CHAPTER 1

INTRODUCTION

1. Literature Reviews of Chloramphenicol

Chloramphenicol is believed to be the most chemically stable of the antibiotics in common use. It has been reported that no significant degredation occurs after boiling for five hours with distilled water or after standing for twenty-four hours at a pH range form 2 to 9, in prolonged standing, however, it does undergo significant degradation with consequent loss of activity (1).

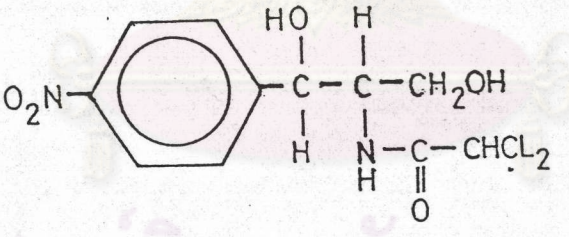


Figure 1 Molecular Structure of Chloramphenicol

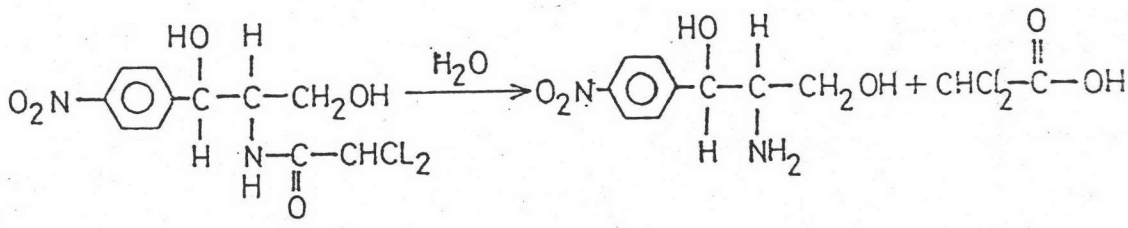
Because of the multiplicity of functional groups in chloramphenicol structure, (Figure 1) the degradative mechanism could be extremely complicated. From the structure, it will be seen that degradative may occur by :- (i) amide hydrolysis, (ii) chloride hydrolysis, (iii) oxidation to the ketone or aldehyde and (iv) reduction of

the nitro group (1,2). Shih I.K. reported that an aqueous solution of chloramphenicol was degraded upon exposure to sunlight, UV, and tungsten light by oxidation, reduction and condensation reactions. The major photodegradative products were hydrochloric acid, p-nitrobenzaldehyde, p-nitrobenzoic acid, 4,4-azoxybenzoic acid and p-aminopheny 1-2-acetamido-1,3 propandiol (3). Mubarak, *et al.*, investigated the photodegradation pathway of chloramphenicol in clark lubs borate buffer at pH 6.8. The result showed that the reaction pathway for the photolysis was the same as its pathway in aqueous solution. The degree of degradation could easily be seen by the development of a yellow color of solution (4,5).

From the report of Higuchi *et al.*, in 1954, showed that the rate of degradation of chloramphenicol molecule in aqueous solution were first order over a wide range of hydrogen ion concentration. The data obtained indicated clearly that the rate of degradations independent of the ionic strength of the medium employed for degradation. In addition, the rate of degradation appears to be substantially independent of the hydrogen-ion concentration of the system within the pH range 2-7.

Very strong evidence exists to indicate that chloramphenicol degradation is general acid-base catalyzed, and in the study of Higuchi (6), indicated that one of the major cause of chloramphenicol degradation can be

attributed to the hydrolytic clevage of the amide linkage in the drug according to the following Scheme.



Chloramphenicol is not only having chemical stability problem, but it has also chloramphenicol solubility problem in aqueous solution, especially, in eye-drop preparation. The concentration of chloramphenicol in the eye-drop preparation was 0.5% w/v which exceed the water solubility of chloramphenicol. Therefore, heat must be employed to solubilize chloramphenicol and hence degradation may be accelerated.

In 1983, Limpiti (7) analyzed eight chloramphenicol eye-drop preparations which she found that only one preparation contained over 90.0% of labeled amount. Limpiti also found that all preparations contained degraded product (1-p-nitrophenyl-2-amino-1,3-propanediol) over the B.P.C. (1973) limit of 5.0%. As result of her work, limpiti concluded that the expiration date erroneous.

In 1985, Suwanna Luangchonlatan (8) reported that the average apparent shelf-life of seven eye-drop preparation was 2.5 months. This was shorter than the standard of B.P.C. 1973 (The limit of B.P.C. 1973 is 4 months at 25°C).

In 1986, Aboutaleb, A.E., *et. al.*, (9) found that the aqueous solubility of chloramphenicol increased by complexation with cyclodextrin. The stoichiometric ratio for chloramphenicol in β- and α-CD were found to be 1:1 and 1:2 (guest : host) respectively. The apparent formation constant (K_C) indicated a particularly good fitness of the chloramphenicol molecule with the β-CD cavities.

2. Classification of Complexes

The term "complex" covers a multitude of compounds in which the bonding is simple (can be described by classic theory of valency between atoms), intricate or combinations there of such bonds, which create the situation referred to as "intricate", may be of ion-dipole, dipole-dipole, dipole induced dipole, or even of the covalent or coordinate type (10).

The classification of complex compounds in accordance with the types of bonds and compounds are as follows :

2.1 Metal Complexes

2.2 Molecular Complexes

(a) Donor-Acceptor Complexes

(b) Hydrogen-Bonded Complexes

2.3 Inclusion Complexes

(a) Clathrates

(b) Channel-Lattice Types

(c) Layer Types

More discussion will be given on the inclusion complexes. Metal complexes and the molecular complexes will not be discussed here.

Inclusion Complexes

The spatial configuration of the molecules rather than the chemical affinity is the primary contributing factor in the inclusion complexes. One or more guest molecules are trapped within the lattice of the host molecules giving rise to a stable arrangement.

(a) Layer types : many hydrocarbons, glycols and alcohols can be trapped between the layers of lattices of such compounds as the clay, montmorillonite.

(b) Clathrates : the term "clathrate compound" refers to a particular form of a molecular compound in which one component forms a cage-like structure and "imprisons" the second component. The components of a clathrate do not react chemically with each other. The underlying principle of the clathrate formation is the spatial configuration and the molecular size of the encaged component. Weak forces such as the van der Waals and the

hydrogen bonds may contribute to hold the two components together.

(c) Channel lattice types : the first channel inclusion complexes identified appear to be those of the cholic acids. Such compounds as paraffins, organic acids, ester, ketones, aromatic compounds, ether, alcohol and dioxane may be trapped within the channels in the crystals of the deoxychloic acid. Other compounds which are known to form channel lattice type complexes are urea, thiourea, and the Schardinger cyclodextrin.

3. Literature review of Cyclodextrins and Inclusion Complexes

The α, β, and γ-CDs are cyclic oligosaccharides consisting of six, seven and eight glucose units which can be obtained on a large scale from starch (11). The ring formed is externally very hydrophillic and relatively apolar internally. CDs were interested in the pharmaceutical sciences due to their ability to interact with many drugs molecules to from complexes which can possess desirable properties on the drugs. The pharmaceutical applications of CDs complexes and complexation phenomena may include (12,13,14) :

a. Conversion of a liquid materials into a solid product
b. Masking an unpleasant taste or odour of a compound

c. Avoidance of an incompatibility of uncomplexed compound with other drugs or excipients in a formulation.

d. Stabilization of a compound which could be sensitive to temperature, hydrolysis, autooxidation, photodegradation etc.

e. Increasing aqueous solubility and ease of emulsification of low aqueous solubility.

f. Enhancing the in vivo absorption and bioavailability of a drug with low aqueous solubility (because of an increase in dissolution rate).

g. Specific reactions (such as catalysis) can be made more selective by the inclusion of specific functional groups etc.

3.1 Preparation, Structure and Properties of Cyclodextrins

CDs were isolated by Villiers (11) from the degradation products of starch and were characterised as cyclic oligosaccharides by Schardinger (11). They were comprised of α-1,4-linked D-glucopyranose units.

The CDs are produced by the hydrolysis and cyclisation of starch through the action of CD transglycosylase (CTG) enzyme, most usually from certain species of Bacillus microorganism e.g. *Bacillus macerans*. The biosynthesis of CDs has been discussed in detail by

Sejtli (11), French *et. al.*, (15), Lane and Pirt (16). The chemical synthesis of CDs has not been reported. The yields from the biosynthesis of the various types of CDs are in the rank order $\beta > \alpha > \gamma$ and this is reflected in their relative prices.

From NMR and X-ray diffraction studies indicated that the CDs were torus shaped, as shown in Figure 2, the secondary OH groups on the C-2 and C-3 atoms of the glucose units being located on one side of the torus.

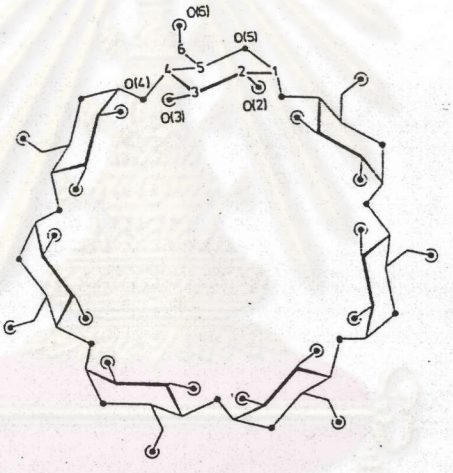


Figure 2 molecular structure of B-cyclodextrin

● = hydroxy group

● = oxygen atom

The hydroxy groups on C-2, C-3, and C-5 are available as points of structural modification without danger of eliminating the "central void" which is available for the accommodation of guest molecules. The interior of the torus consists of two rings of C-H groups, comprising

H-3, H-5, and H-6, and a ring of glycosidic "ether" oxygen atoms. The interior of the torus of each type of CD therefore offers an enviroment of much lower polarity than is present in water, and may be termed a "hydrophobic cavity" The cavities of CDs are slightly cone-shaped, with the C-2, C-3 side more open than C-6 side, the C-H groups comprising H-1, H-2 and H-6 are located on the exterior of the molecule.

3.2 Toxicity of Cyclodextrins

According to FAO Nutrition Meetings series No. 46A WHO/FOOD ADD/70.36, toxicological studies are not needed in cases of starches modified by enzymes. However, Szejtli and Sebestyen (17,18) studied some toxicity test on rats and dogs by the parenteral and oral administration the result showed that CDs do possess a toxic effect if asministered parenterally, but only in fairly high doses. If a drug was efficacious in low concentrations (e.g. prostaglandins) a CD complex could be dissolved and administered parenterally without expecting to see any toxic effect. However, all toxicity tests have shown that orally administered CD is harmless (12).

3.3 Cyclodextrin Inclusion Complexes

CDs have the ability to form complexes with a great variety of molecules, espacially for organic molecules both in the solid state and in solution, particularly aqueous (19). Most of these complexes are inclusion complexes

which are named because the CD molecules act as "hosts" into whose cavities foreign "guest" molecule may fit.

Bergernon *et. al.*, (20) investigated the binding parameters which showed that cycloamylose-substrate binding energy is not associated with a normal hydrophobic interaction, i.e. and entropy controlled phenomenon, but rather an entropy controlled equilibrium. Analysis of the binding indicated that van der Waals-London dispersion forces are responsible for the differences in binding energy between the α- and β-CD complexes.

Cramer, Dietsche, Brosed and Lautsch (19) suggested that the CD cavity acts as a lewis base due to its high electron density. On the other hand, they proposed that the cavity was electrophilic, since the stability of inclusion compounds in solution increases as the basicity of the guest increase. In summary, many CD inclusion compounds owe their existence (a) to van der Waals interactions between guest and host in the cavity of the CD host and (b) to the ability of the guest molecule of suitable shape and size to be physically accommodated in the CD cavity.

The structure of the CD inclusion complexes in solution may differ appreciable from that in the crystalline state. A great deal of varied and recent experimental evidence reveals that in solution the guest molecule fits wholly or partially into the central cavity

of the CD host molecule and the whole complex is surrounded and solvated by solvent molecules, such as water. In the crystalline state, on the other hand, the guest molecule may fit into a void space or interstice in the crystal lattice and not necessarily into the central cavity of the CD torus. A crude analysis would be to imagine the guest molecule fitting into the central cavity of a daughnut shaped host molecule. Many X-ray Diffraction studies of the crystalline compounds of α-CD with various guest molecules have indicated that some are clathrates in which the guest malecule is physically trapped in a cage of guest molecules whereas some are of the channel type in which the guest molecules are located in channels between the host molecules. For example X-ray diffraction studies indicated that p-disubstituted benzene derivatives are orientated with their axes coincident with the axis of the CD cavity. These inclusion compounds may be nonstoichiometric.

Many CD inclusion complexes which are stable in the solid state are dissociated into guest and host in solution. This ocurs when the total free enery of interaction (host-solvent, guest-solvent) is more negative than that for solvent-guest. Since the solid state is more ordered than the solution, the entropy change will tend to favour dissociation of the complex in solution. However, some inclusion complexes are stable in solution, suggesting a favourable enthalpy of interaction (host-guest).

3.4 Synthesis of Cyclodextrin Inclusion Complexes

The method used for the synthesis of cyclodextrin inclusion complexes depends on the properties of the guest molecules :

1) Equimolar or a ten-fold excess of water-soluble substances are dissolved directly in concentrated hot or cold aqueous solutions of the cyclodextrins. The inclusion complexes crystallize immediately or on slow cooling or evaporation.

2) Substances which are not water-soluble are dissolved in organic solvent and are then shaken with or layered solution. In the case of layering, crystals formed must washed with an organic solvent to remove any adhering substances.

The first two procedures are suitable for the laboratory preparation of cyclodextrin inclusion complexes. For the industrial synthesis, the kneading process will be used.

3) Details of the kneading procedures are : the liquid or dissolved guest molecular are added to a slurry of the CD and 2 to 5 times as much water. On stirring in a mixer, the viscosity of the mixture usually increase, giving a paste which can then be dried powdered, and washed. If the inclusion complexes are readily soluble in water or decompose on drying, it is advisable to

lyophyilize them. They then retain their finely dispersed form which is of advantage, especially in the pharmaceutical industry, as if has a high solubility.

3.5 Cyclodextrin Complexation in Solution

Most studies involving the binding of guests to CD hosts have been carried out in aqueous solution. Several fundamental properties of the guest species are often greatly modified by complexation with CDs in solution. These include chemical reactivity such that CD can act as a positive or negative catalyst and thermodynamic activity which determines the vapour pressure and solubility. Since most pharmaceuticals have extremely low vapour pressure, much more sensitive methods are required for the exploitation of the property than are at present generally available. The solubility of guest molecules can often easily be measured, and phase solubility analysis, as applied by Higuchi and Connors (21), then enables the formation constant (stability constant) of the complex to be readily determined. Since CDs do not absorb in the UV the total concentration of a drug in solution is often determined by UV spectrophotometry.

Hydrophobic, solvophobic or apolar interactions, such as occur in the formation of an enzyme-substrate complex, are characterised by a very favourable entropy change. The formation of CD inclusion complexes, however, is associate with a favourable enthalpy change and an

unfavourable (or slightly favourable) entropy change, and so cannot be attributed to classical solvophobic interactions. The following explanations of the very favourable entropy change have been proposed :

a) Van Der Waals electrostatic interactions between guest and host (22).
b) hydrogen bonding between guest and host (23).
c) release of high energy water molecules in complex formation (22).
d) release of strain energy in the macromolecular ring of the CD (24).

All these factors may be involved, but the relative contributions of each may vary according to circumstances and are at present unknown.

3.6 Phase Solubility Analysis

Organic compounds, such as drugs, which are sparingly soluble in water, frequently display an increased aqueous solubility in the presence of CD. This is due to the formation of water soluble complex between the drug and the dissolved CD. The complexation equilibrium lowers the activity of the dissolved drug. Consequently, more drug dissolves until the activity of the free drug, which is in chemical equilibrium with the complex, becomes equal to the thermodynamic activity of the pure solid drug. Phase solubility analysis is used to determine the relationship between the total concentration of dissolved drug and the

concentration of added CD.

A phase diagram is constructed by plotting the molarity of the substrate found in the solution phase against the molarity of ligand added to the system.

3.6.1 Classification of the Solubility Curve

a. Soluble type e.g. A_L, A_P, A_N which were not discussed here.

b. Insoluble type (type B_S) :

Type B_S Solubility Curve

In case of insoluble complex formed, a type B Solubility diagram is found (see Figure 3). From point S_0 to a, the solubility of substrate increase until the point a the solubility limit of the complex is reached. Any further addition of ligand will result in the formation of the complex which must then precipitate out of the solution.

The application of the phase rule to the descending portion of the curve leads to the conclusion that only two phase are presented. From visual observation, it is obvious that at least two phase must exist in the b-c region. However, since the degree of freedom at any point on the b-c curve is equal to one (temperature, pressure and concentration of ligand are fixed by the system. The phase rule indicate that the total number of phase in the system

is two. Thus, there can not be two solid phases. Thus, only one complex may precipitate out at any given point on the b-c curve (10).

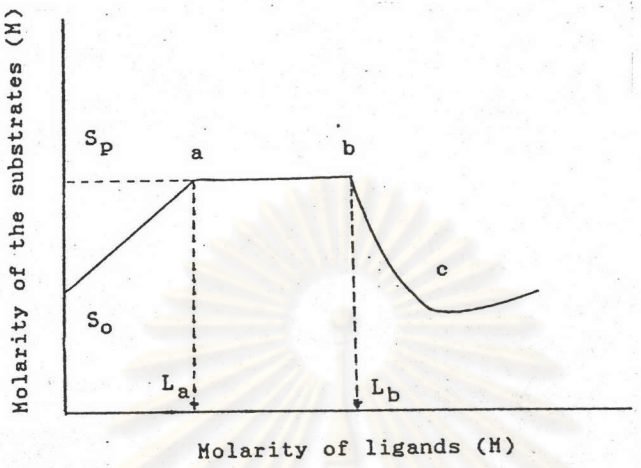


Figure 3 Phase Solubility Diagram as the B_S-Type.

This technique reveals both the stoichiometry and the formation constant (K_C) of the complex (21).

Lach and Cohen (25) investigated the interaction of α- or β-CD in aqueous solution with 19 drugs. The solubility diagram showed that all drugs formed complexes with CD, and they found that the less soluble or smaller drugs showed the greater value of K_C and the stronger interaction.

In a similar study using the aminobenzoic acids or the monohalogenated benzoic acids as guests, Lach and Chin (26) showed that (a) each of the acids interacted with α- or β-CD, (b) the K_C values of the monohalogenobenzoic acids decreased in the rank order CL > Br and (c) also in the rank order -m > -p > -o, and (d) K_C value with β-CD were

greater than with α-CD. The trends (b) and (c) were attributed to size effects.

Extending the solubility method to the interaction of β-CD in water with various phenyl-substituted carboxylic acids, Pauli and Lach (27) showed that all the acids interact with β-CD in the stoichiometric ratio 1:1 or 2:1 (guest : host).

A similar series of experiments was carried out by Lach and Pauli (28) using β-CD as host in water and the following drugs as the guest : adiphenine, antazoline, cortisone acetate, lidocaine, meperidine, morphine, procaine, reserpine, testosterone, tripelennamine. The following points were made : (a) all the drugs interacted even though their molecules were too large for complete inclusion in the CD cavity (b) the larger the molecule the smaller the K_C value and so the weaker the interaction, (c) the complexaiton of the large molecules depended on the presence of suitable group or ring capable of inclusion in the CD cavity, (d) the molar ratio drug β-CD was 1:1 (For instance; adiphenine or tripelennamine) or 2:1 (For instance; cortisone acetate or testosterone).

Summarily the solubility method used by Lach and co-workers showed that the drug-CD complexations were not simply inclusion phenomena, but involved intermolecular forces such as hydrogen bonding and electrostatic interactions.

3.7 Methods Used for Investigating of Inclusion Complexes

3.7.1 Infrared spectra

Complexation formation may be proved in some cases by IR studies, but this method is of limitted use. This is due to the bands of CD representing the majority of the complex being hardly influenced by complex formation but some investigations showed the changing of the chemical shift and the broadening peak (11).

3.7.2 X-ray Powder Diffraction

Since guest molecules have their diffraction patterns, if the diffractogram differs slightly from that of uncomplexed CD, complex formation can be established. Thus, the X-ray diffraction is especially needed to confirm the formation of inclusion complexes (11).

3.7.3 Methods Involving Heating

This method consists of many techniqes, such as, (a) thermal analytical system (TAS), (b) thermo evolution analyser (TEA), (c) differential scanning calorimetry (DSC), (d) thermogravimetry (TG), and (e) differential thermal analysis (DTA) which was used to confirm in this study.

The sensitivity of the techniques can detect any change in the crystalline state of a material. So, this technique are useful to confirm the formation of

inclusion complexes.

3.7.4 Other techniques eg. nuclear magnetic resonance spectra (NMR), circular dichroism (CD) and chromatography may be used in confirming the inclusion complexes.

4. The Application of Cyclodextrin Inclusion Complexes Concerning the Drug Stability

The use of CD inclusion complexes in pharmaceutic brings about the following improvements concerning the drug stability, (a) an increased thermal stability, (b) resistance to oxidation, (c) a resistance to hydrolysis and degradation in solution.

a) Influence on thermal stability

Uekame *et. al.*, (29) carried out stability studies which prove the significantly retardation of the decomposition of 16,16-dimethyl-trans-Δ^2-prostaglandin E_1 by inclusion formation. The stability tests were conducted at 60°C in a moisture-free enviroment. Furthermore, thermal gravimetric analysis showed that the volatility of 16,16-dimethyl-trans- Δ^2-prostaglandin E_1 was extremely depressed by binding to β-CD.

The stability of camomile oil can be significantly enhanced by inclusion complexation with cyclodextrins. Szente *et. al.*, (30) showed that in complexed state heat and oxygen sensitive components of camomile oil do not

suffer significant decomposition even at 150°C in vacuum or at 37°C in an atmosphere of pure oxygen. The complex is a fine powder which seems to be applicable with advantage in various pharmaceutical preparations.

Kernoczi *et. al.*, (31) use thermography and classical or overpressured thin layer chromatographic techniques to show the stability against heat and the diminution of volatility of thymus, dill and sage oil in complexes with cyclodextrins.

Lengyel *et. al.*, (32) prepared a menadione-β-cyclodextrin complex. The result were an enhanced stability to heat and sublimation. This make it possible to applicate the complex in feeding and premixing for verterinary use.

Koch (33) showed that the evaporation of perfumes from detergent powders is strongly inhibited by clathration with cyclodextrins. Perhaps these investigations can be of help, for instance in formulations in dusting powders, ointments or other perparations where perfumes can be used.

Uekame *et. al.*, (34) investigated the effects of cyclodextrin complexation on the thermal behavior of benzaldehyde. The effects were examined by thermal analysis since benzaldehyde is a volatile liquid. Besides the reduction of the volatility of benzaldehyde there is also a decrease of the photooxidation.

(b) Resistance to oxidation

Uekama *et. al.*, (34) described the total inhibition of oxidation of benzaldhyde by complexation. The photodegradation of benzaldehyde under aerobic conditions was significantly retarded by the complex formation, and completely suppressed by α-cyclodextrin. The difference in photochemical stabilities observed for the 3 complexes can be explained on the basis of the structure and stoichiometries of the solid complexes.

In the case of 1:1 α-cyclodextrin complexs, the benzaldehyde molecule can be suitably oriented within the cavity of cyclodextrin to prevent the oxidation reaction. On the other hand, in the case of 3:2 β-cyclodextrin complex or 2:1 γ-cyclodextrin complex, the benzaldehyde molecule could not be tightly included within the cavity of β- or γ-cyclodextrin because of the larger cavities. In addition, the crystal lattice of α-cyclodextrin complex seems to be stronger than those of β- and γ-cyclodextrin complexes since it is harder to release the crystalline or adhesional water molecule from the α-cyclodextrin complex than from the β- and γ-cyclodextrin complexes. This consideration is supported by the crystal structure of benzaldehyde-α-cyclodextrin hexahydrate complex. The data indicated that the thermal and photochemical stabilities of benzaldehyde were improved by all three cyclodextrins, and particularly by α-cyclodextrin. Therefore, the formation of solid (powder) inclusion complexes of benzaldehyde with

cyclodextrins improved the ease of handling and decreased the problems encountered upon prolonged storage of benzaldehyde.

c) Resistance to Hydrolysis and Degradation in Solution

Uekame *et. al.*, (35) examined the effects of cyclodextrins on the hydrolysis of prostacyclin and its methylester in aqueous solution. The rates of hydrolysis of prostacyclin (PGI_2) and its methylester (PGI_2Me) were significantly retarded by α-, β- and γ-cyclodextrins. The deceleration effects of cyclodextrins on the hydrolysis of PGI_2Me were about three times larger than those on the hydrolysis of PGI_2. The importance of the spatial relationship between the host and guest molecules was reflected in the kinetically determined stability constant for these inclusion complexes. To elucidate the deceleration mechanism of the cyclodextrins, the effects of pH, solvent and temperature on the hydrolysis rate were studied. Other investigations also performed by Uekama *et. al.*, which showed an improved thermal stability of prostaglandin $F_{2\alpha}$ in complex with γ-cyclodextrin in solution and solid phase. Dissolution and thermal behaviors of the complex were examined and compared with the drug itself (36). The dehydration rate of prostaglandin E_2 (PGE_2) and the isomerization rate of prostaglandin A_2 (PGA_2), in complex with methylated-β-cyclodextrin. Hirayama *et. al.*, (37) carried out there studies in aqueous alkaline

solutions. In contrast to the acceleration effect of β-cyclodextrin both dimethyl-β-cyclodextrin and trimethy-β-cyclodextrin significantly retarded the reaction rates. The stabilizing effect of dimethyl-β-cyclodextrin was larger than that of trimethyl-β-cyclodextrin. Stability constants and rate constants of the complexes were kinetically determined on the basis of 1:1 inclusion complex formation.

Some data offered by Uekama *et. al.*, (38) suggested that variation in oral bioavailability of digoxin owing to poor dissolution and acid hydrolysis in stomach may be improved by cyclodextrin complexation, without lowering the pharmacological activity. The hydrolysis was suppressed by cyclodextrins in the order of β- > γ- > α-cyclodextrin. These results indicated that either a smaller (α-cyclodextrin) or a larger (γ-cyclodextrin) cavity is unfavourable for preventing the acid hydrolysis of digoxin.

Fugioka *et. al.*, (39) showed that the degradation of bencyclane was damaged by complex formation with cyclodextrins. The delaying effect increased in the order of α-, γ- and β-cyclodextrin. The investigations on the stability of the freeze-dried bencyclane-fumarate cyclodextrin complexes were made compared with bencyclane-fumarate alone in 0.1 N HCl solution at 37°C.

Uekame *et. al.*, (40) proved that the formation of inclusion complexes of proscillaridin with cyclodextrins in water and in solid state brings various advantages. The apparent stability constants for proscillaridin cyclodextrin, suggesting that the larger the cyclodextrin cavity, the more profitable it would be to cyclodextrin cavity, the more profitable it would be to include the bulky prosciliaridin molecule. The acid hydrolysis of proscillaridin was significantly suppressed by β- and γ-cyclodextrin, while no appreciable inhibition was obtained with α-cyclodextrin.

Metronidazole benzoate was found to form an inclusion complex with β-cyclodextrin in aqueous solution and in the solid phase. Andersen and Bundgaard (41) found that by inclusion complexation of metronidazole ester with β-cyclodextrin, the phase transition of the clinically used with an β-cyclodextrin, the phase transition of clinically used anhydrous form of the compound, to the monohydrate, occuring in aqueous suspensions, was inhibited, as was the marked crystal growth resulting from the phase transition. Besides increasing the physical stability of metronidazole benzoate in suspensions, the complexation with β-cyclodextrin protected the drug against photochemical degradation and decreased the rate of hydrolysis.

To explore the possibility of improving the stability of betamethasone-17-valerate by inlcusion complexation with various cyclodextrins, the rate of degradation of the steroid was measured in an aqueous borate buffer (pH 9.00) in the presence of varying amounts of the cyclodextrins. For all solutions Andersen and Bundgaard (42) found that the disappearance of betamethasone-17-valerae displayed strict first-order kinetic behavior. The decrease of betamethasone-17-valerate was in all cases accompanied by the formation of 21-valerate in stoichiometric amounts as revealed by HPLC analysis. The result showed the effect of concentration on the observed pseudo-first-order rate constants for the loss of betamethasone-17-valerate. β-cyclodextrin accelerated the rate of degradation whereas γ-cyclodextrin as well as 2,6-O-dimethylated-β-cyclodextrin have a pronounced stabilizing effect. In contrast, α-cyclodextrin did not exhibit any significant effect on the stability of the steroids (42).

Concerning the stability of acetaminophen, Lin *et. al.*, (43) came to the following conclusions by kinetic studies : Acetaminophen degrades according to pseudo-first order kinetic at 37°C in the presence or absence of cyclodextrins and glucose. The rate of degradation of acetaminophen decreased with the increase of cyclodextrin or glucose concentration. The stability of acetaminophen in an acid or alkaline solution could be improved by

forming α - and β-cyclodextrin complexation, however, the stability of acetaminophen in an acid or alkaline glucose solution was dependent on the degradation of glucose. The included acetaminophen dued to the inhibition of hydrogen and hydroxyl ion attack. Thus, the hydrolytic rate was dependent on the amount of free drug and reversible rate of complexation in the solution. Acetaminophen, whose molecule was more suitable for inclusion into the β-cyclodextrin than the α-cyclodextrin dued to the size of the cavity space, leaded to larger value of stability constant of β-cyclodextrin (43).

The interaction of β-cyclodextrin with ampicillin, methicillin and phenytoin has been investigated by Hsyu *et. al.*, (42). For methicillin the significant extension of the hydrolytic half-life for the complex made the oral route an possiblility for this drug, with was normally given intravenously because of stability problems. The interaction with ampicillin resulted in an improved bioavialability, a retarded hydrolysis and a reduction in the incidence of gastro-intestinal side effects incomparison with the uncomplexed drug. Due to an enhanced solubility of the phenytion-β-cyclodextrin complex there was a greatly improved bioavailability and a reduction in intra- and intersubject level variation (44).