are reviewed and discussed in this section.

Infrared (IR) absorption spectroscopy: IR absorption spectroscopy contains informations about the vibrations of functional groups in a solid and is often site-specific in nature. The acquisition of high-quality IR spectra on the solid material is most amenable with the Fourier-transform IR (FTIR) spectroscopy method because this approach minimizes transmission and beam attenuation problems (Brittain, 1997).

Although this technique is commonly used for the characterization of solid organic substances, it is generally not suitable for detecting the inclusion complexes. This is because the CD molecule is the main part of the complex and therefore, the complex bands are similar to those of the CD bands. The bands of CD and its water of crystallization often mask the bands of the guest molecule. However, some studies reported the shifts of absorbance bands, the changes in intensities and band widths (Szejtli, 1988; Althal, Udupa, and Sreenivasan, 1995).

Thermal analysis: Thermoanalytical methods determine whether a guest molecule undergoes some changes before CD thermally degrades. This change of the guest molecule may be evaporation, decomposition, oxidation, melting or polymorphic transition. Suitably applied methods include thermal analytical system (TAS), thermal evolution analysis (TEA), differential scanning calorimetry (DSC), thermogravimetry (TG),

and differential thermal analysis (DTA). The effect of CDs on the thermogram obtained by DTA and DSC are the broadening, shifting, and appearing of new peaks or disappearing of certain peaks (Szejtli, 1988; Althal et al., 1995).

Powder X-ray diffractometry: This technique is very suitable for determining the complexation of non-volatile liquid guest molecules with CDs because these liquid guest molecules do not produce diffraction patterns. For a solid guest substance, a diffractogram comparison has to be made between the complex and the mechanical mixture of the guest and CD molecules because some inclusion complex preparation methods such as freeze-drying, spray-drying, or grinding may change the crystallinity of the pure substances and this may also lead to different diffraction pattern. The diffraction patterns of inclusion complexes are apparently different from each constituent. These differences are normally characterized as the disappearance of at least one of the component peaks, the appearance of a few new peaks, and/or the shift of certain peaks. While the diffraction pattern of the physical mixture is often the sum of those of the components (Szejtli, 1988; Althal et al., 1995).

Nuclear magnetic resonance (NMR) spectroscopy: Proton NMR is a useful technique for detection of drug-CD inclusion complexes in a solution. If the guest molecule is accommodated in the CD cavity, then the hydrogen atoms located in the interior cavity, C_3-H and C_5-H, will be considerably shielded by the guest, however, the hydrogen atoms on the outer surface, C_2-H, C_4-H, and C_6-H, will not be affected. A typical structure inference is that if only C_3-H undergoes a shift in the presence of substrate then the cavity penetration is shallow, where as if C_5-H also shifts, the penetration is deep (Szejtli, 1988; Althal et al., 1995; Connors, 1997).

Pharmaceutical Applications of Cyclodextrins

The major focus of this section is the effect of CDs on the physicochemical properties of a drug molecule included in their cavities. From the pharmaceutical point of view, these include the drug stability, side effects, solubility, and bioavailability.

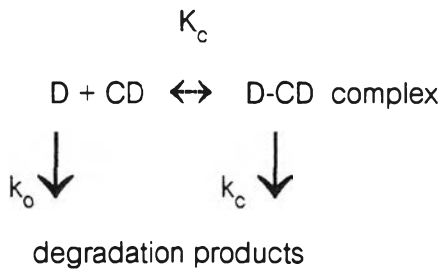
Effects of Cyclodextrins on Drug Stability

Numerous researches on drug-CD inclusion complexes are concerned with the effects of CDs on the chemical stability of drugs. The CD interaction with labile compounds can result in either degradative retardation or degradative acceleration. However, some drug degradative rates of the inclusion complexes are comparable to those of the drug alone.

In a solid state, the inclusion of drug in a CD molecule is similar to a molecular encapsulation or molecular coating. This structure may contribute to the protection of included molecule from oxidation accelerators in the ambient air, such as oxygen (Duchêne and Wouessidjewe,1996). This has been demonstrated especially on vitamin D_3, by which heat, light, and metal salts (copper sulphate) all increase its oxidative degradation. An inclusion of vitamin D_3 in β - CD can reduce its oxidation (Duchêne et al., 1986). Chen et al. (1996) found that HP-β-CD decreased the observed apparent-first-order rate of photochemical decomposition of alkannin/shikonin in pH 9 phosphate buffer. The photodegradation rate of the complex is approximately half of that of the free drug.

In a solution, due to the equilibrium of the complex and free drug and CD as mentioned earlier, the degree of stabilization/destabilization of a drug upon CD complexation is dependent not only on the rate of drug degradation within the complex (the value of k_c) but also on the fraction of the drug residing within the complex (the

value of K_c). For the formation of a 1:1 complex, the following equilibrium applies (Loftsson and Brewster, 1996):



Yoshida et al. (1988) prepared digitoxin inclusion complexes with β - CD and their derivatives. These β - CDs suppress the hydrolysis rates of digitoxin species in an acidic medium (pH 1.2). Famotidine undergoes specific acid catalysis in strongly acidic solutions. An inclusion complex of famotidine and 2HP-β-CD increases famotidine stability by 22-fold in pH 2.02 solution at 37 °C (Islam and Narurkar, 1991). Doxorubicin hydrochloride is poorly stable in a solution and undergoes acid-catalyzed glycosidic bond hydrolysis. An addition of 5%w/v 2HP-β-CD to the aqueous buffer solution of doxorubicin results in a stabilizing effect. 2HP-β-CD reduces the rate of doxorubicin degradation, at a constant ionic strength (μ = 0.5) and temperature (75 °C), by 17%, 32%, 18% and 51.1% at pH 1.01, 1.84, 5.9, and 7.72, respectively (Brewster et al., 1992). Helm, Muller, and Waaler (1995) showed that the degradation rates of dihydroergotamine mesylate in β - CD solutions are comparable to those of pure dihydroergotamine mesylate solution. The $t_{90\%}$ values of the degradation of dihydroergotamine mesylate with and without 2HP-β-CD in 0.05 M KH_2PO_4 solution at pH 5, 20 °C are 262 and 289 days, respectively.

Loftsson et al. (1989) reported decreases in degradation rates of chlorambucil and melphalan in aqueous solutions by forming inclusion complexes with 2HP-β-CD. When 5% (w/v) of 2HP-β-CD is added to the reaction medium, about 19-fold increase in the aqueous stability of chlorambucil and about 5-fold increase in that of melphalan are

obtained at neutral pH at 40°C and 60°C, respectively. Jarho, Urtti, and Jarvinen (1995) studied the effect of 2HP-β-CD on the aqueous stability of pilocarpine prodrug at pH 7.4. Shelf-lives ($t_{90\%}$, calculated by the Arrhenius equation) of the prodrug in 72.5 mM 2HP-β-CD solution increase by 5.1-fold and 6.1-fold at 25 °C and 4 °C, respectively. Antoniadou-Vyza et al. (1997) investigated the chemical stability of methocarbamol. A 2-fold increase in the stability of an inclusion complex with HP-β-CD compared with the free methocarbamol in pH 7.4 buffered solution at 37 and 60 °C was observed. This is due to the fact that the susceptible moiety of methocabamol is concluded in the hydrophobic cavity of the HP-β-CD and is protected from the attack of the aqueous buffered media.

The inhibitory effect of β - CD on the alkaline hydrolysis of benzocaine was reported by Lach and Chin (1964). They attributed this effect to the complete inclusion of the ester in the β - CD cavity; this protected the ester linkage from attack. The degradation of benzocaine depended on the amount of uncomplexed drug in solution, but not on the total amount present.

Effects of Cyclodextrins on Drug Side Effects

Various attempts have been made to reduce the irritant effect of a number of drug substances. An encapsulation of these drugs in the CD molecules is one of the useful techniques that tends to decrease the undesirable effect of drug. A bitter or irritant taste of bencyclane fumarate can be decreased by including it in a CD molecule (Fujioka et al., 1983).

The ulcerous effect on the gastrointestinal tract of nonsteroidal anti-inflammatory drugs may be decreased by including them in CDs. However, this is only the local, not systemic, effect and incomplete, as a result of the equilibrium existing between the dissolved complex and the free drug and CD (Duchêne and Wouessidjewe,1996).

Effects of Cyclodextrins on Drug Solubility

For a poorly water soluble drug, its increase in water solubility can be accomplished by forming an inclusion complex with a water soluble CD. This results from the hydrophilic external part of the CD which has a higher solubility than the free drug molecule.

The solubility of sulindac is increased by a formation of an inclusion complex with β - CD. The solubility of the inclusion complex at pH 6, 25 °C is 2.5 times that of sulindac alone. At pH 2, the solubility of the inclusion complexes are 6, 4, and 3 times those of sulindac alone at 25, 30, and 37 °C, respectively (Tros de Ilarduya et al., 1998).

In fact, the solubility observed is not that of a drug molecule but of an inclusion complex. If the stability constant of the inclusion complex is low, it can dissociate rapidly; the dissociation of the inclusion complex can occur in an aqueous solution leading to a reprecipitation of the drug molecule (Duchêne and Wouessidjewe,1996).

In the case of a highly soluble drug, a decrease in its water solubility can be resulted from an inclusion complex with a hydrophobic CD derivative. Complexed with heptakis (2,6-di-O-ethyl)-β-CD, isosorbide dinitrate dissolves and releases from capsule and tablet significantly slower than the noncomplex drug does (Hirayama et al., 1988). Similarly, the release rate of diltiazem, a water soluble calcium antagonist, from a compressed tablet is significantly retarded by the complexation with ethylated β-CD. The results suggest that diltiazem is released slowly from the hydrophobic matrix consisting of diethyl-β-CD following water penetration (Horiuchi, Hirayama, and Uekama, 1990).

Effects of Cyclodextrins on Drug Bioavailability

If a poor drug bioavailability is the consequence of low water solubility without absorption problems, an improvement of apparent solubility can then solve the bioavailability problem. For this reason, CDs are extremely valuable (Duchêne and Wouessidjewe,1990).

The mechanism involved is as follows. In the presence of biological fluids, the solid inclusion complex dissolves and degree of dissociation depends on its stability constant. Therefore, the inclusion complex, free CD, and free drug are in equilibrium and in contact with the biological membrane. Only the poorly water soluble drug, after it has released from the inclusion complex, is available for absorption through the lipid membrane. While both the inclusion complex and free CD are too hydrophilic at their external part to be absorbed significantly by the membrane.

Moreover, relatively low water solubility and relatively high stability constant of the inclusion complex may result in the slow release of included drug and finally the poor bioavailability of drug. If the drug is highly water insoluble, the stability constant must not be too low, otherwise a large amount of released drug may reprecipitate in the biological fluids before it is absorbed (Duchêne and Wouessidjewe,1996).

Abundant studies have shown bioavailability increment by drug-CD inclusion complexes after they have been administered orally. Some examples are cinnarizine inclusion complexes with SBE4-β-CD (sulfobutyl ether derivative of β - CD) and with 2HP-β-CD (Jarvinen et al., 1995), ketoprofen inclusion complexes with β - CD and with 2HP-β-CD (Ahn et al., 1997), carbamazepine inclusion complex with 2HP-β-CD (Brewster et al., 1997), and griseofulvin inclusion complex with β - CD (Dhanaraju et al., 1998).

Chemical Stability of Ranitidine Hydrochloride

The chemical structure of ranitidine HCl is shown in Figure 5. The hydrolytic degradation of ranitidine HCl is described by Haywood et al. (1987). The reactive properties of the substituted 2-nitro-1,1-vinyldiamino entity allow different hydrolytic behavior of the ranitidine molecule under acidic and alkaline conditions.

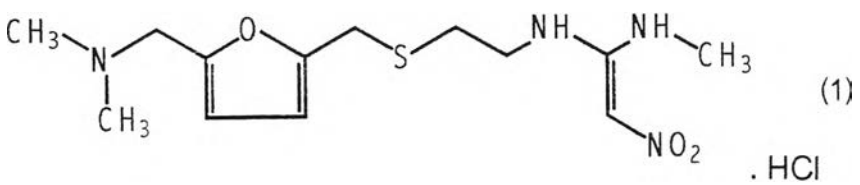


Figure 5. Structure of ranitidine HCl.

Hydrolysis at pH <1: At this condition, ranitidine HCl is resistant to hydrolysis even on prolonged heating. This behavior is attributed to the nucleophilicity of the nitro group-bearing carbon atom, which is protonated (Figure 6).

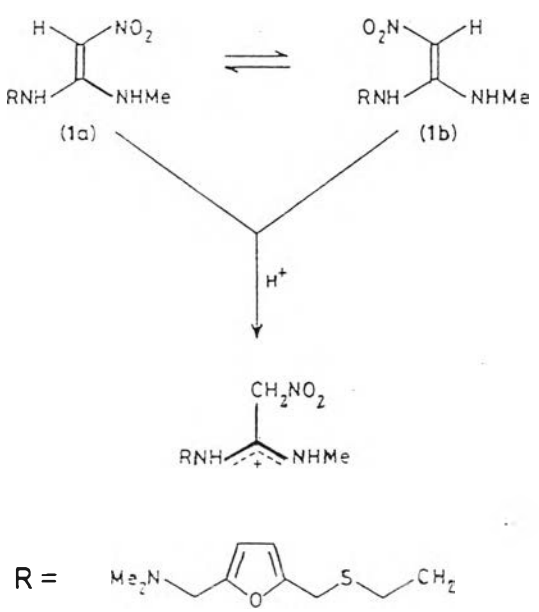


Figure 6. Protonation of the 2-nitro-1,1-vinyldiamino moiety.

Hydrolysis between pH 2 and 4: In the pH range of 2-4, hydrolytic cleavage of ranitidine is rapid at elevated temperatures, hydrolysis being completed in 6 hours at a refluxed temperature. The nature of isolated degradation products (Figure 7) suggests a proton-induced degradation involving the nitro group and a double bond shift (Figure 8). Nucleophilic attack followed by ring closure between the sulphur atom and the carbon atom bearing the original nitro group would lead to the substituted dihydrothiazin-2-one oxime hydrochloride (2).

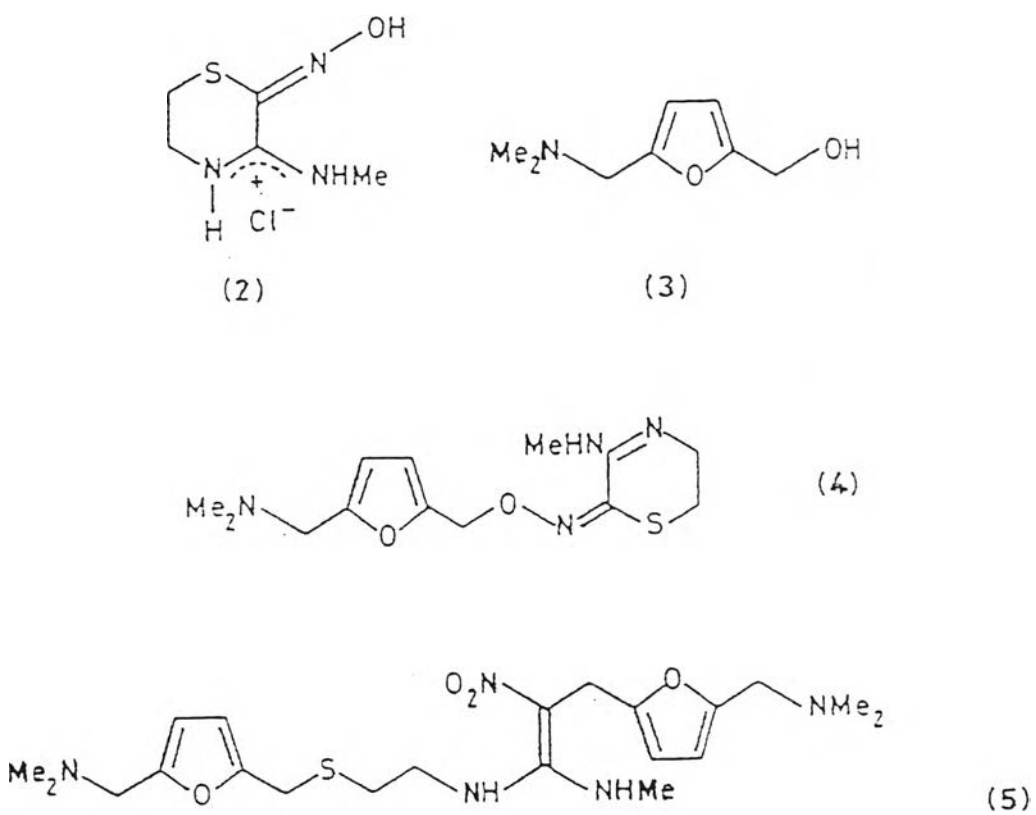


Figure 7. Hydrolysis degradation products of ranitidine HCl between pH 2-4.

It would be expected that the solvent (water) acting as a nucleophile would yield compound (3), any nitroso form of the free base of the compound (2) as nucleophile would yield compound (4), and ranitidine (1) itself acting as a nucleophile would yield compound (5). Independent experiments reveal that under acid conditions there is no reaction between compounds (3) and (2) or between compounds (3) and (1) to yield compounds (4) and (5), respectively.

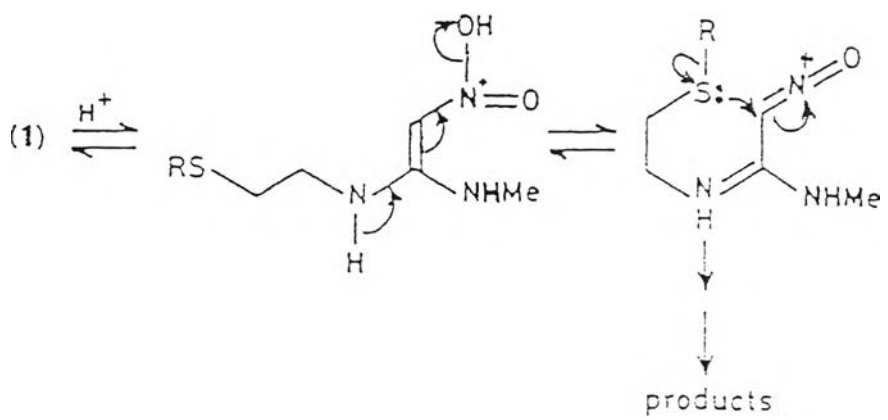


Figure 8. Mechanism of the hydrolysis degradation of ranitidine HCl between pH 2-4.

Hydrolysis at pH > 9: The hydrolysis of ranitidine at pH > 9 is again rapid at an elevated temperature. The complete degradation occurs in about 4 hours at a refluxed temperature. Hydrolysis under strongly alkaline conditions proceeds via a different path to give different products from those formed by the acid-catalyzed path at pH 2-4.

The four products (6) - (9) isolated are shown in Figure 9. The structures of these compounds suggest that the hydrolytic cleavage of ranitidine under strongly basic conditions occurs via hydroxyl ion attack on the β-carbon atom of the nitrovinyl group followed by alternative elimination from the diamino-alcohol intermediate (Figure 10).

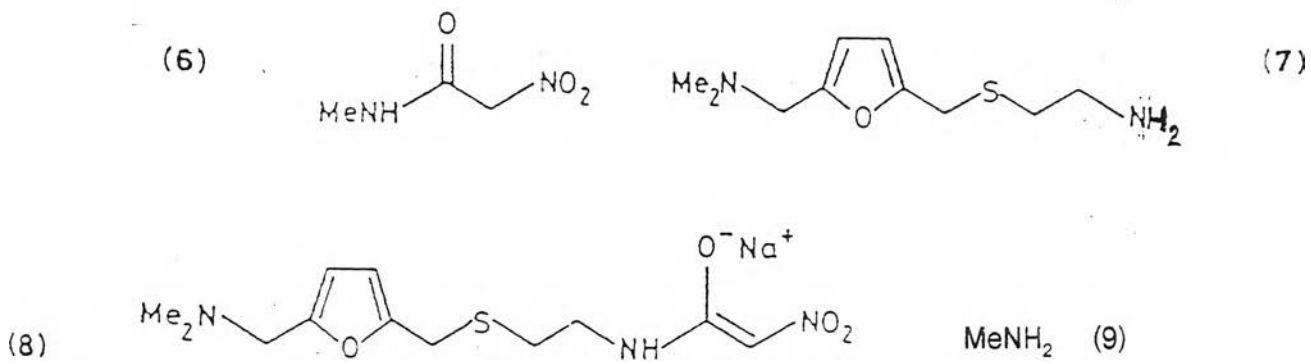


Figure 9. Hydrolysis degradation products of ranitidine HCl at pH > 9.

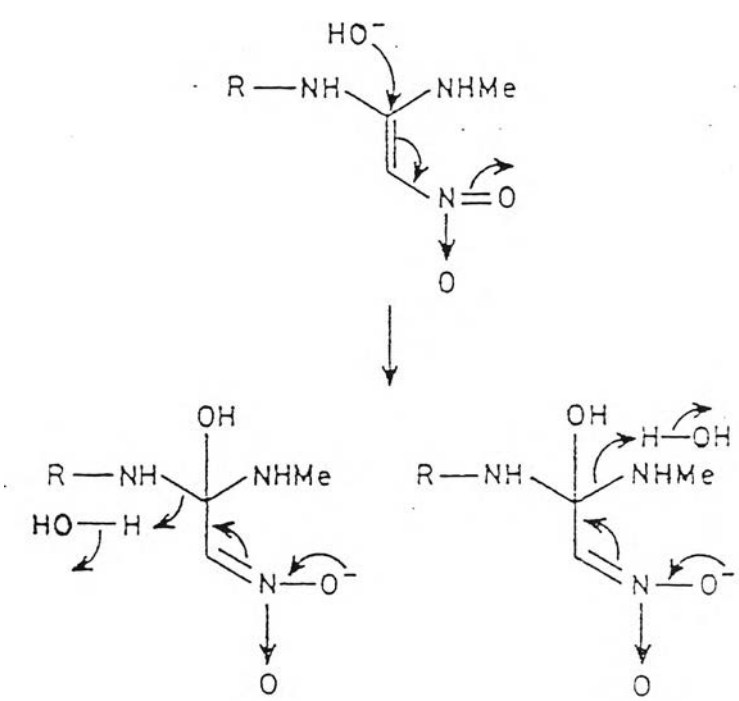


Figure 10. Mechanism of the hydrolysis degradation of ranitidine HCl at pH > 9.

Hydrolysis between pH 5 - 8: In the pH range of 5 - 8 and in particular around neutrality, the hydrolysis of ranitidine is slow; only 20-50% of the reaction is completed (dependent upon actual pH) after 5 - 8 days under reflux. Under these conditions, the presence of compounds (2), (3), (6), (7), and (8), indicates that the hydrolytic degradation by the simultaneous operation of the paths showed in Figure 8 and 10 or of Figure 8 and a modification of Figure 10 involves intercession of protons in a push-pull variation. The products were not, however, isolated. The hydrolysis of drug in neutral pH buffered solutions is particularly slow having proceeded to < 5% after 2 years at 30°C, with the basic pathway predominating over the acid pathway.

Because commercial solid dosage forms of ranitidine HCl are not stable against humidity, they are required to be strip packaged. Teraoka et al. (1993) studied the effects of temperature and relative humidity on the solid state chemical stability of ranitidine HCl. They found that the critical relative humidity (CRH) of ranitidine HCl bulk powder is about 67% relative humidity (RH). After the stability test, the sample powders have changed to the liquid state above the CRH, but they have not changed below 50%RH. It seems that below 50%RH, the water molecules are adsorbed onto the crystal surface. In contrast, above CRH, the powder dissolves in the adsorbed water and the amount of water adsorbed onto the sample is proportional to the RH level. The percent degradation of ranitidine HCl bulk powder at various RHs and 45°C are demonstrated in Figure 11. It may be concluded that ranitidine HCl bulk powder does not degrade in the solid state below 50%RH, the percent degradation is maximum at 60-70%RH, and the percent degradation above 70%RH is less than that at 60-70%RH.

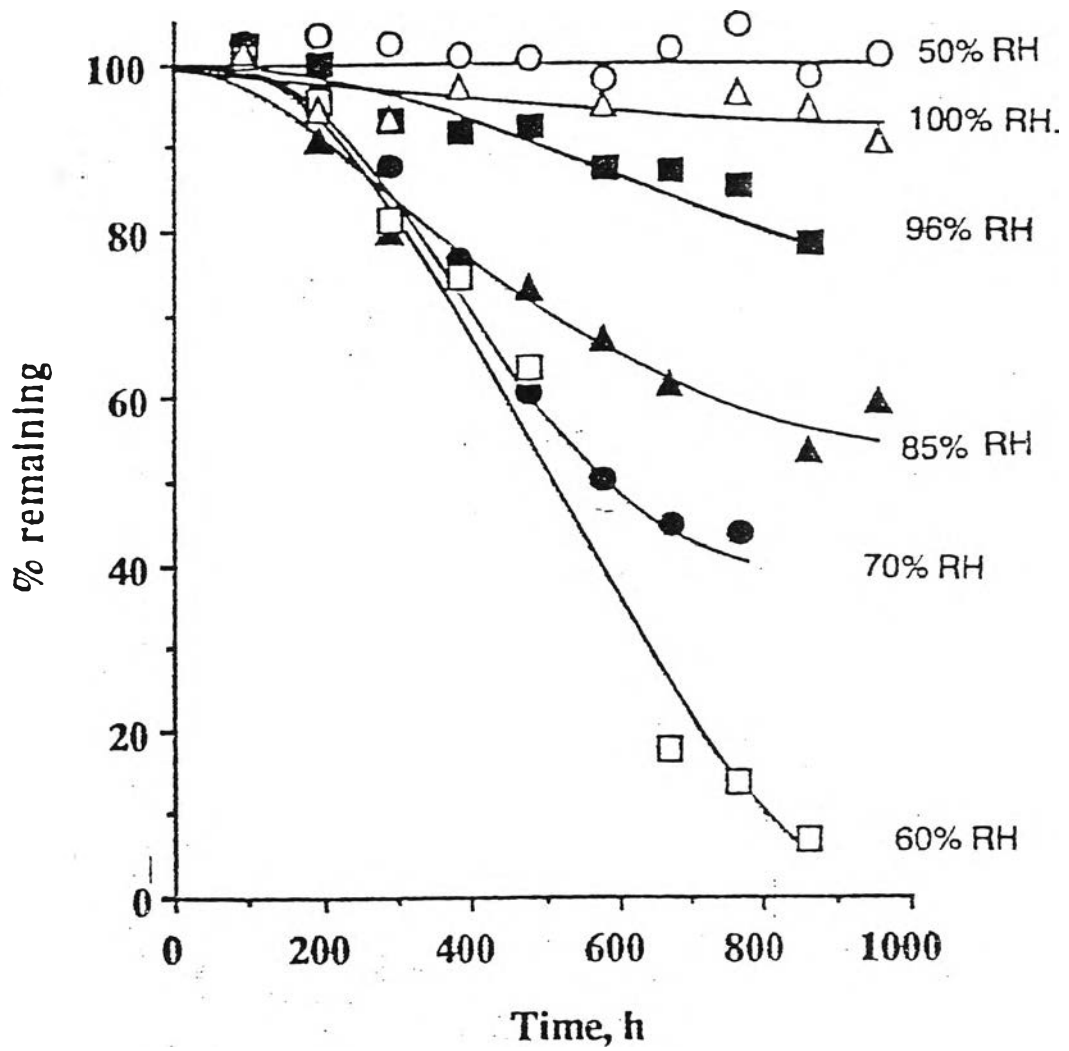


Figure 11. Relative humidity effect on the degradation of ranitidine bulk powder at 45 °C.