CHAPTER I

GENERAL BACKGROUND

INTRODUCTION

Chloramphenicol eye drop is a preparation of broad spectrum antibiotic, which is effective against a wide range of gram-negative and gram-positive micro-organisms. It is used in the treatment of a wide variety of eye infections, and for treatment of superficial skin infections such as impetigo, sycosis barbae and furunculosis.

Chloramphenicol is a poorly soluble drug with a saturation solubility of 2.5 mg/ml in water (25°C). In aqueous solution, it undergoes hydrolysis to 2 - amino - 1 - (*p*-nitrophenyl) - 1, 3 - propanediol (AMPD). Therefore, both the British Pharmaceutical Codex (BPC) 1973 and the British Pharmacopoeia (BP) 1993 specified the concentration of chloramphenicol, its degradation products and shelf - life. The major degradation product, AMPD, is not more than 5.0 % of content of chloramphenicol. When kept at a temperature of 2° - 8°C it may be expected to retain its potency for 18 months after manufacturing date. When kept at a temperature not exceeding 25°C, it may be expected to retain its potency for 4 months after manufacturing date.

Chloramphenicol eye drops are well distributed in Thailand. Most manufactures restricted the expiration date about 2 years after manufacturing date, however, their shelf - lives are doubtful.

In 1983, Doungsamorn Limpiti analyzed eight commercial chloramphenicol eye drop preparations. It was found that only one preparation contained over 90.0 % of labeled amount at the time of assay. All preparations contained degradable product (AMPD) over the BPC 1973 limit of 5.0%. The expiration date claimed on the label by manufacturer was, therefore, unreliable.

In 1985, Suwanna Luangchonlatan studied the stability of chloramphenicol in seven marketted eye drops by accelerated thermodegradation process. It was found that the average apparent shelf-life of seven eye drop preparations at room temperature (30°C) was about 2.5 months. This was shorter than the shelf - life specificd by BPC 1973 (4 months at 25°C).

Several attempts have been made to increase solubility and stabilizing chloramphenicol solution. Siriwan Ruengsawad (1989) improved solubility and stability of chloramphenicol eye-drops by using non-ionic surfactant, poloxamer 407 as a solubilizer, adjusting buffer, pH, viscosity, tonicity of vehicle and partial replacement of water with cosolvent to reduce hydrolysis of chloramphenicol solution. The adjustment of the pH to 6.0 did not improve the stability, nor did the HPMC and PVP (viscosity inducing agent). Its shelf - life according to the standard of BP 1980 at 25°C was 11.8 months and 21.62 months according to the standard of USP XXI.

Aboutaleb et al., (1986) found that the aqueous solubility of chloramphenicol was increased by complexation with cyclodextrin. The stoichiometric ratio for chloramphenicol in β-and α-cyclodextrins 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-cyclodextrin cavities.

In 1990, Anong Patmasiriwat investigated the interaction of chloramphenicol with β-cyclodextrin and found that the predicted and the apparant shelf-life of the complex values were 10.8-14.0 months and 8.04 - 11.2 months, respectively. Chloramphenicol : β-cyclodextrin complex showed significantly longer shelf-life than chloramphenicol about four times.

It is well known that cyclodextrins have capability to increase solubility of many water insoluble drug substances by inclusion in their molecular cavity. In recent years a number of cyclodextrin derivatives have been developed and studied. For their properties, 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) is one of such derivatives possessing high water solubility (>50 g/100 ml), which is nearly 30 times greater than that of β-cyclodextrin. In addition, it has been proved to be non toxic and most often used in sterile dosage form. So in this work, 2-HP-β-CD was investigated for its applicability in preparation of the reconstituted chloramphenicol powder for eye drops. 2-HP-β-CD was employed to increase the solubility of chloramphenicol and the solution of this compound was, then, dried to reconstituted powder using lyophilization technique.

Lyophilization technique is a drying process and significantly useful in the pharmaceutical technology for manufacturing of certain heat labile pharmaceutical products, or otherwise unstable in solution form for prolonged storage periods but that are stable in the solid stage. Stabilization can be applied during preparation of the products prior to freeze dried. The porous structure of freeze dried powder obtained usually increased the subsequent rate of solution as compared with the original materials.

It is expected that the chloramphenicol eye drop preparation which is produced in reconstituted powder may provide the shelf-life of the product longer than when kept in solution forms.

In order to overcome the aforementioned problems of chloramphenicol eye drop, this research study is aimed to develop the preparation of chloramphenicol as reconstituted powder for eye-drops using 2-HP-β-CD as solubilizer and stabilizer.

THE PURPOSES OF THIS STUDY

The aims of this study are :

A. To study the solubility of chloramphenicol when combined with 2-hydroxypropyl-β-cyclodextrin as solubilizer.

B. To study the preparation of reconstituted powder for eye drops of chloramphenicol from chloramphenicol and 2-hydroxypropyl-β-cyclodextrin solution using lyophilization technique.

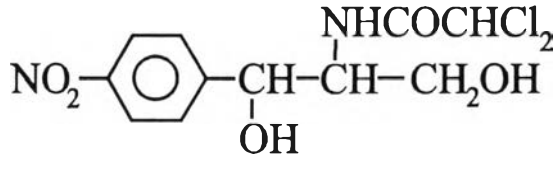
C. To study the stability and shelf-life of reconstituted powder for eye drops of chloramphenicol and after reconstitution with suitable vehicle.

D. To test antimicrobial activity of chloramphenicol eye drops by agar diffusion method.

LITERATURE REVIEW

In general, chloramphenicol is one of the most popular antibiotics. It is a broad spectrum antibiotic, effective against a wide range of gram-negative and gram-positive micro-organism and is popularly prescribed in eye infections. It is now listed in the National List of Essential Drugs. The general and physical properties of chloramphenicol are shown as follow :

Empirical formula : $C_{11}H_{12}C_{l2}N_2O_5$
Structure formula



Molecular weight : 323.13
Appearance, color, odor and taste :
Fine white to greyish white or yellowish white crystals, needles or elongated plates from water or ethylene dichloride with very bitter taste.
Melting point : 150.5°-151.5°
Solubility : 1 g dissolves in about 400 ml of water (2.5 mg/ml); freely soluble in alcohol, acetone, butanol, propylene glycol, and ethyl acetate; slightly soluble in ether and chloroform; insoluble in benzene and petroleum ether.

Stability of Chloramphenicol

1. Crystalline solid and solid dosage forms

Chloramphenicol in the solid state as a bulk drug or in solid dosage forms is a very stable antibiotics. Reasonable precautions taken to prevent excessive exposure to light or moisture are adequate to prevent significant decomposition over an extended period (Abdullah and Humeida, 1975).

2. In solution

Because of the multiplicity of functional groups in chloramphenicol, the degradation machanism could be extremly complicated. From the structure formula (Figure 1) it can be seen that degradation may occur by hydrolysis of the amide, chloride hydrolysis, by oxidation to the ketone or aldehyde, by reduction of the nitro group (Higuchi et al., 1954).

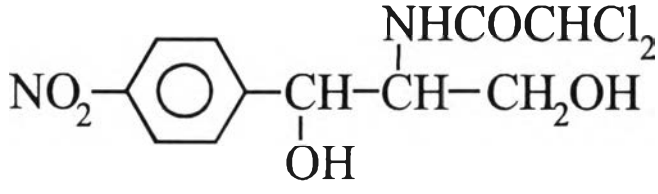


Figure 1 Molecular structure of chloramphenicol.

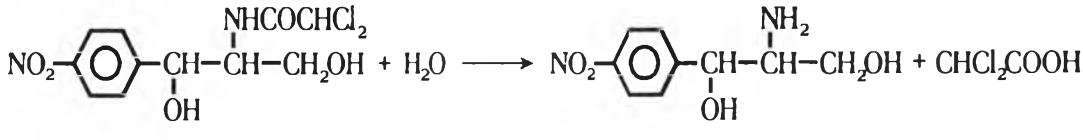
The degradation of chloramphenicol in aqueous solution occurs via several reactions such as follow :

1. Hydrolysis

Two primary routes of hydrolytic process have been determined to be

1.1 Amide hydrolysis

The primary pathway for the degradation of chloramphenicol is the hydrolysis of the amide linkage, forming 2-amino-1-(*p*-nitrophenyl)-1-3-propanediol (AMPD) and dichloroacetic acid (Higuchi et al., 1954).



The hydrolytic cleavage of the amide linkage is the major cause of chloramphenicol degradation in aqueous media. The rate is first order with respect to the drug and is independent of the ionic strength of the medium

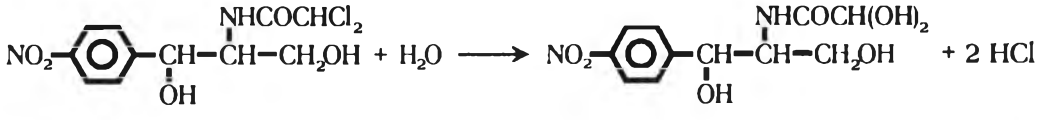
and concentration of hydrogen ion. In pH range 2-7, the hydrolysis is general acid/base catalyzed. Catalyzing species are general acids and bases present in buffer used, specifically, monohydrogen phosphate ion, undissociated acetic acid and mono-and dihydrogen citrate ions.

1.2 Chloride hydrolysis

It is the hydrolysis of the carbon-chloride linkage, forming hydrochloric acid (Higuchi and Bias, 1953). Three pathways of the degradation follow :

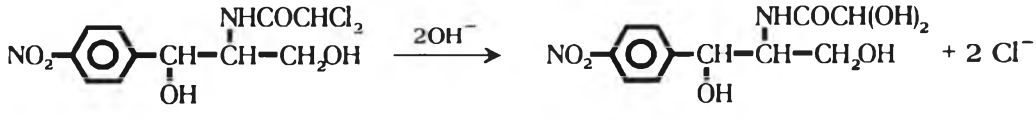
1.2.1 Direct uncatalyzed chloride hydrolysis

It will be observed at pH 6 or below. It is pH-independent reaction



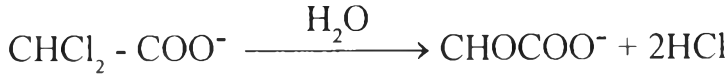
1.2.2 Hydroxyl ion catalyzed chloride hydrolysis

This hydrolysis is pH - dependent that occured at pH over 6



1.2.3 Hydrolyzed dichloroacetic acid

Chloramphenicol undergoes initially a hydrolysis reaction forming an amine and dichloroacetate ion. Subsequently, dichloroacetate ions which are undergoes further hydrolysis giving off chloride ion. It is pH-independent reaction.



2. Oxidation and reduction

Aqueous solution of chloramphenicol, ranging from 1-14, after standing at room temperature over a period of 24 days or longer yields detectable amouts of *p*-nitrobenzaldehyde (an oxidation product) and arylamine (a reduction product), while freshly prepared solutions fail to show the presence of these products. Identical degradation products are also found in certain dosage forms (creams and capsules), although they are not found in ophthalmic ointment (Shin, 1971).

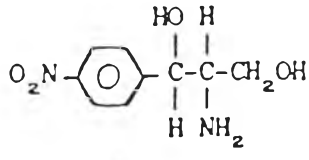
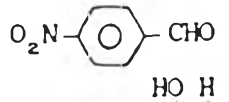
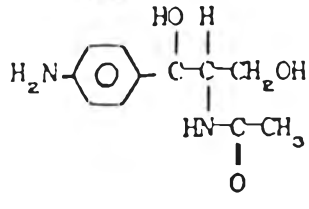
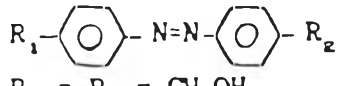
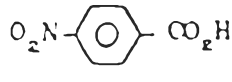
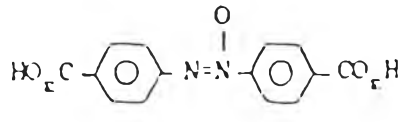
3. Photodegradation

Shin, (1971) reported that, an aqueous solution of chloramphenicol was degraded upon exposure to sunlight, UV and tungsten light. The major photodegradation products were hydrochloric acid, *p*-nitrobenzaldehyde, *p*-nitrobenzoic acid, 4, 4'-azoxybenzoic acid and *p*-aminophenyl-2-acetamido -1, 3-propanediol.

Photolysis of chloramphenicol in other solvents such as ethanol, benzene, ethanolamine and acetic acid was also similar to that in water. The chemistry of the degradation products suggested that chloramphenicol in water under the influence of light undergo oxidation, reduction and condensation reaction.

Products isolated from partially or completely decomposed chloramphenicol solutions exposed to a variety of conditions are given in Table 1.

Table 1 Degradation product of chloramphenical
(Daleszulczewski and Fred, 1975).

No.	Compound	Environmental Conditions
1.		Acidic or basic aqueous solution.
2.	Cl_2CHCO_2H	Acidic or basic aqueous solution.
3.		Aqueous solution, ambient temperature.
4.		Aqueous solution, ambient temperature.
5.	 $R_1 = R_2 = CH_2OH$	Aqueous alkaline solution, high temperature.
6.	$R_1 = R_2 = CO_2H$	
7.	$R_1 = R_2 = CHO$	
8.	$R_1 = R_2 = OH$	
9.	$R_1 = CO_2H$; $R_2 = OH$	
10.	$R_1 = CO_2H$; $R_2 = CH_2OH$	
11.	$R_1 = OH$ $R_2 = CH_2OH$	
12.	$R_1 = OH$ $R_2 = CHO$	
13.		Aqueous solution after exposure to light.
14.		Aqueous solution after exposure to light.
15.	HCl	Aqueous solution; high temperature.

Degradation of Chloramphenicol

1. Reactions and rate equations

The observed rate constant represents the sum of a number of terms. For an aqueous solution, k_{obs}, the observed rate constant, may be given as

$$k_{obs} = k_{H_2O} + k_H [H^+] + k_{OH} [OH^-] + k_{HB} [HB] + k_B [B] \quad ...(1)$$

Where k_{H2O}, k_H, k_{OH}, k_{HB} and k_B are constants for the uncatalyzed, hydrogen-ion-catalyzed, hydroxide-icn-catalyzed, general-acid, and general-base-catalyzed reactions, respectively.

Since rate constant of chloramphenicol depends upon the concentration and nature of the catalyst. In the case of degradations carried out in the presence of moderate concentrations of totally dissociated acid, the above equation may be written

$$k_{obs} = k_{H^+} [H^+] \quad .....(2)$$

Order of the degradation reaction of chloramphenicol in aqueous solution is first order as shown in Figure 2.

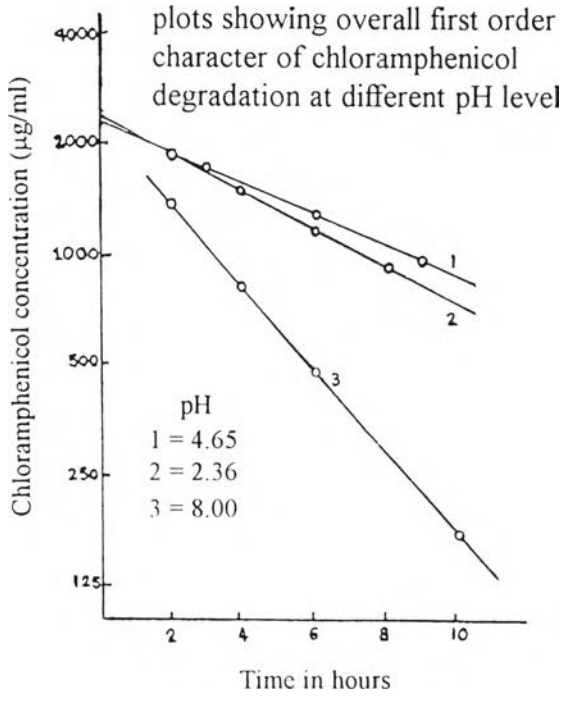


Figure 2 The first order character of chloramphenicol degradation in different buffers (Higuchi et al., 1954).

The degradation rate of chloramphenicol within pH range 2-7 is not specifically influenced by hydrogen ions, but depended on type of the buffer because of general acid-base catalyzed hydrolysis as shown in Figure 3.

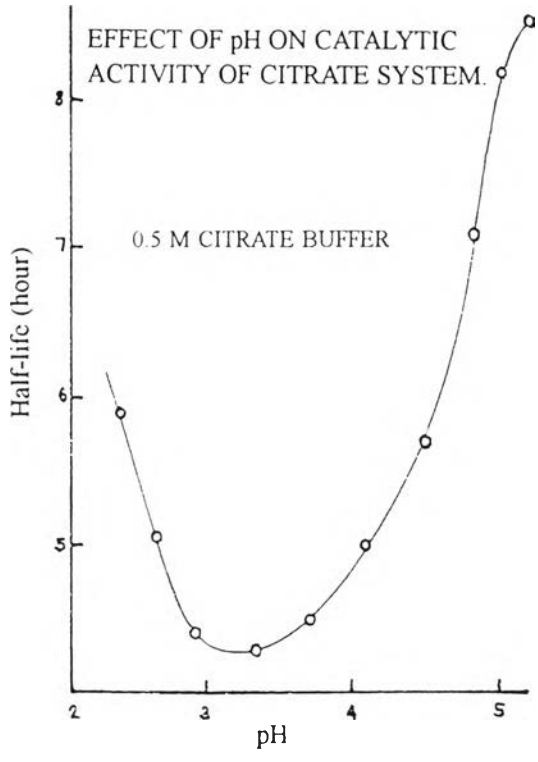


Figure 3 The effect of hydrogen-ion concentration upon the ability of a citrate buffer system to catalyze the degradation of chloramphenicol (Higuchi et al., 1954).

2. pH-rate profile

Figure 4 is the log k vs pH plot for the hydrolysis of chloramphenicol at 91.3°C in perchloric acid solutions. Specific acid catalysis is seen below pH 2 and the curve levels off as pH approaches 2. In universal buffer (containing citric, phosphoric, boric and hydrochloric acids and sodium hydroxide), and at 80°C, hydrolysis of chloramphenicol is independent of pH between 2 and 7. The rate constant in this pH range is 6.3×10^{6} s^{-1}.

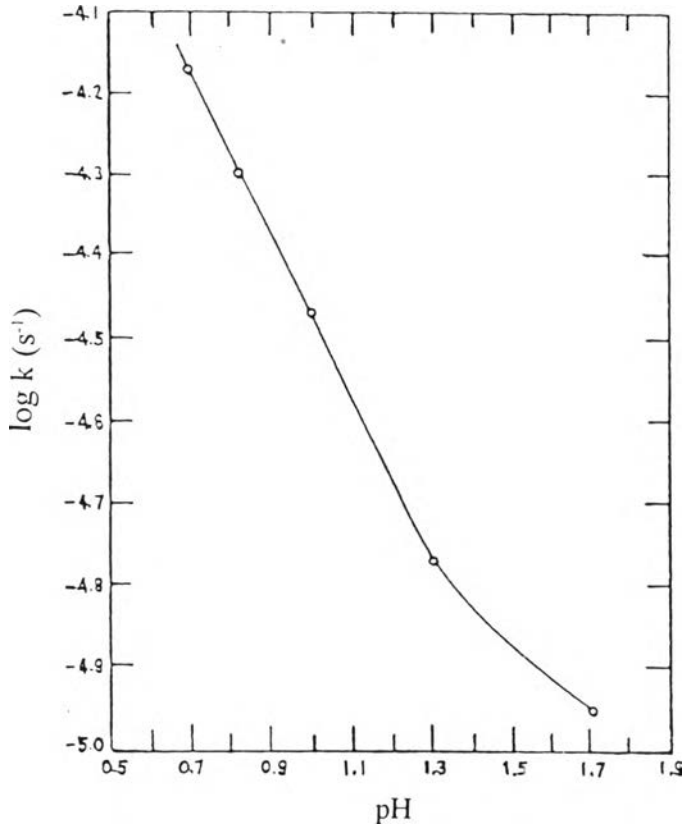


Figure 4 pH rate profile of chloramphenicol
(Higuchi and Marcus, 1954).

3. Activation energy

The Arrhenius plot for the hydrolysis at pH 6, corresponding mainly to the uncatalyzed (water) reaction, is shown in Figure 5. The activation energy at this pH is 24 kcal/mol.

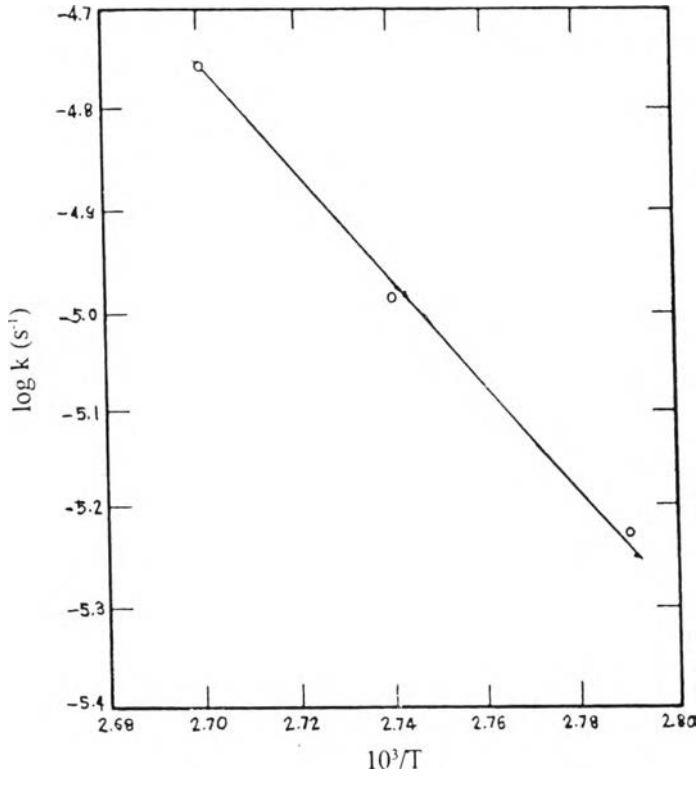


Figure 5 Arrhenius plot for hydrolysis of chloramphenicol at pH 6. (Higuchi et al., 1954).

Chloramphenicol Eye Drops in Pharmacopeia

In this review literature, it was stated only the newest volumes of all pharmacopeia. They are

1. Chloramphenicol Eye Drops BPC 1973.
2. Chloramphenicol Eye Drops BP 1993.
3. Chloramphenicol Ophthalmic Solution USP XXII.
4. Chloramphenicol For Ophthalmic Solution USP XXII.

They were compared in Table 2

Table 2 Comparison of Chloramphenicol Eye Drops in Pharmacopeia.

	Chloramphenical Eye-drops BPC 1973	**Chloramphenical Eye-drops BP 1993**	**Chloramphenicol Ophthalmic Solution USP XXII**	**Chloraphenicol for Ophthalmic Solution USP XXII**
1.Formulation	Chloramphenicol 0.5 g Phenylmercuric Acetate or Nitrate 0.002 g Borax 0.3 g Boric Acid 1.5 g Purified Water to 100 ml	The formulation was not stated, it was deseribed that, Chloramphetcol Eye Drops are sterile solution in Purified Water of Chloramphenicol, containing suitable buffering agents and 0.002 per cent w/v of either phenylmercuric acetate or phenylmercuric Nitrate.	The formulation was not stated, it was described that, Chloramphenicol Ophthalmic Solutin is a sterile buffered solution of Chloramphenicol.	The formulation was not stated it was described that Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluent and preservatives.
2. How to prepare	Dissolve the boric acid the borax, and the phenyl mercuric slt in 90 ml of the purified water with the aid of heat:adjust the temperature of the solution to 60°, add the chloramphenicol, and maintain the temperature at 60° until the chloramphenicol is dissolved. Cool the solution, and sufficient purified water to produce the required volume, and mix. Then, either (I) sterilise the solution by filtration, and transfer by means of an aseptic technique to sterile containers, which are then closed so as to exclude			

	micro-organisms (method B) or (ii) clarify the solution by filtration, transfer it to the final containers, which are then closed to exclude micororganisms, and sterilise it by maintaining at 98° to 100 ° for thirty minutes (Method C).			
3. Strength	0.5% w/v	0.5% w/v	-	-
4. content	0.45-0.55% w/v	90-110% of the prescribed or stated amount.	90-130% of the labeled amount.	90-130% of the labeled amount.
5. Limitation of 2-Amino-1(4-nitro-phenyl)propane-1,3-diol	not more than 5.0% of content of chloramphenicol.	Not more than 5.0% of content of chloramphenicol	-	-
6. pH	-	7.0-7.5	7.0-7.5	7.1-7.5
7. Storage	It should be protected forom light. When stored at a temperature of 2° to 8°, it may be expected to retain its potency for eighteen months form the date of preparation. When stored at a temperature not exceeding 25°, it may be expected to retain its potency for four months from the date of preparation.	Chloramphenicol Eye Drops should be protected from light. When stored at a temperature of 2 ° to 8° ,they may be expected to retain their potency for eighteen moths from the date of preparation. When stored at a temperature not exceeding 25° they may be expected to retain their form the date of preparation.	Preserve in tight containers.	Preserve in tight containers.

Cyclodextrin Inclusion Compound

Cyclodextrins are capable of forming complexes with a variety of compound by trapping various external molecules (guest molecules) inside the cavity of a cyclodextrin (host). The minimum requirement for this inclusion complex formation is that the guest molecule must fit,entirely,or at least, partially, into the cyclodextrin cavity. Not only the stereochemistry but also the polarity of the guest molecules determines whether inclusion may occur. In general, hydrophobic molecules have higher affinity to the cyclodextrin cavity in aqueous solution than hydrophilic molecules.

The structure of cyclodextrin inclusion complexes differ significantly in the crystalline state and in solution. In solution the guest molecule occupies in the cavity, and the whole complex is surrounded by a solvate shell of water molecules. In the crystalline state, the guest molecules can be accommodated not only in the cavity of the molecule, but also in the intermolecular cavities formed by the crystal lattice, or sandwich-like between two complex molecules. Some of the cyclodextrin molecules remain unoccupied or they include water. This arrangement may result in the formation of nonstoichiometric inclusion compounds.

The interaction force for inclusion complex formation cannot be a classical non-polar binding. The cyclodextrin complexes formed should be stabilized by various intermolecular forces such as :

1. Van der Waals interactions between the guest and host. The Van der Waals forces include both permanent induced-dipole-dipole interactions and London dispersion forces.
2. Hydrogen bonding between the guest and host.
3. Release of high energy water molecules in complex formation.
4. Release of strain energy in the macromolecular ring of cyclodextrin.

1. Preparation of inclusion complex

There are different methods to prepare inclusion complex such as :

1.1 In the case of a water-soluble active ingredient, the guest product is added to a saturated aqueous solution of cyclodextrin, and agitated for several hours or even days, until spontaneous precipitation of the inclusion is achieved. Sometimes precipitation dose not occur spontaneously, and it is necessary to cool the medium at ambient temperature or even lower or evaporation

by freeze-drying or spray drying (Gandhi et al., 1988; Helm et al., 1991; Kurozumi et al., 1975; Lin and Kao, 1989; Lin et al., 1991; Oguchi et al., 1990).

1.2 In the case of an insoluble active ingredient or when the active ingredient is susceptible to hydrolysis, a grinding method can be used. If the grinding is carried on for a sufficiently long period, the yield could be 100 %. Furthermore, the product obtained is a microfine powder which can dissolve better than large crystals. This is very interesting method for industrial purpose.

1.3 Kneading method

This is a method applicable to poorly water-soluble active ingredient. It consists of adding the active ingredient to a slurry of cyclodextrin, and kneading throughly to obtain a paste, which is then dried. The product is washed with an organic solvent to remove the free active ingredient mixed with the inclusion compound. This method is far from being recommended for the obtention of a pure inclusion. Washing by the organic solvent can remove a variable quantity of active ingredient already included, due to its partition coefficient in favour of the organic solvent.

1.4 Heating in a sealed container

This new method of preparing inclusion compounds has been described for benzoic acid and α- or β-cyclodextrin. It seems that the inclusion phenomenon may occur for a temperature over 70°C. When the products are under nitrogen pressure and heated to 127°C, the combination ratio is higher than for unpressurized samples (Nakai et al., 1987).

2. Detection of inclusion complex formation

One of the most interesting properties of cyclodextrins is their ability to form inclusion complexes with a wide variety of guest molecules. Molecular encapsulation may occur both in solution and in the solid state. Upon inclusion within the cyclodextrin cavity, a guest molecule changes in its physicochemical properties. These changes provide methods to detect whether guest molecules are really included in the cyclodextrin cavity. This section will discuss on the detection of inclusion complexation in solid state. Detection of inclusion complexation in solution will not be discussed here.

3. Detection of inclusion complexation in solid state

3.1 Infra- red (IR) spectroscopy

IR spectroscopy is used to assess the interaction between cyclodextrin and guest molecules in the solid state (Chow and Karara, 1986; Erden and Celebi, 1988; Glomot et al., 1988; Hassan, 1989; Kedzierewich et al., 1990; Nakai et al., 1987). This technique is not generally suitable to detect inclusion complexes due to the characteristic bands of cyclodextrin reporting the overwhelming part of the complex being hardly influenced by complex formation. However, some investigations showed the changing in the chemical shift and the broadening peak.

3.2 Thermo-analytical method

Thermo-analytical methods determine the guest substance undergoes some change before the thermic degradation of cyclodextrin (Chow and Kardra, 1986; Amdidouche et al., 1989; Erden and Celebi, 1989; Glomot et al., 1989; Takahashi et al., 1988).

The change of the guest may be melting, evaporation, decomposition, oxidation or polymorphic transition. This method consists of many techniques, such as thermo analytical system (TAS), thermo evolution analyzer (TEA), differential scanning calorimetry (DSC), thermogravimetry (TG) and differential thermal analyzer (DTA) which was used to confirm in this study.

3.3 Powder X-ray diffraction

Complex formation is indicated when the pattern of a newly formed substance and uncomplexed cyclodextrin is different. This method is very useful in the case of liquid guest molecule since liquid has no diffraction pattern of their own. When the guest compound is a solid substance, a comparison has to be made between the diffractogram of the complex and the mechanical mixture of the guest and cyclodextrin molecules. Comparison of the diffractograms is possible because cyclodextrin inclusion complex preparation processes such as freeze drying or grinding may change the crystallinity of the pure substances and this may also lead to different diffraction pattern. A diffraction pattern of physical mixture is often the sum of those of each component, while the diffraction patterns of cyclodextrin complexes are apparently different from each constituent and lead to a new solid phase with different diffractogram (Amdidouche et al., 1989; Hassan et al., 1989; Uekama et al., 1985).

3.4 Scanning electron microscopy

Scanning electron microscopy is used to study the microscopy aspects of the cyclodextrin, guest substance and the product obtained by co-precipitation or evaporation. Even if there is a clear difference in crystallization state of the pure substance and the product obtained by coprecipitating this method is inadequate to confirm inclusion complex formation, but helps to assess the existence of a single component in the preparation obtained (Amdidouche et al., 1989; Glomot et al., 1988).

Phase Solubility Analysis

Organic compounds, such as drugs which are sparingly soluble in water, frequently display an increase aqueous solubility in the presence of cyclodextrin. This is due to the formation of water soluble complex between the drug and the dissolved cyclodextrin. The complexation equilibrium lowers the thermodynamic 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 activitiy 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 cyclodextrin. This technique reveals both the stoichiometry of complex formation and the stability constant (or formation constant, k_C) of the complex.

Several different phase diagrams may be obtained from systems which form complexes. These are examined in some detail by Higuchi and Connors (1965). They have divided the systems into two major classes; type A and type B diagram.

Figure 6 shows one general class of phase diagram. A-type curves indicate the formation of soluble complexes. This type is further subdivided. If a plot of cyclodextrin concentration versus the concentration of drug solubilized is linear, an A_L-type is obtained. Positive deviation from linearity gives A_P-type. A_P-systems generally reflect high order complexation at high cyclodextrin concentrations meaning that more than one cyclodextrin molecule is complexing with the guest. The remaining A-type, A_N, shows a negative deviation which represents a decreasing dependence on ligand added at higher ligand concentrations. This type is the least frequently encountered system, and its occurrence may be explained on the basis of self-association of the ligand at high concentration.

The type B curves (Figure 7) are observes when insoluble complexes are formed. If the complex exhibits some solubility, the diagram shows an initial rise in the concentration of guest, a B_S-type is obtained. If the complex of drug and cyclodextrin is not soluble,a B_i-type curve is generated.

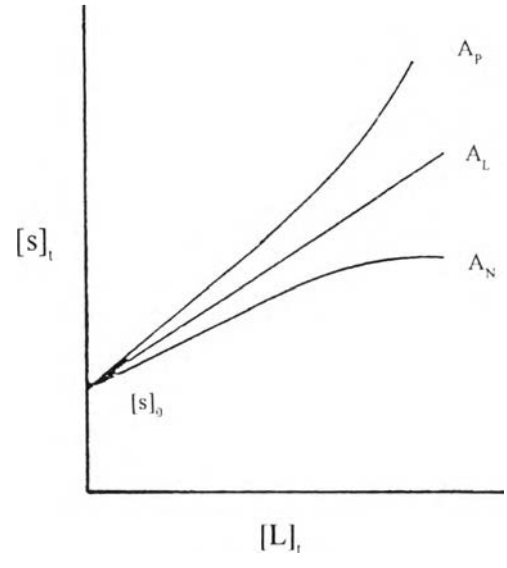


Figure 6 Schematic representation of the A-type phase diagrams ($[S]_t$= the concentration of the total substrate in the solution and $[L]_t$=the concentration of the total added ligand) (Higuchi and Connors, 1965).

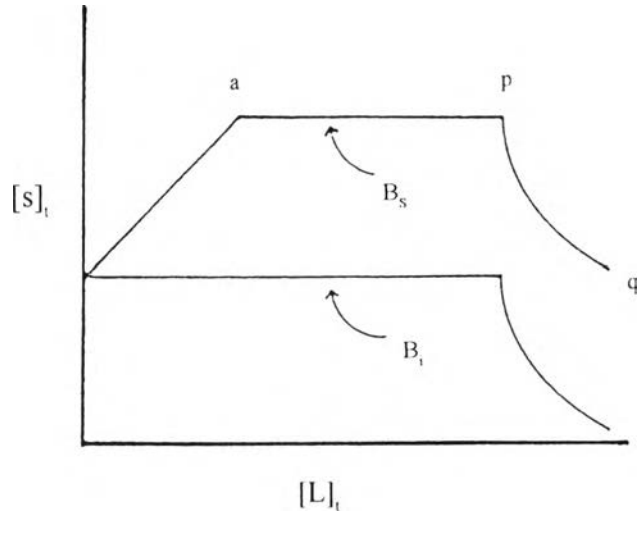


Figure 7 Schematic representation of the B-type phase diagrams (Higuchi and Connors, 1965).

2-Hydroxypropyl-β-Cyclodextrin (2-HP-β-CD)

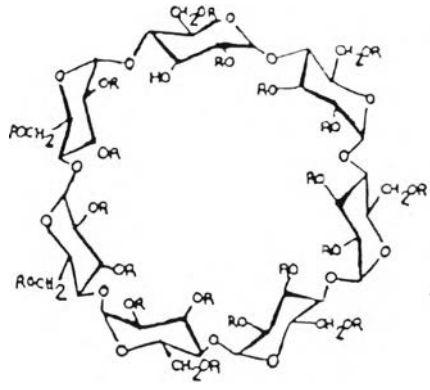
Cyclodextrins are cyclic carbohydrates consisting of 6, 7 or 8 glucose units and called α, β and γ-cyclodextrin, respectively. It has been known for long time in pharmaceutical circle that the cyclodextrins alter physicochemical properties of the guest molecule. It may be useful to improve drug properties such as solubility, dissolution rate, bioavailability, stability or to reduce side effect (Beker et al., 1991). β-cyclodextrin is most useful for incorporating a number of drug. Unfortunately, β-cyclodextrin is poorly water soluble (1.8 g/100 ml at 25°C). This low aqueous solubility of β-cyclodextrin is associated with cytoplasmic crystals in rat renal tubules and severe nephrosis after parenteral administration (Frank et al., 1976).

Several attempts have been made to improve the water solubility of β-cyclodextrin. Hydroxyalkylation of the cyclic oligomer disrupts hydrogen bonding which destabilizes the crystal lattice. In addition, these manipulations can transform the crystalline material into amorphous mixtures of isomeric cyclodextrin (Pitha, 1985). The amorphous of modified β-cyclodextrin as well as the hydroxyalkylated moiety contribute to an increase in aqueous solubility of cyclodextrin. Pharmaceutically important cyclodextrin derivatives are hydroxyalkylated-β-cyclodextrin (HA-β-CD). One of the HA-β-CD used for the preparation of amorphous complexes is 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD).

2-Hydroxypropyl-β-cyclodextrin (2-HP-β-CD) is very water-soluble not only as a result of their chemical nature, but also because of their amorphous structure. Their dissolution is endothermic so there is no decrease in solubility with increasing temperature. Its water solubility at 25°C is higher than 50g/100 ml.

Manufacturing of 2-HP-β-CD

Manufacturing methods use a base-catalyzed substitution reaction (Pitha et al., 1986). The reagent reacted with β-cyclodextrin is usually propylene oxide. The resulting product is purified by ion exchange chromatography, acetone extraction and lyophilization. These cyclodextrin mixtures are characterized by soft-ionization mass spectral techniques such as Californium-252 plasma desorption.



$R = [-CH_2CH(OH)CH_2]_nH$

n = 0, 1, 2, 3,...

Degree of substitution :		7.0
Residue on ignition :		0.02%
Residual acetone (%) :		<0.01%
Residual propylene glycol (%) :		<0.15%
Unreacted β-cyclodextrin :		<0.1%
Arsenic (ppm) :		<0.1
Heavy metals (as lead) (ppm) :		<10
Bromide (ppm) :		<5
Chloride (ppm) :		<5
Microcombustion analysis :	C, %	46.80
	H, %	7.65
Specific rotation [α] :		130° (589 nm RT)

Figure 8 Structure and characteristics of 2-hydroxypropyl-β-cyclodextrin (Brewster et al., 1990).

Table 3 Solubility of cyclodextrins and cyclodextrin derivatives (Janssen Pharmaceutical Industry).

PRODUCT	AQUEOUS SOLUBILITY (g/100 ml 25°C)
α-cyclodextrin	15
β-cyclodextrin	1.8
γ-cyclodextrin	2.3
dimethyl-β-cyclodextrin	57
trimethyl-β-cyclodextrin	31
hydroxypropyl-β-cyclodextrin	50

Toxicity of 2-HP-β-CD

The low solubility in water of β-cyclodextrin generally limits it use to oral applications. Orally administered cyclodextrin are not absorbed by the gastrointestinal system. Although β-cyclodextrin may be useful to improve oral formulations, the most challenging problem with insoluble therapeutic molecules are met in parenteral and local applications. Intravenously administered cyclodextrin are not degraded by common amylases. Therefore, they are eliminated unchanged through the kidneys. Consequently, high dose or chronic administration of β-cyclodextrin may reach higher renal concentration than its limited solubility allows and may crystallize locally causing irriversible cell damage.

The amorphous 2-HP-β-CD is readily water soluble, hydrophilic, easily and reproducible prepared and extensive chemical characterization. 2-HP-β-CD has been applied to numerous biomedical tasks and it is not acutely toxic after oral, IP, IV, IM, IC (intracerebral) or topical administration (Anderson et al., 1988; Brewster et al., 1988; Carpenter et al., 1987; Pitha et al., 1986; Pitha et al., 1988). Moreover, no dermal, ophthalmic or muscle irritation has been shown in aqueous 2-HP-β-CD concentration that are as high as 20, 40 or 50% w/v, respectively.

Mutagenicity testing using the Ames-Salmonella method and the mouse micronucleus method was negative. Embryotoxicity and teratogenicity are shown to be negative in rats and rabbits. Long-term carcinogenicity testing is under way with the full expectation of no unfavorable reports.

Physicochemical Characteristics of 2-HP-β-CD

1. Solubility

2-HP-β-CD is very soluble in water; 75% w/w solution can be made by 10-20 minutes stirring. It has very high water solubility, as expected of amorphous compound. It is also 50-60% w/w soluble in ethanol (95%). HP-β-CD with degree of substitution less than 7 have limited solubility in acetone, this with degree of substitution 11-14 is soluble in acetone or dichloromethan but insoluble in cyclohexane (Pitha et al., 1986).

Table 4 2-hydroxypropyl-β-cyclodextrin dosages given to human and routes of administration use* (Strattan, 1992).

	Intravenous (infusion)	Intravenous (injection)	Nasal	Buccal Sublingual	Per Os	Dermal
Total Dose	**30 g**	**3 g**	**600 mg/day**	**120 mg/kg**	**0.2 mg/kg**	**-**
Single Dose	**-**	**150 mg/kg**	**40 mg/use**	**120 mg/kg**	**0.2% mg/kg**	**-**
Form	**5% continuous infusion for 4 days**	**20% aqueous solution**	**10% aqueous solution**	**Tablet**	**Tablet**	**45% aqueous solution**

* No adverse clinical effects observed.

Table 5 Physicochemical properties of β-Cyclodextrin and its hydroxypropylated derivatives (Duchêne and Wouessidjewe, 1990).

Molecule	Substituent	Degree of Substitution	Average Molecular Weight (D)	Solubility in Water at 25°C (g/100 ml)	Surface Tension (mN/m)
β-CD	-	-	1355	1.85	71
2-HP-β-CD	$-OCH_2CH(OH)-CH_3$	2.5	-	> 50	69
2-HP-β-CD	$-OCH_2CH(OH)-CH_3$	5.6	-	> 50	64
2-HP-β-CD	$-OCH_2CH(OH)-CH_3$	6.8	-	> 50	61
2-HP-β-CD	$-OCH_2CH(OH)-CH_3$	8.0	-	> 50	60
2-HP-β-CD	$-OCH_2CH(OH)-CH_3$	11.3	-	> 50	52
3-HP-β-CD	$-OCH_2CH_2CH_2OH$	1.8	1239	> 50	71
3-HP-β-CD	$-OCH_2CH_2CH_2OH$	2.8	1297	> 50	70
3-HP-β-CD	$-OCH_2CH_2CH_2OH$	4.5	1396	> 50	71
3-HP-β-CD	$-OCH_2CH_2CH_2OH$	6.1	1489	> 50	70
2,3-HP-β-CD	$-OCH_2CH(OH)-CH_2OH$	2.6	1327	> 50	71
2,3-HP-β-CD	$-OCH_2CH(OH)-CH_2OH$	4.7	1483	> 50	71
2,3-HP-β-CD	$-OCH_2CH(OH)-CH_2OH$	5.9	1572	> 50	71
2,3-HP-β-CD	$-OCH_2CH(OH)-CH_2OH$	9.3	1823	> 50	70

2. Specific rotation (α)

Specific rotation (α) of 2-HP-β-CD tended to decrease with increasing degree of substitution (Yoshida et al., 1988).

3. Hygroscopic

2-HP-β-CD is less hygroscopic than the parent crystalline β-cyclodextrin. Low hygroscopicity of 2-HP-β-CD may be of advantage in pharmaceutical applications since the moisture sorption often initiates hydrolytic decomposition of drugs in solid state.

4. Hemolytic activity

At higher concentrations of natural cyclodextrins cause hemolysis and shape change of human erythrocytes even when isotonic solutions are used (Irie et al., 1982). The cyclodextrin induced hemolysis probably due to the membrane disruption elicited by dissolution and removal of membrane components. Hemolytic activity of 2-HP-β-CD is lower than parent cyclodextrin due to the decrease of ability of 2-HP-β-CD to remove membrane components. Furthermore, the hemolytic activity of 2-HP-β-CD decreases with increasing degree of substitution (Yoshida et al., 1988).

Pharmaceutical Application of 2-Hydroxypropyl-β-Cyclodextrin

Physicochemical properties of guest molecules may be altered if they are surrounded by the hydrophobic environment of a 2-HP-β-CD cavity. These alterations may lead be suitable formulations for potential drugs. A drug may dissolve better and faster, have a better bioavailability, fewer side effect and also be more stable. The most obvious alteration is an enhancement of the solubility and in pharmacy, this generally intends to improve bioavailability.

The pharmaceutical applications of 2-HP-β-CD may include :

1. Increase water solubility

HP-β-CD is highly water soluble and can be expected to provide a greater increase in water solubility of the guest molecule than the natural cyclodextrin. In the case of HP-β-CD with a low degree of substitution are better solubilizers than those with a high degree of substitution.

2-HP-β-CD has been used successfully to solubilize various active ingredients, including many steroids, non steroidal anti-inflammatory agents such as ibuprofen and indomethacin, digoxin, benzodiazepines, retinoic acid, nitrogen mustard, nimodipine, salbutamol, dihydropyridine derivatives and fragrance materials (Backensfeld et al., 1990; Brewster et al., 1988; Cebral et al., 1990; Loftsson et al., 1989; Matsuda et al., 1991; Menard et al., 1988; Muler Albers, 1992; Yoshida et al., 1988; Yoshida et al., 1990).

2. Improvment in stability

The inclusion of an active ingredient in 2-HP-β-CD is a means of improving its stability, especially in an aqueous medium. However, the results are highly dependent on the nature of the active ingredient and the cyclodextrin used.

An estradiol chemical derivery system consisted of a lipophilic, biooxidizable molecular carrier that was covalently attached to the 17-position of the estrogen, but this presented the drawback of oxidative lability and hydrolytic instability. These limitations can be solved by inclusion in 2-HP-β- CD (Brewster et al., 1988).

The aqueous stability of chlorambucil can be improved significantly by inclusion in 2-HP-β-CD (Loftsson et al., 1989). 2-HP-β-CD is also efficient in improving the aqueous stability of melphalan.

Indomethacin is a poorly soluble drug with a saturation solubility of 0.4 mg/ml in water. In aqueous solution, it undergoes pH-dependent hydrolysis to 5-methoxy-2-methyl-indol-3-acclic acid and *p*-chlorobenzoic acid. The former can be further brokendown to 5-methoxy-2,3-dimethyl indol. The hydrolysis of indomethacin in aqueous solution is decelerated by 2-HP-β-CD (Backensfeld et al., 1990).

3. Increase in Bioavailability

Most of the previous works with 2-HP-β-CD have been carried out to improve the bioavailability of the active ingredient in the formulations.

Flunarizine is a selective Ca^{2+} blocker which can protect brain cells against hypoxia resulting from stroke. In order to be effective, sufficiently

high flunarizine level must be obtained in the brain as soon as, possible after a stroke. Flunarizine is insoluble in the normal aqueous media used for intravenous injection. Thus, it can not be achieved by rapidly building a high concentration in circulation. An intravenous formulation based on 2-HP-β-CD allows a sufficiently high concentration of flunarizine to be injected as a bolus, immediately resulting in brain levels which can only be achieved hours after oral administration.

For transdermal delivery, 2-HP-β-CD is an effective transdermal permeability enhancer (Loftsson and Bodor, 1989; Vollmer et al., 1994).

In sublingual administration of testosterone, 2-HP-β-CD is very good absorption enhancer and result in high serum drug levels (Loftsson et al., 1994). 2-HP-β-CD can be used with the same drug by subcutaneous administration (Usayapant et al., 1991). 2-HP-β-CD also can improve the extent of ocular absorption of dexamethasone in albino rabbits (Usayapant et al., 1989).

The water solubility of acetazolamide and ethoxyzolamide limit their ocular bioavailability. It is believed that 2-HP-β-CD improve ocular bioavailability of drugs by keeping the water-insoluble drug molecules in solution and delivers them to the surface of corneal barrier where they partition into the eyes (Loftsson et al., 1994). 2-HP-β-CD also improved ocular bioavailability of dexamethasone and dexamethasone acetate (Usayapant et al., 1991).

4. Decrease in irritating properties

Cisapride is a new and very efficient gastro-intestinal prokinetic agent. Compared to the normal oral route, rectal administration shortens the time to a therapeutic response in chronic constipation. Conventional PEG (polyethylene glycol) suppositories often cause local irritation. A new cisapride suppository formulation based on 2-HP-β-CD and fatty acids solves the irritation problem. Moreover, the cisapride bioavailability proves to be about 40% higher than with PEG suppositories.