การพัฒนาตำรับไซโปรฟลอคซาซินเจลและการทดสอบฤทธิ์ต้านเชื้อซูโดโมแนสแอรูจิโนซาต่อไซโปรฟลอคซาซินเจล

นางสาวเอื้ออาภา หาญวานิช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ปีการศึกษา 2551

DEVELOPMENT OF CIPROFLOXACIN GEL AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN GEL AGAINST *PSEUDOMONAS AERUGINOSA*

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	Development of ciprofloxacin gel and antibacterial activity of ciprofloxacin gel against *PSEUDOMONAS AERUGINOSA*
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เอื้ออาภา หาญวานิช: การพัฒนาตำรับไซโปรฟลอคซาซินเจลและการทดสอบฤทธิ์ต้านเชื้อซูโดโมแนสแอรูจิโนซาต่อไซโปรฟลอคซาซินเจล (DEVELOPMENT OF CIPROFLOXACIN GEL AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN ON *PSEUDOMONAS AERUGINOSA*). อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วลัยศิริ ม่วงศิริ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร. พิณทิพย์ พงษ์เพ็ชร, 93 หน้า.

ในงานวิจัยนี้ได้การศึกษาความคงตัวทั้งทางกายภาพและเคมีรวมถึงฤทธิ์ฆ่าเชื้อของสูตรตำรับเจลของไซโปรฟลอคซาซินไฮโดรคลอไรด์ที่อยู่ในรูปสารประกอบเชิงซ้อนกับไฮดรอกซีโพรพิลเบต้าไซโคลเดกซ์ตริน (HP-ß-CD)ที่เตรียมได้ การเกิดสารประกอบเชิงซ้อนระหว่างไซโปรฟลอคซาซินไฮโดรคลอไรด์กับไฮดรอกซีโพรพิลเบต้าไซโคลเดกซ์ตรินสังเกตได้จากแผนภูมิเฟสซนิด A_L และการเพิ่มขึ้นของการเรืองแสงฟลูออเรสเซนต์ของไซโปรฟลอคซาซินไฮโดรคลอไรด์ในภาวะที่มี HP-ß-CD ไซโปรฟลอคซาซินไฮโดรคลอไรด์และไซโปรฟลอคซาซินแลคเทตเกิดสารประกอบเชิงซ้อนกับ HP-ß-CD ได้เหมือนกัน ดังนั้นจึงเลือกใช้ไซโปรฟลอคซาซินไฮโดรคลอไรด์ในการเตรียมสารประกอบเชิงซ้อนในการศึกษาต่อไปเนื่องจากมีราคาถูกกว่า การเตรียมเจลที่ประกอบด้วยสารประกอบเชิงซ้อนของไซโปรฟลอคซาซินไฮโดรคลอไรด์และ HP-ß-CD ทำโดยผสมสารประกอบเชิงซ้อนกับสารก่อเจลพอลอกซาเมอร์ 407 ความเข้มข้น 18 เปอร์เซนต์โดยน้ำหนัก และคาร์โบพอล อีทีดี 2020 ความเข้มข้น 1 เปอร์เซนต์โดยน้ำหนัก ความคงตัวทางด้านกายภาพและเคมีต่อแสงของเจลสารประกอบเชิงซ้อนไซโปรฟลอคซาซินไฮโดรคลอไรด์ที่เตรียมจากพอลอกซาเมอร์ 407 ที่มีความเข้มข้นเท่ากับ 0.15 มิลลิกรัมไซโปรฟลอคซาซินไฮโดรคลอไรด์ ต่อ 1 กรัมเจล ดีกว่าเจลของสารประกอบเชิงซ้อนไซโปรฟลอคซาซินไฮโดรคลอไรด์ที่เตรียมจากคาร์โบพอล อีทีดี 2020 ที่ความเข้มข้นเดียวกัน ในที่มืดที่อุณหภูมิ 40 องศาเซลเซียส ปริมาณของไซโปรฟลอคซาซินไฮโดรคลอไรด์ในพอลอกซาเมอร์ 407 เจลและคาร์โบพอล อีทีดี 2020 ลดลงในปริมาณ 16 และ 26 เปอร์เซนต์ ตามลำดับ ในเวลาสามเดือน ที่อุณหภูมิโดยรอบในที่มืดเจลของ สารประกอบเชิงซ้อนของไซโปรฟลอคซาซินไฮโดรคลอไรด์ในพอลอกซาเมอร์ 407 มีความคงตัวมากกว่าเจลสารประกอบเชิงซ้อนของไซโปรฟลอคซาซินไฮโดรคลอไรด์ในคาร์โบพอล อีทีดี 2020 การศึกษาการปลดปล่อยยาผ่านแผ่นเมมเบรน (Spectrapor® MWCO 1,000) พบว่าสารละลายของสารประกอบเชิงซ้อนและเจลของสารประกอบเชิงซ้อนในพอลอกซาเมอร์ 407 สามารถปลดปล่อยไซโปรฟลอคซาซินไฮโดรคลอไรด์โมเลกุลอิสระได้ รูปแบบของการปลดปล่อยเป็นไปตามฮิกูชิโมเดล ค่าคงที่ของการปลดปล่อยของไซโปรฟลอคซาซินจากเจลคือ 2.82 มิลลิกรัมเซนติเมตร$^{-2}$ ชั่วโมง$^{-½}$ การทดสอบฤทธิ์การฆ่าเชื้อซูโดโมแนสแอรูจิโนซาจำนวนยี่สิบเก้าสายพันธุ์แบบนอกกายของ พบว่าเจลสารประกอบเชิงซ้อนของไซโปรฟลอคซาซินไฮโดรคลอไรด์ สามารถออกฤทธิ์ฆ่าเชื้อซูโดโมแนสแอรูจิโนซาชนิดที่มีความไวต่อไซโปรฟลอคซาซินได้ดีกว่า

ภาควิชา	เภสัชกรรม	ลายมือชื่อนิสิต.....
สาขาวิชา	เภสัชกรรม	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
ปีการศึกษา	2551	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEY WORD: CIRPFOFLOXACIN / HP-ß-CD / PHOTOSTABILITY / GEL /IN VITRO ANTIMICROBIAL ACTIVITY.

EUA-APHA HARNVANICH: DEVELOPMENT OF CIPROFLOXACIN GEL AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN GEL AGAINST *PSEUDOMONAS AERUGINOSA*. THESIS PRINCIPAL ADVISOR: ASSIT. PROF. Pol. Lt. WALAISIRI MUANGSIRI, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. PINTIP PONGPECH, Ph.D., 93 pp.

In this research, the topical gel of ciprofloxacin HCl- HP- ß-CD inclusion complex was prepared and evaluated for physical and chemical stabilities and antimicrobial activity. The observed A_L type phase solubility diagram and the observed fluorescence enhancement of ciprofloxacin HCl in the presence of HP- ß-CD indicated that ciprofloxacin HCl- HP- ß-CD inclusion complex was formed. Ciprofloxacin HCl and ciprofloxacin lactate could form inclusion complex with HP- ß-CD at the same extent. Thus, ciprofloxacin HCl was chosen for complex formation in further studies due to its low cost. To prepared ciprofloxacin HCl gels, ciprofloxacin HCl inclusion complex was incorporated in gel bases making from 18% w/w Poloxamer 407 and 1% Carbopol ETD 2020. Poloxamer 407 gel containing 0.15 mg/g of ciprofloxacin HCl inclusion complex showed better physical and chemical stability than the product making from 1% Carbopol ETD 2020 after photo stress. In the dark at 40 °C, gradually loss of ciprofloxacin HCl in Poloxamer 407 and Carbopol ETD 2020 gels were estimated to be 16 and 26 %, respectively, over 3 months. At ambient temperature in the dark, ciprofloxacin HCl inclusion complex in Poloxamer 407 preparation was more stable than ciprofloxacin HCl inclusion complex Carbopol ETD 2020 gels. The release studies through a membrane (Spectrapor® MWCO 1,000) showed that the complex solution and the ciprofloxacin-complex-Poloxamer 407 gel preparation could release free ciprofloxacin. It was found that ciprofloxacin HCl could be released from its complex and gel. The release profile was described by Higuchi model. The release rate constant from gel was found to be 2.82 mg cm^{-2} $hr^{-½}$. The *in vitro* antimicrobial activity against 29 clinical isolates of *Pseudomonas aeruginosa* showed that inclusion complex ciprofloxacin HCl gel could inhibit all ciprofloxacin susceptible strains of *P.aeruginosa*.

Department :.....Pharmacy..... Student's Signature: Euaapha Harnvanich

Field of Study :.....Pharmaceutics...Principal Advisor's Signature: W. Muangsiri

Academic Year : .....2008.....Co-Advisor's Signature: Pintip Pongpech

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ANOVA	=	analysis of variance
°C	=	degree Celsius
CI	=	confidence interval
CDs	=	cyclodextrin
CV	=	coefficient of variation
df	=	degree of freedom
DSC	=	differential scanning calorimetry
g	=	gram
hr	=	hour
HPLC	=	high performance liquid chromatography
HP-ß-CD	=	2-hydroxypropyl-beta-cyclodextrin
mg	=	milligram
min	=	minute
mm	=	millimeter
mM	=	millimolar
M	=	molar
MW	=	molecular weight
n	=	sample size
nm	=	nanometer
PBS	=	phosphate buffer saline
pH	=	the negative logarithm of the hydrogen ion concentration
R^2	=	coefficient of determination
RH	=	relative humidity
SD	=	standard deviation
USP	=	The United States Pharmacopoeia
UV	=	ultraviolet
w/w	=	weight by weight

CHAPTER I

INTRODUCTION

The most common opportunistic and problematic pathogen in wound infection is *Pseudomonas aeruginosa* (Todar, 2004). Systemic treatment of *P. aeruginosa* infected wound has not yet reached satisfied outcome because the infected wounds are avascular and are covered by biofilm produced by the microorganism (Ghaffari et al., 2006). Thus, effective topical preparation is necessary as a co-treatment.

Topical gels are preferred to other topical formulations since they possess attractive appearance and give non-greasy feeling. Also, gels show better therapeutic efficiency as drug molecule can be released at a faster rate than other dosage forms such as creams and ointments (Tas et al., 2003).

Ciprofloxacin is a fluoroquinolone antimicrobial which is effective against *Pseudomonas* and *Staphylococci*. Ciprofloxacin inhibits DNA gyrase resulting in abnormality of cell replication (Hardman, Limbird and Gilman, 2001). Ciprofloxacin is sparingly soluble in water and its solubility depends on pH and temperature. Formation of needle crystal is a sign of physical instability of ciprofloxacin preparation when solubility limit is reached (Firestone, Dickason and Tran, 1998). Hydrochloride and lactate salts posse better solubility values than the free base.

Cyclodextrins (CDs) are cyclic oligosaccharide which consist of (α-1,4)-linked α-D-glucopyronose units. CDs are often employed to increase drug solubility by formation of inclusion complex, to protect active ingredient from photodegradation, to mask unpleasant taste of drug and to prevent drug-drug or drug-exipient interaction (Funasaki et al., 1999). The inclusion complex forms when whole or part of drug molecule resides in hydrophobic cavity of CDs (Loftsson and Masson, 2001).

CDs used in pharmaceutical applications are classified as natural and semisynthetic CDs. Alpha, beta and gamma CDs are natural CDs with 6, 7 and 8 glucopyranose units, respectively. Hydroxypropylbetacyclodextrin (HP-ß-CD) is an example of the modified CDs frequently used in pharmaceutics (Loftsson and Masson, 2001).

Inclusion complexes of ciprofloxacin with ß-CD and HP-ß-CD have been prepared but photostability and release of such complexes have never been studied (Jianbin et al., 2002, 2004). Therefore, the aims of this study were to develop and evaluate gels containing ciprofloxacin-HP-ß-CD inclusion complex.

The objectives of this study were as followed;

1. To prepare HP-ß-CD inclusion complex with ciprofloxacin HCl or ciprofloxacin lactate.
2. To characterize and to determine release and photostability of the inclusion complex.
3. To formulate ciprofloxacin- HP-ß-CD gels.
4. To evaluate physical and chemical stability of ciprofloxacin gels under stressed conditions.
5. To determine *in vitro* ciprofloxacin release of ciprofloxacin- HP-ß-CD gel.
6. To study the in vitro antimicrobial activity of ciprofloxacin- HP-ß-CD gels against *Pseudomonas aeruginosa*.

CHAPTER II

LITERATURE REVIEWS

A. Background

Skin infection easily occurs when the self-defensive barrier of skin is destroyed. The most common pathogenic microorganism in skin infection is *Pseudomonas aeruginosa*, a negative gram bacillus.

As a standard treatment, antibacterial agents are intravenously or orally administered in order to cure the infection. Avascular nature of the wound impedes drug distribution to the infected site; therefore, treatment efficacy of the standard treatment is low (Ghaffari et al., 2006). Topical preparation is needed to deliver high drug concentration at the infected site. Ciprofloxacin possess a bactericidal effect against *Pseudomonas aeruginosa*. Ciprofloxacin eyedrop, the only topical formulation in the market, shows physical instability, i.e. formation of needle crystal upon administration. Thus, stable topical formulation for skin infection is needed.

There are various kinds of gelling agent used in pharmaceutical products. To formulate the topical gels used in wound infection, the safety of usage has to be considered.

Poloxamer 407 is nonionic synthetic copolymer of ethylene oxide and propylenoxide. Poloxamer gel possesses unique thermoreversible property. Poloxamer 407 is non irritant material and non mutagenic agent (BASF safetydatasheet). Thus, Poloxamer 407 was applied as a gelling agent in many preparations such as naproxen gel (Suh and Jun, 1996), propanolol gel (Pandit and Wang, 2003) and ceftiofur gel (Zhang et al., 2002).

Various grades of Carbopol polymer are well known in pharmaceutical field as gelling agents. Carbopol ETD 2020 is cross-linked polyacrylic acid copolymer. It is superior to other conventional Carbopol because of its "easy-to-disperse" property. Carbopol ETD 2020 is considered as non-irritant to a very slight irritant (safetydatasheet).

B. Ciprofloxacin

1. General data

Ciprofloxacin, 1-cyclo-propyl-6-fluoro-1, 4-dihydro-4-oxo-7-(piperazinyl)-3-quinolinecarboxylic acid, is a member of quinolones (Figure 1). Ciprofloxacin possesses bactericidal activity against both gram positive and gram negative microorganisms including *Pseudomonas* species. The mechanism of action of ciprofloxacin is inhibition of DNA gyrase enzyme (topoisomerase) which is vital in bacterial DNA production. Ciprofloxacin is frequently used in *P. aeruginosa* infection such as in burn patients due to its efficacy and safety (Lesne-Hulin et al, 1999). Various dosage forms of ciprofloxacin are available; for instance, tablets, injections, ophthalmic solutions and ophthalmic ointments.

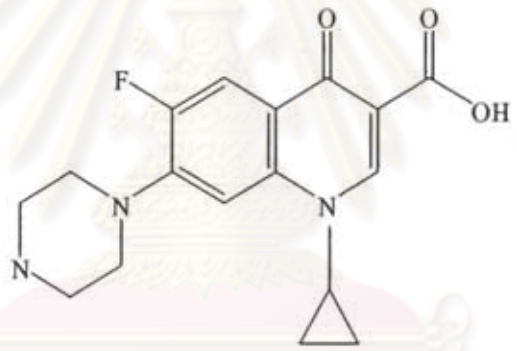


Figure 1 Chemical structure of ciprofloxacin

2. Pharmacokinetics (Davis, Markham and Balfour, 1996)

2.1 Absorption

Ciprofloxacin is well absorbed after oral administration. In healthy volunteers, a single dose oral administration of ciprofloxacin (250 to 750 mg) gives C_{max} and T_{max} of ciprofloxacin in ranges of 0.8-3.9 mg/L and 1 to 2 hr, respectively. The absorption is affected by some disease or illness, for example, decrease of ciprofloxacin absorption is observed in patients with major abdominal surgery.

2.2 Distribution

The distribution of ciprofloxacin in healthy volunteer is rapid. Ciprofloxacin distributes throughout the body.

2.3 Metabolism

Ciprofloxacin shows low first pass effect (5%) which is considered to be insignificant. About 10-20 % of the drug is partially modified at the piperazine group in the liver. Four metabolites have been identified, i.e. desethylene ciprofloxacin, sulfociprofloxacin, oxociprofloxacin and N-formylciprofloxacin. All of these metabolites have lower antibacterial activity than that of the parent. The primary metabolites are oxociprofloxacin and sulfociprofloxacin.

2.4 Excretion

Renal clearance is the major way of ciprofloxacin elimination. About 25-35% ciprofloxacin is excreted unchanged in the urine.

3. Physicochemical properties of ciprofloxacin

Ciprofloxacin is pale yellow crystalline powder. It is an amphoteric compound with 2 pK_a values, 6.09 and 8.74 for the carboxylic group and the nitrogen on the piperazine ring, respectively (Ross and Riley, 1990). Ciprofloxacin contains fluorophore with excitation and emission wavelengths of 330 and 450 nm, respectively.

Ciprofloxacin is practically insoluble in water especially at pH close to 7. Salt formation is employed to increase its solubility. Solubility of ciprofloxacin hydrochloride depends on temperature and pH. At 25 °C, solubility of ciprofloxacin hydrochloride at pH 5 is 3.46 mg/ml and at pH 7 is 0.09 mg/ml (Ross and Riley, 1990). Ciprofloxacin lactate is used in preparation of injection formulation due to its superior solubility.

pH change leads to change in solubility of ciprofloxacin. Ciprofloxacin precipitates and forms needle shape crystal in saturated solution.

4. Stability

Numerous studies on ciprofloxacin stability indicate that ciprofloxacin undergoes photolysis. Therefore, it is necessary to keep ciprofloxacin away from light (USP31).

Degradation scheme is shown in Figure 2. Ciprofloxacin ethylenediamine analog (compound I) is a major degradation product when pH of solvent is less than 5 whereas compound II is a main product if pH is less than 2 (Torniainen, Askolin and Mattinen, 1997). Numbers of degradation products are detected when pH is increased. The compound II is identified as 7-amino-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-3-quinolone carboxylic acid (Torniainen et al., 1997). Rate of photodegradation depends on pH. Ciprofloxacin is most stable in a pH range of 3-4.

Buffer effect on ciprofloxacin photodegradation was investigated at pH 5 using acetate, citrate and phosphate buffers. The result confirmed that buffer species has no effect on degradation process. (Torniainen,Tammilehto and Ulvi, 1996). Temperature has no effect on photodegradation product formation (Tiefenbacher et al., 1994).

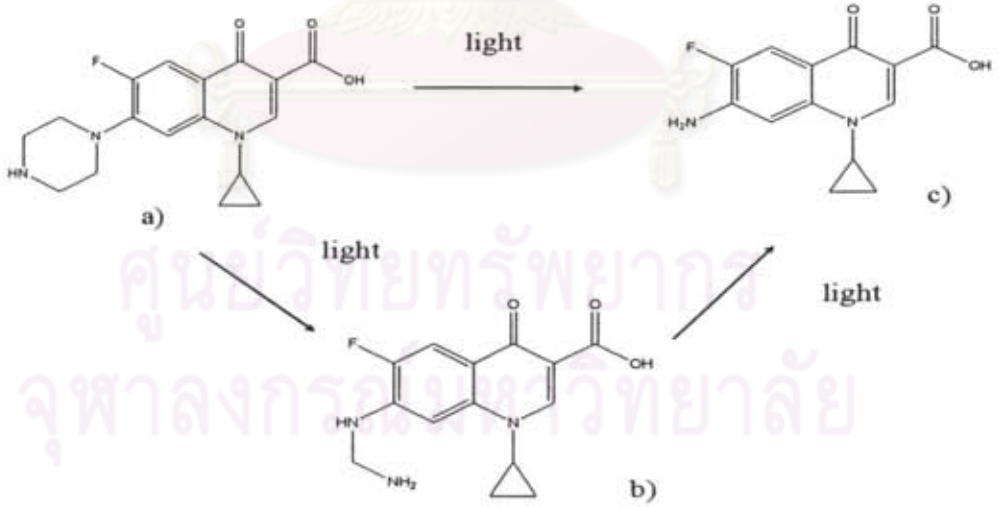


Figure 2 The photodegradation scheme of ciprofloxacin (a), compound I (b) and compound II (c) (Redraw from Torniainen, Askolin and Mattinen, 1997)

C. CYCLODEXTRINS

1. Structure and physicochemical properties of CDs (Loftsson and Masson, 2001)

Cyclodextrins (CDs) are cyclic oligosaccharide containing glucopyranose units which are linked by α-1, 4 linkages. Each glucose unit arranges itself so that secondary alcohol at C-2 and C-3 and primary alcohol at C-6 face outward while hydrogen atoms at C-3 and C-5 and glycosidic oxygen atoms position inside the cavity. For this reason, the central cavity is non-polar. CDs has a truncated cone shape because of the chair conformation of glucopyranose unit.

Natural CDs are α, β and γ CDs containing 6, 7 and 8 glucopyranose units, respectively (Figure 3). The numbers of glucose unit affect on the diameter and complex formation ability of CDs. The inside diameter of α, β and γ CDs are 5.3, 8.3 and 9.5Å, respectively.

β-CD has superior properties than other natural CDs; for example, good complexing ability and low cost. However, its low water solubility is the limitation. The intramolecular hydrogen bonding between secondary hydroxyl groups of β-CD reduces opportunity to form H-bonding with water molecule; thus, the solubility of β-CD is limited.

Numerous derivatives of CDs have been modified by adding substituents such as hydroxypropyl (HP), methyl (M) and sulfonyl ether (SBE). Not only the solubility problem was solved but the toxicity is also reduced by modified CDs' structure. The modified CDs that are currently applied in pharmaceutical research are methylated β-CD (RM-β-CD), sulfobuthylether β-CD (SBE-β-CD) and hydroxypropyl β-CD (HP-β-CD).

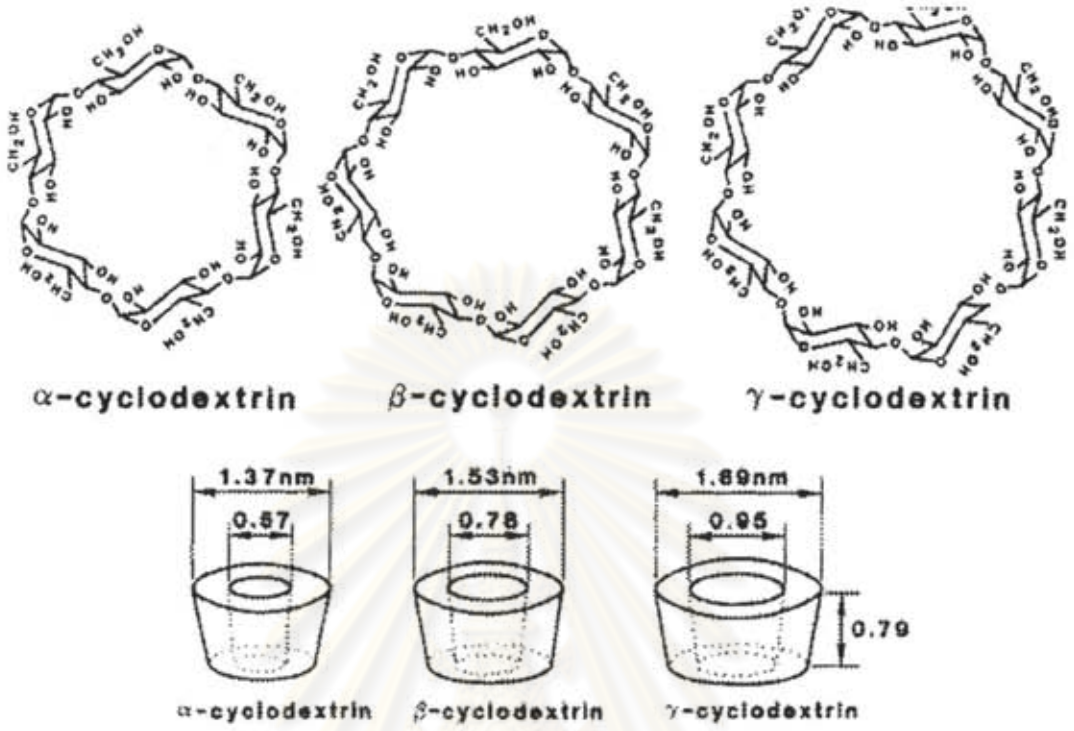


Figure 3 The molecular structure of natural CDs and their molecular dimension (Li and Purdy, 1992)

HP-β-CD is prepared by dissolving β-CD in alkali solution and treating with propylene oxide. The HP-β-CD has very superior aqueous solubility (>600 mg/ml) than the β-CD (18.5 mg/ml) at 25 °C (Brewster and Loftsson, 2007). Thus, HP-β-CD has been used in I.V. infusion products due to its great solubility (Loftsson and Duchene, 2007).

Thermoanalytical profile of β-CD and its derivatives could be divided in three part. At ambient temperature to 120 °C, loss of water molecule could be observed. From 120-300 °C, β-CD fuses and becomes liquid. β-CD decomposed above 300 °C. This information is useful for detecting complex formation by applying thermal analysis such as Differential Scanning Calorimetry (Giordano, Novak and Moyano, 2001).

2. Driving force in complex formation

During complex formation, enthalpy–rich water molecules are expelled from the CDs cavity. Then, either whole molecule or only lipophillic structure is resided in CDs cavity (Figure 4). Other involving forces are van der Waals, hydrogen bonding and hydrophobic interactions. Release of structural strain and changes in surface tension may be engaged in complex formation (Loftsson and Masson, 2001). However, no covalent bond is formed during complex formation. Therefore, drug molecules located in the cavity are easily released and are in the dynamic equilibrium with free drug molecules.

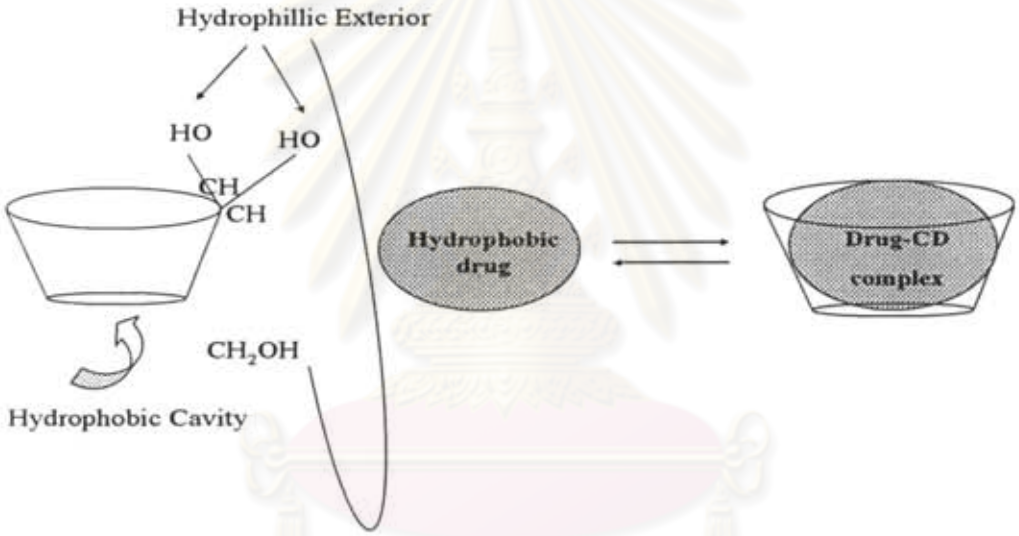


Figure 4 Complex formation between drug and CDs (redraw from Swarbrick and Boylan, 2000)

3. Complex preparation.

There are various methods of complex preparation such as kneading, melting, co-evaporation and freeze drying. The technique frequently employed is freeze-drying since this technique does not involve organic solvent and heat. Thus, the obtained complex is quite safe and less degraded.

4. Detection of complex formation

There are several techniques which can be applied to detect complex formation such as fluorescence, differential scanning calorimetry (DSC), fourier transform infrared spectroscopy, X-ray crystallography and nuclear magnetic resonance. In this review, only fluorescence and DSC will be mentioned.

4.1 Fluorescence Spectrometry

Changes in fluorescence intensity and/or emission wavelength are monitored up on complex formation. Therefore, the guest molecule must contain polarity sensitive fluorophore. In other words, fluorescence characteristic is changed according to polarity of its micro-environment. This technique is very sensitive and requires low sample concentration. However, the stoichiometric ratio of complex can not be obtained by this method. Fluorescence technique has been used to examine complexes between CDs and ciprofloxacin (Jianbin et al., 2002, 2004), cinalukast and montelukast (Meras, Mansilla and Rodriguez, 2007).

4.2 Differential Scanning Calorimetry (DSC)

DSC has been applied to investigate inclusion complex formation in solid state of various drugs; such as miconazole (Wang and Cai, 2008) and spironolactone (Rajabi et al, 2008). The observed melting endotherm of drug is disappeared when drug forms complex because drug is changed from crystalline state to amorphous state. To confirm the result, physical mixture of drug and CDs at the same concentrations as present in the complex is tested as a control. Normally, the melting endothermic peak of drug still presents in the thermogram of the physical mixture. This technique requires small amount of sample. However, there is a limitation due to its sensitivity.

5. Phase solubility study (Brewster and Loftsson, 2007)

The phase solubility study was described by Higuchi and Connors (1965) as the relationship between concentration of CDs added and the dissolved amount of drug (Figure 5). Information from the phase solubility study can be used to confirm

complexation. Generally, excess amount of drug is added in CD solutions with various concentrations. The slurry is stirred at a constant temperature. At appropriate times, samples are filtered and analyzed for concentration of drug dissolved in the slurry. Then, the phase solubility was constructed by plotting concentration of dissolved drug in molar on Y-axis against molar concentration of CDs on X-axis. Phase solubility diagram is classified into 2 major types.

Firstly, type A diagrams depict that the dissolved drug is linearly increased with CDs concentration, A_L. This type of phase solubility diagram could be observed when the complex contains only one drug molecule in the CDs. However, positive and negative deviations (A_P and A_N type, respectively) can be found. A_P type diagram is observed when CDs cavity can contain more than one guest molecule; A_N type diagram is obtained when the complex is self-associated at high CDs concentration.

Secondly, type B diagrams represent formation of complex with limited solubility. Curve B_S indicates that solubility of drug is increased until the complex reaches it solubility. Curve B_I shows that the complex is insoluble so that no increase of solubility is observed.

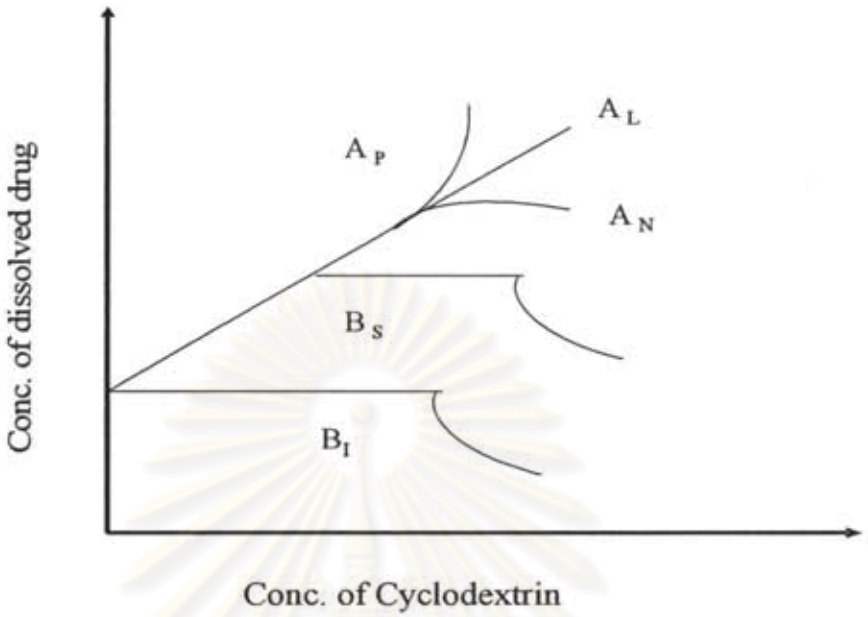


Figure 5 Types of phase solubility diagram (redraw from Brewster and Loftsson, 2007)

Association constant of inclusion complex is a measure of drug affinity for CDs. For complex with the stoichiometry of 1:1, the association constant ($K_{1:1}$) can be determined from slope of phase solubility by using the following equation.

$$K_{11} = \frac{\text{slope}}{S_0(1-\text{slope})} \quad \text{Equation 1}$$

where:

Slope is obtained from the phase solubility study

S_0 is intrinsic solubility of drug

6. Advantages of complex formation

CDs are very interesting compounds in pharmaceutical research because of their unique ability to form inclusion complex. Properties of complex are different from those of free drug molecules and contribute great benefits.

6.1 Control of solubility

The solubility of guest compound can be either increase or decrease upon complexation with CDs. The hydrophobic part of the guest molecule usually is included into the cavity of CDs while the hydrophilic outer surface of the CDs interacts with solvent. As a result, the complexation can promote solubility of the guest molecule as described by the A-type profiles in phase solubility diagram (Yusuff and York, 1991; Loftsson et al., 1994; Nijhawan and Agarwal, 2003; Fernandes, Vieira and Veiga, 2002; Liu and Zhu, 2006). However, decrease in solubility of the guest molecule observed in the B-type phase solubility is seen especially when CDs itself have limited solubility.

6.2 Improve stability

CDs can be used to enhance drug stability especially when reactive part of the guest molecule is hindered inside the CDs cavity. There are several reports stated that CDs could improve drug stability, such as mitomycins (Bekers et al., 1999), doxorubicin HCl (Brewster et al., 1992) and astaxanthin (Yuan et al., 2008)

6.3 Prevent of unpleasant interaction

According to the assumption, only free drug exhibits its taste; thus, CDs inclusion complex is employed to mask the taste of drugs in solution; for example, CD on bitter taste suppression of oxyphenonium bromide by CD complex (Funasaki et al., 1999).

7. Toxicological consideration (Loftsson and Duchene, 2007)

Natural ß-CDs are well known for its limit solubility and cause hemolytic; thus, they are not allowed to be used in parenteral preparation. While, modified CDs, HP-ß-CD, can be used in parenteral dosage form because it has superior solubility. Several

marketed products containing HP-ß-CD such as Sporanox® (Itraconazole), Mitozytrex® (Mitomycin).

D. Photodegradation

Some drug substances or drug products are light labile. The presence of some functional groups in drug molecule leads to the photodegradation, such as carbonyl, nitroaromatic, n-oxide, alkene, aryl chloride, sulfides, weak carbon-hydrogen and oxygen-hydrogen bonds and polyenes.

Classification (Piechocki and Thoma, 2007)

1. Direct photodegradation

Direct photodegradation takes place when a drug molecule absorbs photons and undergoes either bond breaking resulting in product formation or energy transfer giving rise to a molecule at the ground state.

Once the drug molecule absorbs photo energy, it needs to reduce the energy via fluorescence, phosphorescence, internal conversion or energy transfer. These mechanisms are competitive with photoreaction. Therefore, the drug which absorbs photo energy may or may not experience photodegradation.

2. Photosensitized degradation

Photosensitized degradation occurs when a component other than drug molecule, such as impurity or additive, absorbs the photonic energy. These molecules are called photosensitizer. The excited photosensitizer molecules become reactive species and pass the energy to drug molcecules resulting in drug degradation.

Adverse effects of photodegradation

Photodegradtion not only directly affects drug substance and drug product but affects human who ingest or contact its degradation products as well. Photodegradation leads to changes in appearance and efficacy.

Structure of drug molecule is changed resulting in discoloration. Drug efficacy is likely to be altered according to photodegradation. Ciprofloxacin infusion solution lost its antibacterial activity after exposure to the light (Tiefenbacher et. al, 1994).

Patients, who consume products contaminated with photodegradation products, may suffer from photosensitization, phototoxicity and cancer. Photosensitization is defined as the abnormal skin reaction when exposed to sunlight. Ciprofloxacin is well known drug causing photosensitization (Tiefenbacher et al, 1994). Photodegradation product of fenofibrate can induce hemolysis (Vargus and Canudus, 1993).

Factors affecting photodegradation

Many factors affect drug photostability. pH, buffer and ionic strength effects are needed to be individually evaluated for each drug. The photodegradtion rate constant of doxorubicin, daunorubicin and epirubicin are increased in the alkaline solution (Wood, Irwin and Scott, 1990) while the rate constant of flurosemide is decreased in alkaline solution (Bundgaard, Nargaard and Nielson, 1988).

Light source including light intensity and wavelength has direct effect on photodegradation. In addition, exposure time and distance between light source and tested samples also affect extent of degradation; thus, these factors should be controlled to obtain the consistency result.

Temperature effect on photodegradation is reported, for example, tetracycline HCl (Asker and Habib, 1991) and adriamycin (Habib and Asker, 1989). However, some quinolones were found to be temperature independent (Tiefenbacher et al., 1994).

Packaging material plays an important role in protecting drug from light. Amber glass and aluminium container are well known as a shield for light transmission.

Protective techniques, such as complex formation, addition of antioxidant, addition of chelating agent, are employed to retard photodegradation. To enhance photostability, drug complex are formed with various compounds, for example, CDs and cetylethylmorpholinium ethosulfate (Kowarski and Ghandi, 1974).

Autoxidation is likely to occur in photodegradation. Autoxidation is a free radical chain reaction of drug with oxygen. During the process, free radicals are generated. Prevention of autoxidation could be done by an addition of antioxidant. In addition, chelating agent; such as sodium EDTA, is used to retard the photodegradation of minoxidil and promethazine HCl (Chinnian and Asker, 1996; Cox, Meakin and Davies, 1976).

CHAPTER III

MATERIALS AND METHODS

Materials

1. 2-Hydroxypropyl-β-cyclodextrin with degree of substitution ~ 4 molecular weight ~ 1380 (Nutrifirst Biotech Inc., Lot No. 070902, China)
2. Acetronitrile HPLC grade (Labscan Asia Co., Thailand)
3. Carbopol ETD 2020 (Noveon™,, USA)
4. Ciprofloxacin hydrochloride (a gift from Siam Pharmaceutical, Thailand)
5. Ciprofloxacin lactate (Supplied by Millimed Co., LTD., Thailand, Lot No. CL015J06)
6. Disodium hydrogenphosphate (Ajax Fine Chemical, Australia)
7. Hydrochloric acid
8. Magnetic stirrer (Model RCT basic, KIKA Works Guaunghou, China)
9. Mueller Hinton agar (Merck, Germany)
10. Nylon Syringe filters, 13mm, 0.45μm, Ventripure
11. Phosphoric acid (Mallinckrot Baker Inc., USA)
12. Poloxamer 407 (BASF, Lot WPMZ527C, Germany)
13. Sodium chloride (Merck, Lot No.K28555404 049, Germany)
14. Spectra/ Por® Dialysis membrane MWCO 1,000 (Lot No. 3233067, USA)
15. Triethanolamine
16. Triethylamine (Carlo Erba Reactifs, Lot No. 4E230094G)

Equipment

1. Analytical balance (Sartorius model 1615, Germany)
2. Differential scanning calorimeter (NETZCH DSC 822, Mettler Toledo, Switzerland)
3. Fluorescence spectroscopy (Jasco FP-777, Japan)
4. Franz diffusion cell
5. High-Performance Liquid Chromatography (HPLC) equipped with :
   - a solvent delivery module (LC10AD, Shimadzu Corp., Japan)
   - a variable wavelength UV detector (SPD10A, Shimadzu Corp., Japan)
   - a data integrating software (LC10, Shimadzu Corp., Japan)
   - an automatic sample injector (SIL-10A, Shimadzu Corp., Japan)
   - a communications bus module (CBM-10A, Shimadzu Corp., Japan)
6. Lyophilizer, Dura-Dry FTS. Systems™
7. Moisture balance (Model HB43, Mettler Toledo, Switzerland)
8. pH meter (Orion model 420A, Orion Research Inc., USA)
9. Stability chamber (Eurotherm Axyos, Germany)
10. Turbidity meter, Crystal Spec (Becton Deckinson, USA)
11. UV-cabinet
12. Viscometer with the cone C35/1° Ti (RotoVisco RV 1, Germany)
13. Water bath (Model WB22, Becthai Co., Ltd., Thailand)

Methods

A. Validation of analytical procedures of drug substance and drug product

1. Chromatographic conditions

Ciprofloxacin HCl and ciprofloxacin lactate were analyzed using HPLC method described in USP 31.

Column	:	Luna Phenomenex® C18 (2) 5 µm 150×4.6 mm
Guard column	:	Security Guard ® HPLC Guard Cartridge system
Mobile phase	:	0.025 M phosphoric acid pH 3: acetronitrile (87:13 v/v)
Injection volume	:	20 µl
Flow rate	:	1.5 ml/min
Analytical wavelength	:	278 nm
Temperature	:	30 ± 1 °C
Run time	:	10 min

The mobile phase was freshly prepared, filtered through 0.45 µm nylon membrane filter and degassed by sonication for 30 min prior to use.

2. System suitability testing of ciprofloxacin raw material

2.1 Preparation of ciprofloxacin standard solution

About 25 mg of ciprofloxacin HCl was accurately weighed and dissolved in the mobile phase then adjusted volume to 50 ml.

2.2 Theorethical plate number, tailing factor and % RSD

Theoretical plate number, tailing factor and %RSD were estimated based on 5 replicate injections.

2.3 Resolution

Resolution is necessary in order to ensure that ciprofloxacin peak was completely separated from ciprofloxacin ethylenediamine analog. Ciprofloxacin ethylenediamine analog was prepared by dissolving 1 mg of ciprofloxacin HCl in 1 ml of 0.1 N HCl and

diluting with ultrapure water to make an appropriate concentration. The ciprofloxacin solution was kept in the UV- A cabinet at 30 °C, 75% RH for 6 hrs. The sample was analyzed by HPLC.

2.4 Acceptance criteria

Table 1 shows the acceptance criteria for of chromatographic system of ciprofloxacin HCl.

Table 1 The acceptance criteria for ciprofloxacin HCl HPLC analysis

Parameters	Requirement
Theorethical plate number	not less than 2500
Tailling factor	not more than 4
% RSD	not more than 1.5 %
Resolution	not less than 6

3. Linearity of HPLC Method

Stock solutions were prepared by weighing 4 mg of ciprofloxacin HCl or ciprofloxacin lactate and dissolving in the mobile phase to make 100 ml. The stock solution (0.04 mg/ml) was then diluted with the mobile phase to make standard solutions at concentrations of 0.006, 0.012, 0.016 and 0.024 mg/ml, respectively. Three replicates of each concentration were analyzed. Calibration curves were constructed by plotting peak areas against drug concentrations and analyzed by linear regression. The coefficient of determination (R^2) was used to determine the linearity. The acceptable R^2 is not less than 0.999.

4. Determination of ciprofloxacin HCl in finished products using HPLC (Q2R1)

HPLC conditions were the same as described in 1. "Placebo" was defined as either the physical mixture of 0.15 g/ml HP-ß-CD and 18% w/w Poloxamer 407 or the physical mixture of 0.15 g/ml HP-ß-CD and 1% w/w Carbopol ETD 2020 in PBS pH 7.4.

4.1 Specificity

The placeboes and the spiked placeboes containing of 0.012 mg/ml ciprofloxacin HCl were prepared and analyzed using HPLC.

Acceptance criteria:

Under the chromatographic conditions applied, the ciprofloxacin peak must not be interfered by any other components in the sample.

4.2 Linearity

Appropriated volume of ciprofloxacin HCl stock solution was spiked in the placeboes. pH values of the solutions were adjusted to 3 with 0.01 N HCl. Then, the solutions were further diluted with the mobile phase to the concentration range of 0.006-0.04 mg/ml. Each concentration was analyzed in triplicate. Calibration curves were constructed by plotting peak areas against drug concentrations and analyzed by linear regression. The coefficient of determination (R^2) was used to determine the linearity. The acceptable R^2 is not less than 0.999.

4.3 Accuracy

Five sets of three concentrations were prepared by spiking ciprofloxacin HCl stock solution in the placeboes to obtain final concentration of 0.008, 0.010 and 0.02 mg/ml prior to analyze with HPLC.

Acceptance criteria:

The percentage of recovery should be within 98-102 % for each nominal concentration.

4.4 Precision

a) Within run precision

The within run precision was determined by analyzed three sets of three concentrations of ciprofloxacin HCl at 0.008, 0.010 and 0.02 mg/ml in the presence of placeboes in the same day. Concentrations of ciprofloxacin were estimated from peak

areas and the percent of coefficient of variation (%CV) of each concentration were determined.

b) Between run precision

The between run precision was determined by analyzed of three concentrations of ciprofloxacin HCl at 0.008, 0.010 and 0.02 mg/ml in the presence of placeboes on three different days. Concentrations of ciprofloxacin were estimated from peak areas and the percent of coefficient of variation (%CV) of each concentration were determined.

Acceptance criteria:

The percent of coefficient of variation (%CV) for both within run precision and between run precision should be less than 2%.

B. Determination of equilibrium time of ciprofloxacin HCl and ciprofloxacin lactate and HP-ß-CD complex formation

HP-ß-CD in PBS pH 7.4 (0.15 g/ml) was placed in a double vessel jacket controlling temperature at 37 ± 2 °C using a water bath. Excess amount of ciprofloxacin HCl or ciprofloxacin lactate was added to the HP-ß-CD solution. The slurry was kept stirring by a magnetic stirrer in the dark. At appropriate time, samples were taken and filtered using 0.45 µm nylon membrane filters. The filtrate was diluted with the mobile phase to make a suitable concentration before analyzed by HPLC. The studies were done in triplicate.

C. Characterization of ciprofloxacin HCl - HP-ß-CD complex

1. Phase solubility study of ciprofloxacin HCl or ciprofloxacin lactate with HP-ß-CD

The studies were performed as mentioned by Higuchi and Connors (1965). Excess amount (20 mg) of ciprofloxacin HCl or ciprofloxacin lactate was added to 0-100 mM HP-ß-CD solutions. Then, each slurry was kept continuously stirring by a magnetic stirrer and temperature was controlled by a water bath at 37 ± 2 °C for 24 hrs. The suspensions were filtered through 0.45 µm nylon membrane filters and diluted with the mobile phase prior to HPLC analysis. The studies were done in triplicate.

The phase solubility diagram was constructed by plotting the concentration of dissolved ciprofloxacin HCl or ciprofloxacin lactate in molar against the molar concentration of HP-ß-CD. Linear regression analysis was employed to determine the relationship. The slope was obtained and used to estimate association constant ($K_{1:1}$) according to the Equation 1.

2. Determination of complex formation using fluorescence spectroscopy

Slurry of ciprofloxacin HCl and HP-ß-CD obtained from phase solubility study was filtered through 0.45 µm nylon membrane filters and was diluted to 4 mcg/ml with PBS pH 7.4. The solutions were then analyzed using fluorometer at excitation and emission wavelength of 273 and 420 nm, respectively. Ciprofloxacin HCl solution (4 mcg/ml) in PBS was used as a control. Fluorescence spectra were obtained. Fluorescence intensities and characteristics of fluorescence spectra of inclusion complex were compared to that of free drug.

3. Determination of complex formation using Differential Scanning Calorimetry (DSC)

DSC thermograms of ciprofloxacin HCl, HP-ß-CD, inclusion complex and a physical mixture of ciprofloxacin HCl and HP-ß-CD were obtained by using a DSC apparatus with a heating rate of 10 °C/min. To prepare the physical mixture of ciprofloxacin and HP-ß-CD, ciprofloxacin HCl and HP-ß-CD at the same amount as in the inclusion complex were thoroughly mixed in porcelain mortar. Nitrogen gas was used as purging gas. About 3 mg of each sample powder was weighed and crimped in aluminum pan. Empty sealed aluminum pan was used as reference.

D. Formulation of ciprofloxacin HCl-HP-ß-CD gels

1. Selection of gelling agent concentration in the preparations

The study was conducted to select desired gelling agent concentrations. Recommend concentration of Poloxamer 407 is in a range of 15-50 % (Rowe, Sheskey and Weller, 2003). Therefore, series of Poloxamer 407 gel bases were prepared in PBS pH 7.4. Various amount of Poloxamer 407 were added in cold water giving 15-40 %w/w

Poloxamer 407 solution. The Poloxamer 407 solutions were kept in a refrigerator overnight to promote the clear gels. Carbopol ETD 2020 solutions in a concentration range of 1 to 2 % w/w were prepared by addition of the polymer in water. Triethanolamine (TEA) was added to the polymer solutions to adjust the pH value to 7.4. Then, the gels were left on the bench at ambient temperature until it was fully hydrated. Appearance and hardness of the prepared gels were evaluated.

2. Preparation of ciprofloxacin HCl-HP-ß-CD gels

The complex solution was prepared by dissolving 30.4 g HP-ß-CD in PBS pH 7.4 and adjusted to final volume 200 ml. Then, 34 mg ciprofloxacin HCl was dissolved. The mixture was kept stirring for at least 24 hr until clear solution was obtained. Then the complex solution was freeze-dried. Concentrations of ciprofloxacin before and after lyophilization were determined using HPLC and present as % recovery. % moisture content was evaluated using Moisture analyzer balance. The experiments were performed in triplicate.

To prepare 30 g of gel, about 5.5 g of lyophilized ciprofloxacin inclusion complex powder was accurately weighed. Then, the powder was reconstituted with about half volume of water to make a clear complex solution (Portion A).

Gel base (Portion B) was prepared using either Poloxamer 407 or Carbopol ETD 2020. About 5.4 g of Poloxamer 407 was accurately weighed and made weight to about 15 g by cold water. The poloxamer 407 solution was kept in a refrigerator for 24 hrs to promote the clear gel base. In the mean time, 0.3 g of Carbopol ETD 2020 was dispersed in water and titrated with 75% TEA to pH 7.4. The Carbopol ETD 2020 gel was left on the bench until it was fully hydrated prior to use.

Ciprofloxacin HCl-HP-ß-CD gels were prepared by an addition of portion A in to portion B. The final weight was adjusted to 30 g by addition of water. The preparations were gently stirred to avoid air bubble until homogeneous gels were obtained.

E. Photostability of ciprofloxacin HCl-HP-ß-CD gels (ICH Q1B)

Ciprofloxacin HCl inclusion complex in Poloxamer 407 gel (cip-cpx-Poloxamer) and ciprofloxacin HCl inclusion complex in Carbopol ETD 2020 gel (cip-cpx-Carbopol) were prepared to contain 0.15 mg of ciprofloxacin per 1 g gel.

Ciprofloxacin HCl solution, ciprofloxacin HCl inclusion complex solution and the two preparations were subjected to photostability testing. Briefly, samples were filled in clear and well-closed glass vials. Samples were exposed to UV-A with light intensity of 8×10^{-4} watt/cm^2 or kept in the dark at 30 °C for 21 hr.

At appropriate time, samples were accurately weighed and diluted with the mobile phase. The sample pH value was adjusted to 3 with 0.01 N HCl if necessary. Solutions were filtered through 0.45 µm membrane prior to analyze by HPLC. pH values of samples were measured at the beginning and at the end of experiment using a pH meter. Appearance was evaluated by eyes. Viscosity at 37 ± 2 °C was determined at the beginning and at the end of experiment. All experiments were done in triplicate.

F. Stability studies of ciprofloxacin HCl-HP-ß-CD gels (ICH Q1AR2)

Physical and chemical stabilities of cip-cpx-Poloxamer 407 and cip-cpx-Carbopol ETD 2020 gel were studied. Generally, each gel formulation was weighed and packed in vials. Samples were kept in the stability chamber at 40 °C and 75% RH for 90 days. Gel base and complex solution were also kept under the test conditions as controls. The preparations were kept at ambient temperature in order to be used as negative controls. Samples and control were properly protected from light and water loss.

Chemical stability was evaluated by determining drug remaining at day 0, 5, 15, 30, 45, 60 and 90. Samples were prepared by weighing 0.5 g of gels, diluting with the mobile phase, adjusting pH to 3 with 0.01 N HCl and adjusting volume to 5 ml. Solutions were filtered through 0.45 µm nylon membrane prior analyzed by HPLC. Physical stability of gels was assessed by determination of pH, color and viscosity (at 37 ± 2 °C) on day 0, 30, 60 and 90.

G. *In vitro* release study of ciprofloxacin from gel

The release of ciprofloxacin HCl from cip-cpx-Poloxamer gel was determined using Franz diffusion cells. The membrane applied in this study was cellulose acetate (Spectrapor® MWCO 1,000). The membrane were rinsed by distilled water and soaked in PBS pH 7.4 for an hour prior to use.

Briefly, 2.5 g of 0.15 mg/g cip-cpx-Poloxamer gel was loaded in the donor compartment. Spectrapor® MWCO 1,000 was mounted on the Franz cell with the receiver compartment containing PBS pH 7.4. Each cell was equipped with a magnetic bar. The temperature was controlled at 37 °C by circulating water through a double vessel jacket surrounding the receiver compartment. At the appropriate time, samples were withdrawn from the receiver compartment by syringe and immediately replaced with fresh PBS. The known amount of free ciprofloxacin HCl was spiked to the samples in order to make analyzable concentration, and then samples were diluted with the mobile phase. Release of ciprofloxacin HCl-HP-ß-CD complex solution and free ciprofloxacin HCl solution were also performed as control studies. All experiments were done in triplicate (n=3).

H. *In vitro* antimicrobial activity of ciprofloxacin complex gel against *Pseudomonas aeruginosa*

Medium was prepared by weighing thirty eight grams of Mueller Hinton agar in a two liter flask. Purified water was added to dissolve the agar and the volume was adjusted to 1,000 ml. The medium was boiled until completely dissolved. The agar solution was sterilized at 121 °C under 15 pounds per square inch pressure for 15 min. Twenty five milliliters of the sterile Mueller Hinton agar was dispensed into sterile glass petri dishes of 90 mm diameter. The agar was allowed to solidify on a flat level surface. The plates were dried for 20 min at room temperature.

Twenty-nine strains of *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* ATCC 27853 were included in the study. The well-isolated colony from each strain was

inoculated into 7 ml of normal saline (85% w/v) to obtain the same turbidity as Mac Farland 0.5 turbidity standard using the turbidity meter.

A sterile swab was dipped into the culture suspension; the excess volume was removed by pressing and rotating the swab against the inside of the tube above the suspension level. The surface of Muller Hinton agar was thoroughly swabbed. The process was repeated three times by rotating in the direction of 60° to the previous inoculation.

Sterile stainless cups with approximately 6.8 mm diameters were filled with cip-cpx-Poloxamer or cip-cpx-Carbopol gel (about 0.35 ± 0.02 g). These cups were then placed on the surface of the inoculated plates by sterile forceps. Antibacterial efficiency of the formulations were compared to that of Dermazin® cream (1% Silver Sulfadiazine).

All plates were incubated at 37 °C for 18-20 hrs. The diameters of inhibition zone were measured.

CHAPTER IV

RESULTS AND DISCUSSION

A. Validation of analytical procedures of drug substance and drug product.

1. System suitability testing of ciprofloxacin raw material

Typical chromatograms of ciprofloxacin HCl and ciprofloxacin lactate showed a single peak with retention time of 6.7 minutes and 6.6 minutes, respectively (Figure 6). Theoretical plate number, tailing factor, %RSD and resolution were obtained from five replicate injections of ciprofloxacin HCl solution and reported in Table 2.

Table 2 The mean and standard deviation of theoretical plate number, tailing factor, %RSD and resolution. (n=5)

Parameters	Values
Theoretical plate number	2518 ± 7.694
Tailling factor	2.12 ± 0.007
% RSD	0.17 %
Resolution	6.14 ± 0.02

Ciprofloxacin HCl solution was stressed under UV-A for 6 hr to generate ciprofloxacin ethylenediamine analog which is a known as photodegradation product of ciprofloxacin (Torniainen, Askolin and Mattinen., 1997). The degraded solution was analyzed by HPLC. Chromatogram of the degraded solution showed 2 major peaks with retention time of 6.70 and 4.53 min corresponding to ciprofloxacin and its ethylenediamine analog, respectively (Figure 7). Therefore, the HPLC analytical method was indicated to be a stability-indicating assay.

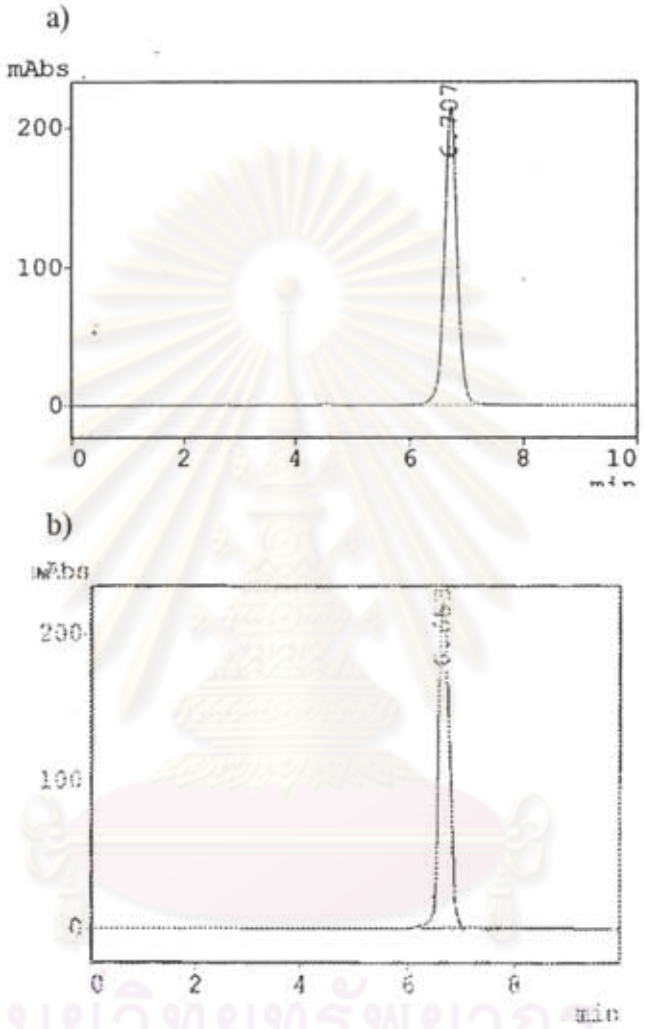


Figure 6 Chromatograms of ciprofloxacin HCl standard solution in the mobile phase (a) and ciprofloxacin lactate standard solution in the mobile phase (b)

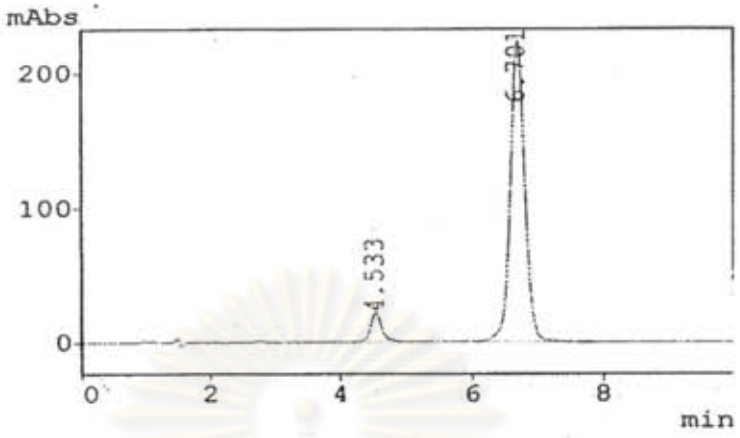


Figure 7 Chromatogram of ciprofloxacin HCl standard solution in the mobile phase after exposure to UV-A. Retention times of ciprofloxacin and its ethylenediamine product are 6.70 and 4.53 min, respectively.

2. Linearity of HPLC method

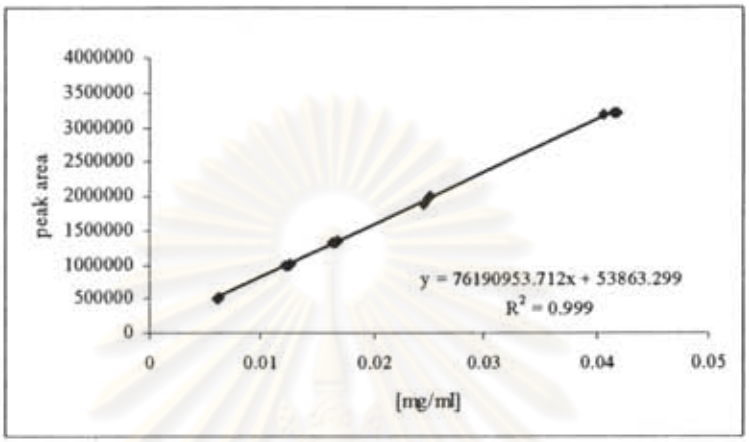
The calibration curve of ciprofloxacin HCl and ciprofloxacin lactate were showed in Figure 8. The plots of peak areas versus ciprofloxacin HCl or ciprofloxacin lactate concentrations showed the linear correlation in the concentration range of 0.006-0.04 mg/ml. The coefficient of determination was 0.999 and 0.999 for ciprofloxacin HCl and ciprofloxacin lactate, respectively. These results indicated that the HPLC method was acceptable for quantitative analysis of ciprofloxacin amount in the range studied.

3. Determination of ciprofloxacin HCl in finished products using HPLC

3.1 Specificity

The chromatograms of the two placebo showed no peak (Figure 9). In the presence of ciprofloxacin HCl, a single peak with the retention time of 6.6 min corresponding to the ciprofloxacin peak was observed (Figure 10). The result indicated that the HPLC peak of ciprofloxacin was not interfered by other compounds present in the formulations.

a)



b)

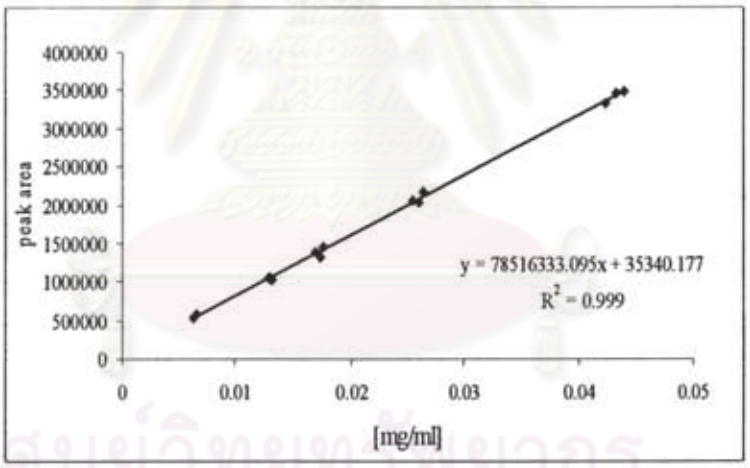


Figure 8 The calibration curves of ciprofloxacin HCl (a) and ciprofloxacin lactate (b)

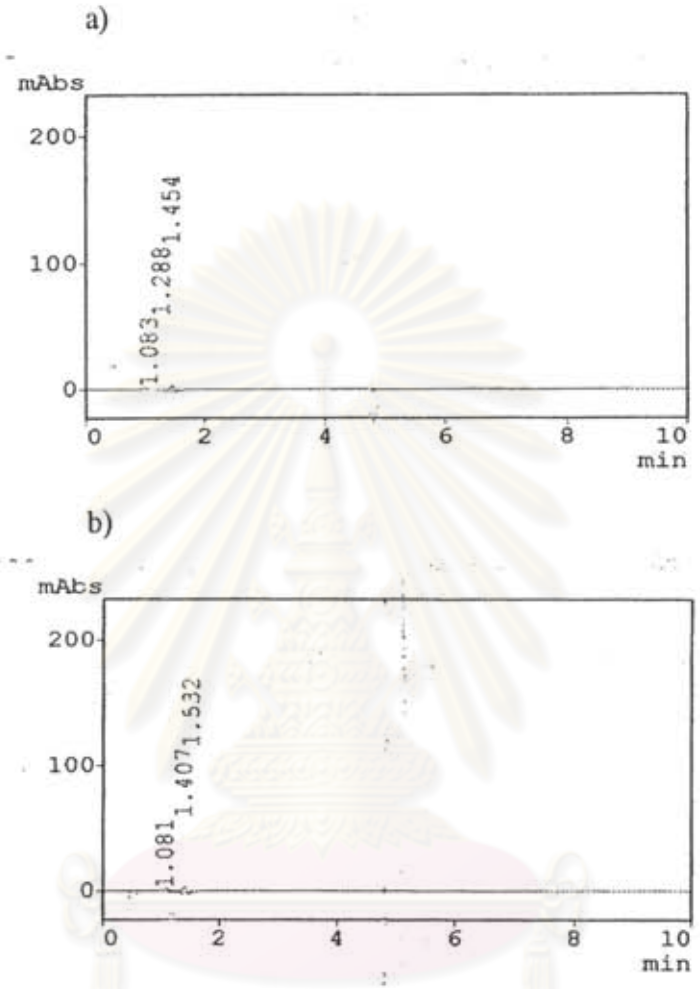


Figure 9 Chromatograms of "Placebo". Physical mixture solution of Poloxamer 407 and HP-ß-CD (a) and physical mixture solution of Carbopol ETD 2020 and HP-ß-CD (b)

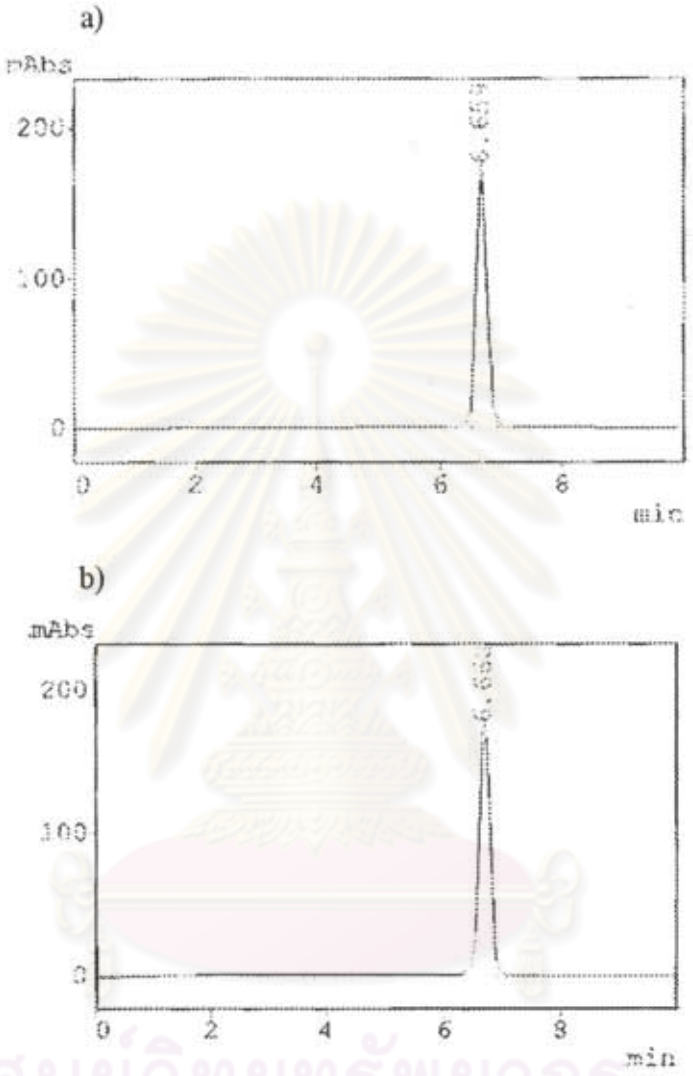


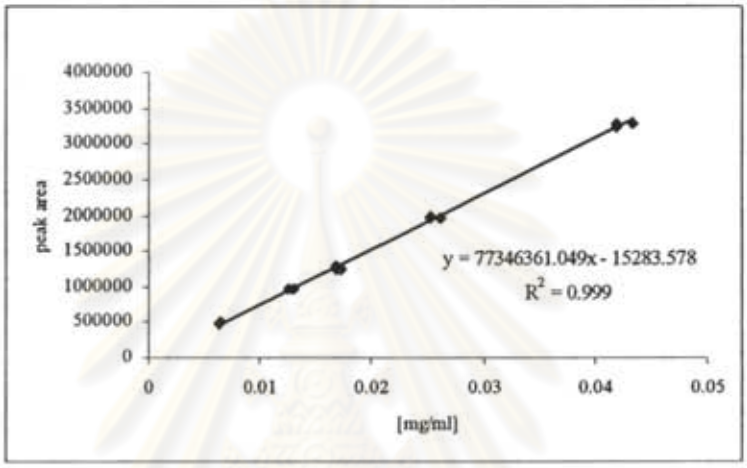
Figure 10 Chromatograms of spiked placebo. Ciprofloxacin HCl in the physical mixture solution of Poloxamer 407 and HP-ß-CD (a) and ciprofloxacin HCl in the physical mixture solution of Carbopol ETD 2020 and HP-ß-CD (b). Peaks with retention time of 6.6 min represent ciprofloxacin.

3.2 Linearity

The calibration curves of ciprofloxacin HCl in the presence of placebo were showed in Figure 11. In a concentration range of 0.006-0.04 mg/ml, peak areas were linearly related to ciprofloxacin concentration with the coefficient of determination of

0.999 regardless of types of polymer. Three calibration curves of ciprofloxacin with and without other additives in formulation were superimposable. Therefore, this can be implied that HP-ß-CD, Poloxamer 407 or Carbopol ETD 2020 did not interfere with the absorbance of ciprofloxacin.

a)



b)

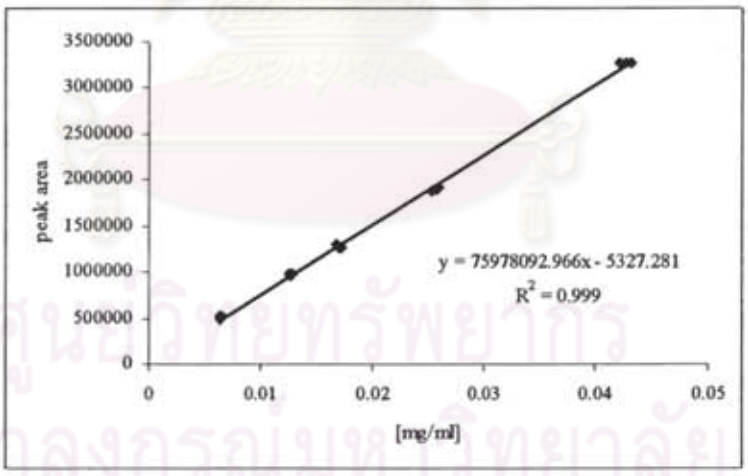


Figure 11 The calibration curves of ciprofloxacin HCl in the physical mixture of HP-ß-CD and 18% w/w Poloxamer 407 (a) and in the physical mixture of HP-ß-CD and 1% w/w Carbopol ETD 2020 (b)

3.3 Accuracy

Accuracy of the analytical method is closeness of test results obtained by the method to the true values. Accuracy is calculated as percent recovery of the assay to the known concentration of analyte. In the presence of Poloxamer 407, the percent analytical recovery of ciprofloxacin was in the range of 98.66-101.12 % (Table 3). In the presence of Carbopol ETD 2020, the percent analytical recovery of ciprofloxacin was in the range of 98.66-101.36 % (Table 4). Thus, this analytical method can be used to determine ciprofloxacin concentration in gels with high accuracy.

Table 3 The percentages of analytical recovery of ciprofloxacin HCl in the presence of Poloxamer 407 and HP-ß-CD by HPLC method

	% Analytical recovery			
Conc. (mg/ml)	**1**	**2**	**3**	**Mean ± SD**
0.008	98.09	99.59	98.30	98.66 ± 0.81
0.010	98.30	100.33	99.85	99.49 ± 1.06
0.020	100.44	101.99	100.92	101.12 ± 0.79

Table 4 The percentages of analytical recovery of ciprofloxacin HCl in the presence of Carbopol ETD 2020 and HP-ß-CD by HPLC method

	% Analytical recovery			
Conc. (mg/ml)	**1**	**2**	**3**	**Mean ± SD**
0.008	100.63	99.32	99.47	98.66 ± 0.81
0.010	101.32	101.90	100.39	99.49 ± 1.06
0.020	101.30	100.80	101.96	101.36 ± 0.58

3.4 Precision

The within run and between run precision of ciprofloxacin HCl were verified. Table 5 and 6 showed the data of within run precision of ciprofloxacin HCl analysis in the presence of Poloxamer 407 and Carbopol ETD 2020, respectively. All of coefficients of variation values were within acceptable range 0.70-1.17 % and 0.69-0.94 %. The result indicated that the HPLC method showed within run precision.

In the same manner, Table 7 and 8 showed the data between run precision of ciprofloxacin HCl analysis in the presence of Poloxamer 407 and Carbopol ETD 2020, respectively. All of coefficients of variation values were within acceptable range, 0.54-1.39 % and 0.93-1.78 %. The result indicated that HPLC method used in this study posse the satisfactory between run precision.

Table 5 The within run precision of ciprofloxacin HCl in the presence Poloxamer 407 and HP-ß-CD by HPLC method

Conc. (mg/ml)	1	2	3	Mean	SD	%CV
0.008	0.0084	0.0086	0.0085	0.0085	0.0001	1.1765
0.01	0.0106	0.0108	0.0107	0.0107	0.0001	0.9346
0.02	0.0216	0.0219	0.0217	0.0217	0.0002	0.7028

Table 6 The within run precision of ciprofloxacin HCl in the presence Carbopol ETD 2020 and HP-ß-CD by HPLC method

Conc. (mg/ml)	1	2	3	Mean	SD	%CV
0.008	0.0084	0.0083	0.0083	0.0083	0.0001	0.6928
0.01	0.0106	0.0107	0.0105	0.0106	0.0001	0.9434
0.02	0.0212	0.0211	0.0214	0.0212	0.0002	0.7194

Table 7 The between run precision of ciprofloxacin HCl in the presence Poloxamer 407 and HP-ß-CD by HPLC method

Conc. (mg/ml)	Quantitative analysis of ciprofloxacin HCl			Mean	SD	%CV
	Day 1	Day 2	Day 3			
0.008	0.0084	0.0082	0.0082	0.0083	0.0001	1.397
0.01	0.0106	0.0107	0.0107	0.0107	0.0001	0.541
0.02	0.0216	0.0211	0.0212	0.0213	0.0003	1.242

Table 8 The between run precision of ciprofloxacin HCl in the presence Carbopol ETD 2020 and HP-ß-CD by HPLC method

Conc. (mg/ml)	Quantitative analysis of ciprofloxacin HCl			Mean	SD	%CV
	Day 1	Day 2	Day 3			
0.008	0.0084	0.0086	0.0087	0.0086	0.0002	1.7831
0.01	0.0106	0.0107	0.0108	0.0107	0.0001	0.9346
0.02	0.0212	0.0213	0.0207	0.0211	0.0003	1.5259

B. Determination of equilibrium time of ciprofloxacin HCl and ciprofloxacin lactate and HP-ß-CD complex formation

Figure 12 (Appendix B; Table B.1.1 and Table B.1.2) depicted the solubility of ciprofloxacin HCl with or without HP-ß-CD in PBS pH 7.4 at 37 ± 2 °C. The solubility of ciprofloxacin HCl in the absence of HP-ß-CD was determined to be 0.12 mg/ml. In the presence of HP-ß-CD, the solubility of ciprofloxacin HCl was about 0.14 mg/ml at 24 hr. At the beginning, the solubility of ciprofloxacin HCl was increased as expected. Later on, the solubility of ciprofloxacin HCl stayed almost the same over the studied period. Figure 12 illustrated that inclusion complex was formed and reached equilibrium

when drug solubility was constant. Therefore, the equilibrium time for ciprofloxacin HCl complex formation was determined to be 24 hr.

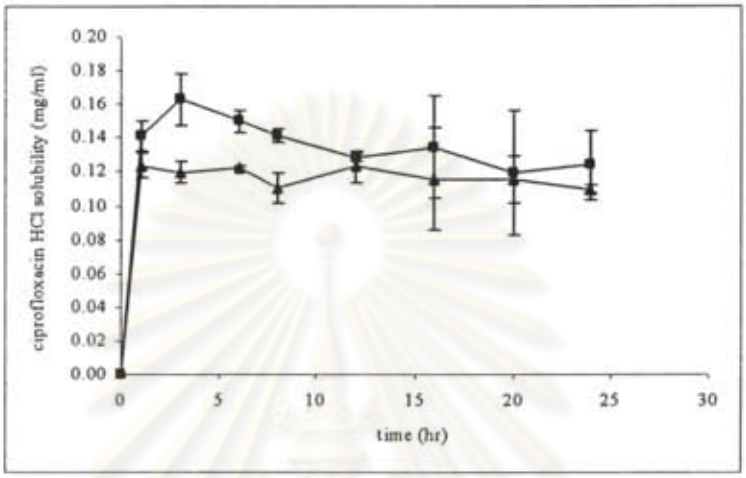


Figure 12 The solubility of ciprofloxacin HCl with 0.15 g/ml of HP-ß-CD (■) and without HP-ß-CD (▲) in PBS pH 7.4 at 37 ºC (n=3)

Ciprofloxacin lactate gave the same result as that of ciprofloxacin HCl. Figure 13 (Appendix B; Table B.1.3 and Table B.1.4) showed the solubility of ciprofloxacin lactate with or without HP-ß-CD in PBS pH 7.4 at 37 ± 2 ºC. The solubility of ciprofloxacin lactate without HP-ß-CD was determined to be 0.12 mg/ml. Ciprofloxacin lactate is well known for its superior aqueous solubility than ciprofloxacin HCl. However, salt type of ciprofloxacin did not significantly affect its solubility at pH 7.4 ($p>0.05$). Equilibrium time of complex formation can be determined by constant solubility of drug. The data showed the fluctuation of ciprofloxacin solubility at the early time then it trended to be stable. Therefore, preparation time of inclusion complex formation was 24 hr same as ciprofloxacin HCl.

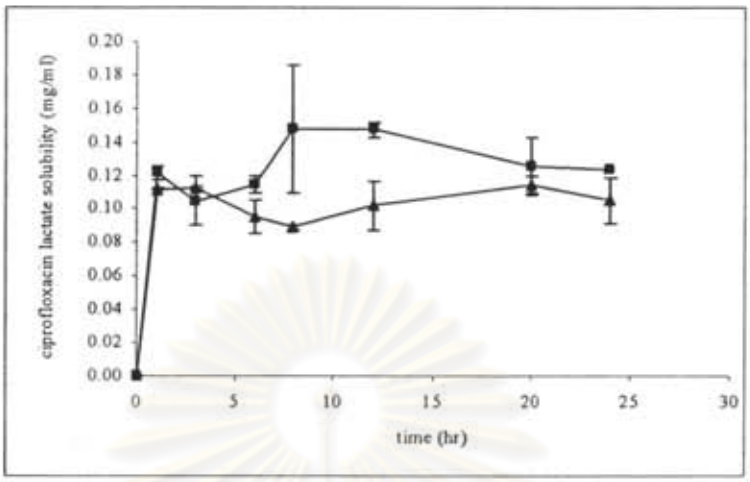


Figure 13 The solubility of ciprofloxacin lactate with 0.15 g/ml of HP-ß-CD (■) and without (▲) HP-ß-CD in PBS pH 7.4 at 37 °C (n=3)

C. Characterization of ciprofloxacin HCl-HP-ß-CD complex

1. Phase solubility study of ciprofloxacin HCl or ciprofloxacin lactate with HP-ß-CD

Solubility of ciprofloxacin HCl or ciprofloxacin lactate was linearly increased as HP-ß-CD concentration was increased (Figure 14, Appendix B; Table B.2.1 and Table B.2.2). The observed phase solubility diagram corresponded to A_L type profile defined by Higuchi and Connors (1965). The linear phase solubility profile between ciprofloxacin HCl and HP-ß-CD was consistent with previous observation indicating that the stoichiometric ratio was 1:1 (Nijhawan and Agarwal, 2003). In this study, $K_{1:1}$ of ciprofloxacin HCl or ciprofloxacin lactate and HP-ß-CD complex were calculated to be $1.01 \times 10^{-5}\ M^{-1}$ and $9.64 \times 10^{-6}\ M^{-1}$, respectively.

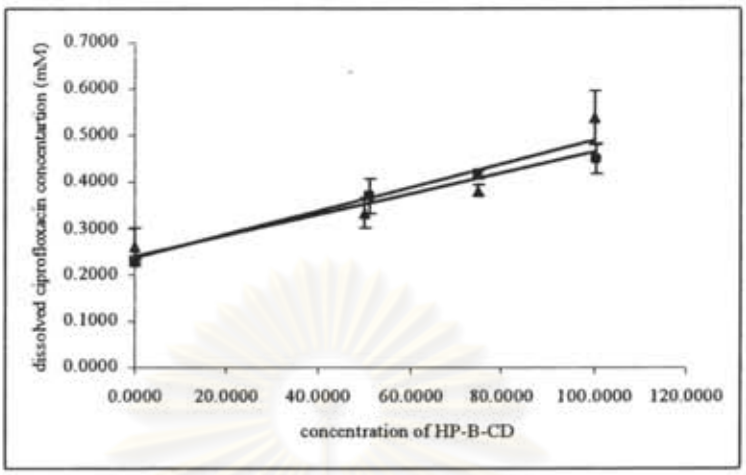


Figure 14 Phase solubility study of ciprofloxacin HCl (■) and ciprofloxacin lactate (▲) in PBS buffer pH 7.4, 37 °C (n=3)

$K_{1:1}$ of ciprofloxacin HCl and HP-ß-CD for complex formation estimated in this study (1.01×10^{-5} M^{-1}) was inconsistent with the value previously reported, i.e. 66.05 M^{-1} (Nijhawan and Agarwal, 2003). The observed difference was due to difference in experimental conditions mainly pH value of the solvents. Nijhawan and Agarwal studied ciprofloxacin HCl complex formation in water while this study investigated the complex formation at pH 7.4. $K_{1:1}$ is inversely proportional to intrinsic solubility while $K_{1:1}$ is directly proportional to slope (Equation 1). In addition, solubility of ciprofloxacin HCl in water is superior to that of in PBS pH 7.4. Slope obtained from the latter was smaller than the former. Thus, $K_{1:1}$ at pH 7.4 is much lower than $K_{1:1}$ in water.

The $K_{1:1}$ of complex formation between ciprofloxacin lactate and HP-ß-CD was 9.64×10^{-6} M^{-1} which was comparable to that of ciprofloxacin HCl. Solubility of ciprofloxacin lactate at pH 7.4 was statistically insignificant to that of ciprofloxacin HCl (ANOVA, $p>0.05$). In addition, phase solubility diagram of ciprofloxacin HCl almost superimpose to that of ciprofloxacin lactate meaning that slope almost the same (Figure 14). Thus, $K_{1:1}$ of both ciprofloxacin HCl and ciprofloxacin lactate complex formation were approximately the same. This can be implied that salt types did not affect complex

formation. In the other words, ciprofloxacin base was the moiety group used to form complex.

Normally, $K_{1:1}$ is used to evaluate the affinity of drugs for CDs (Loftsson, Hreinsdottir and Masson, 2005). Ciprofloxacin possesses two ionizable groups at the carboxylic group and the nitrogen on the piperazinyl ring with pKa values of 6.09 and 8.74, respectively (Ross and Riley, 1990). Since, the microenvironment inside CDs cavity is hydrophobic, zwitterion form is a preferred form in complex formation. At pH 7.4, fraction of zwitter ionic form of ciprofloxacin HCl and ciprofloxacin lactate were equal, resulting in formation of ciprofloxacin complex in the same extent. Ciprofloxacin HCl is less expensive than ciprofloxacin lactate. Therefore, ciprofloxacin HCl was chosen for further studies.

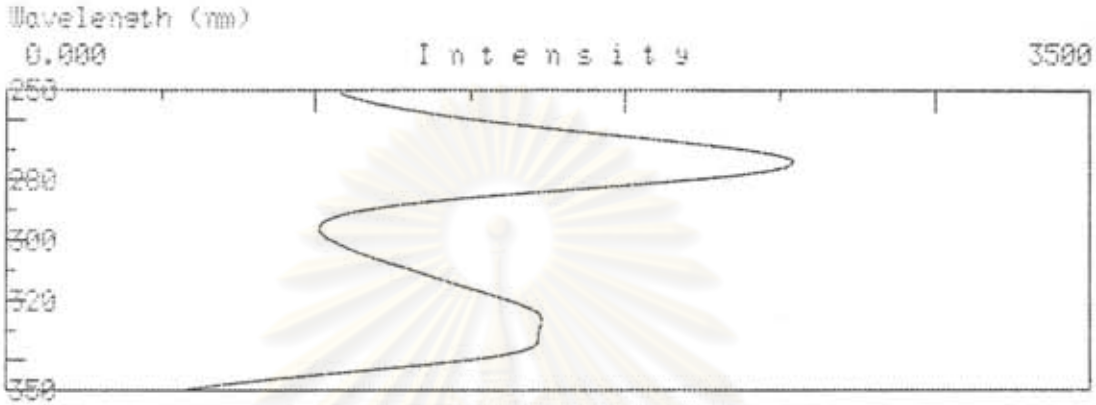
2. Determination of complex formation using fluorescence spectroscopy

In this study, the excitation and the emission wavelength of ciprofloxacin HCl in PBS pH 7.4 was determined to be 273 and 420 nm, respectively (Figure 15) which were different form previously reported, 330 and 450 nm, respectively (Jianbin et al., 2003; 2004). In this study, the excitation spectra of ciprofloxacin HCl showed two absorption maxima at 273 and 330 nm. Ciprofloxacin was excited at either wavelength, fluorescence maxima was obtained at 420 nm. The excitation wavelength 273 nm was chosen because it gave maximum fluorescence intensity at 420 nm. Shift in emission wavelength from 450 to 420 nm was probably due to differences in medium type.

Fluorescence spectra and fluorescence intensity of ciprofloxacin solution in the absence and in the presence of HP-ß-CD were compared. HP-ß-CD gave no fluorescence signal at the excitation and the emission wavelengths used (Figure 16). The samples of ciprofloxacin HCl at 0-100 mM HP-ß-CD were diluted with PBS pH 7.4 to contain 4 mcg/ml of ciprofloxacin HCl. Figure 17 shows that in the presence of HP-ß-CD, ciprofloxacin HCl fluorescence intensities were increased. Upon complex formation, ciprofloxacin was included in the hydrophobic environment of CDs cavity resulting in an

increase of fluorescence intensity. The observed fluorescence enhancement was consistent with the previous observation reported by Jianbin et al., 2003 and 2004.

a)



b)

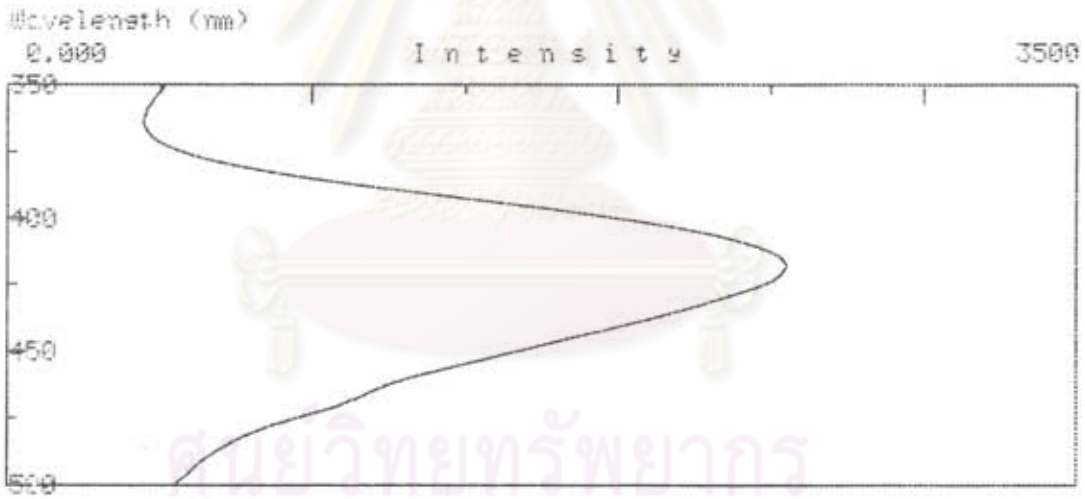


Figure 15 The excitation (a) and emission (b) scan of 4mcg/ml ciprofloxacin HCl in PBS pH 7.4

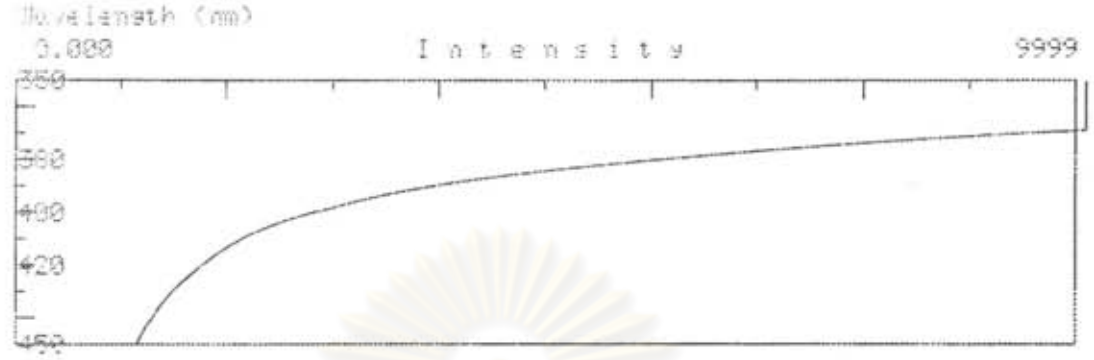


Figure 16 The emission scan of HP-ß-CD in PBS pH 7.4

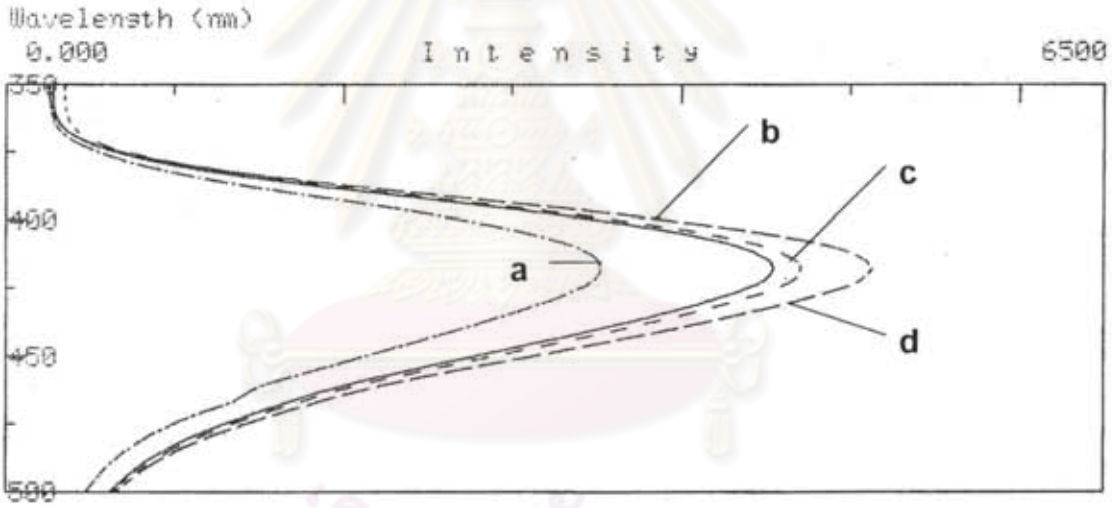


Figure 17 The fluorescence intensity of the same concentration ciprofloxacin HCl (4 mcg/ml) in the absence of HP-ß-CD (a) and in the presence of HP-ß-CD at 50 mM (b), 75 mM (c) and 100 mM (d)

3. Determination of complex formation using Differential Scanning Calorimetry (DSC)

DSC thermograms of ciprofloxacin HCl, HP-β-CD, ciprofloxacin HCl/ HP-β-CD solid complex and ciprofloxacin HCl/ HP-β-CD physical mixture are shown in Figure 18. The thermogram of ciprofloxacin HCl showed a sharp endothermic peak at 325 °C corresponding to its melting temperature (Nijhawan and Agarwal, 2003) whereas thermograms of HP-ß-CD showed a broad endothermic peak at 80 °C corresponding to water desorption (Giordano, Novak and Moyano, 2001).

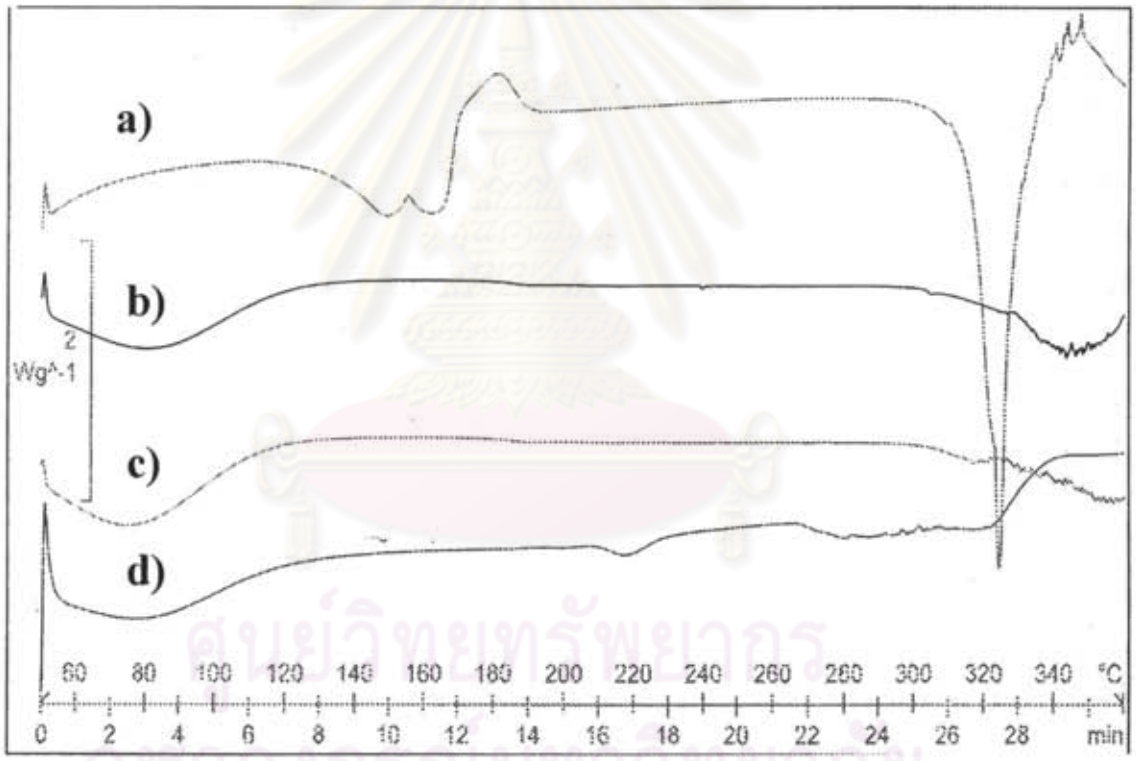


Figure 18 The thermograms of ciprofloxacin HCl (a), HP-ß-CD (b), physical mixture (c) and inclusion complex (d)

The absence of endothermic peak at 320 °C of thermogram of inclusion complex powder could be explained by 2 possibilities, complex formation and limitation of analytical technique. The physical mixture of ciprofloxacin HCl and HP-ß-CD at the same concentration as present in the solid complex was prepared. The endothermic peak of ciprofloxacin HCl was not observed in the DSC thermogram of the physical mixture. The result suggested that the ciprofloxacin HCl concentration in the mixture and in the solid complex were too low to be able to detect by DSC.

In this study, A_L type of phase solubility profile and fluorescence enhancement of the complex solution were evidences of complex formation while DSC was not a proper technique in determination of complex formation.

D. Formulation of ciprofloxacin HCl-HP-ß-CD gels

Appropriate concentration of gelling agent was determined based on appearance and gel hardness. Appearance of 15% w/w Poloxamer 407 gel was clear viscous but flowable at ambient temperature. While concentration of Poloxamer 407 was higher than 25% w/w, hard semisolid gels were obtained (Table 9). Then, the gels Poloxamer 407 with concentration 18, 20 and 22% w/w were prepared. 18% w/w Poloxamer 407 gel base gave a clear and soft semisolid gel. Therefore, the concentration of Poloxamer 407 in the preparation was chosen to be 18% w/w. Carbopol ETD 2020 gel was prepared by addition of 75% triethanolamine to reach pH 7.4. The gel containing 1% w/w gave clear gel with desirable appearance.

White bulky powder of inclusion complex was obtained after lyophillization. The % moisture content and % recovery of dried powder were determined to be 3.14 ± 0.24 % and 80.52 ± 1.57 %.

Poloxamer 407 gel containing 0.15 mg/g of ciprofloxacin HCl-inclusion complex (cip-cpx-Poloxamer gel) was a semisolid, clear and transparent gel with no drug precipitation. In the same manner, Carbopol ETD 2020 gel containg 0.15 mg/g of

ciprofloxacin HCl inclusion complex (cip-cpx-Carbopol gel) exhibited the same appearance as cip-cpx-Poloxamer gel. It was less likely to compare with free ciprofloxacin HCl gel at the same amount of drug because of its solubility.

Table 9 The preliminary study determining appropriate concentration of Poloxamer 407

% Poloxamer (w/w)	Hardness
15	gel could not be formed
18	+
20	++
22	+++
25	+++
30	++++
40	+++++

E. Photostability of ciprofloxacin HCl-HP-ß-CD gels

Solutions of free ciprofloxacin HCl and ciprofloxacin HCl inclusion complex solution at the same concentration (0.08 mg/ml) were stressed under UV-A at 30 °C to evaluate the effect of HP-ß-CD. Under UV-A with light intensity of 8×10^{-4} watt/cm^2 at 30 °C , both solutions degraded and gradually changed its color to yellow (Figure 19). In the dark, concentration of ciprofloxacin HCl in the solution and in the complex solution was unchanged throughout 20 hrs. The initial and final pH of both controls were monitored and showed that pH was not changed during period of study.

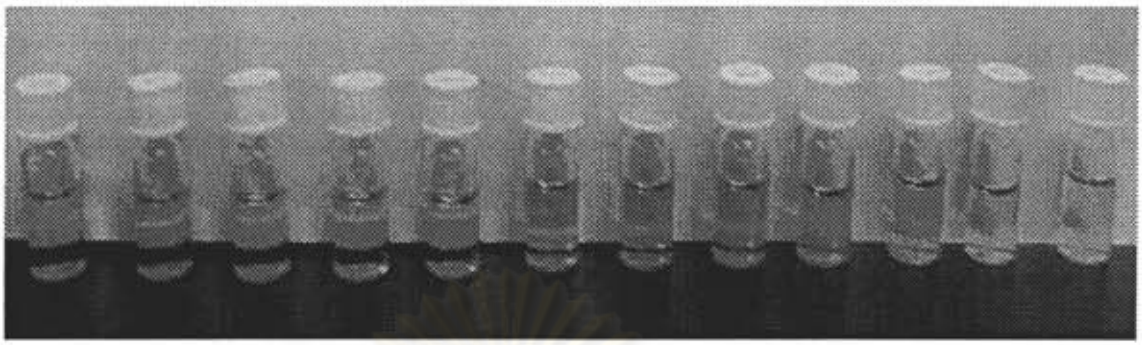
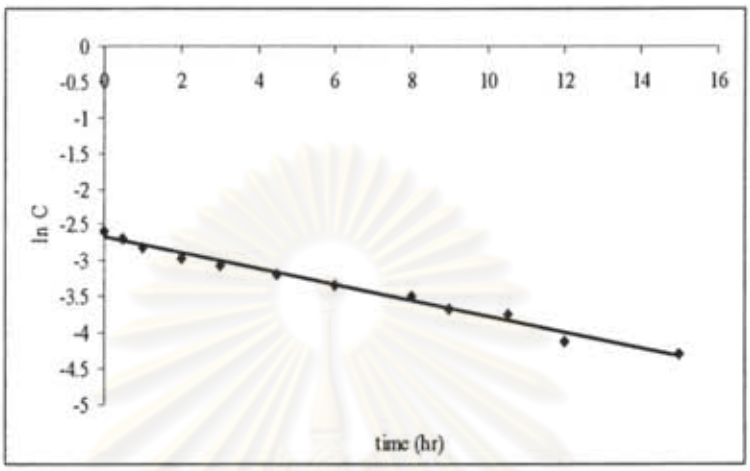


Figure 19 The color change of ciprofloxacin HCl solution when exposed to UV-A

Concentration time profiles of free ciprofloxacin HCl solution and complex solution showed exponential loss of ciprofloxacin HCl over the experimental period. First order plots of both solutions were linear corresponding to first order kinetic (Figure 20, Appendix B; Table B.3.1 and Table B.3.2). Photolysis reaction is typically observed to follow first order kinetic when light intensity is much greater than drug concentration. k_{obs} of free ciprofloxacin HCl and ciprofloxacin HCl inclusion complex degradation were obtained from slope of the plot log remaining concentration versus time to be 0.117 ± 0.008 and 0.099 ± 0.004 hr^{-1}, respectively.

Significant decrease in viscosity and synereis were observed when blank Carbopol ETD 2020 was exposed to UV-A. It is well known that Carbopol polymer is degraded by light. Color of cip-cpx-Carbopol gel turned yellow after light exposure (Figure 21). The decrease of viscosity and syneresis was also observed. However, pH values were remained throughout the experimental period (Table 10). Concentration of ciprofloxacin HCl in cip-cpx-Carbopol gel was exponentially decreased over time. The linear first-order plot indicated that loss of ciprofloxacin HCl in the preparation followed first-order kinetic with k_{obs} of 0.09 ± 0.02 hr^{-1} (Figure 22, Appendix B; Table B.3.6).

a)



b)

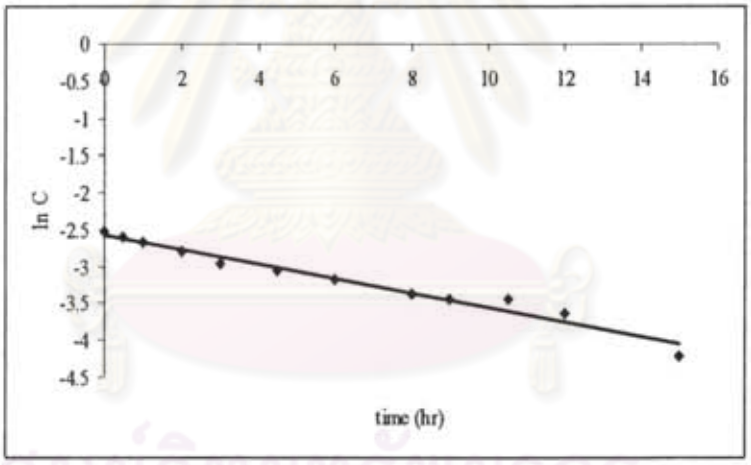


Figure 20 The first order plot of free ciprofloxacin HCl solution (a) and ciprofloxacin inclusion complex solution (b) at 30 °C under UV-A

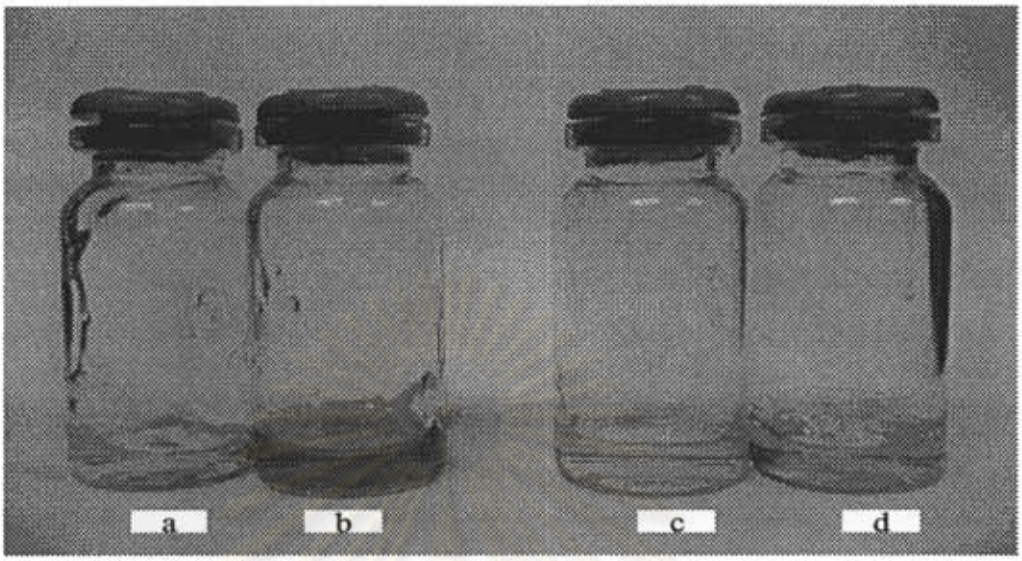


Figure 21 Appearance of preparations before and after UV-A exposure. Cip-cpx-Carbopol gel (a and b), cip-cpx-Poloxamer gel (c and d)

Table 10 Appearance, viscosity and pH values of blank Poloxamer 407 gel, blank Carbopol ETD 2020 gel, cip-cpx-Poloxamer gel and cip-cpx-Carbopol gel before and after UV-A exposure (mean ± SD, n=3)

Formulation	Appearance		Viscosity (cP)		pH		k_{obs} (hr^{-1})
	t=0 hr	t=21 hr	t=0 hr	t=21 hr	t=0 hr	t=21 hr	
I	clear	clear	2,345 ± 74	2,314 ± 81	7.46 ± 0.03	7.38 ± 0.01	NA
II	clear	clear	1,845 ± 148	NA	7.42 ± 0.01	7.48 ± 0.02	NA
III	clear	yellow	2,343 ± 71	2,187 ± 95	7.48 ± 0.02	7.38 ± 0.04	0.07 ± 0.01
IV	clear	yellow	1,997 ± 84	NA	7.44 ± 0.06	7.42 ± 0.06	0.09 ± 0.02

I = blank Poloxamer 407 gel, II = blank Carbopol ETD 2020 gel, III = cip-cpx-Poloxamer gel and IV= cip-cpx-Carbopol gel

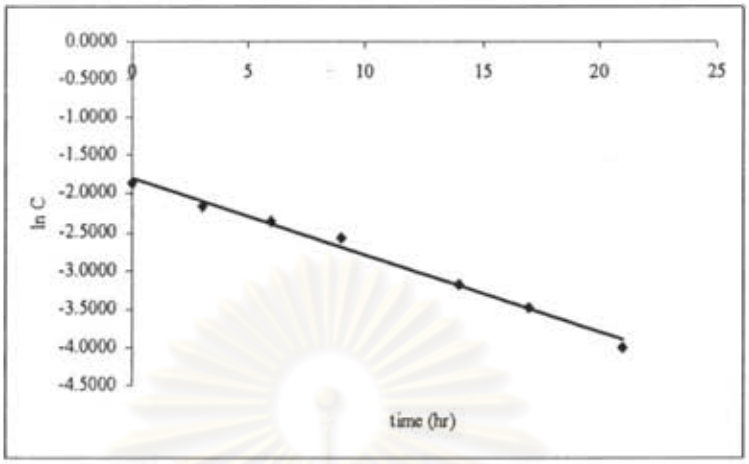


Figure 22 The first order plot of ciprofloxacin degradation in cip-cpx-Carbopol gels after UV-A exposure at 30 °C (n=3)

Poloxamer 407 blank gel showed good physical stability under UV-A light. Appearance, pH values and viscosity of gel were not changed after light exposure for 21 hrs. Cip-cpx-Poloxamer gel turned off-yellow when it was exposed to UV-light (Figure 21). The viscosity was slightly decreased from 2,343 ± 71.73 to 2,187.36 ± 95.96 cP as light exposure time was increased. pH values were remained throughout the studied period (Table 10). Concentration time profiles showed loss of ciprofloxacin HCl in the preparation. The linear first order plot indicated that loss of ciprofloxacin HCl in the preparation followed first order kinetic with k_{obs} of 0.07 ± 0.01 hr^{-1} (Figure 23, Appendix B; Table B.3.12).

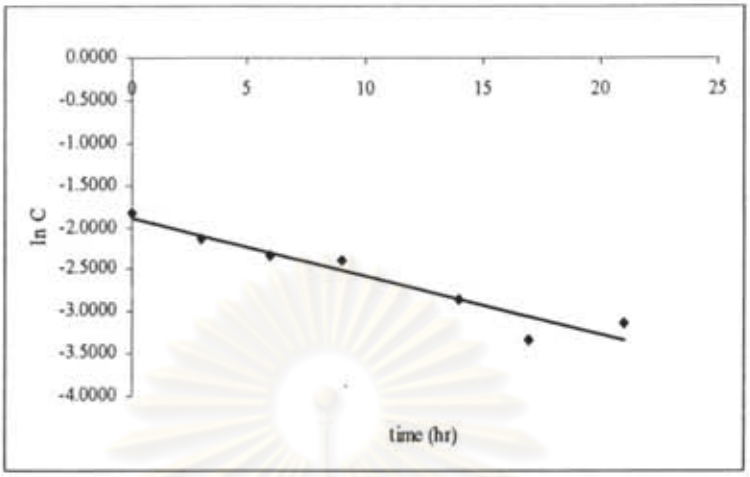


Figure 23 The first order plot of ciprofloxacin degradation in cip-cpx-Poloxmer gels after UV-A exposure at 30 °C (n=3)

In the dark, both preparations showed physical and chemical stabilities. No significant loss of ciprofloxacin was observed over the studied period. Moreover, there were no change in pH value and viscosity. This was an evidence that the preparations should be kept in a container protected from light.

ANOVA analysis was employed to determine whether ciprofloxacin photostability was influenced by HP-ß-CD. The P-value indicated that the k_{obs} of free ciprofloxacin solution, complex solution, cip-cpx-Carbopol gel and cip-cpx-Poloxamer gel are significantly different (ANOVA; $p< 0.05$). Tukey post hoc analysis showed that the rate constant of free drug solution and cip-cpx-Poloxamer gel is significantly different. The k_{obs} of free drug solution was greater than the k_{obs} of cip-cpx-Poloxamer gels. The k_{obs} of free ciprofloxacin HCl was surprisingly comparable to the k_{obs} of ciprofloxacin HCl inclusion complex. CDs complex formation is shown to promote drug photostability (Glass et al., 2001, Tonnesen, Masson and Loftsson, 2002, Scalia et al., 2002, Pomponio, 2004 and Voulgari, 2007). The 3D structure of ciprofloxacin inclusion complex obtained by NMR showed that the photolabile part of the molecule; i.e. the piperazine ring; was not protected inside the HP-ß-CD's cavity (Figure 24; Jianbin, et al.,

2004). Moreover, the $K_{1:1}$ value also implied that most of ciprofloxacin molecules were presented as free drug. Thus, the complex could not totally protect ciprofloxacin from photolysis.

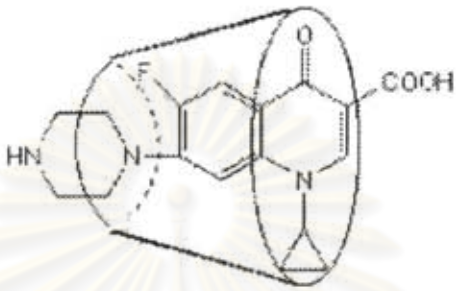


Figure 24 3D structure of ciprofloxacin HP-ß-CD inclusion complex (Jianbin et al., 2004)

F. Stability studies of ciprofloxacin HCl-HP-ß-CD gels

Concentration of ciprofloxacin HCl inclusion complex solution kept in the dark at 40 ºC, 75 % RH was not altered over the 90 days (Figure 25). Moreover, no degradation product peak was shown in chromatograms. pH values and color changes were not observed. These findings implied that ciprofloxacin inclusion complex was stable in the dark at 40 ºC, 75 % RH for at least 90 days.

Color of blank Carbopol ETD 2020 gels at 40 ºC, 75% RH was not changed over 90 days. However, syneresis was observed on day 60 and worsens over time (Table 11). Concentration of ciprofloxacin HCl in the cip-cpx-Carbopol gel at 40 ºC, 75% RH was decreased (Figure 26, Appendix B; Table B.4.8 and Table B.4.10). However, no any other peak was observed except ciprofloxacin HCl peak probably due to the dilution effect during sample preparation. In addition, syneresis was also observed. At ambient temperature, cip-cpx-Carbopol gel also showed instabilities. Concentration time profile of ciprofloxacin HCl in the Carbopol gel showed gradually lost of ciprofloxacin HCl (Figure 26). Linear regression with two-sided 95% confidence interval was employed to

determine preparation shelf life (ICH Q1AR2). The product shelf-life could not be estimated due to the physical instability of the product.

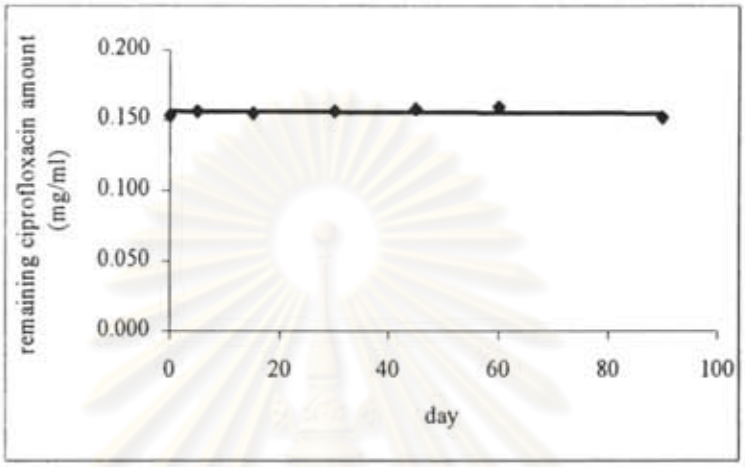


Figure 25 Concentration time profile of ciprofloxacin complex solution in PBS buffer pH 7.4 at 40 ºC, 75% RH in the dark

Table 11 Appearance, viscosity and pH values of, blank Poloxamer 407 gel, Carbopol ETD 2020 blank gel, cip-cpx-Poloxamer gel and cip-cpx-Carbopol gel at 40 ºC, 75% RH at day 0 and day 90 (mean ± SD, n=3)

	appearance		pH		viscosity (cP)	
formulation	day 0	day 90	day 0	day 90	day 0	day 90
I	clear gel	clear gel	NA	NA	2343.83 ± 75.59	2227.50 ± 123.71
II	clear gel	syneresis	NA	NA	2115.43 ± 100.91	NA
III	clear gel	clear gel	7.47 ± 0.07	7.41 ± 0.05	2252.20 ± 91.55	2259 ± 72.52
IV	clear gel	syneresis	7.49 ± 0.03	7.40 ± 0.02	2216.36 ± 105.79	NA

I = blank Poloxamer 407 gel, II = blank Carbopol ETD 2020 gel, III = cip-cpx-Poloxamer gel andIV= cip-cpx-Carbopol gel

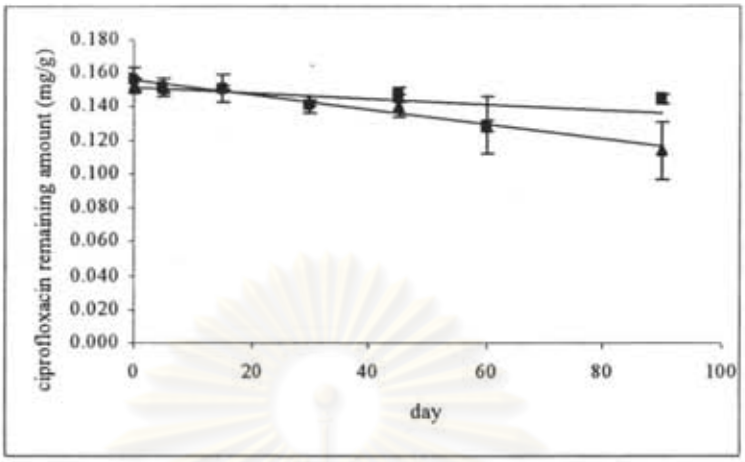


Figure 26 Concentration time profiles of ciprofloxacin in cip-cpx-Carbopol gel at ambient temperature (■) and at 40 °C (▲) in the dark

The viscosity, color and clarity of blank Poloxamer 407 gels at 40 °C, 75% RH did not change over 90 days (Table 11). Blank Poloxamer 407 gel was physically stable throughout the experiment period. Poloxamer polymer is known to be a stable material with thermoreversible properties. Therefore, Poloxamer 407 gel showed good physical stability. For cip-cpx-Poloxamer gels, change in color, clarity, pH values and viscosity were not observed after it was stressed at 40 °C, 75% RH for 90 days (Table 11). Concentration of ciprofloxacin HCl in the preparation was decreased over time (Figure 27, Appendix B; Table B.4.3 and Table B.4.5). Formation of degradation product was not observed in the chromatograms probably due to dilution effect during sample preparation. At ambient temperature, the cip-cpx-Poloxamer gel was physically and chemically stable. Appearance, color, pH values and viscosity of the preparation was not changed over the study period (Table 12). Concentration time profile of ciprofloxacin showed a linear profile with a slope close to zero (Figure 27). The result indicated shelf-life of the preparation was more than 3 months at ambient temperature.

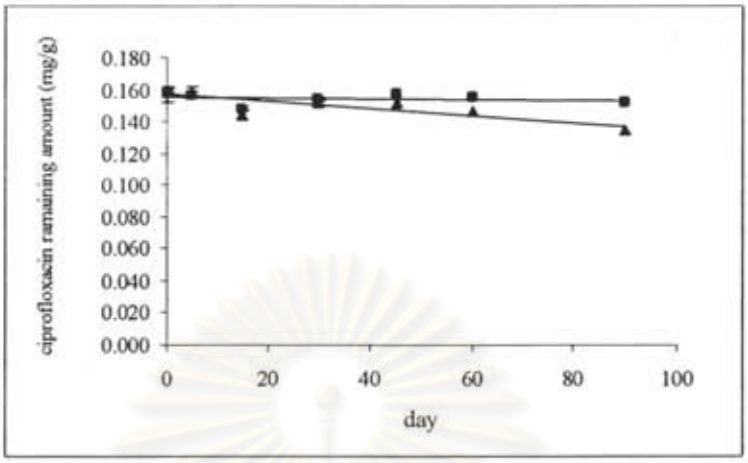


Figure 27 Concentration time profiles of ciprofloxacin in cip-cpx-Poloxamer 407 at ambient temperature (■) and at 40 ºC (▲) in the dark (n=3)

Table 12 Appearance, viscosity and pH values of, cip-cpx-Poloxamer 407 gel and cip-cpx-Carbopol ETD 2020 at ambient temperature at day 0 and day 90 (mean ± SD, n=3)

	appearance		pH		viscosity (cP)	
Formulation	day 0	day 90	day 0	day 90	day 0	day 90
III	clear gel	clear gel	7.42 ± 0.05	7.48 ± 0.04	2326.32 ± 81.73	2280.63 ± 98.07
IV	clear gel	syneresis	7.52 ± 0.05	7.54 ± 0.08	2141.31 ± 54.48	NA

III = cip-cpx-Poloxamer gel and IV= cip-cpx-Carbopol gel

Loss of ciprofloxacin HCl in the cip-cpx-Carbopol and cip-cpx-Poloxamer gels at 40 ºC, 75%RH were greater than 5% over 90 days. The observed chemical instabilities suggested that the storage conditions and the excursion conditions of these preparations should be controlled at a temperature lower than 30-40 ºC. Although cip-cpx-Poloxamer gel showed both physical and chemical stabilities at ambient temperature over 90 days, the product should be kept away form heat which was consistent with the recommended conditions suggested in USP 31; i.e. 25 and 15-30 ºC, for storage and excursion conditions, respectively.

G. *In vitro* release study of ciprofloxacin from gel

Diffusion of free ciprofloxacin HCl through the Spectrapor® MWCO 1,000 was investigated. The profile illustrated that %cumulative amount increased as time increased and reached about 41.73 ± 3.10% after 24 hr (Figure 28, Appendix B; Table B.5.3). The low observed %cumulative amount implied that the membrane with MWCO 1,000 might not be a proper membrane because free ciprofloxacin HCl could not freely transverse across the membrane. Changing the membrane with higher values of MWCO; i.e. 3,000, would result in diffusion of complex, free HP-ß-CD and free ciprofloxacin HCl through the membrane. In other words, presence of free ciprofloxacin HCl and release of free ciprofloxacin HCl from complex could not be studied by the use of membrane with higher MWCO number.

Release profile of ciprofloxacin HCl from complex solution depicted that %cumulative amount of free ciprofloxacin HCl gradually increased with time (Figure 28, Appendix B; Table B.5.2). %cumulative release reached 39.76 ± 5.31% after 24 hr. During the first to twentieth hr, the profile of complex solution showed that the amount of free drug in the receiver compartment was lower than that of free drug solution. Complexation is a well known technique in order to control drug release. This observation was consistent with the fact that release rate of drug from complex is lower than that of preparation containing free drug.

Release profile of ciprofloxacin HCl from cip-cpx-Poloxamer 407 gel showed that % cumulative amount of ciprofloxacin HCl was the lowest among the three samples (Figure 28, Appendix B; Table B.5.1). %Cumulative release reached 32.63 ± 0.44% at 24 hr. The reasons for the lowest release profile were two folds. Firstly, free ciprofloxacin HCl had to be release from the complex. Secondly, the free drug diffusion rate was retarded by viscosity of gel preparation. No lag time was observed in any profile implying that free ciprofloxacin HCl was present in equilibrium with ciprofloxacin HCl complex in the system at the initial time.

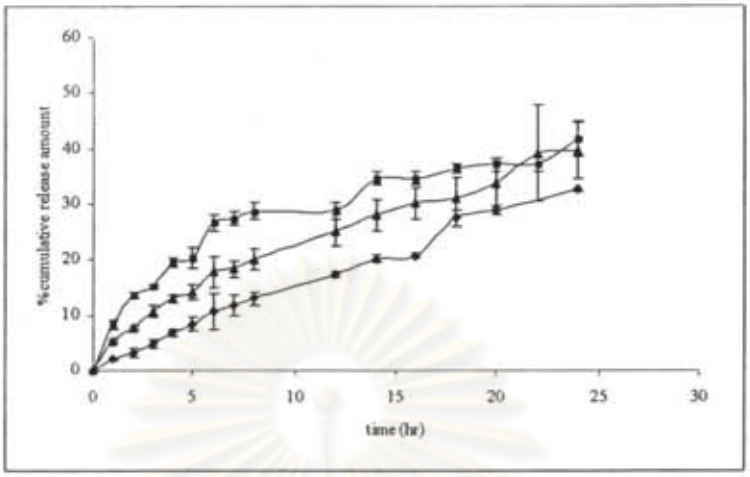


Figure 28 The %cumulative amount of ciprofloxacin HCl passing through a membrane MWCO 1,000 to the receiver compartment from cip-cpx-Poloxamer 407 gel (♦), from complex solution (▲) and from free ciprofloxacin HCl solution (■) versus time (n=3)

Cumulative amount of drug release was plotted against square root of time. Higuchi equation was employed to estimate release rate constant (Figure 29).

$$Q_t = k_H t^{1/2} \quad \text{Equation 2}$$

where:

Q_t is the amount of drug release at time t

k_H is the release rate constants of Higuchi

t is the release time

The release rates were determined from slopes of the plots to be 3.29 and 2.82 mg cm^{-2} $hr^{-½}$ for complex solution and gel preparation, respectively.

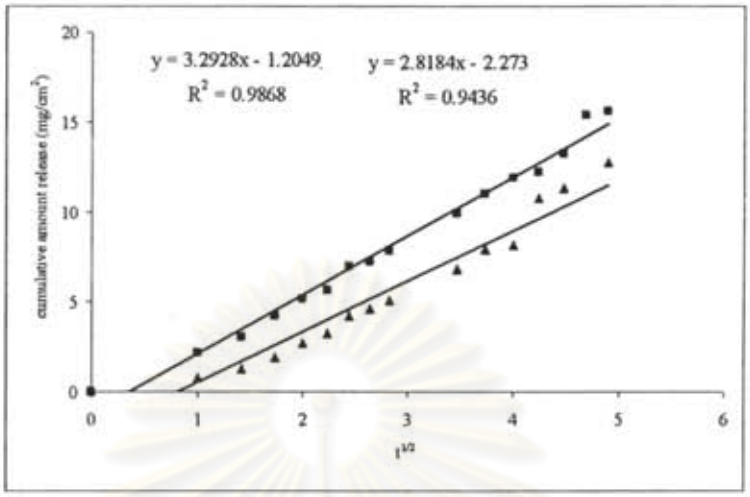


Figure 29 Higuchi plot of ciprofloxacin release from cip-cpx-Poloxamer 407 gel (▲) and from complex solution (■)

H. *In vitro* antimicrobial activity of ciprofloxacin complex gel against *Pseudomonas aeruginosa*

The antimicrobial activity test of ciprofloxacin-complex-Polxamer 407 and ciprofloxacin-complex-Carbopol ETD 2020 preparations were performed. One standard and 29 clinical isolated strains of *Pseudomonas aeruginosa* were included in this study. The diameter of clear zone was measured to indicate antibacterial activity.

The result of the study was shown in Table 13. No inhibition zone was observed when tested with blank gel bases (Figure 30). The inhibition zones were observed when tested with ciprofloxacin HCl inclusion complex against all ciprofloxacin-sensitive strains. While no zone or very small zone size was observed when tested with Dermazin™ cream.

The diameter of inhibition zones were observed when tested with cip-cpx-Poloxamer and cip-cpx-Carbopol. It was shown that both gels had antibacterial activities against susceptible *Pseudomonas aeruginosa*. However, among the 29 tested strains,

strains were determined to be ciprofloxacin resistant strains. It was shown that ciprofloxacin gels as well as Dermazin™ could not inhibit such strains.

Dermazin™ cream is 1% silver sulfadiazine. Silver sulfadiazine has an antimicrobial activity toward both gram positive and negative microorganisms including *Pseudomonas aeruginosa* and is considered as a gold standard treatment in topical burn wound (Atiyeh et al., 2007; Waasbergen et al., 2007). However, silver sulfadiazine was reported to delay the wound-healing process and to posses the serious cytotoxic activity on various cells (Atiyeh et al., 2007).

To evaluate the antimicrobial activity of the two preparations, the diameters of clear zone were measured and recorded as shown in Table 13. Cip-cpx-Poloxamer gel showed larger diameter inhibition zone sizes zone than the cip-cpx-Carbopol gel. This can be implied that the ciprofloxacin HCl inclusion complex Poloxamer 407 preparation had better efficacy against *Pseudomonas aeruginosa*. However, statistic was not included to estimate the difference due to the limitation of study.

Comparing the inhibition zone sizes, ciprofloxacin HCl inclusion complex gels showed much better result than Dermazin™ cream. It is widely known that different vehicle may affect release of drug. Creams has slower release rate than gels (Csoka et al., 2005). Thus, Dermazin™ cream showed no inhibition zone or very small zone. However, it is less likely to find ciprofloxacin creams. This may be the limitation of this study because it is impossible to find others gels used in topical burn wound treatment.

In short, ciprofloxacin inclusion complex Poloxamer 407 gels seem to be an appropriate in the treatment of burn wound. More intensive clinical studies are needed to be evaluated before applying to human.

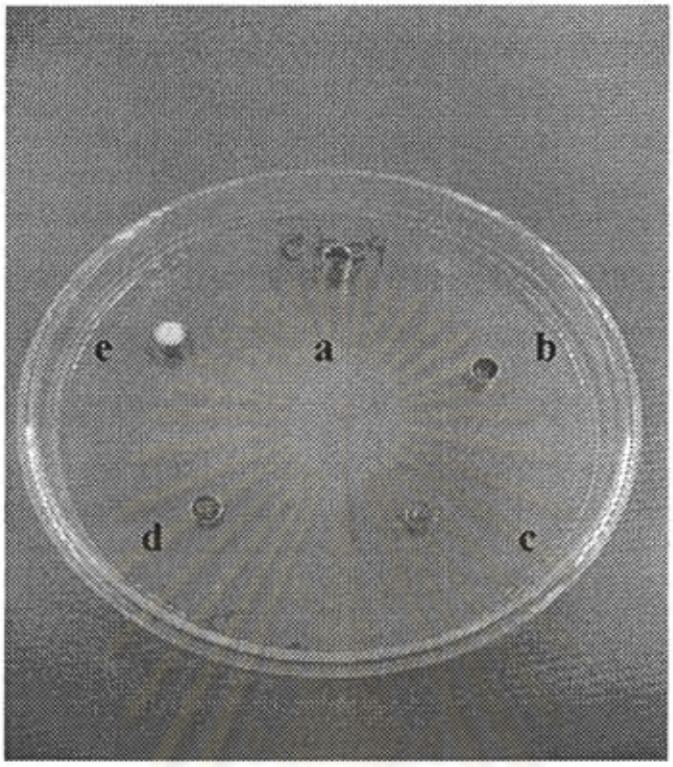


Figure 30 *In vitro* antimicrobial activity of cip-cpx- poloxamer gel (a), blank Poloxamer gels (b), cip-cpx-Carbopol gel (c), blank Carbopol ETD 2020 (d) and Dermazin™ cream (e)

Table 13 Inhibition zone size observed when tested against *Pseudomonas aeruginosa*

Strain	Diameter of inhibition zone (mm)				
	blank Poloxamer gel	**blank Carbopol ETD 2020**	**ciprofloxacin-complex-Poloxamer 407 gel**	**ciprofloxacin-complex-Carbopol ETD 2020 gel**	**Dermazin™**
ATCC	nz	nz	30.37	25.46	9.45
Ps 2	nz	nz	31.66	30.96	13.11
Ps 5	nz	nz	33.75	27.21	9.15
Ps29	nz	nz	24.04	32.09	12.50
Psd 2	nz	nz	33.45	26.27	12.65
Psd 3	nz	nz	31.91	26.52	11.67
Psd 4	nz	nz	33.58	30.39	11.85
Psd 5	nz	nz	34.50	31.30	12.29
Psd 13	nz	nz	30.08	29.53	nz
Psd 15	nz	nz	36.63	35.58	12.97
Psd 16	nz	nz	29.25	26.82	nz
Psd 15	nz	nz	36.63	35.58	12.97
Psd 16	nz	nz	29.25	26.82	nz
Psd 15	nz	nz	36.63	35.58	12.97
Psd 16	nz	nz	29.25	26.82	nz
Psd 23	nz	nz	30.46	25.69	9.88
Psd 24	nz	nz	21.08	23.20	nz
Psd 25	nz	nz	34.93	30.98	13.32
Psd 26	nz	nz	28.82	26.65	10.19

Table 13 Inhibition zone size observed when tested against *Pseudomonas aeruginosa* (cont.)

Strain	Diameter of inhibition zone (mm)				
	blank Poloxamer gel	**blank Carbopol ETD 2020**	**ciprofloxacin-complex-Poloxamer 407 gel**	**ciprofloxacin-complex-Carbopol ETD 2020 gel**	**Dermazin™**
Psd 27	nz	nz	24.90	24.85	11.68
Psd 1*	nz	nz	nz	nz	nz
Psd 7*	nz	nz	nz	nz	nz
Psd 8*	nz	nz	nz	nz	nz
Psd 10*	nz	nz	nz	nz	nz
Psd 12*	nz	nz	nz	nz	nz
Psd 14*	nz	nz	nz	nz	nz
Psd 17*	nz	nz	nz	nz	nz
Psd 28*	nz	nz	nz	nz	nz

* ciprofloxacin-resistant strains, (nz)= no zone

CHAPTER V

CONCLUSIONS

This research aimed to develop HP-ß-CD inclusion complex gels as a secondary treatment for patients with topical *Pseudomonas* infection. The inclusion complex was expected to improve photostability of ciprofloxacin HCl and release adequate amount of free drug in order to inhibit the pathogen.

In PBS 7.4, A_L type of phase solubility study was evidences of complex formation. The estimated $K_{1:1}$ of $1.01\times10^{-5}\,M^{-1}$ and $9.64\times10^{-6}\,M^{-1}$, for HCl and lactate salt, respectively, indicated that majority of ciprofloxacin was present as free drug. Furthermore, phase solubility studies of both salts were superimposable; thus, salt type of ciprofloxacin showed no effect on complex formation. The observed fluorescence enhancement of ciprofloxacin HCl in the presence of HP-ß-CD confirmed that the inclusion complex was formed.

Ciprofloxacin HCl inclusion complex gels were successfully prepared using 18% w/w Poloxamer 407 and 1% w/w carbopol as gel bases. Under UV A light with intensity 8×10^{-4} watt/cm^2, both preparations showed chemical instability with 88% and 75% degradation for cip-cpx-Carbopol and cip-cpx-Poloxamer gel, respectively. Furthermore, the observed syneresis indicated that the cip-cpx-Carbopol gel was physically unstable.

In the dark at 40 °C, about 16% and 26% of ciprofloxacin HCl, in cip-cpx-Poloxamer and cip-cpx-Carbopol gels, respectively, gradually degraded. Moreover, viscosity of the cip-cpx-Carbopol formulation was decreased due to syneresis. In dark at ambient temperature, ciprofloxacin HCl concentration and viscosity of cip-cpx-Poloxamer preparation was not significantly changed over 3 months.

Ciprofloxacin HCl could be released from its inclusion complex solutions and from the cip-cpx-Poloxamer preparation. The release profiles were in agreement with Higuchi model. The release rate constants were 3.19 and 2.82 mg cm^{-2} $hr^{-½}$ for inclusion complex solution and Poloxamer 407 gel, respectively.

Ciprofloxacin HCl inclusion complex gels were able to inhibit ciprofloxacin susceptible strains of *Pseudomonas aeruginosa*. This was confirmed with the result from the release study that ciprofloxacin HCl could be released from complex. In addition, ciprofloxacin HCl inclusion complex Poloxamer 407 gel showed the better antimicrobial activity against *Pseudomonas aeruginosa* than Carbopol ETD 2020 gel base.

In conclusion, inclusion complex between ciprofloxacin HCl and HP-ß-CD could slightly enhance its solubility and photostability. Moreover, needle crystal of ciprofloxacin was not found in the preparation pH 7.4. Polxamer 407 possessed the appropriate properties which were suitable for further product development. Pre-clinical and clinical studies are further required to evaluate safety and efficacy of the preparation.

REFERENCES

Asker, A.F. and Habib, M.J. 1991. Effect of certain additives on photodegradation of tetracycline hydrochloride solutions. Journal of Parenteral Science and Technology 45: 113-115.

Atiyeh, B.S., Costagliola, M., Hayek, S.N. and Dibo, S.A. 2007. Effect of silver on burn wound infection control and healing: review of the literature. Burns 33: 139-148.

BASF. The Chemical Company. 2007. Safety data sheet Pluronic F127 NF Prill Poloxamer 407[Adobe Acrobat].BASF. The Chemical Company. Available from http://worldaccount.basf.com/wa/NAFTA~en_US/Catalog/ChemicalsNAFTA/info/BASF/PRD/30085239 [2008, August 16].

Bekers, O., Beijnen, J.H., Otatiri, M., Bult, A. and Underberg, W.J.M. 1999. Effect of cyclodextrins on the chemical stability of mitomycins in alkaline solution. Journal of Pharmaceutical and Biomedical Analysis 9: 1555-1560.

Brewster, M. E. and Loftsson, T. 2007. Cyclodextrins as pharmaceutical solubilizers. Advanced Drug Delivery Reviews 59: 645-666.

Brewster, M.E., Loftsson, T., Estes, K.S., Lin, J., Fridriksdottir, H. and Bodor, N. 1992. Effect of various cyclodextrins on solution stability and dissolution rate of doxorubicin hydrochloride. International Journal of Pharmaceutics 79: 289-299.

Bundgaard, H., Nargaard, T. and Nielson, N.M. 1988. Photodegradation of hydrolysis of furosemide and furosemide ester in aqueous solution. International Journal of Pharmaceutics 42: 217-224.

Chinnian, D. and Asker, A.F. 1996. Photostability profiles of minoxidil solutions. Journal of Pharmaceutical Science and Technology 50: 94-98.

Cox, N., Meakin B.J. and Davies, D.J. 1976. The influence of some antioxidants on the photochemical decomposition kinetics of promethazine hydrochloride. Journal of Pharmacology 28: 45.

Csoka, I., Csanyi, E., Zapantis, G., Nagy, E., Feher-Kiss, A., Horvath, G., Blazso, G. and Eros, I. 2005. In vitro and in vivo percutaneous absorption of topical dosage forms: case studies. International Journal of Pharmaceutics 291: 11-19.

Davis, R., Markham, A. and Balfour, J.A. 1996. Ciprofloxacin: an updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs: 1019-1074.

Fernandesa, C.M., Vieira, M.T. and Veiga, F.J.B. 2002. Physicochemical characterization and in vitro dissolution behavior of nicardipine–cyclodextrins inclusion compounds. European Journal of Pharmaceutical Sciences 75: 88-79.

Firestone, B.A., Dickason, M.A. and Tran, T. 1998. Solubility characteristics of three fluoroquinolone ophthalmic solutions in an in vitro tear model. International Journal of Pharmaceutics 164: 119-128.

Funasaki N., Kawaguchi R., Hada, S. and Neya, S. 1999. Ultraviolet spectroscopic estimation of microenvironments and bitter tastes of oxyphenonium bromide in cyclodextrin solutions. Journal of Pharmaceutical Sciences 88: 759-762.

Ghaffari, A., Miller, C.C., McMullin, B. and Ghahary, A. 2006. Potential application of gaseous nitric oxide as a topical antimicrobial agent. Nitric Oxide 14: 21-29.

Giordano, F., Novak, C. and Moyano, J.R. 2001. Thermal analysis of cyclodextrins and their inclusion compounds. Thermochimica Acta 380: 123-151.

Glass, B.D., Brown, M.E., Daya, S., Worthington, M.S., Drummond, P., Antunes, E., Lebete, M., Anoopkumar-Dukie, S. and Maharaj, D. 2001. Influence of cyclodextrins on the photostability of selected drug molecules in solution and the solid state. International Journal of Photoenergy 3: 205-211.

Habib, M.J. and Asker, A.F. 1989. Photostabilization of doxorubicin hydrochloride with radioprotective and photoprotective agents: Potential mechanism for enhancing chemotherapy during radiotherapy. Journal of Parenteral Science and Technology 43: 259-261.

Hardman J.G., Limbird, L.E. and Gilman, A.G. 2001. The Pharmaceutical Therapeutics. 10th ed. New York: Mc-GrawHill.

Higuchi, T. and Connors, K. 1965. Phase Solubility Techniques. Advanced Analytical Chemistry and Instrumentation 4: 117-212.

International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Photostability testing of new drug substances or drug products, Q1B [Adobe Acrobat]. [ICH (producer)] Available from: http://www.ich.org/LOB/media/MEDIA412.pdf [2008, August 16].

International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Stability testing for new dosage forms, Q1AR2 [Adobe Acrobat]. [ICH (producer)] Available from: http://www.ich.org/LOB/media/MEDIA419.pdf [2008, August 16].

International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of analytical procedures: Text and Methodology, Q2(R1) [Adobe Acrobat]. [ICH (producer)] Available from: http://www.ich.org/LOB/media/MEDIA417.pdf [2008, August 16].

Jianbin, C., Liang, C., Hao, X. and Dongpin, M. 2002. Preparation and study on the solid inclusion complex of ciprofloxacin with ß-cyclodextrin. Spectrochemica Acta Part A 58: 2809-2815.

Jianbin, C., Dongpin, M., Jiangsong L., Hao, X. and Shuping, H. 2004. Preparation and study on the novel solid inclusion complex of ciprofloxacin with HP-ß-cyclodextrin. Spectrochemica Acta Part A 60: 729-734.

Kowarski, C.R. and Ghandi, H.I. 1974. Complex formation between menadione and cetylethylmorpholinium ethosulfate: Effect on UV photodegradation of menadione. Journal of Pharmaceutical Sciences 64: 696-698.

Lesne-Hulin, A., Bourget, P., Ravat, F., Goudin, C. and Latarjet, J. 1999. Clinical pharmacokinetics of ciprofloxacin in patients with major burns. European Journal of Clinical Pharmacology 55: 515-519.

Li, S. and Purdy, W.C. 1992. Cyclodextrins and their applications in analytical chemistry. Chemical Reviews 92: 1457-1470.

Liu, L. and Zhu, S. 2006. Preparation and characterization of inclusion complexes of prazosin hydrochloride with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. Journal of Pharmaceutical and Biomedical Analysis 40: 122-127.

Loftsson, T., Frioriksdottir, H., Thorisdottir, S. and Stefansson, E. 1994. The effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations. International Journal of Pharmaceutics. 104: 181-184.

Loftsson, T. and Masson, M. 2001. Cyclodextrins in topical drug formulations: theory and practice. International Journal Pharmaceutics 225: 15-30.

Loftsson, T., Hreinsottir, D. and Masson, M. 2005. Evaluation of cyclodextrin solubilization of drugs. International Journal of Pharmaceutics 302: 18-28.

Loftsson, T. and Duchene, D. 2007. Cyclodextrins and their pharmaceutical applications. International Journal of Pharmaceutics 329: 1-11.

Meras, I.D., Espinosa-Mansilla, A. and Rodriguez, D.A. 2007. Complexation study of Cinalukast and Montelukast with cyclodextrins. Journal of Pharmaceutical and Biomedical Analysis 43: 1025-1032.

Nijhawan, R. and Agarwal, S.P. 2003. Development of an ophthalmic formulation containing Ciprofloxacin-Hydroxypropyl-b-Cyclodextrin complex. Bollettino Chimico Farmaceutico 142: 214-219.

Noveon™. 1996. Carbopol™ ETD Polymer Toxicology studies[Adobe Acrobat]. Noveon™. Available from http://www.personalcare.noveon.com/Toxicology/TOX-003.pdf [2008, August 16].

Pandit, N.K. and Wang, D. 1998. Salt effects on the diffusion and release rate of propranolol from Poloxamer 497 gels. International Journal of Pharmaceutics (167): 183-189.

Piechocki, J. T. and Thoma, K. 2007. Pharmaceutical Photostability and Stabilization Technology. New York: Informa, Healthcare.

Pomponio, R., Gotti, R., Fiori, R., Cavrini, V., Mura, P., Cirri, M. and Maestrelli, F. 2004 Photostability studies on nicardipine-cyclodextrin complexes by capillary electrophpresis. Journal of Pharmaceutical and Biomedical Analysis 35: 267-265.

Rajabi, O., Tayyarib, F., Salari, R. and Tayyaric, S.F. 2008. Study of interaction of spironolactone with hydroxypropyl-β-cyclodextrin in aqueous solution and in solid state. Journal of Molecular Structure 878: 78-83.

Ross, D. L. and Riley, C. M. 1990. Aqueous solubilities of some variously substituted quinolone antimicrobials. International Journal of Pharmaceutics 63: 237-250.

Rowe, R.C., Sheskey, P.J. and Weller, P.J. 2003. Handbook of Pharmaceutical Excipients 4th ed. London: Pharmaceutical Press.

Scalia, S., Casolari, A., Iaconinoto, A. and Simeoni, S. 2002. Comparative studies of the influence of cyclodextrins on the stability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. Journal of Pharmaceutical and Biomedical Analysis 30: 1181-1189.

Suh, H. and Jun, H.W. 1996. Physicochemical and release studies of naproxen Poloxamer 407. International Journal of Pharmaceutics 129: 13-20.

Swarbrick, J. and Boylan, J.C. 2000. Encyclopedia of Pharmaceutical Technology. Volume 19 Supplement 2. New York: Marcel Dekker, Inc.

Tas, C., Ozkan, Y., Savaser, A. and Baykara T. 2003. In vitro release studies of chlorpheniramine maleate from gels prepared by different cellulose derivatives. II Farmaco 58: 605-611.

The United States Pharmacopoeia Convention, Inc. 2008. The United States Pharmacopeia 31 and the National Formulary 26: USP 31/NF 26. Philadelphia: National Publishing.

Tiefenbacher, M., Haen, E., Przybilla, B. and Kurz, H. 1994. Photodegradation of some quinolones used as antimicrobial therapeutics. Journal of Pharmaceutical Sciences 83: 463-467.

Todar, K. 2004. Todar's Online Textbook of Bacteriology [Online]. Department of Bacteriology: University of Wisconsin-Madison. Available from: http://textbookofbacteriology.net/pseudomonas.html[2008, July 18]

Tonnesen, H.H., Masson, M. and Loftsson, T. 2002. Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. International Journal of Pharmaceutics 244: 127-135.

Tomiainen, K., Askolin, C. and Mattinen, J. 1997. Isolation and structure elucidation of an intermediate in the photodegradation of ciprofloxacin. Journal of Pharmaceutical and Biomedical analysis 16: 439-445.

Tomiainen, K., Tammilehto, S. and Ulvi, V. 1996. The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin. International Journal of Pharmaceutics 132: 53-61.

Tomiainen, Mattinen, J., K., Askolin, C. and Tammilehto, S. 1997. Structure elucidation of a photodegradation product of ciprofloxacin. Journal of Pharmaceutical and Biomedical Analysis 15: 887-894.

Vargus, F. and Canudus, N. 1993. Photolability and photohemolytic studies of fibrates. Pharmazie 12: 900-904.

Voulgari, A., Benaki, D., Michaleas, S. and Antoniadou-Vyza, E. 2007. The effect of ß-cyclodextrin on tenoxicam photostability, studied by a new liquid chromatography method; the dependence on drug dimerisation. Journal of Inclusion Phenomenon and Macrocyclic Chemistry 57: 141-146.

Waasbergen, L.G., Fajdetic, I., Fianchini, M. and Rasika Dias, H.V. 2007. Antimicrobial properties of highly fluorinated tris(pyrazolyl)borates. Journal of Inorganic Biochemistry 101: 1180-1183.

Wang, J. and Cai, Z. 2008. Investigation of inclusion complex of miconazole nitrate with β-cyclodextrin. Carbohydrate Polymers 72: 255-260.

Wood, M.J., Irwin, W.J. and Scott, D.K. 1990. Photodegradation of doxorubicin and epirubicin measured by high-performance liquid chromatography. Journal of Clinical Pharmacology Therapy 15: 290-300.

Yuan, C., Jin, Z., Xu, X., Zhuang, H. and Shen, W. 2008. Preparation and stability of the inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin. Food Chemistry 109: 264-268.

Yusuff, N. and York, P. 1991. Spironolactone-cyclodextrin complexes: phase solubility and ultrafiltration studies. International Journal of Pharmaceutics 30: 9-15.

Zhang, L., Parson, D.L., Navarre, C. and Kompella, U.B. 2002. Development and in-vitro evaluation of sustained release Poloxamer 407 (P407) gel formulations of ceftiofur. Journal of Controlled Release. 85: 73-81

APPENDIX

A. System suitability and validation

A.1 The calibration curve of ciprofloxacin HCl in the mobile phase

set 1		set 2		set 3	
Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area
0.042	3196160	0.042	3211298	0.041	3183749
0.025	2002267	0.025	1985128	0.024	1871759
0.017	1339038	0.017	1324995	0.016	1330266
0.013	1023644	0.012	977936	0.012	988639
0.006	518840	0.006	505622	0.006	514087

A.2 The calibration curve of ciprofloxacin lactate in the mobile phase

set 1		set 2		set 3	
Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area
0.043	3459931	0.042	3331112	0.044	3488581
0.026	2022671	0.025	2060333	0.026	2157130
0.017	1326911	0.017	1375687	0.018	1456312
0.013	1047638	0.013	1054738	0.013	1023548
0.006	553478	0.006	541196	0.007	577046

A.3 The calibration curve of ciprofloxacin HCl in the presence of HP-ß-CD and Poloxamer 407 in the mobile phase

set 1		set 2		set 3	
Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area
0.042	3271859	0.043	3292936	0.042	3246003
0.025	1997211	0.026	1954691	0.025	1976820
0.017	1287790	0.017	1254856	0.017	1239429
0.012	973157	0.013	971845	0.013	976701
0.006	492234	0.007	500896	0.006	476438

A.4 The calibration curve of ciprofloxacin HCl in the presence of HP-ß-CD and Carbopol ETD 2020 in the mobile phase

set 1		set 2		set 3	
Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area	Conc. (mg/ml)	peak area
0.042	3272026	0.043	3271034	0.043	3272377
0.025	1873551	0.026	1903238	0.026	1910969
0.017	1293681	0.017	1264534	0.017	1259071
0.013	976552	0.013	960298	0.013	988323
0.006	495982	0.006	519291	0.006	523123

B. Experimental data

B.1 Determination of equilibrium time of ciprofloxacin HCl and ciprofloxacin lactate and HP-ß-CD

Table B.1.1 The solubility of ciprofloxacin HCl without HP-ß-CD in PBS 7.4 at 37 ± 2 °C

Time (hr)	Solubility of ciprofloxacin HCl (mg/ml)			Mean	SD
	Set 1	Set 2	Set 3		
0	0	0	0	0.00	0.00
1	0.12	0.12	0.13	0.12	0.01
3	0.12	0.13	0.11	0.12	0.01
6	0.12	0.12	0.12	0.12	0.00
8	0.10	0.11	0.12	0.11	0.01
12	0.13	0.12	0.12	0.12	0.01
16	0.10	0.10	0.15	0.12	0.03
20	0.10	0.13	0.11	0.12	0.01
24	0.11	0.11	0.11	0.11	0.00

Table B.1.2 The solubility of ciprofloxacin HCl in the presence of 0.15 g/ml HP-ß-CD in PBS 7.4 at 37 ± 2 °C

Time (hr)	Solubility of ciprofloxacin HCl (mg/ml)			Mean	SD
	Set 1	Set 2	Set 3		
0	0	0	0	0	0
1	0.15	0.13	0.14	0.14	0.01
3	0.15	0.16	0.18	0.16	0.02
6	0.15	0.14	0.16	0.15	0.01
8	0.14	0.14	0.15	0.14	0.00
12	0.13	0.13	0.13	0.13	0.00
16	0.17	0.12	0.12	0.13	0.03
20	0.11	0.08	0.16	0.12	0.04
24	0.11	0.11	0.14	0.12	0.02

Table B.1.3 The solubility of ciprofloxacin lactate without HP-ß-CD in PBS 7.4 at 37 ± 2 °C

Time (hr)	Solubility of ciprofloxacin lactate (mg/ml)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.00	0.00	0.00	0.00	0.00
1	0.11	0.11	0.11	0.11	0.00
3	0.11	0.11	0.11	0.11	0.00
6	0.11	0.10	0.09	0.10	0.01
8	0.09	0.09	0.09	0.09	0.00
12	0.09	0.10	0.10	0.10	0.01
16	0.11	0.09	0.10	0.11	0.01
20	0.12	0.11	0.11	0.11	0.01
24	0.09	0.12	0.10	0.11	0.02

Table B.1.4 The solubility of ciprofloxacin lactate in the presence of 0.15 g/ml HP-ß-CD in PBS 7.4 at 37 ± 2 °C

Time (hr)	Solubility of ciprofloxacin lactate (mg/ml)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.00	0.00	0.00	0.00	0.00
1	0.12	0.13	0.12	0.12	0.00
3	0.09	0.11	0.11	0.10	0.01
6	0.11	0.11	0.12	0.11	0.01
8	0.12	0.13	0.19	0.15	0.04
12	0.14	0.15	0.14	0.15	0.00
16	0.12	0.13	0.10	0.13	0.05
20	0.16	0.15	0.13	0.13	0.02
24	0.12	0.11	0.11	0.12	0.03

B.2 Phase solubility study

Table B.2.1 The solubility of ciprofloxacin HCl in PBS pH 7.4 at 37 ± 2 °C after 24 hr in the presence of 0-100 mM HP-ß-CD

HP-ß-CD conc. (mM)	Solubility of ciprofloxacin HCl (mM)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.23	0.22	0.23	0.23	0.00
50	0.36	0.34	0.41	0.37	0.04
75	0.43	0.41	0.43	0.42	0.01
100	0.49	0.43	0.43	0.45	0.03

Table B.2.2 The solubility of ciprofloxacin lactate in PBS pH 7.4 at 37 ± 2 °C after 24 hr in the presence of 0-100 mM HP-ß-CD

HP-ß-CD conc. (mM)	Solubility of ciprofloxacin lactate (mM)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.29	0.22	0.27	0.26	0.04
50	0.30	0.34	0.37	0.33	0.03
75	0.39	0.37	0.38	0.38	0.01
100	0.55	0.47	0.59	0.54	0.06

B.3 Photostability of ciprofloxacin HCl-HP-ß-CD gels

Table B.3.1 The remaining amount of free ciprofloxacin HCl solution when exposed to light

Set 1		Set 2		Set 3	
Time (hr)	Conc. (mg/ml)	Time (hr)	Conc. (mg/ml)	Time (hr)	Conc. (mg/ml)
0	0.076	0	0.076	0	0.086
0.5	0.068	0.5	0.068	1	0.078
1	0.060	1	0.060	2	0.062
2	0.051	2	0.052	3	0.054
3	0.047	3	0.046	4.5	0.046
4.5	0.041	4.5	0.038	6	0.037
6	0.035	6	0.025	8	0.031
8	0.030	8	0.023	9	0.026
9	0.026	9	0.021	10.5	0.022
10.5	0.024	10.5	0.021	12.5	0.018
12	0.016	12	0.016	14	0.014
15	0.013	15	0.013		

Table B.3.2 The remaining amount of ciprofloxacin HCl complex solution when exposed to light

Set 1		Set 2		Set 3	
Time (hr)	conc. (mg/ml)	Time (hr)	conc. (mg/ml)	Time (hr)	conc. (mg/ml)
0	0.080	0	0.080	0	0.086
0.5	0.074	0.5	0.072	0.5	0.075
1	0.069	1	0.071	1	0.073
2	0.062	2	0.060	2	0.072
3	0.052	3	0.055	3	0.060
4.5	0.047	4.5	0.048	4.5	0.042
6	0.042	6	0.041	6	0.035
8	0.035	8	0.038	8	0.033
9	0.032	9	0.033	9	0.024
10.5	0.032	10.5	0.030	10.5	0.18
12	0.025	12	0.020		

Table B.3.3 The viscosity of Carbopol ETD 2020 blank gel after exposed to the light

	Viscosity (cP)				
Time (hr)	Set1	Set2	Set3	Mean	SD
0	2013.45	2198.67	2215.02	1845.99	148.47
8	560.32	495.51	505.23	476.31	115.24
21	NA	NA	NA	NA	NA

Table B.3.4 The viscosity of cip-cpx-Carbopol gel in the dark

	Viscosity (cP)				
Time (hr)	Set1	Set2	Set3	Mean	SD
0	2156.84	1986.73	2198.67	2114.08	112.25
21	2023.43	2189.34	1989.25	2067.34	107.03

Table B.3.5 The viscosity of cip-cpx–Carbopol gel after exposed to light

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	1963.78	2093.37	1934.81	1997.32	84.43
8	483.88	357.47	587.58	476.31	115.24
21	NA	NA	NA	NA	NA

Table B.3.6 The remaining amount of ciprofloxacin HCl in cip-cpx –Carbopol gel the in dark

Time (hr)	Ciprofloxacin HCl remaining amount (mg/g gel)			Mean	SD
	Set1	Set2	Set3		
0	0.14	0.15	0.15	0.149	0.004
21	0.15	0.15	0.15	0.149	0.002

Table B.3.7 The remaining amount of ciprofloxacin HCl in cip-cpx-Carbopol gel when exposed to light

Time (hr)	Ciprofloxacin HCl remaining conc. (mg/g)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.15	0.16	0.16	0.16	0.00
3	0.10	0.12	0.13	0.11	0.02
6	0.11	0.08	0.09	0.09	0.01
9	0.09	0.06	0.08	0.08	0.02
14	0.04	0.04	0.05	0.04	0.00
17	0.03	0.04	0.02	0.03	0.01
21	0.02	0.03	0.01	0.02	0.01

Table B.3.8 The viscosity of Poloxamer 407 blank gel when exposed to light

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2356.40	2414.52	2266.40	2345.77	74.63
8	2239.96	2137.33	2374.10	2250.46	118.73
21	2310.06	2397.69	2234.45	2314.06	81.69

Table B.3.9 The viscosity of cip-cpx-Poloxamer gel in the dark

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2282.14	2124.45	2031.34	2145.98	126.78
21	2317.23	2093.41	2313.26	2241.30	128.09

Table B.3.10 The viscosity of cip-cpx-Poloxamer gel when exposed to the light

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2424.82	2314.14	2290.44	2343.13	71.73
8	2412.98	2431.26	2251.29	2365.18	99.05
21	2109.22	2158.97	2293.90	2187.36	95.56

Table B.3.11 The remaining amount of cip-cpx-Poloxamer gels in the dark

Time (hr)	Ciprofloxacin HCl remaining amount (mg/g)			Mean	SD
	Set1	Set2	Set3		
0	0.15	0.16	0.16	0.16	0.01
21	0.15	0.14	0.15	0.15	0.01

Table B.3.12 The remaining amount of ciprofloxacin HCl in cip-cpx-Poloxamer gel when exposed to light

Time (hr)	Ciprofloxacin HCl remaining conc. (mg/g gel)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.16	0.16	0.16	0.16	0.00
3	0.11	0.13	0.12	0.12	0.01
6	0.10	0.10	0.09	0.10	0.01
9	0.09	0.09	0.09	0.09	0.00
14	0.06	0.05	0.06	0.06	0.00
17	0.05	0.05	0.01	0.04	0.02
21	0.05	0.05	0.03	0.04	0.01

Table B.3.13 pH values of ciprofloxacin formulations before and after light exposure

Formulation		1	2	3	average	SD
ciprofloxacin solution	initial	7.31	7.24	7.37	7.31	0.07
	final	7.21	7.25	7.23	7.23	0.02
ciprofloxacin complex solution	initial	7.28	7.3	7.31	7.3	0.02
	final	7.3	7.19	7.27	7.25	0.06
cip-cpx-Poloxamer gel	initial	7.48	7.5	7.47	7.48	0.02
	final	7.39	7.41	7.34	7.38	0.04
cip-cpx-Carbopol gel	initial	7.44	7.49	7.38	7.44	0.06
	final	7.36	7.47	7.42	7.42	0.06

Table B.3.14 The k_{obs} of ciprofloxacin photodegradation in various dosage forms

Formulation	k_{obs} (hr^{-1})			Mean	SD
	Set1	Set2	Set3		
I	0.1106	0.1147	0.1256	0.1170	0.0078
II	0.098	0.0968	0.1031	0.0993	0.0033
III	0.0553	0.071	0.0733	0.0665	0.0098
IV	0.0926	0.0783	0.1182	0.0964	0.0202

Table B.3.15 ANOVA for the k_{obs} of ciprofloxacin photodegradation in various dosage forms

Source of Variation	df	Sum of Squares	Mean Square	F	p-value
Between Groups	3	0.004	0.001	9.120	0.006
Within Groups	8	0.001	0.0001096		
Total	11	0.005			

Table B.3.17 Tukey HSD test of the k_{obs} of ciprofloxacin photodegradation in various dosage forms

Formulation	Formulation	p-value
I	II	0.338
	III	0.004
	IV	0.231
II	I	0.338
	III	0.041
	IV	0.990
III	I	0.004
	II	0.041
	IV	0.062
IV	I	0.231
	II	0.990
	III	0.062

* The mean difference is significant at the 0.05 level.

I = blank Poloxamer 407 gel, II = blank Carbopol ETD 2020 gel, III = cip-cpx-Poloxamer gel and IV = cip-cpx-Carbopol gel

B.4 Stability studies of ciprofloxacin HCl- HP-ß-CD gels

Table B.4.1 Viscosity of Poloxamer 407 blank gels at 40 ºC, 75% RH

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2288.50	2313.02	2429.96	2343.83	75.59
30	2155.73	2234.74	2353.74	2248.07	99.68
60	2414.52	2457.48	2244.09	2372.03	112.86
90	2289.13	2308.28	2085.08	2227.50	123.71

Table B.4.2 Viscosity of cip-cpx-Poloxamer gel at 40 ºC, 75% RH

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2320.03	2148.07	2288.50	2252.20	91.55
30	2157.72	2251.29	2384.42	2264.48	113.92
60	2414.52	2246.41	2343.29	2334.74	84.38
90	2290.38	2176.53	2311.29	2259.40	72.52

Table B.4.3 The concentration of ciprofloxacin HCl in cip-cpx-Poloxamer gel at 40 ºC, 75% RH

Time (days)	Ciprofloxacin HCl remaining conc. (mg/g gel)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.16	0.16	0.16	0.16	0.00
5	0.16	0.16	0.16	0.16	0.00
15	0.14	0.14	0.15	0.14	0.01
30	0.15	0.15	0.15	0.15	0.00
45	0.15	0.15	0.15	0.15	0.00
60	0.14	0.16	0.14	0.15	0.01
90	0.14	0.12	0.14	0.13	0.01

Table B.4.4 Viscosity of cip-cpx-Poloxamer gel at ambient temperature.

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2331.03	2242.33	2405.59	2326.32	81.73
30	2311.97	2276.86	2204.56	2264.46	54.77
60	2311.98	2289.13	2030.67	2210.59	156.24
90	2169.19	2353.74	2318.95	2280.63	98.07

Table B.4.5 The concentration of ciprofloxacin HCl in cip-cpx-Poloxamer gel at ambient temperature

Time (days)	Ciprofloxacin HCl remaining conc. (mg/g gel)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.15	0.16	0.16	0.16	0.01
5	0.16	0.16	0.16	0.16	0.00
15	0.15	0.15	0.15	0.15	0.00
30	0.15	0.15	0.15	0.15	0.00
45	0.16	0.16	0.15	0.16	0.01
60	0.15	0.15	0.15	0.15	0.00
90	0.15	0.15	0.15	0.15	0.00

Table B.4.6 Viscosity of Carbopol ETD 2020 blank gel at 40 °C, 75% RH

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2228.17	2033.54	2084.58	2115.43	100.91
30	1778.88	1641.68	1639.37	1686.64	79.89
60	1295.30	1126.46	1218.70	1213.49	84.54
90	NA	NA	NA	NA	NA

Table B.4.7 Viscosity of cip-cpx-Carbopol gel at 40 °C 75% RH

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2103.07	2228.17	2017.83	2116.36	105.79
30	1828.79	1842.16	1641.68	1770.87	112.09
60	1259.13	1015.91	1086.52	1120.52	125.13
90	NA	NA	NA	NA	NA

Table B.4.8 The remaining amount of ciprofloxacin HCl in cip-cpx-Carbopol gel at 40 °C, 75% RH

Time (days)	Ciprofloxacin HCl remaining amount (mg/g gel)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.15	0.15	0.15	0.15	0.00
5	0.15	0.15	0.15	0.15	0.00
15	0.15	0.15	0.15	0.15	0.00
30	0.14	0.14	0.14	0.14	0.00
45	0.13	0.14	0.14	0.14	0.01
60	0.12	0.13	0.13	0.13	0.01
90	0.11	0.09	0.13	0.11	0.02

Table B.4.9 Viscosity of cip-cpx-Carbopol gel at ambient temperature.

Time (hr)	Viscosity (cP)			Mean	SD
	Set1	Set2	Set3		
0	2128.45	2094.41	2201.07	2141.31	54.48049
30	1931.34	2004.23	1895.13	1943.567	55.56817
60	1343.03	1430.25	1289.36	1354.213	71.10765
90	NA	NA	NA	NA	NA

Table B.4.10 The remaining amount of ciprofloxacin HCl in cip-cpx-Carbopol gel at ambient temperature

Time (days)	Ciprofloxacin HCl remaining amount (mg/ g gel)			Mean	SD
	Set 1	Set 2	Set 3		
0	0.15	0.15	0.16	0.15	0.01
5	0.15	0.15	0.16	0.15	0.01
15	0.15	0.15	0.14	0.15	0.01
30	0.14	0.14	0.15	0.14	0.01
45	0.15	0.15	0.14	0.15	0.01
60	0.13	0.11	0.15	0.13	0.02
90	0.13	0.13	0.14	0.13	0.01

Table B.4.11 pH values of tested samples at 40 °C 75 % RH

Formulation		1	2	3	Mean	SD
I	initial	7.46	7.56	7.53	7.52	0.05
	final	7.42	7.39	7.48	7.43	0.05
II	initial	7.47	7.50	7.42	7.46	0.04
	final	7.47	7.56	7.54	7.52	0.05
III	initial	7.48	7.53	7.39	7.47	0.07
	final	7.36	7.42	7.45	7.41	0.05
IV	initial	7.47	7.52	7.48	7.49	0.03
	final	7.38	7.40	7.42	7.40	0.02

I = blank Poloxamer 407 gel, II = blank Carbopol ETD 2020 gel, III = cip-cpx-Poloxamer gel and IV = cip-cpx-Carbopol gel

Table B.4.12 pH values of tested samples at ambient temperature

Formulation		1	2	3	Mean	SD
I	initial	7.38	7.41	7.48	7.42	0.05
	final	7.44	7.49	7.52	7.48	0.04
II	initial	7.54	7.47	7.56	7.52	0.05
	final	7.45	7.55	7.61	7.54	0.08

II = ciprofloxacin HCl complex in Poloxamer 407 gel and II = ciprofloxacin HCl complex in Carbopol ETD 2020 gel

B.5 *In vitro* release study of ciprofloxacin from gel

Table B.5.1 The release data of ciprofloxacin HCl from cip-cpx-gel

Time (hr)	% Cumulative release of ciprofloxacin HCl			Mean	SD
	Set 1	Set 2	Set 3		
0	0.00	0.00	0.00	0.00	0.00
1	1.94	2.08	2.09	2.04	0.09
2	3.77	2.26	3.42	3.15	0.79
3	4.25	4.40	5.70	4.78	0.80
4	6.28	7.57	6.93	6.93	0.64
5	8.94	9.17	6.93	8.35	1.23
6	11.23	13.78	7.41	10.81	3.21
7	11.61	13.78	10.00	11.80	1.90
8	12.41	14.43	12.24	13.03	1.22
12	16.84	17.90	17.64	17.46	0.55
14	19.39	20.44	20.71	20.18	0.70
16	20.45	21.04	20.71	20.73	0.30
18	27.96	28.70	25.74	27.46	1.54
20	28.14	29.70	28.99	28.94	0.78
24	32.12	32.94	32.82	32.63	0.44

Table B.5.2 The release data of ciprofloxacin HCl from inclusion complex solution

Time (hr)	% Cumulative release of ciprofloxacin HCl			Mean	SD
	Set 1	Set 2	Set 3		
0	0	0	0	0.00	0.00
1	5.31	5.71	5.05	5.36	0.34
2	8.11	7.69	7.41	7.73	0.35
3	11.65	10.94	9.61	10.73	1.03
4	12.42	13.78	13.12	13.10	0.68
5	12.94	15.50	14.34	14.26	1.28
6	19.81	19.06	14.73	17.86	2.74
7	17.92	20.04	17.30	18.42	1.44
8	18.00	21.57	20.72	20.10	1.87
12	23.65	28.68	23.60	25.31	2.92
14	26.06	31.09	26.87	28.01	2.70
16	28.13	33.42	29.19	30.25	2.80
18	28.42	35.12	30.05	31.20	3.50
20	29.59	37.43	34.09	33.70	3.94

Table B.5.3 The release data of free ciprofloxacin HCl solution

Time (hr)	% Cumulative release of ciprofloxacin HCl			Mean	SD
	Set 1	Set 2	Set 3		
0	0.00	0.00	0.00	0.00	0.00
1	7.53	9.25	8.12	8.30	0.88
2	13.50	14.20	13.11	13.60	0.55
3	15.28	15.50	14.87	15.22	0.32
4	19.46	20.45	18.91	19.61	0.78
5	19.61	22.49	18.91	20.34	1.90
6	27.00	27.85	25.14	26.66	1.38
7	28.31	27.85	26.09	27.42	1.17
8	28.31	30.50	27.49	28.77	1.56
12	28.31	30.50	27.59	28.80	1.52
14	33.20	35.54	35.24	34.66	1.27
16	33.20	35.54	35.24	34.66	1.27
18	37.46	35.79	36.19	36.48	0.88
20	37.46	35.79	38.19	37.15	1.23
22	37.46	35.79	38.19	37.15	1.23
24	38.72	41.54	44.91	41.73	3.10

VITA

Miss Eua-apha Hamvanich was born in July 2, 1981 in Bangkok, Thailand. She has finished her highschool in Saint Joseph Convent School, Bangkok, Thailand in 1999. After that, she has attended the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. In 2004, she received her Bachelor Degree of Science in Pharmacy with second class honor. Fortunately after graduation, she got an IAESTE (the International Association of Exchange Student Technical Experience) scholarship to be a pharmacist trainee at Novo Nordisk A/S, Copenhagen, Denmark. As soon as she returned to Thailand, she was entering the Master's Degree Programme in Pharmacy at Chulalongkorn University. She participated in Chulalongkorn-Chiba University exchange student program in 2005. In 2006, she was selected to be given *the student of the year award* by the Faculty of Pharmaceutical Sciences.